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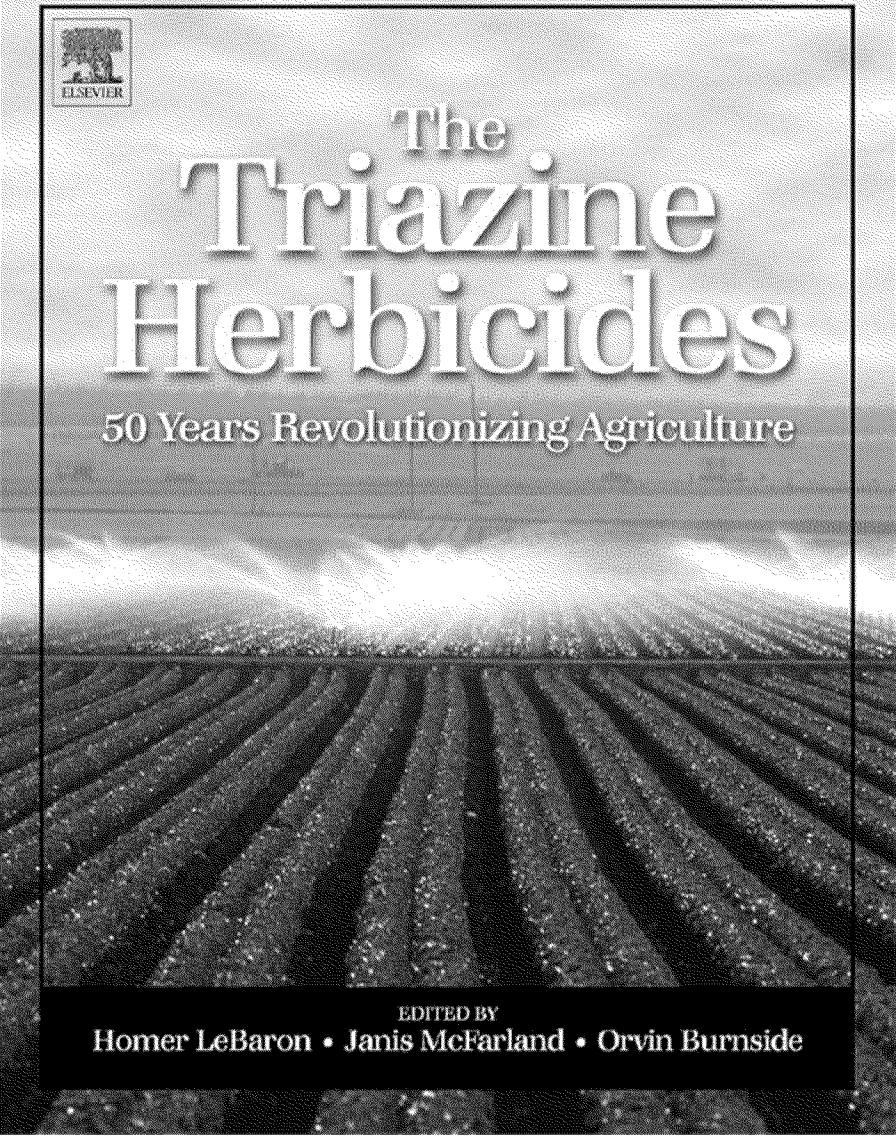
The Triazine Herbicides

50 Years Revolutionizing Agriculture



EDITED BY
Homer LeBaron • Janis McFarland • Orvin Burnside

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and Orvin C. Burnside

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Contents

Foreword ix
List of Contributors xi

Chapter 1
The Triazine Herbicides: A Milestone in the Development of Weed Control Technology 1
Homer M. LeBaron, Janis E. McFarland, and Orvin C. Burnside

Chapter 2
History of the Discovery and Development of Triazine Herbicides 13
Gustav Müller

Chapter 3
Production, Development, and Registration of Triazine Herbicides 31
Walter Heri, Frank Pfi ster, Beth Carroll, Thomas Parshley, and James B. Nabors

Chapter 4
Weed Control Trends and Practices in North America 45
David R. Pike, Ellery L. Knake, and Marshal D. McGlamery

Chapter 5
Farming Trends and Practices in Northern Europe 57
James H. Orson

Chapter 6
Biology and Ecology of Weeds and the Impact of Triazine Herbicides 63
Homer M. LeBaron and Gustav Müller

Chapter 7
Plant Uptake and Metabolism of Triazine Herbicides 73
Bruce J. Simoneaux and Thomas J. Gould

Chapter 8
The Mode of Action of Triazine Herbicides in Plants 101
Achim Trebst

Chapter 9
Basis of Crop Selectivity and Weed Resistance to Triazine Herbicides 111
Amit Shukla and Malcolm D. Devine

Chapter 10
Distribution and Management of Triazine-Resistant Weeds 119
Homer M. LeBaron

Chapter 11
Weeds Resistant to Nontriazine Classes of Herbicides 133
Homer M. LeBaron and Eugene R. Hill

Chapter 12
The Use of Economic Benefit Models in Estimating the Value of Triazine Herbicides 153
Gerald A. Carlson

Chapter 13
Benefits of Triazine Herbicides in Corn and Sorghum Production 163
David C. Bridges

Chapter 14
Benefits of Triazine Herbicides in Ecofallow 175
David L. Regehr and Charles A. Norwood

Chapter 15
Weed Control in Sugarcane and the Role of Triazine Herbicides 185
Dudley T. Smith, Edward P. Richard Jr., and Lance T. Santo

Chapter 16
Benefits of Triazine Herbicides and Other Weed Control Technology in Citrus Management 199
Megh Singh and Shiv D. Sharma

Chapter 17
Triazine Herbicides for Weed Control in Fruit and Nut Crops 211
Clyde L. Elmore and Arthur H. Lange

Chapter 18
Benefits of Triazine Herbicides in the Production of Ornamentals and Conifer Trees 225
John F. Ahrens and Michael Newton

Chapter 19
Benefits of Triazine Herbicides in Turf 235
G. Euel Coats, James M. Taylor, and Steven T. Kelly

Chapter 20
Methods of Analysis for Triazine Herbicides and Their Metabolites 243
Richard A. McLaughlin, V. Michael Barringer, James F. Brady, and Robert A. Yokley

Chapter 21
Triazine Soil Interactions 275
David A. Laird and William C. Koskinen

Chapter 22
Microbial Degradation of *s*-Triazine Herbicides 301
Raphi T. Mandelbaum, Michael J. Sadowsky, and Lawrence P. Wackett

Chapter 23
Nonbiological Degradation of Triazine Herbicides: Photolysis and Hydrolysis 329
Allan J. Cessna

Chapter 24
Soil Movement and Persistence of Triazine Herbicides 355
William C. Koskinen and Philip A. Banks

Chapter 25
Hazard Assessment for Selected Symmetrical and Asymmetrical Triazine Herbicides 387
Charles B. Breckenridge, Christoph Werner, James T. Stevens, and Darrell D. Sumner

Chapter 26
Mode of Action of Atrazine for Mammary Tumor Formation in the Female Sprague-Dawley Rats 399
J. Charles Eldridge and Lawrence T. Wetzel

Chapter 27
Dietary Exposure Assessment of the Triazine Herbicides 413
Leslie D. Bray, Arpad Z. Szarka, Nina E. Heard, Dennis S. Hackett, and Robert A. Kahrs

Chapter 28
Probabilistic Assessment of Laboratory-Derived Acute Toxicity Data for the Triazine Herbicides to Aquatic Organisms 425
Keith R. Solomon and Dennis Cooper

Chapter 29
Atrazine and Simazine Monitoring Data in Community Water Systems in the United States during 1993 to 2000 439
Dennis P. Tierney, B. R. Christensen, Cheryl Dando, and Kendra M. Marut

Chapter 30
A Decade of Measuring, Monitoring, and Studying the Fate and Transport of Triazine Herbicides and their Degradation Products in Groundwater, Surface Water, Reservoirs, and Precipitation by the US Geological Survey 451
E. Michael Thurman and Elisabeth A. Scribner

Chapter 31
Probabilistic Risk Assessment Using Atrazine and Simazine as a Model 477
Robert L. Sielken Jr., Robert S. Bretzlaff, and Ciriaco Valdez-Flores

Chapter 32
Progress in Best Management Practices 501
John F. Hebblethwaite and Carol N. Somody

Chapter 33
Environmental Benefits of Triazine Use in Conservation Tillage 519
Richard S. Fawcett

Chapter 34
Role of Triazine Herbicides in Sustainable Agriculture: Potential of Nonchemical Weed Control Methods as Substitutes for Herbicides in United States Corn Production 527
Leonard P. Gianessi

Chapter 35
Environmental Stewardship: The Roots of a Family Farm 541
Jere White

Appendix 549

Index 575

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Foreword

By Dennis T. Avery, Center for Global Food Issues, Hudson Institute

'Growing more crops and trees per acre leaves more land for Nature. We cannot choose between feeding malnourished children and saving endangered wild species. Without higher yields, peasant farmers will destroy the wildlands and species to keep their children from starving. Sustainably higher yields of crops and trees are the only visible way to save both.'

Dr. Norman Bourlaug
1970 Nobel Peace Prize laureate and father of the Green Revolution, in 'Growing More per Acre Leaves More Land for Nature,' Center for Global Food Issues, www.highyieldconservation.org
April 30, 2002

This is an important book containing a great deal of solid information about the triazine herbicides, one of the most important families of chemicals that support human society and protect our wildlife.

Just as chemistry protects children from disease, farmers are using chemistry to feed twice as many people as they did 50 years ago – without using more land. They have tripled the yields on the planet's best cropland using high-powered seeds, chemical fertilizers, irrigation, and pesticides. Without higher yields, people would already have cleared all of the world's 16 million square miles of forest to get today's food supply. Virtually every forest tree and creature alive on the planet today owes its existence to high-yield farmers and their chemicals. If we ban the pesticides, we almost literally ban forests and wildlife.

Pesticides have played a key role in the world's rising crop yields. As the authors in this book note, the Green Revolution's plant breeding miracles and fertilizers might have failed to prevent massive human starvation and wildlands destruction if the higher yield potential of our crop fields had simply nourished more bugs and weeds.

Even though birth rates are dropping all over the world, thanks to increases in food security, affluence, and urbanization, the world's population will probably exceed 8 billion (up from today's 6.5 billion) by 2030 and might reach 9 billion by 2050.[1] Rising incomes indicate that we'll provide high-quality diets (resource-costly meat, milk, and fruit) for perhaps 8 billion people in 2050, instead of for the one billion who can afford them today. There will even be a pet food challenge, with perhaps 500 million companion cats and dogs in an affluent, one-child China alone.

Overall, we will need to harvest nearly three times as much farm output in 2050 as we harvest today – and we're already farming half the global land area not under deserts or glaciers. Pest control will remain vital to both people and wildlife.

Interestingly, if we chart the pesticide usage in various countries for the past 70 years alongside life expectancy, they rise in parallel. At the same time, age-adjusted cancer risks for nonsmokers have been declining. The use of chemistry in medicines and public health interventions has had more direct human health impact, but pesticides help reduce the real cost of fruits and vegetables. That's vital, because the 25% of people who eat the most produce have only half the total cancer risks of the 25% who eat the least!

Dr. Bruce Ames, who received the National Science Medal from President Clinton, documented that we get 100 000 times as much cancer risk from the natural chemicals in the foods we eat as from the tiny traces of pesticide on our foods and in our drinking water.

The Soil and Water Conservation Society of America has declared that modern high-yield farming is the most sustainable in history. This is in substantial part because of pesticides, and particularly because of conservation tillage made possible by herbicides.

You will read a great deal in this book about herbicides and soil conservation because the triazine herbicides have helped create a soil conservation miracle. Soil erosion for thousands of years was the greatest risk to the sustainability

[1] United Nations Department of Economics and Social Affairs, Population Division. World Population Prospects: The 2006 Revision, Population Database. http://esa.un.org/unpp.

of human society. The soils of the Mediterranean Basin were nearly ruined in ancient times by plowing and low-yield cropping. But because we have tripled crop yields, we need to plant less than one-third as much land to get our food supply, and that lets us use the safest, least-erodible soils. (That's why my hilly, rocky Shenandoah Valley is now in grass for dairy and beef cattle, while the corn is grown on the deeper, more level soils of the Corn Belt.)

Equally important, modern herbicides like the triazines allow us to substitute low-till farming systems for the ancient, erosion-inviting 'bare-earth' farming techniques such as plowing, hoeing, and 'clean-fallow.' (Fallow keeps land bare for a whole season with repeated tillage to reduce the number of lurking weed seeds.)

The development of herbicides that consistently and cost-effectively kill both grasses and broad-leaved weeds through the crop-growing season has enabled farmers to adopt low-till farming on millions of hectares of land around the world. Low-till farming cuts water runoff and soil erosion by up to 95% and can double the moisture retained in the field for crop growth. Dr. Stanley Trimble of UCLA reported that the Coon Creek watershed (a famously erodible hilly region in southern Wisconsin) is now suffering only 6% as much soil loss as it did during the Dust Bowl days of the 1930s. Low-till farming systems have played a key role in enabling today's Coon Creek farmers to build topsoil in the midst of the highest-yield cropping in the region's history.

Weeds are our real competitor for space on the planet, so there is no end in sight to our need for herbicides. To those who warn that weeds are developing resistance to the various widely used herbicides, I say that resistance makes it even more important to have a variety of herbicides on the shelves, ready for rotational use against the weeds. We must encourage still more research in new herbicides with different modes of action. We must also maintain our use of those safe and effective herbicides already on the market. In fact, after a decade-long, comprehensive scientific evaluation, the USEPA has recently reaffirmed the safety of two important herbicides in the triazine family – atrazine and simazine.

It has been only a half-century since better weed-control technology allowed most of us to escape from the drudgery of stoop labor. Without the triazine chemistry, there is little question that millions of us would have to go back into the fields with short-handled hoes. Our society would today be substantially less sustainable and our wildlands and wild species would face far greater pressures from the plows of low-yield farmers.

Only with weed control can we both feed our growing population and protect critical environmental resources.

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Chapter 1

The Triazine Herbicides: A Milestone in the Development of Weed Control Technology

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Summary

This book is about the revolutionary impact of the triazines herbicides, likely the most important class of agricultural chemicals ever developed. For five decades the triazines have provided weed control in more than 50 crops around the world and have helped farmers boost yields and produce enough food to feed a rising global population. The triazine herbicides, and especially atrazine, are the most well-researched herbicides in history, with thousands of scientific studies on their safety to humans and the environment. Data from studies on the triazines have been evaluated extensively by regulatory authorities around the globe to ensure their safe use.

The first triazine was discovered in 1952 at J.R. Geigy, Ltd. in Switzerland and led to major advances in agricultural practices, basic research, safety testing, and environmental stewardship. Today one or more of the triazine herbicides is registered in more than 100 countries around the globe to provide broad-spectrum weed control in a variety of crops and noncrop sites. They provide application flexibility, are extensively used in conservation tillage programs that are integral to sustainable agriculture, and are important contributors to the management of weed biotypes that have developed resistance to other classes of herbicides.

The triazine herbicides are essential for high-yield, sustainable agriculture. They are critical to integrated pest management (IPM) and conservation tillage practices in corn and other crops – reducing the devastating environmental impact of erosion, reducing fuel costs, and retaining moisture in soil.

Changes in Agriculture and the Importance of the Triazine Herbicides

Since the 1900s, there have been significant improvements in agriculture yields, with average increases ranging from 238% to 811% for corn, cotton, sorghum, soybean, wheat, potato, and tomato (Table 1.1). From an average corn yield of 2.76 metric tonnes/ha during 1950–1959, yields of 8.87 metric tonnes/ha were obtained during 2000–2004. Since the late 1950s, the triazine herbicides have contributed significantly to improvements in yields in crops around the world.

The historical record reveals that herbicides have replaced or reduced the use of hand weeding and cultivation for weed control, with an associated reduction in cost and an increase in yield. Today herbicides are used routinely on more than 90% of the area of most US crops, representing 87 million ha of cropland (Gianessi and Reigner, 2007).

There is a need for continued increases in yields not only to feed a growing world population, but also for greater fuel production (OECD-FAO, 2007). For example, US ethanol production, predominately based on corn, is expected to double between 2006 and 2016 (Figure 1.1). By 2016, ethanol is expected to represent a full one-third of corn production. Corn used for fuel in China is expected to increase from 3.5 million tons in 2006 to 9 million tons in 2016 (Figure 1.2). Ethanol production in Brazil is predominately based on sugarcane and is expected to increase by 145% between 2006 and 2016 (Figure 1.3).

The first triazine was discovered in 1952 at J.R. Geigy, Ltd. in Switzerland. Today one or more of the triazine herbicides are registered in more than 100 countries around the world and are key to the production of more than 50 crops. Table 1.2 shows the major triazine herbicides today and their key uses.

The use volumes in the United States by major crops are shown in Figure 1.4 for atrazine and Figure 1.5 for simazine.

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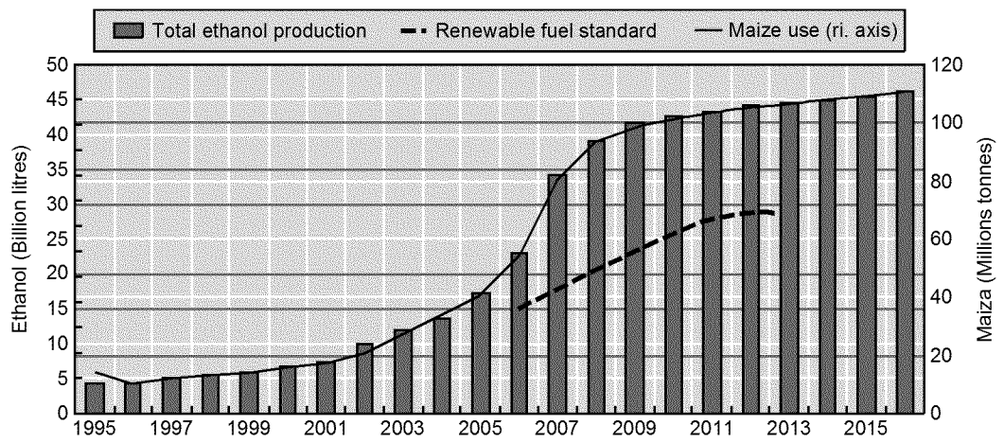
Table 1.1 US average yield in metric tonnes/ha and percent change for 10-year periods through 1999 and for the 5-year period of 2000 through 2004[a]

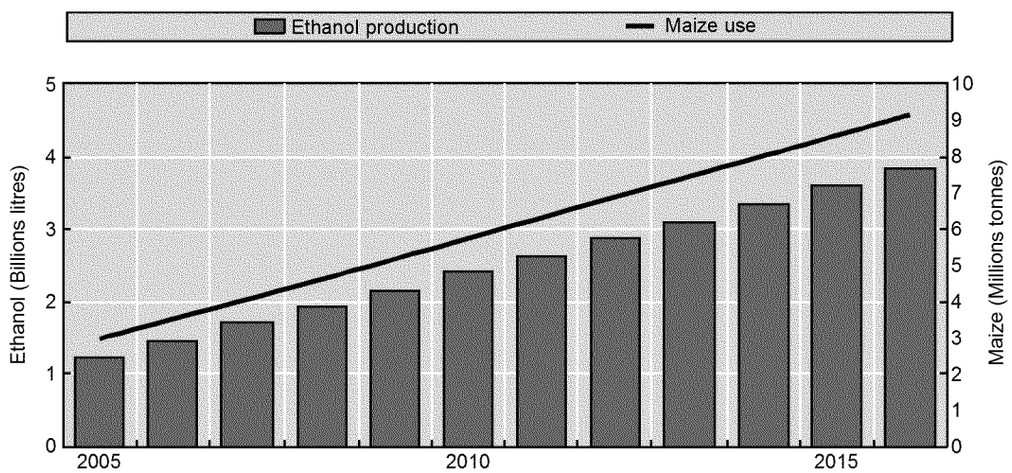
Period	Corn for grain	Wheat for grain	Sorghum for grain	Soybean for beans	Lint cotton	Potato	Processing tomatoes
1900–1909	1.69 (100)[b]	0.97 (100)[b]	NA	NA	0.207 (100)[b]	6.4 (100)[b]	NA
1910–1919	1.63 (96)	0.95 (98)	NA	NA	0.206 (100)	6.5 (102)	NA
1920–1929	1.69 (100)	0.94 (97)	NA	NA	0.183 (88)	7.5 (117)	10.1 (100)[b]
1930–1939	1.51 (89)	0.89 (92)	0.80 (100)[b]	1.08 (100)[b]	0.231 (112)	7.6 (119)	9.4 (93)
1940–1949	2.13 (126)	1.15 (119)	1.10 (138)	1.27 (118)	0.298 (144)	11.3 (177)	13.2 (131)
1950–1959	2.76 (163)	1.32 (136)	1.49 (186)	1.44 (133)	0.406 (196)	18.5 (289)	24.0 (238)
1960–1969	4.46 (264)	1.78 (184)	3.00 (375)	1.67 (155)	0.536 (259)	22.9 (358)	36.8 (364)
1970–1979	5.59 (331)	2.11 (218)	3.39 (424)	1.88 (174)	0.532 (257)	27.8 (434)	48.4 (479)
1980–1989	6.65 (394)	2.41 (248)	3.75 (469)	2.04 (189)	0.647 (313)	31.8 (497)	59.2 (586)
1990–1999	7.76 (459)	2.60 (268)	4.12 (515)	2.47 (229)	0.725 (350)	37.0 (578)	73.2 (725)
2000–2004	8.87 (525)	2.75 (283)	3.95 (493)	2.58 (238)	0.79 (381)	42.8 (669)	81.9 (811)

[a] This table has been modified and updated from Warren (1998) as averages from USDA National Agricultural Statistics Service data.
[b] The numbers in parentheses are percentages of increases or decreases based on the average yields of the crops in the first decade given.



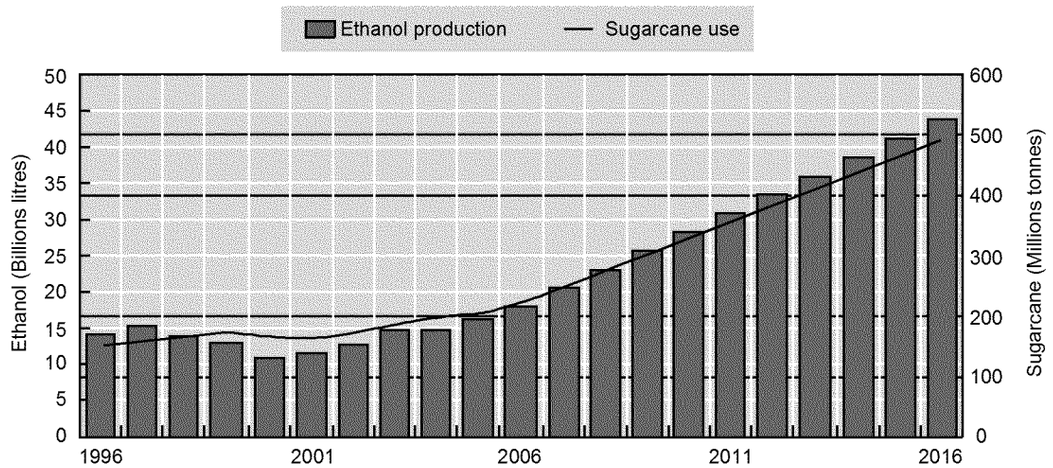
Source: ERS.

Figure 1.1 Expansion of US ethanol production and corresponding use of corn (maize) (figure from OECD-FAO).



Source: ERS.

Figure 1.2 Expansion of Chinese ethanol production and corresponding use of corn (maize) (figure from OECD-FAO).



Source: OECD and FAO Secretariats.

Figure 1.3 Expansion of Brazil ethanol production and corresponding use of sugarcane (figure from OECD-FAO).

Table 1.2 Major triazine herbicides and a partial listing of key uses

Triazine herbicide	Uses
Ametryn	Sugarcane, corn, pineapple
Atrazine	Corn, sorghum, sugarcane
Hexazinone	Alfalfa, sugarcane, forestry, noncropland
Metamitron	Sugarbeet, other beet crops
Metribuzin	Sugarcane, potato, soybean
Prometon	Noncropland
Prometryn	Cotton, celery
Simazine	Corn, citrus, grape, apple, almond, walnut, peach, filbert, pear
Terbuthylazine	Corn, sorghum, grape
Terbutryn	Sugarcane, cereal

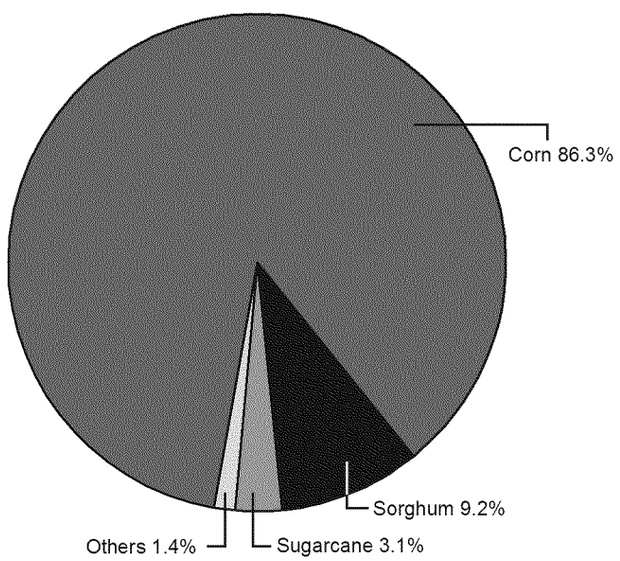


Figure 1.4 Average atrazine use by crop in the United States for 2000–2002 (Doane Marketing Research, Inc.).

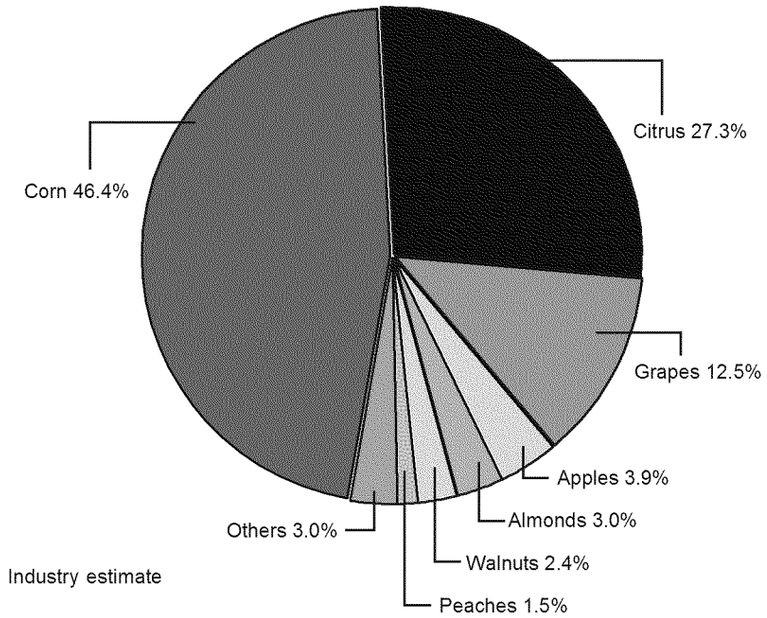


Figure 1.5 Average simazine use by crop in the United States for 2000–2002 (Doane Marketing Research, Inc.).

Table 1.3 Major corn production countries in the world (thousands of metric tonnes)[a]

Country	2003/2004	2004/2005
United States	256278	299917
China	115830	128000
Brazil	42000	37500
Mexico	21800	22000
Argentina	15000	19500

[a] US Department of Agriculture (USDA) Foreign Agricultural Service (2005).

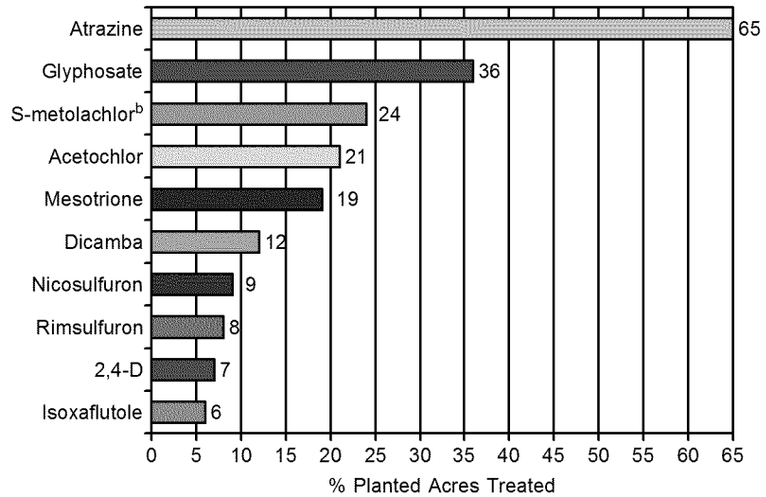
Atrazine is by far the mostly widely used of the triazines, and corn is its major crop use. Table 1.3 shows the top five corn-producing countries in the world. Atrazine is a critical component in the herbicide programs of each of these countries.

One of the reasons the triazines are so important in corn and other crops around the world is their application flexibility and their ability to mix with other herbicides for broad-spectrum weed control. Figure 1.6 demonstrates the relative importance of atrazine in corn compared to other herbicides.

Using atrazine again as an example, Table 1.4 shows a list of nontriazine herbicides used in US corn and the percentage of acres treated with nontriazines that also receive an atrazine treatment.

Many of the active ingredients in Table 1.4 were developed to be atrazine alternatives, but are more valuable to the farmer and provide broader-spectrum weed control when used with atrazine. Specifically, the broadleaf products 2,4-D, bromoxynil, clopyralid, dicamba, flumetsulam, halosulfuron, mesotrione, and prosulfuron are combined with atrazine on 69–82% of their acres. The grass products, *S*-metolachlor, acetochlor, dimethenamid, and nicosulfuron are used with atrazine on 87–97% of their acres. Even the nonselective products glyphosate and glufosinate that are used in genetically modified corn also use atrazine on a large percentage of their acres. Through the use of atrazine with the above herbicides, the average application rate of atrazine in the United States has declined from approximately 2 lb/A (2.24 kg/ha) in 1984 to 1.1 lb/A (1.24 kg/ha) in 2005.

Just as atrazine is important in corn, simazine is a pre-emergence triazine that provides broad-spectrum residual weed control in many of the important fruit and nut crops when applied either alone or in combination with a contact product such as glyphosate to control weeds at the time of application (Figure 1.5).



[a] Based on data from Doane Market Research.
[b] Also includes metolachlor products.

Figure 1.6 Top 10 corn herbicides in United States in 2005 as base acres treated (% of planted acres).[a]

Table 1.4 Percentage of the corn base acres treated with nontriazine herbicides that are also treated with atrazine in 2005

Herbicide	Also treated with atrazine (% acres)
2,4-D	69
Acetochlor	87
Bromoxynil	81
Carfentrazone	80
Clopyralid	73
Dicamba	70
Dimethenamid	97
Flumetsulam	73
Glufosinate	64
Glyphosate	45
Halosulfuron	74
Imazethapyr	51
Isoxaflutole	66
Mesotrione	82
Nicosulfuron	70
Pendimethalin	74
Primisulfuron	76
Prosulfuron	82
Rimsulfuron	72
S-metolachlor	89

[a] Based on data from Doane Marketing Research.

Environmental Benefits of the Triazines

The triazines provide excellent residual pre-emergence weed control and can also be applied with burndown products for control of existing vegetation in no-till or conservation tillage programs. Some of the triazines, such as atrazine and metribuzin, can be used early post-emergence for control of broadleaf weeds and grasses. These unique biological properties of the triazines enable farmers to use no-till and conservation tillage systems that greatly reduce soil erosion and minimize the damage erosion and pollution cause to our lakes, rivers, reservoirs, and water supplies.

Much of today's understanding of the importance of conservation tillage in agriculture began with the US Dust Bowl of the 1930s, an event largely precipitated by extensive plowing to convert grassland acres to wheat and other crops. Though conventional tillage practices were used successfully during times of adequate rainfall, after several droughts, plowing promoted significant wind erosion (Worster, 1979). On April 14, 1935, the powder-dry soil of the

Table 1.5 Estimates of annual off-site damage from soil erosion by damage category in the United States[a]

Damage category	Off-site damage in $ (millions)
Freshwater recreation	2080
Municipal and industrial use	1196
Water storage	1090
Flooding	978
Municipal water treatment	964
Navigation	749
Marine recreation	599
Roadside ditches	535
Marine commercial fishing	390
Irrigation ditches	118
Freshwater commercial fi shing	60
Steam power cooling	24
Total	8783

[a] From Riboudo, 1989.

Table 1.6 Conservation tillage in the United States as a percent of total crop acres[a]

Tillage system	1990	1996	2004
No-till	6.0	14.8	22.6
Ridge-till	1.1	1.2	0.8
Mulch-till	19.0	19.8	17.4
All conservation tillage	26.1	35.8	40.7

[a] Conservation Technology Information Center (2004).

Great Plains created what was described as a 'black blizzard' (Hurt, 1977, 1981). The disastrous events of the Dust Bowl led to the US Soil Erosion Service Act of 1935, which declared soil erosion a national menace and directed the US Department of Agriculture to establish the Soil Conservation Service (Wehrwein, 1938).

Soil erosion continues to be one of the greatest threats to the sustainability of agriculture around the world. Erosion caused by water and wind reduces rich topsoil and crop yields. Soil erosion also produces a variety of adverse off-site impacts, including increased sedimentation of lakes and streams and transport of nutrients and pesticides to surface waters (Ribaudo and Johansson, 2006) (Table 1.5).

Due to the adoption of conservation tillage systems by farmers around the world, great strides have been made to reduce erosion and its adverse impacts. Using herbicides in conservation tillage has significantly reduced topsoil erosion by more than 50% (Ray and Guzzo, 1993) and in some cases by more than 90% (Laflen *et al.*, 1978). The 2001 National Resources Inventory (Natural Resources Conservation Service, 2003) showed dramatic decreases in erosion in the United States since 1982, much of it due to adoption of conservation tillage. Sheet and rill (water) erosion fell from an average 4.0 tonnes/A/year in 1982 to 2.7 tons/A/year in 2001, a 33% drop. The average wind erosion rate dropped 36% during the same period.

The growth in conservation tillage continues today. In fact, the percentage of no-till acres in the United States grew from 6.0% to 22.6% between 1990 and 2004 as shown in Table 1.6 [Conservation Technology Information Center (CTIC, 2004)]. Conservation tillage was used on more than 40% of all crop acres.

Herbicides, especially the triazine herbicides, have played an essential role in the adoption of conservation tillage by substituting for intensive conventional tillage. For example, atrazine is used on 61.7% of conventional tillage corn in 2004, but on 84.1% of conservation tillage corn (Fawcett, 2007). A 2000 US Doane AgroTrak survey shows that 82% of no-till corn was treated with atrazine, compared to 70% under conservation tillage and 68% under conventional tillage. These results show that atrazine's importance increases as tillage decreases. It is estimated that erosion would increase by 252 million tonnes/year (Fawcett, 2007) if current conservation tillage practices in US corn reverted to conventional tillage.

By enabling conservation tillage, the triazine herbicides also help significantly reduce fuel use since fewer tillage trips are made across the field. A conventional tillage system consumes about 5.3 gal fuel/A, a mulch tillage system uses about 3.3 gal/A, and no-till uses about 1.4 gal/A (Ayers, 1989; Jasa *et al.*, 1991). Conversion from conventional tillage to no-till for row crops results in a savings that is equivalent to 3.9 gal/A of diesel fuel, for a reduction of 74%.

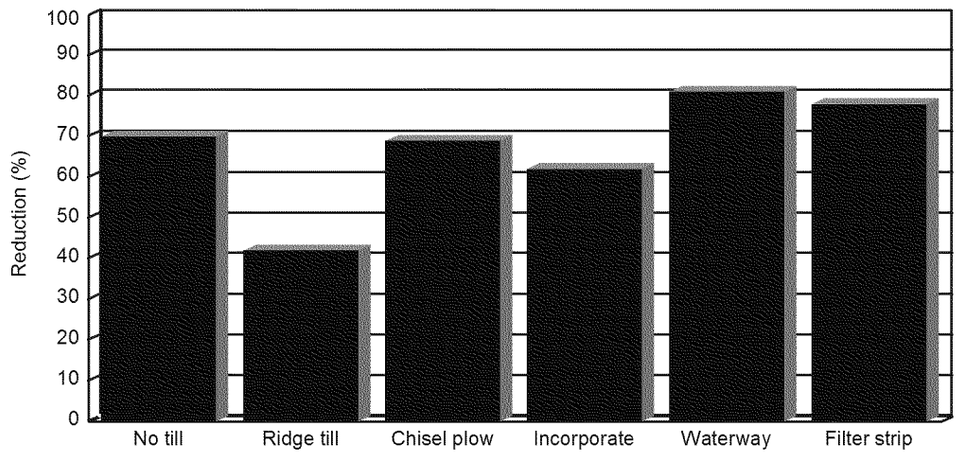


Figure 1.7 Best management practices reduce herbicide runoff (from Ciba-Geigy Technical Report: 10–92).

It is estimated that fuel use would increase by 89 million gallons/year if all corn crop acreage in the United States alone were conventionally tilled (Fawcett, 2007).

Besides saving soil and fuel, the triazines are important tools in conservation tillage systems that conserve soil moisture by reducing evaporation caused by tillage (Fawcett, 2007). Moisture conservation is especially important in the semi-arid areas around the world where grain crops can be grown only by 'fallowing' land and storing soil moisture for all or part of a growing season. When crops are not present and the land is fallow, weeds must be controlled to prevent reductions in soil moisture. The triazines are key to controlling weeds under these conditions and maximize moisture conservation by removing the need for repeated tillage.

Converting from conventional to conservation tillage also can increase the organic matter in soil, rather than continuing to deplete it. During a 10-year study, conservation tillage treatments in a corn–wheat–soybeans–wheat rotation accumulated organic matter at a rate of about 1700 lb/A/year (about 1900 kg/ha/year) faster than conventional tillage treatments (Reicosky *et al.*, 1995). Besides improving soil properties, conservation tillage has the potential to sequester as much as 107 million metric tons of carbon annually in the United States (USDA, 2004).

Conservation tillage and no-till often produce dramatic decreases in water runoff and increases in water infiltration, which results in a reduction not only in soil erosion, but also in pesticide and nutrient runoff into water (Glenn and Angle, 1987; Hall *et al.*, 1991). In several studies of best management practices (BMPs), no-till was shown to reduce herbicide runoff by an average of 70%, while ridge till showed a 40% reduction (Figure 1.7).

Because soil sediment has an extremely negative impact on streams, rivers, and lakes, erosion reductions credited to conservation tillage provide major benefits to aquatic ecosystems. Additionally, conservation tillage benefits wildlife by providing more crop residues for cover, more food sources (grain and weed seed left on the soil surface, as well as a greater number and variety of invertebrates), and less field disturbance.

By making it possible to produce more food and feed on fewer acres, the triazines have provided a direct benefit to the environment. As a result, much of our more vulnerable and erodible land has remained undisturbed as wildlife refuges, wetlands, or other natural ecosystems because the triazines have been critical in increasing yield on acres already in production.

IPM and Resistance Management

The unique action of the triazines also makes them vital to IPM, sustainable agriculture, and resistant weed management strategies. Triazines are an excellent option in IPM programs because of their effectiveness on a broad-spectrum of weeds. In the case of atrazine, its flexibility to be used in early pre-plant, preemergence or postemergence applications, and its utility in combination with other products are also key to IPM. For particularly invasive weeds already known to have resistant biotypes to other herbicides (kochia, common cocklebur, smooth pigweed, Palmer pigweed, tall waterhemp, common waterhemp, and wild sunflower), atrazine is the only product that can be applied either pre- or postemergence and provide effective control.

While newer herbicides are continually being developed, weeds are evolving resistance to these new alternatives very quickly. In addition, weeds that do develop resistance to nontriazine herbicides are generally more difficult

Table 1.7 Role of triazines in management of weeds with resistant biotypes

Family/mode of action	Triazines effective on some resistant biotypes
ACCase Inhibitors (A/1)[a]	Yes
ALS inhibitors (B/2)	Yes
Ureas and amides (C2/7)	Yes
Nitriles and others (C3/6)	Yes
Bipryidiliums (D/22)	Yes
PPO inhibitors (E/14)	Yes
Glycines (glyphosate) (G/9)	Yes
Dinitroanilines (K1/3)	Yes
Thiocarbamates (N/8)	Yes
Synthetic auxins (O/4)	Yes
Organoarsenicals (Z/17)	Yes
Pyrazoliums (Z/8)	Yes

[a] Herbicide Resistance Action Committee (HRAC) group designation, shown in parentheses.

to control than triazine-resistant weeds. Fortunately, the triazines are very effective in controlling many weeds resistant to acetolactate synthase (ALS) and other herbicides, making them an essential component in effective weed management strategies (Table 1.7). The triazines also are very important in controlling the growing number of weeds resistant to glyphosate.

Yield and Economic Improvements Using Atrazine as an Example

The benefits of the triazines in multiple cropping systems range from their application flexibility, effective weed control, soil residual activity, and crop selectivity to their important role in resistance management and conservation tillage. The triazines also have made a major impact on agricultural sustainability and crop yields, as evidenced by the use of atrazine, especially in corn.

The US Environmental Protection Agency (USEPA, 2003a) analyzed the impact of atrazine in corn and found that yields improved on average by approximately 9 bu/A with atrazine as compared to replacement herbicides. Taking into account the yield advantage and alternate herbicide costs, USEPA estimated the value of atrazine in corn at $28/A. This translates into a benefit of $1.6 billion annually nationwide. The USEPA also estimated a 10–40% yield advantage for US sugarcane when atrazine is used, as well as a cost advantage over alternative herbicides.

Corn yields from 236 university trials reported by the North Central Weed Science Society (NCWSS) between 1986 and 2005 showed that atrazine treatments resulted in an average 5.7 bu/A advantage (Fawcett, 2006). These trials used atrazine rates averaging 1.17 lb a.i./A in 1986 and 0.61 lb a.i./A in 2005, which are significantly lower than the maximum label rate. Combining the higher yield from atrazine and the lower herbicide cost with atrazine treatments resulted in added grower income of $25.95/A in 2005.

The yield benefits of atrazine and other triazines vary by tillage type, and field studies have shown that the impact is higher under no-till than under conventional tillage systems (Carlson, 1998).

The National Corn Growers Association in the United States represents more than 32 300 growers from 48 states and each year sponsors a corn yield contest. There are nine production classes varying by geographic region, tillage type, and irrigation. Table 1.8 summarizes the results of the 2006 contest and shows that the impact on yield of treatments containing atrazine in the top five entries in each production class ranged from a 11.5 bu advantage in irrigated corn to a 46.9 bu advantage in no-till/strip till irrigated production. These 2006 results using available tools to maximize yields further support that there are significant advantages with atrazine-containing treatments.

Recent Scientific Reviews and Reregistrations

Hundreds of triazine-containing products continue to be reviewed, registered, and used throughout the world, with regular reregistrations and safety reviews. While several of the triazines have been recently reviewed, the most comprehensive of these reviews in multiple countries involved atrazine and simazine.

In 2006, after a comprehensive science review of chlorotriazines, the USEPA determined 'there is reasonable certainty that no harm will result to the general US population, infants, children, or other major identifiable subgroups of consumers, from the use of simazine, atrazine, and propazine' (USEPA, 2006a, b). The review shows that the chlorotriazines are 'not likely' to cause cancer in humans and that dietary exposure is extremely low, with wide margins

Table 1.8 2006 US National Corn Growers Yield Contest results, including the average yield of the top five in each class, with and without atrazine.[a]

Class	# Grower entries	# of Top 5 entries (by yield) using atrazine	With atrazine		Without atrazine		Average bu/A advantage in top 5 yields with atrazine
			# Entries with atrazine	Average of top 5 yields (bu/A)	# Trials without atrazine	Average of top 5 yields (bu/A)	
Nonirrigated[b]	249	5	177	277.22	72	255.87	21.35
Nonirrigated, seven states[c]	361	5	230	277.93	131	258.23	19.70
No-till/strip till, nonirrigated [b]	236	4	194	270.20	42	257.24	12.96
No-till/strip till, nonirrigated, seven states[c]	155	5	121	277.00	34	250.04	26.96
No-till/strip till, irrigated[d]	183	5	138	316.58	45	269.62	46.96
Ridge till, nonirrigated [b]	47	5	32	241.46	15	213.66	27.80
Ridge till, nonirrigated, seven states[c]	42	4	31	255.35	11	235.53	19.82
Ridge till irrigated[d]	83	4	56	292.66	27	266.24	26.42
Irrigated[d]	263	3	196	298.01	67	286.46	11.55

[a] The 1619 contest entries were not side by side comparisons. 1175 used atrazine as part of their herbicide treatments and 444 did not. Entries with no yield or no herbicide treatments were not included.
[b] Includes continental US states EXCEPT Illinois, Indiana, Iowa, Minnesota, Missouri, Ohio, and Wisconsin.
[c] Includes Illinois, Indiana, Iowa, Minnesota, Missouri, Ohio, and Wisconsin.
[d] Includes all states.

of safety. Additionally, a government-sponsored study conducted by the National Institute of Health, the National Cancer Institute, the National Institute of Health Science, and the USEPA has found no association between cancer incidence and atrazine exposure (Alavanja *et al.*, 2003; Rusiecki *et al.*, 2004; Engel *et al.*, 2005). The USEPA (2007) also determined that atrazine does not impact amphibian gonadal development.

Reviews by the Australian Pesticides and Veterinary Medicines Authority (APVMA) in 1997 and again in 2004 concluded that properly used and applied, atrazine and simazine are safe for humans and the environment [Australian Pesticides and Veterinary Medicines Authority (APVMA), 1997, 2004]. The APVMA also reviewed additional data on potential effects of atrazine on amphibians and concluded that 'taken together, these data indicate that it is unlikely that atrazine is impacting adversely on populations of Australian amphibians at current levels of exposure' (APVMA, 2004).

In 1996 the United Kingdom, which was selected to conduct the scientific review of atrazine for the European Union (EU), concluded: 'It is expected that the use of atrazine, consistent with good plant protection practice, will not have any harmful effects on human or animal health or any unacceptable effects on the environment' (UK Rapporteur Monograph, 1996). In 2000, the United Kingdom for the European Commission also concluded it is not appropriate to classify atrazine as a carcinogen (UK Rapporteur Monograph, 2000).

Much misinformation exists with regard to the European Union's 2003 decision to not reregister atrazine and simazine despite favorable EU reviews of their safety. In 1980, European countries adopted the European Drinking Water Standard, which set an arbitrary limit value of 0.1 ppb for any pesticide in drinking water. This arbitrary limit was applied to all pesticides, irrespective of their safety profiles, and was not scientifically determined. The health-based limit established by the European Union for atrazine based on a preponderance of scientific evidence was 150 times higher. Note that the United Kingdom for the European Union established the health-based limit for water at 15 ppb for atrazine parent. Australia established a 40 ppb health-based limit for parent atrazine and metabolites. In USEPA (2003b) estimated a range of health-based limits (drinking water levels of comparison) for atrazine and its chlorometabolites that ranged from 12.5 to 68 ppb, depending on dietary and water intake estimates for different sub-populations. These health-based limits are more than 125 times to 680 times greater than the arbitrary 0.1 ppb limit adopted by the European Union.

The European Union did recognize that the exceedances of the 0.1 ppb limit in groundwater were based mainly on outdated high rate uses and noncropland uses. Even the 0.1 ppb limit in groundwater would not be exceeded today in most corn-growing regions. Despite these facts, atrazine was not reregistered in the European Union. However, limited uses have been retained until 2007 in some of the member states, such as Ireland, the United Kingdom, Spain, and Portugal (*Official Journal of the European Union (EU) Decision*, 2004). Terbuthylazine, another key triazine herbicide and a product very similar to atrazine, was introduced in Europe almost two decades later than atrazine. As a result, terbuthylazine was never used at high rates or in noncropland applications and remains an important herbicide in Europe for both corn and grape crops. Terbuthylazine also has recently received a favorable science review in the European Union's reregistration process (UK, 2007).

The World Health Organization's International Agency for Research on Cancer (IARC) reviewed atrazine in 1998 and concluded that new toxicology information provides strong evidence that the mechanism responsible for tumors in a specialized type of rat (Sprague–Dawley) is not relevant to humans. As a result, IARC changed its classification of atrazine to 'not classifiable as to carcinogenicity in humans' (IARC, 1999). IARC also reached these same conclusions with regard to simazine.

In 2001, the French Toxicity Research Commission on Pesticide Products cited conclusions by IARC, USEPA, and EU that atrazine is not carcinogenic to humans (French Republic Ministry of Agriculture, 2001). The Commission further stated, 'Considering all these factors, the concentration of the triazines in water, even elevated levels identified in the field both in transitory and localized form, do not represent a public health risk.'

Contribution of Triazines to Agricultural Practices, Basic Research, Safety Testing, and Stewardship

The development of triazine technology resulted in the pioneering of several new agricultural advances, including the development of selective preemergence weed control practices in several crops, the first herbicides with application flexibility (preemergence, postemergence, incorporated, banding, broadcast applications), and the first extensive farmer education programs on weed control. New application techniques using additives were also first developed with the triazines, including surfactants, oils, and liquid fertilizer. New breakthrough formulations discovered with triazine technology included flowable formulations, water dispersible granules, and the first prepacks of herbicides. Packaging advances included the first recyclable containers and the first bulk containers for herbicides.

Advances in science and basic research using triazines as a tool included:

- New developments in the understanding of photosynthesis in plants.
- The first genetic sequencing to explore herbicide resistance.
- Breakthroughs in genetic engineering of plants.
- The first discovery of certain enzymes and metabolites in chemical degradation pathways.
- The discovery of new bacterial genes for pesticide degradation.
- The development of immunoassay methods for herbicides.

Advances in safety testing and chemical risk assessment methodologies attributed to research conducted with the triazines include toxicology mode of action tests, new enzyme and chemical analyses, new methods for analyzing metabolites, exposure monitoring for applicators, water monitoring and analysis methodologies, probabilistic Monte Carlo risk assessment, environmental modeling and mapping, and methodology for amphibian and ecological safety tests.

Widespread adoption of several stewardship practices attributed to the triazines include implementation of conservation tillage practices and many best management practices, including rural well set-backs, vegetative buffers, filter strips, and set-backs from streams and reservoirs. The stewardship practices have been effective and several studies on atrazine levels in water have shown declines in both surface and groundwater. Comprehensive triazine education and research programs to develop and implement best management practices and site-specific watershed management processes have resulted in water quality improvements not only for the triazines, but also for other chemicals, sediment, and nutrients.

Conclusions

The Triazine Herbicides: 50 Years of Revolutionizing Agriculture deals extensively with the research, development, and use of triazine herbicides in the United States, since this is where much of the work on the products was centered.

It is hoped that this book will serve not only as an update and expansion on the agricultural and environmental sciences of the triazine herbicides, but also as a model for the discovery, development, and extensive research needed for future classes of agricultural chemicals and technology. Among the topics it covers are:

- An introduction to the triazines, including their discovery, development, and registration.
- The evolution of weed control in crop production.
- The weed control mode of action of the triazines.
- Benefits of the triazines in crop production.
- Environmental fate of the triazines.
- Human health and environmental risk assessments.
- Environmental stewardship, conservation tillage, and IPM.
- Detailed appendix on triazine nomenclature, chemical structures, and properties.

Today, 50 years since their discovery, the triazine herbicides continue to be critical tools for sustainable and efficient agricultural technology throughout the world.

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Chapter 2

History of the Discovery and Development of Triazine Herbicides

Gustav Müller
Basel, Switzerland

Summary

The initial discovery and development of triazine herbicides took place between the years 1950 and 1970. During that period, a group of leading scientists and agricultural experts of the former chemical company J.R. Geigy, Ltd., developed an idea for a new family of herbicides that would support modern agriculture. A small team of Geigy chemists, biologists, and agronomists made excellent progress in turning that initial concept into not only new compounds, but a new field of weed control research.

Geigy's principal scientist for chemical synthesis, Dr. Enrico Knüsli, started modifying existing chemicals known to have an effect on plants, utilizing new molecular concepts that might be more successful in controlling weeds. Knüsli altered a triazine ring with new active groups and discovered a tremendous source for new compounds with herbicidal properties: the 4,6-dialkylamino-*s*-triazines. Within a short time period, researchers were able to select the most active compounds with respect to both weed control activity and selectivity for corn and other crops. The limits of substitution were soon defined, and work initially concentrated on the use of the unique compounds simazine and atrazine on weed control in corn.

The Geigy scientists developed several new research methods in the areas of biological evaluation of weed control and crop tolerance. They also developed new science methodologies to investigate areas of toxicology, mode of action, and dissipation in soils and plants.

The discovery and development of triazine herbicides were important scientific achievements and a significant example of cooperation among chemists, biologists, and agronomists from around the world. Development of the triazines led to unprecedented success in crop weed management.

Introduction

The triazine herbicides were discovered in the laboratories of J.R. Geigy, Ltd., an international chemical company founded in 1758 and based in Basel, Switzerland. A careful evaluation of the needs in agriculture inspired two researchers in Geigy's Agrochemical Division to focus on discoveries important for weed control. In autumn 1950, Dr. George R. Ferguson, then head of the Technical Department of Geigy Agrochemical Division in the United States, developed ideas on how agrochemical research could be diversified to meet new food production and agricultural challenges. These ideas attracted the attention and support of Dr. Hans Gysin, a chemist and group leader of an organic synthetic research team and later head of Geigy's Basel Agrochemical Research Department. Dr. Gysin worked in the United States during the summer of 1951 to understand the weed control challenges farmers faced and to explore future research in the field of agrochemicals to meet those needs.

For Geigy, the beginning of the herbicide project in 1952 was an ambitious venture in a new field of research. Dr. Albert Gast was given the responsibility for biological evaluation of the herbicides, and Dr. Enrico Knüsli, who had joined the company in 1952, was responsible for the systematic synthesis of potential new herbicides. A good description and source of elucidating information on this early period was presented and published by Knüsli at an American Chemical Society (ACS) Symposium in 1977. A part of this firsthand information is reported *in extenso* below.

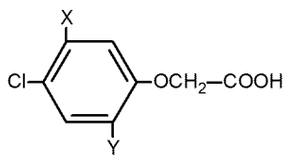
How young an art was chemical weed control then! For a long time man had evidently not felt himself so helpless against weeds as against other pests. It is not by chance that neither thorns nor thistles but mosquitoes, gadflies

ED_001334_00003007-00030 EPA-HQ-2017-008866

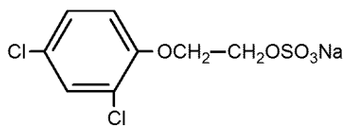
and grasshoppers figure in the range of the ten biblical plagues. Pyrethrum, nicotine, copper, sulfur were chemical control measures long before chemistry entered the field of weed control. In the late thirties, chemistry – and organic chemistry in particular – made a decisive follow-up in the field of insecticides and fungicides, while the field of herbicides was in its infancy.

In the mid-fifties the range of practically-used organic herbicides was dominated by phenoxyacetic acids; in this country (USA) the production of 2,4-D had reached an output of 34,000,000 pounds with a sales value of 28ffi10^6 \$ out of a total herbicide market of 38ffi10^6 \$ and out of a total pesticide market of 260ffi10^6 \$. The range offered to interested herbicide users included, in 1951, besides 2,4-D the 0-alkyldinitrophenols, pentachlorophenol, trichloroacetic acid, sodium isopropylxanthate, additional chlorophenoxyacetic acids, isopropyl-N-phenylcarbamate, endothal, maleic acid hydrazide and p-chlorophenyldimethylurea. The concept of a preemergence treatment of weeds had just been inaugurated by the last-mentioned compound.

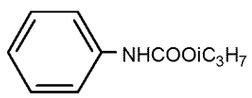
Herbicides, 1951



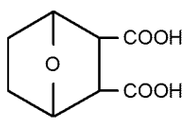
2,4-D
2,4,5-T
MCPA



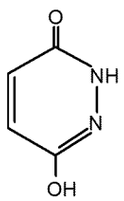
2,4-Dichlorophenoxyethylsulfate, Na salt



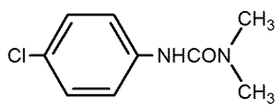
Isopropyl N-phenylcarbamate



3,6-Endoxohydrophthalic acid
ENDOTHAL

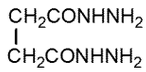


6-Hydroxy-3-(2H)-pyridazinone
MH

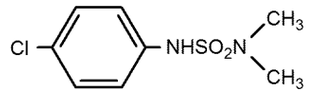


3-(4ffl-Chlorophenyl)-1,1-dimethylurea
CMU

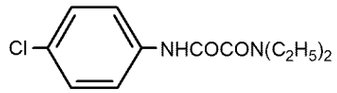
This was the status when we commenced, in 1952, a project for the discovery and the development of herbicides and defoliants. The decision to initiate such a project was taken by the management of our company, then J.R. GEIGY Ltd., a year earlier. The company had at that time experience in the field of pharmaceuticals, dyestuffs, insecticides, moth-proofing agents, and fungicides. It is a pleasure, and an expression of gratitude, for me to recall that Dr. Hans Gysin was the inspiring and enthusing leader of the project and that Dr. Albert Gast cared, with high expertise, for a major part of the greenhouse and field evaluation. How did we attack the problem? In the conventional way: by establishing work hypotheses, by synthesizing, by screening, by discarding many compounds.



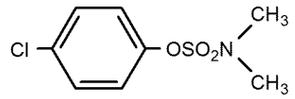
G-25264



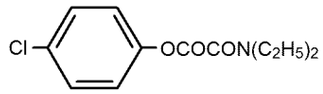
G-25490



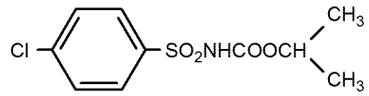
G-25374



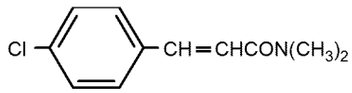
G-25491



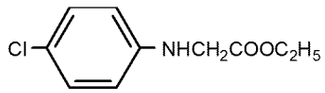
G-25377



G-25494

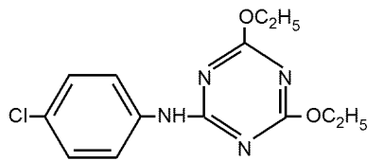


G-25486

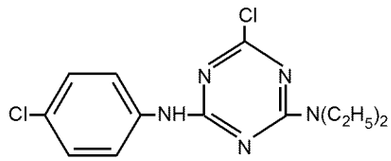


G-25795

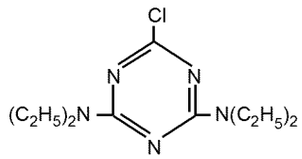
In a first round, we tried to obtain, through structural variation of known active molecules, new and superior biological effects. We were particularly interested to check the consequences of the isosteric replacement of structural elements in chlorophenyl derivatives as shown above. In the greenhouse, during biological evaluation G-25486 showed defoliant properties which led to structural variation work. However, no compound useful under practical conditions could be found. G-25795 demonstrated remarkable root-promoting activity so that many further analogues and homologues were synthesized.



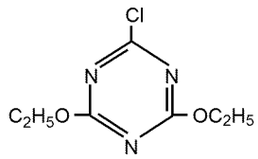
G-25798



G-27902

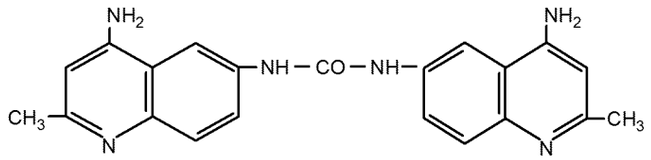


G-25804

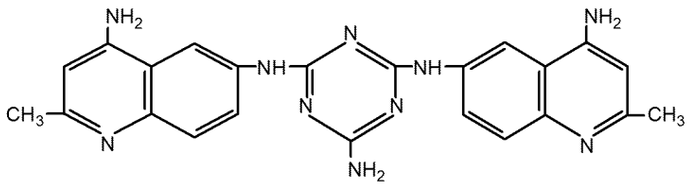


G-25814

G-25804 revealed substantial herbicidal activity and in quite early tests a distinct selective behaviour versus corn and cotton. Why, you may ask, did they include, rather unexpectedly, this s-triazine ring system? The background has already been reported repeatedly. We knew that in the field of dyestuffs and pharmaceuticals the substitution of an urea bridge by a bis-amino-s-triazine group had on occasion not fundamentally changed the respective properties. Surfene shows, as an example, such a structural combination having protozoidicidal activity, developed by a German scientist.



Surfene



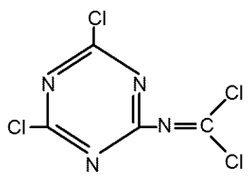
Surfene C or Congasine
Jensch (1937). *Agnew. Chem.*, 50: 891

So we were induced to try this approach, too, and we started synthesis work in the field of s-triazines. The result of our primary working hypothesis was disappointing; derivatives bearing anilino radicals showed no herbicidal effects. Surprisingly, however, the herbicidal activity reappeared in the structure 2-chloro-4,6-bis-diethylamino-s-triazine, compound G-25804 shown previously. The awareness that we were confronted with a completely new herbicidal matrix with apparently superior usefulness led us to intensive work around the s-triazine ring system.

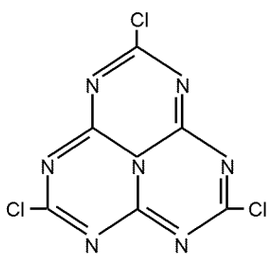
What a beautiful tool is cyanuric chloride for the chemist working in chemical synthesis! Three chlorine atoms offer reaction with a large proportion of the chemicals listed in the Beilstein Handbook or the Chemical Abstracts Index. Not only that: the chlorine atoms are reasonable enough not to react simultaneously but, under adequate conditions, stepwise, allowing myriads of potential combinations. Furthermore: cyanuric chloride has been and is a relatively cheap key material; it can be produced quite easily from such basic materials as chlorine and hydrocyanic acid.

As we assemble under the auspices of the American Chemical Society, you may ask whether it has not been a boring task to deal with this chemistry where the reaction scheme is usually quite transparent. No doubt, the major attractiveness has been and is the structure/activity evaluation and the respective deductions. But now and then it occurred that a rather nice unexpected chemical offspring resulted from this work, and the chemical accent of our meeting may justify the quoting of some examples.

We identified the structure of a side product obtained in a liquid phase process for the production of cyanuric chloride; this tetramer of chlorocyan had not been described before and we studied its reactivity.

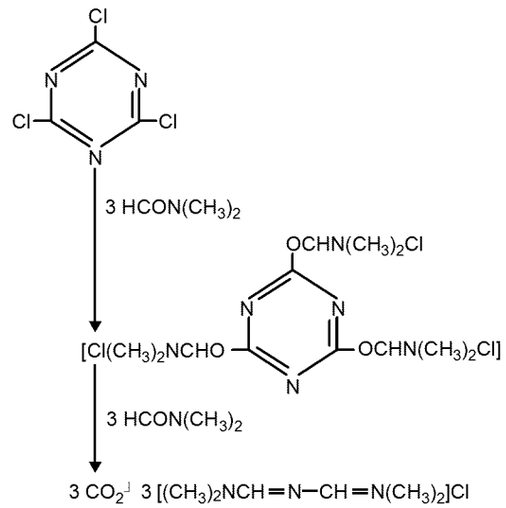


We identified a yellow compound which poisoned for a certain time the carbon-catalyst in the trimerization of chlorocyan as cyameluric chloride.



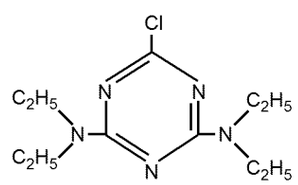
$C_6Cl_3N_7$
Cyameluric chloride

We found that cyanuric chloride reacts easily but in a controllable manner with dimethylformamide and CO_2 being evolved. The reaction was fully elucidated later by H. Gold (1960).

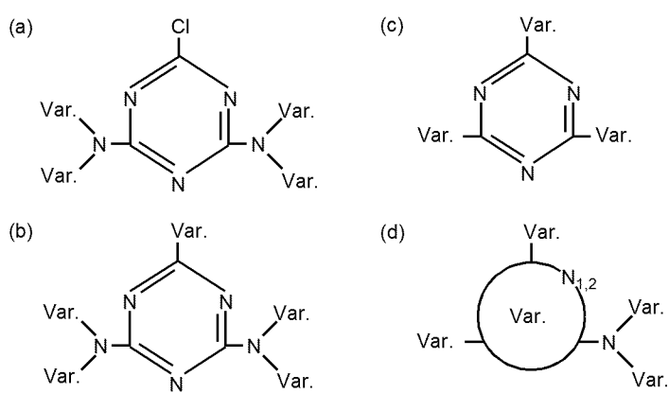


Gold, H. (1960). *Agnew. Chem.*, 72: 956

But let us return to the problem of selecting, out of the myriads of possible 2,4,6-s-triazine derivates, those which have herbicidal activity and from these, those which would be useful under practical conditions. Starting from the structure of G-25804 we initiated variation along four main lines in order to explore the consequences with regard to the biological characteristics.



G-25804



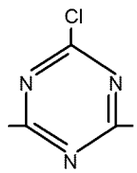
(a) *by varying the N-alkyl radicals;*
(b) *by substituting the chlorine atom by other suitable groups;*
(c) *by permuting most different radicals on the three ring positions allowing substitution;*
(d) *by replacing the s-triazine ring by other N-heterocycles, mainly provided with halogen and alkylamino radicals.*

After having synthesized and tested many representatives we can conclude now that, in general, the following criteria must be fulfi lled in order to obtain substantial herbicidal activity:

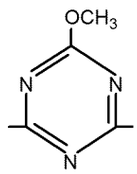
- *Two nitrogen functions bound to ring carbon atoms are essential for the typical triazine activity pattern.*
- *The presence of one to three N-alkyl substituents is needed, those compounds bearing one alkyl group on each nitrogen function being of special interest.*
- *Alkyls C_1 to C_4 are most suitable, including methoxyalkyls.*
- *Substitution of the chloro atom by alkoxy and alkythio groups, preferably methoxy and methylthio, conserves the high herbicidal activity but leads to a change of the crop selectivity pattern.*

Substitution of the chloro atom by bromine, by fl uorine, by nitrilo-, hydrazion-, alkyl-, haloalkyl-, alkoxyalkoxy groups leads very often to remarkable herbicidal but seldom – from the practical point of view – to superior activity. It is thereby obvious to everybody active in this field that the qualification "superior activity" can never relate to one parameter alone; activity against the target organisms is, of course, an absolute prerequisite but this activity can, outside the field of industrial weed control, only be made valuable by a complementary suitable crop selectivity pattern.

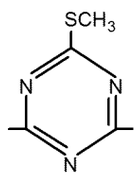
The following compounds resulting from our project reached the level of practical use:



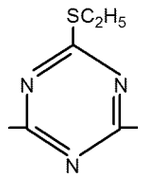
			Common Name
G-27692	C_2H_5NH ^	^ NHC_2H_5	Simazine
G-27901	C_2H_5NH ^	^ $N(C_2H_5)_2$	Trietazine
G-30027	C_2H_5NH ^	^ $NHiC_3H_7$	Atrazine
G-30028	iC_3H_7NH ^	^ $NHiC_3H_7$	Propazine
GS-13528	C_2H_5NH ^	^ NH*sec.*C_4H_9	Secbuthylazine
GS-13529	C_2H_5NH ^	^ NH-*t.*C_4H_9	Terbuthylazine



			Common Name
G-31435	iC_3H_7NH ^	^ $NHiC_3H_7$	Prometone
G-32293	C_2H_5NH ^	^ $NHiC_3H_7$	Atratone
GS-14254	C_2H_5NH ^	^ NH*sec.*C_4H_9	Secbumetone (proposed)
GS-14259	C_2H_5NH ^	^ NH-*t.*C_4H_9	Terbumetone (proposed)



			Common Name
G-32911	C_2H_5NH ^	^ NHC_2H_5	Simetryn
G-34161	iC_3H_7NH ^	^ $NHiC_3H_7$	Prometryn
G-34162	C_2H_5NH ^	^ $NHiC_3H_7$	Ametryn
G-34360	CH_3NH ^	^ $NHiC_3H_7$	Desmetryn
G-36393	iC_3H_7NH ^	^ $NHCH_2CH_2CH_2OCH_3$	Methoprotryn
GS-14260	C_2H_5NH ^	^ NH-*t.*C_4H_9	Terbutryn



			Common Name
GS-16068	iC_3H_7NH ^	^ $NHiC_3H_7$	Dipropetryn (proposed)

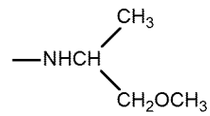
They differ, of course, substantially as to the importance they assumed. As an example, G-27901, trietazine, was sold once in a quantity of a couple of thousand pounds for weed control in chrysanthemums in Japan and can, therefore, not be put in line with for example G-30027, atrazine.

In 1977, when the above review was presented and published, the history of the s-triazine herbicides was already 25 years old, with the first synthesis of these chemicals completed in 1952. The filing of the first basic triazine patent case in Switzerland was on August 16, 1954, and the first commercial products appeared on the market in 1956, following the approval of simazine for use in corn by federal authorities in Switzerland on December 3, 1956. Several other agrochemical companies started immediately to work with their own s-triazine variations, using other radicals or amino-functions on the s-triazine ring. This further research was also briefl y reviewed in Knüsli's 1977 paper.

No research group, be it academic or industrial, can expect unlimited exclusivity after having identified a field which invites further exploitation. The compilation and analysis of the main contributions, experimental or sales products, developed by groups other than ours show the following picture:

(a) *Our conclusion that interesting activity is mainly connected with the presence of two monosubstituted amino radicals, and a halogen, halogenoid, alkoxy or alkylthio group has been confi rmed.*
(b) *One tendency centered around the grafting of a hydroxy or alkoxy group directly on the amino function or into the alkyl radical.*

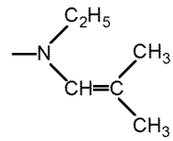
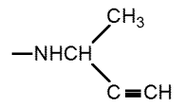
Hydroxy or alkoxyalkyl radicals

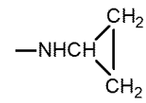
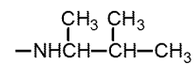
DuPont (1957/1965)	C1 ^	^ $NHCH_2CH_2CH_2OCH_3$ ^ $NHCH_2CH_2CH_2OCH_3$	
Monsanto (1963)	CH_3S ^	^ $NHCH_2CH_2CH_2OCH_3$ ^ $NHCH_2CH_2CH_2OCH_3$	Lambast
Allied (1969)	Cl ^	^ $NHiC_3H_7$ ^ $NHCH_2OH$	ACD 15M
BASF (1967)	Cl	^ NHC_2H_5 	55547

Further lines comprise:

(c) *The insertion of unconventional alkyl, alkenyl or alkynyl substitutes.*

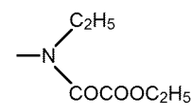
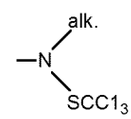
Unconventional hydrocarbon radicals

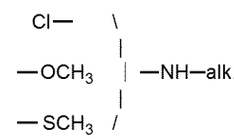
Monsanto (1971)	CH_3S ^	^ NHC_2H_5		MON 0385
BASF (1967)	C1 ^	^ NHC_2H_5		BASF 54187

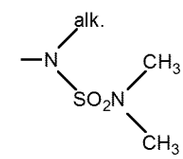
Gulf (1966)	Cl ˆ	ˆ $NH\mathit{i}C_3H_7$		Cyprazine
Ciba (1967)	CH_3S ˆ	ˆ NHC_2H_5		Dimethametryn (proposed)

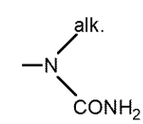
(d) *The introduction of acyl radicals.*

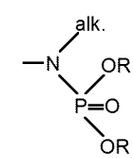
Acylation

Matolcsy *et al.* (1959, 1961)	Cl ˆ	ˆ $NH\mathit{i}C_3H_7$	ˆ $NHCON(C_2H_5)_2$
	Cl ˆ	ˆ NHC_2H_5	ˆ $NHCON(CH_3)_2$
Stauffer (1973)	Cl ˆ	ˆ $NH\mathit{i}C_3H_7$	
Degussa (1959/1964)			



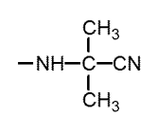
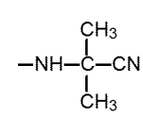






(e) *The introduction of cyanoalkyl radicals.*

Cyanoalkyl radicals

				Common Name
Matolcsy *et al.* (1959, 1960)	Cl ˆ	ˆ NHC_2H_5	ˆ $NHCH_2CN$	
Degussa/Shell (1967)	Cl ˆ	ˆ NHC_2H_5		Cyanazine
Degussa/Shell (1966)	CH_3S ˆ	ˆ NHC_2H_5		Cyanatrine

Because of the susceptibility of the 1-cyano-1-methylethylamino group to hydrolysis cyanazine had a relatively short residual activity. A further possibility of variation on the nonamino positions is illustrated by the next example:

Variation in the nonamino function

Degussa (1958/62)	N_3	ˆ $NH iC_3H_7$	$-NH-C(CH_3)_2-CN$
Degussa (1959)	ˆ SCN	ˆ NH-alkyl	ˆ NH-alkyl
Degussa (1960)	ˆ SCH_2CN	ˆ NH-alkyl	ˆ NH-alkyl

The azido group was also able to substitute for one of the two alkylamino groups:
N_3 – as a replacement for an alkylamino group

Ciba (1963)	CH_3S	ˆ N_3	ˆ $NH iC_3H_7$	Aziprotryn

Additional information on these early discoveries is available in Knüsli's ACS Symposium presentation (Knüsli, 1977).

Selection and Brief History of the First Triazine Herbicides

Within a short time, the discovery of and screening for the herbicidal properties of dialkylamino-*s*-triazines led to selection of the most promising candidates for field development and eventual commercial use.

Before describing the important compounds that reached the farmer, it is helpful to look back to the 'conditions of success,' i.e., the close cooperation between the chemist and the biologist. Beginning with the herbicide project in 1952 and the creation of a new group for synthesis, it was essential to provide simple, quick, and correct answers to questions involving the behavior of screening compounds on plants. The following methods were employed for the primary screen:

1. *Growth inhibition activities*: Epicotyls of bean seedlings were treated and changes in the geotropic reaction were recorded.
2. *Germination inhibition*: Seeds of cucumber, mustard, oat, and onion were placed in thin layers of soil that previously had been mixed with the chemical at rates of 5 and 10 kg of active ingredient per hectare (kg a.i./ha). Influences on seed germination and early growth of seedlings were recorded.
3. *Foliar tests*: Cotton and other plants grown in the greenhouse received a foliar treatment in early stages. Effects such as growth inhibition, distortions (epinasty), chlorosis, discoloration, etc., were recorded.

The same tests also were employed with a somewhat different collection of plants, such as radish, cucumber, soybean, and ryegrass. These approaches seem simple and basic to weed scientists today, but they were revolutionary in the early 1950s and essential to discovering the biological properties and effectiveness of the triazine herbicides.

All synthesized compounds were initially submitted to these primary screening tests. Dr. Gast soon determined that in order to understand all possible herbicidal activities, extended observation time would be necessary for both the germination and foliar tests. Furthermore, biological units in both Switzerland and the United States found it necessary to add – especially for very active compounds – greenhouse experiments including other weeds and crop plants of importance. The idea of herbicides acting after pre-emergence applications was beginning to be considered by researchers (Robbins, *et al.*, 1952). In fact, the study of effects on initial stages of young plants had been included in the triazines research program very early in their development, as can be seen in the very first publication on the new triazines.

In the first public announcement of the triazines, submitted to the scientific review *Experientia* on December 13, 1954, Gast *et al.* (1955) described the biological activity of compound G-25804 (chlorazine) with respect to germination activity and its influence on the growth of young plants 10 and 20 days after treatment. The compound showed activity similar to monuron (CMU), which had recently been discovered. Special attention was given to preemergence activity and crop selectivity in field trials. Gast *et al.* (1956) then compared G-27692 (simazine), G-27901 (trietazine), and two other *s*-triazines with chlorazine. They emphasized the effects of these biological compounds on young plants (similar to CMU) and tested their tolerance to corn and cotton.

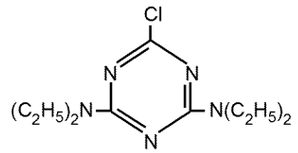
In view of the importance of this history of triazine herbicides as a model for the period, we will briefly review the timing of some important events:

1952
March: Experimental synthesis work was initiated for the herbicide project.
October: The first *s*-triazines were ready for screening.

1953

March: Biologists reported selective herbicidal activity of G-25804 (chlorazine) and analogs. G-25804 was conceived initially as an intermediate compound for further substitution of the remaining chlorine atom, but was also screened for herbicidal activity.

G-25804 (chlorazine)

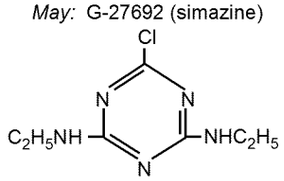


August: Chlorazine was clearly recognized as an herbicide with pre-emergence activity. Its first recorded selectivity was on carrots.

1954

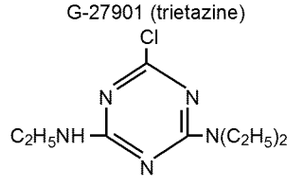
February: Chlorazine showed good activity on ryegrass, crabgrass, and parsley. Tolerance by cotton and bean was also observed.

May: G-27692 (simazine)



and

G-27901 (trietazine)



Simazine and trietazine were submitted for screening with other analogous compounds.

August 14: Geigy filed the basic patent Triazine Case No. 941 in Switzerland. (The US patent, NR. 2,891,855, was granted on June 23, 1959.) In this application, Geigy specifically described the herbicidal activity of chlorazine, simazine, and trietazine. In the same month, Dr. Gast reported that simazine and trietazine were fully tolerated by corn at the rate of 10 kg a.i./ha. Research emphasis was now on field trials for the remainder of the 1954 season, both in Switzerland and the United States.

December 13: The first public announcement on chlorazine was submitted for publication in Switzerland.

1955

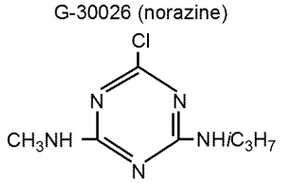
January 17: The first publication in the United States on chlorazine was presented at the Southern Weed Control Conference by Antognini and Day (1955).

March: From early in 1952 to March 1955, some 500 compounds had been screened simultaneously in the United States and Europe. From these, three compounds entered fi eld development.

June: Compound G-27692 (simazine) was found to be two to four times more herbicidally active than the first selected lead compound, G-25804 (chlorazine). The selectivity for corn of G-27692 was confirmed in new tests with outstanding results. The selection of simazine as the new corn herbicide was a decisive step towards the first marketable triazine herbicide.

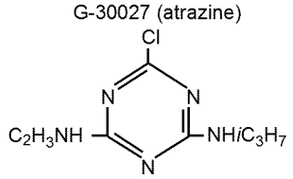
December: Two very promising new candidates were selected for field evaluation:

G-30026 (norazine)



and

G-30027 (atrazine)



These two chemicals were among 35 active derivatives selected for further evaluation in the field in order to differentiate between the various compounds, establish a complete and accurate profile for each, and separate the promising ones from less than superior candidates with respect to their potential as commercial herbicides. Between the various triazine analogs, several were found to be identical in some biological characteristics. With many on-farm successes or failures dependent on the decision, each test and minute observation or difference was of critical importance. So the testing of four test plants (oat, mustard, cucumber, and onion) was expanded to nine, adding sugar beet, corn,

cotton, ryegrass, and common vetch (*Vicia sativa* L.) to the primary screening tests. With this more extensive bioassay, it was possible to differentiate chemicals and to reduce the number of chemical candidates from the preliminary screening to an acceptable number for further evaluation.

Another change that became necessary after this early experience was the revision of rates used for early evaluation. The screening originally started with rates of 5 and 10 kg a.i./ha. The first compounds reaching the stage of field testing resulted in excellent weed control for many weeds at rates below 5 kg a.i./ha. This was especially true for simazine (G-27692), which initially was recommended at a maximum use rate of 4 kg a.i./ha in corn, while corn had a tolerance far above 10 kg a.i./ha. It was discovered that the rates needed for acceptable weed control in corn and other crops could be far below the amounts tolerated by the crops. These rates were also below the use rates of other commercial or promising herbicides at the time. Later, it was discovered that triazines of any kind would generally have use rates less than or equal to 4 kg a.i./ha, but those selected for general or industrial (nonselective) weed control would require use rates of 5 to 10 kg a.i./ha or higher.

1956: The work of the biologists in Basel continued with the screening of new *s*-triazines and with field trials of the compounds G-30026 (norazine), G-30027 (atrazine), G-30028 (propazine), and analogous compounds.

Simazine was submitted to federal, state, and other research institutions for experimental use in corn and to European authorities for testing. Simazine received its first registration in Switzerland in 1956.

In the United States, approval of new herbicides depended not only on performance and toxicity data, but also on a clear picture of eventual crop and soil residues. The Geigy Analytical Department began immediately to develop a reliable residue method for simazine and other triazines. Working methods for residues in plants and soil were available by the end of 1956.

Plans were made to field test G-30026 (norazine), G-30027 (atrazine), G-30028 (propazine), G-30031 (ipazine), and G-27901 (trietazine) as herbicides for corn and approximately 10 other crops. These compounds would be extensively compared with simazine.

New *s*-triazines were developed at a very rapid pace in the project. Therefore, by 1956 there were many compounds in various stages of development. New information on triazine derivatives and herbicidal activity was released to the public at the British Weed Control Conference (Gysin and Knüsli 1956). Table 2.1 (Gunther and Gunther 1970) provides the internal codes and common names, chemical structure and properties of some of these compounds.

1957: Simazine was approved in 1957 in the United States for use on rights of way and noncropland uses. In Basel, field screening trials of several new triazines yielded a favorable profile for G-30026 (norazine) and G-30027 (atrazine). They were found to be superior to simazine in their activity against deep-rooted weeds and were also efficacious as postemergence applications. Both compounds were well tolerated by corn and less rainfall was needed to activate these two triazines, resulting in superior weed control performance (Gysin and Knüsli, 1956).

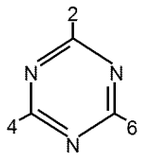
Research had confirmed that no parent simazine residues were found in treated corn plants, and additional data on the dissipation pathway of simazine needed to be developed. Research also indicated that triazines interfered with the photosynthetic process on susceptible growing weeds, as evidenced by the appearance of chlorotic leaves. Steps were undertaken to elucidate simazine's dissipation pathway and herbicidal mode of action. In Basel, Dr. Gast (1958) showed that the accumulation of starch by common coleus (*Coleus blumei* Benth.) plants was inhibited from treatment with 2-chloro-4,6-bis-(alkyl-amino)-triazines due to the inhibition of sugar synthesis. At the same time, Moreland *et al.* (1958) found weed control activity could be reduced by supplying carbohydrates to the plants through their leaves and that simazine was a strong inhibitor of the Hill reaction in photosynthesis. Exer (1958) found that triazines inhibited the Hill reaction as strongly as urea of the CMU (monuron) type.

At the University of Strasbourg, Roth (1958), a member of Geigy's research staff preparing his Ph.D. thesis, observed that *Elodea*, a submerged water plant, was affected by simazine like *Coleus* in Dr. Gast's experiment, and that the consumption of oxygen was increased due to higher respiration. Roth continued to study the uptake of simazine by sensitive plants (such as wheat) and nonsensitive plants (such as corn). The experiments gave a good indication that the simazine-tolerant corn was able to prevent the photosynthetic action of the herbicide. This was clearly shown when corn received simazine through soil uptake. No simazine could be found in the stems and leaves, hence corn was able to metabolize the herbicide. Roth also showed in a simple experiment with freshly pressed plant juice that juice from corn leaves metabolized simazine to a high degree, while juice from sensitive plants was not able to induce simazine metabolism.

In the United States, V.H. Freed at Oregon State University, G.J. Rodgers at Purdue University, and D.E. Davis at Auburn University worked independently with radiolabeled simazine and largely confirmed the previous findings of Drs. Gast and Roth:

- Simazine sensitive and nonsensitive plants take up similar amounts of simazine.
- The uptake is higher when applied to nutrient solution than to soil.
- In sensitive plants, unmetabolized simazine could be isolated and confirmed.

Table 2.1 Code numbers, structures, and properties of some triazine derivatives[a,b]



Code number	Common name	Substitution on triazine ring at positions 2	4	6	pK value	Solubility in water (ppm at 20–25°C)
G-25804	Chlorazine	Cl	$N(C_2H_5)_2$	$N(C_2H_5)_2$	1.74	9
G-27692	Simazine	Cl	NHC_2H_5	NHC_2H_5	1.65	6.2[c]
G-27901	Trietazine	Cl	NHC_2H_5	$N(C_2H_5)_2$	1.88	20
G-30026	Norazine	Cl	$NHCH_3$	$NHiC_3H_7$	–	260
G-30027	Atrazine	Cl	NHC_2H_5	$NHiC_3H_7$	1.68	33
G-30028	Propazine	Cl	$NHiC_3H_7$	$NHiC_3H_7$	1.85	5.0[c]
G-30031	Ipazine	Cl	$NHiC_3H_7$	$N(C_2H_5)_2$	1.85	40
G-30044	Simeton	OCH_3	NHC_2H_5	NHC_2H_5	4.17	3200
G-31430	–	OCH_3	$N(C_2H_5)_2$	$N(C_2H_5)_2$	4.76	–
G-31432	–	OCH_3	NHC_2H_5	$N(C_2H_5)_2$	4.51	40
G-31435	Prometon	OCH_3	$NHiC_3H_7$	$NHiC_3H_7$	4.28	750
G-31717	Ipaton	OCH_3	$NHiC_3H_7$	$N(C_2H_5)_2$	4.54	100
G-32292	Noraton	OCH_3	$NHCH_3$	$NHiC_3H_7$	4.15	3500
G-32293	Atraton	OCH_3	NHC_2H_5	$NHiC_3H_7$	4.20	1654
G-32911	Simetryn	SCH_3	NHC_2H_5	NHC_2H_5	–	450
G-34161	Prometryn	SCH_3	$NHiC_3H_7$	$NHiC_3H_7$	4.05	33[c]
G-34162	Ametryn	SCH_3	NHC_2H_5	$NHiC_3H_7$	–	200[c]
G-34360	Desmetryn	SCH_3	$NHCH_3$	$NHiC_3H_7$	–	580
G-36393	Methoprotryn	SCH_3	$NHiC_3H_7$	$NH(CH_2)_3OCH_3$	–	320
GS-11348	–	SCH_3	$NHiC_3H_7$	$N(C_2H_5)_2$	4.43	–
GS-13529	Terbuthylazine	Cl	$NHCH_2CH_3$	$NHC_3(CH)_3$	1.90	8.5
GS-14254	Secbumeton	OCH_3	NHC_2H_5	$NHtC_4H_9$	–	620
GS-14260	Terbutryn	SCH_3	NHC_2H_5	$NHtC_4H_9$	–	22[c]

[a]Modified from Table II, page VII, Gunther and Gunther (1970).
[b]This table includes all candidates that were at least in the fi eld development stage between 1952 and 1968. Chemical names, structures, and properties of these and all other triazine herbicides and many metabolites are also given in Appendix in Tables 1, 2 and 3.
[c]Water solubility at ffi20–C and pH 7.0.

- In corn, a nonsensitive plant, no simazine was present.
- Corn is able to metabolize simazine.
- Hydroxy-simazine and other metabolites were formed in corn plants.
- A metabolic pathway for simazine in the corn plant was described.

1958: Simazine, which had been approved in 1957 for United States rights of way and noncropland uses, was approved for agricultural use in the United States as a fully selective broad-spectrum pre-emergence herbicide for corn. It immediately set a new standard for weed control in this important worldwide crop. For Geigy, a newcomer in herbicide research, the introduction and success of simazine were due to:

- The pattern of herbicidal activity and selectivity was defined in a relatively short time through screening and special field trials conducted by small development teams in Switzerland and the United States.
- Botanists, physiologists, and chemists were able to develop sensitive methods for residue analysis (Delley *et al.*, 1967). They also developed a clear understanding of the herbicidal mode of action and the metabolic pathway of *s*-triazines in plants, soil, and animals (Knüsli *et al.*, 1969). These early investigations were supported by cooperation from universities in the United States and Europe where scientists recognized that these compounds would be very important tools for worldwide agriculture and to manage weeds in noncropland.
- Since Geigy already had a well-established Pharmaceutical Division, the required toxicological studies were completed in a timely manner.

The experience obtained with simazine, the first product coming out of the herbicide project of 1952, laid the foundation for other *s*-triazine developments that immediately followed. Among the candidates reaching the stage of field screening and development in 1958, G-30027 (atrazine) became the popular choice for weed control in corn. Atrazine demonstrated extremely good performance when applied either pre- or post-emergence. In some very early field trials, it was found that compared to simazine, atrazine was more evenly distributed in the upper soil layer where weed germination takes place. Hence, atrazine was often superior to simazine, giving several important agricultural advantages, especially under dry surface conditions. Atrazine generally out-performed simazine as a more versatile herbicide, especially for corn, sorghum, and other uses where crop tolerance was not a factor.

The experiences gained with simazine between 1955 and 1958 were now a great benefit for the handling of atrazine research and registration by governmental authorities, especially with respect to understanding and developing the more in-depth documentation and comprehensive reviews being required. These relevant experiences included residue analysis, metabolism, herbicidal mode of action, production, formulation, preparing directions for use, and other labeling requirements in the registration and marketing of simazine.

During the early years of the herbicide project, the new *s*-triazines, attracted the attention of countless agricultural scientists and researchers due to their new, unique qualities, and characteristics. Numerous universities and plant and soil research institutes became involved with basic research on their behavior in the soil, modes of action, metabolism, and their effects and fate in the environment. Geigy's Agricultural Research Department soon started to cooperate with leading institutes, mainly in the United States. In 1968, Geigy decided to organize an international symposium on the triazine herbicides, aimed at learning how to use these herbicides in the safest, most effective, and optimum way. Leading scientists were invited to present their findings on the behavior and metabolism of the new *s*-triazines in soil and the influence of climate and environmental factors. This successful symposium was held in Riverside, California, in February 1969, and the papers were published in *Residue Reviews,* Volume 32 (Gunther and Gunther 1970). This publication serves as a summary and review of much early research and data on these herbicides up to 1970.

Status of the Triazines as of 1969

Hundreds of external practitioners and scientists around the world devoted their time and talents to the development, introduction, training, and use of this new chemical weed control technology. The biological characteristics and qualities of these compounds with respect to their potential commercial uses and performance as seen by Geigy's Research and Development Department in 1969 are outlined below. The overview given here on these products is based largely on the information given by Dr. Gast (1970) and by other Geigy scientists from that period.

The Chloro-*s*-Triazines as of 1969

G-25804, chlorazine: This was the first *s*-triazine to show powerful weed control activity at a rate of 8–12 kg a.i./ha from pre-emergence applications. The compound did not show useful post-emergence activity. In retrospect, taking chlorazine through to field trial stage in corn was a significant landmark, leading to the ultimate success of the *s*-triazine group. Some subsequent analogs developed within months of chlorazine development demonstrated a considerable increase in biological activity and provided good control of young weeds below 5 kg a.i./ha, even though they generally had more limited crop tolerance (except for corn). Therefore, further development of chlorazine was not pursued.

G-27692, simazine: With a considerable increase in activity (i.e., providing control of germinating or young weeds with rates below 5 kg a.i./ha), simazine immediately received enthusiastic attention. Equally exciting, it showed a tolerance to corn up to 50 kg a.i./ha, as demonstrated in special trials. In addition to high corn tolerance, simazine had another major advantage in its broad spectrum of activity by controlling nearly all important dicotyledonous weeds and some important grasses in young stages. At rates up to 4 kg a.i./ha, season-long weed control could be obtained. Simazine was applied primarily pre-emergence after planting or was incorporated into the soil prior to planting and had almost no post-emergence activity. Its water solubility was low, so leaching by rain or irrigation was limited. This property made simazine a welcomed product for industrial (nonselective) weed control and for use in fruit orchards, grape vineyards, and forestry. Simazine was often used in mixture with a partner from the group of methylthiotriazines or other herbicides.

G-27901, trietazine: Coming out of early synthesis together with simazine, trietazine showed an increased selectivity in potatoes and tobacco. However, tests over several further seasons showed an unreliable crop tolerance safety margin, which led to a decision that this compound would not be developed for general use. For several years, though, trietazine was used in Japan to control weeds in chrysanthemums.

G-30026, norazine: Norazine reached the level of field screening together with G-30027, atrazine. It showed a typical triazine activity pattern, without distinct advantages over simazine, atrazine, or propazine. In addition, its

relatively high water solubility of 260 ppm represented a new dimension for chloro-*s*-triazines. Since atrazine, with 33 ppm water solubility, resulted in an excellent distribution in the upper soil layer, norazine soon was dropped from further development because of the potential to leach.

G-30027, atrazine: Like simazine, this compound demonstrated an outstanding tolerance for corn and excellent activity against almost all weeds appearing in this important crop. At first, atrazine was offered as an alternative to simazine in special situations where simazine showed a weakness on specific weeds. Two features of atrazine were considered important in these special circumstances: its distinctly superior post-emergence activity and its more reliable performance under temporary dry surface conditions. During the first year simazine was used in corn in the United States (1958), fields in many areas were rather dry during the planting and germination period, leading to a number of claims of insufficient herbicidal activity. On the other hand, all atrazine trials that same year in the United States and in Europe demonstrated a much better performance. After the 1958 season, atrazine quickly became the preferred corn herbicide of growers. Also, atrazine soon entered other segments of the general weed control market. In Europe it was initially introduced at high rates in railways and noncropland, and then into corn. Its use was expanded to include control of deep-rooted perennial weeds in vineyards, orchards, and forests. Atrazine also performed well and became rapidly accepted for use in sugarcane and grain sorghum production. Whereas simazine gave good control of the prevalent annual grasses, e.g., panicum and foxtail species, atrazine showed less efficacy on those weeds.

The rates used for atrazine in corn for most soil and rainfall conditions were up to 4 kg a.i./ha. This was very similar to the use rates of simazine. After introduction in 1958 in Switzerland and the United States, and by 1960 in several other countries, atrazine became an agricultural chemical of unprecedented dimensions. Atrazine's popularity and demand by growers of corn and sorghum have made it by far the most depended upon triazine ever developed.

G-30028, propazine: Propazine was introduced in field trials together with atrazine and norazine. Corn was also tolerant to propazine, and propazine had almost the same spectrum of activity as atrazine and simazine, but with little post-emergence activity. Propazine had low water solubility and was not a powerful herbicide when compared to the other chlorotriazine analogs. It was less active than atrazine and simazine against problem annual grasses. One property, however, kept propazine in commercial use. Propazine was very selective to grain sorghum and became the product of choice for this crop, primarily in areas of the United States where atrazine could not be used because of potential crop injury. Another advantage over atrazine and simazine was the tolerance of propazine by umbelliferae species, permitting propazine's use on carrots and celery. In general, propazine is useful only as a pre-emergence herbicide and has a residual action in soil.

G-30031, ipazine: When ipazine was compared directly with simazine and atrazine, no special use or advantage could be defined. The product was discontinued early.

GS-13529, terbuthylazine: A chlorotriazine similar to atrazine and simazine, terbuthylazine was first introduced to the scientific community in 1966. Terbuthylazine also provided broad-spectrum weed control in corn. Studies comparing efficacy showed that generally atrazine was more effective than terbuthylazine on both broadleaf and grassy weeds. Since terbuthylazine was less efficacious than atrazine in weed control trials conducted in the United States in the late 1960s, it was not commercially developed for corn in the United States. However, development for use in corn and vines continued for Europe and other countries where the weed control needs differed and the weed control differences between atrazine and terbuthylazine were not limiting.

The Methoxytriazines as of 1969

This group of herbicides can be characterized as having: relatively high water solubility; marginal selectivity towards annual field crops; relatively rapid action through both leaf uptake and root uptake; and broad spectrum of activity after pre- and post-emergence treatments.

G-30044, simeton: With a water solubility of 3200 ppm, this first representative of the methoxytriazines tested was prone to rapid, excessive leaching and was not developed further.

G-31430 and G-31432: These compounds were not given common names since they were dropped after only a few field trials due to insufficient weed control performance.

G-31435, prometon: Prometon was the first methoxytriazine to demonstrate an acceptable performance in the field for nonselective and general weed control. Though it has relatively high water solubility of 750 ppm, prometon controls a very broad spectrum of weeds and has a rapid action when applied post-emergence. It also performs well against deep-rooted perennial weeds. For industrial or general weed control, it has been used alone or as a versatile partner to simazine. Prometon also was initially applied as a brush killer for a limited range of species. One particular weakness was its poor control of bedstraw (*Galium aparine* L.), a very common weed in arable fields in Europe. However, bedstraw is usually well controlled by herbicides other than methoxytriazines at very low rates. Other weeds that are poorly controlled with prometon and other methoxytriazines include woodsorrel (*Oxalis* spp.). After treatments with prometon, a dense population of these weeds would sometimes appear.

G-31717, ipaton and G-32292, noraton: These compounds offered no particular practical weed control advantage over prometon, and further development was halted shortly after ipaton and noraton reached the field trial stage.

G-32293, atraton: Atraton showed some interesting features in its activity spectrum that proved useful compared to prometon. Atraton was used alone and in mixtures with other herbicides on roadsides and railway tracks in Europe and in the United States. Atraton found limited commercial use in the late 1950s and early 1960s.

GS-14254, secbumeton: In the beginning, secbumeton gave hope of a better herbicide success due to selectivity exhibited in several crops. After a great number of tests and research during several seasons, it demonstrated sufficient crop safety for use on grape, established alfalfa, and sugarcane. It gave good activity against several perennial weeds that were only partially controlled by the chlorotriazines (i.e., simazine, atrazine) and urea-type herbicides (e.g., diuron) widely used in vineyards. However, after a short initial success, better herbicide alternatives with other chemistries were discovered for vineyards. Further development of secbumeton for alfalfa was halted due to secbumeton's slow metabolism, which resulted in excessive residues. In sugarcane, a crop planted in high rainfall areas, secbumeton did not outperform other triazines already in use. Also, in industrial weed control, it was used only for a short time in special niches.

The Methylthiotriazines as of 1969

The methylthiotriazines reached the market immediately after the first Cl-triazines. Compared with the chloro- and methoxytriazines, their residual activity in the soil is shorter in temperate climates. In acidic tropical soils their weed control activity and persistence may be as long as or longer than that of the Cl-triazines. An early recognized property of this group was rapid action through leaf uptake when applied post-emergence. Most methylthiotriazines have a rather unique and specific pattern of crop tolerance. Unlike corn's tolerance to simazine or atrazine, corn generally does not have a high-level tolerance to methylthiotriazines. Therefore, their use in corn is limited to relatively low rates when applied pre-emergence, or to directed applications between the rows in post-emergence situations.

Methylthio-*s*-triazine group candidates reaching the herbicide market were the following:

G-32911, simetryn: Simetryn was one of the first methylthiotriazine candidates tested, but was developed slowly until it was confirmed that rice had a higher crop tolerance to simetryn than to prometryn. It found its commercial place in the transplanted rice of Japan and in other countries in the subtropical rice belt. Simetryn is used to control broadleaf weeds in mixtures with other herbicides that are active against grasses.

G-34161, prometryn: Prometryn is the most versatile of the methylthio-*s*-triazines. It has been used commercially, at least for limited periods, in the following crops: cotton, sunflower, bean, pea, peanut, lentils, carrot, celery, leek, rice, and common vetch. In combinations with simazine and later with terbuthylazine, it also has been used in potato crops. Substantial use was attained in several crops in a large number of countries. Prometryn was the first effective herbicide for several crops, making it a true pioneer herbicide in the methylthiotriazine class of chemistry.

G-34162, ametryn: Ametryn is the most powerful methylthio-*s*-triazine as far as weed control spectrum and initial herbicidal activity are concerned, especially under warm conditions. In its early development it was tested as a defoliant or burndown agent, but it soon found various other uses and applications. An important commercial use of ametryn was in sugarcane as a basic and important herbicide shortly after the cane is harvested and weed growth emerges. It is used again a second time before canopy closure (i.e., lay-by). Ametryn could be used both pre-emergence and post-emergence in sugarcane crops as a versatile, selective herbicide and as a post-directed treatment in corn. Ametryn often has a much longer persistence and activity in tropical laterite soils than Cl-triazines based on a different pathway of metabolism for methylthiotriazines in these soils. Other uses for ametryn in tropical and subtropical crops emerged in the early years, including its use on pineapple, banana, and palm.

G-34360, desmetryn: Desmetryn showed a distinct tolerance toward cruciferous plants and has been successfully introduced and used in vegetable crops such as cabbage and kale.

G-36393, methoprotryn: Methoprotryn was introduced in the fall-sown small grain cereals, such as wheat and barley. Its application was always post-emergence in relation to both crop and weeds. In most cases it was combined with low rates of simazine in order to provide residual control of late germinating annual grasses. This combination made it possible for the first time to control broadleaf weeds and grasses with one application.

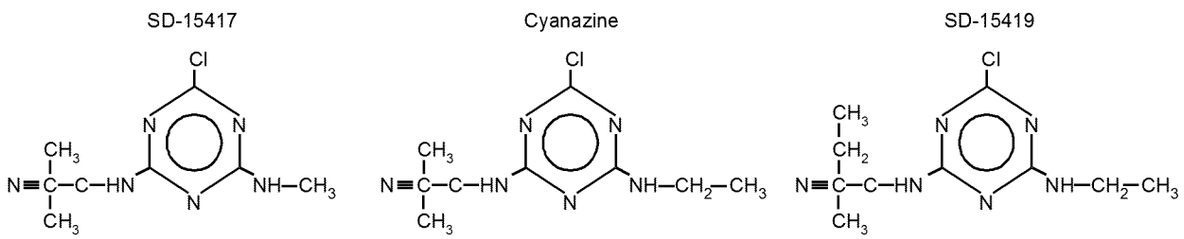
GS-14260, terbutryn: Terbutryn was the first triazine that could be applied pre-emergence to winter-sown small grain cereals, especially barley and wheat. Its spectrum of activity comprised a wide range of fall-germinating broadleaf weeds and grasses. As European grain production increased, annual weedy grasses, i.e., bentgrasses (*Agrostis* spp.), blackgrass (*Alopecurus myosuroides* Huds.), and others, became weed control challenges.

Terbutryn soon found markets in various crops and occupied important niches. Mixtures of low rates of terbutryn with near normal rates of atrazine have been used. Further opportunities for terbutryn were found in pea, broad bean (*Vicia faba* L. var major (Harz.)), common vetch, potato (always in mixture with simazine, or later with terbuthylazine), and sunflower, as well as grain sorghum and sugarcane in special situations. Terbutryn was the last candidate to reach the market during the first 15 years of the herbicide project.

A Brief History of Cyanazine

Shell's Agricultural Division produced and sold fertilizers, insecticides, and soil fumigants. The company had bought the Julius Hyman Company of Denver, Colorado, and in addition had conducted its own research. In July 1962, the Shell laboratories near Modesto, California, developed a comprehensive herbicide research program. The program was designed to guide new compound synthesis, as well as to evaluate efficacy of new herbicides. The first commercial product to emerge from this program was nitralin (Planavin), a methylsulfonyl dinitroaniline herbicide. It was sold for 4 years (1969 to 1973) and provided a basis for future development of herbicides.

In 1965, Shell signed a research agreement with the Degussa Company of Germany. Early in 1966, Degussa submitted a group of herbicidal compounds, three of which were even more active than atrazine on watergrass and crabgrass. These were coded SD-15417, SD-15418, and SD-15419. SD-15418 ultimately became cyanazine. SD-15417 was the methyl-amino analog of cyanazine, and SD-15419 was the (1-methyl-1-cyano-*n*-propyl)-amino analog of cyanazine. SD-15417 proved to have less activity and/or a shorter soil residual than cyanazine. SD-15419 was found to be a little less active than cyanazine and somewhat more expensive to manufacture. The two methyl groups of the (1-methyl-1-cyano-ethyl)-amino radical of cyanazine provided just the right amount of steric hindrance to slow down the hydrolysis of the cyano group. Cyanazine had a little more activity toward some grasses than atrazine, although it had somewhat reduced tolerance by corn. The faster inactivation in soils of the cyano group hydrolysis was important in soils with high pH and certain other soils where atrazine's use was limited due to carryover (Schwartz, 1966).



British and United States (No. 3505325) patents were issued for cyanazine on October 30, 1968 and April 7, 1970, respectively. Cyanazine was announced to United States weed scientists in 1967 (Hughes *et al.*, 1967) and to European weed scientists in 1968 (Chapman *et al.*, 1968). Degussa and several other companies later evaluated thousands of compounds, many of them triazines, but few possessed the necessary herbicidal activity and other properties needed for commercialization.

Building on these early developments, research in triazine chemistry has continued to result in the discovery of new compounds, including asymmetrical triazine herbicides and other classes of chemistry containing the triazine ring structure that are important for weed control around the world.

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Chapter 3

Production, Development, and Registration of Triazine Herbicides

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Summary

The first registered triazine herbicide was simazine (produced by J. R. Geigy, Ltd.), which was approved in Switzerland in 1956 for use on railroad tracks and rights-of-way and in corn, asparagus, and grape root stocks. At that time in Europe and in other countries (except the United States), sales permits were granted on the basis of biological performance and toxicological data. The United States required additional data on triazine residues in corn and in soil and on environmental degradation. Subsequently, and for all herbicide reregistrations, a full package of data became a standard requirement – including performance, toxicological studies, residues, degradation, and environmental behavior. Simazine was registered in the United States in 1957 for noncropland uses and for weed control in corn, ornamentals, and nursery crops in 1958.

By the end of the 1950s, atrazine was introduced for weed control on railroad tracks and rights-of-way and in corn production in Europe. The initial federal approval in the United States occurred in December 1958 when the US Department of Agriculture (USDA) registered Geigy Atrazine 50 W for use in corn and for nonselective weed control in noncrop areas. In 1959 Atrazine 80 W was registered, which in 1970 was trademarked as AAtrex® and quickly became a leading herbicide in the United States.

Geigy started production of simazine in 1956 in Schweizerhalle, near Basel, Switzerland; 2 years later, atrazine also was produced. Use of triazines for weed control to improve crop yields grew quickly, and a production facility was installed at McIntosh, Alabama in 1959. Smaller production units were operated in Mexico, Brazil, and Australia, and important development work by Geigy led to improved production processes. In 1970 a continuous process production unit was built by Geigy Agricultural Chemicals in the United States.

The triazine herbicides brought a new era of preemergence weed control to the agricultural technology of row crops, vineyards, and orchards. Field work became less tedious, and higher yields were achieved. Preemergence or at-planting herbicide treatments were new, revolutionary concepts in corn weed control, and Geigy developed extensive education and stewardship programs for growers, dealers, extension agents, and other crop advisors.

After Geigy's initial discovery of the chlorotriazines, simazine and atrazine, several additional chlorotriazines and other classes of triazine herbicides were researched and developed. These other classes included methoxytriazines, methylthiotriazines, and asymmetrical triazines. The chlorotriazines are selective in a large number of crops, but not in soybean, sugar beet, and most small grains. The asymmetrical (*as*) triazines, metribuzin (Bayer, DuPont) and metamitron (Bayer), proved to be a great benefit in crops not tolerant to other classes of triazines. In the early 1970s, Shell launched cyanazine as a new chlorotriazine, which was important in corn grown on Clarion-Webster and other clay soils where atrazine had the potential to cause carryover injury to soybean and cereal grains in rotation. Cyanazine

[1] Dr. Walter Heri of Basel, Switzerland has unfortunately passed away. He was a gifted scientist and colleague and is greatly missed.

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also was used for weed control in small grain cereals, cotton, sorghum, and sweet corn. Hexazinone (DuPont, 1976), a late comer in the *s*-triazine class, became a critical weed control tool in plantation crops and forestry.

Due to their broad-spectrum weed control, low cost, importance in conservation tillage systems, and flexibility in timing of application, the triazines quickly became important weed control tools for farmers. Even today, after more than 60 corn herbicide active ingredients have been developed and registered in the United States, more than two-thirds of the corn and sorghum acres in the United States are treated with an atrazine product. In addition, several other triazines remain key weed control tools today, including: simazine in more than 30 fruit and vegetable crops; ametryn in sugarcane; prometryn in cotton; terbuthylazine in corn and vineyards; and metribuzin in soybean.

Science and regulatory reviews conducted by the United Kingdom for the European Union in 1996 and 2000 (UK, 1996a, 1996b, 2000), Australia in 1997 and 2004, the International Agency for Research on Cancer (IARC) in 1999, and the US Environmental Protection Agency (USEPA, 2003, 2006b) all support the safety and continued availability of atrazine for weed control. In 2006, after a comprehensive science review of chlorotriazines, the USEPA determined 'there is reasonable certainty that no harm will result to the general US population, infants, children, or other major identifiable subgroups of consumers, from the use of simazine, atrazine, and propazine (USEPA, 2006a, b).'

Production: A Brief History of Triazine Manufacturing

Commercial production of simazine started in 1956 at the Geigy Schweizerhalle plant near Basel, Switzerland. Two years later, atrazine also was produced. Smaller production units were placed in operation in Mexico, Brazil, and Australia. In the fall of 1960, manufacturing of the triazines was started in the United States in McIntosh, Alabama. Examples of production pathways are included in Figure 3.1.

Due to increasing demand for atrazine, in 1969 Geigy began production at a new plant in St. Gabriel, Louisiana. As part of the planning for the new facility, intensive research and development work was performed in Alabama and Basel to improve the production process. Instead of the batch process used previously, a new continuous production process was developed. The new and efficient Louisiana facility has been the recipient of numerous production, safety and environmental awards and recognitions, and is now the production site for all Syngenta atrazine, simazine, and terbuthylazine used worldwide.

Production by Other Companies

As the initial triazine patents expired between 1970 and 1976, several companies (Fisons in the United Kingdom, Makhteshim-Agan in Israel, Oxon in Italy, and Sanachem in South Africa) began to manufacture triazines. These and other companies also became technical registrants and gained approval to sell the triazines in countries around the world. Worldwide there were soon 22 or more triazine plants, including atrazine production in the agricultural countries of Hungary, Czechoslovakia, Romania, and Yugoslavia. In 1970, Industria Prodotti Chimici (I.Pi.Ci.) in Italy started cyanazine production. By 1981, Shell had built its own plant for cyanazine and atrazine. This product and its marketing were transferred to DuPont in 1987.

Generic producers and a number of companies registered and sold technical atrazine under their own trade names to other formulators. There are more than 140 atrazine products in the United States sold by 41 companies.

Most producers concentrated their production on the major triazines (e.g., atrazine, simazine, terbuthylazine, ametryn, and terbutryn). The producers of triazine herbicides through the 1990s are presented in Table 3.1, and producers since 2000 are listed in Table 3.2.

Registrations of Chlorotriazines

Simazine

Simazine was the first triazine to be developed under the Geigy trade name Unkrautvertilger™ and was first sold in the spring of 1956 for noncrop uses on Swiss railroad tracks and rights-of-way. The compound had been widely tested. Additional tests on simazine as a selective herbicide were conducted at experimental stations in Switzerland and abroad, primarily in corn, cotton, asparagus, and grape vineyards. Official approvals for commercial use of simazine in corn, asparagus, and established grapevines were received on December 3, 1956.

Simazine was also the first triazine herbicide registered and sold in the United States. Registered April 11, 1957, simazine was initially approved for total vegetation control in noncropland areas, including rights-of-way. In addition to European data requirements, the United States required information on simazine metabolites and on the presence or fate of simazine residues in crops and soil. Based on these expanded data, simazine was approved by the US Food and Drug Administration (USFDA) and US Department of Agricultural (USDA) for use in corn in 1958. Simazine has a broad spectrum of weed control in corn and a wide range of other crops. In particular, simazine's excellent

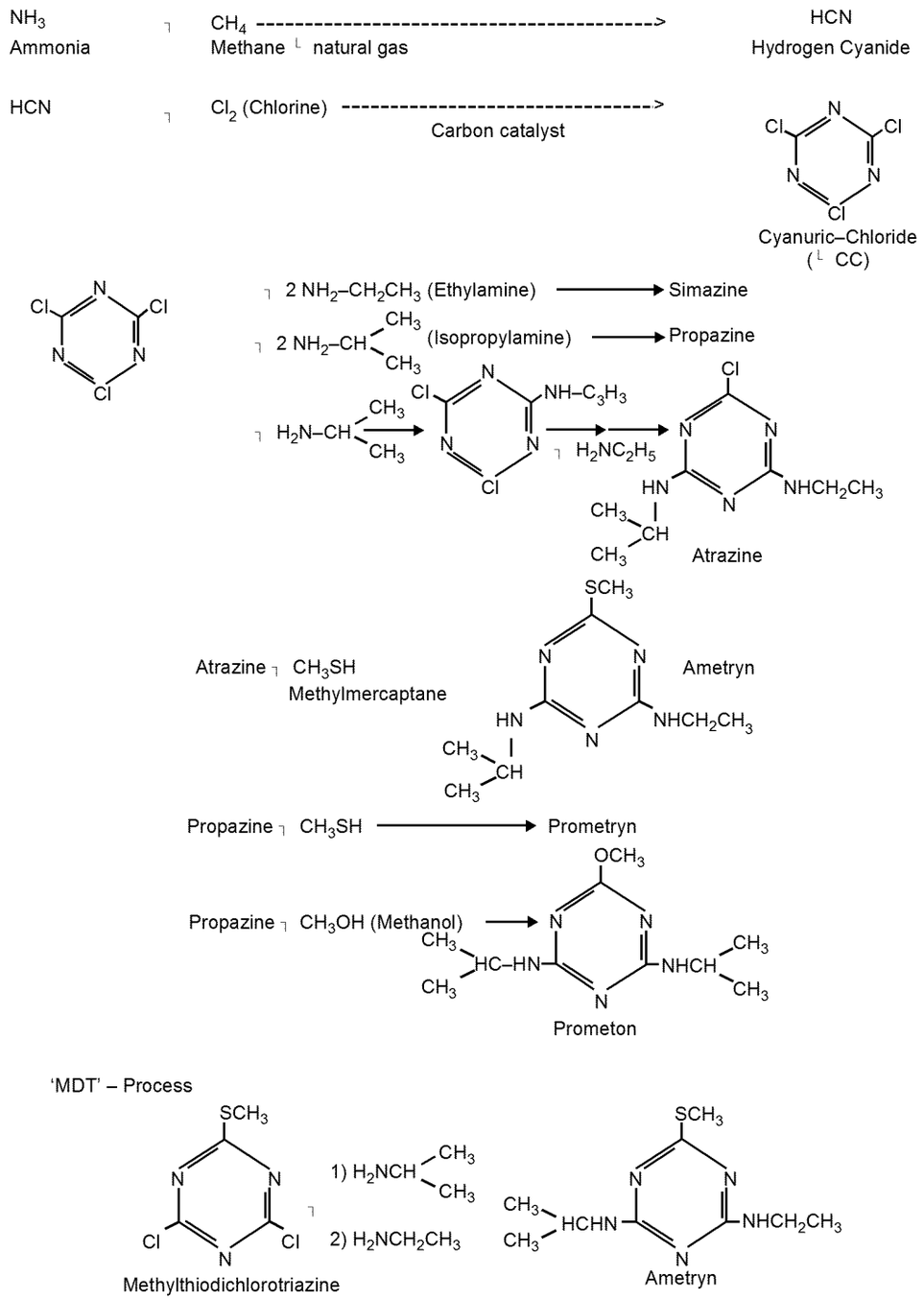

Figure 3.1 Examples of triazine production pathways.

broadleaf weed control when used preemergence made it the product of choice for many growers of fruits, nuts, berries, and citrus. Ornamental and nursery uses were added to the US simazine label in 1958.

During that same year, various granular formulations (10%, 8%, and 4%) of simazine were approved and introduced in the United States for ornamentals, nursery stock, and corn and for total vegetation control in noncropland. Also, a more concentrated (80 W) wettable powder formulation of simazine was approved for the same uses in December 1958.

Major uses added to the Simazine 80 W label included US southern turfgrass species for sod production, sugarcane, pineapple, and strawberry (1961); apple, sour cherry, macadamia nut, asparagus, orange, lemon, perennial grass grown

Table 3.1 Producers of symmetrical triazine herbicides worldwide

Late 1970 through the 1990s[a,b]			
Ciba-Geigy/Novartis – United States	(1–10,12)[b]	Shell/DuPont – United States	(1,12)
Oxon Italia – Italy	(1–6)	Fisons – United Kingdom	(1,11)
Makhteshim-Agan – Israel	(1–6)	Zorka – Yugoslavia	(1,4,6)
Agbro (Sanachem)/Dow – RSA[c]	(1–3)	Nitrokemia – Hungary	(1,4,5)
Radonja – Yugoslavia	(1,4,6)	Dimitrova – Slovakia	(1,2)
Local – CIS[d]	(2)	Local – Bulgaria	(1,2)
Local – China	(1,2)	Local – Romania	(1)
C. Quimica[e]/Atanor – Argentina	(1)	CNDA/Rhodia – Brazil	(1)
Farmland – United States	(1,12)	I.Pi.Ci.[f] – Italy	(1)

[a] Known major producers. Manufacturing not necessarily continuous for all companies listed.
[b] Legend for production: atrazine └ 1, simazine └ 2, terbuthylazine └ 3, ametryn └ 4, terbutryn └ 5, prometryn └ 6, desmetryn └ 7, simetryn └ 8, dimethametryn └ 9, terbumeton └ 10, trietazine └ 11, and cyanazine └ 12.
[c] RSA └ Republic of South Africa.
[d] CIS └ Commonwealth of Independent States.
[e] C. Quimica └ Company Quimica.
[f] I.Pi.Ci. └ Industria Prodotti Chimici, Milano.

Table 3.2 Producers of symmetrical triazine herbicides worldwide

After 2000
Syngenta[a] – United States
Sipcam/Oxon – Italy
Makhteshim-Agan – Israel
Dow – South Africa
Atanor – Argentina
Herbos – Croatia
Changxing Zhongshan – China
Changxing First Chemical – China
Yingkow Organic – China
Hebei – China

[a] In 2000, Syngenta was formed from the merger of the Crop Protection and Agricultural Products divisions of Novartis and Astra Zeneca, respectively.

for seed, and alfalfa grown for seed (1962); blueberry and caneberry (1963); almond, artichoke, avocado, olive, peach, pear, plum, sweet cherry, walnut, grapefruit, and lime (1964). Use on pecan crops was added in 1973. A US import residue tolerance for banana was granted in 1978, allowing simazine to be used for weed control on banana plantations in countries where it was approved. Grape and lemon were added to the label in 1979, along with expanded geography for a number of fruit and nut crops. The first uses for weed control in forests were added in 1977, followed in 1982 with turfgrass for fairways, lawns, and other grassy areas in the southern United States. Simazine is the standard for weed control in nursery crops and Christmas tree production in the United States.

Simazine also became widely used in industrial weed control. It was effective, stable, and did not leach as readily as alternatives. Highway departments sprayed simazine under guardrails to eliminate the need for mowing. Railroads and utilities used simazine in mixes with other herbicides to get longer-lasting control of shallow germinating weeds.

After extensive testing, Simazine 80 W was approved in the United States (1967) for algae and submerged aquatic weed control in fish hatchery ponds. As part of the process for obtaining approval for aquatic uses, a potable water tolerance and a tolerance for fish were established by the USDA. Additions to the label for other aquatic uses occurred over the next few years. In particular, use for algae control in swimming pools was approved by the USEPA in 1972. Simazine proved quite effective as an aquatic herbicide, and in 1975 a water-dispersible granule (WDG) formulation was added. The product carried the Aquazine® brand and quickly became a standard for algae and weed control in aquariums, ponds, and fish hatcheries. In the 1990s this use was limited to aquariums and very small ponds.

The trade name Princep® was introduced in 1969, and the US registration for technical simazine was granted in 1974. This allowed the technical material to be used by other companies to formulate and market products containing simazine. A water-based flowable liquid also was registered in 1974 and soon became the leading formulation of simazine sold in the United States.

Table 3.3 Initial registrations/approvals for use of simazine

Country	Uses	Year approved
Switzerland	Noncropland/rights-of-way, corn, asparagus, grape	1956
United States	Noncropland/rights-of-way	1957
United States	Corn, ornamental, nursery	1958
Germany	Noncropland/corn	1957
Holland	(Provisional) no specification on crops	1957
Austria	Noncropland/corn	1958
France	Noncropland/corn	1958

Table 3.4 Initial registrations for use of atrazine

Country	Uses	Year approved
Switzerland	Noncropland/rights-of-way	1958
Switzerland	Corn, asparagus, vineyards	1959
United States	Noncropland/rights-of-way and corn (50 W)	1958
United States	Corn (80 W)	1959
Austria	Noncropland/corn	1959
Germany	Noncropland/corn	1959
France	Noncropland/corn	1960
Holland	Corn, asparagus, general weed control	1960

With the approval of simazine in 1957 by the USFDA, USDA, and USEPA, the basis and procedures for successful introductions of other chlorotriazines were established. Although additional development work was necessary for approval and registration of the subsequent chlorotriazines, the procedures to optimize the production, formulation, and directions for use and the protocols to analyze and understand metabolism and toxicology remained similar. Approval for the first commercial uses of simazine and atrazine in various countries are given in Tables 3.3 and 3.4, respectively.

Although simazine was the first triazine to be developed and marketed in corn as well as other crops, the more versatile atrazine quickly became the standard herbicide in corn. Simazine, however, has remained very valuable and is important on forage crops, ornamentals, turf, and several other vegetable, fruit and nut crops, including almond, apple, artichoke, avocado, berries, cherry, citrus, grape, hazelnut, peach, and walnut. There also remains a strong demand for simazine use in corn in some areas based on specific weed pressure. Simazine is manufactured and sold by several companies today in more than 25 countries around the world, with Brazil, the United States, Australia, and Japan ranked as the top four.

Atrazine

By the end of the 1950s, atrazine was introduced in Europe and the United States for commercial weed control on noncropland (including rights-of-way) and on corn. For optimum growth and development, corn requires adequate space (rows of 75–90 cm width) and good availability of water and nitrogen. The crop canopy between the rows is open for at least 2 months and offers ideal conditions for weed growth. Before atrazine was available, most growers cultivated their corn many times, and when time or labor was available, used hand weeding to control the weeds the cultivator had missed. Since corn is a crop of major worldwide importance, atrazine became by far the most significant member of the *s*-triazine family and revolutionized the technology of corn production. Indeed, the availability of atrazine resulted in more farmers being able to grow corn throughout the world. Atrazine, more than any other single factor, made it possible to increase the maximum number of acres a corn farmer in the United States could grow and manage from about 100 to 400 A (40–163 ha) or more. In other areas of the world, atrazine often made corn growing possible and economically feasible.

The initial federal approval of an atrazine formulation in the United States occurred December 1, 1958, when the USDA registered Geigy Atrazine 50 W for preemergence and postemergence control of several broadleaf weeds on corn and for nonselective weed control in noncrop areas. A wettable powder was the most popular choice for herbicide formulations at that time and could easily be sprayed uniformly across a field.

On October 21, 1959, a more concentrated wettable powder formulation, Atrazine 80 W, was registered and quickly became the leading corn herbicide in the United States. Other formulations of atrazine, including various granulars

(4%, 8%, and 20%) were also introduced for use on corn and for nonselective weed control. These granular formulations provided farmers alternatives that did not require spray application, but instead were spread evenly over the soil surface or applied as a band over the crop row – often lightly incorporated or activated by rainfall.

The next major crop use in the United States added to the Atrazine 80 W label was weed control in sugarcane, approved by the USDA June 6, 1961. The 80 W formulation soon became the broadleaf weed control product of choice for sugarcane and remained so for many years until improved formulations of atrazine were developed.

In subsequent years, additional uses were approved in the United States for Atrazine 80 W: fall application for quackgrass control in corn (1961); macadamia nut, chemical fallow following wheat or ecofallow and perennial ryegrass (1962); southern turfgrass species for sod production (1963); pineapple (1964); sorghum and conifer (1965); and rangeland (1975).

Other formulations were approved in the United States in 1965 for nonselective weed control in noncropland areas. Those formulations, which carried the Atratol® brand, included a combination of atrazine, sodium chlorate, and sodium metaborate. This unique combination of ingredients, which became popular with highway departments, utilities, and railroads, provided quick burndown of weeds and residual weed control.

Changes in the laws governing pesticides by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetics Act (FFDCA) initiated a process to establish residue tolerances for pesticides, including those for products already registered. Tolerances are levels of the chemicals that are safe for human consumption and allowed on raw agricultural commodities. After residue trials were conducted in 1969, the first US atrazine tolerances were granted for corn, sorghum, macadamia nut, pineapple, sugarcane, wheat, and perennial ryegrass.

Up to the late 1960s, only dry formulations of atrazine were available. Ciba-Geigy Corporation later developed the first water-based, flowable concentrate formulation of an herbicide, AAtrex 4 L, which was approved on January 15, 1970. This development launched a new era in herbicide formulation technology. The water-based formulation improved the manufacturing process, minimized dust, was easier to package and to load into application equipment, and easier to mix. Gradually, more and more farmers who used atrazine switched to the liquid formulation, which is now the preferred formulation.

Shortly after the introduction of AAtrex 4 L, Geigy made an innovative packaging change. Liquid pesticides generally were packaged in 1- and 5-gallon (3.8 and 18.9 L) cans, as well as 30- and 55-gallon (114 and 208 L) drums that had few disposal options. The improved AAtrex 4 L package was a 2.5-gallon (9.5 L) plastic container molded in a rectangular shape. It had a 'built-in' handle and stacked efficiently, especially side by side. The plastic was lightweight and ultimately would have various disposal options, including recycling. In 1973, bulk packaging of AAtrex 4 L became available, and the first railroad tank cars of the product were delivered to be transferred into smaller bulk containers or into spray trucks for custom application.

Ciba-Geigy also improved atrazine dry formulations, developing a 90% WDG – AAtrex Nine-O® – that greatly minimized dust. Approved by the USEPA on December 10, 1975, it was the first WDG formulation sold in the United States.

In the United States in 2005, approximately 88% of all atrazine was used on corn, 8% on sorghum, 2% on sugarcane, and the remainder for minor uses. Atrazine is used by growers in more than 70 countries, with the top five being the United States, Brazil, Argentina, Mexico, and China.

Cyanazine

Cyanazine, a chlorotriazine (*Cl*-triazine) with a cyano group on the *N*-isopropyl radical, was launched in the early 1970s by Shell under the trade name Bladex®. In just a few years, cyanazine became the second highest volume symmetrical triazine herbicide used in agriculture. Its main features were its shorter soil residual effect and its selectivity in corn. Labels for use in corn and sorghum in the United States and elsewhere were obtained in time for introduction in the 1972 crop season. In Europe, cyanazine had commercial uses mainly in small grain cereals and triazine-resistant rapeseed. In the United States, however, it found a significant market in corn grown on soils that were heavy or had high organic matter, such as the Clarion-Webster and Nicollet series found extensively in central and northern Iowa, southern Minnesota, and other states. Cyanazine also was used on high pH soils of the western Great Plains, where atrazine use was minimal due to potential carryover to soybean and other rotational crops. Cyanazine use in cotton for specific weeds became very popular in some states. Cyanazine also was found to be useful in mixture with atrazine, allowing the atrazine rate to be reduced to eliminate carryover in sensitive soils. Shell introduced atrazine plus cyanazine prepack products, and E. I. du Pont de Nemours and Company (DuPont) continued and expanded their uses. Postemergence treatments of cyanazine with various surfactant and vegetable oil additives were important to enhance weed control activity under dry conditions.

In 1995, after 23 years of agricultural use, DuPont announced an agreement with the USEPA to discontinue cyanazine. The company voluntarily phased out production of cyanazine by the end of 1999 (Schieferstein, 1999), and all stocks were used by the end of 2002.

Propazine

Among the four major US commercial *Cl*-triazines (atrazine, simazine, cyanazine, and propazine), propazine was used on less acreage due to its specific niche in sorghum. Known as Milogard® in the United States and as Gesamil® in Europe, propazine was selective in corn, sorghum, and umbelliferae vegetables. In the United States from the West Texas panhandle northward through Oklahoma, Kansas, and parts of Nebraska, Milogard was sold as an 80 W or 4 L formulation. Later, a WDG called Milogard™ MaXX® 90 was added. Propazine also was used in carrot and fennel. In the early 1990s agricultural uses were discontinued due to high reregistration costs and as more efficient combination products were introduced. However, it was marketed by Griffin Corporation as Milo-Pro® until 1997 within four US states under an Emergency Exemption as authorized by Section 18 of the FIFRA, and propazine remained registered for indoor greenhouse use. Propazine was registered in sorghum in 2007 by the USEPA (2007).

Terbuthylazine

In the 1970s, a new *Cl*-triazine terbuthylazine was launched by Ciba-Geigy and sold outside the United States under the Gardoprim® brand. Terbuthylazine was first used together with terbutryn in potato and with terbumeton in vineyards and orchards. It also found important use in corn and partially replaced atrazine in parts of Europe and in South Africa, where it controlled *Tagetes* spp. (marigolds). Marigolds conveyed a bad flavor to corn and were not controlled by atrazine. Though terbuthylazine also had excellent broad-spectrum weed control, it is less efficacious than atrazine on certain weeds and under certain conditions. Terbuthylazine was introduced at lower rates than the initial atrazine rates and is not used on railroads or noncropland. European registrations for terbuthylazine use in corn were obtained in Germany in 1983, Austria in 1984, Italy in 1987, the Netherlands in 1990, and Denmark in 1993. Terbuthylazine has become the key triazine in Europe where atrazine use was discontinued due to detections in groundwater greater than the arbitrary, nonhealth-based 0.1 ppb groundwater limit for any pesticide in Europe. Exceedances of this standard were mainly due to the initial high rates of atrazine used in railroads, noncropland, and on some crops.

In US corn field trials, terbuthylazine was less efficacious than atrazine on certain broadleaf and grassy weeds. Therefore, terbuthylazine was not sold in the United States for use on corn. Due to terbuthylazine's activity against algae, it was registered in the United States in 1986 for use as an algicide and microbiostat in water coolant systems.

In 2004, terbuthylazine continues to be a major component of herbicide programs in Europe, especially in corn. At a country level, the Netherlands treats almost 100% of corn, while on the low end, Austria treats 35% of corn hectares with terbuthylazine. Approximately 60% of the combined area in corn production in Europe received terbuthylazine, including Germany, Italy, and Belgium. Terbuthylazine is used in more than 45 countries and remains a key weed control tool in crops such as corn, sorghum, pea, bean, lupin, grape, pome fruit, citrus, and vine.

Trietazine

Trietazine, a *Cl*-triazine, was discovered by Geigy in the 1950s. Like some other triazines, it showed selectivity in potatoes. Fisons, Ltd. in England developed the product. There were limited sales in England through Fisons, but due to better weed control candidates, trietazine production was discontinued. For several years, though, trietazine was used in Japan to control weeds in chrysanthemums.

Registrations of Methoxytriazines[2]

Atraton. Atraton was one of the first methoxytriazines developed for commercial use and was marketed in the early 1960s for weed control in tropical plantations. However, it was only sold for a few years and then dropped because of poor control of perennial weeds, such as cogongrass. Atraton also was used alone and in mixtures with other herbicides on roadsides and railway tracks in Europe and in the United States.

Prometon. Prometon was used in the United States for weed control on nonagricultural sites under the trade names Pramitol® and Primatol®. Offered as an 80 W or 25E (emulsifiable concentrate) formulation, Pramitol mixed readily with water and other herbicides. Alone, Pramitol controlled a wide variety of weeds and could be sprayed on soil before the application of asphalt to inhibit weed breakthrough in driveways or parking areas. A 5P (pelleted) formulation

[2] Research and development of methoxytriazines was spearheaded by Geigy in the late 1950s.

included 5% prometon, sodium chlorate, and sodium metaborate. Later, simazine was added to the formulation to control oxalis and other shallow-rooted annual weeds. Prometon is still an important weed control tool for certain industrial uses.

Secbumeton. Secbumeton was developed for use in alfalfa (lucerne) in the early 1970s. It was sold in Europe under the trade name Etazin™, where it remained a minor use product until it was discontinued in the 1980s. Further development in grape and sugarcane and continued until better alternative chemistries were developed.

Terbumeton. Terbumeton served as a partner product to terbuthylazine for weed control in vineyards and orchards outside the United States under the trademark Caragard®. Its role in these mixtures was to control deep-rooted perennial weeds. As better alternatives were developed, its use was discontinued.

Registrations of Methylthiotriazines[3]

Ametryn. Ametryn was introduced in 1962 and is still an established preemergence and early postemergence herbicide sold under the trade name Gesapax® and Evik®. Ametryn is effective against annual broadleaf weeds and grasses and is often used in tank mixes and in prepacks with other products. Labeled for use on field corn, popcorn, pineapple, and sugarcane in the United States in 1964, ametryn (Evik®) is sold as an 80 W formulation. It also is used in banana, citrus, palm, and coffee and has had limited use as a postdirected spray on corn. Most ametryn is used in sugarcane. It is used in more than 45 countries, with the top five being Brazil, Thailand, Mexico, Chile, and Cuba. Evik DF (dry flowable) is sold in the United States as a water-dispersible granule.

Aziprotryn. Aziprotryn was developed by CIBA and was selective in *Brassica* spp. (mustard) crops. However, desmetryn could be used at a significantly lower rate than aziprotryn and was more efficient to produce. Therefore aziprotryn (trade name: Mesoranil®) was phased out at the end of the 1980s.

Desmetryn. Desmetryn proved to be selective in *Brassica* crops (except cauliflower) at rates as low as 375–500 g a.i./ha. It was first launched in England toward the end of the 1960s under the trade name Semeron® for control of annual weeds in narrow-stem kale. Semeron is important for use in cabbage in Eastern Europe.

Dimethametryn. Dimethametryn was discovered and developed by CIBA and has served as an important partner product with piperophos for use in transplanted rice under the trade name Avirosan®. It is sold in Japan and South Africa.

Dipropetryn. Dipropetryn was first developed for specific broadleaf weed control in cotton grown on sandy soils in the southwest United States, particularly Texas. It was sold in the early 1970s under the trade name Sancap® and used outside the United States under the trade name Cotofor® in cotton and watermelon.

Methoprotryn. Methoprotryn was developed as a selective herbicide in winter cereals. It was applied postemergence at 1.5–2 kg a.i./ha and sold under the trade name Gesaran®. In most cases, it was applied in combination with low rates of simazine to provide control of late-germinating annual grassy weeds. Near the end of the 1960s, methoprotryn became the standard herbicide for the control of blackgrass and bentgrass in northwestern Europe. Its main alternative was metabenzthiazuron (Tribunil®). Methoprotryn became the official reference product or standard for cereal herbicide trials in France. In the following years, new urea herbicides, including chlorotoluron and isoproturon, became the products of choice.

Prometryn. Prometryn, sold as Caparol® in the United States and Gesagard® in the rest of the world, was registered in the early 1960s. Caparol 80 W became a significant herbicide in US cotton production following its labeling in 1964 for control of broadleaf weeds and a limited number of annual grasses. It was often used in combination treatments and eventually marketed as Caparol┐ MSMA (monosodium acid methanearsonate). MSMA provided faster control of weeds in early postemergence treatments. Also labeled for celery, Caparol 80 W eventually was replaced by a 4 L formulation. While it is now mainly used in cotton, it also is often used in sunflower, vegetables (such as bean, carrot, celery, fennel, lentil, leek, parsley, pea, and potato), peanut, and forage crops in various countries. Early use in rice was discontinued as prometryn was replaced by simetryn. Prometryn is sold in more than 50 countries, with the top five being the United States, China, Greece, Japan, and Hungary.

Simetryn. Simetryn found its place exclusively as a mixing partner with thiobencarb and other grass herbicides for rice in Japan. Simetryn is sold mainly in Japan through Hokko and Kumiai.

Terbutryn. Launched in the early 1960s under the trade name Igran®, terbutryn is applied preemergence and shows selectivity in wheat, barley, potato, sunflower, corn, and sorghum. In the United States, its use was limited to wheat and barley, mostly in the Pacific Northwest, and to sorghum in the Southwest. In sugarcane, terbutryn can be applied preemergence and early postemergence. At low rates it also can be applied postemergence in wheat for the control of broadleaf weeds. In the 1960s, terbutryn's main use was in fall-sown winter wheat in northwestern Europe. Used

[3] The methylthiotriazines were developed by Geigy unless otherwise noted.

preemergence, it controls blackgrass, bentgrass, and a large number of broadleaf weeds. A broad spectrum preemergence product in wheat, terbutryn provided excellent benefit to growers and was considered 'the atrazine of the small grain cereals.' At that time, a preemergence herbicide in cereals was considered unique. However, in the early 1970s, Ciba-Geigy developed chlorotoluron (Dicuran™), a urea herbicide that proved to be more versatile in small grains and had improved weed control. Terbutryn is still used in cereals, but at low rates applied postemergence to control broadleaf weeds. Terbutryn is sold in more than 25 countries, with the top being Mexico, Ivory Coast, Spain, Guatemala, and United Kingdom/Ireland.

Registrations of Hexazinone, Metamitron, and Metribuzin

Hexazinone. Hexazinone was introduced in 1974 by DuPont under the trade name Velpar® and became important for postemergence use in plantation crops and forestry. Hexazinone is sold in more than 30 countries, with the top five being Brazil, United States, Australia, Mexico, and South Africa.

Metamitron. In the mid-1970s, Bayer launched metamitron under the trade name Goltix®. Metamitron has application timing flexibility and can be applied both preemergence and postemergence. One of the mainstays in sugar beet production, metamitron is currently sold in more than 25 countries, with the top five being Germany, France, Netherlands, United Kingdom/Ireland, and Belgium.

Metribuzin. Metribuzin was launched in 1970 by Bayer under the trade name Sencor™ and also is sold by DuPont under the trade name Lexone™ for control of certain broadleaf weeds and grassy weed species. It was first introduced in Germany as a new potato herbicide, but within a short time its main use was in soybeans. In the United States, it has a wide range of uses, including vegetable and field crops, turfgrasses (recreational areas), and other noncrop areas. Metribuzin also was introduced with other products (e.g., trifluralin, metolachlor), and these mixtures exhibited good control over the entire soybean weed spectrum. Metribuzin was first registered in the United States in 1973, and currently there are 86 metribuzin products registered. Metribuzin is sold in more than 75 countries, with the top five being the United States, Brazil, Canada, China, and Germany.

Triazine Introduction in the United States

Introduction of simazine and atrazine use in corn production allowed farmers to learn novel technologies of preemergence or at-planting treatments for weed control. This required research, development, and educational programs to provide information farmers needed to modify, replace, or develop new application equipment.

Spraying atrazine in a 12- to 14-in. (30–36 cm)band directly over the corn row, followed by two or three cultivations, became an accepted weed control program. Shortly thereafter, 10G and 20G formulations (10% and 20% granules, respectively) were applied through a hopper box mounted on a corn planter in a band over newly planted corn. This method of application required light incorporation into the soil, though, and these formulations were less consistently efficacious. Broadcast spraying was also used, especially where nutsedge or quackgrass was a problem.

As use of atrazine grew, it became a Geigy priority to train technical representatives to hold education meetings with growers, cooperatives, and distributors and to call on farmers who had questions. Prior to atrazine's introduction, weed control practices were limited to hand hoeing, cultivation, and/or 2,4-D use after weed germination and growth. Atrazine, on the other hand, could be applied at planting with the assurance of early control of both broadleaf and grass weeds and safety to the corn crop. During the introductory years of atrazine and simazine, many educational programs were conducted throughout the world to explain the new methods of applying herbicides and the concept of preemergence weed control in corn production.

In the United States, Geigy representatives held Corn Clinics, most often with Young Farmer groups led by vocational agriculture teachers. They also held pesticide seminars for county agricultural extension agents, pesticide applicators, and farm managers. Part of the educational program included information on the importance of uniform spray nozzle sizes, 50-mesh (300 μn) or larger screens, constant tank agitation, and calibration of the sprayer to ensure accurate application rates. The Corn Clinics and other educational programs reached tens of thousands of farmers each year during the early 1960s and were instrumental in educating growers on the proper use of atrazine.

In addition, Geigy Ag Leader Pesticide Seminars were inaugurated across the United States to provide information and answer questions from groups such as county agricultural extension agents, agricultural editors and broadcasters, farm managers, custom applicators, and vocational agriculture teachers on the use of the triazine herbicides.

Yield Check Program

A coordinated Atrazine Yield Check Program was launched throughout corn-growing areas to quantify atrazine's benefits. In the fall, representatives went to cornfields and picked ears from three random sections of both atrazine-treated

and cultivated untreated areas. The ears of corn were weighed, measured, and adjusted for moisture content. The results were extrapolated into yield per acre.

The representatives made similar yield checks in cornfields where areas treated with atrazine could be compared with areas treated with other herbicides. These comparisons were made in the same field to prevent possible skewing of yield results by differences in soil types, fertilizer applications, or varieties of corn seed planted. Where grown for silage, stalks were cut from random areas (each representing 1/1000th of an acre or 4 m^2), bundled, and weighed.

From 1964 through 1966, more than 14 000 yield checks were made in corn-growing areas. Results showed up to 40 or more bushels (bu) of additional corn production per acre (100 bu/ha) in atrazine-treated versus untreated areas. Comparisons of atrazine versus other herbicide treatments also showed significant yield increases.

The benefits of field tests and understanding weed control on local soils became so apparent that Geigy began large-plot demonstrations using sprayers built by Broyhill with a 100-gallon (380 L) holding tank and a pair of 30-gallon (136 L) saddle tanks. These field trials were used for education and yield checks.

Evolving Weed Management Strategies

As atrazine went beyond trial use, farmers, dealers, and custom applicators fine-tuned application practices. In an effort to decrease cost and soil carryover potential, atrazine rates were decreased. Farmers moved from band to broadcast applications to achieve greater efficiency. They also sought maximum weed control by using atrazine in mixtures, especially for better grass control, tailored to each agricultural situation.

The crop selectivity and season-long effectiveness of the triazines on several weeds were highly useful to farmers, and adoption of the triazine herbicides for use in crop production was rapid. Long-term weed management strategies included:

- New formulation types, including flowables and dry flowables.
- Mixes (prepacks) with partner products.
- New research on integrated pest management (IPM) practices.
- Additional research on herbicide use to facilitate conservation tillage.

Atrazine and Oil: In the western part of the Corn Belt and the Great Plains, the agricultural extension service promoted the practice of applying a mixture of a lower rate of atrazine and oil in an early postemergence spray (when weeds first appeared). A highly refined crop oil was used, similar to that used in fruit-tree sprays. The practice was adopted extensively, especially in the Northern states to reduce the cost of herbicide treatment and lessen the potential for carryover.

During the late 1960s, the atrazine and oil combination gained widespread use in heavier, high pH soils in areas where rain following atrazine application was uncertain. Again, atrazine use practices led the way to innovation with other herbicides, and by the 1970s crop oil and oil concentrates were used as adjuvants for many other postemergence herbicides (McWhorter, 1982).

AAtrex® Nine-O®: Ciba-Geigy next developed AAtrex Nine-O, a 90% concentration of atrazine in a WDG. AAtrex Nine-O attracted immediate interest when launched in 1979 because of its handling qualities. The granules flowed freely from the bag and were easier to measure. This type of formulation was new to growers, and it fit in an important agricultural niche. It now is a major atrazine formulation.

Farm-Pak® Mini-Bulks: By the early 1980s, dealers and farmers had improvised containers ranging from 60 to 250 gallons (227–946 L) in capacity to handle bulk products. Ciba-Geigy designed the first state-of-the-art 'mini-bulk' (Farm-Pak) containers in 150- and 250-gallon (568 L and 946 L) sizes for large volumes of AAtrex, Dual, and Bicep herbicides. Mini-bulk containers are now made for many pesticides by many companies and are sturdy, stackable, and recyclable.

Premixes of Atrazine and Other Herbicides: The first prepackaged mixture of a grass herbicide with atrazine was Primaze®, a combination of two products: prometryn (Caparol) and atrazine. The prepack was first sold in 1968. However the margin of crop safety for prometryn was narrow, and it was marketed for only 2 years.

Another prepackaged herbicide mixture was AAtram®, the combination of Ramrod® (propachlor) and atrazine, formulated as granules. AAtram was introduced in the western Corn Belt and Great Plains in the early 1970s and was sold for only a few years.

A combination of metolachlor (Dual®) and atrazine in a liquid prepack called Bicep® facilitated the growing practice of mixing atrazine with grass herbicides. Test marketed in 1978 and 1979, Bicep was introduced nationally in 1980. In 1997, atrazine was combined with *S*-metolachlor to produce Bicep II Magnum®, since *S*-metolachlor contains more of the active isomer and reduces the amount of herbicide needed for efficacy.

To facilitate premixes in sorghum, Ciba-Geigy developed a seed safener to ensure greater tolerance to the chloroacetanilide mixing partners. The development of the safener had a significant impact on use of atrazine and propazine

on sorghum because it allowed prepacks and tank-mixes of certain acetanilides to be used by lessening the potential for crop damage.

By the late 1970s, corn acres planted in the United States reached more than 80 million (32.4 million ha) – up 15 million A (6.1 million ha) in 15 years. Today there are approximately 90 million acres of corn grown in the United States. Producers continue to treat approximately 65–70% of US corn acres with atrazine. More than 60 new active ingredients have been registered as herbicides for corn, and the top 10 premixes used in corn all contain atrazine. Registrations of atrazine mixture products with herbicides that control grassy weeds are expected, but the high percentage of all broadleaf herbicides used with atrazine confirms that atrazine continues to improve weed control efficacy and is a complementary product for active ingredients with grass and/or broadleaf activity.

There are currently approximately 80 atrazine premixes with 16 active ingredients registered in the United States.

IPM and Conservation Tillage: By 1992, atrazine was the major herbicide used in conservation tillage/notill in corn (Bull *et al*., 1993). As new research on IPM practices became available, several label changes, best management practices, and stewardship directions were implemented for atrazine (Appendix Table A5). Syngenta, one of the manufacturers of atrazine, developed and implemented a many-tiered, proactive approach to environmental stewardship for atrazine – including education on label changes, an information database, numerous cooperative educational and research projects, watershed programs, and comprehensive drinking water and ecological monitoring programs.

Regulatory Reviews

Regulatory reviews and reregistrations involving herbicides in each of the classes of triazine chemistry are ongoing in individual countries around the world. This section will focus on the chlorotriazines since they have recently received the most comprehensive review.

Special Review, Reregistrations, and Worldwide Science Reviews of the Chlorotriazines

In November 1994, the USEPA undertook a thorough safety assessment of the triazine herbicides called a 'Special Review.' This review included atrazine, simazine, and cyanazine. Through the course of the triazine Special Review, hundreds of research studies were analyzed and more than 80 000 public comments were received, mostly from supportive growers, commodity groups, and university researchers. Additionally, during the time the Special Review was being conducted by USEPA, reviews of atrazine were conducted by Australia, the United Kingdom (for the European Union), and France. The World Health Organization's IARC also reviewed both simazine and atrazine. The results of all of these reviews support the safety of the triazine herbicides (Table 3.5).

The United States: After reviewing the scientific data on atrazine since initiation of the Special Review in 1994 and changes in pesticide regulations under the Food Quality Protection Act in 1996, the USEPA in 2000 convened a Scientific Advisory Panel (SAP) to provide scientific advice on the potential for atrazine to be a carcinogen. The SAP concluded that atrazine was not likely to be carcinogenic to humans. In 2000, USEPA published its determination that

Table 3.5 Summary of atrazine health assessments in regulatory reviews worldwide

	EU–UK[a] 2000	Australia 2004	IARC[b] 1999	USEPA[c] 2000, 2003, 2006
Genotoxicity	Not genotoxic	Not genotoxic	Not genotoxic	Not genotoxic
Animal evidence	Mammary – female SD[d] Rat	Mammary – female SD Rat	Mammary – female SD Rat	Mammary – female SD Rat
Mode of action	Adequately explained MOA confined to female SD Rat	MOA unique to female SD Rat	MOA unique to female SD Rat	MOA unique to female SD Rat
Relevance	Not relevant to humans	Not relevant to humans	Not relevant to humans	Not relevant to humans
Classification	Carcinogen classification not appropriate	Absence of any carcinogenic potential	Not classifiable group 3	Not likely to be carcinogenic in humans

[a] European Union–United Kingdom.
[b] International Agency for Research on Cancer, World Health Organization.
[c] US Environmental Protection Agency.
[d] SD └ Sprague–Dawley.

the 'mode of action for the carcinogenic potential in the Sprague–Dawley rat is not likely to be operative in humans' and reclassified atrazine as 'not likely to be carcinogenic to humans' (USEPA, 2000). USEPA's classification has remained unchanged and was published again on October 31, 2003 in its Interim Risk Assessment for atrazine (USEPA, 2003). In 2006, after a comprehensive science review of chlorotriazines, the USEPA determined 'there is reasonable certainty that no harm will result to the general US population, infants, children, or other major identifiable subgroups of consumers, from the use of simazine, atrazine, and propazine' (USEPA, 2006a, b).

Additionally, the Agricultural Health Study, a government-sponsored study conducted by the National Institute of Health, the National Cancer Institute, the National Institute of Health Science, and the USEPA, has found no association between cancer incidence and atrazine exposure (Alavanja *et al.*, 2003; Rusiecki *et al.*, 2004; Engel *et al.*, 2005).

Australia: Australian Pesticides and Veterinary Medicines Authority (APVMA, 1997, 2004) evaluated atrazine's safety in 1997 and 2004. The 1997 APVMA review that evaluated a range of studies conducted in mice, rats, and rabbits concluded that atrazine is not a reproductive or developmental toxicant. The 2004 APVMA review determined that the data regarding the formation of tumors in one species of laboratory rat (Sprague–Dawley) exposed to high levels of atrazine has no relevance to humans, and that epidemiological data support the absence of any carcinogenic potential for atrazine. Additionally the APVMA (2004) stated that: 'Atrazine is unlikely to be an endocrine disruptor in humans based on the known mechanism of action in Sprague–Dawley rats.'

The APVMA also concluded there were no major toxicological concerns relating to atrazine (APVMA, 2004) and established a health-based water standard of 40 ppb for atrazine and its metabolites. The APVMA also reviewed additional data on potential effects of atrazine on amphibians and concluded that taken together, these data indicate it is unlikely that atrazine is having an adverse impact on Australian amphibian populations at current levels of exposure (APVMA, 2004).

European Union: In 1996, the science review conducted for the European Union by regulatory officials in the United Kingdom concluded: 'It is expected that the use of atrazine consistent with good plant protection practice will not have any harmful effects on humans or animal health or any unacceptable effects on the environment' (UK Rapporteur Monograph Volume 1 and Volume 3, 1996a, b). The health-based water level for atrazine also was determined in this review to be 15 ppb, which is 150 times higher than the 0.1 ppb arbitrary limit set for all pesticides. Simazine also received a similar favorable review from the United Kingdom (UK Rapporteur Monograph, 1996).

The European Union determined there is sufficient evidence to conclude that mammary carcinogenesis appears to occur only in female Sprague–Dawley rats and that the mode of action (MOA) for tumorigenicity is considered unique to the female Sprague–Dawley rat and therefore not relevant to humans. The review concluded that the nongenotoxic MOA of atrazine is adequately explained and without consequence for human health. Classification of atrazine as a carcinogen is not appropriate (UK Rapporteur Monograph Volume 1 and Volume 3, 1996a, b; Volume 3 Annex B, 2000).

The EU further added: 'It should further be noted that metabolism of atrazine is extensively investigated in comparison to many agrochemicals, and that uncertainties relating to the quantity of data available should not be taken out of context with the assumptions made in circumstances where fewer data are available. The quantity of data available with atrazine is strongly reassuring.'(UK Rapporteur Monograph Volume 3 Annex B, 2000).

World Health Organization: The World Health Organization's IARC reviewed atrazine before the development of a full database of animal cancer bioassays and mechanistic data (IARC, 1991). In 1999, IARC re-examined the hazard and new mechanistic data for atrazine. The IARC Working Group concluded the mammary tumors associated with exposure to atrazine involve a nonDNA-reactive, hormonally mediated mechanism, only in intact female Sprague–Dawley rats (not in Fischer 344 rats, CD-1 mice, or ovariectomized Sprague–Dawley rats) and does not increase the incidences of other tumor types. Atrazine affects neuroendocrine pathways of the hypothalamus to accelerate the onset of reproductive senescence in female Sprague–Dawley rats, but not Fischer 344 rats. Atrazine does not have intrinsic estrogenic activity, and there are critical interspecies differences in the hormonal changes associated with reproductive senescence. IARC concluded 'there is strong evidence that the mechanism by which atrazine increases the incidence of mammary gland tumors in Sprague–Dawley rats is not relevant to humans.' The IARC Working Group concluded atrazine is *not classifiable as to its carcinogenicity to humans* and placed atrazine in Group 3 (IARC, 1999). IARC also reached these same conclusions with regard to simazine (IARC, 1999).

France: In 2001, the French Toxicity Research Commission on Pesticide Products cited the IARC, the USEPA, and the EU conclusion that there is an absence of carcinogenic effects of atrazine for humans (French Republic Ministry of Agriculture, 2001). The Commission further stated, 'Considering all these factors, the concentration of the triazines in water, even elevated levels, identified in the field both in transitory and localized form, do not represent a public health risk.'

Conclusions

The triazine herbicides have revolutionized agricultural production of corn and more than 40 other crops. The yield increases, less labor-intensive production, and use for erosion control in conservation tillage are all benefits of the triazines, especially atrazine and simazine. Registered since the late 1950s, atrazine is still a mainstay of corn production and likely the most studied herbicide by regulatory agencies.

Many changes have been made to the triazine labels over the nearly 50 years of registration, both due to changes in regulations for all pesticides and to the adoption of stewardship and best management practices. Commercial production of the triazines has also undergone many changes as new technologies have been introduced.

Regulatory bodies in the United States, the European Union, Australia, and France, as well as the World Health Organization, have all given atrazine favorable safety reviews for continued registration. The safe use and resulting benefits of the triazines in worldwide agricultural production are critical as farmers continue to feed our growing population.

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Chapter 4

Weed Control Trends and Practices in North America

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Summary

As with any crop production enterprise, the selection of one weed management technique over another is a matter of improving efficiency, economics, environmental stewardship, and soil conservation. From the earliest use of sharpened sticks and iron hoes to today's use of herbicides – technical innovation, culture, inspiration, hard work, and serendipitous discovery have worked together to produce the efficient crop management system we have today. For North American agriculture, these changes came slowly at first. During the Colonial Period, readily available virgin farmland and a paucity of agricultural institutions to disseminate technical knowledge produced few incentives for farmers to change traditional practices. However, as the demands for food and fiber increased in the 19th and 20th centuries, the pace of agricultural invention rose to produce high yields and increase production efficiency.

Many of our current technical innovations in mechanical weed control can trace their development from earlier forms of the same tillage device. Even the plows and cultivators used through the 1900s tended to be little more than large, hardened steel replacements of the wooden tools used centuries earlier. On the other hand, herbicides radically changed agricultural production within a few years of their introduction. Novel application equipment is now required by the herbicide user, as is the knowledge to use each new herbicide product effectively.

Initially, lack of application equipment and the technical knowledge needed to use herbicides were challenges to growers. Farmer familiarity with fertilizer application boxes on their planters initially resulted in the granular formulation of many herbicides being banded over the crop row. When spray equipment became more widely available, farmers adapted and began to use broadcast applications of postemergence, preemergence, and preplant incorporated herbicides. Evolving tillage practices impacted the timing of herbicide application and whether it was mechanically incorporated.

Crop safety, drift injury to nontarget plants, and hazards to the environment and the operator contributed to the complexity of weed management decisions. Products with difficult handling characteristics or narrow tolerances for crop injury were put aside as improved herbicides became available. Weed scientists were struggling to learn when and how to best control weeds and how to best convey information to growers about this important emerging agricultural discipline.

A principal issue from the grower's perspective was the ease with which an herbicide could be used to control weeds effectively. Herbicides that would be favored had a broad spectrum of weed control, easily mixed with other herbicides to expand the control spectrum, and could be applied using a number of techniques and application timings. Since their introduction in the 1940s and for the next 60 years, products that exhibited these characteristics, such as the triazine herbicides, came to be the mainstays of the new weed control era. Although herbicides have been an efficient tool for crop production, they have also become an agent of change – increasing productivity, decreasing tillage requirements, facilitating an increase in farm size, and permitting economies of scale.

The purpose of this chapter is to examine trends in weed management in North America. A single chapter does not allow us to address in detail all of the important weed management innovations. However, we will point out some of the significant changes, and through the use of examples, suggest some of the factors that have affected the evolution of weed management. For a concise and informative treatment of the history of weed control in Europe from the days of Rome and Greece up to the 19th century, we recommend Smith and Secoy (1981).

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The First 200 Years: 1600–1800

Agricultural innovation has not been a steady process. The abundance of cheap productive farmland during the first two centuries of European settlement in North America provided little incentive to improve crop management techniques on existing farms. From the first permanent settlement at Plymouth Rock down through the early 1800s, land was readily available for the taking. As repeated cropping impoverished the soil, one only had to move to newer, more productive land 50 or 100 miles (80 or 160 km) further west. Thus, the early history of crop production in North America is less of a steady search for improvements in methods and technique, and more of an opportunistic search for 'greener pastures.'

Innovation in the 1800s

By the beginning of the 19th century, the process of scientific discovery in agriculture throughout Europe was evident in its many agricultural journals and societies. During this same period, however, agricultural education in North America was largely limited to some basic instruction at Yale and Harvard universities. In the United States, it was only when open, productive farmland became scarce in the 1830s, beginning in Virginia and Maryland, that agricultural schools were established. Eventually, other states came to recognize the significant economic consequences that resulted from the loss of farm productivity. Within the United States the scientific method was institutionalized in land-grant colleges and research stations through the Morrill Act of 1862 and the Hatch Act of 1887 (Schaefer, 1970).

Although land-grant institutions dominated the dissemination of information on the latest agricultural technologies, many significant innovations of the 19th century came from private individuals. Inventions such as John Deere's steel plow in 1837, Cyrus McCormick's reaper in 1834, and George Brown's horse-drawn corn planter in 1853 each played a role in increasing the efficiency and productivity of the farmer.

The invention of tools used for weed management, however, tended to be less traceable to a single source. Hand hoeing, or 'chopping' as it was known in many regions, was sometimes supplemented by the use of a light plow between crop rows (Schlebecker, 1975). The earliest cultivators, introduced in the 1820s, had iron spikes or shovels attached to a triangular frame that was pulled between the rows by ox, horse, or human power (Figure 4.1). In the 1840s straddle-row riding cultivators, pulled by a pair of horses, made their debut (Figure 4.2). With the addition of attachments to shield the row, and levers to raise the shovels over obstructions, this cultivator doubled the amount of the crop that could be cultivated, and therefore produced, by a farmer.

Innovations Since 1900

By the early 1900s, tractor power was being used to complement horsepower as a time- and labor-saving device. Initially, tractors were used for plowing or as a stationary source of power for threshing equipment, being too large and cumbersome for row cultivation. The shortage of labor, the need for increased production, and the technological developments of World War I encouraged the adoption of tractors. Modifications that permitted greater horsepower with lighter-weight engines allowed tractors to be used for additional kinds of field work. Cultivation of row crops was a principal use to which the new lighter tractors were well adapted. Mechanized power was adopted most readily in the high-intensity and high-value vegetable and fruit crops of the Northeast and in states surrounding the manufacturing center of Chicago. By the end of World War II, nearly 40% of all farms relied in some part on tractor power (Schlebecker, 1975). It was also the introduction of lightweight tractors, outfitted with power-take-off (PTO) driven pumps, which made chemical weed control possible.

Since the beginning of the 20th century, farm size has steadily increased, while the number of individuals engaged in farming has decreased. According to the US Census of Agriculture (1997), average farm size grew little during the first half of this century – from 150 acres (A) (60 ha) in 1900 to 175 A (70 ha) in 1940. In 1997, however, average farm size was estimated at 487 A (200 ha). Farm size was closer to 800 A (325 ha) for farms in the Great Plains. The increase in farm size has been accompanied by a decrease in the available labor force in agriculture. In the 1997 census, less than 3% of the US labor force was directly employed on farms, versus 37% in 1900 (Truesdell, 1961). Tractors and other mechanical devices have increased farmer efficiency and have provided major gains in productivity since the 1940s. However, much of the economies of scale provided by large farm size can be attributed to the labor-saving use of herbicides. Herbicides not only control major agricultural pests (weeds), but they also reduce manpower and horsepower requirements for crop production.

Herbicides have resulted in a great improvement and diversity in weed management techniques among the various crops grown in North America. An example closely correlated with the triazine herbicides is that of row crop production in the Midwest. If we follow the trends of corn and soybean production, we see how the management of weed control has evolved in response to economic, cultural, and social influences and needs. Because the State of Illinois

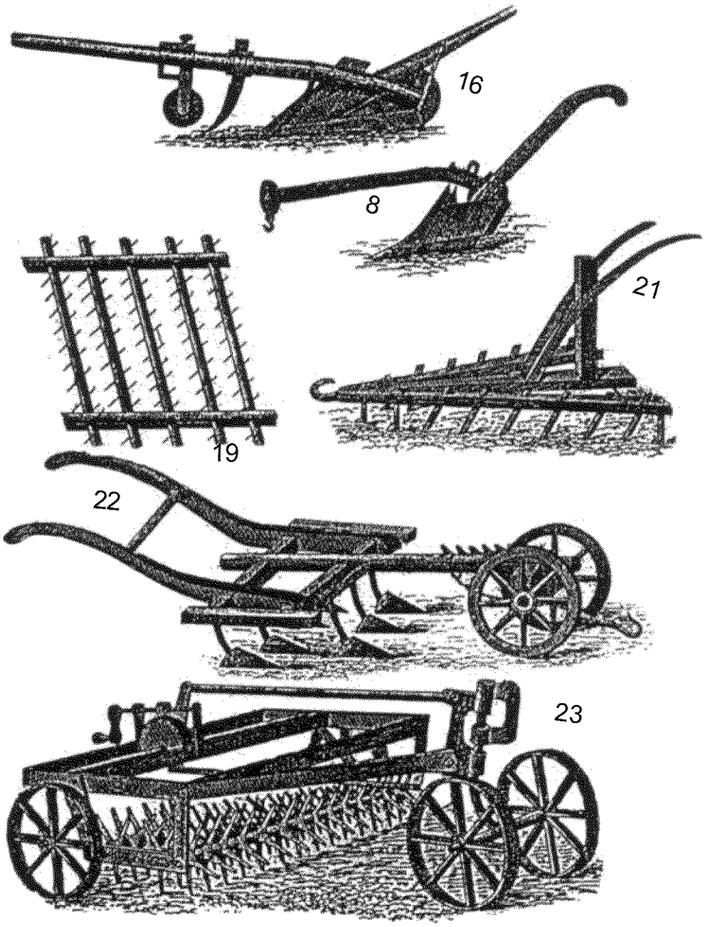

Figure 4.1 Items 8 and 16 are various plows. Items 19, 21, 22, and 23 are various harrows (Heck, 1851).

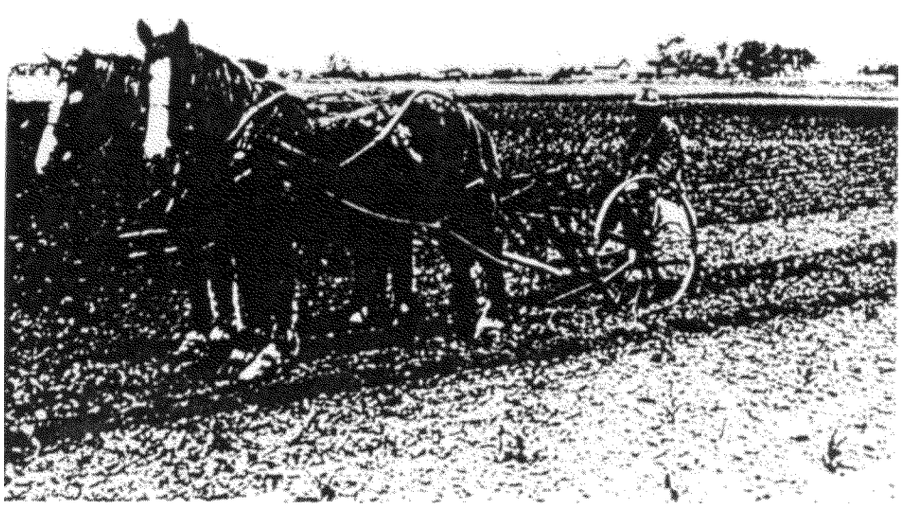

Figure 4.2 Horse-drawn straddle-row cultivator commonly used during the late 1880s in the United States. (Courtesy of Dr. Ellery Knake, Professor Emeritus, University of Illinois.)

Table 4.1 Percent of Illinois corn crop treated with an herbicide

Year	2,4–D	CDAA	Atrazine	Propachlor	Alachlor	EPTC & butylate	Dicamba[a]	Cyanazine	Metolachlor[b]	Glyphosate	Bromoxynil	Bentazon	Nicosulfuron	Primisulfuron	Prosulfuron	Acetochlor	Dimethamid	Halsulfuron
1957	33																	
1958*	39	1																
1959	39	3																
1960	40	4																
1961	40	5	1															
1962*	42	6	2															
1963*	42	8	5															
1964	43	10	10															
1965	46	12	10	1														
1966*	46	14	17	3														
1967	44	17	21	12														
1968	43	10	29	19														
1969	33	7	33	24														
1970*	22	4	38	26	3	1	1											
1971	26	4	48	30	13	1	2											
1972	24	2	48	26	14	1	1	1										
1973*	22	1	55	25	20	1	1	1										
1974*	20		60	20	28	7	2	2										
1975*	19		66	12	36	14	2	3										
1976	19		72	4	40	23	3	4										
1977*	19		76	4	38	30	4	8										
1978	21		81	4	35	41	5	12	3									
1979*	19		80	3	36	40	6	14	7									
1980*	18		76	2	38	38	8	16	12									
1981*	17		75	1	36	36	10	18	18									
1982*	16		73	1	36	35	11	21	26									
1983	16		71	1	33	30	12	17	29									
1984*	17		71	1	31	27	13	16	32									
1985	18		70	1	26	24	14	12	36	1								
1986*	16		68	1	26	20	15	12	36	1		1						
1987*	14		67	1	25	18	16	12	36	1	2	2						
1988	12		76	1	28	16	17	13	36	1	3	3						
1989*	11		78		25	12	19	14	36	1	4	4						
1990	10		84		26	10	19	14	35	1	5	6						
1991	8		78		27	9	20	17	36	1	6	4	2	1				
1992	13		84		27	6	18	25	35	1	6	2	3	1				
1993	15		82		24	7	21	23	38	6	7	3	5	2				
1994	16		82		18	5	22	26	34	4	10	4	14	3		5		
1995	15		70		6	1	18	20	28	4	9	5	12	1		24		
1996	10		76		1		22	21	30	3	7	11	6	9	5	28	11	5
1997	6		79		1		19	15	31	7	6	8	7	5	5	29	8	4
1998	24		78	0	0	0	17	22	32	5	8	3	5	4	4	25	7	0
1999	7		84	0	0	0	23	1	33	7	4	4	14	4	1	24	11	0
2000	13		81				35		45	63			8	20	9	20	14	
2001	14		88				32		24	12		3	13	7	4	35	17	
2002	5		72				25		27	4			11	4		23	14	

* Did not have survey data and were interpolated by the authors
[a] Dicamba: Includes all forms.
[b] Metolachlor: Includes *S*-metolachlor and metolachlor.

is centrally located in the Great Plains and has some of the most complete survey data available on herbicide use, much of our commentary will be supported by data taken from Illinois, but closely parallels similar progress and success stories in other crops, states, and areas of North America. Unless otherwise noted, data detailing product uses are taken from a summary of surveys by the National Agricultural Statistical Service (NASS) and by Pike and others (Pike and Glover 1991; Pike *et al*., 1991), as found in Tables 4.1 and 4.2. Many of the same limitations, incentives, and rationale used to adopt or reject a technology in corn and soybean apply to other crops as well.

Table 4.2 Percent of Illinois soybean crop treated with an herbicide.

Year	Alanap	Chloramben	CDAA	Linuron	Dinitroanilines[a]	Alachlor & metolachlor[b]	Metribuzin	Bentazon	Acifluorfen	Fluazifop	Fenoxaprop	Sethoxydim	Clethodim	Glyphosate	Quizalofop	Chlorimuron	Clomazone	Lactofen	Fomesafen	Imazaquin	Imazethapyr	Thifensulfuron	2,4–D
1960	2	3																					
1961	3	4																					
1962*	3	6																					
1963*	3	9																					
1964	7	12	6	1																			
1965	3	16	8	1	2																		
1966*	7	26	7	1	4																		
1967	2	30	6	1	8																		
1968	2	36	6	1	12																		
1969	1	39	3	3	15	4																	
1970*	1	37		6	16	7																	
1971	1	38		8	17	15																	
1972	1	39		11	21	14	5																
1973*		33		12	18	20	10																
1974*		26		13	36	24	15																
1975*		23		14	40	28	20																
1976		20		15	46	33	30																
1977*		17		15	55	33	45																
1978		15		14	63	34	55	4															
1979*		14		12	65	33	56	6															
1980*		13		10	63	34	54	10															
1981*		12		9	63	35	52	15															
1982*		12		8	64	35	51	20	2														
1983		10		7	64	33	49	22	3														
1984*		8		7	66	31	47	24	5														
1985		5		6	66	29	46	26	6	2		3		3									
1986*		4		6	65	25	45	27	7	1		4		3									
1987*		3		6	65	24	40	28	8	1		5		3									
1988		2		5	65	21	22	30	9	0.7		5.6		3	ffi1	9	13			26			
1989*				1	62	20	20	26	9	1		6		3	1	15	10		0.2	20	6		
1990				0	62	19	17	24	9	1.3		7.5		2	3	17	7		0.5	12	12	5	
1991				0	62	18	15	22	9	5		9		6	4	22	7		0.9	14	26	5	
1992				0	46	15	13	24	11	5	2	9		6	5	20	3		2	15	29	10	
1993				0	56	8	10	22	12	8	3	10		9	7	18	6	2	2	12	38	14	14
1994					51	11	10	23	12	8	5	13	2	10	3	17	3	3	2	14	46	17	17
1995					55		6	10	6	6	5	7	3	7.2	5	21	1	4	3	10	60	12	15
1996					52		7	5	3	1	1	8	10	30	6	13	1	9	5	15	57	11	25
1997					47	4	13	13	10	4	3	11	5	22	1	15	3	4	8	14	47	10	9
1998					24	2	5	7	4	7	6	11	3	59	2	14	4		3	2	12	3	13
1999					23	0	4	2	3	3	3	7	5	58	0	19	4		4	3	16	10	10
2000					26		6	3	3	4	4	4	8	55		18		4	8	4	13	9	11
2001					19				3	4	4		6	72		6			7		9	3	9
2002					9				2	3	3		5	86		7		1	3		10		13

* Did not have survey data and were interpolated by the authors.
[a] Dinitroanilines: Include trifluralin, pendimethalin, oryzalin, and ethalfluralin.
[b] Metolachlor: Includes *S*-metolachlor and metolachlor.

Row Crop Management

One of the principal reasons that crops are planted in rows is to take advantage of the mechanical techniques available to kill the weeds between rows. Weeds growing in the row with the crop are usually not disturbed by mechanical cultivation. Although cultivators have been designed to move some soil into the row to smother small weeds, many weeds escape because this inexact process is affected by soil condition, climate, and equipment variables. As explained in the following paragraph, the limitations of mechanical weed control between the rows became more apparent as a new crop (soybean) and several new weeds were introduced in the Central Plains. We will begin our discussion of the 'state of technology' of row crop weed control by examining one innovation that had been widely adopted for corn husbandry as early as 1870, a procedure known as 'check planting.'

'Check planting' was developed for field corn and required a 'check' wire to be stretched from one end of the field to the other. This wire had knots spaced uniformly along its length, usually about 40 in. apart. The wire was fed through an attachment on the planter, and as the planter moved across the field, the knots in the wire caused the planter to drop corn kernels at evenly spaced distances. The result was a checkerboard pattern of corn with rows in perpendicular directions. Thus, the corn could be cultivated in two directions or 'cross-cultivated.' This planting procedure was common in North America from the late 1800s until about the middle of the 1900s. During this time it was common to cross-cultivate the field three or four times a season to control as many weeds as possible.

During the 1930s to the 1940s, there was a shift from horsepower to the use of tractors for both planting and cultivating. Check wire and checkerboard patterns were used, so tractor-mounted equipment could be used to cross-cultivate. As the size of fields and farms increased, use of the check wire planters and cross-cultivation were replaced by larger and faster planters that 'drilled' the corn kernels in the row without the spacing necessary for cross-cultivation. The advent of these planters, as well as the expanding production of other row crops (e.g., soybean) that did not lend themselves well to cross-cultivation, created a need for controlling the weeds in the row that were out of reach of the cultivator (Figure 4.3).

Soybean made its initial debut in the 20th century as a drilled forage crop. Although soybean production grew during the 1920s and 1930s, it was not until the 1940s that it became well established as a grain crop to be processed for oil, meal, and other by-products. In the Central Plains, corn production became closely tied to soybean production and has been used in crop rotations since the 1940s for three main reasons. First, because soybean does not require nitrogen to be added to the soil to be grown successfully, it left more nitrogen in the soil for corn production in the following season. Second, the size of the soybean seed and the technology of planting and harvesting were similar enough to corn to allow the same equipment to be used for planting, cultivating, and harvesting both crops. And finally, both corn and soybean received a yield advantage of about 10% when grown in alternate years in the same field.

Although the soybean crop was relatively competitive with weeds, it could not be effectively cross-cultivated. To make the crop more competitive, early soybean crops were drilled in very narrow rows or planted by "solid seeding," which scatters the seed more or less evenly but randomly over the soil. But eventually production shifted to planting in rows wide enough to allow for row cultivation. Since row spacing no longer needed to be wide enough to accommodate the width of a horse, row width was narrowed somewhat to allow more efficient utilization of available nutrients and sunlight and to provide greater competitive ability with weeds. Row widths were narrowed from 40 inches (100 cm), to 36 inches (90 cm), to 30 inches (76 cm) and then gradually to even narrower rows of 8- to 20-inches (20–50 cm) spacing, which practically eliminated the possibility of cultivating between rows.

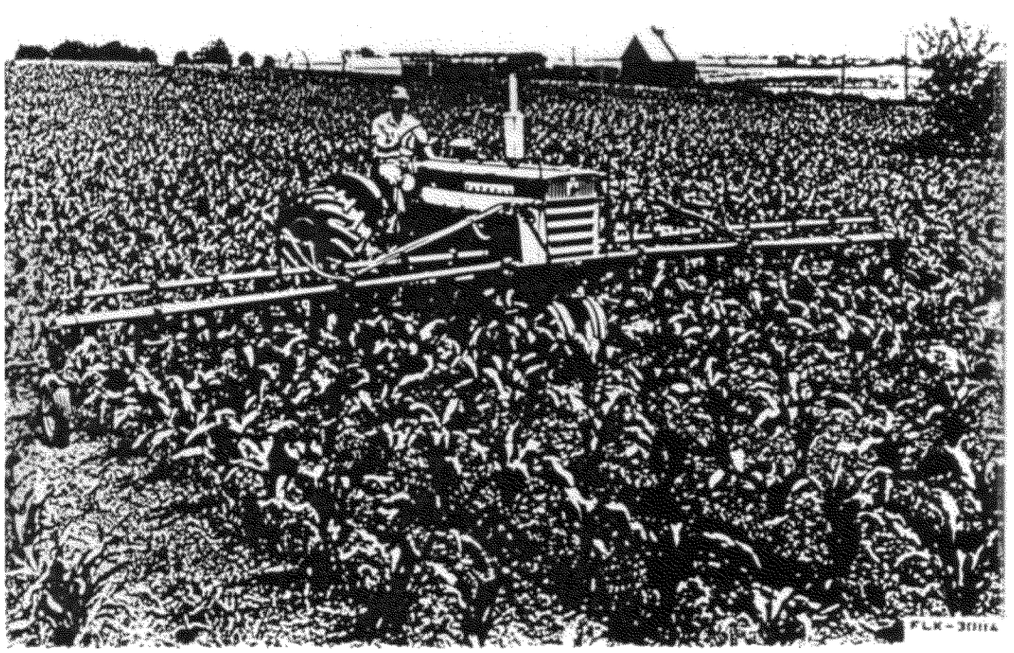

Figure 4.3 Eight-row cultivator being used on check-planted corn (International Harvester).

Also, during the late 1940s and throughout the 1950s, a new weed – giant foxtail – spread rapidly across the fields of the Midwest. This weed was very competitive and invasive, reducing yields and lowering grain quality throughout the Corn Belt. Widespread infestations of this weed were very noticeable, particularly in soybean fields where it was easily seen above the top of the crop (Figure 4.4). The extensive field corn and soybean rotations also led to the proliferation of other annual weeds that were well adapted to row crop culture.

In the 1950s, several crop production factors converged to lay the foundation for a radical shift in weed management. Farmers had discarded check planting and needed an alternative to cross-cultivation. Soybean had become a major grain crop from which giant foxtail and other weeds needed to be removed. A convenient and popular crop rotation of field corn and soybean resulted in changing trends in weed problems with a proliferation of annual weeds well adapted to row crops. There was also a movement toward early planting, narrow rows, and a drive for high yields. These trends promoted the adoption of herbicide use, and by 1975, herbicides were used on 90ffl% of the corn and soybean acreage in the United States.

Chemical Weed Control

Like most innovations, a lack of knowledge and technical support initially constrained herbicide use. During the 1950s the equipment and technology for spraying herbicides at carrier rates of less than 100 gallons per acre (1120 L/ha) were not widely available. When producers began using herbicides, home-built sprayers constructed from 55-gallon drums and rubber hoses were the primary means of application (Figure 4.5). In the Central Plains, pumps, tanks, and the technical knowledge to construct a working sprayer were all in short supply. Approximately one-third of farmers completing a survey in 1959 indicated that they had not used herbicides because satisfactory sprayers were unavailable (Illinois Farm Supply Company, 1960).

The necessary distribution channels for reliable information and farmer education also were not well established. During the late 1950s, herbicide application recommendations were obtained through many of the same sources from which agricultural products were purchased, such as Farm Bureau stores, operators of local grain elevators, and feed stores.

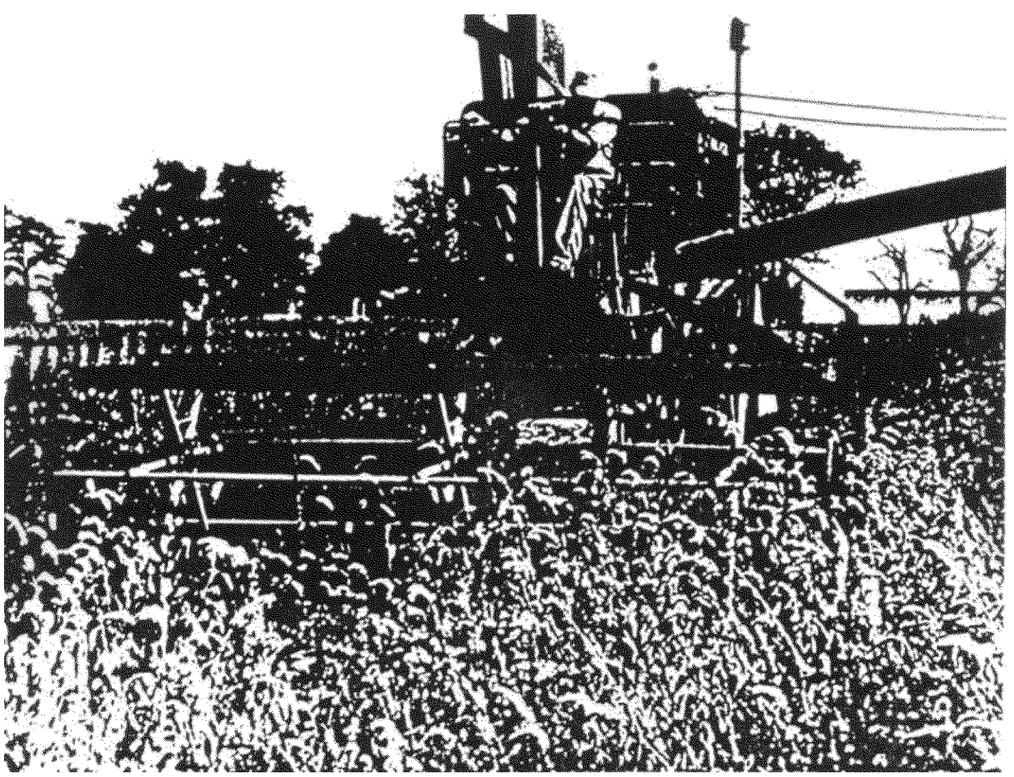

Figure 4.4 Giant foxtail (in foreground) was a very visible and competitive weed, particularly when soybean was harvested in the central Great Plains. (Courtesy of Dr. Ellery Knake, Professor Emeritus, University of Illinois.)

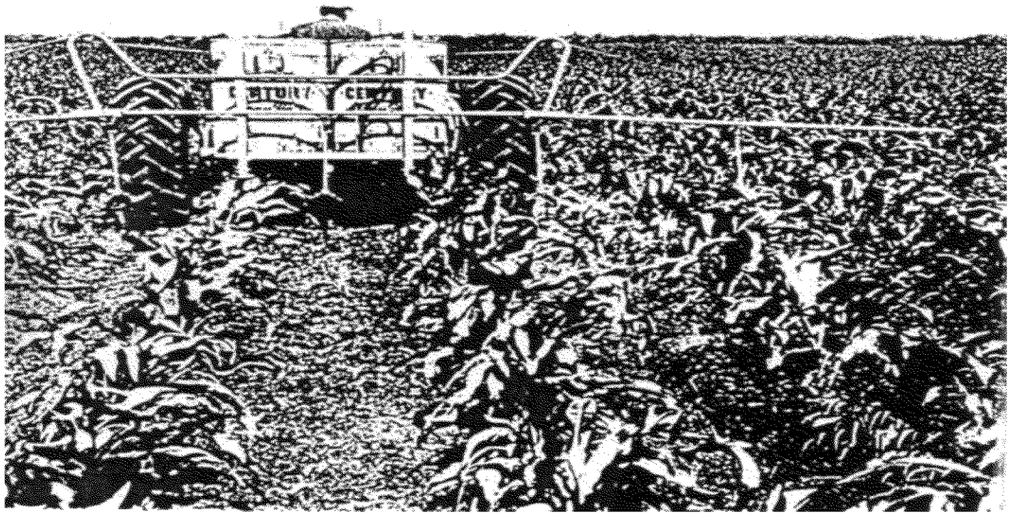

Figure 4.5 Early herbicide sprayers in the United States were often homemade devices constructed with 55-gallon (208 L) drums mounted on the rear of a tractor. (Courtesy of Dr. Ellery Knake, Professor Emeritus, University of Illinois.)

Although larger farm size had resulted in the discontinuation of check planting of corn, tillage and multiple cultivations were still relied upon as the primary means of weed control by producers in the late 1950s. Over half of producers used cultivation and rotary hoeing as the sole methods for controlling existing weed populations, in spite of the fact that herbicides had been used for several years by many early adopters. Most farmers not using herbicides cited product cost as a deterrent to adoption.

The first synthetic herbicide to be used widely in North America was 4,6-dinitro-*o*-cresol (DNOC). Although its use in wheat-growing regions began as early as 1938, in our discussion of row crops we will begin with an overview of 2,4-D. For an excellent detailed discussion of the importance of 2,4-D and its history, Burnside *et al.* (1996) is recommended. Even though used occasionally for preemergence control of weeds, 2,4-D was primarily used in postemergence applications for control of broadleaf weeds. Farmers found it particularly useful for control of two very prevalent vining weeds, field bindweed and, honeyvine milkweed, which fouled cultivators and harvesting equipment. They also found it useful for giant ragweed, a weed whose growth was so prolific that it often made corn production impossible, especially on river bottom ground. As a result of these uses, 2,4-D found a permanent place in row crop (e.g., corn and sorghum) culture.

The recognition of the value of soil-applied herbicide treatments occurred during the early to mid-1960s. One of the most compelling factors for the change in attitude was the rapid proliferation and conspicuous increase of giant foxtail, which often resulted when broadleaf weeds were controlled with 2,4-D. As banding application equipment for herbicides became available, they afforded an opportunity for control of weeds in the crop row at minimal cost, especially control of giant foxtail. And because giant foxtail was so prevalent, the overall emphasis of chemical weed control thereafter quickly shifted to preemergence soil application.

Granular applications were initially more convenient and cost effective than broadcast sprays for applying soil herbicides. Producers had become familiar with banding equipment through the use of fertilizer applicators on planters, and calibration of granular formulation application rates on planter boxes was an accepted practice (Figure 4.6). The herbicide attachments on most two- and four-row planters could be loaded quickly and adjusted to deliver a band of granules over the row at about one-third the cost of broadcast applications (Figure 4.7). When 8-, 12-, and 16-row planters became common, the time required to fill herbicide application boxes became more of an inconvenience during planting. Although cost remained the primary motive for those who continued to band herbicides, broadcast applications were becoming more practical and simpler for most farmers.

During the late 1950s and early 1960s, most soil-applied herbicides typically did not receive mechanical incorporation, and when rain was insufficient, lacked reliability in controlling weeds. The reason for the slow adoption of mechanical incorporation techniques was probably three-fold: (a) banded applications were preferred from a cost standpoint; (b) the advantages of soil incorporation were not universally recognized; and (c) the equipment and techniques for adequate incorporation were not available. Poor soil incorporation not only resulted in poor weed control, but in some cases resulted in crop injury. Simazine, in spite of its good weed control spectrum in corn, was never widely used in the Central Plains. Its lack of acceptance was attributed to its soil residual with the potential to injure

Figure 4.6 Granular application of herbicides was quickly adopted by farmers who were already familiar with granular fertilizer applicators in the Corn Belt. (Courtesy of Dr. Ellery Knake, Professor Emeritus, University of Illinois.)

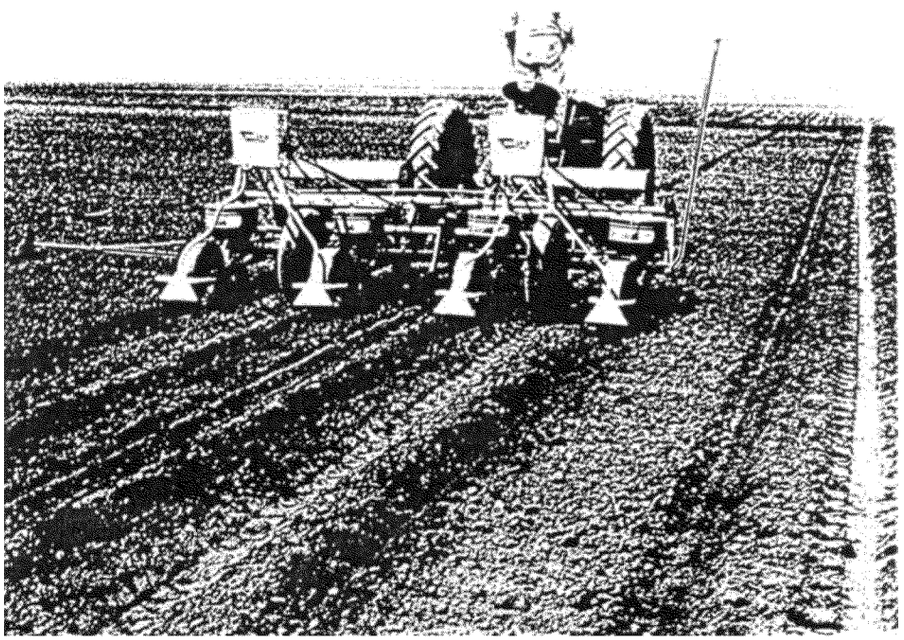

Figure 4.7 A band of herbicide over the row saved farmers the expense of treating the entire soil area in a field. (Courtesy of Dr. Ellery Knake, Professor Emeritus, University of Illinois.)

soybean grown the next year, to the need for mechanical incorporation when the technique was not a common practice, and to its critical need for moisture for effective weed control.

Some herbicides were first accepted by farmers and then rejected in favor of newer compounds that were more agreeable to handle. CDAA was used on up to 17% of the corn and 8% of the soybean crop within 6 years of its

introduction in 1960 (Tables 4.1 and 4.2). Propachlor met with similar success, and its use peaked at 30% of the corn treated in 1971, just 5 years after its introduction. The initial success of both products could be attributed to a number of factors, including effective grass control, consistent performance resulting from adequate water solubility, no corn–soybean crop rotation restrictions, and inexpensive applications as banded granules. But the one significant limitation of these products was that they were unpleasant to handle due to skin and eye irritation. The decrease in the use of CDAA and propachlor is directly due to their substitution by herbicides having similar weed control but with improved handling characteristics. Thus, CDAA was replaced by propachlor, and in turn propachlor was replaced by alachlor and other acetanilide herbicides (Table 4.1).

Although some of the early herbicides were quite effective, their high cost coupled with their narrow weed control spectra resulted in limited acceptance. This was true for naptalam, chlorpropham, and dinoseb, which were some of the first herbicides used for selective weed control in soybean. Use of naptalam and chlorpropham, including use of the first formulated mixtures for soybean, was limited to less than 3% of the soybean crop during their peak years from 1961 to 1966.

On the other hand, the broad weed control spectrum of chloramben, which was introduced in 1959, brought with it widespread acceptance. As an over-the-row band for control of both broadleaf and grass weeds, cost was minimized. With good crop tolerance, chloramben dominated the early soybean herbicide market. During 1972, 85% of the product was applied as the granular formulation (Table 4.2). However, as application practices changed for the soybean crop, banded applications became an inconvenience. Herbicides such as metribuzin (a triazine herbicide), linuron, and bentazon, which could be applied as an affordable broadcast treatment, soon became products of choice, and the marketing of chloramben was eventually discontinued in 1990.

The introduction of atrazine for weed control in corn in 1959 accelerated the adoption of broadcast soil-application technology. Farmers discovered that preemergence broadcast applications of atrazine reduced the need for postemergence treatments of 2,4-D, which sometimes injured corn or drifted to adjacent sensitive crops. Although higher in cost than 2,4-D, atrazine's reliability, excellent crop tolerance, and broad weed control spectrum made the price a reasonable expense. From 1968 to 1970, the increased use of atrazine contributed to a 50% decline in 2,4-D use in corn (Table 4.1). Atrazine continues to be widely used today, although patterns of use tend to closely follow soil type, weather, and cropping patterns. In areas where there is a shorter season, cooler temperatures, low rainfall, or light soils, carryover concerns restrict its use. In the Central Plains where corn or sorghum are grown in mono-cultures or as rotational crops, atrazine remains the foundation of most weed control programs. In 2003 NASS estimated 77% of Illinois corn received atrazine.

The ability of one product to complement the characteristics of another has also had an effect on product acceptance. Although alachlor initially was used by itself as a banded granular grass herbicide on corn and soybean, it was the tank mixes with broadleaf herbicides that propelled its success. Combinations of atrazine–alachlor, metribuzin–alachlor, and linuron–alachlor became very common. Alachlor replaced essentially all propachlor use on field corn over the 4-year period from 1972 to 1976, not only as a result of less hazardous handling characteristics (Table 4.1), but also as a consequence of its success in tank mixes.

A trend toward soil-incorporated herbicide applications began in 1964 with the introduction of trifluralin for control of grass weeds in soybean. Consistent weed control and reasonable product cost resulted in steady, but limited growth in the use of trifluralin from 1964 to 1969 (Table 4.2). A factor restricting its acceptance during this early period was the lack of an economical, complementary broadleaf herbicide that could be applied with trifluralin. After the introduction of metribuzin in 1973, preplant incorporated applications of metribuzin became the catalyst for the increased acceptance of trifluralin. During the 1980s, trifluralin continued to be used as a soil-incorporated treatment for grassy weeds, but was complemented then by postemergence broadleaf herbicides such as bentazon.

In corn, the catalyst for adoption of preplant incorporated applications was not the introduction of an herbicide, but the introduction of safeners. The thiocarbamate herbicide S-ethyl dipropylthiocarbamate (EPTC) for corn weed control was first used in Illinois as early as 1961; however, the propensity for EPTC to injure corn limited its use to 1% or less of the Illinois crop up to 1970 (Table 4.1). Dichlormid, a safener later packaged with EPTC, greatly improved crop safety and resulted in nearly one-quarter of the corn acreage being treated with EPTC throughout the Corn Belt by 1976.

The history of another thiocarbamate, butylate, is similar to that of EPTC. Initially marketed without a safener, butylate's propensity for crop injury resulted in some resistance to its use from farmers. When butylate was released with a safener, this combination product became widely accepted. Because EPTC was more volatile than butylate, it tended to be better suited for the cool soils of the northern Corn Belt. Butylate, on the other hand, was better adapted to the southern corn-growing region of the Central Plains.

Use of butylate and EPTC required mechanical incorporation into the soil. Beginning in the 1970s and 1980s, there was a steady increase in the implementation of conservation tillage practices. Although the thiocarbamates had

performed well, products needing little or no mechanical incorporation, such as alachlor and metolachlor, began to overtake the use of EPTC and butylate.

Since 1980, the discovery of many herbicides with very low application rates has shifted the application timing and chemistry of herbicide applications. The majority of the newly discovered low-rate herbicides were best suited to postemergence applications, and their adoption has resulted in a gradual shift away from preplant or preemergence soil-applied treatments.

A more recent factor affecting weed management has been the introduction of crops genetically altered for tolerance or resistance to herbicides. The first herbicide-tolerant field corn (IMI hybrid corn) was developed as a way to reduce the effects of carryover from imidazolinone and sulfonylurea herbicides applied to soybean in a corn–soybean rotation. These hybrids also soon found use in areas where triazine use was restricted.

Herbicide resistance was also bred into corn lines to permit the use of sethoxydim herbicide. Although these hybrids were widely available to growers, their acceptance was limited by sethoxydim's narrow spectrum of weed control and by concerns over antagonism between the sethoxydim and tank-mixed broadleaf herbicides. Sethoxydim-resistant hybrids are no longer grown.

Other developments include the introduction of hybrid lines resistant to glyphosate and glufosinate. Glyphosate-resistance technology was rapidly adopted for soybean, but was more slowly adopted for field corn due to corn's slower canopy closure. Acceptance of glufosinate-resistant weed control technology faced the same obstacles, but has been even less competitive economically due to product cost.

Sulfonylurea-tolerant soybean was initially introduced to permit growers to use somewhat higher rates of sulfonylurea herbicides than were tolerated by conventional varieties, and they found a niche in many areas where hard-to-control weeds might be present. Historically farmers have been reluctant to adopt herbicide-tolerant and resistant varieties if they are unable to realize an immediate economic advantage. The exception to this has been the glyphosate-tolerant soybean. Although initially there were concerns with variety performance and yield, the simplicity and flexibility of applications offered by the glyphosate-tolerant trait were compelling characteristics for producers. Within 4 years of its introduction in 1995, glyphosate-tolerant soybean had been planted on more than 50% of all soybean acres in the states of Illinois, Iowa, and Indiana. The widespread use of glyphosate-tolerant varieties has not only fostered an increase in glyphosate use, but also has caused a noticeable reduction in the use of most other preplant, preemergence, and postemergence soybean herbicides.

With the culture of soybean and field corn so intimately intertwined throughout the Midwest, changes in soybean weed control affect corn weed control as well. One real effect of the widespread adoption of glyphosate-tolerant varieties has been the disincentive to discover and develop new products and new chemistries. Since the advent of glyphosate-resistant soybean, very few products have been developed for that market.

Environmental concerns and regulatory actions also have had an effect on herbicide use. To reduce herbicide levels in water, voluntary and mandatory label changes have been implemented for several herbicides, including, atrazine, simazine, alachlor, acetochlor, isoxaflutole, and sulfonylureas. Restrictions on maximum use rates have resulted in the development and use of premix combinations with low rates of atrazine and other herbicides. An agreement by the USEPA and the manufacturer (DuPont) to phase out cyanazine (1997–2002) resulted in changes in corn weed control practices throughout the Corn Belt. On the other hand, a regulatory change in 1993 that permitted the application of 2,4-D prior to planting soybean resulted in an immediate increase in the use of that product and fostered the use of no-till practices for soybean.

Another factor that has had a great influence on weed management has been conservation tillage. Where tillage is reduced, there has been a shift away from at-plant soil applications and toward early preplant herbicides (used several weeks before planting to eliminate weeds at planting time), burndown herbicides (used to control emerged weeds present at planting), and postemergence applications. Atrazine, imazethapyr, and similar products have experienced an increase in use as a result of burndown and early preplant applications on conservation tillage fields. In addition, nearly all conservation tillage fields have seen an increase in the use of postemergence herbicides. Although some form of reduced tillage is often practiced throughout the Corn Belt, no-till is more widely adopted on highly erodible lands, and the associated trends in herbicide use are more evident in those regions.

The development of herbicide-resistant weeds has also been an influence on the selection of herbicides used on field corn or soybean. Weed resistance now affects nearly every decision a farmer makes about herbicide selection; either a farmer is trying to control resistant weeds or is selecting herbicides that may reduce the possibility of weed populations becoming resistant. The adoption of the imidazolinone- and sulfonylurea-tolerant corn hybrids mentioned above was in part a response to the presence of atrazine-tolerant pigweeds or kochia in many fields. However, a recent decrease in the use of imidazolinone and sulfonylurea herbicides can also be attributed to the development of populations of weeds that have become resistant to these herbicides.

Several weed species resistant to glyphosate have recently developed in several geographical areas of the United States. This further supports the need for keeping a broad range of herbicides, such as the triazines, available for weed management programs.

Conclusion

In this chapter we have focused on some of the influences that have shaped row crop weed management strategies in North America. As authors we are aware that differences may be found between our example and that of other weed management systems, particularly in areas where high-value crops are grown and in regions of North America where year-round production is possible. However, many of the same principles relating to the adoption of herbicides and transitions between technologies can be similarly illustrated in those situations.

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Chapter 5

Farming Trends and Practices in Northern Europe

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Summary

Technology has had a profound influence on the development of agriculture in Northern Europe, particularly over the last 250 years. The discovery of the corn drill resulted in rotations that used arable land productively every year. In addition, improved communications allowed farmers to share information and for best practices to be adopted extensively. However, the development of the steam engine in the late 19th century resulted in the cheap transport of grain and meat from the North American prairies. This resulted in a prolonged agricultural depression in Europe that was only relieved following World Wars I and II. After the latter war, European governments decided for strategic reasons not only to support agriculture financially, but also to fund extension and to expand research. This encouraged private investment in technologies such as pesticides. Traditional rotations were abandoned in favor of new agricultural developments, and as a result, yields increased dramatically.

The triazines played a significant role in the transformation of agriculture in the latter half of the 20th century – in Europe and around the world. Terbutryn was extensively used at one time for grass weed control in winter cereals. Terbuthylazine has played a major role in corn and in weed control of peas and other broadleaved legume crops. Simazine has been used on a large scale for weed control in crops.

This chapter describes the role played by atrazine, which became commercially available in the 1950s and was used extensively in corn and for amenity (beautification) weed control. Corn area and yields have expanded significantly during the period since the introduction of this herbicide. Atrazine played a significant role in facilitating this expansion.

The extensive and often continuous use of atrazine resulted in two challenges. Atrazine-resistant weeds began to appear in the 1970s, leading to the increased use of alternative herbicides or herbicide mixtures. In addition, both atrazine and simazine were often found in surface and groundwater above the nonhealth-based 0.1 ppb standard established for all pesticides by the European directive, leading to use restrictions in Europe. Although these herbicides were used for weed control in crops, they also were approved for use at high rates on hard surfaces – such as pavement, roads, and railways.

Cyanazine, desmetryn, prometryn, terbutryn, and trietazine were not supported by companies in the recent review of registrations in Europe, and growers had to use their stocks up by the end of 2003 except in some countries where one or more of these herbicides received derogations for some uses until the end of 2007. Despite favorable science and safety reviews (United Kingdom, 1996, 2000), the European Union in 2004 voted not to reregister atrazine and simazine, primarily because past, high-rate uses of the products resulted in levels in groundwater in some areas above the nonhealth-based standard of 0.1 ppb (European Union, 2004). By this time these herbicides had already been withdrawn by some individual country registration authorities. On the other hand, some countries had critical need derogations until the end of 2007 for atrazine and simazine. However, terbuthylazine is still available and used by farmers in several countries in Europe on corn, sorghum, pea, bean, lupin, grape, pome fruit, citrus, and vines.

A Short History of Agriculture in Europe

Crop areas treated and statistical data have not been recorded accurately in many parts of Europe. However, the development of cropping systems and agricultural technology across the continent was similar to that in the United Kingdom (UK), where accurate records are available for at least the last 150 years.

The improved efficiency and competitiveness of European agriculture has always been very dependent on technology. One of the most significant developments was the invention of the corn drill at the beginning of the 18th century

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Table 5.1 Percentage of land in crops, fallow, temporary and permanent pasture devoted to cereal production during 1875–1985 (DEFRA, 2002)[a]

	Land devoted to cereals (%)	
Year	**Cambridgeshire**	**England**
1875	45	28
1935	33	17
1965	55	34
1975	59	36
1985	64	40

[a] The introduction of set-aside areas in the mid-1990s makes it impossible to provide a comparable figure for subsequent years.

by Jethro Tull (1733). Tull was struck by the remarkable effects of cultivation in vineyards, and in 1714 he developed some ingenious theories of plant nutrition. He made a horse hoe to put those theories into practice. His many mechanical inventions, however, were less important than his ideas on how to cultivate for maximum control of weeds and improved crop production.

Perhaps the first great agronomist was the second Viscount Charles Townshend. In the 1730s, he used the discovery of improved cultivation techniques to develop a vigorous rotation that for the first time allowed growers to use all the land productively (Ernle, 1961). The rotation was based on a root crop, followed by a cereal, followed by grasses/clover, and then by another cereal. This became known as the Norfolk four-course rotation. The root crop was in many cases turnips, and the second Viscount Townshend is now known affectionately as Turnip Townshend.

The root and grass crops in the Norfolk four-course rotation were for livestock feed. The resulting manure 'fed' the rotation, while sowing the cereal and root crops in rows enabled manual or mechanical methods to be used to weed the rotation. The adoption of this and similar rotations increased productivity, both to feed an expanding population and to allow population movement from country to town in order to sustain the industrial revolution of the late 18th and early 19th centuries (Ernle, 1961). Corn has remained popular over the last two centuries in Europe due to an expanding market and the ability to grow it in wide rows that facilitated manual or mechanical weed control.

Communications improved during the 19th century, allowing the establishment of farmer discussion groups, farming shows, and educational establishments for agriculture. These, along with investment in field drainage and the development of chemical fertilizers further improved productivity. As a consequence, farming in Europe went through a Golden Age. However, despite the high price of cereals, strict rotation requirements limited them to less than 50% of the area of production in the main arable regions, such as Cambridgeshire (Table 5.1). This time of prosperity was brought to an end in the 1880s by steam technology that enabled the cheap transport of cereals and the refrigeration and cheap transport of meat produced in the North American prairies.

Subsequently, agriculture in much of Europe spent decades in a depression that was only temporarily lifted by the need to provide food during the world wars, particularly World War II. Shortly after this war, governments were more prepared to invest in agriculture in order to achieve food security, and in the last quarter of the past century, the European Union provided significant financial support to agriculture. However, objections to such financial support have been raised in response to food surpluses, concerns about the environment, and concerns about the potential cost of supporting agriculture in the former communist countries that have joined or are preparing to join the European Union. Decreasing direct support measures are exposing European farmers to greater competition in the world markets.

Strict rotational farming was maintained in Europe until after World War II. However, significant government financial support and consequential private investments have resulted in movement away from balanced rotations. The weeding function of the rotation has now been replaced by herbicides and the feeding function by the improved knowledge and use of chemical fertilizers. Hence, crops are now generally grown on the land and in the locations most suited to their production. These changes – along with improved cultivars, machinery, and crop protection chemistry – have contributed to significant increases in yields, particularly since the mid-1970s. For instance, wheat production in the United Kingdom and Northern Europe has become more competitive on world markets because the high cost of land, and in many cases, the high cost of labor can be spread over a greater yield (Figure 5.1). Hence, while steam technology weakened the competitiveness of European agriculture in the latter part of the 19th century, chemical, biological, and mechanical technologies enabled it to become more competitive in the latter part of the 20th century.

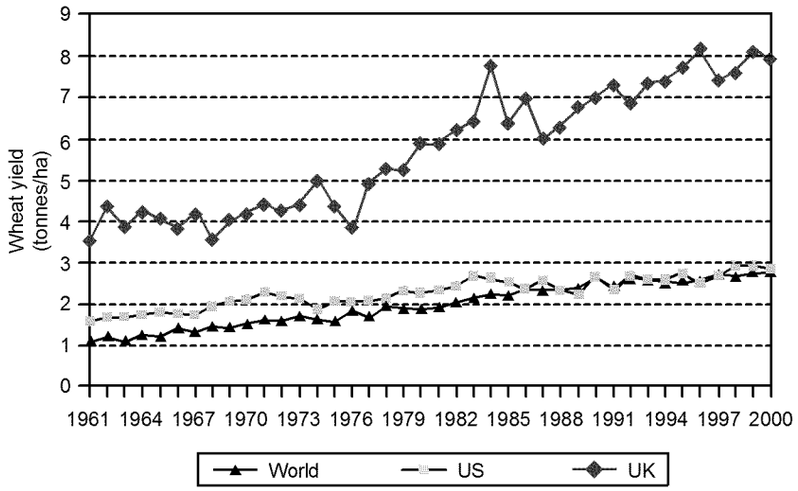

Figure 5.1 Average yields of wheat (tonnes/ha) in the United Kingdom, United States, and World from 1961 to 2000 (FAO, 2001). (See Colour Plate Section).

Table 5.2 Crop area (ha) of corn used to produce grain in the main producing countries of the European Union (FAO 2001)

Countries	1961	1971	1981	1991	2000
European Union (15)[a]	3 371 032	3 964 020	3 778 324	4 036 707	4 313 000
Austria	51 403	125 043	189 049	185 302	164 100
Belgium-Luxembourg	540	3265	5903	9700	36 300
France	980 600	1 655 000	1 569 000	1 769 000	1 808 000
Germany	9024	119 650	129 626	283 052	371 000
Greece	191 000	167 600	175 700	230 300	215 000
Italy	1 197 001	934 000	988 346	858 906	1 104 000
Netherlands	187	2254	162	300	20 100
Portugal	494 577	412 708	291 838	215 347	172 000
Spain	446 700	543 500	428 700	484 800	422 500
United Kingdom	0	1000	0	0	0

[a] The numbers reflect the 15 countries in the European Union as of 2000, including those directly specified in this table.

Corn in Europe

The two main production areas for corn grain in 'old' Europe are France and Italy (Table 5.2). In fact, the major reason for the expansion in the total area cultivated within the European Union over the last 40 years has been the continued popularity of the crop in France (Figure 5.2).

In France, the amount of area devoted to corn crops actually fell between 1840 and 1944, dropping from an estimated 630 000 to 200 000 ha. Much of the corn crop, especially silage corn, was used to feed livestock raised on the farm. The decline in area was not consistent over France and was particularly marked in the southwest, where farming used less technology.

Of the approximately 3 000 000 ha of corn currently grown in France, just under 2 000 000 ha are for grain. The foundations for expansion were laid in southwestern France during the depths of the agricultural depression in the 1930s. A plant breeding station was opened and international contacts were established. However, it was not until 1949 that hybrid varieties were available to growers. Plant breeding proved to be the main reason for the rapid expansion of area planted in corn (Gay, 1999).

Corn grain prices were supported in France from 1952 until recent years. During the following 10 years or so, mechanized sowing and harvesting were introduced, along with weed control with herbicides (notably atrazine) and the improved use of chemical fertilizers. In addition, improvements in plant breeding enabled corn grain production to be expanded further in the north. Also, irrigation was introduced in the drier parts of France, with permanent irrigation facilities eventually encouraging the continuous growing of corn in the southwest and southeast.

The expansion of area planted in corn was usually at the expense of poor pasture land, some of which fell out of arable production during the 19th century. There was, though, some antipathy toward the corn grain crop by farmers.

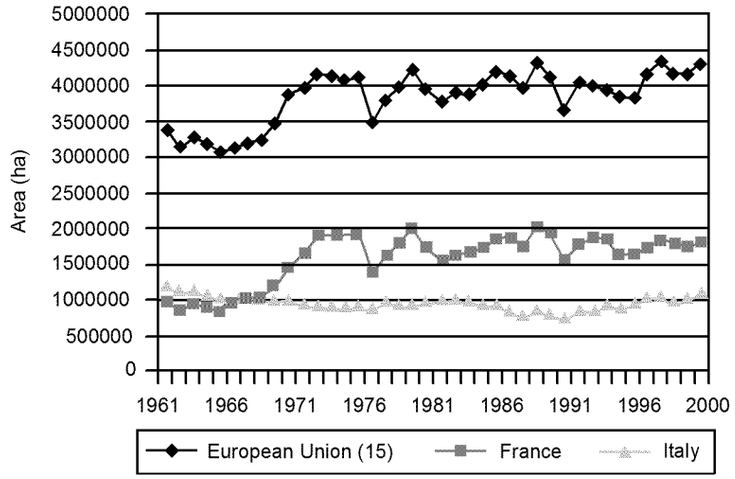

Figure 5.2 Area (ha) of corn grain production in 15 countries in the European Union, France, and Italy from 1961 to 2000 (FAO, 2001). (See Colour Plate Section).

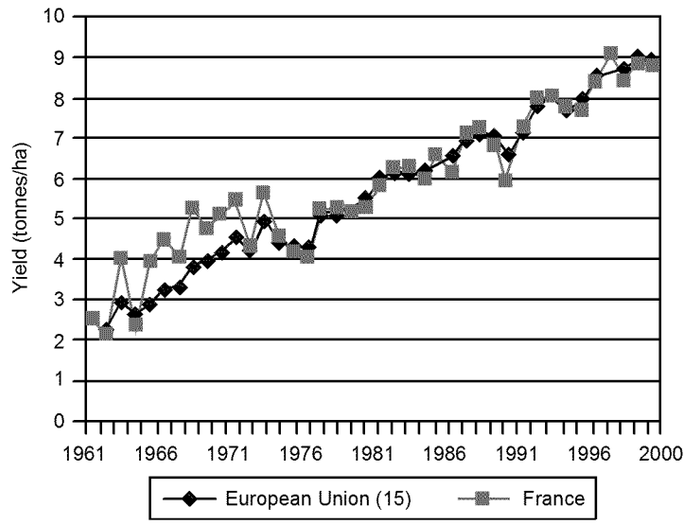

Figure 5.3 Yield (tonnes/ha) of corn grain in 15 countries in the European Union and France from 1961 to 2000 (FAO, 2001). (See Colour Plate Section).

Some did not like moving away from a mainly livestock-based system, where corn was grown from home-saved seed and the rest was fed to the stock. Hybrid corn for grain production was seen as an American technology requiring farmers to borrow money to buy machines, seeds, and fertilizers. Many farmers were reluctant to make that investment or to take advice from technicians, fearing a loss of the independence they valued so highly. In addition, grain storage facilities were lacking, and cooperative stores needed to be constructed. This may explain why there were still less than 1 000 000 ha of corn grain and 300 000 ha of corn silage in France in 1967.

By the 1970s production techniques had been mastered, seed quality had improved, and simpler hybrids allowed a more reasonable seed price. In addition, fertilizer use increased and irrigation became more sophisticated. Yields increased (Figure 5.3) and the area under cultivation rose dramatically. By 1972, there were 1 800 000 ha of grain corn, and by 1983, there were 1 400 000 ha of corn silage. These areas of production have since remained relatively static – primarily as a result of international competition and the resulting fall in prices.

Use of Triazine in Corn

While it is difficult to determine an accurate total for the amount of atrazine previously used in corn in Europe, Table 5.3 describes atrazine use in forage corn in the United Kingdom. The crop expanded rapidly there during the late 1990s due to changes in the method of price support.

Table 5.3 Usage of atrazine in forage corn in the United Kingdom from 1974 to 1997 (Thomas, 2001)

Survey year	Area corn grown (ha)	Area corn treated[a] (ha)	Amount atrazine applied (t a.i.)	Amount atrazine applied to all crops (t a.i.)
1974	17000	12646	56.7	61.7
1982	15715	14696	25.0	47.1
1989	24782	24119	38.1	48.3
1993	72894	71000	94.9	100.8
1997	109413	109578	122.2	125.7

[a] Total area treated in single and multiple applications. For instance, in 1997, 72.9% of the area was treated once with atrazine, 11.3% of the area was treated twice, and 1.4% of the area received more than two applications. This accounts for the area treated matching the area grown, although only 85.6% of the area of the crop grown was treated with atrazine. This compares to 59.4% of the crop area receiving one treatment with atrazine in 1974, and with a further 7.5% of the crop area receiving two treatments.

Terbuthylazine is another novel chloro-*s*-triazine that has found very important uses in Europe for control of weeds in corn, as well as vineyards and orchards. It was introduced at lower application rates than the early atrazine rates and was not registered for use in roads, railways, and noncropland. Terbuthylazine is used in combination with other herbicides and has continued to help replace some uses of atrazine and simazine in many countries of Europe.

Amenity Weed Control

The weed spectrum and persistence of atrazine and simazine made them the mainstay of weed control on hard surfaces (such as pavement, roads, and railway lines) and for some other amenity (beautification) uses. However, these triazines began to be detected in groundwater and surface waters. These detections resulted in the withdrawal of registrations, either for noncrop uses or for all uses, in individual countries of the European Union during the mid- to late-1990s. For instance 137.5 tons of atrazine active ingredient were used for amenity weed control in England and Wales during 1989 (25% of total usage of herbicides in noncrop weed control by weight), but this had fallen to zero by 1995. It should be noted that the weight of atrazine active ingredient used for amenity weed control in 1989 was almost three times that used for weed control within arable crops (Table 5.3) in the same year.

Conclusion

The triazines have played a significant role in the transformation of agriculture in Europe and were key components of weed control systems for many crops within the European Union. In 2005, 21 EU countries were using one or more triazine herbicides. These products are primarily used in combination with other active ingredients for broad-spectrum weed control in various tillage systems employed in the economic production of many crops.

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Chapter 6

Biology and Ecology of Weeds and the Impact of Triazine Herbicides

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Summary

Weeds have presented a serious challenge to crop production since the beginning of civilization. Progress in weed control and management was very limited prior to the 20th century. The discovery and use of triazine herbicides was a major milestone in providing the technology necessary for our modern crop production systems. Our knowledge of weed biology and ecology, and our understanding of the biological effects of triazine herbicides, are very important in making full and optimum use of these valuable resources.

The ecology and biology of weeds involves all aspects of the environment, including climatic, edaphic, and biotic factors. Major weeds possess a high degree of tolerance and adaptability to survive and thrive under a wide variety of environmental conditions and control methods. Genetically and biologically, weeds are very adaptable to both adverse and favorable growing conditions. Weeds that invade and compete with crops cause major yield and quality problems and must be managed.

Historically, tillage systems and hand hoeing have been the main methods of weed control. Most progress on weed management between 1850 and the 1940s was from improved tillage and cultural control methods. Although the era of chemical weed control began soon after the turn of this century, it mostly consisted of nonselective herbicides. The phenoxy herbicides ushered in a chemical weed control revolution in the mid-1940s. This was soon followed by the triazine herbicides and others in the 1950s. With the advent of the synthetic organic herbicides and other modern chemicals in agriculture, weed science became a discipline and a professional career choice. In 1957, there were about 100 weed scientists in North America, but within the next few decades this number grew to several thousand.

To farmers, the benefits of having a uniform field of corn without weeds are numerous, and the desire to have a weedless field prevailed well before the triazine herbicides were introduced. Frequent cultivation, followed by hand hoeing or roguing of remaining weeds, was a well-established practice. However, it did not take long for the growers to discover the outstanding benefits of the triazine herbicides: (1) reduced farm labor, (2) lower costs for fuel and equipment for frequent trips across the field, (3) reduced need for cultivation, especially when rainfall and wet fields prevented timely cultivations, (4) efficient weed control and crop production, (5) easier harvesting due to the control of quackgrass or other weeds that often invaded the crop in late season, (6) increased crop yields and profits, (7) reduced root pruning and other crop injuries resulting from close and frequent cultivation, and (8) improved soil quality due to less erosion of topsoil. With a single application of atrazine or another triazine herbicide, a farmer could avoid many weeds during the entire growing and harvesting season. Such benefits have made the triazine herbicides an extremely important component of efficient weed management and conservation tillage systems in many crops.

Phenomenal progress in weed control and crop management has been made during the past 50 years, leading to greatly improved agricultural technology and efficient crop production. Research has also led to a much greater understanding of weed biology, genetics, and ecology, herbicide mode of action, mechanisms of herbicide resistance, development of herbicide-resistant crops, and weed management systems. As a result, superior weed control can be achieved on various soils and under various weather conditions using lower amounts of herbicides.

Ecology and Biology of Weeds

Ecology of Weeds

The ecology of weeds deals mainly with the effects of climatic, edaphic, biotic, and abiotic factors in their environment. Climatic factors include light (intensity, quality, and day length), temperature (averages, extremes, and frost-free

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periods), water (availability, distribution, percolation, runoff, and evaporation), wind (velocity and duration), and atmosphere (humidity, O_2, O_3, CO_2). Edaphic factors include soil texture, structure, pH, organic matter content, CO_2, O_2, and water drainage and topographic characteristics such as slope, altitude, and sun exposure. Biotic factors include effects from other organisms (competition, diseases, toxins such as allelopathic and stimulating compounds, parasites, soil flora, and fauna). Abiotic factors include machinery, hail, wind, etc. Many of our most common weeds have a high degree of tolerance or adaptability to soil and other ecological conditions. However, weeds having environmental requirements similar to those of major crops form common crop–weed associations (e.g., mustards in small grains, pigweeds in sugar beets, etc.).

To refer to a plant just as a weed is not a satisfactory description in many cases. Frenchman, Jean Rostand stated that, 'In naming a plant a weed, man gives proof of his personal arrogance.' (Young, 1989). Some would rightly claim that no species of plant is always a weed, even thistles. A weed can be defined as any plant that is growing in an unwanted place, in the wrong quantity, or at the wrong time. Even our crop plants can become serious weed problems in some situations. Of the more than 270 000 plant species on the earth (Morell, 1999), only about 250 species are generally considered serious weeds, although Holm *et al.* (1977) report that at least 8000 plant species can be weeds in some situations. Of the 250 major weed species, 70% are found in 12 families, 40% alone being members of the *Gramineae* and *Compositae* families. Interestingly, 12 crops from five botanical families provide 75% of the world's food, and these same five families provide many of our worst weeds. This indicates that our major crops and weeds share certain characteristics and some common origins. For further details on the world's worst weeds, their biology, and distribution (see Holm *et al.*, 1977).

Most plants grow in communities of several plant species. If resources become limiting (i.e., space, water, nutrients, and light), then each species will be forced to compete, not only against other plant species, but also against other plants of the same species. However, weeds often are naturally adapted to a more diverse environment and may compete vigorously with the crop planted in the same land area.

Weed species vary greatly in their biology and life history. The most serious weeds are those that invade fields where crops are grown and interfere with crop establishment, growth, and harvest or invade natural areas where invasive weeds destroy habitat. While most weed species are annuals and reoccur each year from seed, several very difficult weeds in crops such as corn and small grains are perennials and reproduce vegetatively as well as by seed (e.g., field bindweed reproduces by roots, Johnsongrass and quackgrass by rhizomes, and nutsedge by tubers). Weed species have unique traits that contribute to their ability to reproduce, invade, compete, and survive in the various crops and crop rotations. Some weeds have the ability to produce abundant seeds. Examples of the approximate number of seeds produced per weed plant include: leafy spurge – 140; wild oat – 250; common ragweed – 3380; barnyardgrass – 7160; kochia – 14 600; plantain – 36 150; common purslane – 52 300; common lamb's-quarters – 72 450; redroot pigweed – 117 400; mullein – 223 200; and witchweed – 500 000 (Stevens, 1932, 1957).

Work by Norris (1996) shows that seed production per plant can vary greatly if growing conditions and levels of plant competition change. He reported that a barnyardgrass plant produced only 1–5% as much seed (3500) when growing in competition with corn or sorghum as compared to plants growing with no competition (approximately 160 000). Thus, one plant could produce as many seeds as 20–80 plants growing with competition. Norris further stressed that much of the work reported by Stevens (1932, 1957) involved weeds growing in containers, and some shattering of seed occurred before harvest. These factors may have reduced seed production. Norris (1996) also found that a biotype of shepherd's purse from California produced 2- to 3-fold more seed than a biotype from England. He estimated that a common purslane plant could produce as many as 500 000 seeds.

Weed Seed Biology

The species composition and density of weed seed in soil vary greatly and are closely linked to the cropping history of the land and the farming practices. Soil seedbank size may include up to a million seeds/m^2 (Fenner, 1985).

The ability of some weed seeds to remain viable for many years is another feature that allows certain weeds to survive and compete. For example, Indian lotus seeds found in deep mud and very cold water after more than 1000 years were still viable (Klingman and Ashton, 1982). Archeologists who excavated a 14th century English monastery that had been closed by Henry VIII in 1539 found seeds of weld (or dyers rocket, used for yellow dye) and mullein (used for candlewicks) that were still viable (Anonymous, 1997). Toole and Brown (1946) reported that when seeds from 107 species were buried in soil, 71 species could still germinate after 1 year, and 36 species germinated after 38 years including 91% of jimsonweed seed, 48% of mullein seed, 38% of velvetleaf seed, 7% of common lamb's-quarters, and 1% of green foxtail and curly dock seed. In a more recent study, Burnside *et al.* (1996) found that when seed of 41 species of common annual, biennial, and perennial broadleaf and grass weed species of the Great Plains were buried 20-cm deep in soil at two locations, an average of 4% of the annual grass seeds, 11% of the annual broadleaf

Table 6.1 Major characteristics of agronomically important weeds[a]

Reproductive	Physiological	Agronomic
Largely self-fertilized, with some outcrossing	High relative growth rates of seedlings	Weed and crop share morphological and physiological similarities
Copious seed production	High rates of photosynthesis	Seed maturity coincides with crop harvest
Seed will set under a wide range of conditions	Rapid leaf and root development	Resistance or tolerance to herbicides
Pollination by wind or by insects in general	Rapid transition from vegetative to reproductive growth	Vegetative regeneration can overcome mechanical control
	High capacity for acclimation to a changing environment	Prolonged seed viability
		Discontinuousgermination over prolonged periods

[a]From Patterson 1985.

seeds, 30% of the biennial broadleaf seeds, and 8% of the perennial broadleaf seeds still germinated 17 years later. Weed germination was higher in the western Nebraska location with lowest rainfall and more moderate soil temperatures. The greatest seed survival among the 41 species was common mullein, with 95% germination after 17 years. Burnside *et al*. (1996) noted how important it was for weed scientists, computer modelers, and growers to use data on weed seedbank species, depth in the soil, and production practices in evaluating future weed management systems.

Seeds of many weed species have the potential for long-term survival in the soil (Murdoch and Ellis, 1992). However, factors accounting for the loss of weed seed in the soil include germination, decay, predation, and physical movement. The relative importance of these mechanisms varies with species and environmental conditions. Weed management efforts typically are targeted toward seeds that germinate and emerge. These seeds result in new plants that if not controlled reduce crop yields. The ability of seeds to survive over the long term and germinate over a period of years (Forcella *et al*., 1997) allows weeds to survive despite efforts to eradicate them.

Many seeds become dormant as a means of survival and are able to germinate after many years in the soil. Factors that influence seed germination and seed dormancy include: temperature, moisture, oxygen, light, inhibitors (e.g., allelopathic effects), hardness or impermeability of seed coats, mechanically resistant seed coats, immature embryos, and after-ripening requirements (e.g., cool temperatures for several months). Weed seeds may survive and germinate due to several of these characteristics (Pareja *et al*., 1985).

Weeds differ in many respects from insect pests and plant diseases in how they must be managed or controlled. Although weeds are relatively stationary within the field in a given year, there are always many weed species that will germinate and result in serious crop competition if not controlled. With insects and diseases, we are usually faced with only a few that must be controlled or eliminated from a given crop or area, often during a short period. Many weed species can continue to germinate during the growing season if rainfall, temperature, and other conditions are favorable. Much of the injury or competition from weeds occurs while the crop is in the early development stage. Damage from insects and diseases often takes place later, after pests have reproduced. These are some of the reasons that biological controls and breeding of crop varieties to resist insects and diseases often work well in avoiding serious yield and quality losses from these sources, while such methods are seldom successful in managing numerous weed species.

Weeds not only create problems by competing with crops and reducing yields, but also can impact harvesting and reduce crop quality (e.g., poisonous weed seeds in grain crops). Weeds also serve as an important habitat for insect pests and plant pathogens.

Efficient practices for growing food crops in monoculture have exerted a considerable selection pressure in the evolution and invasion of weeds. Many characteristics have evolved that contribute to the success of weeds, as listed by Patterson (1985) in Table 6.1.

Tillage Systems and Weed Biology

The percentage of weed seeds that germinate in a given year is influenced by the species and the soil environment. For common annual species in cultivated soil, approximately 1–40% of the seedbank will emerge in a given year (Roberts and Ricketts, 1979; Wilson and Lawson, 1992; Forcella *et al*., 1997), with great variation both within and among weed species. In field experiments conducted from 1991 through 1994 (Forcella *et al*., 1997), information on weed emergence was collected for 22 site years from Ohio to Colorado and Missouri to Minnesota. Average emergence percentages of some major species were: giant foxtail, 31%; velvetleaf, 28%; common ragweed, 15%; pigweed species, 30%; and common lamb's-quarters, 3%.

Seeds are an important food source for many insects, birds, and small mammals. Seed predation is usually less in agricultural systems due to the intensive soil disturbance, seed burial by tillage, and lack of habitats for predators. However, studies have found significant weed seed loss by predation when seeds remained on the soil surface.

As much as 69% of the weed seed was lost to predation in no-tillage soybean production, as compared with 27% in conventional tillage (Brust and House, 1988).

Tillage systems also greatly affect weed seed production and distribution in the soil. Prior to the use of the triazines and other modern herbicides, farmers typically used the moldboard plow or other preplant tillage to frequently cultivate row crops during the growing season. The use of herbicides along with no-till or conservation tillage systems often led to different dominating weed species, even though the number of weed species and their populations were greatly reduced (Wicks *et al*., 1994). Winter annual and biennial weed species are those most rapidly and commonly observed in no-till systems. In terms of greatest numbers of weed seeds, moldboard plow plots had fewer weeds in the upper 20 cm of soil than chisel plow or no-tillage plots after 5 years (Buhler *et al*., 1998). Moldboard plowing resulted in the most uniform distribution of seed throughout the plow layer. In the no-tillage system, more than 60% of all weed seeds were found in the upper 1 cm of soil, and few seeds were found below 10 cm. The concentration of weed seeds in no-tillage plots decreased logarithmically with increased depth. In the chisel plow system, more than 30% of the weed seeds were in the upper 1 cm of soil, and seed concentration decreased linearly with depth. Buhler and Mester (1991) found that in two soil types over 3 years, mean seedling emergence depths were smallest in no tillage, followed by chisel plow and then moldboard plow systems. At least 40% of the giant and green foxtail emergence was from the upper 1 cm of soil in no-tillage plots, as compared to about 25% in chisel plow and less than 15% in moldboard plow plots. Buhler *et al*. (1996) concluded that vertical seed distribution in the seedbank plays a more important role in weed population shifts among corn tillage systems than does surface residue.

Crop rotation also has a positive influence on weed management. It diversifies the selection pressure, preventing the proliferation of weed species well suited to the practices associated with a single crop. In ridge tillage, soils harbored at least twice as many weed seeds under continuous corn than a corn/soybean rotation (Forcella and Lindstrom, 1988a). Ridging the soil just before canopy closure stimulated germination of weed seed. The resulting weed population produced up to 1000 seeds/m^2 in continuous corn and about 100 seeds/m^2 in the corn/soybean rotation. Forcella and Lindstrom (1988b) further reported that withholding herbicides for 1 year increased weed growth, resulting in 10–27% reductions in yields for continuous corn under ridge tillage. They concluded that reducing seed production from small-seeded weeds may aid in solving the weed problem in the ridge tillage systems. Schreiber (1992) found that growing corn in a soybean/corn or soybean/wheat/corn rotation greatly reduced giant foxtail seed in the soil as compared to continuous corn, regardless of herbicide use or tillage system. He further reported that as tillage is reduced, giant foxtail seed and plant populations increase.

Herbicides and Weed Biology

The use of triazine herbicides resulted in the control of many weed species with one application. Research showed that repeated control of weeds resulted in reductions in the weed seedbank in soil after several years. In a 6-year study in Colorado, Schweizer and Zimdahl (1984) found the number of seeds in the seedbank decreased by approximately 70% after 3 years of annual atrazine application plus interrow cultivation. Atrazine use was ceased in some plots after the first 3 years, and weeds were controlled with one or two cultivations. After 3 years of cultivation only, the weed seedbank was approximately 25 times greater than those where atrazine use and cultivation were continued. A similar study was conducted at five locations in Nebraska (Burnside *et al*., 1986). Broadleaf and grass seed density in the soil declined by 95% after a 5-year weed-free period. During the sixth year, herbicide use was ceased, and seed density increased to 90% of the original level at two of the five locations. These studies demonstrate that weed management has a great impact on the weed seedbank, resulting in a rapid decline in the seedbank when seed introductions are minimized or prevented. However, a small number of seeds of most weed species remain viable for long periods in the soil, and when weed management practices are not entirely effective, these seeds can germinate, mature, and produce enough seed to replenish the seedbank (Buhler *et al*., 1998).

Norris (1992) proposed that with proper use of herbicide and weed management technology, we can eliminate weeds from an area by preventing weeds from producing seed. He further stated that the economic threshold, defined as the pest population at which control action should be initiated in order to prevent the population from increasing to or exceeding the economic injury level, should not be adopted in weed management as it has been in entomology for insect management. Weed management must recognize long-term weed population dynamics, including the nature of the seedbank. He recommended that weed management, especially for serious problem weeds, should adopt a 'no-seed' threshold. This threshold implies that weeds should not be permitted to set seed. He cited several cases where this has worked in California on high-value crops where the same growers are in control of the land for many years. Norris (1999, 2000) further stated that a 'no seed' threshold can only be successful when weed management technologies are integrated, including the use of hand labor for controlling low-weed populations that have not succumbed to other management tools.

Jones and Medd (2000) proposed that a longer-term management approach is needed to manage weed seedbanks and to determine the optimal level of intervention required for a specific weed situation. Managing seedbanks is complex because of the difficulty in preventing seed production and introduction, as well as the persistence of certain seeds in the seedbank and the high seed production potential of many weed species (Buhler *et al.*, 1998). Weed seedbanks are an ever-present component of agricultural land, and resources directed to understanding, interpreting, and predicting seed germination potential can improve agricultural production. Management systems can be devised that minimize the impact of the resultant weeds.

Cousens and Mortimer (1995) confirmed that fields receiving herbicides annually for more than 20 years may be reinfested with damaging weed flora if left unsprayed, often within one or a few years.

Weed populations are never constant, but are in a dynamic state of flux due to changes in climate, environmental conditions, tillage, husbandry methods, use of herbicides, and other means of control. Weeds that were at one time of minor importance, but not controlled by certain broad-spectrum herbicides, have increased to become major problems. Reduction in tillage has sometimes led to the increased occurrence of perennial weeds and annual grasses, particularly of those species that readily establish near the soil surface and have relatively short periods of dormancy. Many perennials have increased in importance under minimal cultivation (e.g., field bindweed and Canada thistle). The occurrence of herbicide-resistant weed biotypes is also a phenomenon of increasing concern. Some research results show that large changes in the seedbank can impact weed control efficacy. Winkle *et al.* (1981) and Buhler *et al.* (1992) found large increases in weed densities reduced weed control with herbicides and mechanical practices.

Webster and Coble (1997) reported on weed shifts in major crops of the Southeastern states over a 22-year period (1974–1995) when herbicides were the major means of weed control. Sicklepod and bermudagrass had become the most troublesome weeds. The largest decreases in weed pressure were found with Johnsongrass, crabgrasses, and common cocklebur. Morningglories and nutsedges remained relatively constant. The weeds of greatest importance in soybean, peanut, and cotton are the pigweeds.

Webster and Coble (1997) listed several factors that may play an important role in the future weed species composition of cropland: (1) Herbicide-resistant weeds represent a change in the weed spectrum in some of the management systems, with almost every state having at least one reported herbicide-resistant weed. (2) Cropping systems that use fewer tillage operations may allow weeds that are unable to survive frequent disturbances (e.g., biennials and simple perennials) to invade and become problem weeds in fields. (3) A reduction of triazine herbicides used in corn and cotton weed management systems may allow previously controlled broadleaf weeds to become major weeds again. (4) The widespread use of herbicide-tolerant crops may have a further significant impact on the weed species composition.

Changes in weed species and populations also cause changes in plant diseases and insect pests since certain weeds serve as their hosts (Bendixen *et al.*, 1981; Manuel *et al.*, 1982; Weidemann and TeBeese, 1990; Norris and Kogan, 2000). Herbicide-resistant weed biotypes are present in our weed populations, although often at very low frequencies, even when herbicides are not used. Weed species have acquired built-in genetic adaptability to survive most control methods used against them. For example, dandelions usually develop a vertical growth habit when growing wild, but when growing in a frequently mowed lawn, more prostrate or flat-growing biotypes evolve. We should continually add to our weed control technology and keep tools available in order to address the adaptability of weeds to different control methods. For further information on the biological characteristics of weeds, including growth strategies, mimicry with crops, plasticity of weed growth, photosynthetic pathways, weed seed reservoir, and vegetative reproduction see Cousens and Mortimer (1995) and Buhler *et al.* (1998).

Development of Weed Management Systems

With the increased urbanization and industrialization following World War II, the farm workforce became scarce and more costly. As other work and occupations in cities became available, the routine, hard work of hand hoeing and farm labor became less desirable. These factors drove increased mechanization in order to reduce labor requirements on the farm. Following land preparation and crop planting, weed control became the predominant labor need. In the early part of the past century, whenever there was not other more pressing work to do, farm workers had to be busy with cultivation, hand weeding, or other methods of removing weeds in production, harvesting, or cleaning operations. Virtually no work in city factories or production plants was as monotonous, low paying, and unattractive to workers as hand-hoeing weeds out of crops. And yet, ironically, hand weeding is actually skilled and exacting labor. It requires constant attention, concentration, and close observation, especially when the seedling weeds and crop plants appear similar in size and form. Weed control by hand hoeing and tillage not only became excessively laborious and expensive to the farmer, but it often resulted in major yield reductions due to carelessness or unavoidable damage to crop plants from the hoe, cultivator, or from weeds that were not controlled.

Since the 1950s, an increasing proportion of our world cereal crop has been regularly treated with herbicides to control a wide variety of weeds. Today, chemical weed control has expanded to virtually every crop in the developed and developing world. Chemical weed control is not only far more economical than traditional methods in most situations, but herbicides also have important technical advantages. Weeds growing closest to the crop – and hence competing most for essential resources – can now be controlled with selective herbicides. Furthermore, when weeds are controlled with herbicides, crops experience little or no root disturbance or injury from mechanical cultivation and hoeing. In addition, far fewer weed seeds are brought to the surface in the process of weed management with herbicides. Conservation tillage is possible and protects topsoil from cultivation erosion. Finally, for the first time in history, farmers now have chemical solutions for most weed problems at an economical price. If herbicides were not used in the United States, an estimated 7.2 million laborers would be required to provide weed control (Gianessi and Reigner, 2006).

For farmers, controlling weeds in crops is absolutely essential. Lacy (1985) summarized weed control objectives as: reducing the competitive ability of an existing population of weeds in a crop; establishing a barrier to the development of further significant weeds within that crop; and preventing weed problems in future crops either from an existing weed reservoir or from additions to the weed flora.

The first two objectives are accomplished primarily by chemical means, and the third relies on agronomy and crop husbandry. Cultural crop production practices continue to change along with the weed spectrum, and it is now increasingly recognized that an integrated approach utilizing both cultural and chemical practices is usually necessary for optimal weed management.

Not only do farmers have to prevent weeds from competing with their crops during the current season, but they also must try to keep new seeds or vegetative parts of weeds from reinfesting the soil and creating future problems. In addition to weed management practices used by the grower, many countries have quarantine regulations or mandatory control legislation to prevent the importation and spread of noxious weeds. In recent years, concerns about invasive weeds have increased, leading to executive orders in the United States to prevent the importation of invasive weeds (Executive Order 13112, 1999). Such legislation is intended to prevent, or at least reduce, invasions by weeds likely to have an impact on agricultural production or native plant communities.

Technology of Chemical Weed Control

Chemical weed control is largely a 20th century technology. Prior to 1900, there was no serious consideration that chemicals could be used selectively to remove weeds from crops. Weeds were too closely related biologically to crop plants and yet too diverse to imagine that they could be chemically removed without killing or injuring the desirable plants. Salt (sodium chloride) was first tested for nonselective control of common hawkweed in Vermont in 1896 and for field bindweed control in Kansas in 1915. Hundreds of carloads were used along highways and railroad rights-of-way in Kansas between 1937 and 1950, usually at rates of 20 tons/acre (45 metric tons/ha) (Timmons, 1970). In about 1900, copper sulfate was used for the control of wild mustard or charlock in oats. Soon after came chemicals such as calcium cyanamide, sodium chlorate, and sulfuric acid. Sulfuric acid was used in France and the United States early in the 1900s for control of annual broadleaf weeds in cereals.

The arsenicals came into limited commercial use as soil sterilants. Sodium arsenite was used extensively by the Army Corps of Engineers for control of water hyacinth in Louisiana from 1902 to 1937 (Timmons, 1970), and much more widely as an aquatic herbicide in lakes and ponds. Sodium chlorate was first used for nonselective weed control about 1926.

Synthetic, organic, selective herbicides first appeared in France in 1932 with the patenting of dinitro-o-cresol (DNOC) for the selective control of annual weeds in cereals. Dinitro-cresols and dinitro-phenols soon appeared, but these compounds had variable effectiveness and could kill animals as well as plants.

While each of these milestones brought forth a renewed interest in chemical weed control and led to more research in the field, the use of chemicals for selective weed control in crops was very limited and not very successful until the discovery and development of 2,4-D, MCPA, and other phenoxyacetic acid herbicides in the 1940s. These compounds were the first truly selective herbicides that could reliably kill broadleaf weeds in cereal crops, including corn, and they quickly developed widespread popularity and use after World War II. With this major milestone, new application technology emerged, including the low-volume sprayer, and new herbicide formulations were developed.

In addition to the phenoxy herbicides used mostly to control broadleaf weeds in cereals and grass crops, by the mid-1950s several of the inorganic chemicals continued to be used at very high rates (e.g., from several hundreds of pounds to a ton per acre) for nonselective control of perennial weed problems. Some organic chemicals had been introduced for control of specific weed problems and were useful in certain crops. The phenoxy herbicides (e.g., 2,4-D

and MCPA) were useful in corn, sorghum, small grains, and grass crops for control of many broadleaf weeds. However, they tended to release grass weeds and cause crop injury. When used in corn and sorghum, the phenoxy herbicides were never considered as replacements for cultivation because they gave poor control of grasses and sedges. Other chlorinated organic compounds found some uses, such as benzoic acids (e.g., benzac), acetic acids (e.g., TCA and fenac), acetamides (e.g., CDAA and CDEC), phenoxy ethyl sulfates (e.g., natrin), substituted ureas (e.g., DCU, CMU, and diuron), and propionic acids (e.g., dalapon). A few nonchlorinated organics also became available for some weed problems, including maleic hydrazide, aminotriazole, dinoseb, and NAA.

While several of these organic chemicals reached a commercial stage, all had major limitations. Among those limitations were marginal crop selectivity, limited weed spectrum, too short duration of activity, serious failures on some soils or under certain weather conditions, offensive smell or touch, corrosion of spray equipment, drift, secondary adverse effects, etc. At best, many of these organic chemicals had to be used at high rates (e.g., 4–10 lb/A or 4.5–11.2 kg/ha), and were often too costly for their limited benefi.

At the 1957 North Central Weed Control Conference (NCWCC), Slife (1957) summarized that since the introduction of 2,4-D, many new materials had been introduced and tested, and some of these had found a place in weed control programs. With each new development came a greater interest in the potential of organic chemicals to help solve the very serious problem of crop losses from weeds. Prior to the 1950s, very few scientists were actively working in weed control in North America. Timmons in 1935 became the first person hired by the United States Department of Agriculture (USDA) as a full-time weed scientist (Timmons, 1970). By the late 1940s, four regional societies and one national scientific society dealing with weeds were established within the United States, and eastern and western sections were organized in Canada. By 1957, about 100 people were employed in this discipline in the United States at the state and federal level (Timmons, 1970). A recent 50-year chronology of the Weed Science Society of America (Appleby, 2006) documents the emergence of the weed science discipline and highlights those who achieved leadership positions and made significant contributions to the profession.

New Approach with Triazines

During the 1957 NCWCC, Minarik reported on early exciting results with simazine and several other promising new herbicides. At rates of from 1 to 4 lb/A (1.1 to 4.5 kg/ha) preemergence, simazine gave excellent control of both broadleaf and grass weeds throughout the growing season, with no drift problems or injury to corn at rates of up to 16 lb/A (18 kg/ha). Results were remarkably consistent throughout the United States and Canada, and corn yields were often much higher than from the standard commercial herbicides then available. Beginning in 1957, similar results were reported with unprecedented excitement at weed science and agricultural extension meetings as simazine and in subsequent years atrazine and other triazine herbicides were introduced and tested.

For example, Buchholtz (1958) reviewed the best of what were then very poor, temporary, and inadequate means of controlling quackgrass – considered the worst weed in most of the northern United States. In corn, the only options at the time included 20–240 lb/A of TCA (22.4–270 kg/ha), 10–20 lb/A of dalapon (11–22.4 kg/ha), 4 lb/A of amitrol (4.5 kg/ha), or 4 lb/A of maleic hydrazide (4.5 kg/ha) (Lee, 1958). In areas where no crop would be grown on the treated soil for several years, from 320 to 1000 lb/A of sodium chlorate (360 to 1 120 kg/ha) or 20 to 40 lb/A of monuron (22.4 to 45 kg/ha) could successfully eradicate quackgrass. Based on limited research, it was suggested that 10–20 lb/A (11.2–22.4 kg/ha) of simazine could give good control, but only corn could be grown on the treated soil for two or more years. Beginning that same year, research results with atrazine led to the conclusion that quackgrass and many other weeds could be controlled at rates of 3–4 lb/A (3.4–4.5 kg/ha), with no injury to the corn crop (Fertig, 1961a, b; LeBaron and Fertig, 1961, 1962; Raleigh, 1961). The best quackgrass control and corn yields resulted from split applications of atrazine, with 2 lb/A (2.2 kg/ha) before plowing and 2 lb/A preemergence or postemergence to the corn (Fertig, 1961a, b).

LeBaron and Fertig (1961, 1962) and Schirman and Buchholtz (1966) found that while simazine and some of the other herbicides would kill the above-ground quackgrass vegetation, they had very little effect on the underground rhizomes, which were the real source of survival and reinfestation. Of all the herbicides studied, only atrazine was found to adequately control the underground rhizomes of quackgrass.

Of all the triazines tested in 1958, atrazine gave the best and most consistent results (Lee, 1958), without negative side effects such as crop injury, drift, serious handling problems, allergic responses, or odors. However, by far the most important characteristic of both simazine and atrazine was their ability to control many weed species for the entire growing season, with no corn injury at even high rates, and under adverse weather conditions (e.g., cold and wet).

In the first research reports on atrazine, McWhorter and Holstun (1961) found that in solution cultures used to provide maximum differentiation and the most rigorous test for selectivity of 29 triazine compounds, the chloro-derivatives were highly selective. These included atrazine, simazine, and propazine.

Later research and use experience showed that the chlorotriazines were stable and readily adsorbed by soil, resisting excessive leaching and lateral movement, and mostly metabolized or degraded before the next growing season. They also were considered among the least toxic to man and animals of all crop protection herbicides. The chloro-*s*-triazine herbicides were found to be selective in corn and sorghum, with the crops rapidly metabolizing or detoxifying the chloro-*s*-triazines before they arrive at the chloroplasts. Chloroplasts are the sites of action where the chloro-*s*-triazine herbicides inhibit the photosynthetic process known as the Hill reaction in weeds.

After much synthesis and testing, it was concluded that many chloro-*s*-triazines are excellent herbicides, expressing activity in most annual broadleaf weeds, many grasses, and some perennials. The remarkable selectivity of corn and sorghum to atrazine and simazine was found to be due to their conjugation with glutathione. The discovery, purification, and characterization of glutathione by Shimabukuro and colleagues (Shimabukuro and Swanson, 1969; Frear and Swanson, 1970; Shimabukuro *et al.*, 1971) were of great historic significance because it established for the first time the existence of this metabolic pathway in plants.

Some chlorotriazine herbicides have postemergence activity on seedling weeds. This foliar absorption and control from atrazine and some of the chloro-*s*-triazines can be enhanced by adding surfactants or emulsifiable oils. Some chloro-*s*-triazines also have good-to-excellent selectivity in sugarcane and sorghum, which can metabolize the herbicides by dealkylation. The selectivity of various other crops – such as conifer trees, ornamentals, fruit and nut crops, turf, and citrus – involves a combination of metabolic ability of the crop and placement selectivity of the herbicide into the root zone. The methylthio-*s*-triazines generally have less crop selectivity in corn and sorghum, but some have good tolerance in other crops. They often give good weed control when applied preemergence and postemergence. For control after both weeds and crops have emerged, post-directed applications are generally required to minimize crop injury. Methoxy-*s*-triazines generally have less selectivity in crops, but have been useful for general or industrial weed control situations.

Other *s*-triazines not in these three groups have been developed, and a few are in commercial use. For example, hexazinone is used for weed control in conifers, sugarcane, and alfalfa, but is mostly used to control annual and perennial weeds and some brush species along railroads and on land not used for crops. A few asymmetrical triazines have been developed with crop selectivity and biological properties that are different from *s*-triazines. Metribuzin controls both broadleaf and grass weeds with preemergence and postemergence treatments and has selectivity in soybean, potato, and several other crops.

Conclusions

The weed control successes of the triazines led to important discoveries about new and better ways to use herbicides. The remarkable biological success of the triazine herbicides has had a tremendous impact on weed control and crop management over a relatively short time. Sumner (1999) told of his uncle in Hastings, Kansas, who looked over his weed-free corn field after he had applied his first atrazine and remarked: 'If I didn't see it with my own eyes, I wouldn't believe it.' Such accounts could be repeated many thousands of times in the late 1950s and 1960s. The triazines are still the most important herbicides for weed control in corn, sorghum, and sugarcane.

Research should continue to be directed to our understanding and management of weeds, including weed biology and their responses to the soil, climate, and biotic factors. Extensive research over the past years on herbicides and their behavior, fate, and effect in the soil, and on their interaction with weeds, has been very useful and has led to efficient systems to control weeds in crop production. However, we must rely on multiple tools for weed control. For maximum future benefit for farmers, agriculture, and our environment, we must develop and use weed management systems, such as integrated pest management (IPM) and integrated weed management (IWM).

Tillage, crop rotation, and weed control practices affect the weed seedbank. Information on the influence of those cropping practices should be useful in IWM and in applying decision-aid models to develop weed control tactics based on estimated weed populations and crop yield loss. Improved understanding of weed seedbank dynamics is also essential to developing better weed management systems. The science of weed management must be integrated with the principles of plant ecology and weed biology to develop future strategies and systems for agronomic crops. A number of computer software programs have been and are being developed to aid in increasingly sophisticated approaches to weed management. The proceedings of a symposium on 'Importance of Weed Biology to Weed Management' include a good review on these subjects by a variety of weed scientists (Oliver, 1997). Research on the triazines has advanced the understanding of the biology and ecology of weeds, and these herbicides continue to be an integral tool in weed management around the world.

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Chapter 7

Plant Uptake and Metabolism of Triazine Herbicides

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Summary

The metabolism of triazine herbicides in plants is very complex and involves a variety of biological reactions. The most active crops metabolically include corn, cotton, soybean, sugarcane, and wheat. Less complex metabolic pathways have been observed in citrus and various fruit crops.

The chlorotriazines are metabolized at the chloro- or 2-position of the triazine ring by hydrolytic dehalogenation via a nonenzyme constituent of plant sap to the corresponding hydroxytriazines. Another important reaction of the chloro group involves an enzyme-mediated conjugation with glutathione to form a series of S-bound amino acid conjugates. These compounds can rearrange to form N-bound amino acid conjugates. A third metabolic reaction involves oxidation of the alkylamino side chains located at the 4- and 6-positions of the triazine ring, prior to sugar conjugation or *N*-dealkylation. In the case where the alkyl amino group contains a cyano group, hydrolysis leads to amide and carboxylic acid formation on the alkyl group. These three competing reactions can result in a complex mixture of Phase I metabolites (simple metabolites) and Phase II metabolites (conjugates of simple metabolites) that can occur either free or bound in various plant matrices.

The methylthiotriazines are metabolized at the 2-position of the triazine ring by oxidation of the sulfur atom to the corresponding sulfoxide, prior to hydrolysis to hydroxytriazines or conjugation with glutathione. As with the chlorotriazines, the S-bound conjugates can rearrange to form N-bound conjugates. In some cases, a further oxidation of the sulfoxide to the sulfone has been observed. Oxidation of the side-chain alkyl groups occurs prior to sugar conjugation or dealkylation reactions.

The metabolism of the methoxytriazines is generally limited to oxidation and conjugation of the side-chain alkyl groups because of the extreme stability of the methyl ether bond of the methoxy group.

The metabolism of the substituted triazinone group of herbicides involves deamination, demethylation, dethiomethylation, hydroxylation, and/or conjugation reactions dependent on the nature of the parent compound. Conjugation reactions can lead to formation of *N*-glucose conjugates and *O*-malonyl-*N*-glucose conjugates. The thiomethyl group can lead to formation of homoglutathione conjugates.

Introduction

The triazine herbicides can be divided into four different structural classes: chlorotriazines, methylthiotriazines, methoxytriazines, and atypical or asymmetrical triazines. The chlorotriazine group includes atrazine, simazine, propazine, terbuthylazine, and cyanazine. The methylthiotriazine group includes ametryn, prometryn, and terbutryn. The methoxytriazine group will include prometon and secbumeton. Hexazinone and metribuzin were chosen to represent the atypical triazine group. The plant metabolism of the most researched member of each triazine group will be discussed in detail to cover all major biological and chemical transformations reported in the literature.

Uptake and Distribution of Triazine Residues

Chlorotriazines are widely used for preemergence and postemergence control of many broadleaf and grass weeds in corn, sorghum, sugarcane, and a variety of other crops. Methylthiotriazines are used for preemergence and post-directed

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control of many broadleaf and grass weeds in banana, corn, cotton, celery, pineapple, sugarcane, and noncrop areas. Methoxytriazines are used primarily for weed control in noncrop areas. Metribuzin and hexazinone belong to the asymmetrical (*as*-) or atypical triazine class. Metribuzin is used for preemergence and postemergence control of many grasses and broadleaf weeds in soybean, potato, sugarcane, corn, cereals, and other crops. Hexazinone is used for selective weed control, primarily as a contact herbicide in alfalfa, pineapple, sugarcane, forestry, and noncrop areas. Annual application rates for most agricultural uses of these chemicals will vary between 0.6 and 12 kg of active ingredient per hectare (kg a.i./ha).*

Metabolism and uptake studies usually employ parent compounds that have the radiolabeled carbon atom(s) incorporated into the most stable portion of the molecule so that extensive biotransformations can be followed in an expeditious manner. These studies can be short term (immature crops) or long term (mature crops) in duration. Methods of application and the number of treatments can be as varied as those specified in the use directions. In fact, most government agencies responsible for pesticide regulation require radiolabeled studies to be done in major crops at the maximum registered use rate in order to obtain uptake and distribution data for all degradates in raw agricultural commodities. Short-term studies may involve the use of cell culture, nutrient uptake, or stem injection to generate a clear picture of the initial phases of metabolism. Long-term studies can be done in the greenhouse or in small field plots and are usually continued to crop maturity.

Overall, treatment of various crops preemergence or postemergence with chloro- or methylthio-*s*-triazines results in low-level or nondetectable residues in forage, grain, or fruit commodities – even under the worst-case scenario of treating crops at maximum use rates and growing them to maturity in small field plots or in the greenhouse. Grain and fruit residues, if detected, are much lower than corresponding forage residues. Polar metabolites are often sequestered in foliar tissues with limited remaining mobile compounds available for translocation to the fruiting bodies.

Early Research

The metabolism of *s*-triazines has been the subject of extensive research since the 1950s to the present time. Much of this research has been the subject of review articles published over the years since *s*-triazines were introduced. The metabolism of *s*-triazine herbicides in animals and plants and their degradation in soil were the subject of a review by Knuesli *et al.* (1969), later updated and revised by Esser *et al.* (1975) as a second edition. The metabolism of *s*-triazines in plants was also reviewed by Shimabukuro *et al.* (1971a). Naylor (1976) published a review of herbicide metabolism in plants that included the *s*-triazines. Lamoureux *et al.* (1998) reported on the identification of several plant metabolites of atrazine and simazine.

Chloro-*s*-triazines have been shown to be metabolized in plants by one of four competing processes: hydrolytic dehalogenation, oxidative *N*-dealkylation, nucleophilic displacement of the chlorine atom with glutathione, and amination or deamination reactions. Much of the early research focused on the first three processes and attempted to determine the relative importance of each process to herbicide tolerance. The relevant research undertaken between 1961 and 1973 will be discussed.

Hydrolytic Dehalogenation: Simazine was shown by Roth (1957) to degrade in the presence of corn extracts, but was stable in the presence of extracts from a susceptible wheat crop. Castelfranco *et al.* (1961) described a similar nonenzyme constituent of expressed corn sap that hydrolyzed simazine to hydroxysimazine [2,4-bis(ethylamino)-6-hydroxy-*s*-triazine]. Wahlroos and Virtanen (1959) and Hamilton (1964) have established that the catalytic conversion of simazine to hydroxysimazine in roots and shoots of resistant species is caused by 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (benzoxazinone) or its 2-glucoside as follows:

$$\text{Simazine} \xrightarrow{\text{Benzoxazinone}} \text{Hydroxysimazine}$$

Montgomery and Freed (1964) reviewed the early research metabolism of triazine herbicides by plants and concluded that there was a good correlation between resistance in plants and the extent of their metabolism. A common pathway of degradation for these chemicals was indicated by the presence of 2-hydroxy analogs.

N-Dealkylation: Montgomery *et al.* (1969) studied the further metabolism of hydroxysimazine in plants. They demonstrated that the primary metabolite was the result of dealkylation to produce 2-amino-4-ethylamino-6-hydroxy-*s*-triazine (GS-17792). There was also chromatographic evidence for a second dealkylation step that possibly produced ammeline, 2,4-diamino-6-hydroxy-*s*-triazine (GS-17791) and ammelide, 2-amino-4,6-dihydroxy-*s*-triazine (G-35713). The authors concluded that the dealkylation of these herbicides appears to be an important pathway of detoxification.

* Some treatments were made in kilograms per hectares, while others were in pounds per acre. To convert to kg/ha, multiply lb/A by 1.12.

Kearney *et al.* (1965) identified 2-chloro-4-amino-6-ethylamino-*s*-triazine as a major metabolite of simazine when incubated with *Aspergillus fumigatus* in culture solution. Ammelide was postulated to be a second metabolite of simazine produced by this soil fungus.

Shimabukuro *et al.* (1966) identified 2-chloro-4-amino-6-isopropylamino-*s*-triazine (G-30033) as a major metabolite in shoots of mature pea plants. These results indicated that a second mechanism for tolerance to atrazine existed in some moderately susceptible plants. Later, Shimabukuro (1967a) was able to demonstrate that atrazine could be metabolized independently in both roots and shoots of young pea plants to 2-chloro-4-amino-6-isopropylamino-*s*-triazine. This metabolite was much less phytotoxic than the parent compound. The metabolism of atrazine in resistant corn and sorghum, in intermediately susceptible pea, and in highly susceptible wheat was reported by Shimabukuro (1967b). This study revealed two possible pathways for metabolism of atrazine in higher plants. All species studied were able to metabolize atrazine by *N*-dealkylation of either of the two alkyl groups present. Corn and wheat that contain the cyclic hydroxyamate (2,4-dihydroxy-7-methoxy-1,4-benzoxazine-3-one) also metabolized atrazine by conversion to hydroxyatrazine (G-34048). Subsequent metabolism was postulated to be by conversion to more polar compounds.

Shimabukuro (1968) studied the metabolism of atrazine in resistant species of corn and sorghum. He concluded that atrazine in corn was metabolized by both the 2-hydroxylation and *N*-dealkylation pathways, whereas sorghum metabolized atrazine via the *N*-dealkylation pathway. The interaction of both pathways in corn resulted in production of three nonphytotoxic 2-hydroxylated derivatives of atrazine.

In a study designed to determine the mode of action of atrazine in higher plants, Shimabukuro and Swanson (1969) concluded that atrazine inhibits the Hill reaction and its noncyclic phosphorylation, while being ineffective against cyclic photophosphorylation. Atrazine readily penetrated the chloroplast of resistant as well as susceptible plants. In tolerant plants such as sorghum, the metabolism of atrazine was postulated to occur outside the chloroplasts to form water-soluble and insoluble residues that reduced the concentration of photosynthetic inhibitors in the chloroplasts.

Shimabukuro *et al.* (1973) identified 2-chloro-4,6-diamino-*s*-triazine (G-28273), which represented complete dealkylation of the triazine ring, as an organosoluble metabolite in sorghum. This metabolite did not inhibit the Hill reaction and cyclic and noncyclic photophosphorylation in isolated pea chloroplasts.

Glutathione Conjugation

The third metabolic pathway discovered for degradation of triazine herbicides in plants was first reported by Shimabukuro *et al.* (1970) and involved conjugation of atrazine with glutathione in corn nutrient uptake, excised leaves, and leaf disc experiments. This new degradation mechanism was postulated to be the primary factor in the tolerance of corn to atrazine.

Frear and Swanson (1970) isolated a soluble glutathione *S*-transferase from corn leaves. Active enzyme preparations were also isolated from leaves of sorghum, sugarcane, johnsongrass, and sudangrass. Appreciable enzyme activity was found only with substituted 2-chloro-*s*-triazines. Substitution of a methoxy, methylmercapto, or hydroxy group in the 2-position of the triazine ring resulted in loss of weed control.

Lamoureux *et al.* (1970) identified a glutathione conjugate and a γ-glutamylcysteine conjugate of atrazine from sorghum leaf disc incubations. The latter metabolite was postulated to form by the action of a carboxypeptidase enzyme on the glutathione conjugate of atrazine. These compounds represented a new class of herbicide metabolites in plants. This pathway appears to be the primary mode of detoxification of atrazine in sorghum and appears to be active in corn – but not in pea, wheat, or soybean.

The metabolism of atrazine and a series of 2-chloro-*s*-triazines were reported by Lamoureux *et al.* (1972) in excised leaf or shoot tissue of barley, corn, sorghum, and sugarcane. The authors found that the primary route of metabolism was the displacement of the 2-chloro group with glutathione or γ-glutamylcysteine. The overall rate of metabolism in susceptible barley was much slower than in tolerant crops.

Shimabukuro *et al.* (1971b) concluded that the primary factor for atrazine selectivity in corn was the activity of a glutathione *S*-transferase enzyme that detoxified atrazine by catalyzing the formation of the glutathione conjugate. All corn lines investigated, except for the susceptible GT112 line, detoxified atrazine by glutathione conjugation. Hydroxyatrazine was found in significant quantities only when introduced via the corn roots.

Beynon *et al.* (1972a) compared the breakdown of cyanazine, atrazine, and simazine in soils and corn. Residues of parent compound in leaf and stem tissue of corn plants 70 days after application at a rate of 1.5 kg a.i./ha to a medium loam soil were barely detectable. Cyanazine metabolized to chlorotriazines and hydroxytriazines, including their dealkylated derivatives. Atrazine and simazine metabolized to hydroxytriazines and unidentified polar components.

Lamoureux *et al.* (1973) reported on the timeline for metabolism of atrazine in sorghum initially grown in treated nutrient solution. Plants were placed in ^{14}C-atrazine-treated nutrient solution for 2 days when seedlings were 22 days old. A portion of the plants were harvested after 2 days of treatment, and most were placed in atrazine-free nutrient solution and harvested in several time intervals up to 30 days after initial treatment. Injection studies were performed

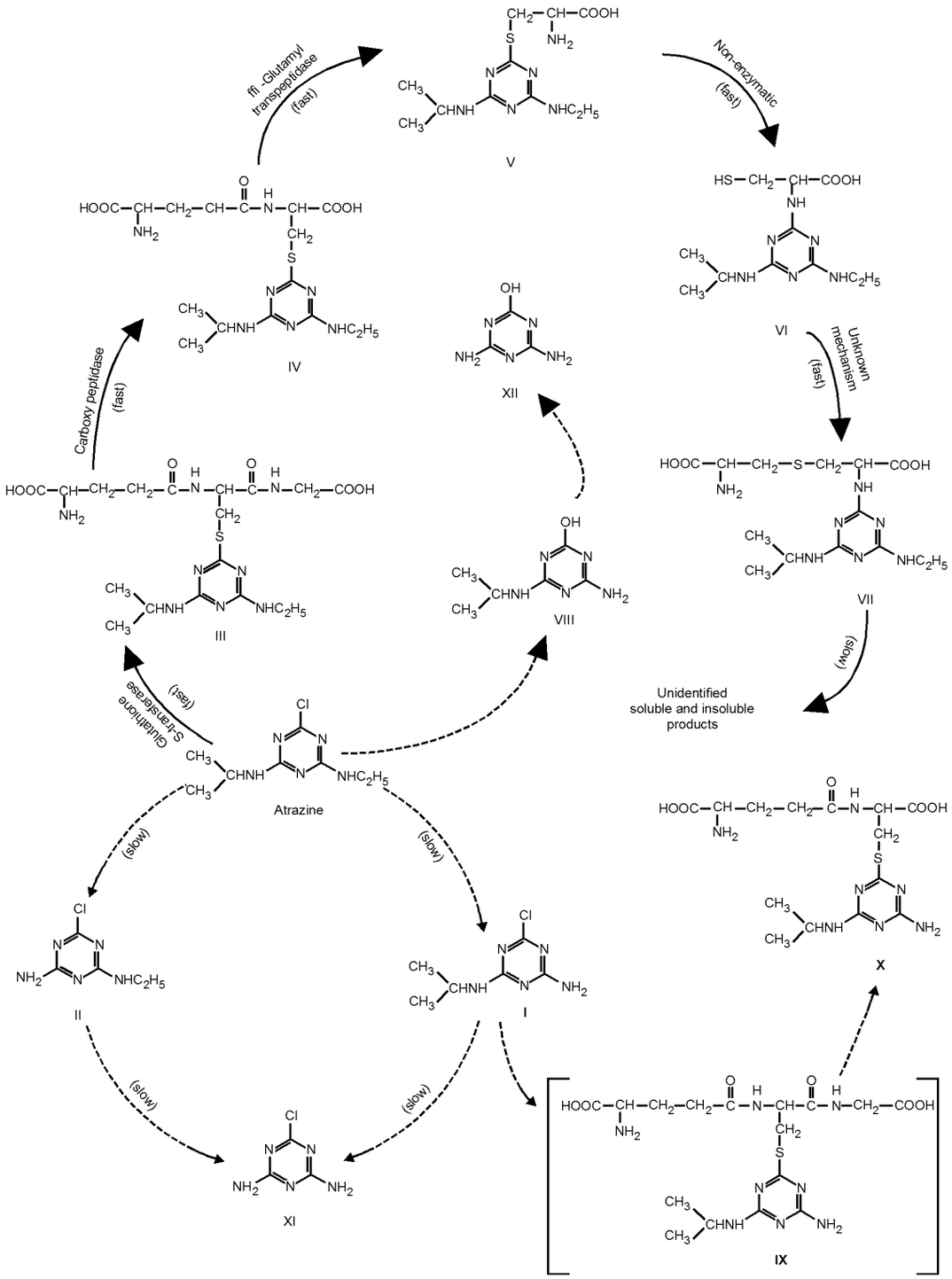

Figure 7.1 The metabolic pathway for atrazine in sorghum. The major pathway is indicated by the solid line with large arrows. The dashed line with large arrows indicates minor pathways, and the dashed line with small arrows indicates a hypothesized reaction(s). The structure in brackets was not identified. Redrawn from Lamoureux *et al.* (1973).

on 25-day-old sorghum seedlings with glutathione, glutamylcysteine, and lanthionine conjugates of atrazine to determine the sequence of events in the proposed metabolic pathway for sorghum. The metabolic pathway presented in Figure 7.1 contains the previously discussed dehalogenation and dealkylation reactions leading to hydroxy-*s*-triazines and dealkylated chloro- and hydroxy-*s*-triazine products. The lanthionine conjugate of atrazine [*N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)lanthionine] was thought to originate as a result of stepwise degradation of the glutathione conjugate of atrazine to the glutamylcysteine, *S*-cysteine, and *N*-cysteine conjugates. The formation of the lanthione conjugate was postulated to occur by condensation of the *N*-cysteine conjugate with cysteine to form the mixed disulfide, mono-*N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)cystine, which then expels one atom of sulfur to yield the corresponding sulfide. In fact, the *N*-cysteine conjugate of atrazine was identified as a chloroform soluble disulfide dimer of atrazine in sorghum by Shimabukuro *et al.* (1973).

Metabolism of Chloro-*s*-triazines

The metabolism of individual chloro-*s*-triazines has been the subject of renewed research in plants as a result of the reregistration process initiated by the United States Environmental Protection Agency (USEPA) in the form of data-call-ins for new study requirements. Atrazine was chosen as a model compound for this class of *s*-triazines because completed research on several crops has greatly expanded our knowledge of the original pathway (Figure 7.1) as proposed by Lamoureux *et al.* (1973).

Atrazine

Studies on the metabolism of atrazine in corn, sorghum, and sugarcane following preemergence and postemergence in small field plots have provided new insight into the metabolic pathways in these plants. Corn and sorghum were grown in Mississippi, Illinois, and New York and treated at an exaggerated rate of 3.0 lb a.i./A postemergence when plants were approximately 1 ft (0.3 m) high. Plants were harvested 30 days after treatment, at silage stage and at maturity. Samples of forage, fodder, and grain were analyzed for the expected chloro- and hydroxy-*s*-triazines and for any unknown nonpolar or polar extractable metabolites in sufficient quantity for identification (Larson and Ash, 1992). A major component found primarily in the aqueous fraction of sorghum samples 30 days after treatment was identified by mass spectrometry as the lanthionine conjugate of atrazine. Later harvests of sorghum and all harvests of corn contained much smaller amounts of this component, indicating that the lanthionine conjugate was the precursor to more acidic polar metabolites.

Additional work on the isolated cation exchange peak associated with the lanthionine conjugate of atrazine from sorghum forage 30 days after treatment revealed three additional components (Larson and Ash, 1994). Two of these components were identified as stereoisomers of *N*-(4-isopropylamino-6-ethylamino-*s*-triazinyl-s)-lanthionine-*S*-oxide (lanthionine sulfoxide conjugate of atrazine). The third component was tentatively identified as a glutamine conjugate of atrazine (*N*-(4-isopropylamino-6-ethylamino-*s*-triazinyl-2)-glutamine).

In a separate study, the metabolism of atrazine was studied in field-grown sugarcane (Larson, 1993; Larson and Ash, 1993). Sugarcane was treated four times with radiolabeled atrazine. The first treatment was a 4-lb a.i./A (4.5 kg/ha) broadcast spray when the seed cane was planted in the autumn. The second treatment was a 2-lb a.i./A (2.2 kg/ha) broadcast spray approximately 1 month later. The third application was a 2 lb a.i./A (2.2 kg/ha) postemergence broadcast spray approximately 7 months after the initial treatment. The fourth treatment was a 2-lb a.i./A (2.2 kg/ha) post-directed spray approximately 4½ months before final harvest. Before the fourth application, sugarcane leaves had enough total triazine residues (69 ppm) to allow for isolation and identification of metabolites at levels down to 0.05 ppm. As a result of these investigations, 12 new metabolites were identified by various mass spectrometry techniques – two organic soluble, three basic, and seven acidic. A summary of the corn, sorghum, and sugarcane metabolic pathways was reported by Lamoureux *et al.* (1998).

Several metabolic pathways have been created to illustrate the complex nature of atrazine metabolism in plants in a sequential fashion. In Figure 7.2, the dealkylation (cycle A) and dechlorination (cycle B) pathways are illustrated. Cycle A led to further metabolism of G-30033 (2-amino-4-chloro-6-isopropylamino-*s*-triazine) as illustrated in Figure 7.3, and cycle B led to an *O*- or *N*-glucoside of GS-17794 (2-amino-4-hydroxy-6-isopropylamino-*s*-triazine). The conjugation of atrazine with glutathione (Figure 7.2, cycle C) leads to formation of an *S*-cysteine conjugate and to its further metabolism as illustrated in Figure 7.4. The *S*-cysteine conjugate of atrazine can rearrange to form an *N*-cysteine conjugate, and through a deamination process can form a postulated thiopyruvate conjugate intermediate. Reduction gives a thiolactate conjugate, before conjugation with glucose to form the glucose–thiolactate conjugate. The *S*-cysteine conjugate can form an *N*-malonylcysteine conjugate by reaction with malonic acid. The monodealkylated product of atrazine (G-30033) can conjugate with glutathione as illustrated in Figure 7.3. Analogous to atrazine, the

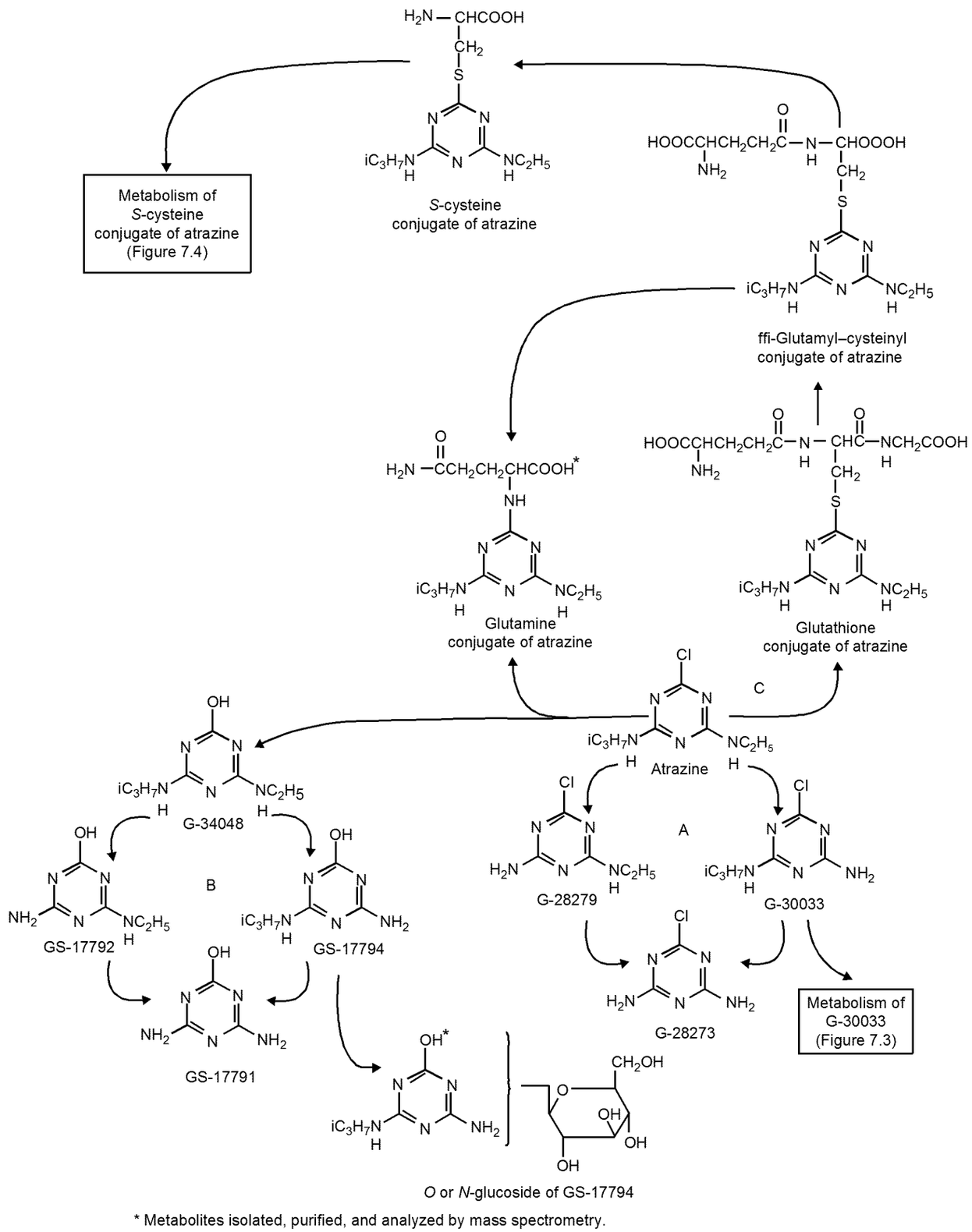

Figure 7.2 Metabolism of atrazine in sugarcane plants by (a) *N*-dealkylation of the side chains, (b) hydrolysis of the 2-chloro group followed by *N*-dealkylation, and (c) displacement of the 2-chloro by glutathione conjugation (Larson and Ash, 1993).

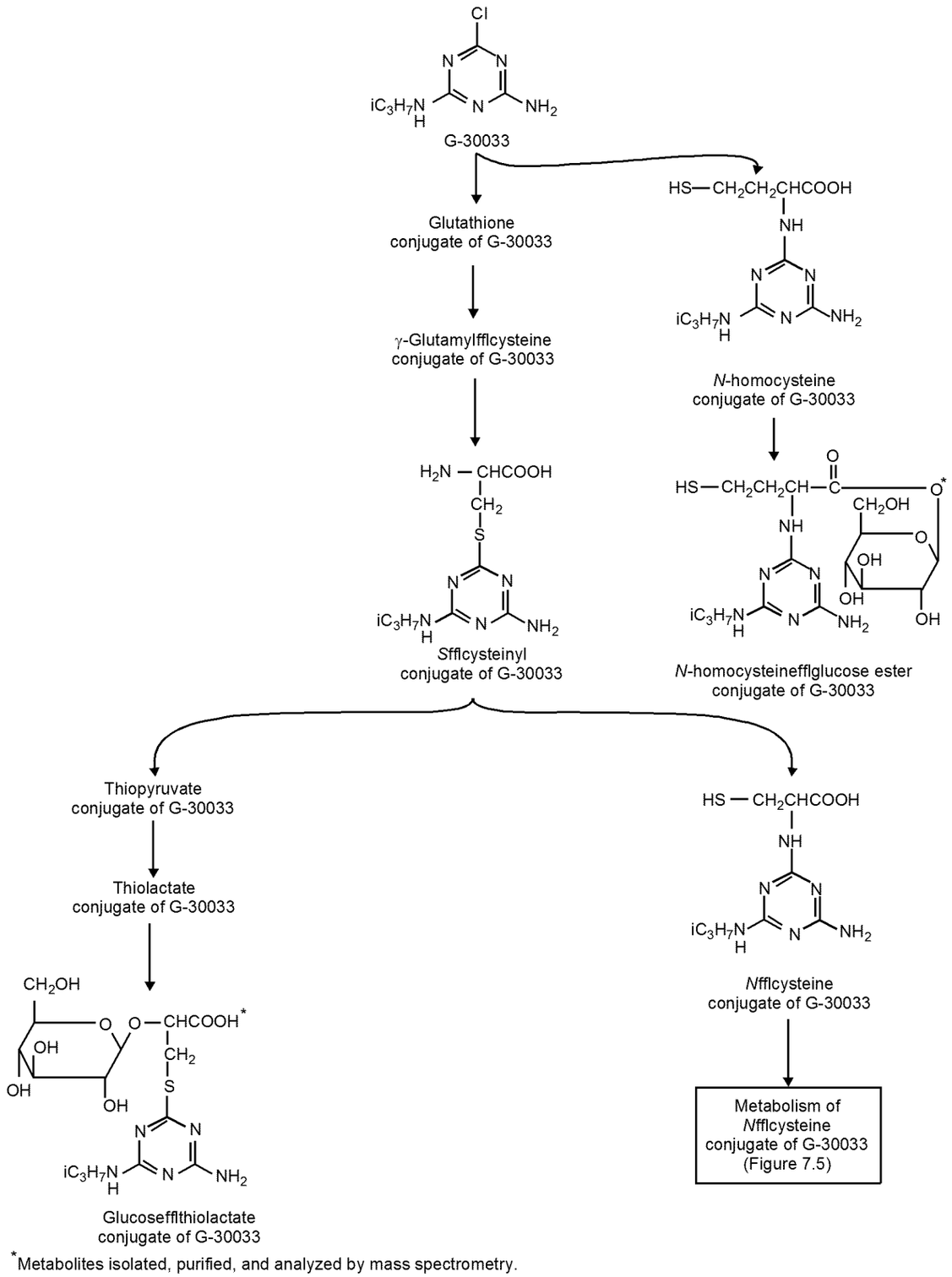

Figure 7.3 Metabolism of G-30033 in sugarcane plants (Larson and Ash, 1993).

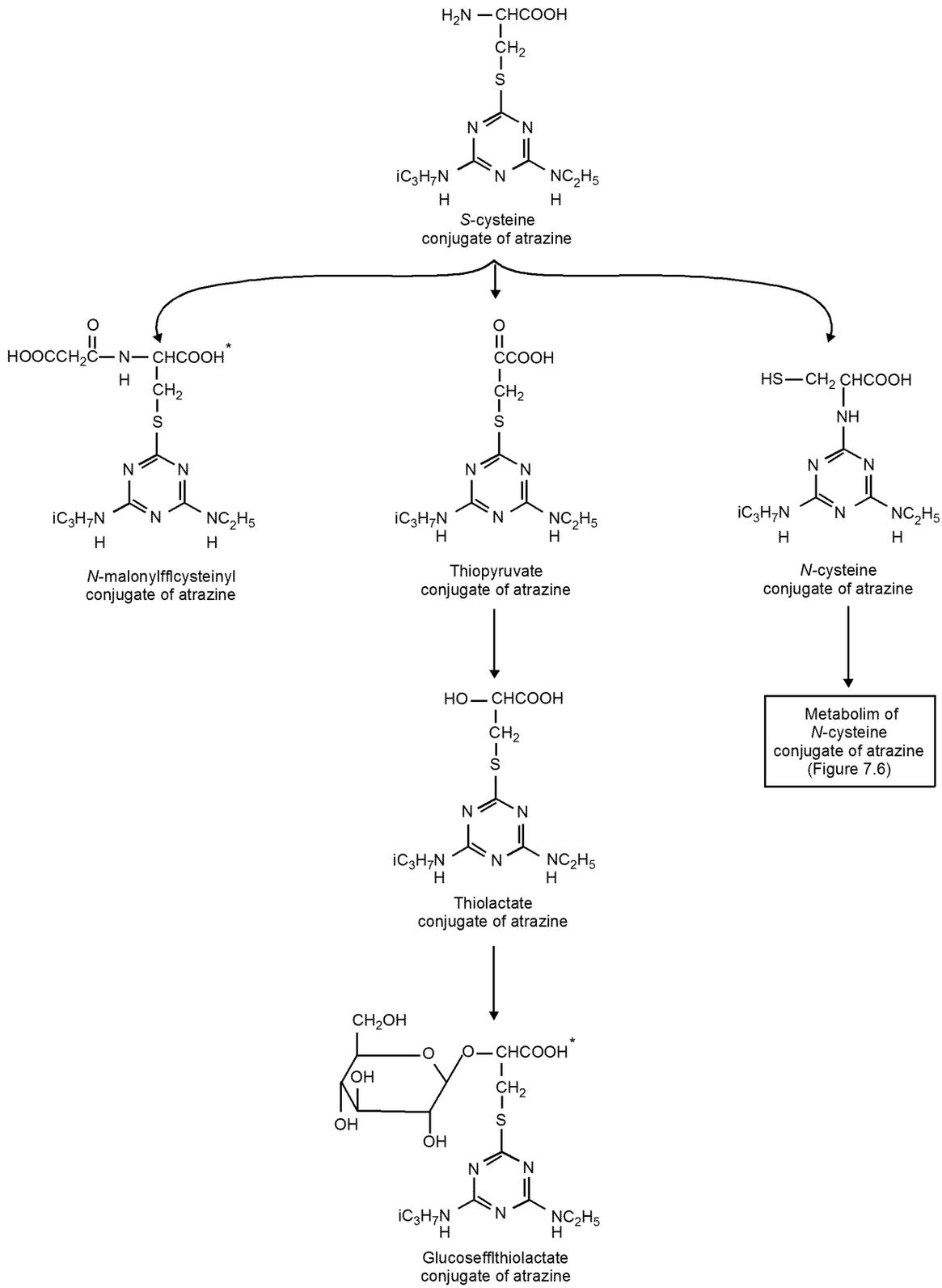

Figure 7.4 Metabolism of the *S*-cysteine conjugate of atrazine in sugarcane plants (Larson and Ash, 1993).

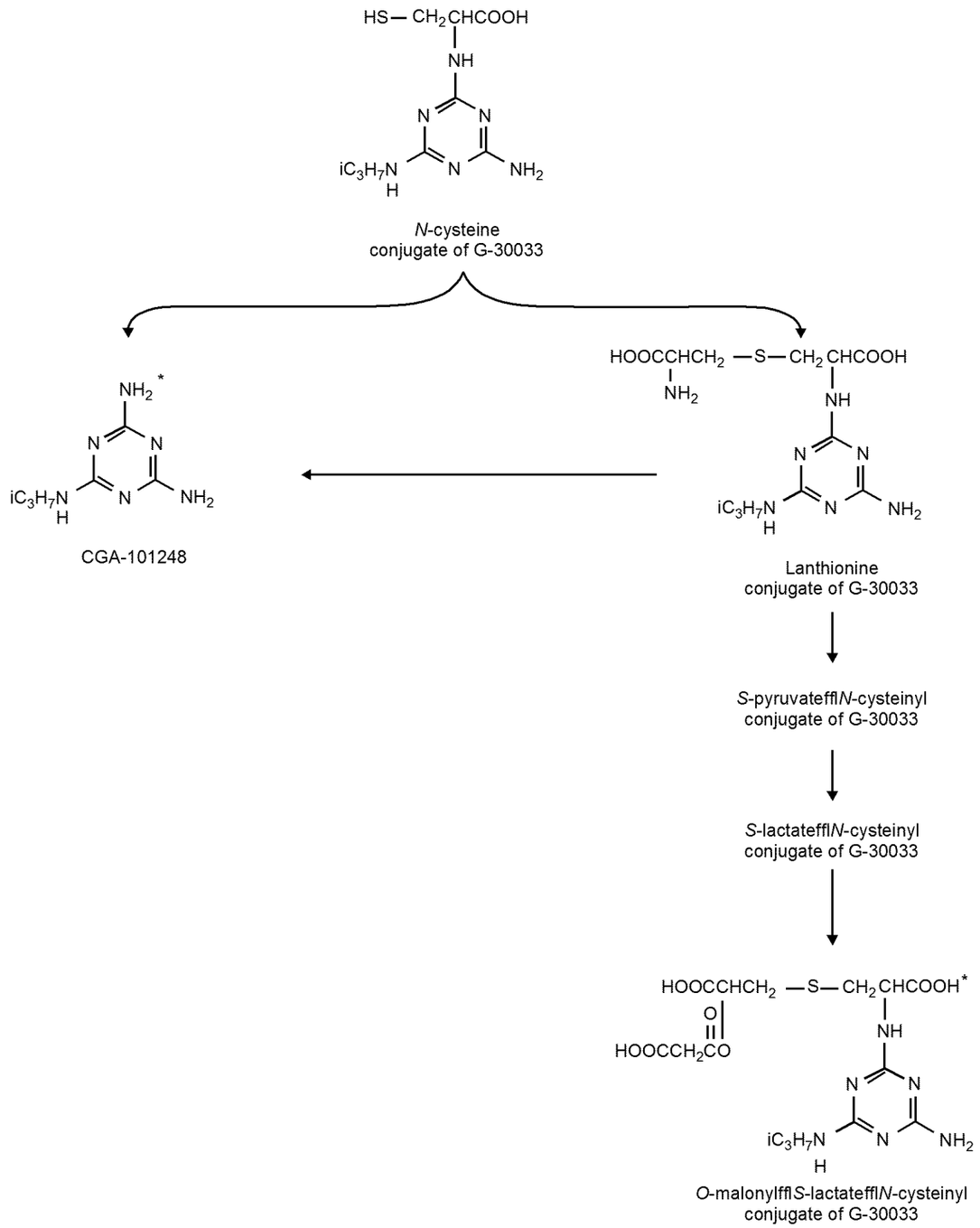

Figure 7.5 Metabolism of the *N*-cysteine conjugate of G-30033 in sugarcane plants (Larson and Ash, 1993).

glutathione conjugate of G-30033 can metabolize to the *S*-cysteine and *N*-cysteine conjugates. The terminal metabolite of the *S*-cysteine pathway is a glucose–thiolactate conjugate. In sugarcane, G-30033 can also form a homoglutathione conjugate that further metabolizes to the *N*-homocysteine–glucose ester conjugate. The terminal metabolites of the *N*-cysteine conjugate of G-30033 (Figure 7.5) are the *O*-malonyl-*S*-lactate–*N*-cysteinyl conjugate and CGA-101248 [2,4-diamino-6-isopropylamino-*s*-triazine]. CGA-101248 may originate by direct amination of G-30033 or as a cleavage product of an *N*-linked conjugate derived from the glutathione or homoglutathione pathway.

The *N*-cysteine conjugate of atrazine (Figure 7.6) can form a cysteic acid conjugate, a lanthionine conjugate, and GS-12517 [2-amino-4-ethylamino-6-isopropylamino-*s*-triazine]. GS-12517 may originate either by direct amination of atrazine or by degradation of an *N*-linked conjugate derived from the lanthionine pathway. The terminal metabolites of the lanthionine pathway are the *S*-lactate–*N*-cysteinyl sulfoxide conjugate and the *S*-acetate–*N*-cysteinyl-sulfoxide conjugate.

Simazine

The metabolism of simazine has been studied in corn (Castelfranco *et al.*, 1961; Larson, 1994a), apple (Larson, 1994b), grape (Larson, 1994c), and citrus (Burnett and Bateman, 1994) using radiolabeled simazine. The major simazine metabolites identified in corn silage and fodder after preemergence treatment were the simple hydroxy-*s*-triazines – G-30414 [2,4-bis(ethylamino)-6-hydroxy-*s*-triazine], GS-17792 [2-amino-4-ethylamino-6-hydroxy-*s*-triazine], and GS-17791 [2,4-diamino-6-hydroxy-*s*-triazine]. Based on comparisons to atrazine profiles, up to 25% of the total radioactive residues chromatographed in regions of the cation exchange profiles, where Phase II metabolites are derived from the glutathione pathway.

The metabolism of simazine in apple and grape was studied primarily in mature fruit. The total radioactive residues were highly extractable (┘ 90%) with neutral solvents. The two major metabolites characterized in apple extracts were ammeline (GS-17791) and the proline conjugate of diaminochloro-*s*-triazine (G-28273). It was proposed by Lamoureux *et al.* (1998) that the proline conjugate of G-28273 could be formed by ring closure of a glutamine conjugate of G-28273, or by attack of proline on either G-28273 or an activated form of this metabolite. Acidic conjugates accounted for approximately 30% of the total radioactive residues. The extracts of grape contained two major metabolites characterized as ammelide (G-35713) and ammeline (GS-17791). Only a trace amount of the proline conjugate of G-28273 was detectable, and acidic conjugates accounted for 4% of the total residues.

The extracts of citrus leaves at fruit maturity contained mostly GS-17791 and the proline conjugate of G-28273. The extracts of the fruit fractions contained only one major residue that was characterized as the proline conjugate of G-28273. Only small amounts of the acidic conjugates were present in the extracts of leaves or fruit fractions.

Propazine

Propazine is a member of the chloro-*s*-triazine group of herbicides that was registered for use on sorghum up until 1997 and was reregistered in 2007. Propazine is also registered for use in greenhouses and may be registered for use in sorghum in the future. A metabolism study (Hermes and Knaak, 1972) was done on sorghum-treated preemergence in the greenhouse at 2 lb a.i./A. Fodder contained 4.0 ppm and mature grain 0.54 ppm total radioactive residues. The grain contained no organosoluble residues, indicating that no measurable chloro-*s*-triazines were present. Total chlorotriazine residues in fodder only accounted for 0.8% of the total. Free hydroxy-*s*-triazines accounted for an additional 5%. At least 12 other metabolites were present in the aqueous phase, but none individually accounted for more than 8% of the total radioactive residues.

In a greenhouse study (Keezer and Hermes, 1971a), wheat was treated with 2.0 lb a.i./A preemergence the previous year and grown to maturity. Fodder contained 2.8 ppm and mature grain 0.10 ppm total radioactive residues. Chromatography of extractable residues from the wheat stalk showed 1.4% total chloro-*s*-triazine residues and 31% total hydroxy-*s*-triazine residues. The predominant hydroxy-*s*-triazines were GS-11526 [2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine] and GS-17794. There were 25% nonhydroxy polar metabolites attributed to conjugates.

Griffin Corporation (Collier, 1997) concluded that propazine metabolism in sorghum occurs by the following three reactions: *N*-dealkylation, hydrolytic dehalogenation, or nucleophilic displacement of the 2-chloro group with glutathione. Dehalogenation and conjugation were the predominant pathways as only small amounts of chloro-*s*-triazines were detected in forage and fodder. No chloro-*s*-triazines were detected in grain in the two metabolism studies that were conducted. The metabolic pathway of propazine appears to be qualitatively the same as that reported for other members of the chloro-*s*-triazine group of chemicals.

Terbuthylazine

Terbuthylazine is a member of the chloro-*s*-triazine group characterized by ethylamino and *tert*-butylamino side chains. It is used throughout Europe and more than 45 countries and is not registered in the United States except for use in cooling towers. In a Nebraska study, sorghum was treated preemergence in the field at 2.5 lb a.i./A and in a greenhouse at 2.0 lb a.i./A (Simoneaux and Knaak, 1972). Total radioactive residues were typical for a preemergence field study (0.4 ppm in mature fodder and 0.02 ppm in grain). Corresponding residues for greenhouse-grown sorghum were 5.8 and 0.10 ppm for fodder and grain, respectively. Fodder from the greenhouse contained approximately 3% chloro-*s*-triazines and 10% free hydroxy-*s*-triazines. Corresponding values for field fodder were 1% and 10% for

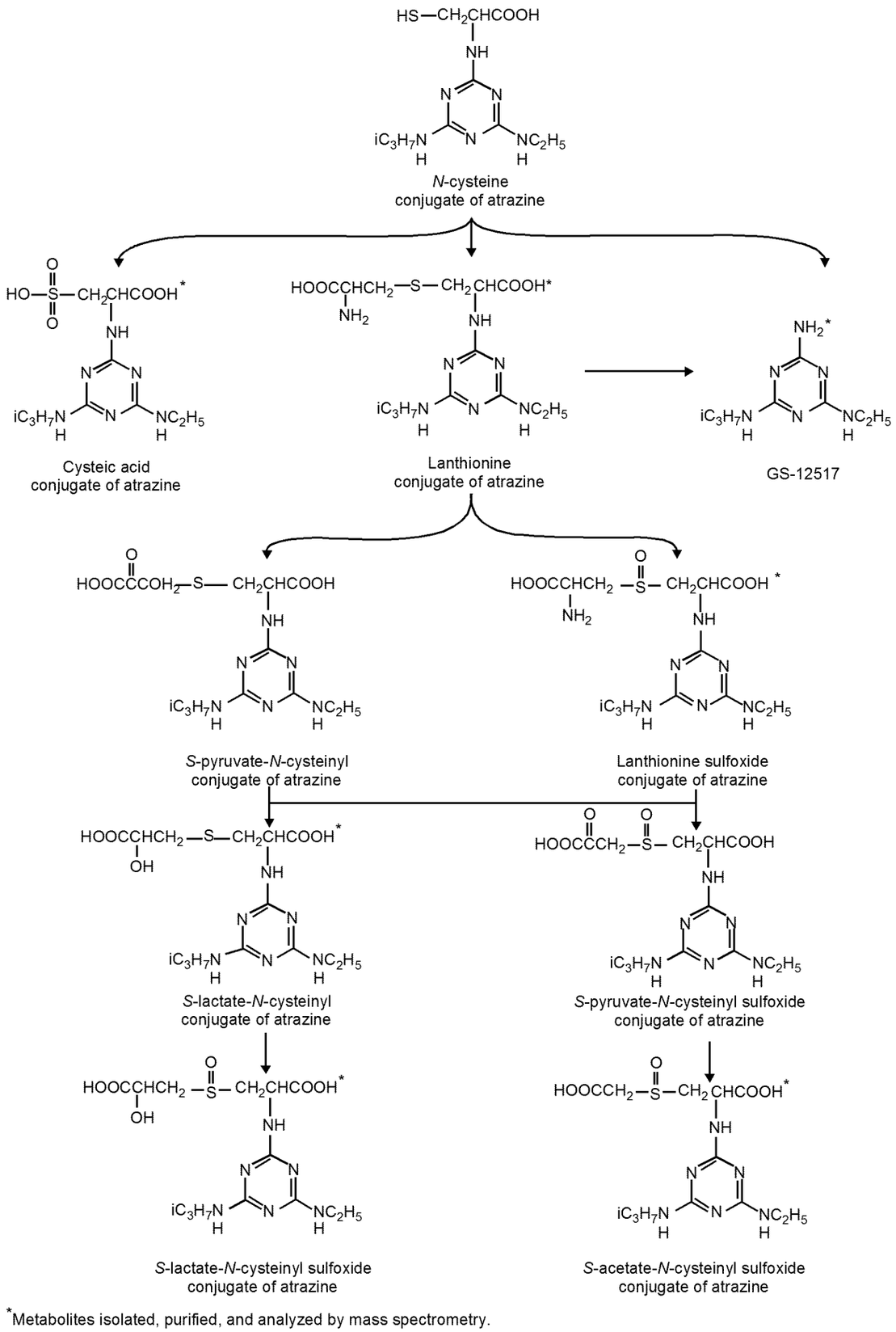

Figure 7.6 Metabolism of the *N*-cysteine conjugate of atrazine in sugarcane plants (Larson and Ash, 1993).

chloro- and hydroxy-*s*-triazines, respectively. Grain residues were too low to be characterized. The major chloro-*s*-triazine residues in fodder were intact parent and G-26379 (2-amino-4-*tert*-butylamino-6-chloro-*s*-triazine). The major hydroxy-*s*-triazine residues were GS-23158 (2-*tert*-butylamino-4-ethylamino-6-hydroxy-*s*-triazine) and GS-28620 (2-amino-4-*tert*-butylamino-6-hydroxy-*s*-triazine).

Cyanazine

Cyanazine is different from the other chloro-*s*-triazines already discussed in that it has a labile cyano group as part of the alkylamino substituent. A series of papers were published in 1972 describing the metabolism of cyanazine in corn (Beynon *et al.*, 1972a, 1972b), wheat, and potato (Beynon *et al.*, 1972c).

In corn grown in the greenhouse in four different field soils treated preemergence at 2 kg a.i./ha, the magnitude of uptake in stem, leaf, and cobs (whole cob includes husk and core) varied with soil type. Leaves (1.41–2.07 ppm) had greater uptake than stems (0.12–0.21 ppm) or cobs (0.02 ppm) from plants grown in soils ranging in texture from sandy loam to clay loam. Plants grown in peat had considerably less uptake in leaves (0.31 ppm), stems (0.02 ppm), and cobs (| 0.02 ppm) than was observed for the three mineral soils. Metabolites identified (Figure 7.7) included hydrolysis products of the parent, including the chloro amide (compound II) and the hydroxy acid (compound IV), as well as the corresponding metabolites formed by the loss of the *N*-ethyl group (compounds VI and VIII, respectively). The hydroxy acids (compounds IV and VIII) were present predominantly in free form, but there was some evidence of conjugates that could be converted to these acid metabolites.

The metabolism of cyanazine in spring and winter wheat and in potato was studied after preemergence soil treatment at rates varying from 0.25 to 1.0 kg a.i./ha in wheat and at a rate of 1.5 kg a.i./ha in potato. These plants were grown to maturity in the greenhouse. Total radioactive residues in spring wheat and winter wheat plant parts increased with increasing treatment rate. Spring wheat plants were divided into seed, chaff, and leaf with stem fractions. Winter wheat did not form much seed, and plants were divided into seed-head, stem, and leaf fractions. With the highest rate of cyanazine treatment (1.0 kg a.i./ha), leaf and stem residues in spring and winter wheat ranged from 1.29 to 3.60 ppm. The winter wheat seed-head contained 0.75 ppm total residue, and spring wheat seed contained only 0.10 ppm.

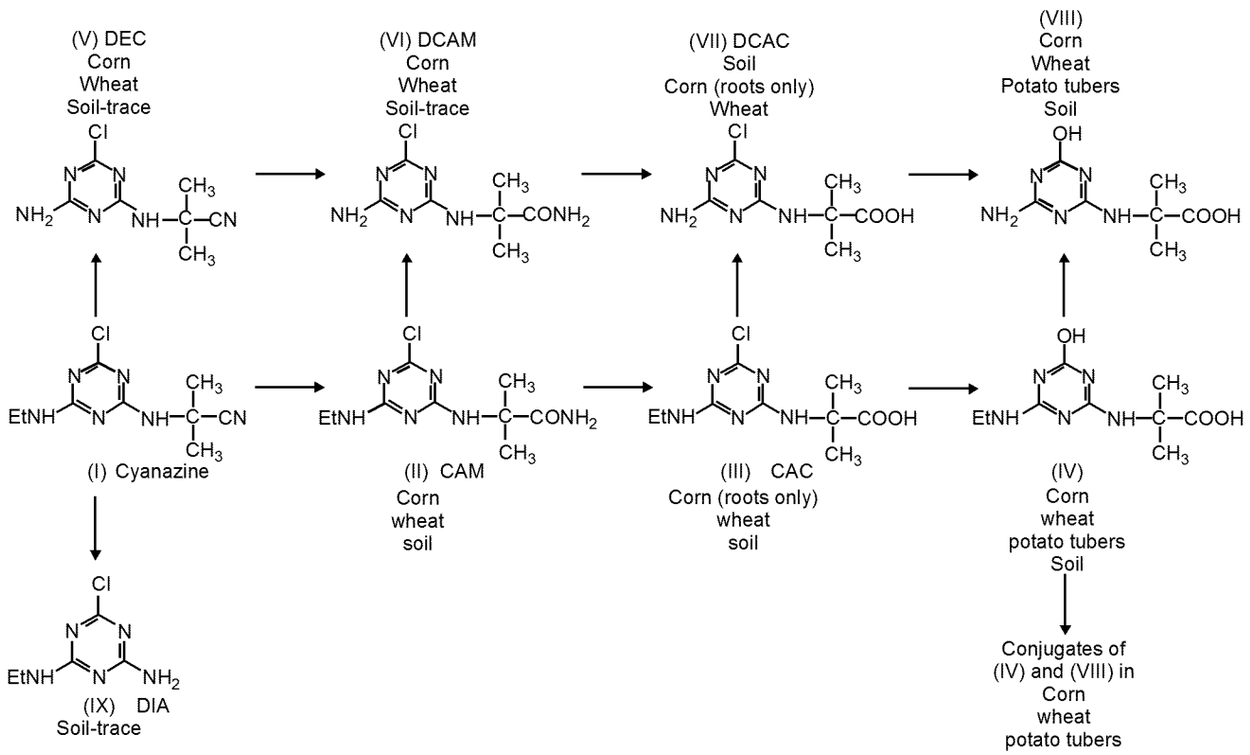

Figure 7.7 The breakdown of cyanazine is soils, corn, potato, and wheat (redrawn from Beynon *et al.* (1972c)).

The chaff associated with the winter wheat seed probably contributed to most of the residues in the seed-head. Chaff separated from the spring wheat seed contained 1.90 ppm, whereas seed contained only 0.10 ppm total radioactive residues. The chloro-acid metabolites (compounds III and VII) were found in the leaves and stems of wheat plants, whereas in corn they were detected only in the roots. Wheat apparently hydrolyzed the 2-chloro group less rapidly than corn. The nature of the residues were similar in spring and winter wheat, except that the hydroxy acids (compounds IV and VIII) were present in spring wheat in free and conjugated forms, but were mostly present in conjugated forms in winter wheat. The conjugates were more readily hydrolyzed in wheat than corn. As previously noted for atrazine and simazine, this might reflect a difference in the relative bond strength at the point of attachment to the triazine ring (*S*-triazine versus *N*-triazine). The lanthionine type conjugates (*N*-triazine) have been shown to require much more stringent acid conditions (greater normality of HCl and longer hydrolysis times) to hydrolyze than corresponding *S*-triazine conjugates. The metabolic pathway for cyanazine in corn, potato, and wheat involves dechlorination, dealkylation, and conjugation reactions as illustrated in Figure 7.7.

Kern *et al.* (1975, 1976) studied the metabolism of cyanazine in corn, fall panicum, and green foxtail. These short-term greenhouse studies involved foliar and root treatment at the four-leaf stage. Five days following foliar treatment, fall panicum and green foxtail contained a larger number of metabolites than corn; following root uptake the opposite was true. Less uptake of cyanazine residues was observed for the foliage of corn than the foliage of weed species, and as a result, a lower concentration of parent cyanazine was evident in corn leaves than in the weed species. Although rapid metabolism of cyanazine occurred in corn roots, the large amount of cyanazine absorbed via the root system resulted in similar internal concentrations of parent cyanazine for all three plant species. Corn rapidly hydrolyzed the nitrile group and hydroxylated the 2-position of the triazine ring. Green foxtail was more sensitive to cyanazine than was fall panicum. Increased phytotoxicity was observed with a combination of foliar and soil treatments. The basis of cyanazine tolerance in corn was attributed not solely to the differential foliar uptake, but also to the proportion taken up by the foliage and roots and the rapid metabolism in the corn root system.

Metabolism of Thiomethyl-*s*-triazines

Ametryn

Ametryn is used for preemergence and postemergence control of both monocotyledonous and dicotyledonous weeds in corn, sugarcane, banana, pineapple, and noncrop areas. The metabolism of ametryn in corn (Detra and Chib, 1990a), sugarcane (Detra and Chib, 1990b), and banana (Thalaker and Ash, 1996) has recently been studied, and the major findings will be summarized by individual crop.

Corn grown in a greenhouse was treated post-directed with ^{14}C-ametryn at a rate of 4 lb a.i./A 32 days after planting. Corn at maturity had total residues of 4.56 ppm in fodder, 0.71 ppm in cobs, and 0.16 ppm in grain. The major organic soluble residues identified in mature stalk extracts were intact ametryn and GS-11355 (2-amino-4-ethylamino-6-methylthio-*s*-triazine). Together, these methylthio-*s*-triazine residues amounted to less than 0.05 ppm in the fodder. Organic soluble grain residues were too low to be successfully chromatographed ($\leq$ 0.01 ppm). Cation exchange profiles of the aqueous solubles of mature corn fodder, cobs, and grain were qualitatively similar, but differed in the relative proportions of individual metabolites. Approximately 15 radioactive zones could be separated by this technique. High-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) were used to identify two of these metabolites as the hydroxy-*s*-triazines GS-17794 and GS-17792. Many of the cation exchange zones eluted in the weakly basic to acidic region of the column were probably polar metabolites derived from the glutathione pathway. These were identified in the previously discussed section on atrazine and simazine metabolism.

Sugarcane was grown in the greenhouse and treated preemergence with ^{14}C-ametryn at 8 lb a.i./A. It was treated two more times postemergence at 4 lb a.i./A, for a total application of 16 lb a.i./A. Total radioactive residues in mature cane and foliage were 0.42 and 3.06 ppm, respectively. Organic soluble residues in cane and foliage were too low to characterize further. Cation exchange profiles of aqueous soluble residues from cane and foliage were similar to those previously noted for corn and contained approximately 15 zones. Two of these metabolite zones were characterized as the hydroxy-*s*-triazines GS-17794 and GS-17792. Other less basic metabolites were probably derived from the glutathione pathway.

Banana grown in the greenhouse received three soil-directed treatments of ^{14}C-ametryn that totaled 24 lb a.i./A. Mature harvest leaves and whole fruit contained 1.59 and 0.087 ppm total residues, respectively. The organic fraction of mature leaves contained three TLC zones – ametryn and GS-11354 (2-amino-4-isopropylamino-6-methylthio-*s*-triazine), G-34048 (2-ethylamino-4-isopropylamino-6-hydroxy-*s*-triazine), and GS-12517. Levels of organic soluble residues ($\leq$ 0.01 ppm) from whole fruit were not high enough for chromatographic analysis. The aqueous solubles of mature leaves and whole fruit contained mostly GS-11957 (2,4-dihydroxy-6-isopropylamino-*s*-triazine) with some G-28251 (cyanuric acid), G-34048, GS-17794, GS-17792, and GS-17791 (ammeline). There was very little evidence for involvement of glutathione pathway metabolites in these profiles. The metabolic pathway for ametryn in banana is presented in Figure 7.8.

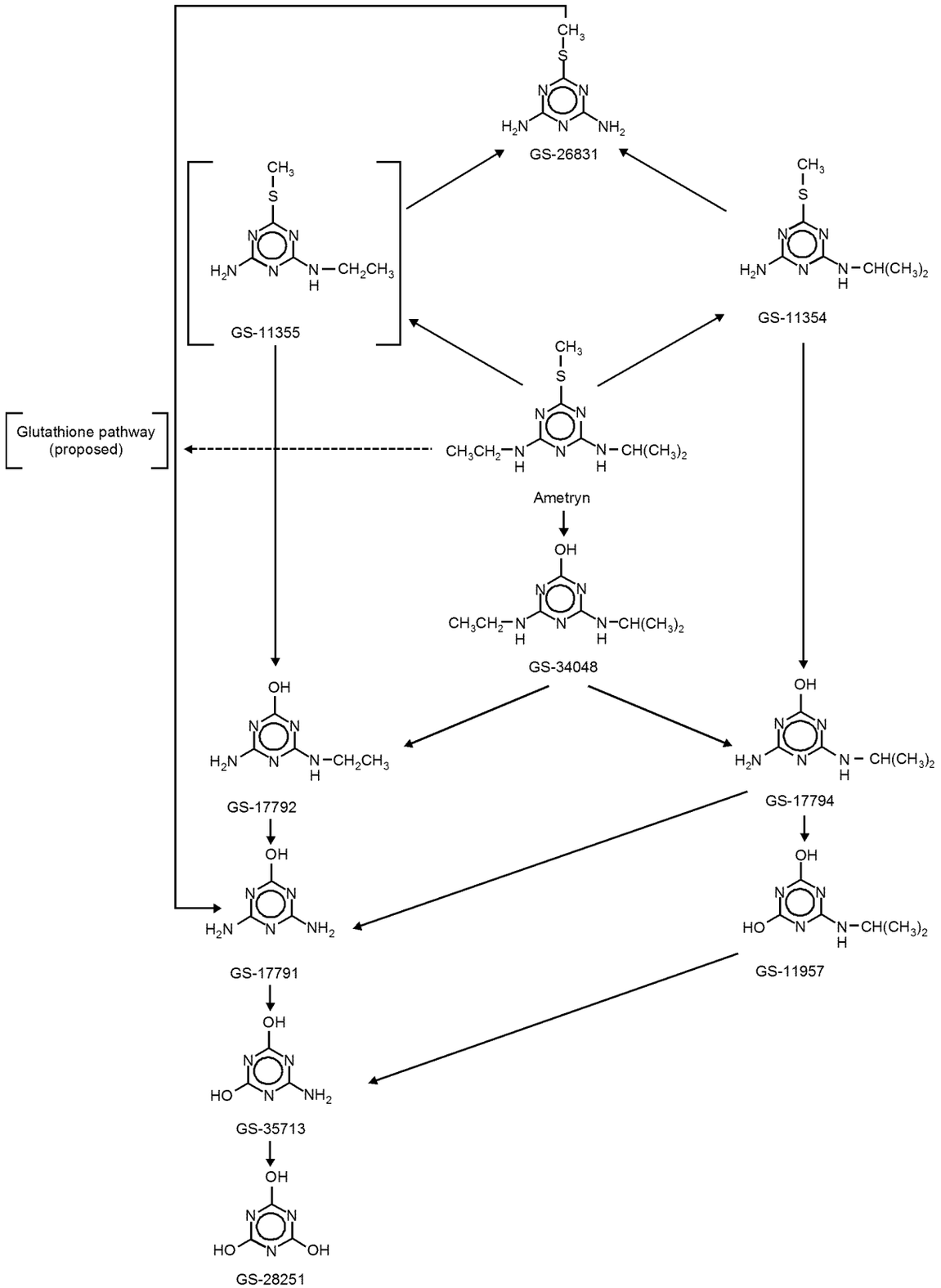

Figure 7.8 The metabolic pathway of ametryn in banana (Thalaker and Ash, 1996).

Prometryn

Prometryn is the active ingredient in herbicides labeled for preemergence and postemergence use on celery and cotton. The metabolism of prometryn in cotton and celery was investigated in greenhouse-grown cotton (Sanson, 1994) and in field-grown celery (Fleischmann *et al.*, 1990; Sanson, 1992). Cotton was treated once preemergence and twice postemergence for a total exaggerated application rate of 10.9 lb a.i./A of ^{14}C-prometryn. The exaggerated rate was employed to try to maximize cottonseed residues to expedite their characterization. Immature cotton stalks had 2.63 ppm residue and mature stalks had 1.28 ppm. Ginned cottonseed had only 0.118 ppm total residue. Organic solubles from immature stalk represented approximately 50% of the total radioactive residue. Intact prometryn, GS-11354, GS-17794, and GS-26831 (2,4-diamino-6-methylthio-*s*-triazine) were characterized as organic soluble residues in immature and mature stalks. Cottonseed contained mostly nonextractable residues (72%) after a hexane reflux and a methanol/water extraction and 10% was water soluble. The aqueous solubles were separated into as many as 40 different metabolite fractions by a combination of anion exchange and cation exchange column chromatography in conjunction with HPLC. Cation exchange profiles of separated anion exchange clusters indicated that although the immature stalk, mature stalk, and seed contained qualitatively similar metabolite patterns, a trend toward more polar and acidic moieties in mature stalks and seed was evident. Two-dimensional TLC and HPLC were used to identify several components of the mature stalk polar fraction: GS-28521 (2,4,6-trihydroxy-*s*-triazine), GS-17794, GS-11957, GS-17791 (ammeline), and GS-11526. Many of the polar components constituted 2% or less of the total residue (| 0.05 ppm).

Celery transplants were treated with a single, over-the-top broadcast spray at rates of 1.6 (normal rate) and 3.2 (twice the normal rate) lb a.i./A. Total radioactive residues in mature celery stalks averaged 0.42 ppm for both treatments, with little difference in total residues between the normal and exaggerated rates. Most of the residues (┘ 85%) were extractable with neutral solvents. The characterized organic soluble residues (Figure 7.9) included intact prometryn, GS-16141 (prometryn sulfoxide), GS-16158 (prometryn sulfone), GS-26831, GS-17794, GS-11526, GS-11957, G-35713 (2-amino-4,6-dihydroxy-*s*-triazine), and GS-17791. Use of a biphasic extraction solvent encouraged carryover of some polar components into the organic phase (chloroform and methanol). The aqueous solubles were characterized by use of anion and cation exchange chromatography, HPLC, and TLC. At least 11 aqueous soluble components could be resolved with these techniques. GS-11957, MCO-III-25 (side-chain alkanol of GS-17794), and its possible isomer were identified as aqueous soluble metabolites in mature stalks treated with an application at twice the normal rate. Two other zones were postulated to be sugar conjugates derived from these side-chain alkanol metabolites.

Further analysis of celery samples taken from the aforementioned study characterized 14 metabolites. These structures included simple hydroxy-*s*-triazines (GS-11526, GS-17794, GS-11957, GS-17791, GS-35713, and cyanuric acid), side-chain oxidized hydroxy-*s*-triazines (MCO-III-25 and MCO-IV-34), oxidized parent metabolites (GS-16141 and GS-16158), and dealkylated thiomethyl-*s*-triazines (GS-11354 and GS-26831). A metabolic pathway for prometryn in celery is illustrated in Figure 7.9.

Terbutryn

Terbutryn is an herbicide previously registered in the United States for use on sorghum and wheat and is currently registered in Europe and other countries throughout the world. The metabolism of terbutryn was investigated in field-grown (Fischer and Cassidy, 1978) and greenhouse-grown (Keezer and Hermes, 1971b) sorghum and field-grown spring wheat (Stockton and Szolics, 1988). A Nebraska field plot of sorghum was treated preemergence with ^{14}C-terbutryn at 2.4 lb a.i./A. Total radioactive residues at crop maturity were only 0.07 ppm in fodder and 0.01 ppm in grain. They were too low for characterization of residues. A greenhouse sorghum study conducted with a preemergence treatment rate of 2 lb a.i./A had fodder residues of 2.18 and 0.17 ppm in grain. Less than 1% of the fodder residues were thiomethyl-*s*-triazines, and 11% of the total residues were identified as hydroxy-*s*-triazines. Thiomethyl-*s*-triazines identified include intact terbutryn, GS-26575 (2-amino-4-*tert*-butylamino-6-methylthio-*s*-triazine), GS-11355, and GS-26831. The hydroxy-*s*-triazines identified included GS-23158 (2-*tert*-butylamino-4-ethylamino-6-hydroxy-*s*-triazine) and GS-28620 (2-amino-4-*tert*-butylamino-6-*s*-triazine).

Spring wheat in a field plot in New York was treated postemergence with ^{14}C-terbutryn at 1.2 lb a.i./A. At maturity, wheat fodder contained 0.52 ppm total radioactive residues, and grain only 0.011 ppm. Aqueous and organic fractions were characterized by TLC and cation exchange chromatography, respectively. The major metabolites identified in fodder were GS-28620 and GS-17792, with lesser amounts of GS-23158, GS-26831, GS-26575, GS-11355, and intact terbutryn (Figure 7.10).

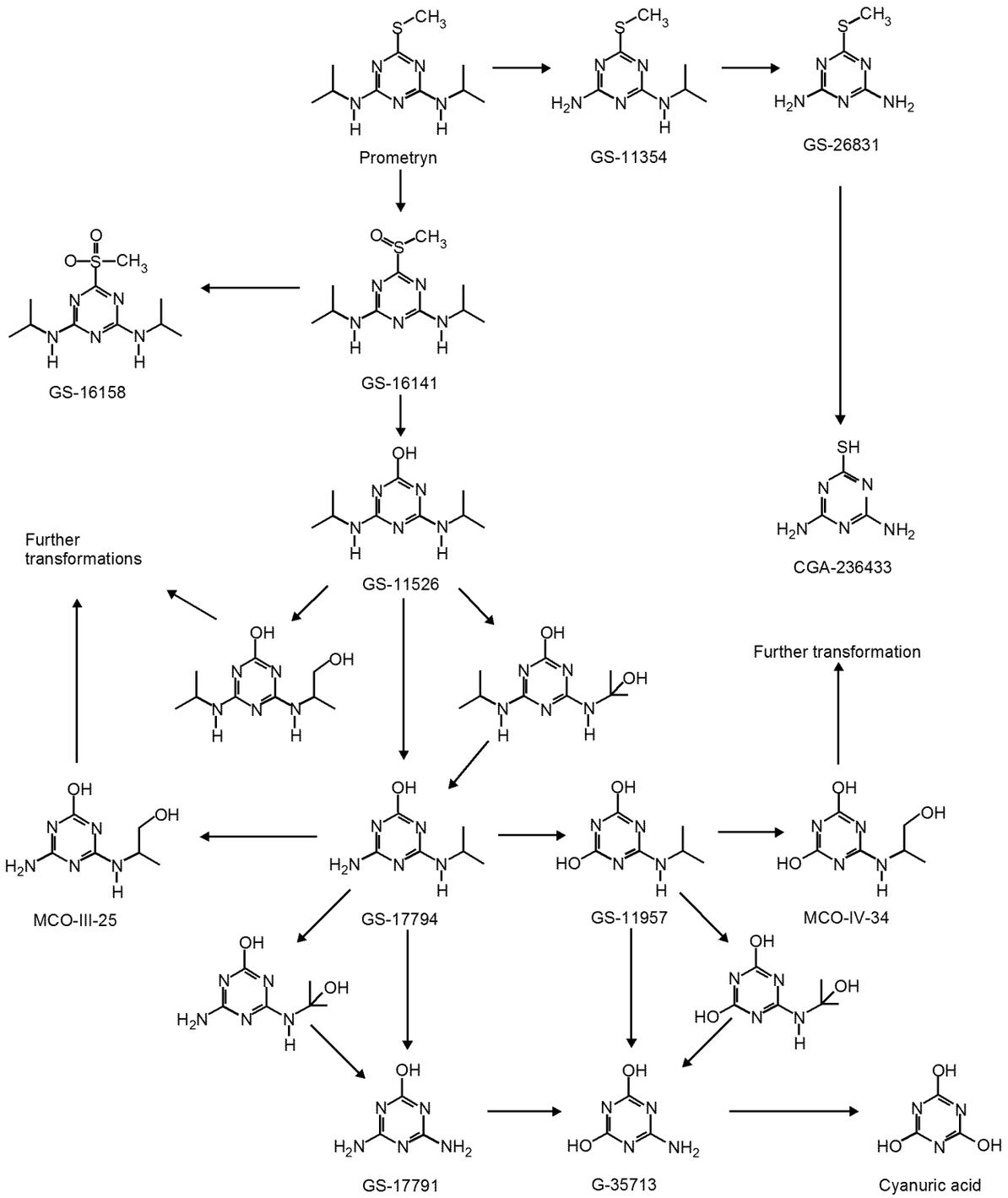

Figure 7.9 The metabolic pathway of prometryn in celery (Sanson, 1992).

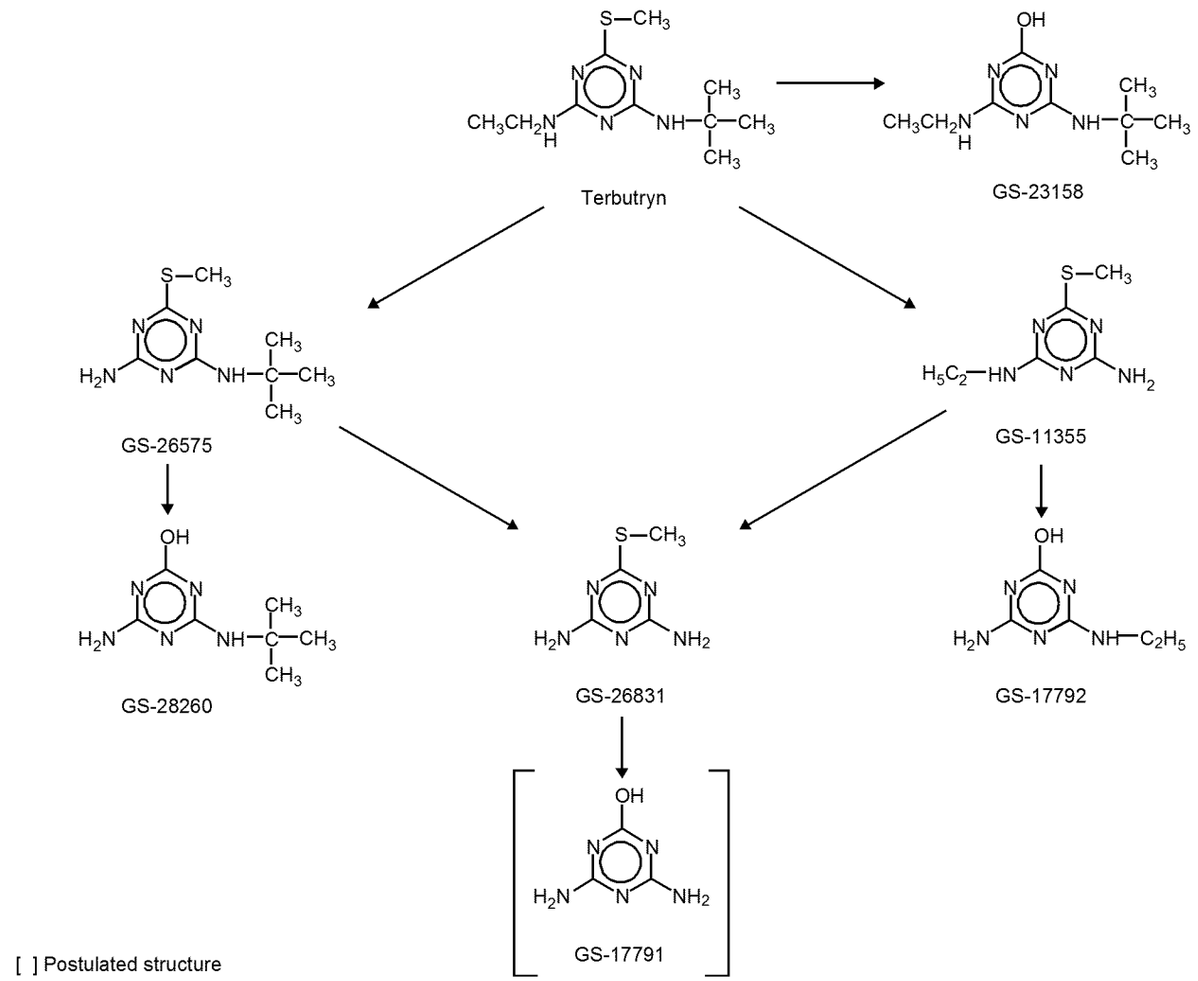

Figure 7.10 The metabolic pathway of terbutryn in wheat (Stockton and Szolics, 1988).

Metabolism of Methoxy-*s*-triazines

Prometon

Prometon is registered for total vegetation control on industrial sites, noncrop areas around the farm, and under asphalt. Although metabolism studies of prometon in plants are not available, the disposition of prometon in rats (Orr *et al.*, 1991) provides some insight into the metabolic processes that might be operative in plants. Male and female rats were dosed orally with the equivalent of 0.5 and 50 mg a.i./kg of ^{14}C-prometon. Most of the dose was excreted in urine for both sexes (77–94%). Analysis of pooled 0 to 24-h urine indicated that approximately 85% of the urine radioactivity was GS-12853 (2,4-diamino-6-methoxy-*s*-triazine). This study shows the relative stability of the methoxy group to demethylation and subsequent conjugation.

Secbumeton

The metabolism of secbumeton was studied in field-grown alfalfa (Cassidy *et al.*, 1969). A small plot of established alfalfa in New York State was sprayed with 1.0 lb a.i./A of ^{14}C-secbumeton following a June cutting. Alfalfa foliage sampled in August and again in September contained 0.23 and 0.15 ppm total residues. These samples contained predominantly aqueous soluble residues (90%). Organic soluble and nonextractable residues together accounted for less than 10% of the residue. The major metabolite accounting for approximately 25% of the residue was GS-12853.

Two other metabolites characterized in the extracts were GS-25433 (2-amino-4-*sec*-butylamino-6-methoxy-*s*-triazine) and GS-31709 (2-amino-4-ethylamino-6-methoxy-*s*-triazine).

Sugarcane (Keezer *et al.*, 1969) was grown in a greenhouse sand culture experiment for 36 days before treatment with 2 mg per container ^{14}C-secbumeton or ^{14}C-GS-12853, a major plant metabolite. Six weeks after treatment the foliage from sugarcane plants was analyzed for metabolite distribution. The GS-12853-treated foliage contained mostly unaltered GS-12853 as the major organic soluble and aqueous soluble residue. At 6 weeks, the secbumeton-treated foliage contained mostly GS-12853, with smaller percentages of GS-37186 (carbinolamine isomer of GS-25433) and GS-31709. The exact site of the oxidation on the side-chain sec-butylamino group could not be determined because of lack of all possible isomeric standards. Dealkylation appears to be the primary metabolic pathway for methoxy-*s*-triazines in plants and animals.

Metabolism of Atypical Triazines

Hexazinone

Hexazinone is an example of an atypical triazine herbicide used for control of woody plants in reforestation areas and for selective weed control in sugarcane, pineapple, and alfalfa. The degradation of hexazinone in the rat, alfalfa, and sugarcane was originally reported by Holt (1981). The mass spectral identification of hexazinone metabolites isolated from rat urine and from sugarcane extracts was reported by Reiser *et al.* (1983). Established sugarcane plants grown in the greenhouse were treated by soil drench with ^{14}C-hexazinone and harvested at plant maturity 6 months later. The total radioactive residues in mature sugarcane were less than 0.1 ppm, and the intact parent was not detected. Three major plant metabolites were identified by gas chromatography/mass spectroscopy (GC/MS) as their trimethylsilyl derivatives. They were metabolites A [3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione], C [3-(4-hydroxycyclohexyl)-6-(methylamino)-1-methyl-,3,5-triazine-2,4(l*H*,3*H*)-trione], and E [3-(4-hydroxycyclohexyl)-1-methyl-1,3,5-triazine-2,4,6(l*H*,3*H*,5*H*)-trione]. The three major rat urine metabolites were A, B [(3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(l*H*,3*H*)-dione)], and C. Minor metabolites in urine were D [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(l*H*,3*H*,5*H*)-trione] and F [3-cyclohexyl-6-amino-1-methyl-1,3,5-triazine(1*H*,3*H*)-dione]. The metabolic pathway for hexazinone in plants and animals is presented in Figure 7.11 and involves the processes of hydroxylation, demethylation, and deamination.

Metribuzin

Metribuzin is a member of the substituted *as*-triazinone group of herbicides. Activity is due to interference with photosystem II electron transport in plant chloroplasts (Dodge, 1983). The metabolism of metribuzin in plants has been the subject of many short-term and long-term studies dating back to the early 1970s.

The short-term treatment studies include hydroponic studies with whole immature plants (Hargroder and Rogers, 1974; Smith and Wilkinson, 1974; Maun and McLoed, 1978; Mangeot *et al.*, 1979; Frear *et al.*, 1981, 1982, 1983, 1985; Abusteit, 1983; Fedtke, 1983, 1986a, 1986b; Fedtke and Schmidt, 1983; Falb and Smith, 1984, 1987; Abusteit *et al.*, 1985; Gawronski *et al.*, 1985, 1986, 1987; Devlin *et al.*, 1987; Smith *et al.*, 1989; Davis *et al.*, 1991), hydroponic studies with excised plant tissues (Schumacher, 1974; Frear *et al.*, 1981, 1982, 1983, 1985; Gawronski *et al.*, 1985; Fedtke, 1986b; Davis *et al.*, 1991), and studies with plant cell cultures (Oswald *et al.*, 1978; Abusteit, 1983; Fedtke and Schmidt, 1983; Davis *et al.*, 1991). Typically, the duration of these studies was measured in terms of hours or days. These experiments were instrumental in delineating the detoxification of metribuzin (Mangeot *et al.*, 1979; Frear *et al.*, 1985; Gawronski *et al.*, 1986), particularly those aspects that lead to tolerant cultivars (Smith and Wilkinson, 1974; Maun and McLoed, 1978; Mangeot *et al.*, 1979; Frear *et al.*, 1982, 1983; Abusteit, 1983; Falb and Smith, 1984, 1987; Abusteit *et al.*, 1985; Gawronski *et al.*, 1985, 1986, 1987; Devlin *et al.*, 1987; Davis *et al.*, 1991). These short-term studies will be reviewed in the context of the observed metabolic pathways.

The long-term treatment studies also include hydroponic studies (Hilton *et al.*, 1974, 1976), as well as preemergence studies (Robinson *et al.*, 1970; Gronberg *et al.*, 1971; Church and Flint, 1973; Morgan, 1974; Lenz *et al.*, 1987) and postemergence studies (Morgan, 1972, 1973; Stanley and Flint, 1974; Maun and McLoed, 1978; Schocken *et al.*, 1987) involving analysis of mature plants. The duration of these studies was usually measured in terms of weeks or months, and their purpose was to determine the nature of the terminal residues in crops in order to set pesticide residue tolerances.

Short-term Metabolism of Metribuzin

The short-term metabolism of metribuzin may involve two major nonconjugative pathways, two major conjugative pathways, and/or production of nonextractable residues.

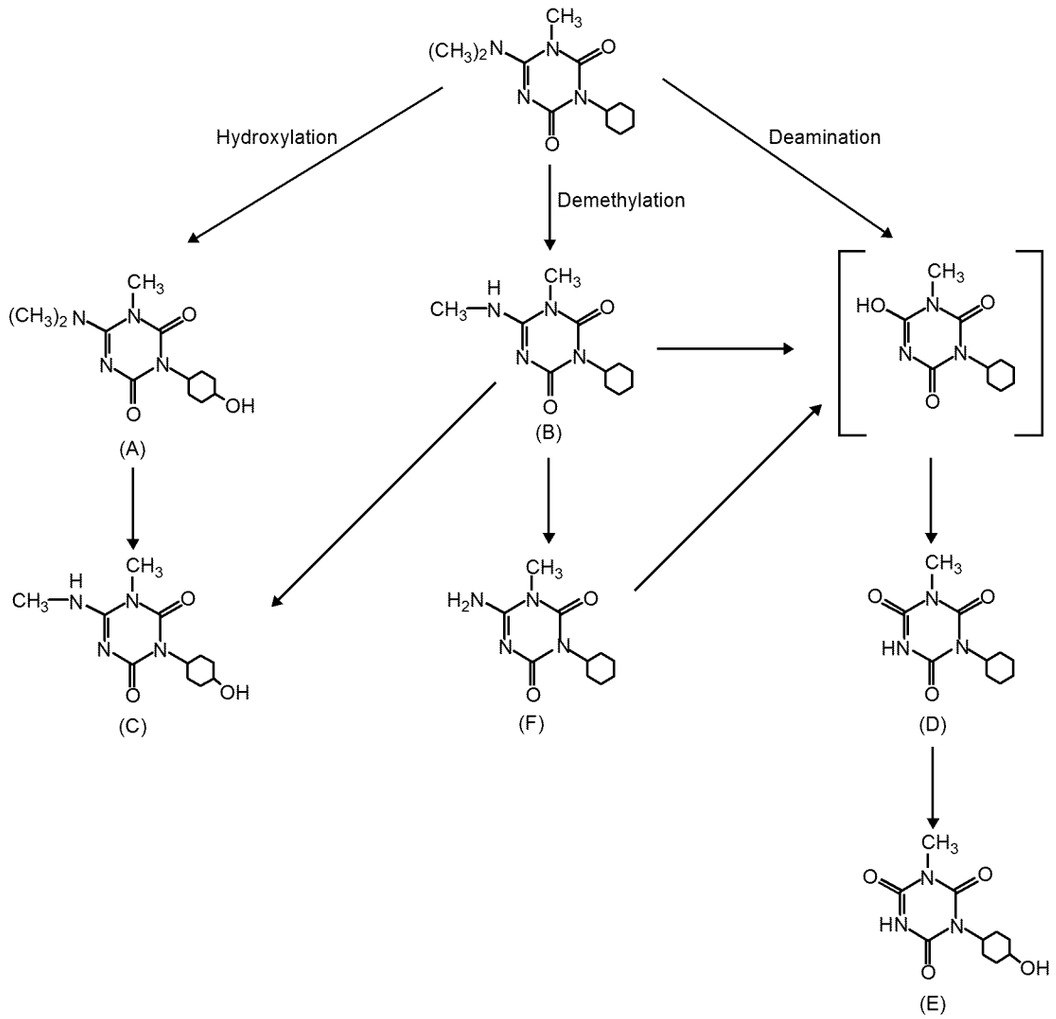

Figure 7.11 Metabolic pathways of hexazinone in rats and sugarcane (redrawn from Reiser *et al.* (1983)).

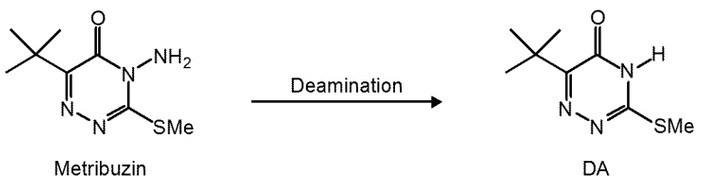

Figure 7.12 The deamination of metribuzin to deaminated metribuzin in various plants.

One nonconjugative pathway is via deamination to give deaminated metribuzin (DA) [6-*tert*-butyl-3-(methylthio)-*as*-triazin-5-(4*H*)-one] as shown in Figure 7.12. This process was documented by research of Schumacher (1974); Mangeot *et al.* (1979); Fedtke (1983, 1986a, 1986b); Fedtke and Schmidt (1983); Falb and Smith (1984); Abusteit *et al.* (1985); Gawronski *et al.* (1986, 1987); and Devlin *et al.* (1987). The amount of deamination in susceptible and nonsusceptible cultivars appeared to be similar (Mangeot *et al.*, 1979; Falb and Smith, 1984; Frear *et al.*, 1985; Devlin *et al.*, 1987; Gawronski *et al.*, 1987), so deamination was not the principal origin of metribuzin tolerance. However, in some reports deamination was considered to be a minor cause of tolerance (Abusteit, 1983; Abusteit *et al.*, 1985; Gawronski *et al.*, 1985, 1986; Fedtke, 1986a, 1986b).

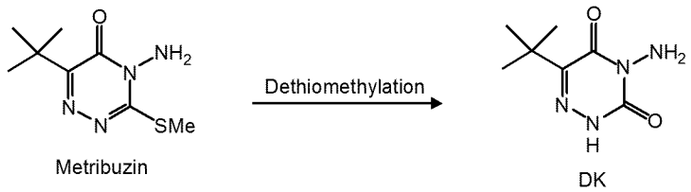

Figure 7.13 The dethiomethylation of metribuzin to diketo metribuzin in various plants.

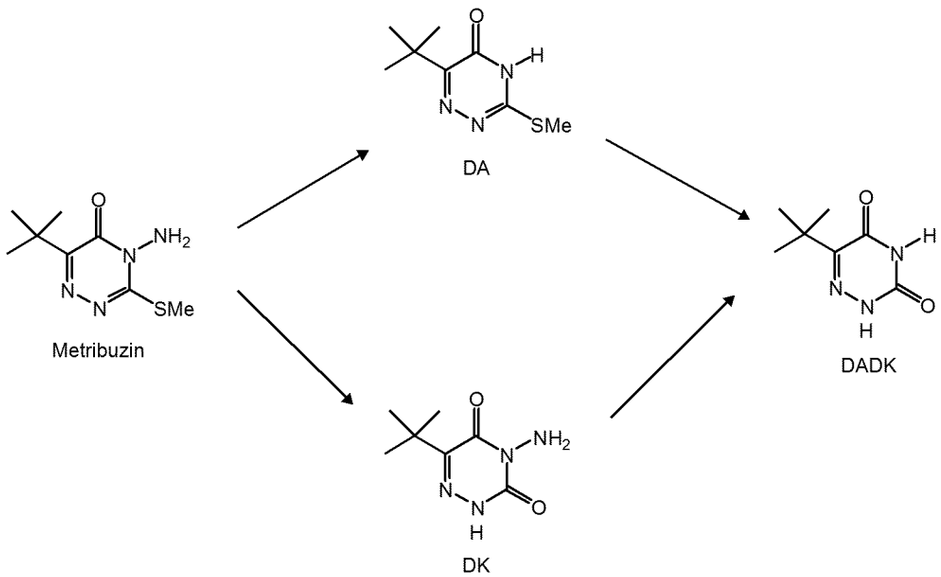

Figure 7.14 Metabolism of metribuzin to deaminated diketo metribuzin in various plants.

The second nonconjugative pathway is dethiomethylation, which results in diketo metribuzin (DK) [4-amino-6-(1,1-dimethylethyl)-1,2,4-(2*H*,4*H*)-dione], as shown in Figure 7.13. This process was observed by Schumacher (1974); Mangeot *et al.* (1979); Abusteit *et al.* (1985); Gawronski *et al.* (1986, 1987); and Devlin *et al.* (1987). The dethiomethylation may proceed through an oxidative pathway via a sulfoxide intermediate, as reported in mice and rats (Saeman and Casida, 1984; Saeman *et al.*, 1985).

In combination, these two processes yield deaminated diketo metribuzin (DADK) [6-(1,1-dimethylethyl)-1,2,4-triazin-3,5-(2*H*,4*H*)-dione], as shown in Figure 7.14. Formation of DADK was documented by Hargroder and Rogers (1974); Schumacher (1974); Smith and Wilkinson (1974); Mangeot *et al.* (1979); Abusteit *et al.* (1985); Gawronski *et al.* (1985, 1986, 1987); and Devlin *et al.* (1987). DADK was shown to be a short-term metabolic sink by Frear *et al.* (1985). When excised soybean leaves were treated hydroponically with ^{14}C-DADK, 74% of the radiocarbon was extracted unchanged with organic solvents at 48 h after the treatment.

Conjugative processes are the primary determinant of tolerance to metribuzin in plants. The most studied conjugative pathway (Frear *et al.*, 1982, 1983; Davis *et al.*, 1991) is the *N*-glucosylation of metribuzin, concurrent with or followed by *O*-malonation of the glucose, as shown in Figure 7.15. Frear *et al.* (1983, 1985) showed that this pathway was operative in tomato and, to a lesser extent, in soybean. The major *N*-glucoside in both species was the *O*-malonated *N*-glucoside. This conjugate lost the *O*-malonyl group upon purification. The resultant *N*-glucoside was identified by ^{1}H-nuclear magnetic resonance (NMR) and GC/MS as the tetraacetate derivative (Frear *et al.*, 1982, 1983). In addition, Davis *et al.* (1991) isolated the *N*-glucotransferase enzyme from tomato tissue culture. Stephenson *et al.* (1976) and Smith *et al.* (1989) also reported the formation of *N*-glucosides in tomatoes. Apparent *N*-glucosides of metribuzin have been found by Schumacher (1974) and Smith and Wilkinson (1974) in soybean and by Gawronski *et al.* (1986, 1987) in potato and barley.

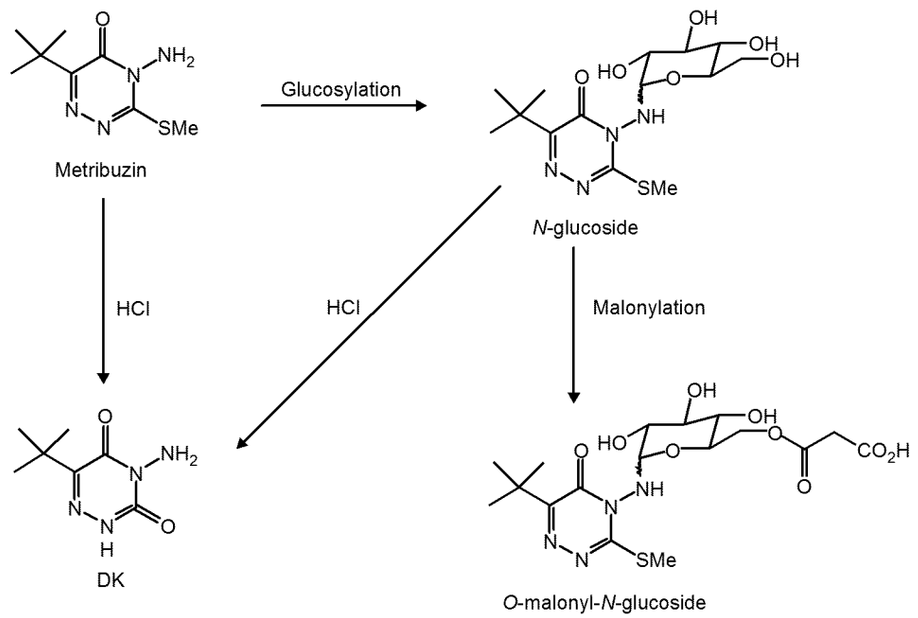

Figure 7.15 The *N*-glucosylation of metribuzin in tomato.

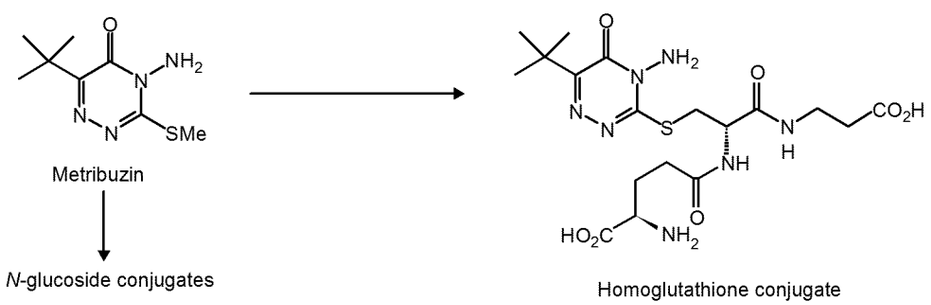

Figure 7.16 Homoglutathione conjugation of metribuzin in soybean.

Frear *et al.* (1982, 1983) showed that the *N*-glucoside was stable to *β*-glucosidase. However, on heating the *N*-glucoside with 1 N HCl at 80ºC for 3 h,Frear recovered DK (Frear *et al.*, 1981, 1982, 1983). Metribuzin was converted to DK under the same acidic conditions (Saeman, 1984; Saeman and Casida, 1984; Saeman *et al.*, 1985). The acid liability of the *N*-glucosides of metribuzin has also been documented by Smith and Wilkinson (1974), Stephenson *et al.* (1976), Gawronski *et al.* (1986), and Smith *et al.* (1989). Stephenson *et al.* (1976) also showed that the *N*-glucoside was hydrolyzed to metribuzin upon refluxing with water for 1 h.

The second major conjugative pathway involves conjugation of metribuzin with homoglutathione, as shown in Figure 7.16. This pathway was documented by Frear *et al.* (1985) as the principal detoxification process in soybean, with *N*-glucosylation being of secondary importance. A possible glutathione adduct was seen as a minor metabolite. Like dethiomethylation, homoglutathione formation proceeds via an oxidative intermediate. In a rat liver microsome model, nicotinamide adenine dinucleotide phosphate (NAPDH) dependent oxidation of metribuzin to an intermediate sulfoxide was followed by conjugation with either glutathione (Saeman, 1984; Frear *et al.*, 1985; Saeman *et al.*, 1985) or *N*-acetylcysteine (Saeman, 1984; Saeman and Casida, 1984). Similar peptidic conjugates in potatoes (Gawronski *et al.*, 1986) and in barley (Gawronski *et al.*, 1987) were observed by comparing relative TLC mobilities of the water-soluble metabolites. Mangeot *et al.* (1979) and Falb and Smith (1984) also have documented peptidic conjugates in soybean.

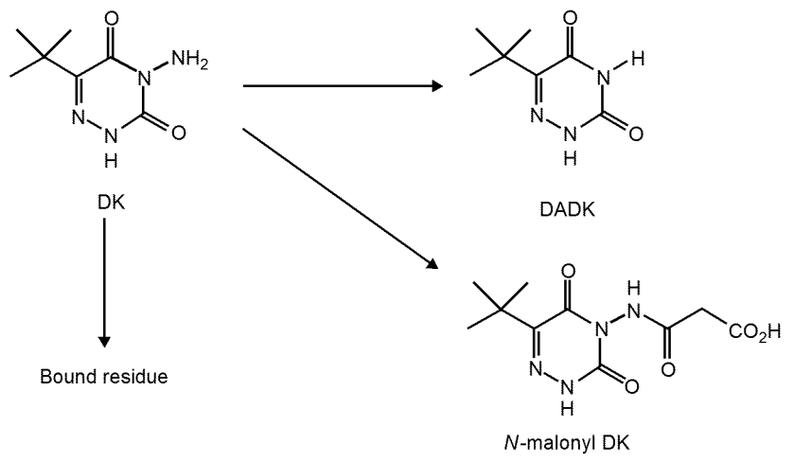

Figure 7.17 The short-term metabolism of DK in soybean.

Mangeot *et al.* (1979), Falb and Smith (1984), Frear *et al.* (1985), and Gawronski *et al.* (1987), have all noted that these peptidic conjugates are acid labile. Saeman and Casida (1984) have shown that the chemically similar *N*-acetyl cysteine conjugates are also acid labile.

The metribuzin metabolite DK is deaminated, conjugated, and rapidly bound to insoluble fractions in soybean, as shown in Figure 7.17. Frear *et al.* (1985) hydroponically treated excised soybean leaves with ^{14}C-DK for 48 h. Only 16% of the total radioactive residues were organosoluble with a 3:1 ratio of DK and DADK. The water-soluble fraction was mainly *N*-malonyl DK, amounting to 17% of the residue. The remainder of the residues was nonextractable. *N*-malonated DK was detected as a minor metabolite in this study.

^{14}C-metribuzin forms nonextractable residues in plants in as early as 24 h after treatment (Schumacher, 1974; Frear *et al.*, 1985; Gawronski *et al.*, 1985, 1986, 1987). In tolerant plants, where the conjugative pathways are very important, radioactivity appears to be more confined to the vascular system of the plant (Hargroder and Rogers, 1974; Abusteit, 1983; Falb and Smith, 1984; Abusteit *et al.*, 1985; Gawronski *et al.*, 1985; Devlin *et al.*, 1987). In susceptible plants, radioactivity in the leaves is more prevalent (Hargroder and Rogers, 1974).

DK forms nonextractable residues more rapidly than metribuzin (Frear *et al.*, 1985). In the previously mentioned soybean hydroponic study on DK (Frear *et al.*, 1985), at 48 h post-treatment, 60% of the radiocarbon remained nonextractable with organic solvents. Only a third of the nonextractable residues could be solubilized with refluxing HCl to yield DK. By comparison, in ^{14}C metribuzin and ^{14}C DADK studies (Frear *et al.*, 1985), only 26% and | 1%, respectively, of the radiocarbon was insoluble in organic solvents at 48 h post-treatment.

In summary, nonconjugative metabolic processes deaminate and dethiomethylate metribuzin to give DA, DK, and DADK. These pathways are minor in most plants and are nonexistent in tomato (Stephenson *et al.*, 1976; Frear *et al.*, 1981, 1982, 1983; Smith *et al.*, 1989; Davis *et al.*, 1991). Far more rapid detoxification occurs via conjugative pathways. In tomato (Frear *et al.*, 1981, 1982, 1983; Davis *et al.*, 1991), *N*-glucosylation of metribuzin occurs. In soybean (Mangeot *et al.*, 1979; Frear *et al.*, 1985), potato (Gawronski *et al.*, 1985, 1986), and barley (Gawronski *et al.*, 1987), homoglutathione conjugation is the predominant detoxification pathway, with *N*-glucosylation being of secondary importance. The glucosidic and peptidic conjugates are acid labile (Schumacher, 1974; Mangeot *et al.*, 1979; Frear *et al.*, 1982, 1983, 1985; Saeman, 1984; Saeman and Casida, 1984). The *N*-glucosides are enzyme stable (Frear *et al.*, 1982, 1983). Finally, formation of nonextractable residues in plants is seen even in short-term studies. A short-term pathway for metribuzin plant metabolism is shown in Figure 7.18.

Long-term Metabolism of Metribuzin

Short-term studies utilized immature plants and plant parts, not the mature raw agricultural commodities consumed by people. In addition, the extent and nature of metabolism has been documented to progress with time (Gronberg *et al.*, 1971; Hilton *et al.*, 1974; Stanley and Flint, 1974; Schocken *et al.*, 1987). Thus, many metabolism studies have now been conducted to address the nature of residues in these foods.

The long-term metabolism of metribuzin is very complex. Given the extended length of these experiments, identifying the individual metabolic pathways has been very challenging. Also, some degradation of metribuzin occurs in the treated soil before plant uptake occurs (Schumacher, 1974; Prestel *et al.*, 1976).

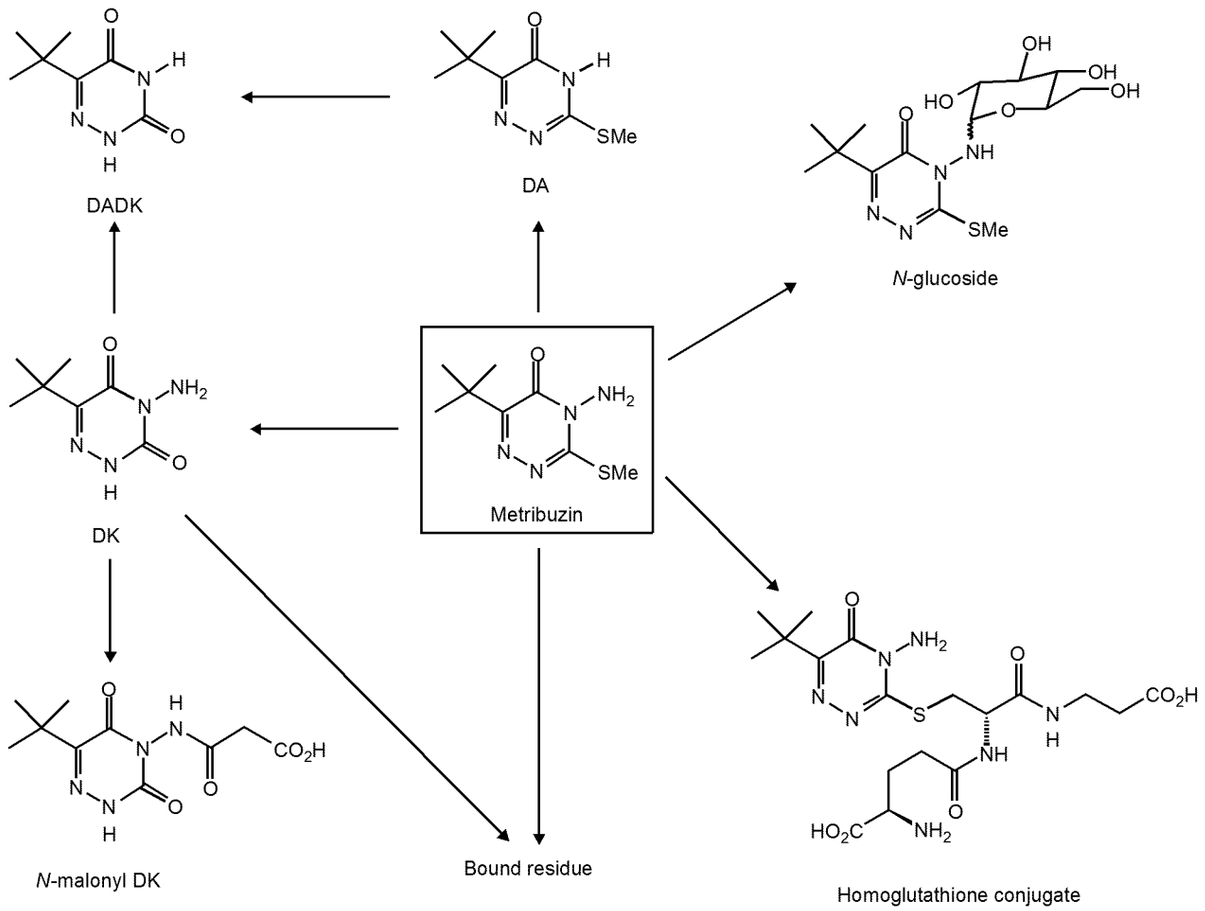

Figure 7.18 The short-term metabolism of metribuzin in soybean.

In long-term plant metabolism studies (Gronberg *et al.*, 1971; Morgan, 1972, 1973, 1974; Church and Flint, 1973; Hilton *et al.*, 1974, 1976; Stanley and Flint, 1974; Lenz *et al.*, 1987; Schocken *et al.*, 1987) very little free metribuzin, DA, or DK were detected (0.1–15%). The majority of the organosoluble material was DADK (2–35%).

Morgan and Lenz (1992) showed that the major water-soluble metabolite in wheat grain was 2-amino-3,3-dimethyl butanoic acid (Figure 7.19). This material accounted for 11% of the total radioactive residue. Degradation of the heterocyclic ring to form the semicarbazone of pyruvic acid was reported in a potato rotational crop study by Prestel *et al.* (1976). However, Scholz (1982) was unable to repeat this observation.

In some studies (Stanley and Flint, 1974; Lenz *et al.*, 1987; Schocken *et al.*, 1987; Morgan and Lenz, 1992), 3-amino-DA compounds hydroxylated on the *tert*-butyl group and ring-cleaved products were identified (Figure 7.19).

In two studies (Lenz *et al.*, 1987; Schocken *et al.*, 1987) the water-soluble metabolites were separated by HPLC. Schocken *et al.* (1987) separated 21 water-soluble metabolites from wheat straw, none of which amounted to ┘ 1.5% of the total residue. Lenz *et al.* (1987) separated 20 water-soluble metabolites from soybean forage and 13 from soybean seed. Two of the five major forage metabolites were shown to be DADK *O*-glucosides by enzyme hydrolysis with α-glucosidase (DADK was detected). The other three major metabolites were resistant to hydrolysis with HCl. The 15 minor forage metabolites were also resistant to acid hydrolysis.

Other studies (Robinson *et al.*, 1970; Gronberg *et al.*, 1971; Morgan, 1972, 1973; Church and Flint, 1973; Stanley and Flint, 1974) also examined the water-soluble fraction from ^{14}C-metribuzin-treated plants. Size-exclusion chromatography (SEC) of the water-soluble fractions indicated molecular weights for the radioactive residues from 300 to 800 atomic mass units (AMU). Stanley and Flint (1974) showed that many of the SEC fractions from metribuzin-treated alfalfa had a peptidic component. This was in accord with the short-term studies of Falb and Smith (1987), who used SEC to show many high molecular weight water-soluble metabolites of both peptidic and glucosidic nature.

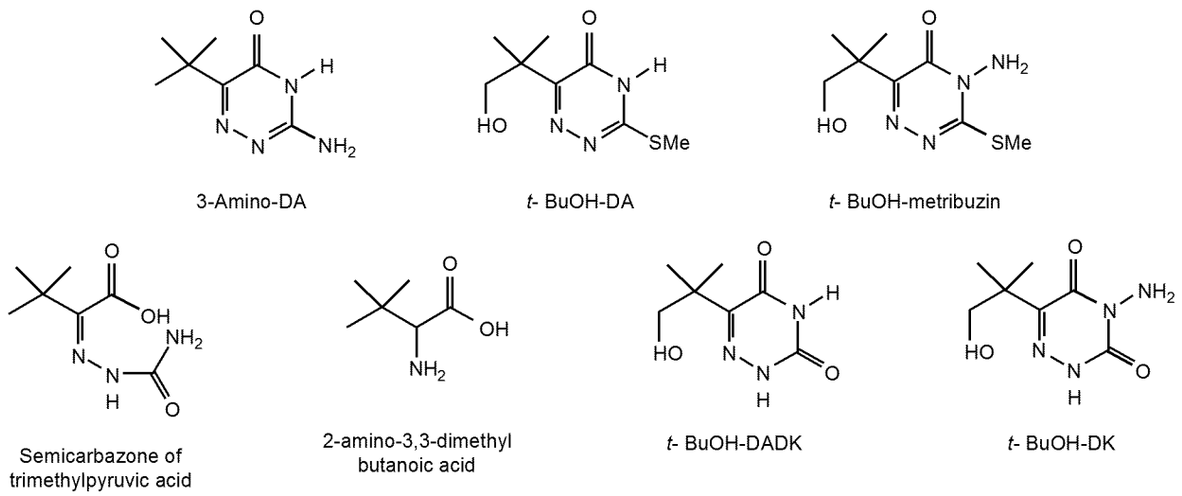

Figure 7.19 Additional metribuzin metabolites found in long-term plant studies.

Gronberg *et al.* (1971) studied the soybean metabolism of metribuzin, utilizing both ^{14}C and ^{3}H. The ^{14}C-label was in the 5-position of the heterocyclic ring, and the ^{3}H-label was in the thiomethyl group. The water-soluble metabolites were fractionated by SEC, and the ^{3}H- and ^{14}C-labels were detected in separate fractions. This indicated that the water-soluble metabolites no longer had the thiomethyl group associated with the heterocyclic ring. Thus, large quantities of water-soluble metabolites were not conjugated to metribuzin, *per se*.

In earlier studies, efforts were made to hydrolyze the water-soluble metabolites. Acid hydrolysis or autoclaving of the water-soluble fraction sometimes yielded small quantities of DADK, indicative of cleavage of DA or DADK conjugates (Robinson *et al.*, 1970; Gronberg *et al.*, 1971; Stanley and Flint, 1974). Only in potato were large quantities of organosoluble metabolites released by hydrolysis. Church and Flint (1973) found that about 80% of the potato residues were water soluble. Only 37% of the total residues were made organosoluble by autoclaving at 120°C without any added acid. Fourteen percent were released as metribuzin, and 16% were released as a mixture of DA, DK, and DADK. The release of metribuzin residues is in accord with the report of Stephenson *et al.* (1976) on the hydrolysis of *N*-glucosides by refluxing with water. Enzyme hydrolysis was largely unsuccessful in release of organosoluble residues from the water-soluble fraction of soybean, alfalfa, and potato (Robinson *et al.*, 1970; Gronberg *et al.*, 1971; Church and Flint, 1973; Stanley and Flint, 1974).

Conclusion

The metabolism of triazine herbicides in plants has been diligently studied since their introduction. Much of what is currently known about the metabolic pathways was obtained only after newer and more advanced methods of chromatography and spectral analysis were discovered. The study of triazine herbicides has resulted in improved methodologies and understanding of plant proteins, biochemistry, and metabolic pathways. These pathways will serve as a reference point for future researchers in their quest for a complete understanding of plant metabolic chemistry.

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Chapter 8

The Mode of Action of Triazine Herbicides in Plants

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Summary

Triazines inhibit photosynthesis in all organisms with oxygen-evolving photosystems. They block photosynthetic electron transport by displacing plastoquinone from a specific-binding site on the D1 protein subunit of photosystem II (PS II). This mode of action is shared with several structurally different groups of other herbicides. The elucidation of the mechanism of the inhibitory action is followed in this review.

The identification of the target in PS II attacked by triazine and other herbicides was actually a part of the general photosynthesis research establishing the principle features of photosynthetic electron transport as of the mid-1950s. This close interaction of photosynthesis and herbicide research in university and industry laboratories remains today. An appreciable part of the biochemical approach was and is to examine the response of the photosynthetic system to inhibitors, in particular those interfering with the role of plastoquinone. The resulting developments over the past 50 years have provided many astounding and unexpected insights in the mechanism of photosynthesis and herbicide inhibition.

After it was established that triazines are displacing plastoquinone from its functional site in the chloroplast membrane, a major breakthrough in 1981 was the identification of the triazine target as a hydrophobic membrane protein of 32 kDa size by photoaffinity labeling with azidoatrazine. This knowledge of the action of triazines on plastoquinone reductions also led to the first identification of a plastoquinone-binding protein (i.e., the 32-kDa protein is a herbicide and plastoquinone (Q_B) binding protein). The trypsin sensitivity of both herbicide binding and of the 32 kDa protein showed an orientation of the protein accessible from the matrix side of the thylakoid membrane. It led also – quite unexpectedly – to the association of the herbicide and Q_B-binding protein with D1, a protein of PS II that turned over very rapidly. This in turn opened the molecular biology of the triazine mode of action. Because it was known that D1 protein was encoded by the *psbA* gene, this gene was localized in the chloroplast genome and sequenced. The *psbA* gene from already discovered triazine-resistant plants could now be sequenced and the amino acid substitution responsible for the change in inhibitor sensitivity identified. With a change of serine 264 to glycine, researchers zeroed in on that part of the D1 protein that forms the specific-binding niche for the herbicides. With more herbicide-resistant mutants available from algae systems, a three-dimensional folding of that amino acid sequence could be attempted. This was greatly aided by the homology of PS II to the reaction center of purple bacteria that was crystallized, and an atomic structure became available in 1985. From this research came the startling conclusion that the herbicide-binding protein is one of the reaction-center proteins of PS II that not only carries plastoquinones, but also six chlorophylls and two pheophytines as well as an Fe (iron), and provides part of the anchor for the Mn (manganese) cluster for oxygen evolution. Many more site-selected and site-directed amino acid substitutions in the D1 protein are now constructed that lead to herbicide tolerance, but some also lead to supersensitivity. This research could allow for the generation of several types of triazine-resistant crops, although the development of these crops requires stable transformations of the chloroplast genome.

The advances from the physiology to the biochemistry, biophysics, molecular biology, and molecular genetics kept this field of photosynthesis herbicides always at the top of scientific progress, and it remains a prime example of the complete and comprehensive clarification of the mode of action of a pesticide.

The triazine herbicide-binding protein has been studied extensively with regard to its biochemistry, molecular biology and genetics, amino acid and DNA sequences, and three-dimensional folding through the thylakoid membrane. Several site-selected and site-directed amino acid substitutions in the D1 protein in triazine-tolerant mutants of algaes and higher plants have been described. The orientation of the triazines and the specific amino acids involved with

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their binding in the topology of the membrane protein can be modeled. This review follows the early steps in the elucidation of the mode of action of triazine and related herbicides and in the identification of the target protein.

Identification of the Target Protein

The principle mode of action of triazine herbicides in photosynthesis was first recognized in the late 1950s (Exer, 1958; Moreland *et al.*, 1959). This mode of action appeared to be similar to that of the then just-described urea herbicides (Wessels and van der Veen, 1956). The inhibitory site for both groups of herbicides was shown to be associated with photosynthesis. But how and why the light-dependent oxygen evolution in the so-called Hill reaction was inhibited could not, at that time, be specified. Indeed, the nature of the target in the thylakoid membrane of the chloroplast remained unclear for many years. Progress depended on advances in understanding photosynthesis. At that time, in the 1960s, the components and reactions in the photosynthetic electron flow system were slowly being discovered. But the study of the triazine and urea inhibition of photosynthesis turned out to be a very useful experimental approach to dissecting the reaction sequence of photosynthesis (Moreland, 1967). The interaction of photosynthesis and herbicide research continues to the present time.

When the concept of two light reactions in photosynthesis was experimentally verified in 1961, the triazines were shown to inhibit the PS II that drives oxygen evolution. The triazines have no effect on PS I, which raises electrons up to the very electronegative potential for NADP^{ffi} reduction. The effect of triazines and diuron (an urea derivative) on the fluorescence of PS II (Duysens and Sweers, 1963) proved to be a powerful noninvasive method that could be used to follow the photochemistry of the primary reactions of this photosystem, as well as the biochemistry of isolated photosynthetic systems. The results showed that the inhibition of oxygen evolution by triazines was not on the water-splitting system *per se*, but on the acceptor side of PS II. The electrons from water splitting arrive on the acceptor side of PS II after going through the light-driven reaction center of the photosystem. The physical separation of PS I and PS II by mild detergent treatment of the membrane in the mid-1960s proved these early notions to be correct. With plastoquinone identified as the electron acceptor of PS II, it was now possible to propose (Van Rensen, 1971) and then show (Velthuys, 1981) that the triazine herbicides removed one of the quinones from this acceptor site (Oettmeier and Soll, 1983; Vermass and Arntzen, 1984), which prevented electron flow from PS II to PS I.

However, the chemistry of the target still remained unclear. In particular, was it in a lipidoidal or in a proteinaceous phase? Good (1961) envisioned a relationship between chemical groups in the photosynthesis-inhibiting herbicides with a peptide bond (see boxes in Figure 8.1). Renger (1976) proposed a proteinous shield above the plastoquinone functional site in PS II. The removal of triazine inhibition of photosynthesis by treating the thylakoid membrane by the protease trypsin (Regitz and Ohad, 1976; Renger, 1976; Trebst, 1979) strongly supported the proteinaceous notion for the triazine target. Slowly the mode of action of triazines was characterized as the displacement by the herbicide of the plastoquinone, which was the one in the so-called Q_B site in PS II. Two plastoquinone-binding sites could be distinguished (see later) from an herbicide-binding protein, also called the Q_B-binding protein (Kyle, 1985).

Still the identity, nature, and size of the herbicide-binding protein remained a puzzle. This putative protein was likely very hydrophobic. A methodology for isolating such membrane proteins was not at that time available. Even more difficult to comprehend was the orientation of such hydrophobic proteins in a membrane that had been a matter of discussion for many decades. The first X-ray structure of a membrane protein complex in 1985 suddenly solved this orientation question, as will be discussed below.

Two developments rapidly advanced the concept and identification of the herbicide-binding protein in PS II in the 1970s and early 1980s: (a) the discovery of triazine resistance, and (b) the technique of photoaffinity labeling. The first triazine-resistant plant (common groundsel) was reported in 1970 (Ryan, 1970). The resistance was subsequently shown (Radosevich and DeVilliers, 1976; Pfister *et al.*, 1979) to be due to a change in the primary target and not in uptake, translocation, or degradation, which could also bring about resistance. It was assumed that resistance was likely due to a change in the postulated herbicide-binding protein. This resistance was maternally inherited, the first indication that the herbicide-binding protein was encoded by the chloroplast genome (Pfister and Arntzen, 1979). Secondly, in 1980 radioactive photoaffinity-labeled azidotriazine (Figure 8.1) was provided by Gardner (1981). This azidotriazine upon UV radiation would generate a nitrene that would immediately react covalently with components in its immediate neighborhood. An azidotriazine bound to the thylakoid membrane would form accordingly a covalently linked radioactive triazine derivative bound to the first specific target. The just-developed polyacrylamide gel electrophoresis allowed the separation of the many hydrophobic proteins of a thylakoid membrane after detergent solubilization. The radioactive labeling by azidotriazine of the thylakoid membrane (Pfister *et al.*, 1981) showed just one labeled band on the gel at about 32 kDa. Furthermore, the labeling of this band did not light up in a triazine-resistant plant. This indicated that the binding site for triazines in that protein seemed to be modified, thus no longer allowing binding. By this method, the herbicide-binding protein (or Q_B) was shown to have a molecular weight of

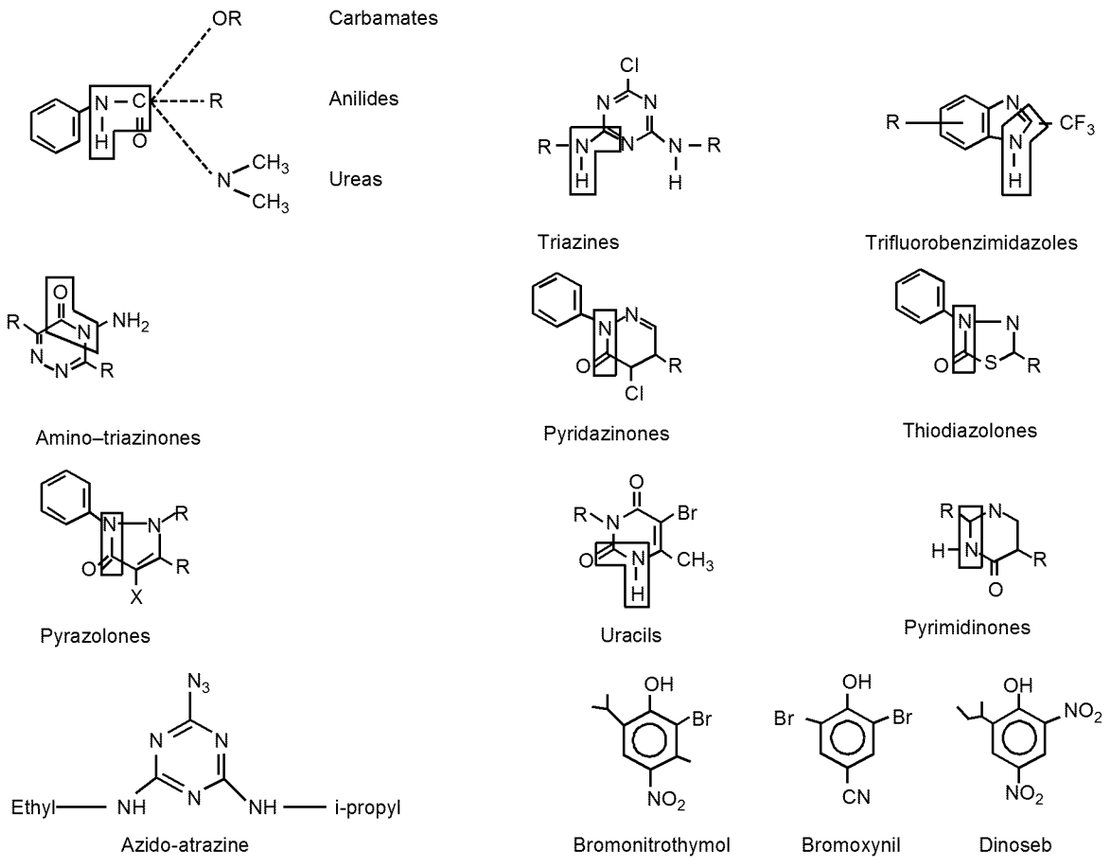

Figure 8.1 Examples of herbicides and herbicide classes containing some compounds that inhibit photosynthesis with a mode of action like that of the triazines. Structures within the boxes are essential elements common to all classical inhibitors of photosynthesis II at the Q_B site. Some phenolic compounds also inhibit in a similar way.

32 kDa. The protein could now easily be followed by gel electrophoresis of thylakoid membrane proteins for further analysis. With this breakthrough, the principal identification and isolation of the triazine target protein was completed.

Further progress, now in the molecular biology and in the protein chemistry of the photosynthetic membrane, provided additional exciting insights in both photosynthesis and herbicide research. Again using the triazine herbicides as tools was a major experimental approach.

When the expression of genes encoded in the chloroplast genome was studied by pulse labeling with radioactive amino acids, one messenger RNA and one protein were labeled very rapidly in the light, but not in the dark (Ellis, 1981; Mattoo *et al.*, 1981). In pulse chase experiments, fast labeling the protein with a radioactive precursor (often ^{14}C methionine) showed a rapid incorporation. If followed by a 'chase' with the ^{12}C precursor, the radioactivity disappeared as quickly. A 'rapidly turning over protein' was discovered, with a molecular weight of about 32 kDa. There was no suspicion at the beginning that this protein had anything to do with herbicides. The likely identification with the herbicide-binding protein came slowly, and perhaps only when it was shown that both proteins shared the same trypsin sensitivity (Mattoo *et al.*, 1981). The RNA messenger for the rapidly turning over protein that was also called the D1 protein (diffuse band No. 1 on the gel) was available before the protein had been purified. 'Fishing' for the corresponding DNA was successful (Zurawski *et al.*, 1982). The identities of the rapidly turning over protein, of the D1 protein, of the Q_B-binding protein, and of the herbicide-binding protein now were established, and the gene for the herbicide-binding protein had been discovered. Because it encodes for a component of PS II, it was called the *psbA* gene, the first gene to be identified for a photosynthetic membrane protein. Of course from the nucleotide sequence, the amino acid sequence could be deduced. The target protein for triazine herbicides could now be described in its primary sequence of 345 amino acids. The next step was to identify those amino acids in the sequence that interact with the triazines.

The Compounds and Quantitative Structure–Function Relationships

Following the discovery of the herbicidal properties of triazines, numerous triazine derivatives were synthesized and tested for herbicidal activity. Many of these were checked for inhibiting photosynthesis, as summarized by Ebert and Dumford (1976). Measurement of a photosynthetic reaction in isolated thylakoid membranes from leaves or unicellar algae provides a convenient, reliable, and precise system to evaluate the inhibitory potency of a triazine herbicide at the primary target, undisturbed by the complex physiology of plants (Fedtke, 1982). The potency is expressed as a pI_{50} value (i.e., the negative logarithm of the concentration that inhibits PS II by 50%). The data obtained allow quantitative structure activity relationships (QSAR) in which the biological property (i.e., inhibition) is correlated to physicochemical parameters of the compounds. The so-called Hansch equation (Hansch and Fujita, 1964) is often used to evaluate chemical substituents for further increase in inhibitory potency after the basic essential chemical element has been recognized (see below for triazines). As previously mentioned, the triazines were shown to have the same mode of action as urea derivatives (e.g., diuron). Many more such compounds were discovered with the same mode of action in photosynthesis, and many were also developed as commercial herbicides.

Examples for compounds are given in Figure 8.1, and the regression analysis equation is provided below for the QSAR of triazine derivatives in photosynthesis (Draber, 1992). The inhibitory potency expressed as a pI_{50} value is equal to a lipophilicity parameter ffl (log of the partition coefficient P), an electronic substitution parameter ┘ (the Hammett constant) and to a lesser degree to a steric component E_S (the Taft constant).

$$pI_{50} \mid 1.070 \text{ ffi } 12.39 \text{ ffl} - 7.94 \text{ ffl}^2 - 3.01 \text{ ┘ ffi } E_S$$

An equation attempting to describe many PS II herbicides is given by Kakkis *et al.* (1984). For comparison, the structures of a few other compounds that have the same mode of action as triazines are given in Figure 8.1. They show that there are essential elements common to many different chemical compounds that have the same mode of action (Trebst and Draber, 1986; Oettmeier, 1992).

Photosynthesis in all photosynthetic organisms is blocked by triazines, as well as by other PS II herbicides, when isolated thylakoid systems are tested. However, in intact plants, they express either different inhibitory potency or no inhibition. This shows that the specificity of these photosynthesis herbicides to certain weeds is not related to a difference in the chemistry of their primary target, but rather is attributed to degradative mechanisms, translocation, and translocation mechanisms.

The phenolic derivatives indicated in Figure 8.1 are also bound to the same binding niche on PS II as the triazines (Oettmeier, 1992). However, they have a somewhat different inhibition pattern than the 'classical' family of PS II herbicides (e.g., triazines and ureas) and, therefore, were regarded as a separate family with a somewhat different mode of action (Van Rensen *et al.*, 1978; Trebst and Draber, 1986). It is now clear that they just orient somewhat differently in the same binding niche, as discussed below. Although the phenolics are photosynthesis inhibitors, dinoseb and the halogenated benzonitriles also inhibit respiration.

Compounds with the same mode of action interact with the same binding site on the protein. Triazines and ureas, as well as the other compounds listed in Figure 8.1, displace plastoquinone Q_B. Therefore, they also displace each other from the target site in PS II, and their inhibitory potency can be evaluated by the procedure introduced by Tischer and Strotmann (1977). This is experimentally followed with a radioactive derivative in which a ^{14}C labeled triazine is bound to the target. The radioactivity will be diluted out of this site by an unlabeled compound of similar potency and mode of action. This method does not require measuring photosynthetic activity, but does require a structurally and functionally intact PS II because binding efficiency is easily lost by improper handling of the membrane.

The Three-Dimensional Orientation of the Triazines in the Herbicide-Binding Protein of PS II

As discussed, the target of the triazine herbicides has been identified as a subunit protein of PS II, called the D1 protein, as well as an herbicide-binding protein, Q_B-binding protein, or rapidly turning over protein. PS II is an integral membrane complex of the thylakoid membrane of the chloroplast. In the light, water is oxidized to oxygen, and a plastoquinone (Q_B) is reduced. The complex consists of at least 24 protein subunits, most of which are hydrophobic, that are integral and span the membrane. A few subunits are hydrophilic and are attached peripherally to the lumen side of the complex. The total size of the PS II complex is about 300 000 kDa. The complex contains about 250 chlorophylls, two pheophytins, several carotenoids, two plastoquinones, one iron, four manganese, and one cytochrome b559. The reaction center of PS II that holds the special P_{680} chlorophylls and catalyzes the light-driven charge separation is much smaller and consists of just two very hydrophobic integral protein subunits. All the other subunits are part of the antenna system and the oxygen-evolving system. These two reaction-center polypeptides – the D1 and the D2 protein of 33 kDa – bind six chlorophylls a, two pheophytins, two •-carotenes, two plastoquinones, and one iron. They have a peripheral-binding site for the manganese cluster. The two reaction-center polypeptides catalyze all of the primary

photochemical reactions of this photosystem. These primary reactions are the charge separation of the reaction center, in which an excited P_{680} chlorophyll gets oxidized to P_{680}^{ffi} and one pheophytin reduced in a few picoseconds, followed by the reduction of Q_A by the pheophytin. Once this is completed, the charge separation is stabilized, as P_{680}^{ffi} and Q_A are about 30 Å apart, the P_{680} being oriented toward the lumen side and Q_A on the matrix side. The P_{680}^{ffi} is rereduced by the manganese of the oxygen evolution system via tyrosine (Tyr_{161}) of the D1 protein. Q_A reduces Q_B to the semiquinone. Now a second cycle with a second light quantum can take place and Q_B is reduced to the hydroquinone and half an O_2 is evolved, with two electrons being transferred. Also, two protons from the water splitting are released into the lumen space, which are used to produce ATP.P_{680} is bound to both the D1 and the D2 proteins. Q_A is bound to the D2, and Q_B is bound to the D1 protein. Numerous reviews have followed these research advances (Satoh, 1996).

The three-dimensional orientation, or the folding of the two PS II reaction-center proteins in the membrane, is deduced from their homology to the two reaction polypeptides of purple bacteria, where they are called the L and M proteins. This reaction center has been crystallized and an X-ray analysis yielded an atomic structure at better than 2-Å resolution (Deisenhofer *et al.*, 1986[1]). The homology in the primary amino acid sequence of the D1 to the L protein, and of the D2 to the M protein, was noted (Youvan *et al.*, 1984), and it was suggested that these four proteins fold through the membrane in the same way (Trebst, 1986; Michel and Deisenhofer, 1988). This prediction that the D1 and D2 proteins are indeed the reaction-center polypeptides of PS II led to a folding model for the amino acid sequence of the two polypeptides (Trebst, 1986, 1987), using data obtained on substituted amino acids in the herbicide-binding protein (i.e., the D1 protein) in triazine resistance (Rochaix and Erickson, 1988), and discussed in detail below. An X-ray structure of terbutryn in a bacterial reaction center (Sinning, 1992) showed how accurate the modeling of the PS II subunits and the herbicide-binding niche according to the bacterial center really is. A schematic drawing of the folding of the herbicide-binding niche on the D1 protein (Trebst, 1987), updated with further amino acids in triazine resistance, is given in Figure 8.2. Finally, an enriched PS II preparation, consisting only of the D1 and D2 proteins and cytochrome b559, settled the question as to the identity of PS II (Nanba and Satoh, 1987). A computer-aided fitting of a triazine in this pocket is shown in Figure 8.3 (Tietjen *et al.*, 1991). A modeling of the D1/D2 protein complex was provided by Sobolev and Edelman (1995), and Xiong *et al.* (1998).

Shown in Figure 8.2 are about 60 amino acids from a total of 345 amino acids of the D1 protein. These 60 amino acids are part of the herbicide and Q_B-binding niche. Methionine (Met_{214}) is at the end of a transmembrane helix (IV) and leucine (Leu_{275}) in transmembrane helix V. A short, almost parallel helix from isoleucine (Ile_{241}) to phenylalanine (Phe_{260}) is on top of the transmembrane helices. The connecting loop is exposed to the matrix space of the chloroplast with arginine (Arg_{238}) easily accessible to trypsin. Arrows indicate some mutations in herbicide-tolerant plants and algae [like Val_{219} (valine), Ala_{251} (alanine), Phe_{255}, Gly_{256} (glycine), Ser_{264} (serine), and Leu_{275}] or amino acids tagged by

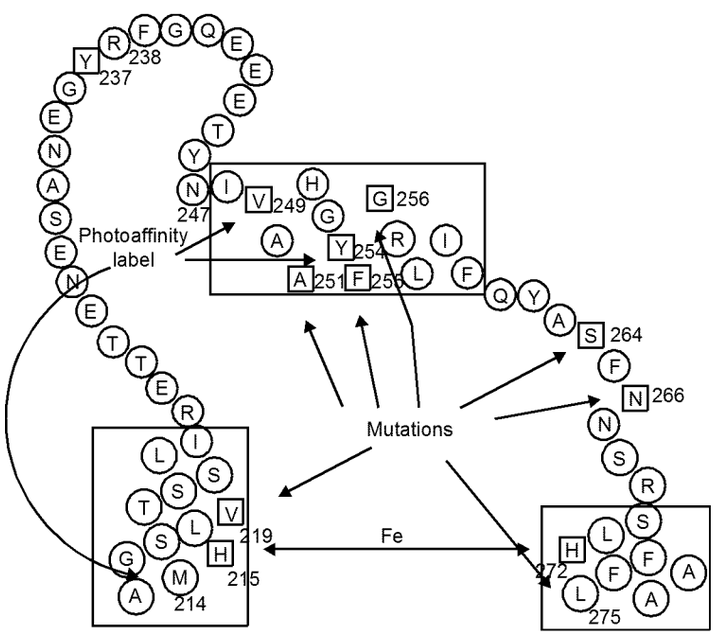

Figure 8.2 Folding of the amino acid sequence of the herbicide-binding niche in the D1 protein of PS II.

[1] This work of Michel and Deisenhofer was honored with the Nobel Prize.

Figure 8.3 Stereo representation of a computer-aided modeling of the three-dimensional structure of the triazine-binding niche, according to Tietjen *et al*. (1991). (See Color Plate Section)

azidoderivatives of herbicides (Met_{214} by azidoatrazine). His_{215} (histidine) and His_{271} are ligands to an Fe, that is further bound to two more histidines of the D2 protein, the other reaction-center polypeptide of PS II.

The sequence of amino acids 211 to 275 is indicated. It is this section of the sequence that forms the Q_B- and herbicide-binding niche, consisting of two hydrophobic transmembrane helices (only part of them is shown), and a small helix almost parallel to the membrane plane. Amino acid substitutions are indicated that confer herbicide tolerance, or are tagged by photoaffinity labeling. Histidines (His_{215} and His_{272}) are involved in both Fe and chlorophyll binding. His_{215} is also hydrogen bonded to the herbicides.

Triazine Resistance: Amino Acid Substitutions in the D1 Protein

The first atrazine-resistant common groundsel (Ryan, 1970) helped identify the herbicide-binding protein (Pfister *et al*., 1979) and made it possible to identify the D1 protein. After the *psbA* gene was found and sequenced (Zurawski *et al*., 1982), the *psbA* gene from an atrazine-tolerant *Amaranthus* was then sequenced by Hirschberg and McIntosh (1983). They found that a base change had led to the substitution of serine (Ser) 264 to glycine (Gly). This base change has now been found in many more triazine-resistant weeds (Morden and Golden, 1989; Van Oorschot, 1991). It is interesting to note that all PS II herbicide-resistant higher plants found to date in the field have only this Ser_{264} to Gly substitution. However, in tissue cultures and in triazine resistances in many algae induced by mutagenesis, many more amino acid substitutions in other positions have been identified (Erickson *et al*., 1985; Hirschberg *et al*., 1987; Wildner *et al*., 1990; Trebst, 1991; Oettmeier, 1999). Furthermore, in the green alga *Chlamydomonas*, Ser_{264} is changed to alanine (Ala), because of the codon usage, affecting the cross-resistance (Hirschberg *et al*., 1987) (see below). Table 8.1 lists some of the many single, double, and triple mutations that confer resistance. All present examples are given in a review by Oettmeier (1999).

All of these amino acid substitutions fall into a relatively narrow range of the amino acid sequence of the D1 protein from phenylalanine (Phe_{211}) to leucine (Leu_{275}). As is indicated in Figure 8.2, they are all in the Q_B-binding niche on the matrix side of the membrane. Indeed the first five substitutions in herbicide-resistant *Chlamydomonas* (Erickson *et al*., 1985; Rochaix and Erickson, 1988), were those that were the basis for modeling the three-dimensional folding of the D1 protein (Trebst and Draber, 1986; Trebst, 1987), and for the prediction that the herbicide-binding protein is part of the reaction center of PS II (Trebst, 1986). The D1 protein is the herbicide and reaction-center-binding protein of PS II, and the L subunit is the equivalent protein in purple bacteria. The Ser_{264} substitution in the D1 protein is equivalent to a Ser_{223} change in the L subunit of purple bacteria leading to triazine resistance in the photosynthetic bacteria (Paddock *et al*., 1988). More examples of mutations in plants and bacteria and a complete evaluation are given in a comprehensive review (Oettmeier, 1999).

Cross-Resistance to Other Herbicides in Triazine Resistance

As stressed above, two PS II classes of herbicides with the same mode of action (e.g., triazines and ureas) share the same binding site and replace each other from that site on the D1 protein (Tischer and Strotmann, 1977). From this it

Table 8.1 Positive and negative cross-resistance of PS II herbicides in a selection of mutants of various organisms

Amino acid substitution in					
D1 protein	**L subunit**	**Resistance to**	**Negative resistance to**	**Organism**	**Reference**
Phe_{211} Ser		Atrazine		*Synechococcocus*	Gingrich *et al.* (1988)
Val_{219} Ile		Diuron		*Chlamydomonas*	Wildner *et al.* (1990)
Ala_{251} Leu		Metribuzin		*Chlamydomonas*	Forster *et al.* (1997)
Ala_{251} Val		Atrazine, diuron, ioxynil		*Chlamydomonas*[a]	Wildner *et al.* 1990; Johanningmeier *et al.* (1987)
Phe_{255} Tyr		Atrazine	Phenols	*Chlamydomonas*	Rochaix and Erickson (1988); Erickson *et al.* (1985)
Ser_{264} Ala		Atrazine, diuron	Phenols	*Chlamydomonas*[a]	Erickson *et al.* (1985); Hirschberg *et al.* (1987); Oettmeier 1999; Wildner *et al.* (1990)
Ser_{264} Gly		Atrazine		*Amaranthus*[b]	Hirschberg and McIntosh (1983)
Asn_{266} Thr		Bromoxynil		*Synechocystis*	Creuzet *et al.* (1990)
Leu_{275} Phe		Diuron		*Chlamydomonas*	Rochaix and Erickson 1988; Erickson *et al.* (1985)
	Ser_{223} Pro	Terbutryn		*R. sphaeroides*	Paddock *et al.* (1988)

[a] Also in other single-celled algae.
[b] Also in *Brassica*, *Chenopodium*, *Senecio*, *Solanum*, and many other higher plants.

should not be concluded that a substitution at Ser_{264} to Gly in triazine resistance should also lead to urea resistance. Indeed the Ser_{264} to Gly change does not lead to DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) insensitivity (Hirschberg and McIntosh, 1983). However, a Ser_{264} to Ala substitution in the D1 protein is insensitive, as is the case in *Chlamydomonas* (Erickson *et al.*, 1985; Hirschberg *et al.*, 1987; Wildner *et al.*, 1990) (Table 8.1), indicating that DCMU is still accommodated by a glycine in that position, but not by the larger alanine. The study of this cross-resistance has lead to many insights into the detailed orientation of individual herbicides in the binding niche and to an understanding of the chemical interaction of the side chains of the amino acids in the protein with the chemical substituent of the inhibitors. Such information is essential for modeling herbicides into the target, and from there to predict new or more inhibitory compounds.

By screening for site-selected or site-directed mutations using the resistance to one herbicide as a marker, usually, but not always, the mutations also show (positive) cross-resistance to many other inhibitors. The PS II system may not be inhibited (or may be less inhibited) by the marker herbicide, as well as by others shown in Figure 8.1. However, there is also sometimes negative cross-resistance (i.e., increased inhibitory potency) when compared with the wild type of the same species. This has been largely noted for phenol-inhibitor derivatives (Oettmeier, 1999). This was first taken as another indication that the binding area of phenolic inhibitors is very different from those of the classical inhibitors (Figure 8.1), and that their inhibition pattern is also somewhat different. However, cases of resistance to phenolics were also observed (Table 8.1). It is now clear that all inhibitory compounds of the Q_B site in PS II occupy the same binding niche, but each herbicide has its characteristic orientation in the target protein touching and interacting with different efficiency of the different amino acids in the protein. The inhibitors can be more closely oriented toward Ser_{264} or toward His_{215} in the binding niche, and from the two families of herbicides of the Q_B site can be formed the classical type with triazine and ureas, and the phenol type with dinoseb and ioxynil (Trebst and Draber, 1986; Trebst, 1987). Whether there is a conformational adjustment of the protein when an herbicide or plastoquinone moves into the binding area remains to be determined.

Significance of the Rapid Turnover of the Herbicide-Binding Protein for the Mode of Action of Triazines

As discussed above, the somewhat surprising property of the rapid turnover of the D1 protein was an important step in the identification of the herbicide-binding protein (Ellis, 1981; Mattoo *et al.*, 1981). The protein is continuously degraded in the light, but also resynthesized such that the function of PS II remains undisturbed. Only when degradation and repair do not balance, as under very high light and other stress conditions, does photosynthesis cease. The turnover is related to photoinhibition and is part of the adaptation of PS II activity to environmental conditions and to down regulation. In high light, the turnover is controlled by the redox state of plastoquinone (as a balance between

the respective ratios of PS I and PS II), and of that in the Q_B site (Schuster *et al.*, 1988). The primary cleavage site in the degradation of D1 protein is likely in the amino acid sequence (Greenberg *et al.*, 1987), which folds for the Q_B and herbicide binding, likely glutamic acid (Glu_{244}) (Figure 8.2).

Triazines and the other PS II herbicides delay the degradation of the D1 protein, because they remove the controlling plastoquinone at the Q_B site (Marder *et al.*, 1984; Jansen *et al.*, 1993; Zer and Ohad, 1995). The role of triplets of the P_{680} reaction center and of singlet oxygen in the degradation is not considered here (Keren *et al.*, 1997). But the singlet oxygens generated in the D1 protein turnover are the cause of the eventual breakdown of PS II and then bleaching of the chlorophylls. This bleaching is the basis for the secondary, but actual, herbicidal action in the mode of action of triazines, whereas the primary action is the arrest of photosynthesis by replacing the plastoquinone at the Q_B site.

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Chapter 9

Basis of Crop Selectivity and Weed Resistance to Triazine Herbicides

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Summary

Triazines are selective herbicides used to control a wide spectrum of grass and broadleaf weeds in cereal, oilseed, and horticultural crops. Triazine herbicides kill weeds by interfering with the electron transport chain in photosystem II (PS II). These herbicides bind to the Q_B protein in the PS II reaction center and block the flow of electrons through the photosynthetic electron transport chain.

The basis for triazine selectivity in most crops is their rapid metabolic detoxification. The major detoxification pathway is conjugation with glutathione (GSH), catalyzed by the enzyme glutathione *S*-transferase (GST) (e.g., in corn, sugarcane, and sorghum). Another metabolism-based tolerance mechanism is replacement of the chlorine with a hydroxyl group, which also inactivates the herbicides (e.g., in corn). Triazines can also be inactivated by removal of the alkyl groups, probably mediated by cytochrome P_{450} (Cyt P_{450}) monooxygenases (e.g., in sorghum). Selectivity to metribuzin, an *as*-triazine (asymmetrical triazine), between crops and weeds is also based on differential rates of metabolism. Another mechanism of selectivity to triazine herbicides is physical selectivity, that is, placement of herbicide on the soil surface where it is not available to deep-rooted crops. Simazine, for example, stays close to the soil surface where it affects shallow-rooted weeds, but not deep-rooted perennial crops.

Triazines were one of the first family of herbicides where weed resistance was widely recognized and documented in the literature. A simazine-resistant biotype of common groundsel was identified in Washington, United States, in 1968. Since then biotypes of at least 66 triazine-resistant weed species have been reported, mostly in the United States, Canada, and Europe (Heap, 2006).

The most common mechanism of triazine resistance in weeds involves an alteration of the target site, the quinone B (Q_B) protein in photosystem II (PS II). Such alterations are caused by changes in the amino acid sequence of the Q_B protein. These mutations result in reduced binding of the herbicide to its target site, thereby making the weed resistant. The most common alteration is replacement of a serine at position 264 (Ser_{264}) by glycine (Gly), but many other mutations in the Q_B protein are also known to confer resistance. The second mechanism of resistance in weeds is the enhanced metabolism of the herbicide to inactive products by GSH conjugation or Cyt P_{450}-mediated oxidation. This mechanism is less common than target-site alteration.

Introduction

Triazine herbicides provide selective weed control in crops such as corn, sorghum, and sugarcane. In addition, some members of the triazine family are used for weed control in orchards, horticultural, and perennial crops, etc. A unique selective use of triazine herbicides is in triazine-tolerant rapeseed. Although triazine herbicides provide control of a wide variety of grass and broadleaf weeds, the long-term, widespread, and repetitive use of triazine herbicides in crop and noncrop situations has led to the selection of many triazine-resistant weeds. The physiological and biochemical basis of triazine selectivity between crops and weeds and of resistance to triazine herbicides in weeds is well understood.

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Triazine Selectivity in Crops and Weeds

Triazine-Tolerant Crops

The basis for triazine selectivity in most tolerant crop species, including corn and sorghum, is rapid metabolic detoxification of the herbicides. Research over the past 35 years has shown that triazine herbicides can be metabolized by three major pathways (Figure 9.1). GSH, catalyzed by glutathione *S*-transferase (GST), is a major route of detoxification of 2-chloro-*s*-triazine herbicides in tolerant crop species (Lamoureux *et al.*, 1972; Shimabukuro 1985; Weimer *et al.*, 1988). GST activity is present in many triazine-tolerant species, including corn, sugarcane, and sorghum, but is absent in sensitive species such as oat, wheat, and barley (Shimabukuro, 1985; Frear and Swanson, 1970). GST is present in multiple forms in many species; the different forms may differ in their affinity for various herbicides (Mozer *et al.*, 1983; Lamoureux *et al.*, 1991). GST catalyzes the nucleophilic attack of GSH on electrophilic sites of many herbicides, including atrazine (Figure 9.1). For example, Shimabukuro *et al.* (1971, 1978) showed that the primary mechanism of tolerance to triazine herbicides in corn was the GST-catalyzed conjugation of the herbicides with GSH, with hydroxylation and *N*-dealkylation playing secondary roles. One corn line (designated GT112) had a very low rate of GSH conjugation, which rendered it susceptible to atrazine (Shimabukuro *et al.*, 1971).

A second mechanism of tolerance to triazine herbicides involves replacement of the chlorine substituent with a hydroxyl group, forming hydroxyatrazine and hydroxysimazine from atrazine and simazine, respectively (Figure 9.1) (Castelfranco *et al.*, 1961; Hamilton and Moreland, 1962; Roeth and Lavy, 1971). This nonenzymatic reaction, which generally occurs following herbicide uptake through roots, is catalyzed by a cyclic hydroxamate, benzoxazinone (DIMBOA; 2,4-dihydroxy-7-methoxy-1,4(2*H*)-benzoxazin-3(4*H*)-one). Since the hydroxyl derivatives are essentially inactive, this pathway also results in complete detoxification of the herbicides.

A third mechanism by which triazine herbicides can be inactivated is by removal of the alkyl groups (Shimabukuro, 1985). This is accomplished by mono-*N*-dealkylation, probably mediated by a Cyt P_{450} (Figure 9.1) (Gronwald, 1994).

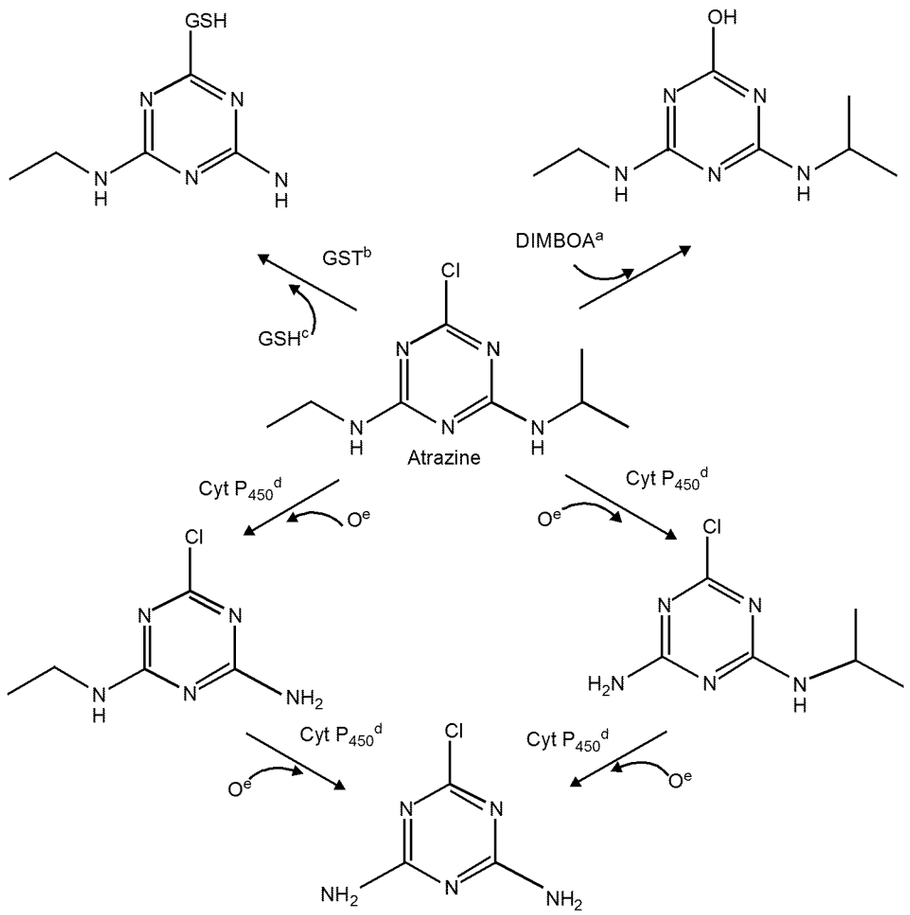

Figure 9.1 Metabolism of atrazine in plants.
[a]DIMBOA ffi 2,4-dihydroxy-7-methoxy-1,4(2*H*)-benzoxazin-3(4*H*)-one.
[b]GST ffi glutathione-*S*-transferase.
[c]GSH ffi glutathione.
[d]Cyt P_{450} ffi cytochrome P_{450} monooxygenase.
[e]O ffi oxygen (adapted from Gronwald, 1994).

Removal of one alkyl group leaves a partially phytotoxic compound, which is then further metabolized to confer total tolerance to the herbicide. For example, Shimabukuro *et al.* (1973) showed that sorghum dealkylated atrazine to 2-chloro-4,6-diamino-*s*-triazine (GS-28273), which was nonphytotoxic (Figure 9.1). The biological activities of the mono-dealkylated products were intermediate between those of atrazine and the diamino derivative (Shimabukuro and Swanson, 1969; Shimabukuro *et al.*, 1973). These differences are considered to be the reason for intermediate tolerance in some species.

Tolerance or susceptibility to metribuzin, an *as*-triazinone herbicide, is also based on differential rates of metabolism of the herbicide to inactive products. Three distinct pathways of metribuzin metabolism have been identified (Figure 9.2). The rates of metabolism in the different pathways contribute to differential tolerance between species and between cultivars within a species. For example, soybean cultivars are differentially sensitive to metribuzin, based on the rate of reductive deamination of the parent herbicide to inactive products (Fedtke and Schmidt, 1983). Other evidence indicates a role for *N*–glucosylation of metribuzin, with subsequent malonylation of the glucose moiety, in the tolerance of soybean cultivars (Figure 9.2) (Falb and Smith, 1984; Frear *et al.*, 1985). Some cultivars metabolize the herbicide to a dione derivative (DADK) or a homoGSH derivative, presumably through a sulfoxidation step (Figure 9.2). Differential rates of metribuzin metabolism have also been implicated in variations in cultivar susceptibility in potato, barley and tomato (Stephenson *et al.*, 1976; Gawronski *et al.*, 1986, 1987).

In 1978, Maltais and Bouchard (1978) reported identification of a birdsrape mustard (*Brassica rapa* or *B. campestris*) biotype that was resistant to triazine herbicides. This trait, which is of cytoplasmic origin, was transferred to the cultivated rapeseed by conventional plant breeding techniques and subsequently backcrossed with *B. napus* to produce triazine-resistant rapeseed lines (Beversdorf *et al.*, 1980). These lines were tolerant of atrazine, cyanazine and, to a lesser extent, metribuzin. Erucic acid content in the seed, time to flowering, and fertility were equal in the resistant and susceptible lines (Beversdorf *et al.*, 1980). One *B. napus* line, registered under the name OAC Triton in 1984, became the first commercially licensed triazine-tolerant spring rapeseed (Beversdorf and Hume, 1984). However, the triazine-tolerant lines proved to be agronomically inferior to the susceptible cultivars (Grant and Beversdorf, 1985). The mechanism of resistance and the physiological consequences of the resistance mutation are discussed in detail in the section on mechanisms of triazine resistance in weeds.

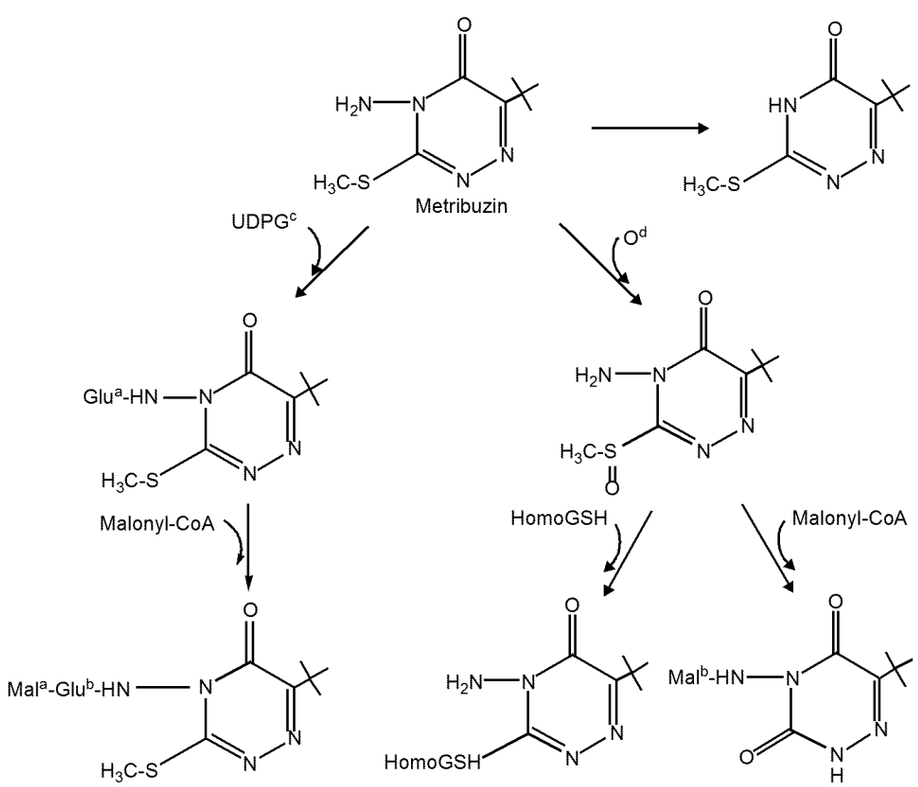

Figure 9.2 Metabolism of metribuzin in plants.
[a] Glu ffi glucose.
[b] Mal ffi malonyl; Mal-Glu, malonyl glucose.
[c] UDPG ffi uridine diphosphate glucose.
[d] O ffi oxygen (adapted from Lamoureux *et al.*, 1991).

Finally, certain triazine herbicides can be used selectively in orchards and in some horticultural crops. In this case, selectivity is not based only on physiological differences between species, but on physical selectivity associated with the location of the herbicide and the roots of the crop and weed species in the soil. Triazine herbicides such as simazine, which has very low solubility in water, remain close to the soil surface in most mineral soils. Careful application of simazine in horticultural or fruit crops can result in the herbicide being available to control shallow-rooted weed species without harming the deeper-rooted perennial species. The success of this use is dependent not only on the relative rooting depths of the tolerant and susceptible species, but also on soil conditions and other factors that may affect herbicide fate and movement.

Weeds

Various grass weed species are tolerant of triazine herbicides based on their ability to metabolize the herbicides to inactive products. The more tolerant panicums and foxtails metabolized atrazine and propazine to their hydroxy derivatives and peptide conjugates. However, both species were susceptible to simazine because they were unable to metabolize it as rapidly or in the same way (Thompson, 1972a). There was no difference in the rate of absorption, translocation, and hydroxylation of atrazine and simazine in shattercane; however, atrazine was conjugated at five times the rate of simazine (Thompson, 1972b). In a study of atrazine detoxification in 53 grass species of the subfamilies *Festucoideae, Panicoideae* and *Eragrostoideae*, GSH conjugation was found to be the major detoxification pathway in the more tolerant grass species (Jensen *et al.*, 1977). In another study, seedlings of big bluestem and switchgrass (tolerant) metabolized atrazine by GSH conjugation, whereas indiangrass and sideoats grama (susceptible) metabolized it by *N*-deethylation (Weimer *et al.*, 1988). GSH conjugation occurred at a higher rate than *N*-deethylation, which may explain the difference in tolerance level between the two pairs of species. These results indicate that tolerance to triazine herbicides in grass weeds can be due to metabolism through different pathways, as well as differences in the rate of metabolism for a particular pathway.

Triazine Resistance in Weeds

Weed resistance to the triazine herbicides was first identified in the late 1960s, with a biotype of common groundsel that was resistant to simazine (Ryan, 1970). Since then, resistance to triazine herbicides has been reported in many weed species (Holt and LeBaron, 1990; LeBaron and McFarland, 1990; Gronwald, 1994). Most cases of triazine resistance have been reported in the US, Canada, and Europe, where triazine herbicides have been used extensively in corn monocultures (LeBaron and McFarland, 1990; Stephenson *et al.*, 1990; LeBaron, 1991). Most of the *s*-triazine-resistant weed species have been selected against atrazine and usually show a high level of cross-resistance to other *s*-triazine herbicides. In most cases, these weeds also show a low level of resistance to *as*-triazinones (e.g., metribuzin). Triazine-resistant weeds are often less vigorous than nonresistant weeds, which facilitates their management.

Target Site-Based Resistance

Triazine (e.g., atrazine, simazine) and substituted urea (e.g., diuron, monuron) herbicides bind to the plastoquinone (PQ)-binding site on the D1 protein in the PS II reaction center of the photosynthetic electron transport chain. This blocks the transfer of electrons from the electron donor, Q_A, to the mobile electron carrier, Q_B. The resultant inhibition of electron transport has two major consequences: (i) a shortage of reduced nicotinamide adenine dinucleotide phosphate ($NADP^{ffl}$), which is required for CO_2 fixation; and (ii) the formation of oxygen radicals (H_2O_2, OH, etc.), which cause photooxidation of important molecules in the chloroplast (e.g., chlorophylls, unsaturated lipids, etc.). The latter is the major herbicidal consequence of the inhibition of photosynthetic electron transport.

Target-site-based resistance in triazine-resistant weeds is conferred by changes in amino acid residues in the Q_B-binding niche on the D1 protein that reduce herbicide-binding affinity at this site (Devine *et al.*, 1993; Gronwald, 1994; Devine and Eberlein, 1997). This protein is a thylakoid-membrane spanning protein, coded by the *psb*A gene. In almost all cases where such resistance has been identified, resistance is conferred by a Ser_{264} to Gly mutation that alters the conformation of the Q_B (and herbicide) binding niche (Gronwald, 1994). In the absence of the herbicide, Q_B can still access this site and transfer electrons from the PS II reaction center to the cytochrome b_6/f complex. The Ser_{264} to Gly mutation reduces the binding of *s*-triazine and *as*-triazine herbicides to this site, but does not affect the affinity for substituted urea herbicides and other PS II electron transport inhibitors (e.g., hydroxybenzonitriles and phenol-type herbicides) (Devine *et al.*, 1993). Atrazine binding at this site is partially dependent on the hydrogen bonding of the herbicide with the hydroxyl side chain of Ser_{264}. A resistance factor of 1000 at the binding site on the D1 protein and 100 at the whole plant level has been observed in biotypes with this mutation (Pfister and Arntzen, 1979; Fuerst *et al.*, 1986).

Masabni *et al.* (1996) identified a Ser_{264} to Thr (threonine) mutation in a resistant biotype of common purslane. This conferred a high level of resistance to atrazine and also to linuron, a substituted urea herbicide. This was the first report of a Ser_{264} to Thr substitution in higher plants selected under field conditions. Previously, this mutation had only been selected through tissue culture in tobacco and potato (Sigematsu *et al.*, 1989; Smeda *et al.*, 1993).

In contrast, *Ernst et al.*, (1996) have shown that a substitution at Ser_{264} does not necessarily lead to herbicide resistance. They found both Ser and Gly at position 264 in various sensitive and resistant biotypes of common groundsel. However, all resistant biotypes of black nightshade had Gly at position 264, but some of the sensitive biotypes also had Gly at this position. They suggested that the effect of this mutation in sensitive biotypes was overcome by two additional mutations in these biotypes: alanine at position 251 (Ala_{251}) to arginine (Arg) and valine at position 280 (Val_{280}) to leucine (Leu). In sensitive common lambsquarters, only Ser was present at position 264; either Ser or Gly were detected at position 264 in different atrazine-resistant plants (Ernst *et al.*, 1996).

Several substitutions conferring resistance to triazine herbicides have been identified at positions other than Ser_{264}. For example, amino acid changes at positions 219 [Val to isoleucine (Ile)] and 251 (Ala to Val or Thr), without a change at Ser_{264}, were suggested to be responsible for herbicide resistance in various cell culture lines of red goosefoot (Schwenger-Erger *et al.*, 1993). Trebst (1991) has listed a number of amino acid changes between positions 211 and 275, including phenylalanine (Phe) at position 211 to Ser, Gly_{256} to aspartic acid (Asp) and Leu_{275} to Phe, that confer herbicide resistance in various organisms. Finally, a Ser_{268} to proline (Pro) mutation has been identified in soybean cell culture that confers a high level of resistance to both triazine and phenylurea herbicides (Alfonso *et al.*, 1996). To date, these mutations have not been reported in any resistant weed biotypes in the field.

Based on the various models proposed, mutations at or close to positions Ser_{264}, Phe_{265}, Phe_{255}, and His_{215} (histidine) affect the binding of PQ or herbicides and play an important part in the development of resistance (Devine and Eberlein, 1997). These alterations in the Q_B-binding site on the D1 protein reduce the binding of triazine herbicides but have variable effects on the binding of structurally different PS II inhibitors (Gronwald, 1994).

A number of pleiotropic effects have been observed as a result of the Ser_{264} to Gly mutation. In addition to decreasing herbicide-binding affinity, this substitution reduces the rate of electron transfer between Q_A and Q_B (Bowes *et al.*, 1980; Jansen and Pfister, 1990). Galactolipid composition and unsaturated fatty acid levels also differ between resistant and susceptible biotypes (Burke *et al.*, 1982; Chapman *et al.*, 1985; Lehoczki *et al.*, 1985). There is also an increase in grana stacking and a reduction in the chlorophyll *a/b* ratio in the chloroplasts of triazine-resistant plants as compared to susceptible plants (Burke *et al.*, 1982; Vaughn, 1986). Together, these factors reduce the photosynthetic capacity of resistant plants. Hart and Stemler (1990) suggested that the resistant plants containing these mutations are more sensitive to photooxidation under high light conditions. Similarly, Perewoska *et al.* (1994) reported increased sensitivity to high light in mutants of *Synechocystis* PCC 6714.

In practical terms, the Ser_{264} to Gly mutation causing triazine resistance reduces plant productivity and yield. This has two agronomic implications: first, triazine-resistant weeds carrying this mutation are less competitive (often referred in the literature as reduced fitness) on a per plant basis as compared to their susceptible counterparts, and second, triazine-resistant rapeseed cultivars with the mutant *psb*A gene are less productive than near isogenic, susceptible cultivars (Forcella, 1987; Beversdorf *et al.*, 1988; Hart and Stemler, 1990; Hall *et al.*, 1996).

Negative cross-resistance has been reported in some triazine-resistant biotypes. *B. napus* and redroot pigweed biotypes have been identified that exhibit negative cross-resistance to bentazon (Van Oorschot and Van Leeuwen, 1988; Gressel, 1991), and a *Chlamydomonas* mutant (Phe_{255} to Tyr) shows negative cross-resistance to diuron (Trebst *et al.*, 1988). De Prado *et al.* (1992) reported negative cross-resistance to bentazon and pyridate in atrazine-resistant smooth pigweed biotypes. The authors suggested that the enhanced susceptibility to bentazon and pyridate may be due to alteration in the D1 polypeptide subunit of PS II.

Weed Resistance Based on Enhanced Herbicide Metabolism

Triazine resistance based on enhanced herbicide metabolism has been documented in at least two weed species, velvetleaf and rigid ryegrass. An atrazine-resistant biotype of velvetleaf identified in Maryland in 1984 was 10-fold more resistant to atrazine than the susceptible biotype (Ritter, 1986; Gronwald *et al.*, 1989). This biotype was resistant to both atrazine and simazine but not to other PS II inhibitors (Gronwald, 1994). Inhibition of photosynthetic electron transport was similar in chloroplasts of resistant and susceptible biotypes. However, the rate of atrazine GSH was 6-fold higher in leaves of the resistant biotype as compared to the susceptible biotype (Gronwald *et al.*, 1989). The enhanced conjugation was found to be due to the overexpression of two GST isozymes, not due to increased glutathione content. Gray *et al.* (1996) have reported enhanced metabolism-based (increased GSH conjugation) resistance to atrazine in two of these biotypes of velvetleaf from Wisconsin and Maryland.

A biotype (WLR2) of rigid ryegrass was identified in western Australia that was resistant (3- to 9-fold) to chloro-*s*-triazines, methylthio-*s*-triazines, substituted ureas and triazinone herbicides (Burnet *et al.*, 1991). A second biotype (VLR69) showed similar resistance patterns to simazine and chlorotoluron (Burnet *et al.*, 1993). The resistant and susceptible biotypes appeared to metabolize the herbicides by the same pathway, but the rate of metabolism was greater in resistant plants. For example, both resistant biotypes had an enhanced ability to detoxify simazine by removal of the ethyl side chain (*N*–dealkylation) (Burnet *et al.*, 1993). 1-Aminobenzotriazole, an inhibitor of Cyt P_{450} monooxygenases, reduced the rate of *N*-dealkylation in both of the resistant biotypes and enhanced simazine toxicity, indicating that the resistance was mediated by elevated Cyt P_{450} activity (Burnet *et al.*, 1993).

In other weed biotypes, resistance to triazine herbicides is likely conferred by rapid metabolism of the herbicides to inactive compounds. A chlorotoluron-resistant biotype of blackgrass (slender foxtail) was cross-resistant to various other groups of herbicides, including triazines (Kemp *et al.*, 1990). The mechanism of chlorotoluron resistance was Cyt P_{450}-based enhanced oxidative metabolism through *N*-demethylation and ring-methyl hydroxylation (Moss and Cussans, 1991). Consequently, it is likely that resistance to triazines in this blackgrass biotype is also due to enhanced herbicide detoxification.

In summary, triazine resistance in weeds is most commonly due to a target site alteration that confers a very high level of resistance to *s*-triazine herbicides. Although a Ser_{264} to Gly mutation in the D1 protein is most common, additional alterations have been identified that confer resistance to triazines and other classes of PS II inhibitors. Enhanced herbicide metabolism plays a major role in conferring resistance in only a few weed biotypes. In these biotypes, the pattern of resistance may be broader, with some cross-resistance to *as*-trazinones, uracils, heterocyclic ureas and phenyl ureas. The level and pattern of resistance to various herbicides in these biotypes depend, presumably, on the activity and specificity of the enzyme(s) responsible for the enhanced herbicide metabolism.

Conclusions

The successful use of triazine herbicides has been based on their selectivity in several major crops, particularly corn, sugarcane, and sorghum. In general, the margin of safety in these crops has precluded phytotoxic effects on the crops while providing satisfactory control of a broad range of weed species. In certain cases, however, a relatively narrow difference in the rate of metabolism between crops and weeds can occasionally result in some crop injury (e.g., atrazine in sorghum grown on light soils and metribuzin in some potato and tomato varieties).

The high efficacy of triazine herbicides and their repetitive use in crops and noncrop situations has resulted in the selection of weeds that are resistant to these herbicides or are not well controlled at the lower rates now being used. In most instances, triazine resistance is due to an alteration in the herbicide-binding site in PS II. Despite the widespread occurrence of triazine resistance, these herbicides are still widely used, even in fields in which triazine-resistant biotypes are known to occur. The rate of increase in the selection for triazine-resistant weed species depends in part on the integration of alternative weed control strategies, in addition to the use of triazine herbicides, for control of these weed species. Due to their resistance mechanism, many triazine-resistant weeds are less competitive than their susceptible counterparts.

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Chapter 10

Distribution and Management of Triazine-Resistant Weeds

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Summary

The use of modern selective chemicals for the control of weeds and unwanted vegetation faced a new challenge in 1968 when the first weed (common groundsel) to exhibit resistance to the triazine herbicides was found in the state of Washington (Ryan, 1970). Herbicides by then had bypassed insecticides and fungicides as the most extensively used agricultural pest control method. A few cases of weed resistance to herbicides had been reported earlier, and some weed scientists predicted that resistance in weeds would become as common a problem for herbicides as it had earlier with insecticides and fungicides. By this time, the triazines were widely used and provided excellent control of most weeds, while showing remarkable selectivity in various crops. Triazine-resistant biotypes began to be reported in locations where the herbicides were used alone for more than 8 years on the same land. The international survey of herbicide-resistant weeds and the author's surveys show that there have been isolates or biotypes of 78 weed species resistant to triazines in specific fields or regions around the world. With few exceptions, these weeds are resistant due to the selection for an insensitive Q_B protein in the chloroplasts, which is the site of action for triazine herbicides in plants.

The practical or economic importance of triazine-resistant weeds has not proved to be as great as many feared. Triazine resistance is different than the resistance developed to most herbicides because it is intimately connected with a lack of fitness or vigor due to a photosynthetic deficiency in the resistant weed. Therefore triazine-resistant weeds are often easier to control than weeds resistant to alternative herbicides. University researchers have shown that several herbicides are excellent partners in combinations with triazines to control most triazine-resistant weeds, and these combinations expand the range of weeds (especially grasses) successfully treated. In spite of the occurrence of triazine-resistant weeds and the development of many other herbicides in recent years, atrazine and simazine are used as preferred partners in combinations with other herbicides. Triazines are also used to control weeds that are resistant to other herbicides. Approximately 40 herbicides are prepackaged with atrazine as a mixing partner. Due to the implementation of resistance management practices, the triazine herbicides will continue to be important for weed management in corn, sorghum, sugarcane, fruit, nut, turf, and for many other uses in years to come.

History of Triazine-Resistant Weeds

The earliest reports of weeds becoming resistant to herbicides preceded the use of the triazines. In 1950, Blackman gave a convincing report on how the eventual selection for herbicide-resistant weeds was not only possible, but certain. He showed that based on the variation within different crop varieties, there was a bright future for the breeding of herbicide-resistant varieties. But within weed populations, which have much more genetic adaptability, the reverse would be true. Repeated spraying of one type of herbicide would surely select for resistant strains within the weed populations. In California, continued spraying of roadsides with mineral oils killed the original vegetation, only to have it replaced by oil-resistant weeds, and the exclusive use of 2,4-D replaced original broadleaf weeds with grasses (Blackman, 1950). Blackman predicted that both crop rotation and herbicide rotation would be necessary for sound husbandry. Just prior to the commercial introduction of the first triazines, Harper (1956) predicted that weeds would evolve to become resistant to herbicides. This prediction was based on extensive experience in the fields of medicine, bacteriology, and applied entomology, as well as the knowledge then available on the genetic variability or flexibility in many weed species. There were already reports that herbicides used repeatedly on the same populations of weeds were becoming less effective. The very next year, Hilton (1957) reported that spreading dayflower survived after several years of 2,4–D applications in sugarcane, and Switzer (1957) reported on 2,4-D-resistant strains of wild carrot following several applications along roadsides. Differences in susceptibility to 2,4-D and other earlier herbicides

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within weed species and biotypes were well known (Whitehead and Switzer, 1963; Bandeen *et al.*, 1982; Gressel and Segel, 1982), but these initial reports failed to get much attention.

An important agronomic factor was that the phenoxys and other classes of herbicides developed and used prior to the triazines were seldom, if ever, able to give total or season-long control of all weed species. Repeat or supplemental herbicide treatments, cultivation, or other means were needed to control later-germinating or other uncontrolled weed problems.

Triazine-resistant weeds have been extensively researched and documented since 1968 when the first such weed was discovered. Ryan (1970) reported that a previously susceptible biotype of common groundsel was no longer controlled by simazine or atrazine. This original discovery was of great significance not only because the weed had an extremely high level of resistance, but also because that resistance was genetically transferred (Scott and Putwain, 1981; Souza-Machado, 1982). It was predicted that a resistant field population would build very rapidly from a very low initial frequency of the resistant genotype (Gressel and Segel, 1978, 1982). In addition, the triazine herbicides (especially atrazine and simazine) had become the most important class of herbicides in North America and much of the world – able to provide season-long control of most weeds with no detrimental effect on corn and with good safety on many other crops. A powerful statement on this development was made by Holm *et al.* (1997) in their work on *World Weeds*: 'This discovery (i.e., resistance to triazines) has proven to be one of the most important events since the inception of weed science.' Historical accounts of this discovery and of conferences and events that followed are published in greater detail elsewhere (LeBaron and Gressel, 1982; Heap and LeBaron, 2001).

One of the characteristics of herbicides sought by industry and academic weed scientists, biologists, and agronomists is the ability to control several weed species in the treated area. In many fields, from 10 to 20 or more weed species may cause problems for crop production, habitat preservation, or land management. Since most weeds will grow larger to fill any available space and will use the available resources, farmers realize that even if they control all but one species of weed, their crops can still face serious competition.

Development and Distribution of Triazine Resistance

Soon after the discovery of triazine-resistant common groundsel, another equally important discovery was made. Radosevich and DeVilliers (1976) found that the mechanism of resistance in this weed was due to insensitive chloroplasts that were capable of photosynthesis, even in the presence of simazine or atrazine. This was surprising because earlier research had confirmed that there were no differences in plant selectivity or susceptibility due to the origin of chloroplasts. Moreland (1969) had reported that isolated chloroplasts were equally inhibited to simazine whether they came from tolerant corn or susceptible spinach. Radosevich and Appleby (1973) had confirmed there were no differences between the susceptible and resistant biotypes of common groundsel due to herbicide uptake, distribution, or metabolism, whereas it is known that corn metabolizes triazine herbicides (Shimabukuro, 1985).

The study of triazine-resistant weeds greatly increased the knowledge and understanding of photosynthesis, herbicide-binding sites, and modes of action. To have two plants available – identical in every respect except for a different herbicide-binding site in the thylakoid membrane of their chloroplasts – provided a useful and powerful tool to study the mechanisms and processes of photosynthesis, herbicide modes of action, and other physiological and molecular genetic processes (Arntzen *et al.*, 1982; Arntzen and Duesing, 1983; Hirschberg and McIntosh, 1983). Indeed, the target (binding site) has been isolated and crystallized from resistant and susceptible photosynthetic bacteria (Michel and Deisenhofer, 1988). This research led to a Nobel Prize in chemistry in 1988 shared by Deisenhofer, Huber, and Michel for the determination of a three-dimensional structure of a photosynthetic reaction center.

A review of data from a variety of global surveys on resistant weed biotypes shows that 32 weed species (26 broadleaf and 6 grass species) had isolates resistant to triazine herbicides by 1980. By September 1989, this number had increased to 58 (40 broadleaf and 18 grass species), and to 65 by March 1996 (LeBaron, 1998). Based on Heap (2006) and the author's global surveys, there have been 78 (57 broadleaf and 21 grass) species at one time or in at least one location with triazine-resistant isolates. Of these, 30 species (21 broadleaf and 9 grass) have been found in specific fields in the United States, 14 (10 broadleaf and 4 grass) in Canada, 57 (45 broadleaf and 12 grass) in Europe, and 22 species (13 broadleaf and 9 grass) in other countries. Several of the triazine-resistant isolates are no longer known to be resistant. This may be due to the lack of fitness of the resistant biotypes, which were then supplanted by more competitive weedy species (Gressel and Kleifeld, 1994). In 1996, Rubin reviewed the distribution, mechanisms, and significance of herbicide-resistant weeds. The International Survey of Herbicide Resistant Weeds is an excellent resource and can be found on the World Wide Web (Heap, 2006).

Weed species resistant to the triazines that have been found in one or more fields in 10 or more states, provinces, or countries include: smooth pigweed, Powell amaranth, redroot pigweed, common lamb's-quarters, kochia, common groundsel, black nightshade, and annual bluegrass (Tables 10.1(a) and 10.1(b)).

Table 10.1(a) Earliest discovery of biotypes of 57 triazine-resistant[a] dicotyledonous (broadleaf) weeds[b]

Genus and species	Common name	Year discovered	Field location[c]
Abutilon theophrasti[d]	Velvetleaf	1984	Maryland[1]
Amaranthus albus	Tumble pigweed	1985	Spain
Amaranthus arenicola[e]	Sandhills amaranth	1977	Colorado[1]
Amaranthus blitoides	Prostrate pigweed	1983	Israel
Amaranthus bouchonii[e]	Bouchons amaranth	1975	Italy
Amaranthus cruentus	Redshank, red amaranth or Italian amaranth	1975	Italy
Amaranthus hybridus (also *A. chlorostachys*)	Smooth pigweed	1972	Maryland[1]
Amaranthus lividus	Livid amaranth	1978	Switzerland
Amaranthus palmeri	Palmer amaranth	1987	Texas[1]
Amaranthus powellii	Powell amaranth	1968	Washington[1]
Amaranthus retroflexus	Redroot pigweed	1973	Austria
Amaranthus rudis	Common waterhemp	1992	Nebraska[1]
Amaranthus tuberculatus[e]	Tall waterhemp	2003	Illinois[1]
Ambrosia artemisiifolia	Common ragweed	1976	Ontario[2]
Arenaria serpyllifolia	Thymeleaf sandwort	1980	France
Atriplex patula[d]	Spreading orach	1975	Switzerland
Bidens tripartita	Bur beggarticks	1976	Austria
Brassica kaber (or *Sinapis arvensis*)	Wild mustard	1983	Ontario[2]
Brassica rapa (or *B. campestris*)	Birdsrape mustard	1977	Quebec[2]
Capsella bursa-pastoris	Shepherd's purse	1984	Poland
Chenopodium album	Common lamb's-quarters	1973	Austria
Chenopodium ficifolium	Figleaved goosefoot	1978	Germany
Chenopodium gigantospermum (or *C. Hybridum*)	Mapleleaf goosefoot	2000	Yugoslovia
Chenopodium missouriense[e]	Missouri goosefoot	1978	Pennsylvania[1]
Chenopodium pedunculare[e]	Pitseed goosefoot	1999	Czech Republic
Chenopodium polyspermum	Manyseeded goosefoot	1980	France
Chenopodium strictum var. *glaucophyllum*	Lateflowering goosefoot	1976	Ontario[2]
Conyza bonariensis (or *Erigeron bonariensis*)	Hairy fleabane	1987	Spain
Conyza canadensis (or *Erigeron canadensis*)	Horseweed	1981	France
Datura stramonium	Jimsonweed	1992	Indiana[1]
Epilobium adnatum (or *E. tetragonum*)	Square-stalked willowherb	1981	France
Epilobium ciliatum (or *E. adenocaulon*)	American willowweed (or willowherb)	1980	Belgium
Galeopsis tetrahit[e]	Common hempnettle	1993	Bulgaria
Galinsoga ciliata	Hairy galinsoga	1980	Germany
Galinsoga parviflora[e]	Smallflower galinsoga	1993	Bulgaria
Iva xanthifolia[e]	Marshelder	1995	Yugoslavia
Kochia scoparia	Kochia	1976	Idaho[1]
Matricaria matricarioides (or *Chamomilla suaveolens*)	Pineapple-weed (or rayless mayweed or disk mayweed)	1984	United Kingdom
Myosoton aquaticum[e]	Water starwort	1983	Germany
Physalis longifolia[e]	Longleaf groundcherry	1984	Pennsylvania[1]
Plantago lagopus	Plantain	1992	Israel
Polygonum aviculare	Prostrate knotweed	1980	Netherlands
Polygonum convolvulus (or *Fallopia convolvulus*)	Wild or climbing buckwheat	1977	Austria
Polygonum hydropiper	Marshpepper smartweed	1989	France
Polygonum lapathifolium	Pale smartweed	1979	France
Polygonum pensylvanicum	Pennsylvania smartweed	1988	Iowa[1]
Polygonum persicaria	Ladysthumb	1980	France
Portulaca oleracea	Common purslane	1991	Michigan[1]
Raphanus raphanistrum	Wild radish	1996	Australia
Sagina procumbens[e]	Birdseye pearlwort	1975	Belgium
Senecio vulgaris	Common groundsel	1968	Washington[1]
Solanum nigrum	Black nightshade	1975	Italy
Sonchus asper	Spiny sowthistle	1980	France
Stachys recta[e]	Upright hedgenettle or betony	1995	Yugoslavia
Stellaria media	Common chickweed	1974	Germany
Urtica urens	Burning nettle	2002	Australia
Xanthium strumarium[e]	Common cocklebur	1994	Yugoslavia

Table 10.1(b) Earliest discovery of biotypes of 21 triazine-resistant[a] monocotyledonous (grass) weeds[b]

Genus and species	Common name	Year discovered	Field location[c]
Alopecurus myosuroides[d]	Blackgrass	1982	Israel
Brachypodium distachyon	False brome	1975	Israel
Bromus tectorum	Downy brome or cheatgrass	1977	Kansas[1]
Chloris barbata (or *C. infl ata*)	Swollen fingergrass	1987	Hawaii[1]
Chloris radiata[e]	Radiate fingergrass	1988	Hawaii[1]
Digitaria sanguinalis	Large crabgrass	1983	France
Echinochloa crus-gall[d]	Barnyardgrass	1978	Maryland[1]
Heleochloa schoenoides (or *Crypsis schoenoides*)	Swamp timothy	1995	Israel
Lolium rigidum[d,f]	Rigid (or annual) ryegrass	1979	Israel
Lophochloa cristata (or *L. phleoides* or *L. smyrnacea*)	Annual catstail	1978	Israel
Panicum capillare	Witchgrass	1975	Michigan[1]
Panicum dichotomifl orum	Fall panicum	1981	Spain
Phalaris paradoxa	Hood canarygrass	1974	Israel
Poa annua	Annual bluegrass	1976	California[1]
Polypogon monspeliensis	Rabbitfoot polypogon	1979	Israel
Setaria faberi	Giant foxtail	1984	Maryland[1]
Setaria glauca	Yellow foxtail	1980	Nebraska[1]
Setaria verticillata	Bristly foxtail	1992	Spain
Setaria viridis	Green foxtail	1982	France
Setaria viridis var. *major*	Giant green foxtail	1982	France
Urochloa panicoides	Liverseedgrass	1995	Australia

[a] This herbicide class includes all symmetrical (Δ-) triazines such as atrazine, simazine, cyanazine, prometryn, and ametryn, and asymmetrical triazines such as metribuzin, although there may be much variation in biotype response to some of these herbicides.
[b] Some dates and locations do not match the international survey since they are based on author's surveys.
[c] Location: 1. States within United States. 2. Province of Canada.
[d] Some of these biotypes do not appear to have resistant chloroplasts, but are probably resistant due to metabolic detoxification or other mechanisms.
[e] From the author's surveys; not included in International Survey of Herbicide Resistant Weeds (www.weedscience.com).
[f] Resistance in grass has developed under two herbicide programs:
• Selected with amitrole/atrazine combinations with cross-resistance to all triazines, triazinones, ureas, and triazoles.
• Selected with diuron, and cross-resistance to all triazines as well.

Some of these species (e.g., smooth pigweed, redroot pigweed, common lamb's-quarters, horseweed, kochia, common groundsel, black nightshade, barnyardgrass, and annual bluegrass) have biotypes with resistance to the triazine herbicides in many locations. Based on research and observations, many cases of resistance have developed separately (Gasquez and Darmency, 1983; Gasquez *et al*., 1985; Darmency and Gasquez, 1990a; Rola and Rola, 1996). However, from a study on genetic variation and phylogenetic relationships of 25 triazine-resistant and triazine-susceptible biotypes of black nightshade in Poland, France, and the United Kingdom, Stankiewicz *et al*. (2001) concluded that while some resistant populations developed independently, migrating birds have played a role in the spread of resistant black nightshade by seed.

Within North America and a few other countries, most triazine-resistant weed biotypes have been reported after repeated use of atrazine in corn and sorghum. In some areas of Western Europe and other countries, triazine-resistant weeds have been reported after repeated use of simazine in orchards and along roadsides. A few triazine-resistant weeds (e.g., kochia, cheatgrass, and common groundsel) have biotypes with triazine resistance in nurseries and perennial tree crops, as well as along railways and roadsides.

For example, kochia is normally very sensitive to atrazine and can often be controlled with 1.1 kg/ha (1 lb/A) along railroads and on farm lands. However, Burnside *et al*. (1979) reported that after 13 years of atrazine use, Union Pacific personnel were applying up to 15 kg/ha (13 lb/A) with poor control. These high rates are no longer allowed for atrazine uses. The use of mixtures of triazines and herbicides with alternate modes of action has been an effective management strategy to control weeds resistant to triazines.

The science, understanding, and predictability of weed resistance to the different classes of herbicides could benefit greatly from a thorough investigation by molecular biologists. More information is needed on gene frequency and resistant alleles in various populations and on the relationships between the origin, genetic variability, and other

taxonomic characteristics of a weed family, genus or species and the tendency for resistant biotypes to survive and/or compete. Studies of the relationship between the genetics of a population and the resistance within that population have been conducted and reported by Gasquez (1985, 1988, 1991, 1995); Darmency and Gasquez (1983, 1990a, 1990b); Darmency (1994); Putwain (1982); Putwain *et al.* (1982); and Putwain and Mortimer (1995). Holm *et al.* (1997) emphasized that the *Amaranthus* species, which has more triazine-resistant biotypes than other weeds, encompasses a large family of 65 genera and about 900 species. During the Aztec Empire, grain of *Amaranthus* was an important component in the food supply. They are the most widely distributed weed species in arable crops of the world and are listed among the world's worst weeds (Holm*et al.*, 1977; Stallknecht and Schulz-Schaeffer, 1993).

Locations having the greatest number of triazine-resistant biotypes are as follows: France (23), Spain (19), Austria (15), Czech Republic (14), Germany (14), Switzerland (15), Israel (14), Bulgaria (13), Netherlands (11), Poland (11), Yugoslavia (11), Michigan (11), Ontario (11), Belgium (8), Pennsylvania (7), Hungary (7), United Kingdom (7), Nebraska (7), Illinois (6), Italy (6), Maryland (6), Oregon (6), Washington (6), and Denmark (6).

The levels of infestations or seriousness of triazine resistance within each species varies greatly. The author is aware of 19 cases where the resistant weed is no longer present or cannot be identified as resistant to triazine herbicides. In other cases, the current status is unknown. Several triazine-resistant biotypes are likely to be of little or no agronomic importance within a geographical area.

Until recent years, there had been no organized effort to document and confirm resistant weeds. Cases of resistant weeds would be known by the farmers experiencing problems of herbicide failure, or possibly by the local dealer, industry representative, or extension agent. Often, though, these herbicide failures were not known by the state or county weed specialists who, in most cases, would be contacted for surveys on resistant weeds. Also, determining when resistant weeds first appeared or where they were found is subject to considerable error. The person responding to a survey might determine that a resistant weed should be reported when it first escapes control of the herbicide, while others say that it should be reported when first called to the attention of a weed specialist, or when confirmation of the resistant weed is first determined in the greenhouse or laboratory. Others prefer to wait until the taxonomic identification of the resistant weed is confirmed, or when the resistant weed becomes a widespread or significant problem in the area. Because there are so many opinions and subjective judgments involved, the timing and confirmation of resistance development in resistant weed surveys and questionnaires will be subject to differences.

A major limitation of global surveys and confirmations of herbicide-resistant weeds has been the variability in the methods used in identifying resistance. Unless the cases of resistance have been confirmed by laboratory or greenhouse studies and include information on the degree of resistance, the reports of resistance should not be recognized as being confirmed. Van Oorschot (1991); Truelove and Hensley (1982); and others have conducted and reported on extensive research on the best methods for confrming triazine-resistant weeds.

In some cases, even before a weed is confirmed in laboratory tests to be resistant to an herbicide, the farmer has already changed his weed control program and the resistant weed may no longer be easy to find. There have been also cases where the resistant weed was identified as one species, but was later confirmed by a taxonomist as another. In those cases where no qualified weed scientist was available to conduct confirmation tests, the resistant biotypes are not listed as resistant, even though they may no longer be controlled with the once effective herbicide.

A central system for documenting and reporting triazine-resistant weeds has been established by the Herbicide Resistance Action Committee (HRAC), the North American Herbicide Resistance Action Committee (NAHRAC), and the Weed Science Society of America (WSSA). The system is administered by Heap (2006), who conducts the official surveys and publishes data on a continuing basis. The data presented in Tables 10.1(a) and 10.1(b) cannot be exactly compared to those compiled by Heap (1994, 1997, 2006) (Heap and LeBaron, 2001) since the tables include data from earlier surveys and publications (LeBaron and Gressel, 1982; LeBaron, 1989, 1991, 1998; Holt and LeBaron, 1990; LeBaron and McFarland, 1990). Most of these additional species were confirmed by the Ciba-Geigy Corporation in the 1970s and 1980s. Resistant biotypes of some of these species have not been reported in recent years and are no longer found in their original fields and locations. Moss and Rubin (1993) reported on the worldwide distribution of herbicide-resistant weeds and projected future trends and possible solutions to avoid more serious problems.

The common and scientific names of weeds in this book use as our primary source the *Composite List of Weeds – Revised 1989* by Weed Science Society of America, 309 W. Clark Street, Champaign, Illinois (now P.O. Box 7050, Lawrence, Kansas, USA 66044). For all weeds not found in this source we have used the publication by Bayer AG, *Important Crops of the World and Their Weeds* 1992 Second Edition, published by Business Group Crop Protection, Leverkusen, Federal Republic of Germany. For the few weed species not found in either of these sources, we have referred to Richard Jensen at Brigham Young University Library, Provo, Utah, and the Internet.

In almost all cases of triazine-resistant weeds, it has been documented that they are discovered in fields after years of repeated use of triazine herbicides without mixing or rotating with herbicides that have an alternate mode of action. However, a few exceptions have been reported. For example, Lior *et al.* (1996) found that various populations

of resistant plantain and horseweed were found in Israel following many years of simazine applications, with and without other herbicides. The distribution of triazine-resistant horseweed plants indicated that wind dissemination of seeds from resistant plants could impact distribution.

Fitness of Triazine Resistance

In most of the triazine-resistant biotypes tested, it has been confirmed that triazine-resistant weeds show reduced rates of CO_2 fixation and oxygen evolution compared to triazine-susceptible or wild biotypes. When triazine-resistant weed biotypes were found that were closely related to important crops, there were efforts to breed resistance into crop varieties. These efforts were largely unsuccessful, though, due to the reduction in fitness and productivity that were closely associated with resistance and could not be completely overcome. Most triazine-resistant weeds appear to share a common genetic basis and physiological mechanism of resistance, resulting in insensitive chloroplasts to triazine herbicides (Fuerst and Norman, 1991; Beversdorf *et al.*, 1988; Reboud and Till-Bottraud, 1991). Reduced fitness has been characteristic of triazine-resistant biotypes, even within very closely related genetic populations such as isonuclear lines (Gressel *et al.*, 1983; Gressel and Ben-Sinai, 1985; McCloskey and Holt, 1990). In spite of the fitness reduction in triazine-resistant plants, triazine-resistant canola was developed as an agronomic crop and used in Australia because of the prevalence of *Raphanus* and other weed species that are difficult to control without triazines.

Triazine-resistant weeds have an impaired electron transport system. Reduced electron transport, in turn, reduces photosynthetic activity and fitness. Under controlled conditions, most triazine-resistant biotypes exhibit impaired photosynthesis. Significant fitness costs from resistance (10–50%) have been reported in most studies (Warwick, 1991). This substantial fitness cost or 'handicap' has been important in the management of triazine-resistant weeds (Radosevich *et al.*, 1991; Bergelson and Purrington, 1996). Anderson *et al.* (1996b) found that the competitive advantage of triazine-susceptible waterhemp over triazine-resistant waterhemp isolated from one field in Nebraska was equal to or less than that for other species or isolates. This indicates that additional factors contributed to the slow and limited distribution of resistance for this waterhemp biotype.

Research results from Williams *et al.* (1995) indicate that triazine-resistant jimsonweed is less fit when growing in the midst of a vigorous crop than in a more open habitat. The authors suggested that efforts to manage field crops to enhance crop interference will be an effective tool for management of resistant populations. This lack of fitness helps to explain why crop and herbicide rotations have proven to be more effective in managing resistance to the triazine herbicides than models predict (Gressel and Segel, 1990b). Use of narrow row spacing, planting of tall and vigorous crop varieties, and placement of nutrients to maximize crop uptake are also management options to enhance crop competition (Jordan, 1993). Various simulations of herbicide resistance dynamics show that fitness differentials of the magnitude observed by Williams *et al.* (1995) could lead to rapid decreases in the frequency of resistance alleles in the absence of the herbicide (Maxwell *et al.*, 1990).

This characteristic, however, is not universally found in all triazine-resistant weeds. Gray *et al.* (1995a, b) found that velvetleaf resistance to atrazine in Wisconsin was not associated with a reduction in fitness, productivity, or intraspecific competitive ability. This triazine-resistant species found in Maryland and Wisconsin does not have D1 level resistance in the chloroplasts, but instead has a more rapid metabolic detoxification of triazines in these biotypes. The extent of the rapid metabolic resistance in other velvetleaf-resistant biotypes is unknown.

Clay *et al.* (1991) found triazine-resistant biotypes from two different weed species (i.e., American willowherb and common groundsel) were also resistant to two powdery mildews. They proposed that the relationship may be due to the gene responsible for triazine resistance being closely linked to the inability of the mildews to infect those weeds.

Genetics of Triazine Resistance

Shortly after the introduction of the triazine herbicides, it was confirmed that their target site in the photosystem II (PS II) complex was in the thylakoid membranes. Triazines displace plastoquinone at the Q_B-binding site on the D1 protein, thereby blocking electron flow from Q_A to Q_B. This in turn inhibits $NADPH_2$ and ATP synthesis, preventing CO_2 fixation.

Except for *Abutilon* (velvetleaf) species and a few grasses, genetic resistance typically results from a single point mutation at the *psb* A locus of the chloroplast genome, which codes for a protein component (D1) that is a 32 kDa quinone-binding protein of photosystem II (PS II) (Holt *et al.*, 1993). The *psb* A gene is highly conserved with great homology throughout the plant kingdom (Morden and Golden, 1989). The resistance mutation has been characterized by DNA sequencing studies. In nearly all triazine-resistant weed populations examined, the same single amino acid substitution has taken place in which glycine (Gly) replaces the serine (Ser) at codon 264 (Bettini *et al.*, 1987;

Eberlein *et al.*, 1992; Gronwald, 1994). The substitution eliminates a hydrogen bond, which greatly reduces triazine binding at D1, conferring resistance (Mattoo *et al.*, 1989; Holt and LeBaron, 1990; Fuerst and Norman, 1991; Trebst, 1991). This type of resistance is maternally inherited and is, therefore, primarily spread by seed and only rarely by pollen (Bettini *et al.*, 1987; Eberlein *et al.*, 1992). Many weed species have high paternal levels of DNA transfer (Darmency and Gasquez, 1990b; Putwain and Mortimer, 1995).

Smeda *et al.* (1993) reported that in a mutation of the *psb* A gene in a photoautotropic potato, atrazine resistance was attributable to a mutation from AGT (ser) to ACT (threonine) in codon 264 of the *psb* A gene that encodes the Q_B protein. Although the mutant cells exhibited extreme levels of resistance to atrazine, no concomitant reductions in photosynthetic electron transport or cell growth rates were detected compared to the unselected cells. This is in contrast with the losses in productivity observed in atrazine-resistant mutants that contain a Ser to Gly 264 alteration. Research has shown that triazine resistance by various algae and photosynthetic bacteria has been due to changes in many different binding sites (Oettmeier, 1999).

Other reviews have been published on the mechanisms of triazine resistance and the biochemistry, genetics, and molecular biology of PS II (Jansen and Pfister, 1990; Oettmeier *et al.*, 1992; Gronwald, 1994). Oettmeier (1999) provides information on the impact of various mutations on protein binding and, therefore, the herbicidal activity of the several triazines and other PS II herbicides. While most triazine-resistant higher plants have the Ser to Gly mutation, many photosynthetic purple bacteria and algae have been found to have other mutations that contribute to varying degrees of resistance to the triazines and other PS II herbicides. Cases of PS II resistance due to serine-to-threonine substitutions and valine to isoleucine substitutions have been documented in higher plants (Masabni and Zandstra, 1999; Mengistu *et al.*, 2000). Each mutation contributes unique differences in binding and resistance. Based on the known structure of the PS II reaction center, Oettmeier (1999) describes the binding mechanisms involved. The loss of the hydroxymethyl group in Ser_{264} upon changing to Gly leads to dramatic differences in triazine binding due to the loss of the hydrogen bonding with the alkylamino group on the triazine. Within the other mutants, it can now be understood how some possess differential resistance to atrazine, diuron, metribuzin, and other photosynthesis inhibitors. For example, some alterations are supersensitive to atrazine. In most of the resistant weeds, resistance to atrazine and metribuzin run parallel. Most triazine-resistant weeds are supersensitive to ioxynil and a few other PS II herbicides. Although all inhibitors compete with the native plastoquinone for binding in the Q_B site, it is clear that no common binding pattern exists, and each herbicide has to be studied separately.

Role of Herbicide Metabolism in Triazine Resistance

In a few cases, resistance in weeds has been reported to be due to more rapid detoxification of the triazine herbicide (Andersen and Gronwald, 1987; Andersen, 1988). Gronwald *et al.* (1989) and Anderson and Gronwald (1991) found that the chloroplasts were still PS II sensitive and that a 10- to 100-fold increase in triazine resistance in velvetleaf was nuclear encoded and was due to enhanced metabolism by glutathione-S-transferase (GST) to form N-dealkylation. This is the same mechanism responsible for the corn and sorghum tolerance to atrazine. Differential metabolism is one of the most important factors in determining crop selectivity and tolerance (Shimabukuro, 1985). Gray *et al.* (1995a) reported that triazine-resistant velvetleaf biotypes from Wisconsin and Maryland were about 100-fold more resistant to atrazine and simazine than the normal triazine-susceptible accession, but there was no cross-resistance or negative cross-resistance to other herbicides, including ametryn, cyanazine, metribuzin, and terbacil. Gray *et al.* (1995b) and Balke and Stoltenberg (1998) further found that none of the biotypes metabolized atrazine in their roots, as in the case of corn, but both the stem and leaves of triazine-resistant biotypes contained greater quantities of atrazine glutathione conjugates and its metabolites than did the susceptible velvetleaf plants. Burnet *et al.* (1993) found that triazine resistance in Australian rigid ryegrass was due to more rapid and complete metabolism of simazine by oxidative enzymes in the resistant biotypes.

Yerkes *et al.* (1996) reported that although chlorophyll fluorescence measurements and CO_2 assimilation in resistant jimsonweed leaves were affected within 1 day of atrazine application, they returned to normal (at rates and levels equivalent to those in untreated leaves) within 5 days. Atrazine-resistant jimsonweed was cross-resistant to simazine, but was susceptible to prometryn, metribuzin, terbacil, and other herbicides. Chlorophyll fluorescence was unaffected in triazine-resistant pigweed, which showed cross-resistance to some triazines, moderate resistance to metribuzin and terbacil, and negative cross-resistance to bentazon and pyridate.

In some triazine-resistant species where resistance is due to more rapid metabolism of the herbicide, the weeds develop resistance gradually and may be only slightly resistant. This is especially true with some of the monocot or grass weeds that are already partially inherently resistant to atrazine (Thompson *et al.* 1971; Gressel *et al.*, 1982, 1983). DePrado *et al.* (1995) found that fall panicum has the capacity for rapid detoxification, which is slightly greater in plants from fields that have been repeatedly treated with atrazine.

Control and Management of Triazine-Resistant Weeds

There are several reasons why triazine resistance has not led to serious and widespread weed infestations and why the triazines remain extremely effective weed control tools.

1. There is generally a lack of fitness or ability in the triazine-resistant biotypes to compete with the crop or with other nontriazine-resistant weeds as a result of the altered triazine binding site at the Dl protein in PS II.
2. There are several other herbicides with different modes of action and a broad range of activity that are used in combination with triazines. The acetanilide herbicides have been effective combination partners since they are often more effective on grass weeds, whereas triazines are more effective on broadleaf weeds. Herbicide combinations have become common practice for weed management in corn and sorghum and other crops to broaden the range of weeds controlled and to allow the use of lower rates of each individual herbicide in a mixture. There are no known cases of triazine resistance among *Amaranthus* (pigweed) or *Chenopodium* (lamb's-quarter or goosefoot) species where mixtures of atrazine and chloroacetanilide herbicides have been used, even though chloroacetanilides alone are not as effective at controlling these weeds. Chloroacetanilides apparently weaken the occasional triazine-resistant biotype, thus rendering the weed uncompetitive (Owen and Gressel, 2001).
3. Because of the major dependence of farmers on triazine herbicides, researchers, extension personnel, teachers, and industry rapidly developed management information and programs in order to avoid more serious and widespread problems.

For the reasons above, triazine-resistant weeds have been managed well. Growers have often continued to use herbicide combinations that include triazines, even where triazine-resistant biotypes are present, because of the many other susceptible weeds needing to be controlled. In some cases, however, where other classes of selective herbicides have not been available, economical, or effective, the crop of choice has been replaced with another crop due to triazine-resistant weeds. Lopez-Garcia *et al*. (1996) reported that when 14 corn-growing areas in Spain (i.e., Aragon) were resurveyed in 1994 – 2 years after 52 triazine-resistant biotypes of smooth pigweed, redroot pigweed and common lamb's-quarters had been found – many of the farmers had switched their farms to sunflower and alfalfa, thus providing a temporary solution to the spread of resistant weeds. Stephenson *et al*. (1990) reported that cultural and agronomic practices had a positive impact on limiting the occurrence and distribution of triazine-resistant weeds in Ontario. Crop and herbicide rotations and other integrated pest management (IPM) programs are the best solutions to avoid or delay herbicide-resistant weeds (Gressel and Segel, 1990a; Gressel, 1991).

Ritter and Menbere (1997) have reviewed the history and control of triazine-resistant weeds – especially common lamb's-quarters, smooth pigweed, barnyardgrass, velvetleaf, and giant foxtail – in the mid-Atlantic region of the United States. They concluded that the factors influencing the presence of the resistant weeds included lack of crop rotation and lack of herbicide rotation.

Common lamb's-quarters is one of the triazine-resistant weeds that occurs most frequently. Resistant varieties of common lamb's-quarters were first reported in western Washington in 1973 (Bandeen *et al*., 1982), but were found in the same year in Ontario and Austria. These resistant varieties since have been reported in fields in at least 40 US states, six provinces of Canada and 28 other countries. Several studies have included evaluations of effective weed control programs for triazine-resistant weeds including: common lamb's-quarters (Menbere and Ritter, 1995; Glenn *et al*., 1997; Ritter and Menbere, 1997, 2001); black nightshade and common lamb's-quarters (Himme *et al*., 1984, 1986; Bulcke and Desmet, 2005); *Amaranthus* species (Eberlein *et al*., 1992; Birschbach *et al*., 1993; Rola and Rola, 1996; Foy and Witt, 1997); kochia (Wicks *et al*., 1993, 1994); and American willowherb (Bailey and Hoogland, 1984, Himme *et al*., 1984, 1986). Other weeds that have frequently exhibited resistance to triazine herbicides include species of *Amaranthus* (pigweed). Twelve species of *Amaranthus* have been found to be triazine resistant in localized areas or fields, the most common being redroot pigweed and smooth pigweed.

As had been predicted by many weed scientists who understood the biological cause and nature of herbicide-resistant weeds, resistance to the ALS inhibitors and other newer herbicides developed more rapidly than resistance to the triazine herbicides.

Potential Risk from Cross-Resistance and Multiple-Resistance

Cross-resistant plants have the ability to survive herbicides from different chemical classes. Cross-resistance may be conferred either by a single gene or, in the case of quantitative inheritance, by two or more genes influencing a single mechanism. Two types of mechanisms for cross-resistance are:

1. Target site cross-resistance, in which a change at the site of action of one herbicide also confers resistance to herbicides from a different class (e.g., selection by triazine-resistant D1 protein that is also less sensitive to triazinones).

2. Nontarget site cross-resistance, in which a mechanism other than resistant enzyme target sites is involved (e.g., reduced herbicide uptake, translocation, activation, or enhanced herbicide detoxification).

The term 'negative cross-resistance' is used when a herbicide-resistant biotype is more sensitive to and more easily controlled by classes of herbicides other than the class to which it is resistant.

Multiple-resistance is when more than one mechanism conferring resistance to herbicides in different chemical classes is active in an individual weed or population of weeds. Plants with multiple resistance may possess two or more distinct resistance mechanisms. Two grass species that display both cross- and multiple-resistance are rigid (or annual) ryegrass and blackgrass (Hall *et al.*, 1994).

Some have reported intermediate resistance to metribuzin in triazine-resistant plants, and negative cross-resistance to other nontriazine herbicides (e.g., Yerkes and Weller, 1995). There are great variations in the levels of resistance within biotypes of various species and from various locations. These biotypes are often moderately resistant to metribuzin and other triazines, and often vary greatly in sensitivity to other herbicides. Arntzen *et al.* (1982); Fuerst *et al.* (1986); Van Oorschot and Van Leeurwen (1988); and others found that resistant factors (the ratio between the herbicide concentration in nutrient solution giving 50% growth inhibition in triazine-resistant versus triazine-sensitive biotypes) ranged from very high with atrazine (125–1500), intermediate with metribuzin (3–15), low with diuron (1–3), and almost always negative (less than 1) with pyridate, bromoxynil, ioxynil, and sometimes bentazon.

In order to better avoid and manage the spread of resistance, research efforts have focused on the classification of herbicide-resistant weeds according to herbicide class. Although there have been exceptions to the general rules governing herbicide resistance, generally a weed biotype that is resistant to one triazine herbicide (e.g., atrazine) will very likely have target site cross-resistance to other herbicides in the chemical class. There are occasionally varying degrees of sensitivity between the other triazine and triazinone herbicides. The classification of herbicides based on sites of action, reported by Retzinger and Mallory-Smith (1997), place the triazine herbicides in WSSA Group 5. Schmidt (1997) places the triazines in HRAC Group Cl. The WSSA group not only includes all conventional triazine herbicides, but also other herbicide classes including triazinones, uracils, pyridazinones, and phenyl-carbamates, all of which are considered to have common binding sites. Due to the great differences in crops, weed problems, and use patterns between the different herbicide classes, only resistance to the triazines, and to some extent the triazinones (e.g., metribuzin), are included here. Triazine-resistant biotypes may be resistant to the other herbicides in these groups. For example, Clay and Underwood (1989) found in cross-resistance studies that triazine-resistant biotypes (i.e., American willowherb, horseweed, annual bluegrass, and common groundsel) were more resistant to uracil herbicides compared to triazine-sensitive biotypes, but not to any other herbicide tested.

The first case of metabolic cross-resistance involving triazine herbicides was reported in rigid ryegrass following 10 consecutive years of single annual applications of atrazine plus amitrol on railway rights-of-way in western Australia (Powles and Matthews, 1992). These two biotypes of rigid ryegrass were also resistant to diuron, simazine, and metribuzin (Burnet *et al.*, 1993), due to an increased detoxification mechanism in this biotype. Walsh *et al.* (2004) reported multiple resistance in wild radish across at least three different modes of action, including the triazines.

Until the mid-1990s, multiple-resistance (i.e., resistance to more than one herbicide mode of action within the same biotype) had not been reported within North America. However, Foes *et al.* (1996) found a kochia biotype from western Illinois resistant to atrazine and several ALS-inhibiting herbicides. Lopez-Martinez *et al.* (1996) reported that a triazine-resistant *Echinochloa* species found in atrazine-treated corn also showed cross-resistance to quinclorac. Clay and Underwood (1989) and Clay (1989) reported that one triazine-resistant biotype of American willowherb was also resistant to paraquat from a hop garden in the United Kingdom treated annually for 25 years with simazine and paraquat.

Common waterhemp has increasingly become a very serious weed throughout the Corn Belt states. Triazine-resistant biotypes of this weed have been reported in the Midwest. Even more alarming, however, has been the rate at which biotypes resistant to ALS inhibitors have emerged. Common waterhemp is a dioecious species (separate male and female plants) that must cross-pollinate with nearby common waterhemp plants. This mandatory out-crossing maintains the genetic diversity within the species and may partially explain the rapid development of common waterhemp biotypes (Horak and Peterson, 1995; Anderson *et al.*, 1996a, b) that are resistant to triazine or ALS-inhibiting herbicides.

Foes *et al.* (1998) reported that a common waterhemp biotype not controlled by triazine or ALS-inhibiting herbicides was isolated from a field in Illinois in the fall of 1996. Patzoldt *et al.* (2004, 2005) have reported on a tall waterhemp biotype in Illinois that has multiple resistance to ALS inhibitors, PPO inhibitors, and atrazine in the same plants. Maertens *et al.* (2004) reported a smooth pigweed biotype from southern Illinois confirming multiple resistance to both atrazine and ALS inhibitors.

The development of these cross-resistant biotypes of waterhemp reduces the number of effective options a grower has to manage this difficult weed because of the widespread, increased, and repeated use of ALS-inhibiting herbicides. In addition there are reports of waterhemp biotypes resistant to glyphosate (Heap, 2006). Multiple resistance to both triazine and ALS herbicides has been confirmed in prostrate pigweed in Israel (Sibony and Rubin, 2004). The development of weed biotypes with cross or multiple resistance to herbicides shows the importance of resistance management programs.

A resistance management labeling initiative is an important part of an integrated approach to the prevention of herbicide resistance in weeds. Labeling initiatives by Canada's PMRA and by USEPA on resistance and rotating or mixing alternative modes of action are important milestones to managing herbicide resistance (Canada Pest Management Regulatory Agency Regulatory Directive, 1999; US Federal Register Notice, 2000).

Conclusions

Excellent progress has been made in the understanding of the cause, nature, genetics, mechanism and solutions of herbicide-resistant weeds since the first triazine-resistant common groundsel was reported more than 35 years ago. Resistance management programs have been extremely successful in controlling most weeds that have developed resistance to the triazine herbicides. However, research is critical to better understand the rapid increase and spread of many new weed biotypes resistant to several classes of herbicides.

Herbicide and crop rotations and mixtures of herbicides with alternative modes of action are essential for the management of weeds resistant to herbicides. The understanding of the site or mechanism of actions of herbicides and record keeping and planning are integral to the development of effective resistance management programs, which are key components of sustainable agriculture.

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Chapter 11

Weeds Resistant to Nontriazine Classes of Herbicides

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Summary

The triazines serve an extremely important role in controlling weeds resistant to other classes of herbicides. In recent years, there has been an extremely rapid growth in the number of biotypes that are resistant to nontriazine herbicides, especially to glyphosate and the acetolactate synthase (ALS) and acetyl coenzyme A carboxylase (ACC) inhibitors. The ALS and ACC inhibitor herbicides are now used frequently in corn, soybean, cereal grain, and in other crop rotations. In contrast to triazine-resistant weeds, these biotypes are more difficult to manage and are usually as competitive as the susceptible weeds. As a result, alternative herbicides or control methods can be much more costly than controlling weeds resistant to the triazines. The continuous use of glyphosate now occurs on an increasing number of crop acres. The expanded use of glyphosate-tolerant crops has contributed to the increase in glyphosate-resistant weeds. Several weed species have recently been discovered to have resistance to glyphosate. Without the triazines and other tools with alternative modes of action, the repeated use of 'single target site' herbicides such as ALS and ACC and now glyphosate on the same weeds can render the products useless, jeopardizing efficient agricultural systems.

Triazine-resistant weeds have been controlled successfully in many countries by the use of alternative herbicides often in combination with atrazine, but the number of weed species resistant to ALS-inhibitor herbicides now greatly exceeds the triazine-resistant species and is increasing much faster. By combining the Heap (2006) survey with Dr. LeBaron's global survey and a review of the literature, we find that there are at present 108 weed species with biotypes resistant to ALS inhibitors, including 70 dicotyledonous and 38 monocotyledonous weeds in 19 countries.

In addition, biotypes of 35 weed species are resistant to ACCase inhibitors in 26 countries. Heap warned that resistance to these herbicides in *Lolium* and *Avena* species threaten cereal production in Australia, Canada, Chile, France, Saudi Arabia, South Africa, Spain, the United Kingdom, and the United States. Twenty-one weed species are resistant to urea herbicides. Also of major economic importance are isoproturon-resistant little seed canary grass infesting wheat fields in northwest India and chlorotoluron-resistant blackgrass in Europe. Although 22 weed species are resistant to bipyridilium herbicides and 24 to synthetic auxins, the limited areas of their infestation and the availability of alternative herbicides have kept their impact minimal. The lack of alternative herbicides to control weeds with resistance to multiple herbicide classes, such as rigid or annual ryegrass and blackgrass, make these extremely challenging resistance problems.

Until recent years, it was speculated that glyphosate-resistant weeds would not be a major challenge. This was in part because of glyphosate's unique mode of action [inhibition of the 5-enolpyruvylshikimate-3 phosphate synthase (EPSPS) enzyme] and its metabolism and lack of soil residual activity (Padgette *et al.*, 1995; Bradshaw *et al.*, 1997). However, in 2002 Baerson *et al.* confirmed that a glyphosate-resistant goosegrass found in Malaysia in 1997 was the first evidence of an altered EPSPS enzyme as an underlying component of glyphosate resistance in plant species. Wakelin and Preston (2004) and Lorraine-Colwill *et al.* (2003) reported glyphosate-resistant populations that were 7-fold resistant, and reduced translocation of glyphosate appeared to be the major mechanism of resistance. Feng *et al.* (2004) also reported that glyphosate resistance in horseweed is likely due to reduced translocation, as well as reduced inhibition of EPSPS. Monquero *et al.* (2004) reported evidence that susceptibility to glyphosate in morningglory is influenced by translocation.

Strict, rapid, and sound herbicide-resistant management strategies will need to be implemented due to the increased adoption of glyphosate-resistant crops (Heap, 1999, 2006). Important reminders of this urgency are the discovery of glyphosate-resistant rigid ryegrass in Australia (Pratley *et al.*, 1996), California in 1998, South Africa in 2001 and France in 2005, Italian ryegrass in Chile in 2001 (Perez and Kogan, 2002), Brazil in 2003, Oregon in 2004, goosegrass in Malaysia (Lee and Ngim, 2000), and horseweed in Delaware (Van Gessel, 2001), followed quickly by similar reports from more than a dozen other states, and hairy fleabane and buckhorn plantain in South Africa

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(Cairnes and Eksteen, 2004). Hairy fleabane resistance to glyphosate has recently been found in Brazil and Spain, common ragweed in Missouri and Arkansas, giant ragweed in Indiana and Ohio, common waterhemp in Missouri, johnsongrass in Argentina, wild poinsettia in Brazil, and Palmer amaranth in Georgia (Heap, 2006). Triazine herbicides are integral to the weed management programs used to control weeds resistant to other herbicides.

Introduction

Herbicide-resistant weed biotypes have already had greater impact on agricultural technology and economics than resistance from all other pests. The future impact will be much greater if we do not properly manage weeds and the valuable chemical resources we have. Weeds require longer reproductive cycles, usually with a relatively low number of plants, and do not travel as far or as readily as insects and pathogens. Therefore, with the availability of several herbicides having different modes of action, we will be more successful in avoiding or managing resistant weed biotypes.

If multiple cross-resistance increases in major weed populations and leaves growers without effective herbicides, they will be forced to use mechanical and other weed management systems. Nalewaja (1999) advised that cultural practices, such as delayed crop seeding, tillage, black fallow, crop rotation, hand weeding, and competitive crops, provide an opportunity to reduce the selection pressure to herbicides. Since herbicides, on the other hand, reduce the pressure that causes selection of weeds that are highly adapted to particular cultural practices, rotation of different management programs should delay the development of resistance. The extent of the delay will depend upon the genetics of resistance, weed reproduction characteristics, weed seed survival, and fitness of resistant weeds. An understanding of the basic aspects of weeds and herbicides, as well as their interaction with the environment, will help predict the delay in resistance to an herbicide from use of cultural practices in the rotation. A grower's final choice of a weed control practice will involve risk of soil erosion, available equipment, timing, and agronomic impact of the resistant weeds.

Duke (1995) contended that resistant weeds have driven growers to develop and use integrated pest management (IPM) or integrated weed management (IWM) methods, such as biocontrol, cover crops, more crop and herbicide rotations, more selective use of herbicides, weed thresholds, etc.

Research is essential to inform growers of the cost of resistance and to predict with accuracy the risk of resistance to specific herbicides in weed populations and biological cultural systems and how best to use IWM for long-term weed management. Orson (1999) described a method of calculating the cost to farmers of herbicide resistance in weeds, with examples to demonstrate the inherent difficulties and benefits in preventing resistance. Prevention can cost significantly less than dealing with resistance once it fully develops. The true challenge is defining those cropping systems/practices and herbicide strategies that prevent the development of resistance.

Global Status and Importance of Herbicide Resistance

Distribution of Herbicide-Resistant Weeds

Biotypes of weed species are resistant to virtually all classes of herbicides previously used for their control. Most weeds resistant to triazine herbicides have appeared after the triazines alone were used for 8–10 years of consecutive treatments, sometimes much longer (Eleftherohorinos *et al.*, 2000; Gressel, 2002). Weed biotypes resistant to the ALS inhibitors have often been reported after only 3 to 5 years of repeated use, and in some cases after only 1 or 2 years (Kendig and Barrentine, 1995; Jeffers *et al.*, 1996; Lovell *et al.*, 1996b; Sprague *et al.*, 1997a; Hall *et al.*, 1998).

Weeds are rapidly becoming resistant to some of the newer herbicides, and it is important to realize the consequences. For example, in Missouri, Bader *et al.* (1995) conducted a study on corn–soybean rotations in which they used only ALS-inhibitor herbicides, that is, imazethapyr in soybean and primisulfuron-methyl in corn. Within 4 years, a common waterhemp biotype resistant to 5-fold higher rates of ALS inhibitors was flourishing. Greenhouse tests confirmed that ALS-resistant common waterhemp biotypes were present in several plots in the experiment. Within the same state, Bader *et al.* (1994) reported one case of atrazine-resistant common waterhemp, which developed where a farmer grew continuous corn and used only atrazine for more than 10 years. Table 11.1 shows trends in the numbers of herbicide-resistant weeds.

In Table 11.2, a listing of known herbicide-resistant biotypes is given according to the type of weeds (dicots and monocots), and herbicide class.

Table 11.3 gives a listing of the total number of all known herbicide-resistant biotypes in each of the countries where they have been reported.

Significance of Herbicide-Resistant Weeds

Data in our tables are not entirely in agreement with those reported by Heap (2006) for triazine-resistant and ALS-resistant weeds due to variations in data collection techniques. Also, some triazine-resistant biotypes, mostly within

Table 11.1 Number of weed species that have biotypes with resistance to major classes of herbicides over time[a]

Herbicide class/example	1981	1989	1996	2006
Triazines[b] (atrazine)	36	58	65	78
Ureas (diuron)	2	7	19	21
Phenoxys (2,4-D)	6	11	16	24
Bipyridiliums (paraquat)	5	13	21	23
Dinitroanilines (trifluralin)	2	5	8	10
Carbamates (triallate)	1	2	8	8
ACCase[c] inhibitors (diclofop)	1	5	18	35
ALS[c] inhibitors (chlorsulfuron)	0	11	52	108
Glycines (glyphosate)	0	0	0	12

[a] Compiled from Heap (2006) and LeBaron's surveys.
[b] First introduction in the late 1950s.
[c] First introduction in the 1980s.

Table 11.2 Occurrence of resistant weed biotypes to different herbicide groups up to 2006

			Resistant weed species		
Herbicide class/example	WSSA[a] code	HRAC[b] code	Dicots	Monocots	Total
ALS inhibitors (chlorsulfuron)	2	B	70	38	108
Triazines (atrazine)	5	C1	57	21	78
ACCase inhibitors (diclofop-methyl)	1	A	0	35	35
Bipyridiliums (paraquat)	22	D	15	8	23
Synthetic auxins (2,4-D)	4	O	17	7	24
Ureas/amides (chlorotoluron)	7	C2	8	13	21
Dinitroanilines (trifluralin)	3	K1	2	8	10
Thiocarbamates (triallate)	8	N	0	8	8
Glycines (glyphosate)	9	G	8	4	12
Pyrazoliums (difenzoquat)	8	Z	0	1	1
Chloroacetamides (metolachlor)	15	K3	0	2	2
Triazoles (amitrole)	11	F3	1	3	4
Chloro-carbonic-acids (dalapon)	26	N	0	1	1
Organoarsenicals (MSMA)	17	Z	1	0	1
Benzoflurans (ethofumesate)	16	N	0	1	1
Nitrites (bromoxynil)	6	C3	1	0	1
PPO inhibitors (oxyfluorfen)	14	E	3	0	3
Arylamino propionic acids (flampropmethyl)	25	Z	0	2	2
Carotenoid biosynthesis inhibitors (flurtamone)	12	F1	1	1	2
Mitosis inhibitors (propham)	23	K2	0	1	1

[a] Retzinger and Mallory-Smith (1997); Mallory-Smith and Retzinger (2003).
[b] Schmidt (1997).

the United States, had been confirmed by Ciba-Geigy but were never studied by others. Some of these resistant weed biotypes can no longer be found because of use of other herbicides or combinations. We have used a combination of these sources to compile the number of weeds that are or were at some time triazine resistant or ALS resistant, but we have used data from Heap (2006) for all other cases of resistance.

Heap (1999) accurately predicted that due to the economic importance of ALS and ACCase inhibitor herbicides worldwide and the ease with which weeds become resistant to them, it is likely that the weeds resistant to these herbicides will present farmers with greater problems in the next 5 years than triazine-resistant weeds have in the past 25 years.

In a review on the global status of herbicide-resistant weeds, Rubin (1996) concluded that the number of weeds possessing resistance to herbicides throughout the world would increase at a threatening pace. Resistance is usually associated with high selection pressure imposed by lack of herbicide and crop rotations. Although the majority of cases were found in intensive arable cropping systems where farmers rely heavily on herbicides for weed control, an increasing number of reports come from small farms and mixed farming systems. The major reported resistance mechanisms are altered herbicide target site, enhanced detoxification, and sequestration of the herbicide away from

Table 11.3 The number of herbicide-resistant weed biotypes in each of 52 countries in 2006

Country	Number	Country	Number	Country	Number
United States	113	South Africa	14	Ireland	2
Australia	47	China	13	Mexico	2
Canada	44	Poland	12	Portugal	2
France	31	Hungary	10	Sri Lanka	2
Spain	31	Chile	11	Ecuador	1
United Kingdom	24	New Zealand	10	Egypt	1
Israel	22	Denmark	7	Ethiopia	1
Germany	19	South Korea	6	Fiji	1
Belgium	19	Bolivia	5	India	1
Japan	17	Costa Rica	5	Indonesia	1
Switzerland	17	Norway	5	Kenya	1
Brazil	16	Thailand	5	Paraguay	1
Italy	16	Romania	4	Philippines	1
Malaysia	16	Colombia	3	Saudi Arabia	1
Austria	15	Sweden	3	Slovenia	1
Bulgaria	15	Turkey	3	Taiwan	1
Czech Republic	15	Argentina	2	Tunisia	1
Netherlands	14				

its target site. Resistance to ALS and ACCase inhibitors has developed at a much faster rate than resistance to triazine herbicides, indicating a high frequency of the resistance trait in weed populations.

Most of the ALS and ACCase herbicides have been introduced and used commercially only within the past 10–15 years and are often used repeatedly on the same land area. For example, several ALS inhibitors are used on corn, while others in this class are used on soybean. Even though the crops are rotated and different herbicides are used, the different herbicides have the same mode or site of action, which increases the selection pressure for resistant weed populations. Various ALS inhibitors are now being used in many crops, including corn, sorghum, soybean, and cereal grain.

ALS Inhibitor Resistance

Although the ALS inhibitor herbicides have been used for approximately 20 years, the number of resistant weed biotypes for this group now exceeds those for all other types of herbicides. Singh and Shaner (1995) and Boutsalis (2001) reported that a total of five chemical families or herbicide classes are commercially marketed as inhibitors of ALS, and that these herbicides comprise more than 50 active ingredients for selective use in many different crops. They include sulfonylureas, imidazolinones, triazolopyrimidines, sulfonylamino-carbonyl-triazolinones, and pyrimidinyl (thio)benzoates.

ALS inhibitor herbicides act on ALS (Shaner and Lym, 1991; Shaner, 1995), which catalyzes the first reaction in the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine (Holt *et al.*, 1993). All of these herbicides are in the Group B as defined by the Herbicide-Resistance Action Committee (HRAC) and in the Group 2 as defined by the Weed Science Society of America (WSSA). Branched-chain amino acids are essential for plant growth and development, and inhibition of their synthesis is lethal to many plant species. These herbicides have gained popularity in the agricultural community because of their high activity on many broadleaf weeds (such as common cocklebur, velvetleaf, and some grass species), low use rates, low mammalian toxicity, and in some instances, extended soil persistence (Beyer *et al.*, 1988; Brown, 1990; Newhouse *et al.*, 1991; Gerwick *et al.*, 1993; Saari *et al.*, 1994). Selectivity of these herbicides in crops such as soybean and wheat is based primarily on the plant's ability to metabolize the herbicide rapidly to nonphytotoxic forms (Sweetser *et al.*, 1982).

However, several weed species populations that were originally controlled with these herbicides have developed resistant biotypes. These ALS-resistant weeds are spreading faster, causing more economic impact, and presenting a greater risk to our present weed management systems than any other herbicide-resistant weeds, especially in the United States (Brown *et al.*, 1995). In all but a few instances, resistance to the ALS-inhibiting herbicides is due to a less sensitive ALS. In contrast to triazine resistance, target-site-based resistance to the ALS-inhibiting herbicides can be conferred by a number of different point mutations (Preston and Mallory-Smith, 2001). Weed biotypes that are resistant to an herbicide within one of these five herbicide classes or within the group may show reduction in control from others within the class or group. The level of this cross-resistance varies greatly, especially among the five classes, so cross-resistance is difficult to predict.

The HRAC, the joint chemical industry consortium working to avoid and manage weed resistance, is proactively involved in research and educational efforts. Jutsum and Graham (1995) reported that HRAC is supporting surveys, proposing management strategies, organizing educational initiatives, setting up monitoring programs, and sponsoring fundamental research. Legislation is also being introduced in different countries to delay resistance and to aid in the management of resistant weeds. Management techniques advocated by HRAC include the use of mixtures, alternating herbicide modes of action, and adopting specific cultural practices to improve the longevity of current herbicides.

The first ALS-resistant weeds were reported in 1987 when prickly lettuce (Mallory-Smith, 1990; Mallory-Smith *et al.*, 1990b) and kochia (Primiani *et al.*, 1990) control failures occurred in Idaho and Kansas, respectively, after 5 consecutive years of chlorsulfuron use. The kochia biotype proved to be cross-resistant to six other ALS-inhibitor herbicides, including sulfonylureas and imidazolinones. Within 5 years, sulfonylurea-resistant kochia had been identified at 832 sites in 11 states of the United States and in three Canadian provinces (Saari *et al.*, 1994). ALS inhibitor-resistant kochia and Russian thistle have become widespread problems in cereal-producing regions of northwestern United States and Canada. The mobility of these tumble weeds as plants with mature seeds or pollen carried by wind has undoubtedly contributed to the rate at which resistance has spread.

Numerous cases of resistance to the ALS inhibitors have now been reported in other broadleaf weed species, including pigweed and cocklebur, as well as grasses such as shattercane. Walsh *et al.* (2001) reported that only a few years after the first case of ALS-resistant wild radish, a major weed in Australian wheat fields, 21% of randomly collected wild radish populations were found to be resistant to chlorsulfuron. Patzoldt and Tranel (2002) reported that cloransulam resistance was found in an Indiana population of giant ragweed during the first year of that herbicide's commercialization in 1998, and that the resistant plants were cross-resistant to imazethapyr and chlorimuron. Since 1989, the number of species resistant to ALS inhibitors has increased almost 10-fold in crops and on roadsides. The total ALS-resistant weed species now number 108, as seen in Tables 11.4a and b).

Note that all common and scientific names of weeds in this book use as a primary source the *Composite List of Weeds – Revised 1989* by WSSA, 309 W. Clark Street, Champaign, Illinois (now P.O. Box 7050, Lawrence, Kansas, USA 66044). For all weeds not found in this source, the publication by Bayer A.G., *Important Crops of the World and Their Weeds* 1992 Second Edition, published by Business Group Crop Protection, Leverkusen, Federal Republic of Germany was used. For the few weed species not found in either of these sources, Richard Jensen at Brigham Young University Library, Provo, Utah, and the Internet were referenced.

Currently, there are six ALS-resistant weed species that can be found in one or more fields in 10 or more states, provinces, or countries. These weed species include: Palmer amaranth, common chickweed, common cocklebur, kochia, redroot pigweed, and common waterhemp.

The ALS inhibitors are at the highest risk for the selection of resistance in weeds because they have a single target site, are effective against a wide spectrum of weeds, are now used extensively on many crops, and are relatively persistent – often providing season-long control of germinating weed seeds (Brown *et al.*, 1995). Also, the various sites of mutations for resistance are not near the active site of the enzyme. As a result, there is no fitness loss due to a lower affinity for the normal substrates (Christoffoleti *et al.*, 1997).

Fitness of ALS Resistance

There are very few examples of biotypes resistant to herbicides other than the triazines that have reduced fitness. Data on the ALS inhibitor-resistant biotypes show that they are as equally fit and vigorous as the susceptible native populations (Thompson *et al.*, 1994; Jeffers *et al.*, 1996; Tierney and Talbert, 1996; Poston *et al.*, 2002). Poston *et al.* (2002) found that the susceptible biotypes of smooth pigweed displayed an advantage in vegetative growth and development over three out of four imidazolinone-resistant biotypes during the early stages of plant development, but competitive differences were not confirmed in the field. Several inheritance mechanisms appear to be involved in ALS-inhibitor resistance, but all cases involve an insensitive acetolactate synthase enzyme system (Saari *et al.*, 1990, 1994; Mallory-Smith, 1990b; Devine *et al.*, 1991; Christopher *et al.*, 1992; Thill *et al.*, 1993; Barrentine and Kendig, 1995; Eberlein *et al.*, 1997; Anderson *et al.*, 1998a; Manley *et al.*, 1999; White *et al.*, 2002). Ma *et al.* (1997) indicated that a combination of differential rates of translocation and metabolism may account for the differing insensitivity of these weeds at the whole plant level in common lambsquarters or common cocklebur, but not in sicklepod. Resistance to ALS herbicides is inherited by a single nuclear dominant or semidominant gene (Mallory-Smith *et al.*, 1990a). Since this resistance is dominant, both the heterozygous (RS) as well as the homozygous (RR) individuals are resistant.

Genetics, Mechanisms, and Spread of ALS Resistance

In Australia, two types of sulfonylurea resistance have been reported (Burnet *et al.*, 1994). Rigid ryegrass exhibited cross-resistance to certain sulfonylurea and imidazolinone herbicides following selection for resistance to other

Table 11.4a First discovery and distribution of ALS inhibitor (B-2) resistant broadleaf biotypes to date (2006)[a]

Dicotyledonous weeds (70)		
Common name	**Year found**	**Location[b]**
Amaranth, livid	1993	New Jersey[c]
Amaranth, Palmer	1991	Kansas[c]
Amaranth, Powell (or green pigweed)	1996	Ohio[c]
Ammannia, purple or long-leaved loosestrife	2000	California[c]
Azena (J)[f]	1995	Japan
Azetogarashi (J)[f]	1996	Japan
Beggarticks or amor seco (S)[f]	1996	Brazil
Beggarticks, hairy	1993	Brazil
Buckwheat, wild or climbing	1993	Queensland[e]
Chamomile, mayweed	1997	Idaho[c]
Chickweed, common	1988	Alberta[d]
Chrysanthemum, Garland or crown daisy	2000	Israel[(gffi2)]
Cleavers, false	1996	Alberta[d(gffi2)]
Cocklebur, common	1989	Mississippi[c]
Paterson's curse or salvation jane	1997	South Australia[e]
Dodder, field	1993	New South Wales[e]
Falseflax, smallseed	1999	Oregon[c]
Falsepimpernel, low	1995	Japan
Falsepimpernel major low	1995	Japan
Falsepimpernel, shortstalked	2000	Korea
Fleabane, hairy	1993	Israel
Fleabane, tall	1998	Spain
Flixweed	2005	China
Hempnettle, common	1995	Manitoba[d]
Horseweed	1993	Israel[(gffi2)]
Iceplant	2005	South Australia[e]
Kochia	1987	Kansas[c]
Lambsquarters, common	1994	Yugoslavia
Lettuce, prickly	1987	Idaho[c]
Limnophila	1997	Japan
Marigold, corn	1997	Ireland
Marshelder	2003	North Dakota[c]
Marshweed	2002	Malaysia[(gffi2)]
Marshweed, Asian	1996	Japan
Mustard, African or wild turnip	1992	Western Australia[e]
Mustard, ball	1998	Alberta[d]
Mustard, Oriental or Indian hedge	1990	South Australia[e]
Mustard, wild	1991	North Dakota[c]
Nightshade, eastern black	1999	North Dakota[c]
Oxtongue, hawkweed	2000	Russia
Parthenium, ragweed	2004	Brazil
pennycress, field	2001	Alberta[d]
Pigweed or quitensis(S)[f]	1996	Argentina
Pigweed, prostrate	1991	Israel[(gffi2)]
Pigweed, redroot	1991	Israel
Pigweed, smooth	1992	Kentucky[c]
Poinsettia, wild	1992	Brazil[(gffi2)]
Poppy, corn	1993	Spain[(gffi2)]
Radish	2001	Brazil
Radish, wild	1996	Western Australia[e]
Ragweed, common	1994	Yugoslavia
Ragweed, giant	1998	Illinois[c]
Redstem	1983	California[c]
Rocket, wall	2004	South Australia[e]
Sheepbush or karoobush	2004	South Australia[e]
Sida, prickly	1993	Georgia[c]
Sowthistle, annual	1990	Queensland[e]
Sowthistle, spiny	1996	Alberta[d]
Starwort, water	1993	China
Sunflower, common	1996	Kansas[c]
Thistle, Canada	1996	Hungary
Thistle, Russian	1987	Montana[c]

(*Continued*)

Table 11.4a (Continued)

(a) Dicotyledonous weeds (70)		
Common name	**Year found**	**Location[b]**
Toothcup, Indian or kikashigusa (J)[f]	1997	Japan
Turnipweed	1996	Queensland[e]
Turnipweed, African	1996	Queensland[e]
Velvetleaf	1994	Yugoslavia
Waterhemp, common	1993	Illinois[c(gffi3)]
Waterhemp, tall	2000	Michigan[c]
Waterhyssop, disc or ukiazene (J)[f]	2000	Malaysia
Waterwort or mizohakobe (J)[f]	1996	Japan

Table 11.4b First discovery and distribution of ALS inhibitor (B-2) resistant grass biotypes to date (2006)[a]

Moncotyledonous weeds (38)		
Common name	**Year found**	**Location[b]**
Arrowhead, California	1992	California[c]
Arrowhead, dwarf or urikawa (J)[f]	2004	Korea
Arrowhead-lily or swamp-potato	2000	Malaysia
Barley, wall	2005	Australia
Barnyardgrass	2002	Yugoslavia
Blackgrass	1984	United Kingdom
Brome, downy	1997	Oregon[c]
Bulrush, Japanese or inuhotarui (J)[f]	1997	Japan
Bulrush, ricefield	1993	California[c]
Bulrush, river	1997	Spain
Canarygrass, littleseed	1999	South Africa[(gffi3)]
Crabgrass, large	1993	South Australia[e(gffi2)]
Foxtail, giant	1996	Minnesota[c]
Foxtail, green	1996	Minnesota[c]
Foxtail, Japanese or setogaya (J)[f]	1996	China
Foxtail, robust white	1996	Minnesota[c]
Foxtail, Spanish or hatico (S)[f]	1988	Costa Rica
Foxtail, yellow	1997	Minnesota[c]
Goosegrass	1988	Costa Rica
Johnsongrass	2000	Texas[c]
Junglerice	1998	Costa Rica[(gffi3)]
Konagi (J)[f]	2003	Korea
Mizuaoi (J)[f] or moolokzam (K)[f] or monochoria, Japanese	1994	Japan[(gffi2)]
Monochoria	2003	China
Monochoria, arrowleafed	1999	Japan
Oat, sterile	2005	South Australia[e]
Oat, wild	1986	South Africa[(gffi2)]
Ryegrass, Italian	1991	Mississippi[c]
Ryegrass, perennial	1989	California[c]
Ryegrass, rigid or annual	1982	South Australia[e(gffi7)]
Sedge, smallflower umbrella	1992	California[c]
Shattercane	1994	Nebraska[c]
Sloughgrass, American	1995	China
Starfruit	1994	New South Wales[e]
Velvetleaf, yellow	1998	Malaysia[(gffi2)]
Waterplantain, common	1994	Italy
Windgrass or silky bentgrass	2005	Czech Republic
Fringerush, globe	2001	Brazil

[a] From the author's survey and files and the International Survey of Herbicide Resistant Weeds (www.weedscience.com).
[b] Location indicates where the species was first reported to be resistant to ALS inhibitors.
[c] States of the United States.
[d] Provinces of Canada.
[e] Provinces of Australia.
[f] Common name in: J ffl Japan, K ffl Korea, S ffl Spanish language.
[g] These biotypes show multiple resistance to X – modes of action (e.g., [g – 2] ffl 2 modes of action).

herbicides (Christopher *et al.*, 1991). The mechanism of resistance in one biotype involved enhanced detoxification without any change to the ALS protein. This detoxification mechanism is similar to that in wheat, which is sensitive to ALS inhibitors but has the ability to rapidly detoxify some ALS inhibitors – including chlorsulfuron, metsulfuron, and triasulfuron (Meyer and Muller, 1989). Both the resistant rigid ryegrass biotype and wheat are susceptible to sulfometuron, which is not rapidly metabolized by wheat. Thus, both wheat and this biotype are resistant only to the wheat-selective sulfonylureas and not to the nonselective analogues. Richter and Powles (1993) treated pollen from two herbicide-resistant biotypes of rigid ryegrass, one resistant to ALS inhibitors and the other resistant to ACCase-inhibitors. Pollen produced by resistant biotypes grew uninhibited when challenged with the respective herbicide, whereas the pollen from a susceptible biotype was inhibited. Since resistance to ALS inhibitors can be spread both by seed and pollen, once the initial incident of resistance occurs, the resistant subpopulation will dominate the population in a very short period of time.

Stallings *et al.* (1995a) studied the Russian thistle plant movement and seed dispersal in order to follow the spread of chlorsulfuron-resistant biotypes. They found that 48% to 68% of the Russian thistle seed were lost as the plants tumbled across the fields, and they were carried by varying winds from 60 m to more than 4 km. Pollen-mediated transfer of chlorsulfuron resistance in kochia was observed in greenhouse and field studies at frequencies of approximately 31% and 4%, respectively (Stallings *et al.*, 1995b). They showed that resistant kochia pollen can spread the sulfonylurea-resistant trait at least 30 m during each growing season. Although the initial frequency of resistance-conferring alleles for ALS inhibitors is not known, it is considerably higher than the frequency for the triazine herbicides.

Target-site insensitivity has been used to develop sulfonylurea-resistant crops. More than 12 resistant crop species have been introduced – including varieties of wheat, oilseed rape, soybean, tomato, and other crops – either by selection for resistant lines or by introductions of resistant ALS genes through transformation (Gressel, 2002; James, 2003). The level of resistance depends on the specific mutation in the ALS gene, the degree of resistant gene expression, and the herbicide used. Several mutations in the ALS gene have been identified that confer the resistance phenotype, but the gene most frequently used for transformation encodes ALS with a substituted amino acid at a proline, equivalent to Pro_{197} in *Arabidopsis thaliana*. With the prospect of increasing use of ALS-inhibiting herbicides, we should expect the more extensive evolution of resistant weed biotypes to follow quickly.

More than 80% of the soybean area in the northern Corn Belt is rotated with corn (Pike, 2002). A farmer who formerly rotated herbicides along with soybean–corn crop rotations can now use ALS inhibitors or glyphosate continuously. The same populations of weeds will, therefore, be exposed to the same herbicide chemistry year after year, increasing the probability of the evolution of herbicide-resistant weed biotypes. The situation is further exacerbated by the availability of sulfonylurea herbicides in both soybean and corn where they are registered for preemergence and postemergence applications on both crops. Use in this rotational pattern of ALS-products or glyphosate without a combination partner, such as atrazine in corn, or without rotating modes of action of herbicides, significantly increases the risk of herbicide resistance.

In studies with a sulfonyl-resistant biotype of redroot pigweed, the first weed in Israel to exhibit ALS resistance, Sibony *et al.* (2001) found it was cross-resistant to all other classes of ALS herbicides. From nucleotide sequencing, they concluded that a proline to leucine change in Domain A at position 248 is the only difference in the amino acid primary structure of the regions sequenced, indicating that it is responsible for all ALS inhibitor resistance observed.

Cross-Resistance to ALS Inhibitors

Christopher *et al.* (1992) reported that a chlorsulfuron-resistant rigid ryegrass in Australia was resistant to most other sulfonylurea and imidazolinone ALS inhibitors. However, a common cocklebur biotype resistant to several imidazolinone herbicides was not resistant to sulfonylurea herbicides (Saari *et al.*, 1994). It is, therefore, difficult to generalize as to patterns of resistance within the five classes of ALS inhibitors. Weed biotypes resistant to one herbicide will usually show some level of resistance to most herbicides within the same class, and may in addition show some resistance to ALS inhibitors in other classes.

Some of the above rigid ryegrass biotypes first developed resistance to an ACCase herbicide in WSSA Group 1 (i.e., diclofop-methyl) through its continuous use in wheat. They then showed nontarget site cross-resistance to chlorsulfuron and other ALS inhibitors (Matthews *et al.*, 1990; Christopher *et al.*, 1991). The basis of this cross-resistance is rapid metabolic detoxification, usually by cytochrome P_{450} monooxygenase or glutathione S-transferase (GST) enzymes (Cotterman and Saari, 1992; Christopher *et al.*, 1992), although in some resistant weed biotypes, more than one mechanism exists in the same biotype (Saari *et al.*, 1994). Burnet *et al.* (1994) reported that some ALS-resistant rigid ryegrass biotypes have at least two mechanisms of sulfonylurea resistance, which occur at different frequencies within the population.

In 2001, Llewellyn and Powles reported a survey of fields in the Western Australian Wheat Belt, conducted to determine the extent of rigid ryegrass resistance to commonly used herbicides (i.e., diclofop-methyl, clethodim, chlorsulfuron, and sulfometuron). Of the randomly collected populations, 46% exhibited resistance to diclofop-methyl and 64% to chlorsulfuron, with 37% exhibiting resistance to both herbicides.

In 1987, Moss first reported that a blackgrass biotype resistant to chlorotoluron and isoproturon (urea herbicides in WSSA Group 7) was also resistant to the ALS inhibitor chlorsulfuron. Menendez *et al.* (1997) also found that a chlorotoluron-resistant blackgrass biotype in Spain was resistant to ALS inhibitors (e.g., chlorsulfuron and imazamethabenz), and that the resistance was due to its greater ability to metabolize the herbicides.

The ALS inhibitor-resistant weed biotypes documented to date are often cross-resistant at varying levels to members of the same herbicide family or class, but they display varying patterns of cross-resistance to members of other ALS-inhibitor herbicide families or classes (Hall and Devine, 1990; Mallory-Smith *et al.*, 1990a; Powles and Howat, 1990; Primiani *et al.*, 1990; Devine *et al.*, 1991; Saari *et al.*, 1994; Horak and Peterson, 1995; Lovell *et al.*, 1996a, 1996b; Schmenk *et al.*, 1996; Manley *et al.*, 1998). Owen (2001) reported that about 75% of all ALS-resistant biotypes in North America are cross-resistant. Zelaya and Owen (2004) studied the nature of resistance and cross-resistance in several weeds to ALS inhibitors in Iowa. They concluded that resistance in shattercane, common sunflower, and giant ragweed developed independently with much variation in cross-resistance.

Gaeddert *et al.* (1997) found that cross-resistance of the ALS-resistant Palmer amaranth biotype occurred among 16 postemergence ALS herbicides evaluated. Sprague *et al.* (1995) found that two suspected resistant populations of Palmer amaranth and common waterhemp were resistant to imazethapyr and cross-resistant to the sulfonylureas, chlorimuron and thifensulfuron, at 10 times higher than labeled rates.

Ferguson *et al.* (2001) confirmed resistance to ALS inhibitors (imazethapyr or flumetsulam) in populations of Powell amaranth and redroot pigweed in Ontario. High-level cross-resistance to thifensulfuron was found in two populations of each species. On the other hand, Poston *et al.* (2001) found that a population of imidazolinone-resistant smooth pigweed was 10-fold more sensitive to cloransulam-methyl (another class of ALS inhibitors) than the sensitive population.

Devine *et al.* (1991) and O'Donovan *et al.* (1994) reported that chlorsulfuron-resistant chickweed populations were also resistant to other sulfonylurea herbicides. Primiani *et al.* (1990) reported cross-resistance to several sulfonylurea and imidazolinone herbicides in chlorsulfuron-resistant kochia. Lovell *et al.* (1996a) also documented that chlorsulfuron-resistant kochia biotypes from Idaho and Montana were cross-resistant to imazethapyr.

Sprague *et al.* (1997a), however, found that of five imazethapyr-resistant biotypes of common cocklebur, only four of these biotypes were also resistant to imazaquin. Ohmes and Kendig (1999) studied the crossing of ALS-cross-resistant and susceptible biotypes of common cocklebur and concluded that the cross-resistance trait is dominant to semidominant. In all cases, the resistance exhibited at the whole plant level was associated with an insensitive ALS enzyme.

After only 2 years of use of imazethapyr and thifensulfuron for weed control in Kansas soybean, poor control of pigweed species (e.g., Palmer pigweed and common waterhemp) was reported. Horak and Peterson (1995) confirmed that both species had developed resistance to these herbicides. Sprague *et al.* (1997b) reported that the imidazolinone-resistant common waterhemp was cross-resistant to other ALS inhibitors (i.e., thifensulfuron and chlorimuron). Lovell *et al.* (1996b) found that the imidazolinone-resistant common waterhemp biotypes showed a high degree of cross-resistance to sulfonylurea herbicides. Sprague *et al.* (1997b, c) further reported that these and other imazethapyr-resistant biotypes of both Palmer amaranth and common waterhemp were cross-resistant to other ALS inhibitors. Hinz and Owen (1997) also found that an ALS-resistant biotype of common waterhemp was cross-resistant to both imidazolinone and sulfonylureas herbicides, but not to lactofen. Finally, McNaughton *et al.* (2005) found distinct ALS mutations in geographically separated populations of redroot pigweed and Powell amaranth, suggesting simultaneous occurrences and that resistance to ALS inhibitors is easily selected in many weed populations.

Anderson *et al.* (1995, 1998a, b) reported that a primisulfuron-resistant shattercane biotype found after 3 years of consecutive treatments was cross-resistant at all rates of primisulfuron, nicosulfuron, and imazethapyr. Lee *et al.* (1999) found that when shattercane from three resistant accessions were intercrossed, all the F_2 populations were resistant to primisulfuron, indicating that the ALS-resistant alleles in the three accessions were at the same locus, or possibly linked loci. When the accessions were crossed with the wild type, comparisons between the F_1, susceptible, and resistant populations showed that primisulfuron resistance was expressed as a dominant, partially dominant, and additive trait.

Nandula and Messersmith (2001) found that a wild oat accession with metabolism-based resistance to imazamethabenz, an ALS inhibitor, was cross-resistant to flucarbazone-sodium (BAY MKH 6562).

Christoffoleti *et al.* (1996) reported that after 7 years of annual application of imazaquin to control weeds in soybeans in Brazil, imazaquin failed to control hairy beggarticks. Further field experiments with other sulfonylureas

show the hairy beggarticks were resistant to imidazolinone and sulfonylurea herbicides, and that no ALS inhibitors controlled the resistant biotypes. Smit and Cairns (2001) reported that chlorsulfuron-resistant wild radish, a major weed challenge in oil seed crop protection, has been found in South Africa.

Baumgartner *et al.* (1999) conducted studies to determine the cross-resistance of imazethapyr-resistant common sunflower to selected imidazolinone, sulfonylurea, and triazolopyrimidine herbicides. Whole plant herbicide dose–response curves and *in vitro* enzyme studies showed that the resistant common sunflower was also highly resistant to imazamox, slightly resistant to thifensulfuron and chlorimuron, but not resistant to cloransulam. Marshall *et al.* (2001) and White *et al.* (2001) studied the movement potential of imazethapyr resistance in common sunflower. Gene flow from resistant to susceptible biotypes occurred with movement up to 15.5 m. Mallory-Smith *et al.* (1999) reported that a downy brome biotype was resistant to primisulfuron after two applications during two successive years and was cross-resistant to sulfosulfuron. Park and Mallory-Smith (2004) observed that downy brome ALS-resistant biotypes from different locations in Oregon responded quite differently to some ALS inhibitors.

Multiple Herbicide Class Resistances in ALS-Resistant Biotypes

Multiple-resistance mechanisms, defined as resistance due to more than one mode of action or class of herbicide, have been reported in several ALS-resistant weed biotypes – including false cleavers, wild oat, common waterhemp, kochia, rigid ryegrass in Australia (Powles and Matthews, 1992; Preston and Mallory-Smith, 2001), and wild radish (Walsh *et al.*, 2004a).

Diebold *et al.* (2003) concluded that multiple resistance in a Powell amaranth biotype in Ontario was due to the presence of altered target sites for triazine and imidazolinone herbicides.

Hall *et al.* (1998) reported that an ALS-resistant biotype of false cleavers was cross-resistant to a broad range of ALS inhibitors, as well as to an auxin-type herbicide, quinclorac, which had never before been applied to these fields. A similar case of quinclorac multiple resistance in smooth crabgrass has been reported in California when plants were previously treated with ACCase herbicides. Data suggest a target site-based mechanism of resistance involving the accumulation of cyanide derived from stimulated ACC synthesis, which is a precursor of ethylene (Abdallah *et al.*, 2004).

Multiple resistance to two or more classes of herbicides within the same biotypes will be a great challenge for farmers and weed scientists of the future. At the *4th International Weed Science Congress* in Durban, South Africa, June 20–24, 2004 and at the 2004 and 2005 meetings of the WSSA, there were many reports of weeds evolving resistance to herbicides having several different modes of action, including resistance to ALS and ACCase inhibitors in *ryegrass* spp. in Italy (Bravin *et al.*, 2004); to ALS and ACCase in Italian ryegrass in Oregon (Perez-Jones and Mallory-Smith, 2004); to ALS and ACCase in rigid ryegrass in Greece (Kotoula-Syka *et al.*, 2004); to ALS and triazines in prostrate pigweed in Israel (Sibony and Rubin, 2004); to ALS, triazines and phenoxys in wild radish (Walsh *et al.*, 2004b) and to ALS, ACCase, and trifluralin in rigid ryegrass (Hawthorn-Jackson *et al.*. 2004) in Australia; to ALS and ACCase in *ryegrass* spp. (Pieterse and Kellerman, 2004); to ALS, ACCase, glyphosate, and paraquat in rigid ryegrass (Eksteen *et al.*, 2004) and to glyphosate and paraquat in rigid ryegrass and hairy fleabane (Cairns and Eksteen, 2004) in South Africa; glyphosate and ACCase in goosegrass in Taiwan (Chiang *et al.*, 2004); ALS, diuron, and metribuzin in kochia in Minnesota (Mengistu *et al.*, 2004); and ALS, triazines and PPO (lactofen) in tall waterhemp in Illinois (Patzoldt *et al.*, 2004). Patzoldt *et al.* (2005) reported further that this biotype from Adams County, Illinois, which is the first reported weed population in the United States with resistance to herbicides inhibiting three unique sites of action, showed resistance to all three herbicides in individual plants, not merely within the population.

Glyphosate-Resistant Weeds

About 80% of soybean planted in the United States in 2003 was glyphosate-tolerant soybean, treated with only glyphosate for weed control. In addition, more acres of corn are expected to be glyphosate-tolerant over the next years.

Glyphosate resistance in weeds has already been reported in many areas (at least 15 US states, three Australian states, and seven other countries) and in at least 12 weed species (Pratley *et al.*, 1996; Lorraine-Colwill *et al.*, 1999; Lee and Ngim, 2000; Van Gessel, 2001; Perez and Kogan, 2002; Heap 2006) as summarized in Table 11.5.

It has been easy to develop glyphosate-resistant lines in plant species and bacteria using various laboratory procedures (Dyer, 1994). Furthermore, there is much variation among plant species in their ability to metabolize or tolerate glyphosate. Climatic or soil conditions also influence glyphosate performance. Gressel (2002) has documented the intraspecific biotype variability in susceptibility to glyphosate at the whole plant and cellular level, as well as known mechanisms that could confer resistance. He warns that genetic variation occurring in crops will surely occur in some weeds. Dyer (1994) warned: 'Given human nature as it is, farmers may be tempted to rely exclusively on one resistant

Table 11.5 Discovery history of weeds resistant to glyphosate[a]

Dicotyledonous weeds			Moncotyledonous weeds		
Common name	**Year found**	**Location**	**Common name**	**Year found**	**Location**
Amaranth, palmer	2005	Georgia	Johnsongrass	2005	Argentina
Waterhemp, common	2005	Missouri	Ryegrass, Italian	2001	Chile
Poinsettia, wild	2005	Brazil	Goosegrass	1997	Malaysia
Ragweed, common	2004	Arkansas, Missouri	Ryegrass, rigid	1996	Australia
Ragweed, giant	2004	Ohio			
Fleabane, hairy	2003	South Africa			
Plantain, buckhorn	2003	South Africa			
Horseweed	2000	Delaware			

[a] From the International Survey of Herbicide Resistant Weeds (www.weedscience.com).

cultivar for successive years, with the accompanying temptation to apply multiple glyphosate treatments during the growing season to control successive weed flushes.' Such a trend is causing an increase in glyphosate resistance.

A biotype of rigid ryegrass from a field in Australia in which glyphosate had been used for 15 years failed to be controlled by recommended rates. This biotype exhibited resistance to three different salts of glyphosate and was nearly 10-fold more resistant compared to the susceptible biotypes (Powles *et al.*, 1998). Pratley *et al.* (1999) reported that glyphosate resistance in rigid ryegrass may be due to natural variability in the population. Because a biotype from Echuca, Victoria was found resistant not only to glyphosate, but also to diclofop-methyl, these researchers suggest that resistance by one herbicide has perhaps aided the evolution of resistance to others. Resistance in rigid ryegrass to selective herbicides already exists in most wheat fields of southern Australia (Matthews and Powles, 1996; Neitschke *et al.*, 1996b; Hawthorn-Jackson *et al.*, 2004; Neve *et al.*, 2004). Further work by Lorraine-Colwill *et al.* (2001, 2003) investigated the inheritance of evolved glyphosate resistance and the mechanism of resistance in *Lolium*. More recent work by Yu *et al.* (2007) confirmed multiple resistance of a *Lolium rigidum* biotype to glyphosate, paraquat, and ACCase herbicides. Glyphosate resistance continues to expand as noted by the evolution of resistant *Sorghum halepense* (johnsongrass) in glyphosate-resistant soybean (Vila-Aiub *et al.*, 2007). These studies have emphasized the importance of IWM and careful use of selective herbicides to preserve the efficacy of glyphosate, one of the most important herbicides in use today.

Comparing the EPSPS activity in both glyphosate-resistant and sensitive biotypes showed about 5-fold higher IC_{50} for the resistant biotype, apparently due to a change from proline to serine at position 106 in the EPSPS mature protein. Ng *et al.* (2004) concluded that glyphosate resistance in goosegrass is inherited as a single, nuclear, and incomplete dominant gene. In an earlier research report by Gruys *et al.* (1999) on glyphosate-resistant rigid ryegrass, the only difference detected in the resistant and susceptible biotypes was the basal activity of EPSPS, where the resistant lines had 2- to 3-fold greater activity.

Use of Weed Thresholds and IWM

Although economic thresholds are often used to make decisions on insect control and occasionally for plant diseases, the use of thresholds for IWM has been limited. Considerable research and effort have gone into measuring the levels of weed infestations that growers should tolerate instead of applying herbicides or other control measures. Many researchers have made recommendations for using weed thresholds as IPM in US agriculture (Shaw, 1982; Thill *et al.*, 1991; Buhler *et al.*, 1992; Hollingsworth, 1994; Buhler, 1996; Cardina *et al.*, 1996; Norris, 1999). Czapar *et al.* (1997) conducted surveys of growers, agricultural chemical dealers, and farm managers/rural appraisers in Illinois to identify limitations to grower acceptance of economic thresholds for IWM, and found that 9% used economic thresholds as a basis for weed control. They reported that the reasons for limited acceptance by growers include:

1. *Weeds interfering with crop harvest*. With fast and efficient modern harvesting equipment and with many acres to harvest in a short time, even a few weeds can be very problematic during harvesting.
2. *Weed seed production*. Growers know that most weed seeds produced in their fields this year will reinfest the same land and will have to be controlled next year and into the future. This is an even greater concern if the escaped weeds are resistant to the herbicide of choice.

However, in some other countries, especially in Australia where multiple- and cross-resistant rigid ryegrass has become such a serious and widespread problem in wheat and grain crops, weed thresholds and IWM are much more successful. Powles and Howat (1990) have researched and promoted various alternatives for managing herbicide-resistant rigid ryegrass, for use alone or in association with wiser use of herbicides. Neitschke *et al.* (1996a, b) emphasized that herbicide resistance will inevitably become more serious as a limiting factor in production of our major crops, and we must adopt strategic, long-term approaches to weed control, including IWM. Moss and Clarke (1994) designed improved control strategies for blackgrass, which is cross-resistant to several classes of ACCase-inhibitor herbicides in small grains in the United Kingdom. Wild oat, a major weed problem in small grains in western Canada, is resistant to several herbicides. Morrison *et al.* (1992) reported on possible options for managing this weed. Even a few resistant biotypes left in the field will quickly reinfest the area with many resistant weeds the next year. Also in glyphosate-resistant canola in western Canada, Upadhyay *et al.* (2006) found that the use of glyphosate at 50% rate when weeds were in the four-leaf stage was more frequently in the risk-efficient IWM strategy set for economic weed control across a range of canola prices.

Buhler (2002) concluded that the adoption of herbicide-based weed management systems enables relatively simple cropping systems and has facilitated changes such as earlier crop planting and increased farm size. The current challenge for producers is to manage herbicides and other inputs in a manner that prevents adapted species from reaching troublesome proportions. The challenge for weed scientists is to develop innovative, economical IWM systems that can be integrated into current and future cropping systems to bring a more diverse and integrated approach to weed management. Because of the diversity and plasticity of weed communities, weed management needs to be viewed as a continuous process. Successful IWM can include increased crop seeding rates, delayed seeding, weed seed capture at crop harvest, and rotating herbicides with different mode of action each year (Diggle and Neve, 2001).

Owen (2001) has written a summary of the past, present, and potential future use of IWM and other herbicide resistance management tactics in corn and soybean, especially within United States. He concluded that herbicides have been, and are likely to remain, the primary management tool for weed control and the management of herbicide-resistant weed populations in the foreseeable future. Growers who adopt alternative strategies for weed control incur a number of risks, including economic issues, time management, and relative efficacy compared to the traditional use of herbicides. The key to herbicide-resistant weed population management is to use multiple strategies that enhance the competitiveness of the crop against the weed population. Cultural and mechanical practices will be most effective when combined with the judicious use of herbicides in an integrated system. However, to be adopted, IWM programs must be economically sustainable.

Use of Modeling in Managing Herbicide Resistance

The rate of evolution of resistant weeds is based on several factors, including characteristics of the weed and herbicide, gene frequency, size and viability of the soil seedbank, weed fitness, herbicide potency, frequency and rate of application, and persistence in soil. Various attempts have been made to use modeling to determine the relative importance of these factors and to predict the probability of resistance, as well as to evaluate how to avoid, delay, or solve the problem (Gressel and Segel, 1990).

Richter *et al.* (2002) have reviewed the use of models to evaluate the dynamics of herbicide resistance and to develop suitable anti-resistance strategies. Herbicide resistance is impacted by a high initial frequency of resistance alleles in a population, out-breeding, dominance of inheritance, a short persistence of the seed bank in the soil, and the lack of a fitness penalty for resistant versus susceptible biotypes of a weed species, along with agronomic factors having a positive influence on weed development. The occurrence of herbicide-resistant weeds in a field usually means the loss of an effective control measure. This is particularly serious if resistance develops in species for which there are few if any effective alternatives. As a rapid increase in the development of herbicides with new modes of action is not likely, and since economic and environmental conditions often will not support cultural control measures or alternative cropping systems, it is important to manage resistance wisely in order to avoid further loss of herbicides.

Using a model to maximize strategies for herbicide-resistant blackgrass, Cavan *et al.* (2000) gave estimates on the effectiveness of various strategy options. Based on research with a long-term model for control of blackgrass and annual bluegrass, Munier-Jolain *et al.* (2002) concluded that threshold-based weed management strategies can be more cost-effective than spraying every year and may enable important reductions in herbicide use. However, the highest long-term profitability was obtained for the lowest weed level threshold tested.

Müller-Schärer *et al.* (2000) reviewed the progress made during 1994–1999 by 25 institutions within 16 European countries on biological weed control. These efforts were aimed at control of major weed species, including common lambsquarters, common groundsel, and species of pigweed, broomrape and bindweed in major crops, including corn and sugar beet. No practical control has yet been reached for any of the five target weeds, however, the authors concluded that potential solutions have been identified.

Triazines are Important Tools to Manage Weeds Resistant to Other Herbicides

Of all the control options, the triazine herbicides offer the greatest assistance where glyphosate and ALS-inhibitor herbicide-resistant broadleaf biotypes are occurring. This is especially true of atrazine in corn, sorghum, and sugarcane, of simazine in orchards and perennial crops, and of metribuzin in soybean and other crops. To prevent, delay, or manage the occurrence of herbicide-resistant weeds, it is important to rotate herbicides with different modes of action or to apply them in mixtures or in sequence to a single crop or succeeding rotational crops (i.e., soybean–corn–soybean). Atrazine, which inhibits photosynthesis, provides an effective alternative mode of action to that of ALS herbicides. Although other nonALS herbicides are registered for use in corn, none provide the combination of benefits provided by atrazine – including broad-spectrum, broadleaf weed efficacy, application flexibility, and maximum crop tolerance. Also, unlike some nonALS herbicides, atrazine is not associated with injury to nontarget plants by herbicide drift. Atrazine is the only nonALS herbicide labeled for preemergence and postemergence control of many confirmed ALS-resistant broadleaf weeds in corn.

The economics of mixtures and dual applications are very important to farmers. The treatment cost would include the cost of the ALS herbicide plus the cost of the nonALS product. Depending on the nonALS herbicide partner, treatment costs per acre could increase by 24% to 98%. These additional costs could be prohibitive to the grower, and the use of the ALS herbicides on the infested acres could decline significantly. Atrazine is the preferred nonALS herbicide alternative because it has preemergence and postemergence flexibility, does not have the shortcomings of other nonALS-alternatives, and is very economical.

Table 11.6 contains results from field trials of atrazine in combination with various ALS-inhibitor herbicides on six ALS-resistant biotypes. This pictorial representation of field data shows the efficacy level of atrazine, ALS-inhibitor herbicides, and atrazine in combination with the ALS herbicide. These trials clearly show the utility and need for atrazine in resistance-management strategies.

Atrazine is the only proven product for control of these six common and economically important broadleaf weed species with ALS-resistant biotypes in corn. Product labels for each of the ALS herbicides recommend tank mixtures with atrazine. When used in the corn–soybean rotation, atrazine use in corn breaks the continuous use of ALS-inhibitor herbicides and delays the spread of ALS-resistant biotypes. For example, Owen *et al.*, (1995) reported that none of the ALS herbicides controlled ALS-resistant common lambsquarters, but atrazine provided excellent control. Sprague *et al.* (1997c) reported excellent control with atrazine both preemergence and postemergence on ALS-resistant, cross-resistant, and susceptible biotypes of common waterhemp.

Tierney and Talbert (1995) found that the best treatments for control of both ALS-resistant and susceptible common cocklebur were triazine herbicides (i.e., atrazine, cyanazine, and metribuzin). Tonks and Westra (1997) reported that mixtures including ALS inhibitors gave no better control of ALS-resistant kochia from Colorado and Kansas than the nonsulfonylurea herbicides alone.

Table 11.6 Field performance of atrazine with and without ALS-inhibitor herbicides on confirmed ALS-resistant biotypes of six economically important broadleaf species in corn and sorghum

		Control of ALS-resistant biotypes[b]				
Application time	**Herbicides used**	**Kochia**	**Smooth pigweed**	**Palmer amaranth**	**Common cocklebur**	**Common & tall waterhemp**
Preemergence applications		(1)[a]	(3)	(3)	(2)	(4)
	Atrazine		Yes[b]	Yes	Yes	Yes
	ALS inhibitor[c]		No[b]	No	No	No
	Atrazine ┘ ALS inhibitor[c]		Yes	Yes	Yes	Yes
Postemergence applications					(3)	(3)
	Atrazine	Yes	Yes	Yes	Yes	Yes
	ALS inhibitor[c]	No	No	No	No	No
	Atrazine ┘ ALS inhibitor[c]	Yes	Yes	Yes	Yes	Yes
	ALS inhibitor[c]	No		No	No	No
	Atrazine ┘ ALS inhibitor[c]	Yes		Yes	Yes	Yes

[a] ( ) ffl Number of trial locations per species/application.
[b] Control class: No ffl control | 25% and Yes ffl control − 85%.
[c] Test dependent product ffl flumetsulam, halosulfuron, imazethapyr, primisulfuron-methyl, or prosulfuron.

As more acreage is planted with glyphosate-resistant crops, there will be a critical need for resistance management practices to be implemented. Several important weeds resistant to glyphosate have been managed in the past because farmers have rotated from glyphosate-tolerant soybean to nonglyphosate-tolerant corn, allowing the triazines to control resistant weeds from the soybean rotation.

Throughout western Canada and the central Great Plains of North America, volunteer wheat is becoming a more serious problem (Leeson *et al.*, 2005). This may become a special concern if the volunteer wheat is glyphosate-resistant (Harker *et al.*, 2005). There are also many examples of integration of traits from weeds into crops, and there is some evidence of spread from herbicide-resistant crops into weeds (Gressel, 2002).

Conclusions

Weed scientists and farmers readily agree that no herbicide, class of herbicide, or tool can ever manage all weed problems. Weeds are far too numerous, variable, and adaptable.

We are learning from experience and research that there are problems in crop production from repeated use of herbicides without mixing partners, or without alternating the use of herbicides with different modes of action. Not only do the 'resistant' weeds become problems, but they lead to additional applications of the herbicides, adding further expense and further pressure to the development of resistant populations.

Modern herbicides have revolutionized the efficient production of most agricultural crops, and they will continue to be essential in feeding our present and future population. Atrazine and the triazine herbicides are critical in the management of weeds resistant to alternative herbicides. We must continue to develop management strategies for triazines and other herbicides as essential tools for weed control in agricultural production.

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Chapter 12

The Use of Economic Benefit Models in Estimating the Value of Triazine Herbicides

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Summary

The US Environmental Protection Agency's (USEPA) Special Review process for evaluating the benefits and alternatives for a product includes an estimation of 'lost benefits' or total costs if a product were not available to farmers. There have been more than 10 economic and biological studies to assess lost benefits if the triazine herbicides were not available. Some of the differences in value estimates arise because different baseline herbicide use patterns are utilized. Baseline crop acres and yields are usually similar. However, several of the studies were not national in scope for the major crops of corn and sorghum. Most of the studies examined costs and benefits of triazine herbicides on corn and sorghum, and not on other crops. The process of selecting the replacement herbicides and nonherbicide weed control methods varies significantly for some of the studies. For example, the Comprehensive Environmental Economic Policy Evaluations System (CEEPES, 1993, 1994) studies from the Iowa State University Center for Agriculture and Rural Development projected a very low use of dicamba, 2,4-D, and bromoxynil postemergence and a high use of EPTC and acetanilides.

Another major difference among past studies was the treatment of aggregate analysis and 'other costs.' The Battelle (1989, 1993) studies included extra cultivation costs and extra yield penalties on no-till corn. Unlike other studies, one US Department of Agriculture study (USDA, 1993) increased unit prices of the replacement herbicides, which would be expected if a major product were no longer available. Increased unit prices of the replacement herbicides, which would be expected if a major product were no longer available, were not included in most studies. Assessments were also conservative because other costs associated with replacement herbicides were not included, such as drift damage, phytotoxic effects, or costs associated with weed resistance. Several of the studies (NAPIAP, 1992; Morrison *et al.*, 1994; Pike *et al.*, 1994) did not include any aggregate analysis. This means that the studies did not include price changes for the crop or farmer and consumer adjustments to these price changes. The differences in aggregate economic models used also help explain some of the differences among study results.

The review of past studies points out the importance of having clear and accurate estimates of baseline herbicide use, inclusion of all costs, accurate estimates of yield changes, a clear and comprehensive method for selecting replacement herbicides by region, and a good aggregate analysis that includes farmer supply response to yield and price changes that would occur if a major herbicide were no longer available.

The Ciba Crop Protection models and results submitted to USEPA in 1995 through 1997 in response to the USEPA Special Review of triazines (Novartis, 1997; Ciba Crop Protection, 1995, 1996) are also summarized. In this case, results from the biological and economic components of the model have been published (Carlson, 1998), so this discussion centers on a few of the major changes versus 10 earlier studies. The yield effects on no-till corn were estimated from field trials where no-till, atrazine, and atrazine-substitute herbicides were used. No-till corn acreage may also decline if atrazine use is restricted, and if it does, this will mean an increase in farmer costs in terms of extra tillage trips and an increased potential for soil erosion. Drift damages were estimated for dicamba and 2,4-D based on a large farmer survey that provided data on frequency and extent of damage on soybean.

Other modifications of the aggregate analysis in the Ciba studies included showing costs to 17 other crops, estimating siltation costs associated with the reduced use of no-till corn, and running the models with new herbicides and with a phase-out of the federal farm program. These results show that these components of costs can change overall results substantially. The Ciba studies show that we must take a careful look at both biological and economic impact if we are to assess accurately the range of likely costs if key herbicides are not available.

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The most recent USEPA (2003) assessment on the benefits of atrazine estimated an annual value of $1.58 billion in the United States for field corn alone, based only on yield decreases and the increased cost of alternative herbicides.

Study Components

Large numbers of methods are available to forecast what will happen if a major herbicide is no longer available in some or all crop markets. Models for assessing the most likely farmer responses to removals of pesticides range from simple 'expert opinion' on costs of replacement pesticides on a given acreage base, to elaborate models with yield and cost changes entered into other models to estimate the impact on both farmers and consumers. To understand why there are wide differences in estimates for the costs and benefits of a product, we must understand the specific components of these models.

At least five major components (or sub models) are involved in estimating the costs of removing a product from a specific crop. These include: (a) estimates of baseline use of the targeted pesticide(s); (b) estimates of the amounts of replacement pesticides currently available (and that might be available in the next 5 years) and nonpesticides (e.g. tillage) that will be used if a product is not available; (c) reliable estimates of changes in crop yields for each replacement pesticide; (d) other cost changes associated with the change in available pesticides; and (e) an aggregate model that will sum the effects across all replacement pesticides, regions, and crops to estimate the costs to farmers, consumers, and taxpayers. More elaborate models, data, and methods are important for the evaluation of high-use pesticides, such as the triazine herbicides.

Past Triazine Studies if Atrazine were Unavailable

Between 1986 and 1994, there were at least 10 national or regional evaluations of the costs of not having the triazine herbicides available in the United States. Some of these studies consider other policy options, such as restrictions of atrazine only or restrictions of the triazines on only certain crops or regions. These past studies were used as background in a USEPA special review of the triazine herbicides on all crops, which was initiated in 1994.

The 10 separate studies are listed in Table 12.1. The authors, time of completion, crops included, and aggregate economic costs based on atrazine or triazine availability are shown when they were available. Because most of the atrazine used is on corn and sorghum, these studies focus on these crops.

The studies have very different cost estimates for the loss of availability for either atrazine or a triazine. The atrazine loss costs range from $458 million (regional) to $3.277 billion annually for corn and sorghum, while the triazine loss had cost estimates of $912 million to $3.350 billion annually. Differences in estimated effects depend upon input data, model types, and factors, such as different geographical regions and government policies.

Table 12.1 Estimates of aggregate economic effects of atrazine and triazines from various studies published prior to 1995

Study	Crops[a]	Atrazine not available (millions of dollars per year)	Triazines not available (millions of dollars per year)
Osteen and Kuchler (1986)	C, S	ffi780	ffi3350
NAPIAP (1992)	C, S	ffi813	ffi1246
Battelle (1989)	C, S	ffi1521/ffi3277[e]	–
Battelle (1993)[b]	C, S	ffi1983	–
CEEPES (1993)[b,c,f]	C, S	ffi715	ffi955
CEEPES (1994)[b,f]	C, S	ffi458	ffi993
Ribaudo and Bouzaher (1994)	C, S	ffi665	–
Danielson *et al.* (1993)	C	ffi635	–
Pike *et al.* (1994)[d]	C	ffi679	ffi912
Morrison *et al.* (1994)[g]	S	ffi58	–

[a] Crops included: C is corn, S is sorghum.
[b] Study includes total gain of taxpayer cost for program payment savings.
[c] Excludes gains to foreign consumers of US exports.
[d] Does not include consumer or aggregate effects beyond the farm gate.
[e] Without and with inclusion of growing use of conservation tillage.
[f] The CEEPES studies focused on 15 Midwest states and estimated atrazine use ranged from 37.6 to 38.9 million pounds, compared to estimates of 53 to 63 million pounds in other studies.
[g] Does not include all sorghum-growing states.

There are also many similarities and interconnections among most of the 10 studies. The earliest study (Osteen and Kuchler, 1986) and several others (NAPIAP, 1992; Danielson *et al.*, 1993; and Pike *et al.*, 1994) used similar information compiled by similar groups of weed scientists. However, the information for the Osteen and Kuchler study was based on conditions and herbicides available in 1985, while the latter three studies used information from 1992 to 1993. The two Battelle studies (funded by Ciba Crop Protection) and the three CEEPES-based studies (CEEPES, 1993, 1994; Ribaudo and Bouzaher, 1994) are more distinct because they used different farm level data and different aggregate models. CEEPES was designed to evaluate various agricultural policy tradeoffs in 15 Midwest states. The three triazine studies based on the CEEPES model were partially financed by USDA and USEPA.

Baseline Conditions

One of the major differences among the past studies is the set of assumptions and the estimates about base or starting production conditions prior to the proposed pesticide cancellation. For detailed benefit assessments it is desirable to have the average pesticide use, crop acreage, and crop yield that would prevail over the next 5 years without a cancellation. Usually, 5-year average crop yields and crop acres as estimated by USDA are utilized without assuming any trend over time. Baseline crop yield is usually not controversial, but crop acres and herbicide use are.

Table 12.2 gives the baseline level of atrazine used in the United States on corn and sorghum as estimated in the various studies, if they were available in acres treated and pounds. An estimate by Gianessi and Anderson (1994) is also shown for comparison purposes. The 52.8 million A (21.4 million ha) treated (about 65–70% of corn grown) and the 60.47 million lb (22.5 million kg) used in corn should be given high credibility, because they agree both with atrazine sales information in the 1990s and with estimates from large surveys of growers by private research firms. In 2003, it is estimated by the National Agricultural Statistical Service (NASS) that approximately 68% of corn acres are treated with atrazine.

Except for the CEEPES studies, total annual use of atrazine in the United States was estimated to be in the 50–63 million lb range (23–29 million kg). The low estimate of total atrazine use in the CEEPES studies (37.6–38.9 million lb; 17.1–17.6 million kg) may be due to a smaller area of corn production being evaluated (15 states). However, the aggregate model used by CEEPES [AGSIM (Taylor, 1993)], assumes that the baseline area of corn to be evaluated is the entire national acreage of 72.5 million A (29.4 million ha).

The Morrison *et al.* (1994) study utilizes the 1992 NASS farmer survey of pesticide use, but they do not include all sorghum-growing states. Likewise, the CEEPES 1994 study includes 4.1 million lb (1.9 million kg) applied to sorghum, even though the 1992 NASS survey shows that 5.25 million lb (2.4 million kg) were applied in Nebraska, Kansas, and Texas alone. Additional surveys show about 8 million lb (3.6 million kg) applied on about 65% of the sorghum area planted, or about 8 million A (3.24 million ha) treated.

Yield and Cost Changes

In the triazine benefits studies, there have been three methods used to estimate the on-farm economic effects. These are: expert opinion of weed specialists, expert opinion combined with farmer surveys, and the CEEPES method. NAPIAP (1992), Danielson *et al.* (1993) Morrison *et al.* (1994), and Pike *et al.* (1994) follow the first method, while Battelle (1989, 1993) uses the second method.

Table 12.2 Treated acres (A) or hectares (ha) and pounds (or kg) of atrazine used in the United States on corn and sorghum as estimated in various studies published prior to 1995

	Areas treated (million)				Pounds or kg a.i. (million)			
	Corn		Sorghum		Corn		Sorghum	
Study	A	ha	A	ha	lb	kg	lb	kg
NAPIAP (1992)	39.50	16.0	3.80	1.5	50.60	23.0	4.10	1.9
Pike *et al.* (1994)	44.11	17.9	–	–	50.10	22.7	–	–
Danielson *et al.* (1993)	42.86	17.4	–	–	53.57	24.3	–	–
CEEPES (1994)	54.34	22.0	3.47	1.4	37.60	17.1	4.10	1.9
CEEPES (1993)	50.30	20.4	2.10	0.9	38.90	17.7	3.30	1.5
Battelle (1989, 1993)	40.74	16.5	6.18	2.5	63.15	28.7	8.28	3.8
Gianessi and Anderson (1994)	52.83	21.4	7.95	3.2	60.47	27.5	7.90	3.6
Morrison *et al.* (1994)	–	–	5.02	2.0	–	–	5.08	2.3

Table 12.3 Yield and herbicide cost changes per area of crop grown if atrazine and the triazines were not available (various studies published prior to 1995)

		Atrazine not available		Triazines not available	
Study	**Crop**	**% yield**	**$ costs (A)**	**% yield**	**$ costs (A)**
NAPIAP (1992)	Corn	ffi1.98	5.70	ffi5.28	3.04
	Sorghum	ffi12.13	4.36	ffi12.56	4.36
Pike *et al.* (1994)[a]	Corn	ffi2.68	4.15	ffi4.46	2.24
CEEPES (1994)	Corn	ffi1.19	1.08	ffi2.60	3.09
	Sorghum	ffi3.43	3.10	ffi10.26	3.04
CEEPES (1993)	Corn	ffi2.80	6.70	ffi4.10	8.25
Battelle (1989)[b]	Corn	ffi6.10	5.52	–	–
	Sorghum	–7.03	–	–	
Battelle (1993)[c]	Corn	ffi10.63	21.40	–	–
	Sorghum	–7.03	–	–	
Danielson *et al.* (1993)	Corn	ffi3.00	6.73	–	–
Morrison *et al.* (1994)	Sorghum	ffi5.70	–	–	–

[a] For consistency in Table 12.3, and because the aggregate models evaluate cancellation costs per area of corn grown, the treated area figures of Pike *et al.* (1994), have been adjusted downward by multiplying by the percent of corn treated with atrazine or triazines.
[b] No yield or cost change effects for atrazine on conservation tillage assumed.
[c] Based on acreage or hectare weighted average across USDA regions at the conclusion of the reduced tillage adjustments.

Table 12.3 gives the national estimates for corn and sorghum acreage of percent yield change and dollar cost per acre or hectare for eight of the studies. The expert opinion method as followed by Pike *et al.* (1994) asks weed scientists in each state to give the number of treatment acres by type of herbicide that would be used to replace atrazine (or all triazines) for the area currently treated. After the number of herbicide applications is compiled, the acre treatments are multiplied by their respective retail prices. This provides a total expenditure and the change in total herbicide expenditure relative to that for the triazines, which includes application costs. Likewise, the experts are asked to estimate percent yield losses on atrazine or triazine-treated areas with use of the replacement herbicides. This lost corn production is multiplied by a constant price per bushel. The baseline atrazine- or triazine-treated areas at the state levels are summed to obtain national yield loss and cost change figures per acre or hectare currently treated with atrazine or triazine.

The Battelle (1989, 1993) studies made three modifications to the expert opinion approach. First, a farmer survey was used to find which replacement herbicides would be used if atrazine were canceled. The resulting changes in cost were computed using estimated retail prices of herbicides. Yield changes based on expert opinion were used together with the estimates of replacement herbicides from the farmer survey. Second, the Battelle (1989, 1993) studies included a cost increase to account for more cultivation on 60% of the area now treated by atrazine. Finally, they found from weed scientist studies that there could be a larger yield penalty from not having atrazine on conservation tillage land. They used the USDA estimate of increases in conservation tillage to estimate likely conservation tillage area.

The yield penalty that Battelle researchers used was an additional 3.2% on reduced tillage and 8.8% on no-till corn based on one experiment (Battelle, 1993). The data in Table 12.3 (Battelle, 1989) were developed when the first two adjustments were made. The 1993 study added a third effect, namely that the yield penalties will also occur on estimated future no-till corn areas. The high total in cancellation costs that Battelle found in a 1993 study can be partly traced to the extra yield losses that would likely occur on no-till corn. Subsequent field trials do show a larger yield reduction in no-till corn than in conventional tillage.

The CEEPES (1993) model describes farmer weed control choices with approximately 500 herbicide and tillage options. The expert opinions of Iowa state economists and weed control specialists were used to obtain the yield and cost changes shown in Table 12.3 for the entire country. The description of the CEEPES model does not explain how replacement herbicides were chosen. However, the actual herbicides chosen are given, and they are very different from those in the Pike *et al.* (1994) and Danielson *et al.* (1993) studies. The state weed scientists in the Pike *et al.* (1994) and Danielson *et al.* (1993) studies estimated that cyanazine, dicamba, 2,4-D, and bromoxynil would replace 80% of the atrazine on corn, while the CEEPES 'experts' said that these four herbicides would only replace 36% of

the atrazine. They placed a much higher reliance on alachlor, metolachlor, EPTC, and bentazon as replacements. In addition, whereas the weed scientists in the other studies believed that the number of replacement herbicide applications will need to be greater than the number of atrazine applications, the CEEPES modelers estimated that there would be an 11% decrease in replacement herbicides applications.

The percent yield loss and per acre or hectare cost changes are shown in Table 12.3. Due in part to the low estimate of atrazine pounds used, the CEEPES model resulted in a 1.19% yield loss and a $1.08 extra production cost per acre ($2.66/ha) of corn grown, which is very small as compared with those by Battelle (1989, 1993) and is less than half of the effects shown by NAPIAP (1992), Danielson *et al.* (1993), and Pike *et al.* (1994). More recently, USEPA (2003) estimated that without atrazine in corn, there would be a yield reduction of 8.8 bu/A, or a 6.4% reduction based on 2001 estimates of 138 bu/A. The total increased production cost estimated by USEPA for corn was $28.31/A, based on decreased yield and increased herbicide cost.

The CEEPES (1993, 1994) and NAPIAP (1992) studies show a higher yield and herbicide cost penalty for loss of atrazine or the triazines on sorghum than on corn. This is due to the fact that there are fewer substitute herbicides registered for use on sorghum.

Other Cost Changes

The loss of availability of a major herbicide like atrazine or all triazines would seriously change weed management options. Extra cultivation costs were included in the Battelle (1989, 1993) studies. None of the studies included any costs of drift damage from use of alternative herbicides in close proximity to other, susceptible crops. Drift costs, extra yield losses, and other costs on no-till corn were included in the Ciba Crop Protection and Novartis Crop Protection studies described later in this chapter.

Other costs which were identified but not included in many of the studies, primarily because of the difficulty in quantifying their effect, are: costs from increased weed resistance to herbicides replacing the triazines; direct labor and management costs of developing and using new weed control practices; and increased erosion damage costs, such as siltation of lakes, subsequent water recreation reduction, and lower land productivity. The economic impact due to weeds becoming resistant to the triazines was found to be minor.

The Osteen and Kuchler (1986) study included an assumed 25% increase in unit prices in replacement herbicides in one scenario.

Aggregate Analysis

Morrison *et al.* (1994) and Pike *et al.* (1994) do not consider any adjustments by corn farmers to higher costs and lower yields following a triazine cancellation. However, farmers indeed can adjust crop areas, and livestock producers and other consumers of corn and sorghum can adjust their feeding practices, to respond to changes in crop production and costs. When production of corn falls or its demand is higher than supply, its price will increase. Farmers may switch some land into soybean if profits from corn decline. Farmers in some regions have different weed control and alternative cropping patterns than farmers in other regions. For these reasons it is necessary to see how yield and cost change in each region will affect crop-area allocation and animal feeding choices. In addition, farm programs in the past have had higher taxpayer costs when crop production is high and prices are low. Therefore, when there are production losses due to a product no longer being available, potential savings to taxpayers need to be accounted for in a complete social cost evaluation of the cancellation policy.

All of the studies reviewed here except Danielson *et al.* (1993), Morrison *et al.* (1994), and Pike *et al.* (1994) use some version of the aggregate economic model known as AGSIM (Techsim, AGSIM-1, AGSIM-2) developed by C. Robert Taylor (Taylor, 1993) at Auburn University. This is a 10-region, econometric-simulation model of the supply and demand for major field crops and livestock products in the United States (Taylor, 1993) and has been used to assess many pesticide cancellation scenarios and other national policies that affect agricultural production and demand.

The particular version of the AGSIM model used does affect the results. AGSIM-1 improved the simulation of farmer behavior as compared with Techsim because it had more realistic supply equations and explicitly included responses to the federal farm program provisions. The higher estimates of triazine cancellation costs in the Osteen and Kuchler (1986) study are partly due to using Techsim. The movement to AGSIM-2 improved the demand equations to reflect the growing importance of international trade. For example, in the 1993 Battelle study switching from AGSIM-1 to AGSIM-2 lowered costs from $3.3 billion to $1.98 billion, while using the same regional yield and cost changes. As indicated in Table 12.1, the Battelle (1993) and CEEPES (1994) studies include taxpayer savings from lower price supports in the aggregate economic effects.

The Ciba Crop Protection and Novartis Crop Protection studies use the AGSIM-2 model. In addition, these studies have shown that a comprehensive study should include all regions and all crops. The baseline herbicide use pattern is based on those weeds that are controlled in all areas on all crops. The Ciba Crop Protection and Novartis Crop Protection studies include cost changes on 19 other crops besides corn and sorghum. In addition, yield changes on sweet corn and popcorn are included because the weed control problems are similar to those in field corn.

The Ciba and Novartis Benefits Studies

During 1995–1997, the information from past studies, almost 5000 field trials, new surveys of growers, and a weed control model developed by Dr. David Bridges (Bridges, 1998) were used to provide USEPA with estimates of the economic benefits of triazine and atrazine on all crops labeled with these products in the United States. A summary of the economic and biological models and some of the components are available (Bridges, 1998; Carlson, 1998).

A concerted effort was made to include additional information in this analysis, along with yield and herbicide cost changes. A farmer survey was available to quantify drift damage from increased use of dicamba and 2,4-D on corn. An effort was made to include all potentially available new herbicides. This was done by running the complete model in all 10 regions with and without new herbicides as potential replacements. In addition, the AGSIM model (Taylor, 1993) was run both with an assumption of a termination of the federal farm program payments, as well as with a continuation of programs. This meant that eight different scenarios were run through the AGSIM model for national corn and sorghum as follows: with atrazine not available or with the triazines not available, with and without farm program payments, and with and without new herbicides.

Compared with the previous studies, the other major changes in the Novartis Crop Protection (1997) and Ciba Crop Protection (1995, 1996) studies were the inclusion of off-farm costs and costs to several minor acreage crops where the triazines are used. In addition, these studies used actual field research data from thousands of trials to estimate yield differences.

The direct weed control costs (yield and herbicide cost changes) are included in the analysis. The cost changes related to drift damage from the replacement herbicides used on-corn and the on-farm costs from reduced no-till corn are also accounted for. The sums of the drift damages, no-till corn trip costs, and weed control costs were entered into the AGSIM model because these are the on-farm costs and yield changes that farmers observe. Effects on farmers, consumers, and taxpayers from the yield and cost changes over the next 5 years were simulated to estimate the corn and sorghum sector costs. To the corn and sorghum sector costs, the off-farm sedimentation costs related to reduced conservation tillage and the changes in weed control costs on 17 other crops were added.

Corn and Sorghum Yield and Weed Control Cost Changes

This comprehensive biological and economic study for the field corn and sorghum sectors included large amounts of data to give separate cost estimates if atrazines or triazines were not available. University weed scientists' efficacy ratings on all major herbicides on all weed species, unit costs of all herbicides and cultivation treatments, acres or hectares planted, weed densities, and current herbicide use patterns were compiled for each of the 10 USDA production regions as described by Ciba Crop Protection (1995) and Bridges (1998). Almost 5000 university and Ciba Crop Protection field trials were compiled in these analyses.

Ciba and university experimental results from large numbers of herbicide trials provided an alternative source of herbicide efficacy data. This information, combined with yield loss relationships, gave yield and treatment cost changes from using replacement herbicides and/or cultivation for either an atrazine or triazines. To obtain regional estimates of yield and cost changes if triazine alternatives were used, the current utilization data for atrazine and all triazines in eight distinct market niches were assembled from Ciba and Maritz Marketing Service surveys. The eight market niches were: atrazine-pre, atrazine-post, atrazine combinations-post, atrazine-broad spectrum, cyanazine and atrazine, cyanazine-pre, cyanazine post, and simazine.

The baseline areas for which the triazines were used were allocated to the best (most profitable) substitute herbicides based on profit rankings and current use shares of the substitutes. Herbicide combinations including all two-way and three-way combinations were used in the profit rankings of herbicides. This was done by market niche and region for each of the four scenarios. Weighted-average yield and cost changes using area shares as weights were then computed for each region for the scenarios where either atrazine or triazines were not available.

Table 12.4 gives the corn and sorghum yield and cost changes for three of the major regions if the triazines were not available. The same analysis was conducted for all 10 USDA regions. The regional analysis allows one to model the different farmer adjustments to no triazines for the particular weed species, crop yields, and use patterns of triazine and other herbicides found in each region. For corn, all regions – except for one minor area – will have higher herbicide

Table 12.4 Regional US corn and sorghum yield and cost changes if atrazine were not available[a]

	Corn			Sorghum		
	Herbicide Cost Change ($/A)	**Yield Change (bu/A)**	**Net Return Change ($/A treated)[b]**	**Herbicide Cost Change ($/A)**	**Yield Change (bu/A)**	**Net Return Change ($/A treated)[b]**
Northern Plains	ffl3.04	ffi3.30	ffi17.72	ffi2.97	ffi4.44	ffi6.75
Corn Belt	ffl3.52	ffi5.64	ffi23.90	ffi4.49	ffi8.54	ffi15.65
Lake States	ffl1.50	ffi2.45	ffi18.79	ffi4.49	ffi8.54	ffi15.65

[a]Data taken in part from Tables 1 and 2 in Carlson, 1998.
[b]Net return based on $2.30 per bushel per acre of corn and $1.90 per bushel per acre of sorghum and includes additional costs as documented in Novartis Crop Protection 1997.

costs and lower yields. Of course, the national effects are most heavily influenced by the changes in the Northern Plains, Corn Belt, and Lake states where most of the corn is grown. The analysis for sorghum is simpler because atrazine is the only triazine currently used.

Conservation Tillage Cost and Yield Changes

The basic yield and cost change evaluation was based on conventionally tilled corn and sorghum. However, much of the corn area (about 41% and increasing over time) receives some form of conservation tillage. There is evidence that the loss of the triazines would impact no-till corn more than conventionally tilled corn. It also would slow or reduce the use of conservation tillage, which is effective in preventing soil erosion. The two primary, quantifiable effects on growers are: (a) an increase in direct tillage costs and (b) an increase in yield losses on no-till corn.

Tillage trips across the field are much higher for conventionally tilled corn relative to that in conservation tillage. The USDA surveyed farmers in the 10 major corn-growing states and found that the average trips per acre are 3.47 for conventional tillage (weighted average of land using and not using moldboard plow), and 1.17 for conservation tillage (1.50 for corn in ridge-till and 1.10 for no-till corn) (Bull *et al.*, 1993; USDA, 1993). According to Doane's farmer surveys (Doane Ag Services, 1994), these trips had a cost of $6.80/A each ($16.80/ha). Therefore, based on 1994 data, land under conventional tillage would have direct tillage costs that are $15.64/A ($38.62/ha) higher than if the land were under no-till.

The amount of land that would be in no-till without the triazines or atrazine is difficult to estimate. Farmer surveys show that the percent of corn under no-till has increased from about 5% in 1989 to 18% in 1994 (CTIC, 1994) to 20% in 2004 (CTIC, 2004). Continued increases are expected, eventually reaching about 30% of all corn acres if the triazines are available. Without atrazine or the triazines, no-till production will not be as attractive to farmers and may in fact be impossible in some cases. A conservative assumption would be that acreage under no-till would decline to 15% if triazines were not available.

Two factors help explain why there will be a decline in no-till corn if the triazines are not available. First, there is a higher dependency on the triazines for no-till corn versus conventionally tilled corn (Carlson, 1998). The dependency of no-till corn on the triazines is an average of 1.20 treatments per acre of corn grown, which is 57% higher than that on land that receives moldboard plowing. In addition, conservation tillage is not only related to conservation benefits. In fact, there has been a faster increase in conservation tillage in land that is not highly eroded.

Secondly, relative to alternatives, the yield gains from triazine weed control are higher on no-till corn than on conventional till. Simple average yields were computed across more than 2000 university field tests, where tillage and herbicides were the two main means of weed control. Changes in yield were then computed by tillage system, comparing treatments using and not using atrazine. The results show a yield advantage with atrazine that increases in less intensively tilled systems. The no-till corn had an 11.4% higher yield (425 field tests) when atrazine was used compared to alternative herbicides, whereas the conventionally tilled corn had a 4.3% (1630 field tests) advantage when atrazine was used (Carlson, 1998).

The regional weed control cost changes were expanded to include extra costs from drift damage and additional trips across the field when triazines were not used on no-till and mulch-till corn. The estimates of cost and yield

changes resulting if atrazine and all triazines were not available were then input into an aggregate analysis using the AGSIM model.

Aggregate Analysis for Ciba and Novartis Models

The AGSIM model (Taylor, 1993) has equations projecting the yield and area planted for 12 major field crops in the 10 USDA production regions of the United States. When there is a change such as a loss of a herbicide, the economic effects are traced through the economy. Lower yields per acre and reduced aggregate corn production mean higher unit prices for corn in the feed, industrial, and export markets. Higher production costs for corn or sorghum relative to alternative crops will lead farmers to allocate more land to alternative crops, such as soybean or wheat. All of these effects are evaluated in the model for each year following the loss of a product or tool. Farmers growing crops and feeding livestock adjust to the changes in feed prices with production in the following years. These changes are carried out year-by-year for 10–12 years beyond the time a product is not available to farmers.

An analysis of average farm income with and without an herbicide available allows us to estimate the costs both to farmers producing the 10 crops and to all livestock producers. One of the largest costs without triazines is an increase of about 12% in corn feed costs and a 14% to 18% increase in sorghum costs. Because of this, net income to livestock producers falls by about $800 million ($777 million to $836 million) per year. The largest livestock cost increases are for hog producers ($192 million to $208 million), but those producing fed cattle, dairy, and broilers will lose more than $100 million per year (Carlson, 1998). Final consumers of meat, milk products, and other products made from corn and sorghum (ethanol, cereals, corn sweeteners, etc.) will also have higher costs if the triazines are not available. However, Farm Program subsidies and disaster payments to farmers could potentially be reduced due to decreased production.

Minor Crop and Off-Farm Effects

There are yield and weed control costs in other commodities besides corn and sorghum. Sweet corn and popcorn have similar weed pests, but fewer herbicide substitutes than field corn. Of course, these crops are more valuable crops per area than field corn, so losses per acre are large relative to field corn. Both yield and cost of production changes were computed for sweet corn and popcorn using the same agronomic and analytical approach as for field corn. Sweet corn losses are estimated to be $80.5 million and $62.4 million if triazines or atrazines were not available, respectively.

Herbicide cost changes were also estimated for 17 other crops. These costs are largest for sugarcane and citrus growers. The large number of commodities where simazine and atrazine are used on relatively large portions of the areas grown makes the losses in these so-called 'minor acreage crops' fairly substantial. The sum of the extra herbicide costs for these 17 commodities (and that from sweet corn and popcorn) result in losses estimated to be $160.8 million without the triazines and $96.1 million without atrazine (Novartis Crop Protection, 1997; Carlson, 1998).

Other costs included in the Ciba and Novartis assessments are the off-farm costs associated with the reduction in area devoted to no-till and ridge-till corn. Economists have studied the damage to freshwater recreation, water storage, navigation, flood control, and water treatment from soil sedimentation from agriculture. A summary of these studies by Smith (1992) finds off-farm costs that average 4.6% of gross crop value per area. Applying this figure to the conservation tillage area results in an off-farm cost of $15.27/A ($37.70/ha) in conventional tillage rather than no-till, and $5.34/A ($13.19/ha) using conventional tillage rather than ridge tillage.

If atrazine or triazines were not available, the total of the off-site costs was estimated to be $155.4 million, and $188.3 million, respectively. This represents costs across the US corn crop for the no-till and ridge-till systems. More details of these calculations are available in studies by Ciba Crop Protection (1995, 1996) and Novartis Crop Protection (1997).

Total Costs if Atrazine and Triazines were not Available in the United States

The overall costs to farmers, consumers, and taxpayers if either atrazines or triazines were not available are extensive. Table 12.5 gives a summary of net costs per year over the first 5 years beginning in 1996. In this case the results from the scenarios with a federal farm program in place were averaged with the case when there are no farm program payments. This average scenario was an attempt to model the federal farm program effects if they were phased out over 7 years.

The sum of corn and sorghum sector costs, off-farm costs and costs to minor crops is $1.66 billion without triazines and $1.47 billion without atrazine. This includes the savings to taxpayers from lower farm program payments, based

Table 12.5 Annual costs if the triazine herbicides were not available in the United States[a]

Costs	Triazines not available	Atrazine not available
Corn and sorghum sector	$1.30 billion	$1.20 billion
Off-farm sedimentation	$188 million	$155 million
Minor crops	$161 million	$96 million
Total costs	$1.66 billion	$1.47 billion

[a] Average of the 'with and without Farm Program' scenarios. Data from Table 7 of Carlson (1998).

on estimates of the farm program provisions. In addition, there are substantial costs to minor crop farmers, increased costs to local citizens faced with more soil sedimentation, increased costs to farmers in avoiding herbicide drift and increased tillage costs for no-till and ridge-till corn. These estimates depend upon comprehensive weed density data, yield damage models and extensive university field trial data on relative herbicide efficacy. The AGSIM model (Taylor, 1993) allows the inclusion of costs to consumers, livestock producers, and taxpayers across most field crops.

Conclusions

The review of triazine benefits studies shows the extensive data collection and analytical effort that is needed to carry out a credible benefits assessment. For the loss of an herbicide used on many crops, and which makes up a large part of the agricultural economy, it is essential to look at effects beyond the farm gate. In this case, losses are substantial because of lower weed control, higher herbicide costs, and indirect costs related to drift damage, sedimentation damage, and losses in reduced tillage corn. In 2003, the USEPA estimated an annual value of $1.58 billion in the United States for corn (USEPA, 2003).

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Chapter 13

Benefits of Triazine Herbicides in Corn and Sorghum Production

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Summary

The introduction of the triazine herbicides in the late 1950s revolutionized weed control in corn and grain sorghum. Probably no other crop production discovery, certainly no other herbicide discovery, transformed agriculture to the extent that atrazine did. By the mid-1960s atrazine had attained a record share of the corn herbicide acreage. In fact, it has been the leading corn herbicide almost since the day it was introduced. Farmer reliance on, confidence in, and loyalty to atrazine is apparent in its remarkably stable presence in the market. Today, almost 50 years since its introduction, approximately 65–70% of US corn acreage is treated with atrazine. Detailed analyses of the use and benefits of triazine herbicides in US corn and grain sorghum production were conducted during the 1990s. These analyses all show that atrazine is irreplaceable to US corn and grain sorghum growers. 'Alternative herbicides' are generally either less effective, more expensive, or pose greater crop injury risks. It has been the often-stated objective of agricultural research scientists to discover, develop, and register products as efficacious and as safe as the triazines. This is a worthy goal, but one not yet accomplished. New herbicides have entered the marketplace, but not as true triazine replacements. Rather, the new herbicides are commonly premixed or tank mixed with atrazine.

The Early Years: 1954 Through 1969

Advances in crop production technology were numerous during the 20th century, and none were more spectacular than the advances in weed science. Until the middle of the century farmers relied almost exclusively on cultural and mechanical approaches to weed control. Weed control involved good sanitary practices, planting quality, weed-free crop seed, and clean tillage or harvesting equipment to prevent the spread of weeds from one field to another. Good cultural practices, such as crop rotation and the use of fallow periods, were used to reduce weed populations. Advancements in farm mechanization provided affordable tractors, tillage, and cultivation equipment that greatly improved the farmer's weeding capability. While these improvements provided some relief from the extensive and laborious hand weeding and tillage of corn and grain sorghum, the hoe was used frequently up to the mid-1960s.

The last half of the 20th century ushered in the agricultural chemical age. The post-World War II introduction of 2,4-D gave many American farmers their first experience with selective weed control, an innovation that truly revolutionized farming in cereal grains and other grass crops. No doubt, the introduction of 2,4-D afforded American corn farmers a weed control tool that was previously beyond their dreams. The major annual increases in corn yields during the late 1940s and 1950s were probably largely attributable to the introduction of DDT and 2,4-D (Decker, 1964). However, a more significant revolution was yet to come, and yields would continue to rise dramatically during the late 1950s and 1960s due to the triazines and other selective herbicides for corn and sorghum (Figure 13.1).

Atrazine was registered in 1958 and its versatility helped revolutionize farmers' thoughts about weed control. It was the most dependable preemergence herbicide used in corn in 1960, yet it also had very good postemergence activity. Weather conditions during the days following application did not have to be perfect. And, most importantly, corn tolerance was excellent. Corn injury that was sometimes associated with 2,4-D use was not an issue with atrazine. Great changes were seen in corn and sorghum production during the first decade of triazine herbicide use. Buchholtz (1962a) reported that in 1950, 4 years after 2,4-D was introduced, less than 3% of Wisconsin's corn crop was treated with an herbicide. By 1960, shortly after the introduction of simazine and one season into the atrazine

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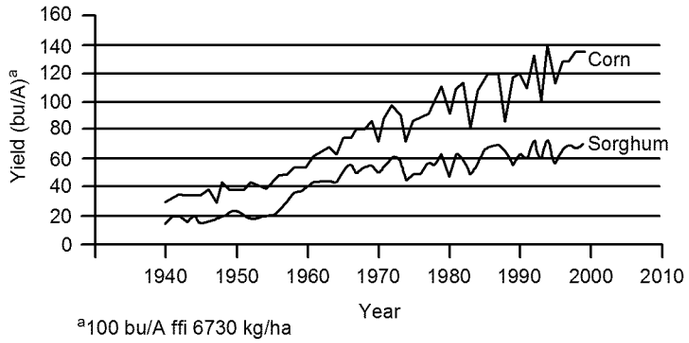

Figure 13.1 Average annual US corn and sorghum yields during the 20th century [adapted from United States Department of Agriculture (USDA), National Agricultural Statistical Service (NASS) data].

era, nearly 36% of Wisconsin's corn crop was treated with an herbicide. Within 2 years of atrazine's introduction, it was becoming an integral part of corn production. Buchholtz (1962a) reported that atrazine with one cultivation increased net returns as compared to several cultivations alone in each of 16 experiments. Thus, atrazine became the standard herbicide for corn worldwide.

The level of weed control achieved in corn with atrazine during the early 1960s was unprecedented. Despite the weed control successes with 2,4-D during the late 1950s and early 1960s, grass weeds like quackgrass (*Elytrigia repens* (L.) Nevski), were becoming increasingly difficult to control. However, with atrazine farmers could readily control this troublesome and widespread perennial cool-season weed. In Buchholtz's (1962b) article, *How to Get the Best of Quackgrass*, he reported:

> 'The control obtained with favorable applications of atrazine appears to be complete enough so that reinfestations will be very slow. The spring following treatment in areas to which atrazine has been applied are often bare of quackgrass.'

The usefulness of atrazine for corn weed control was recognized and reported frequently by weed scientists during the early 1960s. In a summary to the North Central Weed Control Conference, Slife (1964) reported:

> 'Atrazine is so effective in the majority of this region (Southern part of North Central Region) that there is no other compound that gives the spectrum of weed control and the results that atrazine does.'

At the same meeting Behrens (1964) reported:

> 'Atrazine controls most species of annual weeds of the area when used in preemergence or very early postemergence applications.'

Because of its cost, reliability, and superior performance, atrazine's adoption was swift. Surveys of Illinois corn farmers showed that in 1961 approximately 1% of Illinois corn received atrazine, but only 8 years later in 1969, 33% of the crop received atrazine. Average corn yields rose dramatically during the late 1950s and 1960s. What role did improved weed control and the triazine herbicides play in this rise in corn yield? Decker (1964) provided an interesting analysis of corn yield changes in the United States during this period:

> '…the upward trend was evident before 1946, but appeared to be temporarily accelerated slightly at that point. However, the abrupt climb seemed to start about 10 years later when the average yield for the United States jumped from 40.6 bushles per acre (bu/A) (2730 kg/ha) in 1955 to 64.1 bu/A (4310 kg/ha) in 1962, thus affecting in 7 years a rise equal to or greater than that attained in all of the previous 90 years for which records were available. To whom or to what are we to credit this phenomenal burst of progress?'

In partial response to his own question, Decker acknowledged the contributions that hybrid seed corn, fertilizer, and improved insect control technologies made to corn production. He noted that the increased use of insecticides played a role, but he also noted that this unprecedented rise in corn yield coincided with the introduction of the triazine

Table 13.1 Opinions on weed control technology during 1959 and 1962 in the United States (adapted from Shaw, 1964)

		No. of states rating	
Question	**Rating**	**1959**	**1962**
Effectiveness of preemergence herbicides?	Good	15	34
	Fair	15	7
	Poor	2	1
Effectiveness of postemergence herbicides?	Good	24	31
	Fair	13	13
	Poor	0	0
Herbicide usage trend?	Up	37	42
	Stabile	1	3
	Down	0	0
Need for better herbicides?	Urgent	21	11
	Little	17	32

herbicides and the associated improvements in weed control. In his address to the North Central Weed Control Conference (Decker, 1964) he remarked:

> 'In view of this correlation, the entomologist might be carried away with over-enthusiasm if he were not aware the use of herbicides and the use of fertilizers increased at the same time, in fact, at about the same relative rate.'

The dramatic impact of changing weed control technologies during the early 1960s was eloquently captured in Shaw's (1964) paper, *Weed Science – Revolution in Agricultural Technology*. Shaw reported on United States Department of Agriculture (USDA) surveys conducted during 1959, and another survey conducted in 1962, after the third year of atrazine use. These surveys showed remarkable changes in weed control practices; in fact, the use of preemergence herbicides had nearly tripled from 1959 to 1962. Shaw's paper also showed that attitudes about weed control in corn had changed rather dramatically (Table 13.1). Respondents from 15 states reported that preemergence herbicides for corn were 'good' in 1959. That number more than doubled by 1962, when respondents from 34 states reported that preemergence herbicides for corn were 'good.' More states were reporting increasing use of herbicides in corn. The most telling evidence of change from 1959 to 1962 was the change in opinion regarding the need for better herbicides. Twenty-one states reported an 'urgent' need for better corn herbicides in 1959, but only 11 states reported this same need in 1962.

Reliance on the triazine herbicides for weed control in grain sorghum came later and with somewhat less enthusiasm due to more marginal selectivity and soil persistence in low rainfall areas (Burnside *et al.*, 1964). Nonetheless, numerous scientific articles report on the research and development of atrazine and propazine for weed control in grain sorghum. Phillips (1964a) reported that several triazines had been evaluated for weed control in grain sorghum and that good crop tolerance and weed control were achieved with preemergence applications of propazine and postemergence applications of atrazine. Injury was reported at certain rates in some trials in sandy or light soils where atrazine was used preemergence, but propazine was safe to sorghum when applied preemergence. Burnside *et al.* (1964) found atrazine selective when applied preemergence to grain sorghum on the heavier soils of Nebraska, and superior to postemergence applications of atrazine. Their research showed that atrazine plus propachlor provided selective and broad-spectrum weed control in grain sorghum, and this became the herbicide treatment of choice for more than a decade. Over time, though, the slow but steady increase in triazine-resistant weed species forced weed scientists to initiate new weed control research in sorghum. Subsequently, newer acetanilide herbicides began to replace propachlor in the mixture, and these are presently used with atrazine as standard herbicide mixtures for the northern grain sorghum-growing region in the United States.

Phillips (1964b) and Wicks *et al.* (1969) found that atrazine would not only provide selective weed control in grain sorghum, but also controlled weeds during the subsequent fallow year without affecting winter wheat in a wheat–grain sorghum–fallow rotation in central and western Kansas and Nebraska. This use of atrazine in the grain sorghum rotation and in wheat–corn–fallow has been termed chemical fallow or ecofallow and is widely utilized in the Great Plains of the United States and in semi-arid regions around the world. This practice changed the winter wheat–fallow

rotation program, which had long been a popular way of conserving moisture while producing a crop of wheat every other year under dry land conditions. Thus, atrazine changed crop production in these semi-arid regions from one crop every other year to the production of two crops every 3 years. Also, winter wheat–corn–fallow rotation was used in western Nebraska, eastern Colorado, and western Kansas where rainfall or moisture is not adequate to grow corn or sorghum each year. Atrazine continues to be the backbone of these cropping systems, resulting in increased crop yields and production dependability and in improved economics. Atrazine produces these results by conserving moisture through superior weed control and reduced tillage, since tillage tends to dry out the soil in the tilled zone.

The Middle Years: 1970 Through 1994

While the late 1950s and early 1960s gave agriculture the discovery and introduction of the triazine herbicides, the latter part of the 1960s, the 1970s, and the 1980s proved the sustainability and versatility of the triazines. Many factors determine the use and performance of the triazine herbicides, including target crop and weed species and their size, application timing, application rate, carrier type and volume, adjuvants, environmental conditions, and edaphic conditions. These factors became the focus of research during several decades following discovery of the triazine herbicides. It was during this time that weed scientists and farmers discovered triazine's true versatility.

Subtle, but important differences were noted in the behavior and performance of the triazines. For example, it was noted during discovery that simazine was less water soluble than atrazine and therefore requires more rainfall for weed control. Lower water solubility provided some advantages, including keeping simazine near the soil surface and thus providing sustained weed control plus greater selectivity in deeper-rooted crops. Furthermore, it was noted that simazine was superior to atrazine in controlling several grass species (i.e., large crabgrass and fall panicum). However, simazine proved to have weaknesses also. The postemergence activity of simazine is so limited that it prevents its use as a postemergence herbicide.

Atrazine proved to be more versatile in corn and grain sorghum than simazine. Researchers quickly discovered that atrazine gave selective weed control in an almost unlimited number of ways in field corn. It could be applied in the fall for fallow-season weed control. It could be applied in the spring either preplant or preemergence to the crop, or it could be applied postemergence. The selectivity of atrazine-applied postemergence was somewhat unusual. It was readily apparent that both corn and sorghum were quite tolerant of postemergence applications of atrazine and that many weed species were not. In fact, crop tolerance was so good that researchers quickly began investigating ways to use the margin of safety in the crops and increase foliar activity of atrazine on weeds. It was this objective that led to the widespread use of crop oils as agricultural spray adjuvants with herbicides.

From the early 1960s, it was found that nonphytotoxic crop oil plus emulsifiers, when added to atrazine-applied postemergence to small weeds, could enhance weed control. LeBaron (1966) reported on this research and recommended the use of 1–2 gal/A (9–18 L/ha) of such oils containing 1% v/v of emulsifiers for broadcast nondirected postemergence applications of atrazine in corn and grain sorghum. Oil concentrates (containing higher amounts of emulsifiers) applied at about 1 quart/A (2.3 L/ha) soon became popular. This was especially true in Northern states or where atrazine carryover in soil was a problem. McWhorter (1982) reported that by the late 1950s, using adjuvants, emulsifiers, and crop oils to enhance the postemergence activity of atrazine and other herbicides was an important research topic. By the late 1960s, phytobland oils were commonly used by farmers when applying atrazine to corn. By the 1970s, the use of oil-surfactant concentrates, now referred to as crop oil concentrates (COCs), was widespread in corn and grain sorghum. In fact, it was the use of COCs with atrazine that led to their widespread popularity as adjuvants for many other herbicides like the postemergence graminicides.

Postemergence applications of atrazine enjoyed widespread adoption by grain sorghum farmers as well, even though crop tolerance issues limited atrazine's use as a preemergence herbicide on sandy soils but not on heavier soils. Certain edaphic conditions do allow for preemergence use. In southern areas of the Great Plains, grain sorghum farmers used propazine, a related chloro-*s*-triazine, for preemergence applications and atrazine for postemergence applications. During the 1970s, Shell (later DuPont) released cyanazine, another chloro-*s*-triazine, which was ultimately used in corn and cotton. Cyanazine was used widely as a premix partner with atrazine. Cyanazine's spectrum of activity was similar to that of atrazine, but it was less persistent than atrazine. Mixing cyanazine with atrazine permitted lower atrazine application rates and minimized concerns about carry-over to rotational crops, especially in calcareous soils.

Preplant application of atrazine was important in dry areas of the Great Plains. In the semi-arid areas where corn or grain sorghum can be grown, preemergence and postemergence applications of these herbicides are often too late, since early weeds use too much of the soil moisture. Atrazine plus paraquat, often applied several weeks before planting in order to prevent early weeds from depleting soil moisture in semi-arid regions, was used extensively; now glyphosate is commonly used in these regions before planting.

Because of atrazine's versatility, it is widely used as a premix partner with other corn or grain sorghum herbicides. Atrazine is commonly marketed as a premix with acetanilide herbicides – including *S*-metolachlor, alachlor, dimethenamid, and acetochlor – to control broadleaves and to improve control of annual grasses. The products are commonly applied preemergence to corn and weeds. Also, atrazine is commonly mixed with 2,4-D, dicamba, bromoxynil, isoxaflutole, mesotrione, or sulfonylurea herbicides for the control of broadleaf and grass weeds in corn. Adding low rates of 2,4-D to atrazine increased postemergence grass control to such an extent that atrazine rates could be reduced while selectively controlling grass weeds in corn and grain sorghum. Atrazine and paraquat mixtures have been widely used for broad-spectrum weed control in no-till systems, especially in ecofallow. Atrazine is commonly used with glyphosate in genetically modified corn to provide residual weed control.

Research in Texas showed that crop protectants would increase the selectivity of metolachlor to sorghum. Spotanski and Burnside (1973) found that naphthalic anhydride as a seed treatment was the most effective crop protectant used to reduce alachlor injury to sorghum. In the early 1970s, Geigy and Ciba-Geigy scientists realized that a novel source of biological chemicals for agriculture could be crop protectants or safeners in crops sensitive to injury from existing herbicides, and they developed a new bioassay to screen for such activity. In a remarkably short time they discovered that oxime derivatives gave excellent protection to sorghum when applied as a seed treatment against metolachlor and other chloroacetanilide herbicides, which otherwise would cause serious sorghum injury. The original product, cyometrinil (Concep®), was replaced by oxabetrinil (Concep® II) and later by fluxofenin (Concep® III), which was found to be superior. These safeners led to much greater use of metolachlor/S-metolachlor or other chloroacetanilide herbicide combinations with atrazine for weed control in sorghum.

Application versatility, combined with a high level of crop tolerance, led to atrazine being the most widely used corn herbicide in history. In fact, atrazine led the US corn herbicide market within several years of its introduction. Illinois corn farmer surveys show a steady increase in the use of atrazine in corn from its debut in 1960, with 75–85% of corn being treated with atrazine since 1975.

Since the Initiation of Special Review: 1994 to the Present

During late 1994, the United States Environmental Protection Agency (USEPA) published a public document (PD-1) relative to the use of triazine herbicides by American farmers. In doing so, they placed atrazine and simazine in 'Special Review.' USEPA'sPD-1 triggered a benefits study of unprecedented proportions on the following issues: benefits of atrazine and simazine use; economic and biological impact of the loss of these products; feasibility and efficacy of alternatives; environmental benefits associated with atrazine and simazine use; best management practices; and comparative performance of alternatives.

To address these issues, it was obvious that a comprehensive evaluation and assessment of use and benefits would be required, and the magnitude of the task became obvious very quickly.

Soon after its initial registration, atrazine became a widely used herbicide in the United States (Padgett *et al.*, 2000). In 1994 at the time the USEPA Special Review was initiated, atrazine was used on approximately 67%, 65%, and 90% of US corn, sorghum, and sugarcane acreage, respectively. These 1994 percentages of crop treated remain consistent today.

The diversity and extent to which these triazine herbicides are used led to a two-tiered approach for assessing their use and benefits. One set of techniques was developed for triazine use in field corn, sweet corn, popcorn, and grain sorghum, and a second set of techniques was employed for the remaining minor uses or minor acreage crops. Because there was little yield information available for minor uses and minor crops, detailed yield response analyses were not possible with these uses. The remainder of this chapter will focus on the use and benefits of atrazine to field corn and grain sorghum growers as outlined in the most comprehensive and detailed study ever conducted on the benefits of pesticide use.

Analysis approach – corn and sorghum: A two-tiered approach was also used to characterize the benefits associated with uses of atrazine and simazine in corn and sorghum. First, a comparative analysis was made of product labels. The following parameters were considered in this review: performance profiles, including efficacy, spectrum, and crop tolerance; label comparisons; physical and chemical characteristics of the product; hazard profiles; economic benefits; and other relevant issues, such as use restrictions, etc.

This analysis provided qualitative information about possible alternative or competitive herbicides and revealed some important deficiencies that would remain, should the triazines be removed from the marketplace. However, a more quantitative approach was needed to provide detailed estimates of the costs that would be associated with regulation of the triazine herbicides. Due to the complexity and extent of triazine use, it was necessary to conduct a

computer simulation of the benefits. In fact, 12 models were developed for corn analysis and six for sorghum analysis. These included:

- *University Corn Model*: A national model using recommendations from public institutions.
- *Ciba National Corn Model*: A national model using data from Syngenta Crop Protection Inc.'s extensive corn herbicide database.
- *Regional Corn Models*: Ten regionally specific corn models (one for each USDA production region) using data from Syngenta's corn herbicide database.
- *Ciba Sorghum Model*: A national model using data from Syngenta's extensive sorghum herbicide database.
- *Regional Sorghum Models*: Five regionally specific sorghum models (one for each USDA production region) using data from Syngenta's sorghum herbicide database.

Field data from almost 5000 of university and proprietary research studies were used to estimate cost, yield, net return, and consumer effects for herbicide use in corn and grain sorghum in the early 1990s. They were also used to estimate changes that would likely occur under a variety of regulatory scenarios. Regional cost and yield changes were estimated using these models. These data were then used to estimate consumer effects using the Agricultural (AGSIM) model (Taylor, 1993). Similar analyses were conducted for sweet corn and popcorn uses of triazine herbicides, though macroeconomic (consumer) effects were not estimated because these crops were not included in the AGSIM model. Details relative to this part of the analysis are published in an *American Chemical Society Symposium Series* (Bridges, 1998; Carlson, 1998).

Numerous herbicides were included in the corn and grain sorghum analyses (Tables 13.2 and 13.3). All herbicides registered for corn and used on 2% or more of US corn or grain sorghum acreage were included, as well as herbicides whose registrations were immediately forthcoming.

Summary of Findings: Impacts on Atrazine Users

Comparative analyses and computer simulations revealed no true replacement(s) for triazine herbicides. Nonchemical alternatives were limited to cultivation and cultural practices, neither of which is very effective when used alone. The environmental costs of cultivation are simply too great, and repeated cultivation of the nation's corn and sorghum crops is not feasible. Several chemical alternatives were considered, each revealing its own particular weakness (Bridges, 1998). Relative to atrazine use in corn and sorghum, the following characteristics were identified as nearly irreplaceable benefits.

Table 13.2 Herbicides and mechanical methods included in the 1995 triazine corn benefis assessment for the United States

Atrazine	Dicamba	2,4-D
Pendimethalin	Flumiclorac	Halosulfuron
Alachlor	Simazine	EPTC
Bromoxynil	Metribuzin	Propachlor
Nicosulfuron	Dimethenamid	Cyanazine
Butylate	Prosulfuron	Imazethapyr
Primisulfuron	Metolachlor	Acetochlor
Rimsulfuron ffi thifensulfuron	Metolachlor ffi atrazine	Cyanazine ffi atrazine
Dicamba ffi atrazine	Prosulfuron ffi rimsulfuron	Alachlor ffi atrazine
Acetochlor ffi atrazine	Bromoxynil ffi atrazine	Bentazon ffi atrazine
Flumetsulam ffi metolachlor	1 Cultivation	2 Cultivations

Table 13.3 Herbicides and mechanical methods included in the 1995 triazine sorghum benefits assessment for the United States

Atrazine	Dicamba	2,4-D
Metolachlor	Propachlor	Bromoxynil
Alachlor	Propazine	Prosulfuron
Halosulfuron	Metolachlor ffi atrazine	Propachlor ffi atrazine
Alachlor ffi atrazine	1 Cultivation	2 Cultivations

Application flexibility: Farmers can achieve residual control of weeds with either preemergence or postemergence applications of atrazine. Atrazine can be applied during the fallow season, early preplant, immediately prior to or after corn planting, or postemergence. It can be used preemergence or postemergence in many sorghum-producing areas, with postemergence application being a great benefit where edaphic and climatic conditions do not permit preemergence application.

Crop tolerance: The margin of crop tolerance is excellent in both corn and grain sorghum. Corn injury does not occur, even with use at maximum labeled rates. Corn postemergence tolerance is so exceptional that it can be used with a variety of carrier and/or adjuvant systems to enhance activity on target weeds. Grain sorghum tolerance is good, and when applied according to label directions, grain sorghum injury is rare.

Weather insensitivity: Atrazine efficacy is relatively unaffected by weather. Since it is not particularly susceptible to photodegradation or to volatility, it can be applied under a variety of conditions and still be expected to deliver weed control benefits when rain occurs. The mode of action of atrazine in higher plants is such that the sensitivity of susceptible plants is only minimally affected by environmentally induced changes in plant growth, unlike many alternative herbicides that work well only in rapidly growing plants.

Premix and tank-mix compatibility: Atrazine is an excellent mixing partner with many herbicides. Compatibility and antagonism problems are rare. In fact, its premix compatibility is so good that atrazine is used more often than any other herbicide as a premix component in corn herbicide products. Furthermore, because of the tremendous margin of corn safety, mixes do not typically pose a risk for increased crop injury.

Broad-spectrum weed control: Atrazine and simazine control a broad spectrum of broadleaf and grass weeds. In fact, of the 28 weeds species considered in the analysis, atrazine provides a higher level of control of a greater number of them than any other herbicide.

Tillage compatibility: Because atrazine provides both postemergence and residual preemergence weed control, it fits well into conventional, minimum, and no-till production systems. Research indicates that dependency on atrazine increases when tillage is reduced, and in fact, currently available data indicate that 67.6%, 70.1%, and 81.5% of conventional, conservation, and no-till corn acreage, respectively, is treated with atrazine (Doane Marketing Research, 2000).

Economical weed control: Atrazine provides very cost-effective weed control. The per acre cost of atrazine is competitive because the herbicide provides broad-spectrum residual control and minimizes follow-up treatments, making net return to treatment cost very good. No single alternative herbicide included in these analyses returns more value per cost invested than atrazine.

Worker and environmental safety: Atrazine and simazine are safe to apply according to the directions on the label. Nontarget safety margins are good because atrazine is nonvolatile and has low specific activity. In addition, avian, mammalian, and aquatic toxicities are low. Relative safety to nontarget plant species is a positive characteristic that is not always found in alternative products.

Cost, yield, and net return changes for corn: Computer simulations using a substitution analysis revealed that weed control costs for corn producers would increase in all 10 USDA production regions without atrazine (Table 13.4). Corn

Table 13.4 A computer simulation of projected US regional changes to corn growers if atrazine had not been available for use during the 1995 crop year[a]

	Per acre grown					
Region[b]	**Yield[c] (bu)**	**Income[d] ($)**	**Cost[d] ($)**	**Net return ($)**	**Acres treated (%)**	**Net return[d] per acre treated ($)**
Appalachian	ffl8.54	ffl19.64	6.99	ffl26.63	87.00	ffl30.61
Corn Belt	ffl5.64	ffl12.97	3.52	ffl16.49	69.00	ffl23.90
Delta	ffl3.22	ffl7.41	7.52	ffl14.93	76.00	ffl19.64
Lake States	ffl2.45	ffl5.64	1.50	ffl7.14	38.00	ffl18.79
Mountain	ffl4.05	ffl9.32	2.30	ffl11.62	38.00	ffl30.58
Northeast	ffl2.80	ffl6.44	3.39	ffl9.83	65.00	ffl15.12
Northern Plains	ffl3.30	ffl7.59	3.04	ffl10.63	60.00	ffl17.72
Pacific	ffl0.28	ffl0.64	0.49	ffl1.13	3.00	ffl37.67
Southeast	ffl1.13	ffl2.60	0.87	ffl3.47	67.00	ffl5.18
Southern Plains	1.58	3.63	8.40	ffl4.77	74.00	ffl6.45
Weighted Average	ffl4.27	–	–	–	–	–

[a]From Bridges (1998).
[b]USDA production regions.
[c]To convert bu/A to kg/ha, multiply by 67.3.
[d]To convert income, cost, or net return/A to $/ha, multiply by 2.47.

Table 13.5 A computer simulation of projected US regional changes to grain sorghum growers if atrazine had not been available for use during the 1995 crop year[a]

Region[b]	Per acre grown				Acres treated (%)	Net return[d] per acre treated ($)
	Yield[c] (bu)	Income[d] ($)	Cost[d] ($)	Net return ($)		
Appalachian	ffl8.54	ffl16.23	ffl4.49	ffl11.74	78	ffl15.65
Corn Belt	ffl8.54	ffl16.23	ffl4.49	ffl11.74	75	ffl15.65
Delta	ffl2.09	ffl3.97	ffl1.13	ffl2.84	80	ffl3.55
Lake States	ffl8.54	ffl16.23	ffl4.49	ffl11.74	75	ffl15.65
Mountain	ffl0.90	ffl1.71	0.32	ffl2.03	28	ffl7.25
Northern Plains	ffl4.44	ffl8.44	ffl2.97	ffl5.47	81	ffl6.75
Northeast	ffl8.54	ffl16.23	ffl4.49	ffl11.74	75	ffl15.65
Pacific	ffl0.90	ffl1.71	0.32	ffl2.03	28	ffl7.25
Southeast	ffl2.09	ffl3.97	ffl1.13	ffl2.84	60	ffl4.73
Southern Plains	ffl3.81	ffl7.24	ffl1.02	ffl6.22	58	ffl10.72

[a] From Bridges (1998).
[b] USDA production regions.
[c] To convert bu/A to kg/ha, multiply by 67.3
[d] To convert income, cost, or net return/A to $/ha, multiply by 2.47.

yield would also decline in nine of the 10 USDA regions and ranged from 1.5 to 8.5 bu/A (100 to 570 kg/ha) grown, or 1.7 to 9.8 bu/A (114 to 660 kg/ha) treated. The weighted average yield decline was 4.27 bu/A (287 kg/ha) for all corn acres grown. Net returns to corn farmers declined by $5–37/A ($12–90/ha) treated.

How do these results compare with other studies? Data from almost 5000 field trials contained in Syngenta's corn weed control database indicated that corn yield declined approximately 4 bu/A (270 kg/ha) when treatments did not include atrazine. Fawcett (2006) compiled published data from 103 Corn Belt weed control trials conducted from 1986 through 1996 and found that corn yields were approximately 7 bu/A (470 kg/ha) lower without atrazine. A 2-year Wisconsin experiment (Harvey, 1996) showed a nearly 11 bu/A (740 kg/ha) decline when atrazine was omitted from weed control programs. Trials conducted by universities in the United States from 1986 through 1995 showed that corn yields declined by 5.5% and 11% in minimum and no-till experiments, respectively, when atrazine was not used.

These results clearly demonstrate that even though many other herbicides are available for use by corn farmers, they generally either cost more and/or are less effective at controlling weeds – regardless of whether they are used alone or in combination with one another – resulting in lower net returns. USEPA in 2003 estimated the economic impact of atrazine in corn to be approximately $1.6 billion per year, with an 8.8 bu/A decrease in yield and a $28/A increase in costs if atrazine were not available (USEPA, 2003a, b).

Cost, yield, and net return changes for sorghum: Projected impacts to sorghum growers were similar to those for corn growers. On average, yield changes were greater for sorghum growers than for corn growers, primarily because there are fewer weed control options available for grain sorghum producers (Table 13.5). Yield changes ranged from approximately 1 to 8.5 bu/A (67 to 570 kg/ha) grown, or 3.5 to 11.3 bu/A (235 to 760 kg/ha) treated. Alternatives for sorghum growers were often cheaper, but yield declines were greater as compared with corn. Simulated net returns declined in all USDA sorghum production regions with the cancellation of atrazine, ranging from approximately $3.50 to $15.50/A ($8.65 to $38.20/ha) treated. These results demonstrated the irreplaceable value of atrazine to sorghum growers.

Consumer effects: Using a free market agricultural assumption, AGSIM (Taylor, 1993) simulations revealed a variety of impacts for various sectors of the economy if atrazine and simazine were not available for use in corn and grain sorghum. The livestock and consumer sectors were impacted most negatively. The total effects were projected to be a loss of $1.55 billion annually for atrazine only and $1.75 billion annually if all triazines were canceled. Details concerning macroeconomic and consumer effects have been reported by Carlson (1998).

Importance in conservation tillage: Several studies indicate that atrazine use grows with the adoption of conservation tillage practices by corn growers. Market studies indicate that 67.6%, 70.1%, and 81.5% of conventional, conservation, and no-tillage corn acres, respectively, are treated with atrazine (Doane Marketing Research, 2000). Significantly lower acres of corn grown in each of these three major tillage types are treated with nontriazine herbicides that are considered by many to be alternative products (Figure 13.2). All studies conducted to date clearly indicate that atrazine is a critical component for conservation tillage production of corn in the United States.

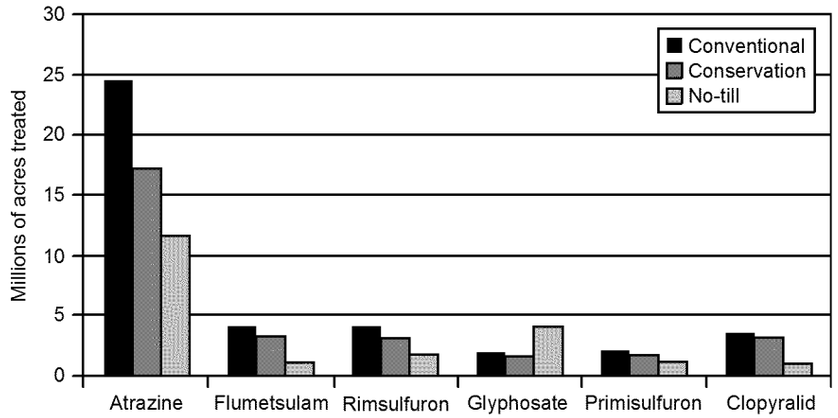

Figure 13.2 Herbicide-treated corn acres (To convert acres to hectares, multiply by 0.405) by tillage type in the United States (Doane Marketing Research, 2000).

Performance and market share for competitive herbicide products: Since the USEPA Special Review was initiated in 1994, acres treated with various corn herbicides have been carefully monitored. No clear alternative has proved to be a possible replacement to atrazine. Several facts are noteworthy. No corn herbicide introduced between 1994 and 2000 reached a 10% market share, nor did the market share increase for putative atrazine replacements like 2,4-D or bromoxynil (Table 13.6). These new products suffered from one or more of the following limitations: limited spectrum of weeds controlled, crop injury potential, or rotational restrictions. Meanwhile, atrazine's total market share remained constant at approximately 70%. To date market retention has been poor when new herbicides are used alone. Virtually all corn herbicides introduced since 1994 are used with atrazine, and the percentage of acreage treated in combination with atrazine is increasing (Table 13.6).

The fact that market share for competing products did not increase and that these products are increasingly being used in combination with atrazine indicates that farmers are not shifting emphasis away from atrazine. In fact, during the same time period, atrazine use did not decline. Total atrazine base acres have remained relatively constant since 1996, and more corn acres received both preemergence and postemergence applications of atrazine than before 1996 (Table 13.7).

Further evidence of corn grower loyalty to atrazine is evidenced by the fact that more, not fewer, herbicide active ingredients are being used for corn weed control. In fact, from 1990 to 2003, the average number of herbicide active ingredients applied per base herbicide acre increased (Figure 13.3). This occurred because virtually all corn herbicides introduced since 1959 work better with atrazine than they do alone. Many of the alternative herbicides do not control a broad spectrum of weeds, nor do they provide residual weed control. Mixing atrazine with these herbicides expands the spectrum of weed control and increases residual activity. Preemergence uses of atrazine alone have declined, as have applications at maximum rates. But atrazine continues to be used both preemergence and postemergence to ensure adequate weed control. It is the use of atrazine postemergence, though, that is growing. Market studies show that in 1996 approximately 3% of US corn acreage received atrazine both preemergence and postemergence. In 1998, approximately 7.1% of the acreage received both preemergence and postemergence atrazine applications, and this percentage has remained fairly constant through 2004 (Table 13.7).

What Will Happen in the Future

New corn herbicides continue to be registered, but if history repeats itself, many will be either premixed with atrazine or will be used in combination with atrazine by America's corn farmers. New product registrations for grain sorghum will likely be fewer in number, but they too will likely be used in combination with atrazine. The triazine herbicides have been, and will continue to be, very important in managing weed resistance to other herbicides. The vast majority of corn and soybean acres in the Corn Belt are rotated with one another. While the use of glyphosate has dramatically increased in soybean acres in this production region, ALS-inhibitor herbicides are still used. Therefore, ALS-inhibitor herbicides are being used in both corn and soybean acres, which presents a challenge with respect to weed resistance management. If atrazine is not available for weed control in corn, reliance on these ALS-inhibitor herbicides will increase and resistance management will become all the more challenging.

Table 13.6 Corn herbicide use as a percent of planted acres and the percent of those applied with atrazine in the United States[a]

	1998		2004	
Herbicide	**Percent planted acres treated**	**Percent of a.i. acres with atrazine**	**Percent planted acres treated**	**Percent of a.i. acres with atrazine**
Broadleaf products				
Atrazine	69.1	100.0	66.7	100.0
2,4-D	7.7	62.6	6.9	70.3
Bromoxynil	4.7	70.4	1.1	83.4
Carfentrazone–ethyl	–	–	0.6	85.8
Clopyralid	7.6	43.3	6.2	65.6
Dicamba	20.1	57.4	13.0	58.9
Difl ufenzoypr	–	–	3.4	51.3
Flumetsulam	9.2	46.6	6.4	66.8
Foramsulfuron	–	–	1.3	48.4
Halosulfuron	1.2	66.3	0.8	78.5
Imazethapyr	2.2	37.6	2.0	41.2
Isoxafl utole	–	–	7.5	65.9
Mesotrione	–	–	17.0	77.1
Prosulfuron	3.5	66.8	1.2	89.8
Rimsulfuron	8.3	72.7	11.1	67.6
Simazine	1.6	91.8	2.9	87.5
Thifensulfuron	0.5	17.4	0.7	58.6
Grass products				
Acetochlor	23.5	75.5	24.0	83.8
Alachlor	4.7	77.3	1.6	83.2
Dimethamid	6.1	69.2	5.9	85.0
Flufenacet	0.2	64.6	2.2	53.3
Metolachlor	25.5	83.8	–	–
S-metolachlor	6.1	58.2	23.2	86.9
Nicosulfuron	16.1	64.2	12.5	68.5
Pendimethalin	3.8	75.7	2.3	78.9
Primisulfuron	5.8	57.8	2.4	73.0
Post contact				
Glyphosate	7.6	69.8	28.9	42.3
Glufosinate	1.9	41.8	4.2	58.4
Paraquat	1.7	87.2	1.7	92.6

[a]From Doane Marketing Research (2004).

Table 13.7 Atrazine use trends in the United States since 1996 by application type[a]

	Atrazine-treated corn acres (%)				
Application type	**1996**	**1998**	**2000**	**2002**	**2004**
PRE[b] only	68.9	64.7	64.3	63.2	64.3
POST[c] only	27.8	28.2	29.3	30.8	28.4
PRE ffi POST	3.3	7.1	6.4	6.0	7.3

[a]From Doane Marketing Research (2004).
[b]PRE ¬ Preemergence.
[c]POST ¬ Postemergence.

The use of glyphosate-tolerant seed is becoming more prevalent in corn and glyphosate-resistant weeds are increasing at an alarming rate. New resistance management and weed shift challenges will be faced when corn and soybeans are grown year after year and farmers rely heavily on glyphosate in both crops for weed control. Atrazine use in corn will be critical to manage specific weeds resistant to glyphosate.

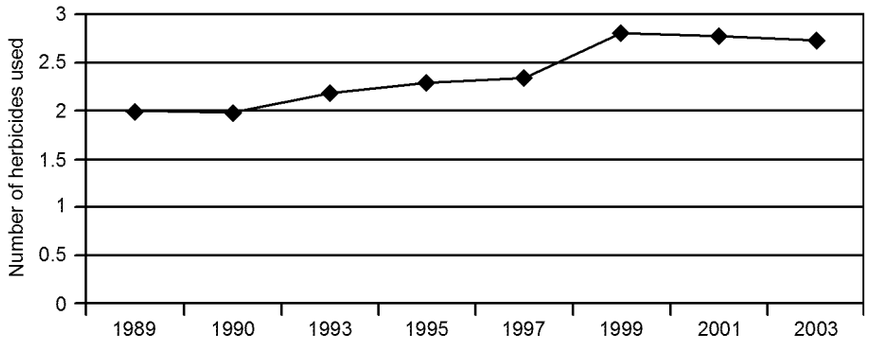

Figure 13.3 Average number of herbicide active ingredients (a.i.) applied per acre of corn grown in the United States (Doane Marketing Research, 2003).

Conclusion

It is a well-known and established fact among weed scientists and most farmers that there are no perfect herbicides. Atrazine in corn and sorghum, though, probably is as close to perfect as any modern herbicide. In earlier years, it often gave virtually complete control of all weeds in many fields, with no crop injury, no drift or handling problems, or other side effects. However, under some conditions and years of continued use, certain weeds began to develop resistance. Fortunately, effective uses of prepack combinations and tank-mixed herbicides has become almost universal and has been important for resistance management. In addition, triazine-resistant weeds are generally less robust and easier to control than weeds resistant to other herbicides.

As new herbicides were introduced over the years, weed scientists and farmers looked for the best mixtures, rates, and ratios to determine where the new ones would fit. The objective was always to provide the grower with the most dependable and efficacious control of major weeds, with the least amount of herbicides and cost, and with little or no risk to the applicator, consumers, and environment. With corn, sorghum, sugarcane, and certain other crops, such mixtures most often included atrazine or other triazine herbicides. Many times as weed scientists or farmers would discuss the virtues and performance of new herbicides, they would state: 'The new products performed well, but it sure helped to add a little atrazine.'

The triazine herbicides provide important benefits to US agriculture, the general economy, and the environment. Benefits reported to date, including those outlined in this report, are considered conservative. Alternatives to the use of these herbicides are generally more costly, less effective, and are often 'add-ons' because they are used in conjunction with the triazines. The triazines – particularly atrazine – quickly captured a large share of the corn and grain sorghum weed control acreage in the United States, and that share has been remarkably sustained for more than four decades. The often-stated objective of agricultural research scientists is to discover, develop, and register products as efficacious and as safe as the triazines. This is a worthy goal, but one not yet accomplished.

The National Corn Growers Association annually sponsors a contest where growers provide production inputs and report their per acre grain yield. There are nine classes, covering variations in geography, tillage type, and irrigation. In 2006, results were reported for more than 1600 sites across the nine classes (National Corn Growers Association, 2006). Atrazine was a component in the top reported yields within all nine classes. Overall, atrazine was a component of the herbicide program in greater than two-thirds of all reported yields. This clearly shows the confidence that the nations top corn growers have in atrazine to provide economical weed control and crop safety for maximum yield potential.

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Chapter 14

Benefits of Triazine Herbicides in Ecofallow

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Summary

Ecofallow is part of a farming and crop rotation system increasingly practiced in more arid regions of the Great Plains of North America. Prior to the development of ecofallow, winter wheat was the dominant rain-fed (non-irrigated) crop in these regions. From Texas to North Dakota, winter wheat was produced on an alternate-year basis, with long periods of 'summer fallow' to accumulate soil moisture between successive wheat crops. During summer fallow, weeds were controlled with repeated tillage.

In ecofallow, herbicides are used to reduce or eliminate mechanical tillage following winter wheat harvest. There is typically a post-harvest application of glyphosate with 2,4-D and/or dicamba to control growing weeds and the first flush of volunteer wheat. Later in summer, atrazine is applied at rates sufficient to control winter annual and other weeds, plus volunteer wheat, up to and after corn or grain sorghum planting the next spring.

Retaining wheat stubble on the soil surface by using reduced or no tillage reduces erosion by wind and water and enhances infiltration of precipitation, thereby increasing soil water storage. Evaporation is reduced, and water use by weeds is stopped, making more water available for crop growth. Faster water accumulation permits shortening of fallow periods and more intensive and diverse crop rotations. The 3-year wheat-grain sorghum-fallow and wheat–corn–fallow rotations use precipitation and soil water far more efficiently than the traditional 2-year wheat–fallow rotation. Summer crops typically have greater yield potential, higher water-use efficiencies, and may be more profitable than winter wheat.

Atrazine is the key herbicide facilitating ecofallow corn and sorghum production in the semi-arid Great Plains, where crop production is often uncertain and profits to farmers are often marginal. The success of atrazine in ecofallow is attributable to its duration of weed control as a soil-applied herbicide, the broad spectrum of weeds controlled, the low cost per area treated, and its safety to crops. In this semi-arid environment, maintaining weed-free fallow with repeated applications of nonresidual herbicides is not an economically viable alternative to atrazine.

Introduction

Dryland farming in the Great Plains of the United States is undergoing a revolution that is based firmly on the role that triazine herbicides play in reducing or eliminating mechanical tillage for weed control during fallow periods. Soil dries out to the depth that is tilled, so reducing or eliminating tillage conserves soil moisture. Because of the unique nature of the Great Plains, the impact of triazine herbicides extends well beyond simply controlling weeds. The burndown and residual weed control that can be achieved with triazine herbicides, especially atrazine, enhances soil water accumulation, which allows fallow periods to be shortened and crop rotations intensified. These benefits result not only in increased short-term profits for farmers, but also in crop production systems that maintain and even increase soil organic matter, decrease soil erosion, and enhance the long-term sustainability and productivity of dryland agriculture in the Great Plains.

The Geographical Setting

To appreciate the contribution of triazine herbicides to sustainable dryland agriculture in the Great Plains, we must examine the geographical setting. The Great Plains region encompasses an area of approximately 700 million A

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(284 million ha), stretching from Texas through Montana, North Dakota, and southern Canada, and from the Rocky Mountains to eastern Oklahoma, Kansas, Nebraska, and the Dakotas. The region is characterized by a marked precipitation gradient, with precipitation increasing from west to east. The more arid part, also with the highest elevation, is known as the High Plains. The region is characterized by an evapotranspiration demand that far exceeds annual precipitation. At Garden City, Kansas, average annual precipitation is 18 in. (46 cm), and open-pan evaporation for April to October averages 74 in. (188 cm) (Norwood *et al.*, 1990). Weather patterns are highly variable, a characteristic of continental climates (i.e., climates with minimal maritime influence).

The High Plains region is characterized by large, very gently rolling uplands that are dissected by ancient river channels. Upland soils in the west are predominantly loams and silt loams that give way to silty clay and clay loams in more humid areas to the east. Nearly all soils are classified as Mollisols (Cannell and Dregne, 1983). Native vegetation consists of the short-grass prairie complex in the west, changing to mixed-grass and tall-grass prairies to the east.

Conversion of Native Prairies to Cropland

The conversion of native prairies to crop production in the Great Plains began in the late 1800s, as settlers from the eastern United States and Europe migrated to the region. These settlers brought with them farming methods and crops better suited to more humid areas. Corn production flourished during wet years, but withered at other times. Winter wheat became the dominant crop, but it also failed at times. Nitrogen levels in soil that ranged from 0.08% to 0.23% in the unbroken prairie declined by 50% in the first 40 years of farming (Haas and Evans, 1957). Soil erosion by wind and water also took its toll, as dramatized by the 'Dust Bowl' and the 'muddy' Missouri River.

Since about 1900, researchers at state and federal experiment stations have worked to develop crop production systems better suited to the Great Plains. 'Summer fallow' systems, mainly wheat–fallow, came into widespread use (Throckmorton and Myers, 1941). In the wheat–fallow system, wheat is grown in alternate years, following a 15-month fallow period during which precipitation is stored as soil water. Although less than 25% of precipitation was stored, this practice helped assure adequate soil moisture for wheat stand establishment, and wheat yields from such alternate-year cropping often exceeded the combined yields from two years of continuous cropping. Thus, the wheat–fallow system helped to stabilize and reduce the risk of wheat production on the Great Plains.

Dust–Mulch Fallow

Peterson *et al.* (1996) summarized the evolution of wheat–fallow systems. During the early years, summer fallow depended on repeated tillage for weed control, using a 'dust mulch' to discourage weed germination. Primary tillage usually was done with moldboard or disk plows (one-way). Shallower secondary tillage with spring-tooth harrows, duck-foot cultivators, or tandem disks destroyed emerged weeds and reestablished the dust mulch following rains. Frequent tillage in dust–mulch fallow fields, combined with decreases in soil structure over time, made the silt loam soils of the High Plains extremely vulnerable to wind erosion. Wind erosion reached its peak in the 1930s when periods of record-setting heat and drought resulted in poor crop establishment. During these years, the region earned its reputation as the 'Dust Bowl' of the United States.

Stubble–Mulch Fallow

Following the devastation of the Dust Bowl of the 1930s, adjustments were made to reduce the depth and frequency of tillage operations in order to protect the soil by leaving more plant residues on the soil surface. Moldboard plows bury 95–100% of the plant residues, and one-way disks bury 50–60% of plant residues on the first pass (Fenster *et al.*, 1977). Moldboard plows and one-way disks gradually gave way to tillage implements that destroyed weeds, but left more plant residues on the soil surface. One such early implement was the Graham Hoeme chisel with 18–24-in. (45–60 cm) sweeps, which was used to undercut standing stubble. The chisel had more residue clearance than spring-tooth harrows or duck-foot cultivators. This evolution in tillage implements eventually led to V-blade sweep plows with 5–7-ft (150–210 cm) wide sweeps that control weeds while leaving about 85% of the plant residues intact on the soil surface. Sweep plows were used for both primary and secondary tillage on the soils of the High Plains. Compared with clean-tilled and dust–mulch fallow, 'stubble-mulch' fallow left more plant residues on the soil surface to improve water infiltration and to help trap snow during fallow periods. Simultaneous with improved machinery and tillage practices, the frequency and intensity of dust storms on the High Plains greatly diminished because of increased rains and lower summer temperatures. Since the Dust Bowl days, there have been years with low rainfall (e.g., 1952–1956), but the combination of low rainfall and high summer temperatures of the 1930s has not occurred again (Phillips, 2001).

The transition from dust-mulch to stubble–mulch fallow was largely complete by the 1960s. With this transition, water-use efficiency climbed from about 0.46 bushels of wheat yield per acre-inch (12.3 kg/ha/cm) of precipitation to about 0.78 bu/A-in. (20.7 kg/ha/cm) (Peterson *et al.*, 1996). Wheat–fallow continued as the primary crop rotation for the High Plains, with wheat acres outnumbering summer crop acres by a 4 to 1 margin (Dhuyvetter *et al.*, 1996). Hybrid grain sorghums were developed during the 1950s, and sorghum became an important summer crop. Sorghum often was produced in a sorghum–fallow rotation with a 19-month fallow period between crops. Government farm programs helped stabilize production and prices, and 'set-aside' provisions of US Department of Agriculture (USDA) farm programs encouraged long fallow periods even in more humid parts of the Great Plains.

Wheat–fallow, sorghum–fallow, and wheat–sorghum–fallow on the High Plains, now called 'traditional fallow,' remained fairly static for the next 30 years. Water-use efficiency had reached an apparent plateau (Greb *et al.*, 1967; Norwood *et al.*, 1990). Some economies of scale came about through adoption of larger machinery and consolidation of land into larger farming units. Increases in yields from improved crop varieties and from better technologies for fertilization and weed control barely kept pace with increased production costs. Farmers increased their reliance on USDA subsidy programs that favored wheat–fallow or sorghum–fallow over alternative crops and production systems (Havlin *et al.*, 1995). With the discovery of groundwater resources such as the Ogallala aquifer, many farmers turned to irrigation to increase production and incomes. Irrigated corn, with much greater yield potential than dryland wheat and sorghum, became the dominant crop on irrigated lands of the Great Plains.

Traditional fallow cropping on the High Plains continued to fall short of meeting expectations for sustainable, dryland farming systems. Mechanical weed control during the 15–19-month fallow required five to seven sweep tillage passes, each diminishing the amount of plant residue cover protecting the soil (Norwood *et al.*, 1990). Soil loss caused by wind and water erosion continued at unacceptable levels. A Kansas study showed that for every inch (2.54 cm) of topsoil lost on Ulysses silt loam soils, organic matter decreased by 0.10%, and wheat yields decreased by 1.8 bu/A (121 kg/ha) (Havlin *et al.*, 1992). Although the efficiency of precipitation storage as soil water was excellent early in the traditional fallow period, it declined rapidly later in the cycle (Peterson *et al.*, 1996).

Replacing Mechanical Fallow with Chemical Fallow

Aasheim (1948) at Havre, Montana, was among the first to reduce tillage trips in a wheat stubble fallow by using chemicals to control weeds. He used 2,4-D and dinoseb, applied in water or diesel fuel, calling this practice 'chemical fallow.' Later, dalapon replaced dinoseb to obtain better grass control (Baker*et al.*, 1956).

Since about 1960, agricultural scientists have studied the potential for triazine herbicides to control weeds with little or no mechanical disturbance of crop residue or soil during fallow periods. Fenster *et al.* (1965) reported that atrazine applied to wheat stubble in a wheat–fallow rotation in western Nebraska injured wheat planted the following year. When atrazine rates applied during the fallow period were reduced enough to avoid injury to succeeding wheat crops, volunteer wheat was not adequately controlled. Wiese *et al.* (1967) reported similar problems with atrazine and propazine in a wheat–fallow rotation on the Texas High Plains, but recognized the potential for triazine chemistry to control weeds and volunteer wheat after wheat harvest in wheat–sorghum–fallow rotations. In 1961, Phillips (1964, 1969) at Hays, Kansas, was the first to use atrazine to reduce tillage of wheat stubble in wheat–sorghum–fallow rotations. Chemical fallow with triazine herbicides was far more effective in wheat–sorghum–fallow rotations than in wheat–fallow rotations because: (a) wheat is highly susceptible to triazine herbicides, but grain sorghum usually tolerates atrazine and propazine very well; and (b) a triazine application to wheat stubble in a three-year wheat–sorghum–fallow rotation allows 24–26 months for the herbicide to dissipate before the next wheat planting. Wicks (1976) expanded the wheat–sorghum–fallow concept in Nebraska by substituting corn for grain sorghum.

Chemical–Fallow, Ecofallow, Ecofarming, Reduced-Till, Lo-Till, and No-Till

Terminology describing today's tillage and fallow systems in the Great Plains is not uniform. Aasheim (1948) used the term 'chemical fallow' in contrast to 'cultivated fallow' and 'idle fallow.' Wicks (1976) used a more descriptive term, 'ecofallow,' to describe vegetation control with atrazine between wheat harvest and the subsequent no-till corn crop in western Nebraska, and 'ecofarming' for the entire wheat–corn–fallow rotation. Here, ecofallow will refer only to winter wheat stubble treated with atrazine, aimed at replacing fallow tillage, and then no-till planting grain sorghum or corn the following spring.

The Conservation Technology Information Center (CTIC) has standardized residue management terminology in order to monitor changes in tillage systems over time. CTIC tillage classification categories are based on the percentage of plant residue cover, together with the degree of soil disturbance up through crop planting. By CTIC definition, no tillage (including ecofallow), ridge tillage, and mulch tillage all have more than 30% plant residue cover after

crop planting and are forms of 'conservation tillage' (CTIC, 1996). 'Reduced tillage' systems have 15–30% cover, whereas 'conventional tillage' systems have less than 15% residue cover after planting. According to these criteria, a wheat–sorghum–fallow rotation typically would include no tillage during the wheat harvest-to-sorghum planting (ecofallow) transition and reduced tillage in the sorghum-to-wheat transition, where several V-blade operations often are used in preparation for wheat seeding. Ecofallow and ecofarming are regional terms not presently included in the CTIC's terminology.

Herbicides for Ecofallow

Atrazine, cyanazine, propazine, and terbutryn were the main triazine herbicides studied for potential use in ecofallow. Atrazine and cyanazine have good foliar burndown activity on small, emerged weeds when applied with appropriate adjuvants. All four have soil-residual activity, with cyanazine and terbutryn giving excellent weed control for 30–60 days, whereas atrazine and propazine applied at 2 lb/A (2.24 kg/ha) at Garden City, Kansas, may control volunteer wheat and weeds in wheat stubble for 10–12 months (Norwood *et al.*, 1990). The soil-residual activity of triazine herbicides is influenced greatly by soil pH, moisture, and texture (Vencill, 2002). Of these triazine herbicides, only atrazine is currently registered and used widely for weed control in ecofallow.

Several nontriazine herbicides also are used widely in ecofallow, either tank mixed with atrazine or applied sequentially. Paraquat is a nonresidual herbicide that burns down emerged weeds more rapidly and completely when applied with atrazine than either herbicide does alone. Thus, the grower may select an atrazine rate to match the desired duration of residual control and complement the atrazine with paraquat for complete vegetation burndown.

Another nonresidual herbicide very widely used in ecofallow is glyphosate. This herbicide can be partially deactivated with some formulations of atrazine, a problem that may be overcome by increasing the rate of glyphosate in the tank mix (Stahlman and Phillips, 1979). However, atrazine is still often applied with glyphosate and other systemic herbicides such as 2,4-D or dicamba to control large weeds during fallow periods. Antagonism can be avoided by using sequential herbicide applications rather than tank mixtures. In Kansas and southward, where the ecofallow season lasts longer than in the north, a glyphosate plus 2,4-D or dicamba application to wheat stubble early in the fallow period controls annual and perennial weeds and volunteer wheat. Thus, the atrazine application can be delayed until late summer, so that the control of volunteer wheat and winter annual weeds is enhanced.

The contribution of nontriazine herbicides to ecofallow is to extend the range of weed species and sizes that are controlled. Because they have little or no soil-residual activity, however, herbicides such as paraquat, glyphosate, 2,4-D, and dicamba need to be applied repeatedly to maintain a weed-free fallow period. The unique contribution of atrazine is that a single application in late summer may control a wide spectrum of late summer and fall germinating weeds, including volunteer wheat, up to the spring planting period for corn or sorghum.

Central Role of Water-Use Efficiency

Behind the evolution of dryland farming in the semi-arid Great Plains is the story of water-use efficiency and the factors that influence it. Water-use efficiency is the ratio of crop plant matter produced per inch of water used by the crop. It is influenced by many factors – including soil texture, soil structure, precipitation patterns, plant residue cover, water infiltration rates, evaporative demand, and effectiveness of weed control. In addition, the inherent water-use efficiency of crops varies. Summer crops such as corn, sorghum, foxtail millet, pearl millet, and proso millet all have higher water-use efficiency than winter wheat (Peterson *et al.*, 1996). Because the growth of these summer crops coincides with the period of highest annual precipitation in the Great Plains, they utilize precipitation for plant material more quickly than winter wheat.

Plant Residue Effects

Maintaining plant residues on the soil surface and reducing tillage during fallow periods enhance the storage of precipitation as crop-available soil water (Greb *et al.*, 1967; Fenster and Wicks, 1982; Norwood *et al.*, 1990). Plant residues play important roles in trapping snow and in reducing soil temperatures, evaporation from the soil surface, and wind and water erosion.

Wheat stubble may physically intercept up to half of the atrazine as it is applied (Ghadiri *et al.*, 1984). Subsequent rains wash atrazine off the stubble and bring it into contact with the soil. In Nebraska, suppression of summer annual weeds in wheat stubble was proportional to the amount of stubble. Crutchfield *et al.* (1985) showed that even though heavier wheat stubble intercepted more metolachlor applied in spring prior to ecofallow corn planting and reduced the amount of herbicide actually reaching the soil, weed suppression by the heavier stubble more than offset the interception effect.

Crop residues vary in quality and quantity. Wheat residues are very fine compared to corn or sorghum residues. Sunflower residues are especially coarse and provide much less soil protection pound for pound and degrade faster than finer residues. Wicks *et al.* (1995) showed that in western Nebraska, winter wheat was a better fit in the three-year cropping system than spring cereals. This was due not only to the value of the grain produced, but also to stem density and biomass of the stubble. However, dense, fine-stemmed stubble like that of wheat can be detrimental to subsequent crop establishment. In the more humid areas of the Great Plains, fields with undisturbed wheat stubble on fine-textured soils are frequently too wet for timely spring row-crop planting.

Storing Precipitation as Plant-Available Soil Water

A key benefit of no-till fallow, as compared with tilled fallow, is the increased storage of precipitation as plant-available soil water. At Garden City, Kansas, as much water was stored during each of the two 11-month fallow periods in a wheat-sorghum–fallow rotation managed with no-till methods as during the 15-month fallow period of a wheat–fallow rotation or during the 19-month fallow period of a sorghum–fallow rotation using stubble-mulch methods (Norwood, 1994).

Shorter fallow periods store precipitation more efficiently for several reasons. First, water is stored more readily when soils are dry, early in fallow periods, than later in fallow periods when soils are wetter. Secondly, when plant residues are preserved with no-till methods, water infiltration is encouraged and runoff and evaporative losses are reduced as compared to tilled soils with less residue. Indeed, mechanical tillage within fallow periods practically assures soil water loss as soils dry out and warm up in the tilled zone.

Water sources vary in their contribution to crop growth. Stored soil water is potentially 100% available to the growing crop, whereas snow melt and rainfall are subject to runoff and evaporation losses before they become available (Greb, 1983). To maximize water-use efficiency, water left in the soil profile at crop harvest must not be lost to weed growth and evaporation from tillage. Protecting stored soil water can be accomplished most readily by using herbicides.

Comparison of Crop Rotations and Tillage Systems

A winter wheat–fallow rotation with stubble-mulch tillage is still a common cropping system on the High Plains. However, even with stubble-mulch tillage, the efficiency of storing precipitation in the soil is usually less than 30% (Greb *et al.*, 1967). Little or no yield benefit results from wheat–fallow or sorghum–fallow cropping with reduced-till or no-till fallow, as compared to growing these crops with stubble-mulch methods. If the soil profile is filled in 10–12 months under no-till fallow management, then nothing is gained by longer 15- or 19-month fallow periods. Furthermore, herbicide costs for weed control increase dramatically during longer fallow periods (Dhuyvetter *et al.*, 1996).

Wheat often is considered the best adapted dryland crop in the Great Plains because it completes much of its lifecycle before summer heat. Of course, soil water must be adequate for stand establishment and growth. However, wheat in the Great Plains does not respond as much as corn or sorghum to the additional soil water obtained with no-till. Wheat yields in the Great Plains are limited more by the short grain-fill period (between the last killing frosts in spring and the onset of summer heat) than by water shortage (Paulson, 1994). For these reasons, winter wheat is simply unable to respond adequately to the extra stored water available in a wheat–fallow system managed under no-till conditions.

Out-of-pocket expenses per trip across the field may be less for mechanical tillage than for herbicides, but soil moisture loss is greater with tillage. No-till fallow is more profitable than mechanical fallow only if no-till fallow makes better use of the improved water infiltration through crop rotations with shorter fallow periods. Peterson *et al.* (1996) compared water-use efficiency for entire fallow cropping rotations. Three-year rotations (two harvested crops in 3 years) had consistently higher water-use efficiencies across the central and southern Great Plains than 2-year rotations (one harvested crop in two years).

Wheat–fallow rotation, although reasonably profitable with stubble-mulch tillage, is not economically viable under no-till. Because wheat fails to respond adequately to the extra water available under no-till, the cost of foliar-applied, nonresidual herbicides for weed control during long fallow periods may not be recovered. In the United States, government subsidies favoring wheat-fallow have been phased out. More intensified crop rotations that can generate more production through better water-use efficiency are required (Tanaka *et al.*, 2002).

Increasing water-use efficiency in the Great Plains depends on: (a) maintaining adequate plant cover to aid water infiltration into soils and reduce evaporation losses, (b) timing crop rotations so that sufficient soil water is available to establish crop stands and to sustain the crops between precipitation events, and (c) preventing water losses from tillage and weed growth. Water-use efficiency by crops is related inversely to the length of the fallow period.

Reducing tillage can have beneficial effects on soil structure and seedbed quality. Phillips (1964) observed that sorghum seedling emergence was better in chemical fallow treatments than in V-bladed treatments. Planter depth control and seed placement into proper soil moisture are achieved more readily in firm, untilled soils than on fluffy, tilled seedbeds. Furthermore, planter colters cut through plant residues more readily on firm soils.

Insect and Plant Disease Control

An added benefit of ecofallow is a reduction in yield loss due to several insect pests and plant diseases. Burton *et al.* (1990) reported lower greenbug numbers in no-till grain sorghum than in tilled sorghum. They found that no-till was a more effective method of insect control during the early stages of greenbug infestation than the use of resistant hybrids. The combination of no-till and resistant hybrids decreased damage below the economic threshold for greenbug. Doupnik *et al.* (1975) observed reduced *Fusarium* stalk rot in grain sorghum grown under ecofallow conditions. Stalk rot averaged 39%, 23%, and 11% with conventional, minimum, and no tillage, respectively. They attributed the reduction to increased water conservation, reduced soil temperature fluctuations, lower mean soil temperature, and better weed control through the use of herbicides. However, Doupnik and Boosalis (1980) reported a buildup of tan spot (caused by the fungus *Pyrenophora tritici-repentis*) and *Septoria* leaf blotch in the reduced tillage wheat–fallow rotation. They emphasize the importance of using at least two different crops, as in the wheat-sorghum or corn–fallow system, to help prevent buildup of these diseases.

Limited Irrigation and High Plains Farming

In light of declining water reserves in High Plains aquifers, scientists have studied the potential for limited irrigation to supplement dryland farming. In the Texas Panhandle, alternate years of irrigated and dryland wheat produced more yield than the same cropping surface area with half under continuous irrigation and half in continuous dryland production (Unger, 1977). Norwood (1995) showed that under limited irrigation, water-use efficiencies were greater for rotated crops than for continuous crops. Hergert *et al.* (1993) showed that with 6 in. (15 cm)/crop/year of supplemental irrigation, corn or soybean has greater potential for yield increases than wheat or sorghum in western Nebraska.

Role of Triazine Herbicides in the Great Plains

The unique fit of atrazine for weed control in wheat stubble became apparent as soon as it was integrated into the ecofallow segment of three-year rotations that contained grain sorghum or corn. Phillips (1964) recognized that atrazine was more effective than propazine for use on wheat stubble prior to planting sorghum. The combination of lower cost, broad weed control spectrum, better foliar uptake, and soil persistence favored atrazine over other triazine herbicides. Where corn or sorghum is to be no-till planted into wheat stubble, atrazine has no rivals.

Cyanazine and terbutryn have shorter residual activity than atrazine and propazine. 'Lo-till' farming in central Oklahoma used cyanazine and terbutryn to control weeds between successive crops of continuous, no-till planted wheat (Stiegler *et al.*). Cyanazine was once used in the spring before sorghum or corn planting to enhance weed control in these row crops, while reducing the risk of triazine carryover to wheat that might be seeded after the next fallow period (Norwood *et al.*, 1990). Neither cyanazine nor terbutryn is now registered in the United States.

Wicks *et al.* (1996) studied the potential for clomazone use in ecofallow corn in western Nebraska. Applied alone, clomazone failed to control emerged cheat and downy brome in autumn and pigweed species in spring. However, clomazone applied with atrazine provided excellent control up until corn harvest.

Atrazine has helped to solve some unique production problems on coarse-textured soils. Along the Arkansas River in central Kansas, cheat and downy brome are problem weeds in continuous wheat production on sands. Deep tillage to bury cheat or downy brome seed is not consistent with soil conservation practices because of soil crusting and excessive wind erosion. Since atrazine has excellent activity on the seedling stage of these grass weeds, the wheat–sorghum–fallow rotation was modified to a wheat–wheat–sorghum–sorghum–fallow rotation, with atrazine applied to stubble following harvest of the second wheat crop. This technique controlled cheat, downy brome, and volunteer wheat, as well as many other weeds in the next sorghum crop. Atrazine rates were reduced or eliminated for the second sorghum crop, permitting rotation back to wheat (TenEyck and Ball, 1984). This is an early example of 'stacked' rotations (Anderson, 2005) with 2-year intervals of cool- and warm-season crops to achieve superior pest control.

An extension of the ecofallow concept in the eastern Great Plains is to use atrazine between summer row crops to control winter annual weeds and to provide residual weed control for the following corn or sorghum crop. Application of atrazine in early spring before corn or sorghum planting is a best management practice (BMP) to reduce potential atrazine loss in surface water runoff in central and eastern Kansas (Regehr *et al.*, 1996, 1998).

Research in northeast Kansas shows that atrazine applied over soybean stubble in the fall has less potential for runoff than atrazine applied to the soil surface during the spring planting season (Rector *et al.*, 2003).

Atrazine use in ecofallow usually is supplemented with other herbicides. For example, the first herbicide application to wheat stubble often uses glyphosate and 2,4-D or dicamba, with the atrazine application postponed until later in summer to coincide with the emergence of volunteer wheat, cheat, and downy brome. Atrazine can be applied with glyphosate, but antagonism with some atrazine formulations is associated with this tank mixture (Stahlman and Phillips, 1979; Wicks and Hanson, 1995) because of physical binding of inert components in the atrazine formulation with glyphosate (Ahmadi *et al.*, 1980). Farmers know that if rainfall does not move atrazine off the wheat residue and into the soil, control of weeds, and volunteer wheat will be unsatisfactory.

Conclusions

Why Atrazine is Essential to Ecofallow

Norwood and Currie (1996) showed that yields and profitability of ecofallow corn at Garden City, Kansas, were superior to yields and profitability where V-blade tillage was used for weed control in the fallow period. They concluded that 'no-till is essential for adequate yields in dry years and usually will result in yield increases even in years with more favorable climatic conditions.' This study was conducted in a region of the central Great Plains where there has been little dryland corn production since early in the 20th century. At present, no other herbicide approaches the economic and biological advantages of atrazine.

Atrazine plays a central role in ecofallow because of its low cost, effective weed control, and extended soil activity. Atrazine controls volunteer wheat and most of the winter annual weed complex – including cheat, downy brome, wild mustards, and henbit, plus many spring annuals. No alternative herbicide has similar characteristics. Repeated applications of nonresidual, foliar-applied herbicides such as glyphosate or paraquat are not as economical.

In ecofarming systems, atrazine use is limited to the wheat stubble or ecofallow portion of the crop rotation. Tillage and/or foliar-applied herbicides may be used for the transition from sorghum or corn back to wheat. Dhuyvetter and Norwood (1994) reported that profitability of wheat-sorghum-fallow in Kansas was greatest when the wheat harvest-to-sorghum planting fallow period was managed with no tillage, and conventional or reduced tillage was used in the subsequent sorghum harvest-to-wheat planting fallow period. No broad-spectrum, soil-residual herbicides currently are registered for use ahead of wheat. However, a combination of decreased prices for glyphosate and a realization of the value of standing stubble have encouraged heavier reliance on nonresidual herbicides for rotating back to wheat in recent years.

Ecofallow: The Heart of Sustainable Dryland Farming in the Great Plains

Using herbicides to reduce or eliminate tillage in Great Plains crop rotations has shown this vast region to be considerably more productive than previously thought possible. The key to sustainable dryland farming in the Great Plains is reducing or eliminating tillage. Reduced or no-till farming retains crop residues on the soil surface, thereby reducing soil loss by wind and water, reducing soil water loss by evaporation or runoff from the soil surface, and enhancing infiltration of precipitation.

Consequences of more rapid replenishment of plant-available soil water are the shortening of fallow periods and a greater selection of crops from which to choose. Corn, sorghum, and millets are crops with higher water-use efficiency than wheat. Sunflower and soybean have deep taproots that can scavenge soil water not extracted by crops with fibrous root systems. Though not tolerant of atrazine, they have a place in more humid regions for double cropping after winter wheat and in drier regions following corn or sorghum, when atrazine residues have dissipated.

No-till methods, with more rapid replenishment of plant-available soil water, have paved the way for more intensified cropping, even to the point of eliminating summer fallow in some areas and some years. As a result, soil organic carbon and nitrogen concentrations are enhanced, especially near the soil surface (Sherrod *et al.*, 2003).

Wheat remains an important crop in the rain-fed Great Plains. It requires modest inputs, risk of crop failure is low, and the residues that remain after harvest play a key role in no-till crop rotations. Wheat residue is fine stemmed and very protective of the soil surface.

Atrazine remains the standard herbicide for making the transition from wheat to sorghum or corn in Great Plains cropping systems. Even where more intensified crop rotations have been developed, they are built around winter wheat followed by ecofallow sorghum or corn. The success of atrazine is due to its persistence as a soil-applied herbicide, to the broad spectrum of weeds controlled, to its low cost per acre, and to its safety on sorghum and corn. In the Great Plains, repeated burndown of weeds in fallow with nonresidual herbicides is not a viable alternative to the role that atrazine plays.

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Chapter 15

Weed Control in Sugarcane and the Role of Triazine Herbicides

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'We control weeds that sugarcane may grow and produce sugar… Tactics vary from weed prevention to keeping fi eld operations to a minimum… Once sugarcane attains a certain height, weed control continues without expense… The form of weed control that is economically best for sugarcane is not easy to decide…'

Dr. H.P. Agee,
Director, Hawaiian Sugar Planters' Association 1909 Plantation Strategy Report

Summary

Sugarcane is produced in mostly tropical regions of the world where year-round weed growth is favored. Before the sugarcane crop emerges, weeds must be managed in order to maximize solar heating and to stimulate new shoots. During early growth the crop is particularly sensitive to competition for light, moisture, and nutrients. Within 6 months the crop canopy expands to a size that shades and suppresses most weeds. Suppression is important because weedy sugarcane impairs harvesting and reduces sugar recovery at the mill.

Selective herbicides are relatively recent developments in the 5000-year history of sugarcane production. After 1945, when phenoxy herbicides were first sold, one person using 2,4-D in a backpack sprayer could accomplish the work of 15 others using hoes and with far more lasting results than simply severing weeds with a steel tool at the soil surface. While three herbicide families (triazines, phenoxys, and dinitroanilines) are of major importance in sugarcane production, the triazines, atrazine and ametryn clearly predominate.

Triazine herbicides were first developed for sugarcane in the 1960s. No other herbicide chemistry has provided the magnitude of global, lasting benefits to weed control in sugarcane as the single class containing atrazine and ametryn. Atrazine is used on greater than 70% of the US sugarcane acreage. While other herbicides have been commercialized for sugarcane, no single chemical or combination offers the advantages of atrazine in terms of consistent performance, low cost, residual control of numerous weeds, flexibility in time and method of application, compatibility with other herbicides, and crop safety.

Furthermore, the triazine herbicides have freed operators from much of the laborious burden of weed control, enabling them to manage other resources to maximize returns of both agricultural and milling operations. Since atrazine and ametryn were introduced, the genetic potentials of sugarcane cultivars have been more fully realized because the soil tillage and water losses have been reduced.

Ametryn is important in sugarcane on a global basis. Ninety-four percent of all ametryn sold is used in sugarcane. Ametryn is used more than any other herbicide on sugarcane; it is applied on more than 7 million ha (17 million A) of sugarcane worldwide. The chemical is especially useful for postemergence control of several grassy and broadleaf weeds and offers residual activity. Pendimethalin and trifluralin, two dinitroaniline herbicides, offer good residual control of seedling grasses and are sometimes applied with the triazine herbicides. However, they provide no postemergence control.

The sugarcane industry in the United States and worldwide is highly dependent on the continued availability of triazine herbicides. In the United States, growers use reduced quantities of atrazine by applying it as a band over the row. Ametryn is strategically important as a postemergence treatment. The loss of any of the most essential sugarcane

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herbicides, including ametryn, atrazine, or 2,4-D, will likely result in more frequent applications of other herbicides to maintain the same level of weed control. Also, risks of crop injury would occur.

Case studies from Hawaii and Louisiana document a dramatic decline in the development of sugarcane herbicides. Evidence shows that the decline in the Hawaiian sugarcane acreage was attributable, at least in part, to delays in commercializing new weed control products (Smith, 1998b).

Crop rotations and tillage are already practiced to the fullest extent feasible in sugarcane. Cultivation is a complementary practice; it is not a substitute for chemical weed control because sugarcane roots are extremely sensitive to tillage and compaction (Matherne, 1974). Some farmers endure a negative cash flow from fallow tillage to control pernicious weeds before replanting. Biological control with beneficial insects or pathogens may be possible for a few specific weed pests (Rozeff, 1997). The development of transgenic herbicide-resistant sugarcane offers a potential for controlling perennial weeds, but several nontechnical ownership equity issues must be resolved (Irvine and Mirkov, 1997). In addition, as more glyphosate-resistant crops are introduced, more weeds are becoming resistant to glyphosate.

Short Course on Sugarcane

Sugarcane (a complex hybrid of *Saccharum* species) and sugar beet are the earth's most efficient plants in producing sucrose for humankind. Sugar is one of nature's purest foods. The sugarcane crop naturally converts the sun's radiant energy into molecular sucrose. Sucrose contains no synthetic chemicals, carries no warning labels, and has less than 16 calories per teaspoon. Historical trends show that as subsistence family incomes rise, sugar is one of the first luxury items purchased by households.

Botanically, sugarcane is a perennial bunch grass that evolved as a wild species in New Guinea in the South Pacific (Deerr, 1949). With human migration, the species spread throughout the Pacific Basin and was introduced to Hawaii. Sugarcane came to the New World, Caribbean Basin, and Latin America by way of European colonists. Today sugarcane is grown either as a plantation crop with central management or on hundreds of diversified tracts by numerous small growers.

Prior to World War II, North America and Europe produced one-half of the world's sugar. Production was limited to rudimentary methods of artisan farming. With the emergence of scientific agriculture, world trade, and stronger economies, production methods improved in nearly all areas (Smith, 1978). Sugarcane today is grown on nearly 20 million ha (50 million A) in more than 100 countries. Although 15 countries account for more than 85% of the world's production (Table 15.1), the crop is produced under highly diverse technologies with a relationship between production efficiency (average yields) and weed control systems. For example, in Brazil, Mexico, Australia, and South Africa, where yields of 70–90 tons/ha are common, chemical weed control is commonly practiced. In other countries, such as Cuba (Alvarez, 2005), Pakistan, and Vietnam, hand weeding and older technologies still prevail, and yields are substantially lower (30–50 tons/ha).

Table 15.1 Global production of sugarcane (2005)[a]

Sugarcane production (15 leading countries)	Production area (1000 ha)	Sugarcane yield (metric tons/ha)
Brazil	5767	73
India	3750	62
China	1340	66
Thailand	1067	46
Pakistan	966	49
Mexico	640	71
Colombia	432	92
Australia	420	91
Cuba	400	31
United States	387	67
Philippines	380	81
Indonesia	350	73
South Africa	312	70
Argentina	305	63
Vietnam	280	54
83 other countries	2 909	–
World production	19 705	65

[a]2005 production data from Food and Agriculture Organization of the United States, Statistics Division. http://FAOstat.FAO.org/.

Sugarcane is vegetatively propagated by placing whole stalk sections with three or more nodes horizontally in the soil (Humbert, 1968). New roots and shoots are generated each year from nodal bands. After the first year's harvest, regrowth or ratoon crops are harvested annually for several years. After 8–24 months of sucrose accumulation, stalks are harvested and crushed to extract juice. Juice is boiled and evaporated to produce a thick syrup that is eventually crystallized to form sugar granules. Sugarcane also produces molasses and bagasse. Molasses is commonly fermented to produce alcohol products for fuel or beverages and is used to supplement animal feedstocks. Bagasse is the fiber constituent remaining after the juice is expressed. It is burned for steam generation for the milling process and in some cases to produce electrical power. Bagasse is also used in various industrial fiber products. Sugarcane also is becoming a crop of choice for the production of biofuels in certain countries. In Brazil, for example, the world's leading producer of sugarcane, nearly half the crop is used to produce ethanol (Bolling and Suarez, 2001).

Weeding Sugarcane: Historical Perspectives

Farmers have struggled with weeds throughout the 8000-year span of cultivated agriculture. Perhaps for the first few thousand years, people in hunter-gatherer societies simply accepted weeds and gleaned the plant products that nature provided (Borlaug, 1997). As seeds of superior crop plants were replanted, people became more aware of food losses caused by weedy vegetation, and hence the battle against weeds began. In the 5000-year history of sugarcane culture, the development of selective herbicides occurred only in the past 50 years. Herbicides have relieved thousands of workers from the drudgery of the oldest and most uninspiring work in humankind's quest for food. Sugarcane, with its long history as a cultivated crop, is an excellent model for examining the evolution of weed control from labor-intensive methods to current chemical- or molecular-based approaches. Economic losses to weeds continue to constitute the biggest threat to sugarcane production.

In the mid-1800s, global changes altered the way sugar was produced. Weary of an English blockade of sugar coming into Europe, Napoleon encouraged sugar beet production on the continent. By 1880 sugar beet production exceeded demand, which led to a worldwide collapse of sugar prices (Lowndes, 1956). At the same time, post-Civil War conditions in the United States led to shortages in farm labor. The world sugar economy did not recover until World War I increased the demand for food and disrupted sugar beet production in Europe (Crafton and Walton, 1970). Periodic shortfalls in price and labor drove new investments in research to cut costs and enhance yields. However, advances in weed control did not occur until after World War II, when newly developed herbicides began to replace callused hands and steel tools.

Weeding by Hand

The early history of sugar was closely tied to servant labor because weeding required year-round attention by plantation workers. Nearly all sugarcane literature up to the late 1800s placed heavy emphasis on management practices that discouraged the development of weeds. Three examples are illustrative of the tremendous cost of weeding sugarcane by hand.

In 1837 in Jamaica, early season hoeing required 25–30 person-days per hectare (10–12 person-days/A), while mid- and late-season weeding took 30 days per hectare, and post-harvest hoeing and shaping ratoons involved 25 person-days per hectare (Crafton and Walton, 1970).

In the 1920s, detailed production records from Java showed that weeding made up 38% of all production expenses (Quintus, 1923). While child and female labor was relatively inexpensive at that time, tremendous crop losses still occurred. In Java, hoe laborers were paid 24 cents per day, or about $26.00/ha ($10.50/A). Those wages, adjusted for inflation, would have been $600/ha in 1995. Labor records for the Mysore Sugar Company in India in the mid-1930s showed that each weeding required 60 person-hours per hectare (Rao, 1961) and was essential for the first 21 days after planting. Weeding costs made up 38–40% of all labor requirements and more than 20% of all production costs.

The capital requirements for weeding were relatively low when only simple tools were involved. Cutlasses or machetes were the early steel tools for weeding. However, weeding with a blade only severed a weed at the soil surface and failed to destroy the root system, resulting in rapid regrowth. Annual grasses retain a growing point near the soil surface and perennials regrow from underground meristems. Early hoes and mallets were crudely made from wood, which wore out quickly from soil abrasion, offered limited effectiveness, and resulted in low productivity by laborers. Deerr (1911) described and photographed numerous early manual implements used for weed control in sugarcane, including cutlasses, hoes, forks, and shovels.

A steel blade on a stick offered durability and was a great advancement for both labor and management. Early hoes were forged by plantation blacksmiths or local artisans and were sometimes crafted to the worker's preference. Hand hoeing is seldom used in US sugarcane production. Independent of the costs, the hard work of hoeing, management

time and logistics, increased potential for erosion, and the lack of residual control are all deterrents to hand-hoeing sugarcane. Experiences in small plots in Hawaii verified that hoeing costs today would be greater than the $600/ha cost extrapolated from Java. However, hand hoeing is still practiced in sugarcane in many parts of the world where labor is plentiful and the lack of capital still limits the use of herbicides.

Animal Power and Mechanization

Plows and animal-powered cultivators were not commonly used on most sugarcane properties until after labor shortages occurred. As the massive hand-hoe crews all but disappeared from sugarcane fields, animal-drawn implements provided weed control. By 1928, one animal-powered riding cultivator did the work of 20 men with hoes (Earle, 1928).

In the subtropics and more temperate regions, mules and horses were the preferred draft animals because of their speed and flexibility in cultivating sugarcane. Draft requirements tended to be less demanding in temperate zone soils, and equines offered more versatility – as in off-farm transportation. Oxen are still commonly used for tillage and weed control in many tropical areas of the world. Oxen and bullocks are heavily muscled, have cloven hooves for good traction, and provide increased draft power required for the clayey, weathered alluvial soils commonly found in the tropics. Sugarcane plantations typically maintained an oxen herd to till fields and to haul sugarcane. With a long yoke, two animals straddled a sugarcane row, and a long hitch line allowed the cultivator to be held close to one side of a row. But the usefulness of oxen for sugarcane cultivation was limited. Oxen work best in teams and, as the crop grew taller, the long yoke between the two animals broke off sugarcane tops (Earle, 1928). However, in many regions of the world, the multipurpose bovine is still important in the local economy. These animals convert local roughage and other feedstuffs into meat and milk, provide manure, and are used for draft and transportation. For more than two centuries, most of the world's sugarcane found its way to the mill by ox carts.

By 1928, tractor cultivation was in experimental stages. Capital needs for equipment and recurring outlays for fuel prolonged the adoption of mechanized agriculture. However, the distribution, mechanical, and logistical difficulties associated with gas- or alcohol-powered rigs were soon overcome. Cultivation in sugarcane was based on field inspections for weeds and was usually required every 2 weeks.

Both Earle (1928) and Deerr (1949) discussed cultivator design features and advantages in detail and included descriptions, drawings, and photographs of horse-drawn cultivators, spike-tooth and spring-tooth harrows, shovels and disks, and other tools for sugarcane.

After World War II, mechanization became virtually essential for weed control and resulted in dramatic adoption of tractors in sugarcane. In Louisiana, the number of tractors on sugarcane farms doubled between 1940 and 1947. During this same period, sugarcane acreage in Louisiana increased 20% while farm labor decreased 40%. Much of this change was due to cultivation with tractors and the introduction of 2,4-D for broadleaf weed control (Conrad and Lucas, 1995).

Flame cultivation was attempted in sugarcane in the 1940s (Conrad and Lucas, 1995), but was soon abandoned. Liquid propane flamers burned broadleaf and grassy weeds as shields partially protected the crop from thermal damage. While flame cultivation was only marginally useful in sugarcane, tractor-mounted weed burners have been important in the transition from dependence on repetitive mechanical cultivation to the concept of chemical energy for weed control.

Weeds and Sugarcane Biology

Scientific investigations of sugarcane date back only one century as a consequence of the geographic diversity and remoteness of the crop (Deerr, 1911). Deerr reviewed the sugarcane writings produced between 1848 and 1903. The works were mostly localized descriptions of production practices in the Caribbean, Java, and other tropical regions, with little scientifically designed research. Most of the early technical work was from colonial areas and predominately written in Spanish, German, French, and occasionally English. The sugarcane world was fortunate to have Deerr's integration of world knowledge of sugarcane biology, production, and processing, which established an historical base for those who followed (Deerr, 1949).

Soil Aspects

Sugarcane grows slowly in the early phase of development. Weed competition imposes serious nonrecoverable consequences; the crop needs a distinct biological advantage until a crop shade canopy can suppress understory weeds. Poor crop stands exacerbate weed control and add to costs because the increased light penetration results in greater weed growth and lower sugarcane yields. Humbert (1968) summarized many of the following scientific and biological aspects of sugarcane culture related to weed management practices.

Control of winter annual ground cover is essential for early season crop growth. Weeds reduce solar heating of soil, delaying crop root regrowth. Root development is particularly critical in ratoon stands because new roots must be generated annually when soils are naturally cool. A minimum soil temperature of 17ºC (62ºF) is required for sugarcane root growth. Accumulation of radiant heat is also essential to reduce crop damage on cold nights. Nutrient uptake is enhanced and less leaching occurs in warmer soils.

Soil compaction from repeated tillage limits essential root functions, such as crop anchorage, root expansion, moisture extraction, and nutrient uptake. After planting or reshaping soil in the ratoon crop, any additional traffic compacts soil and impedes root extension between the rows. While some cultivation is necessary for integrated weed control, the overall control of weeds with residual chemicals is far more desirable than the constant movement of tillage equipment through a field. Cultivation must be viewed as a complementary practice rather than an alternative practice for weed control in sugarcane.

Various tillage methods, tools, smother crops, and other strategies have been researched throughout the last century in order to find ways to suppress perennial weeds or reduce annual weed populations before replanting a sugarcane crop. Improvements in horsepower and the design of tillage implements have resulted in faster response times for field operations. Combinations of mechanical and chemical fallow have enabled growers to remove weeds and start with a clean field during the first year of production. Weed control efforts must be renewed in subsequent ratoons. Fallow-tillage, herbicide-tillage combinations, and other cultural approaches have been researched in Louisiana with little long-term advantage over two or more crops (Richard and Viator, 1989; Richard, 1997).

Losses Imposed by Weeds

In weed competition studies, management and hand harvesting are far more difficult in sugarcane than in annual row crops. Hence, there are a limited number of crop-loss studies. Millhollon (1972) showed that sugar yields were reduced 11% when winter annual weeds remained in sugarcane until mid-March. In Hawaii, crop yields on Maui were reduced 5–30% because uncontrolled vines (most commonly morningglories and balsamapple) interfered with crop growth and harvesting. Unsuccessful control of guineagrass and vines caused growers to destroy and replant some fields of sugarcane. Other experiments showed that sugarcane growth and yield were reduced when grassy weeds were present for 5–6 weeks after the start of the growing season (Bruff *et al.*, 1996; Richard, 1996).

Weeds primarily reduce yield and sucrose content in sugarcane, but they also impose other losses on growers, millers, and surrounding communities. These related deprivations include harborage of other pests, impacts on noncrop areas, harvest losses, and sucrose losses in processing weedy sugarcane.

Weeds host pathogens and nematodes and use soil nutrients. One example is pigweed, a luxuriant extractor of soil nitrogen that causes nitrate deficiencies in the sugarcane plant. Weedy grasses serve as an alternate hosts and reservoirs for systemic viruses, and they harbor insects that carry diseases to sugarcane. Rats find shelter in weedy fields. Because stalk juice alone does not provide an adequate diet, rats are attracted to weed seeds as a source of protein.

Weeds must be controlled around field margins and along irrigation canals to reduce seed reservoirs, weed residues, and fire hazards. Weed propagules in noncrop areas pose problems because seeds move with irrigation or flood water (King *et al.*, 1953). Seed screens have been developed to remove guineagrass and other seeds from irrigation water.

Weedy vines entwine sugarcane stalks, impede both hand and mechanical harvest, and compromise worker safety. Morningglories and other weeds hamper trash removal and reduce the effectiveness of field burns and mechanical harvesting. Any additional trash creates other problems in the field and mills. Weeds may reduce harvesting efficiency by 5–20% and excessive weeds may cause some fields to be abandoned. In processing, mill recovery of sucrose is reduced by extraneous plant material. As a rule of thumb, each 1% increase in extraneous plant material passing through a mill reduces sucrose recovery by 1–2 kg per ton of sugarcane milled (Rozeff, 1999), which seriously affects economic returns for the milling operation.

Field workers also may be injured by weeds with spiny or thorny protrusions, burs, or needles that penetrate the skin. Examples include starbur, spiny amaranth, itchgrass, smooth pricklypoppy, and other weedy species with burs or spines. Some weeds may cause allergies in some workers, resulting in lost productivity.

Herbicide Development and Chemical Control in Sugarcane

Chemical weed control in sugarcane may have started in Hawaii. Sodium arsenite was first used in rubber plantations in 1913, but its most lasting impacts occurred in the sugar industry. At that time the Hawaiian industry was spending $750 000 to $1 million annually for hand hoeing, but growers learned that they could apply sodium arsenite at 5.5 kg/ha in 380 L of water and achieve weed control at one-fourth the cost.

Herbicide use in sugarcane generally developed when advances in chemistry for agriculture took place more rapidly after World War II and changing social conditions (e.g., movement of rural labor to the cities) spurred the use

Table 15.2 Summary of herbicide use in US sugarcane production in 2003–2005[a]

Herbicide	Planted hectares treated %	Chemical cost[b] $/ha	Herbicide use in US sugarcane %	Comments
Ametryn	29	5[c]	2	Limited flexibility; preemergence and post-directed
Asulam	29	87	15	Post only
Atrazine	725	20	45	Flexible; excellent crop tolerance
Dicamba	12	8	ffi1	Post-directed only; drift potential
Diuron	16	20	6	Preemergence and post-directed
Glyphosate	6	41	2	Post-directed only; shield necessary
Hexazinone	7	30	ffi1	Sensitive varieties; limits on soil types
Metribuzin	19	59	4	Short residual
Paraquat	5	15	ffi1	Post-directed only; shield necessary
Pendimethalin	28	30	12	Preemergence
Trifluralin	7	20	3	Preemergence; incorporation
2,4-D	42	7	8	Post to weeds; drift problems
Clomazone	2	63	ffi1	Potential for off-site movement
Halosulfuron	5	63	ffi1	Application flexibility and rotational limits
Trifloxysulfuron	5	46	ffi1	Application flexibility and rotational limits

[a]From Doane AgroTrak.
[b]Average cost based on rate applied per year.
[a]Cost based on average rate of 0.36 kg/ha, which is more typical of post-directed applications.

of selective organic herbicides. Between 1950 and 1980 numerous firms in the United States, Europe, and Japan launched massive searches for new herbicides. As advances occurred in organic chemistry, more effective chemicals evolved. However, regulatory demands increased, along with the time and cost of registering and maintaining products. Some chemicals, such as the aliphatic acids dalapon and trichloroacetic acid (TCA), are no longer available because profit margins did not justify the regulatory maintenance costs. Hanson (1962) and Humbert (1968) provide historical perspectives on the development of herbicides for sugarcane. General benefits of herbicides also have been reviewed (Millhollon, 1970).

Three herbicide families are of major strategic importance for sugarcane in the United States (Table 15.2). In order of importance in the sugarcane industry today, these are the triazine, phenoxy, and dinitroaniline herbicides.

Triazine Herbicides

Since the 1960s, three triazine compounds have been particularly important in the sugarcane industry. These include simazine, atrazine, and ametryn. The strategic and tactical importance of the triazines in sugarcane production cannot be overemphasized.

Starting in 1960, simazine was the first triazine to be sold as a selective preemergence herbicide in sugarcane, and it is still labeled for the crop in some countries. Simazine demonstrated excellent crop safety and was particularly useful in controlling small-seeded broadleaf and annual grass weeds. It was nonhazardous to applicators and easy to handle. This chemical offered excellent soil residual activity, a particularly important benefit because sugarcane is grown in areas that receive frequent rainfall or irrigation, which stimulates numerous flushes of weeds. Simazine is no longer used for sugarcane in the United States. However, atrazine offered the same advantages with the added versatility of foliar activity and complementary uses with other herbicides.

Atrazine was registered for preemergence and postemergence use on sugarcane in 1961 and ushered in revolutionary advantages for producers. Atrazine is the most widely used sugarcane herbicide in the United States and some other countries because it provides residual, broad-spectrum weed control, offers flexibility of application and use with other chemicals, has excellent crop safety before or after emergence of either plant or ratoon sugarcane, and gives consistent, economical control. Atrazine is used by virtually every mainland and offshore sugarcane operation in the United States (Gianessi and Reigner, 2006). It is applied on 70% or more of US sugarcane, accounting for nearly 4% of worldwide atrazine use.

The availability of atrazine enabled sugarcane growers to plan their production systems strategically with greater biological and economic certainty (Hilton and Osgood, 1972). With consistent chemical weed control, repeated soil tillage was no longer necessary. In addition, the genetic yield potentials of new cultivars could be realized, fertilizer efficiency was greater, and less rainfall and irrigation water was lost to weeds. In brief, once operators began to use

atrazine to control most weedy vegetation, managers could concentrate on other production and quality factors to maximize returns.

Atrazine use varies in each region of the United States because of differing soil and environmental characteristics and sugarcane cropping practices. For example, in Florida two or more residual herbicide applications are required for each crop because much of it is grown on muck soils and rainfall is relatively high. In Louisiana and Texas, broadleaf weeds in sugarcane are readily controlled with atrazine, but perennial grass problems require special attention. In Hawaii atrazine is used only in the first few months of the first year of a 2-year production cycle (Santo, 1989). These different use patterns illustrate why atrazine is a foundation tool for weed control in sugarcane in diverse regions throughout the world.

Today atrazine is used primarily for broadleaf weed control. This versatile herbicide may be applied with either ground or aerial equipment, at planting or before ratoon growth emerges, and broadcast or banded after emergence. A total of 11 kg of active ingredient per hectare may be applied per crop year, in some situations, but that total annual rate is seldom necessary (see product label for details). To date no atrazine-resistant weed populations have become a significant problem where sugarcane is monocultured. This is likely due to the use of other herbicides applied in combinations or sequentially.

A sugarcane herbicide use survey was conducted in three states, and findings were extrapolated for the United States (Smith, 1998a). Atrazine was applied on 89% of all sugarcane land and made up 31% of all herbicides used in sugarcane. Atrazine was used by all sugarcane growers and was frequently tank mixed with another herbicide to broaden the spectrum of weed control. The single highest application rate of atrazine was 4.5 kg/ha in Hawaii. Louisiana reported the highest number of applications (three), with the chemical commonly banded at 2.8 kg per treated hectare.

Ametryn was introduced in 1962 and is sold as Evik® or Gesapax®. Globally, more than 94% of all ametryn sold is used in sugarcane. Applied on 2.8 million ha (7 million A) of the crop annually, ametryn is the most widely used sugarcane herbicide. More than 60% of all ametryn is used in Latin America. Ametryn is a versatile, selective herbicide commonly used as a postemergence treatment. Ametryn has excellent foliar activity on grass and broadleaf weeds and offers good, short-term residual activity. Compared with atrazine, phytotoxicity may occur in some sugarcane cultivars, and ametryn is not as effective for preemergence control of large-seeded broadleaf weeds. In the United States, ametryn is applied on 29% of sugarcane acreage and makes up 2% of all herbicide used on the US crop (Table 15.2).

Metribuzin, an asymmetrical triazine (triazinone) herbicide, provides residual control of seedling weeds and is relatively nonphytotoxic to sugarcane. Metribuzin is used on 19% of acres and makes up about 4% of the US market. Hexazinone, a triazinedione herbicide, can give good seedling weed control, but is expensive and may cause damage to sensitive cultivars or sugarcane suffering from cold damage, drought, or insect infestations.

Phenoxy Herbicides

The development of 2,4-D in 1947 ushered in dramatic changes in weed management. Weed control in sugarcane was never the same once managers and laborers saw what could be achieved using chemical weed control methods. The early control of broadleaf weeds with 2,4-D involved teams of 4–10 people using pressurized sprayers, hand lines, or knapsack sprayers. These early devices were heavy and expensive compared with today's lightweight, hand-pumped plastic sprayers. However, the knapsack sprayer was a big advancement for the industry, and workers could be paid more because of greater efficiencies. For example, by 1950 one individual with a hand sprayer and 2,4-D could control weeds at the rate of 3.4–6 person-hours/ha (1.6–2.4 person-hours/A), in contrast to 60 person-hours/ha (24 person-hours/A) required for hoeing weeds.

In 2003–2005, 42% of US sugarcane was treated with 2,4-D, the second leading herbicide in the crop. This chemical is inexpensive, controls a wide range of annual and perennial broadleaf weeds, and complements the spectrum of weeds controlled with triazine herbicides. However, 2,4-D is restricted in many areas to protect nearby sensitive crops, such as cotton and vegetables. If 2,4-D were not available for use in sugarcane, the net economic loss to the US sugarcane industry is estimated to be $51 million annually (Nalewaja, 1996).

Dinitroaniline and Other Herbicides

The two dinitroaniline herbicides pendimethalin and trifluralin are used on 28% and 7%, respectively, of the US sugarcane acres. Both chemicals give residual control of seedling grasses and are commonly used in conjunction with atrazine. Pendimethalin has fewer soil incorporation requirements, while trifluralin is less expensive.

Table 15.3 Ranking of weeds most commonly found in US sugarcane fields in 1997

Florida	Hawaii	Louisiana	Texas
Fall panicum	Guineagrass	Winter annual broadleaves	Common sunflower
Broadleaf panicum	Morningglories	Annual grasses (crabgrass, junglerice, goosegrass)	Pigweeds
Alexandergrass	Swollen fingergrass	Nutsedge	Guineagrass
Goosegrass	Nutsedge	Johnsongrass	Johnsongrass
Crowfootgrass	Showy crotalaria	Morningglories and vines	Winter annual broadleaves
Foxtails	Pigweeds	Itchgrass	Annual grasses (Texas panicum or junglerice)
Nutsedges	Alexandergrass	Bermudagrass	Morningglories and vines
Pigweeds	Bermudagrass	Pigweeds	Nutsedge
Ragweed	Napiergrass	Browntop panicum	Bermudagrass
Florida pellitory	Job's tears	Broadleaf signalgrass	Woolly croton
Itchgrass	Paragrass		
American black nightshade	Goosegrass		

In addition to the triazines, 2,4-D, and the dinitroanilines, several other herbicides are registered for sugarcane and account for the remaining US market (Table 15.2). Asulam, used on 29% of acres, is especially useful for post-emergence control of perennial grasses, but lacks residual activity and is expensive. Dicamba (on 12% of A) and glyphosate (on 6% of A) are used in conjunction with atrazine and help meet special weed control needs, but they do not provide residual control and can present serious drift risks near other crops. Diuron, used on 16% of Asulam, gives good postemergence and short-term residual control of some species. In high rainfall areas diuron is frequently applied in combination with atrazine.

Registrations have been granted for several new herbicides that are being introduced into sugarcane; their use is still relatively small. These are briefly described as to application method, rate, and weed spectrum.

Carfentrazone is an aryl triazinone broadleaf herbicide used at a low rate of 0.1 kg a.i./ha/season as a directed postemergence application. Sugarcane is subject to injury, which may limit product use.

Clomazone is effective on many grass and broadleaf species in the 1.12–1.4 kg a.i./ha/season rate. It has the potential for off-site movement, though, which limits its potential use in sugarcane.

Halosulfuron is a sulfonylurea, labeled for multiple preplant emergence and/or postemergence applications, not to exceed 0.14 kg a.i./ha/season for control of broadleaves and sedges. Grasses are not controlled.

Sulfentrazone is an aryl triazinone broadleaf and sedge herbicide that can be applied preemergence, and/or post-directed up to layby. The maximum total annual rate cannot exceed 0.42 kg a.i./ha.

Trifloxysulfuron is a sulfonylurea for control of grasses, sedges, and broadleaf weeds following preemergence, postemergence over the top of sugarcane, or post-directed applications. The total of all applications should not exceed 0.08 kg a.i./ha per season.

Since these products are in the introductory phase in the sugarcane market, their impact on sugarcane weed control practices is still being defined.

The economic value of selective herbicides in sugarcane is difficult to quantify. However, it can be conservatively estimated that worker productivity increased 142% or more between the time 2,4-D was introduced in 1947 and the widespread use of atrazine in 1965. Sugar yields have increased similarly due to a myriad of technical advancements, all of which were dependent on consistent control of weedy vegetation.

Herbicide Use and Weed Targets

Effective pest management targets specific weed species. In a survey on sugarcane in the United States (Smith, 1998a), participants were asked to rank weeds (a) that most commonly occurred in sugarcane fields (in the absence of any control measure), and (b) in order of difficulty of control. Two 'top ten' weed lists were prepared for each state. The highest-ranking 'most common weeds' were primarily annual species (Table 15.3). Most of the 'common weeds' shown in Table 15.3 were being controlled with atrazine, 2,4–D, or a dinitroaniline herbicide. In contrast, the top three 'most difficult to control weeds' in all states (Table 15.4) were perennial or pernicious grasses that lacked economical, consistent methods of control.

The contrast between the 'most common' and 'most difficult' weeds is particularly significant in considering the herbicides that are labeled for sugarcane. For example, the two most commonly used herbicides (atrazine and 2,4-D)

Table 15.4 Ranking of weeds most difficult to control in US sugarcane fields in 1997

Florida	Hawaii	Louisiana	Texas
Itchgrass	Guineagrass	Bermudagrass	Guineagrass
Giant foxtail	Napiergrass	Itchgrass	Johnsongrass
Yellow foxtail	Alexandergrass	Johnsongrass	Bermudagrass
Alexandergrass	Swollen fingergrass	Morningglories and vines	Nutsedge
American black nightshade	Bermudagrass	Browntop panicum	Common sunflower
	Paragrass	Broadleaf signalgrass	Morningglories and vines
	Morningglories	Winter annual broadleaves	Annual grasses
	Showy crotalaria	Annual grasses	Winter annual broadleaves
	Pigweeds	Pigweeds and summer annuals	Pigweeds
		Nutsedge	

were developed more than five decades ago, and the dinitroaniline herbicide family has been around for four decades (Santo, 1992). Growers everywhere depend heavily on these three chemical groups to control the top-ranked 'most common weeds.' A concern is that the top-ranked weeds on the 'most difficult to control' list are perennial or pernicious grasses with few prospects for new herbicides to provide control.

Weed Challenges and Herbicide Use in Other Countries

Sugarcane is commercially produced in almost 100 countries around the world. Weed problems and herbicide use in four countries are summarized in the sections that follow. These countries represent some of the diversity found in sugarcane production. For example, production in Australia takes advantage of a favorable climate and progressive marketing. Brazil is a world leader in industrial use of sugarcane for both alcohol and sugar production. Mauritius is a small island country with a well-organized, adaptive research program supported by the sugar industry. The Republic of South Africa has a long history in weed management research. These four countries share several similarities in weed problems, but they face diverse social and economic factors in adapting sugarcane herbicides to meet local needs. However, a common thread throughout these and other countries is the predominant use of triazine herbicides for effective and economical control of weeds in sugarcane.

Many herbicides available for use in sugarcane in other countries are not currently labeled for sugarcane in the United States. These herbicides include acetochlor, alachlor, dithiopyr, EPTC, fluazifop-P, ioxynil, MCPA, metazachlor, *S*-metolachlor, MSMA, sulcotrione, sulfentrazone, sulfosate, tebuthiuron, and thiazopyr. Many of these chemicals control specific weed problems or are strategically important in controlling perennial grass weeds and sedges in the perennial grass crop.

Australia

Sugar production in Australia evolved from artisan boiling in open kettles in the mid-1800s to one of the most modern production systems in the industry today. The industry prospered initially from domestic demand, but now 85% of the production is exported to markets in the South Pacific, Southeast Asia, and Canada.

Sugarcane is grown on more than 400 000 ha in Queensland and New South Wales, with average yields of approximately 90 tons/ha. A small industry of 3800 ha exists in the Ord River district in Western Australia. Rainfall from moisture-laden winds off the Pacific Ocean ranges from 110 to 420 cm per year on the eastern side of the continent; however, 50% of the crop receives supplemental irrigation. Sugarcane is grown on soils derived from alluvia or sedimentary rock and some smaller areas of volcanic origin. Soils are generally acidic, highly leached, and low in organic matter.

Historically, tillage and hand weeding were the main methods of weed control. Since the advent of herbicide use in 1950, essentially all sugarcane in Australia now receives one or more herbicide applications. Virtually all of the crop is produced on family-operated, commercial-scale farms and is processed at cooperative or privately owned mills.

The lush growing conditions of Australia create year-round problems with annual and perennial weeds. Major weeds include purple nutsedge, guineagrass, bermudagrass, and crabgrass (McMahon, 1989). Sugarcane is mechanically harvested; 35% is burned and 65% is harvested green, without burning. The green unburned extraneous plant material forms a trash blanket that conserves soil moisture and suppresses weed development. This plant residue reduces the use of preemergence herbicides and cultivation in ratoon cane. However, large-seeded vines and morningglories pose problems because they emerge through the residual plant litter and are not controlled with preemergence herbicides.

Herbicides account for 93% of all pesticide use in sugarcane in Australia. Pesticide use throughout the sugarcane region has been identified by river basin areas within the country (Hamilton and Haydon, 1997). Atrazine, the most commonly applied herbicide, is used on 35–45% of the crop annually. Atrazine provides important residual control, which enables sugarcane to grow without competition from weeds or disturbance from soil cultivation. The versatility of atrazine in application and weed control is an important factor in its widespread use. Diuron is also applied alone or in combination with other herbicides for broad-spectrum control in sugarcane. Broadleaf weeds are controlled in 15% of the crop with 2,4-D. Glyphosate is applied as a directed postemergence spray in the crop and between plantings to control perennial grasses and other weeds. Paraquat, MSMA, and ametryn are used to a more limited extent. Timely, cost-effective weed control will continue to be a high priority for Australia sugarcane growers.

Brazil

Since Portuguese settlers introduced sugarcane in 1530, the crop has become a leading commodity in Brazilian agriculture. Well over 5 million ha are now grown, and this number is expected to increase due to the use of sugarcane for biofuel production. One-half of the production is in the state of São Paulo, with additional production in other south central states and, to a lesser extent, in the tropical northeastern coastal states. In the state of São Paulo, sugarcane is typically grown on dark red oxisols, which are low in organic matter (1–3%), have pH levels of 4.0–6.0, and receive seasonal rainfall from September to March. New plantings are completed before the rainy season begins. The crop is ratooned six or more times. Green manure crops and peanut, soybean, and other vegetable food legumes are frequently grown between replantings for additional income and to improve soil fertility.

Sugar production in Brazil dramatically increased after 1980 when the National Program of Alcohol was initiated to reduce dependence on petroleum imports. Over the past 20 years, sugarcane hectarage has doubled, yields have increased 50%, and ethyl alcohol production has tripled to more than 14 billion liters. A portion of these dramatic increases in Brazil is attributed to chemical weed control, which enabled better yield expression of new cultivars and improved use efficiencies in water and fertilizer.

Part of the increased hectarage in Brazil resulted from sugarcane expansion into lands previously devoted to pastures. Perennial grasses, such as guineagrass, became weed problems in sugarcane. In older, traditional areas of production, major weed problems include Alexandergrass, purple nutsedge, redroot pigweed, tropical spiderwort, wild poinsettia, common purslane, and several species of crabgrass, morningglory, and Sida. Purple nutsedge is a problem during the first 40 days of plant cane establishment (Kuva *et al.*, 1999).

Prior to the 1980 alcohol program and incentives, herbicides were used on less than 50% of the crop. Inexpensive labor and plentiful land enhanced the expansion of sugarcane. However, with the growth of progressive educational systems throughout most of the country, fewer people were available for traditional hand weeding. Consequently, herbicide use steadily increased, and by 1993 more than two-thirds of the sugarcane was treated with an herbicide. Today all plant cane and 70% or more of the ratoon crops are treated with one or more herbicides. Because sugarcane is mechanically harvested without burning the plant trash, 10–15 tons/ha of residual plant material remain on the soil surface, causing a shift in weed flora.

Twenty percent of all herbicide expenditures in Brazil are for weed control in sugarcane. In the 1940s growers started using 2,4-D to control broadleaf weeds in sugarcane. Use of simazine, atrazine, and diuron began when grassy weeds became more troublesome. Today four chemicals – ametryn, tebuthiuron, and a mixture of hexazinone plus diuron – make up 70% of all herbicide use on sugarcane in Brazil.

Ametryn is the leading herbicide in sugarcane in Brazil because of its preemergence and directed postemergence effectiveness against a broad spectrum of grass and broadleaf weeds. New plantings are treated with ametryn prior to the rainy period, which enables the crop to grow undisturbed by weeds for 50–80 days. In ratoon crops, ametryn controls the more common weeds after harvest and provides residual control during crop regrowth. Simazine and atrazine were important in Brazilian sugarcane for many years, but these chemicals are rapidly metabolized to a hydroxy metabolite under high moisture and temperature conditions. Ametryn, a methylthio-*s*-triazine, follows a different, slower path of degradation and provides longer herbicidal activity in sugarcane in Brazil.

Use of tebuthiuron increased when the alcohol program in Brazil expanded sugarcane production. This urea derivative controls several perennial grasses and is often applied in combination with ametryn. Its persistence is important for weed control during dry periods, but can be a limiting factor if legumes are planted between cycles of sugarcane. Hexazinone, an asymmetric triazine, is commonly applied with ametryn or diuron in preemergence or early postemergence treatments. The diuron–hexazinone mixture shows some injury to new sugarcane plantings in sandy soils. Hexazinone is applied during dry periods and still gives lasting control of perennial grasses. Other herbicides that make up the remaining 30% of agricultural chemicals used in the Brazilian sugarcane market include clomazone, isoxaflutole, 2,4-D, MSMA, and atrazine.

Mauritius

Mauritius offers an interesting case study of herbicide use in sugarcane. Although this island country is somewhat geographically isolated in the South Indian Ocean, the industry has a progressive history of weed research. Herbicides and additional weed management techniques from other countries have been adapted to benefit sugarcane productivity in Mauritius. This rapid adoption of new technologies is also common in other regions of sugarcane production.

More than 70 annual and perennial weed species occur on this volcanic island (McIntyre, 1991). None of these weeds are indigenous; they generally have resulted from commerce in earlier centuries as sailing ships plied between Europe, Africa, and Asia. Mauritius was a strategic trade location and its tropical and subtropical environment was a haven for weeds commonly found in other parts of the sugarcane world. Today the major weeds in Mauritius sugarcane include purple nutsedge, bermudagrass, guineagrass, vaseygrass, tiende capote (Spanish), or capim-colchao (Portuguese), blue (or giant) panicgrass, tropic ageratum, Russell rivergrass, black nightshade, and several pigweed species. Annual rainfall ranges from 80 cm on the west coast to more than 400 cm at 700 m elevations. The seasonality of rainfall enhances crop growth and ripening, but exacerbates weed control problems.

Herbicide research started in the 1950s with impetus from the Mauritius Sugar Industry Research Institute. Early adopted herbicides included phenoxys, atrazine, and diuron. Today more than 80% of the sugarcane crop is treated with one or more herbicides. Diuron is the most commonly used herbicide and is applied alone or in mixtures with other herbicides, such as oxyfluorfen, acetochlor, or *S*-metolachlor. In ratoon crops, diuron is frequently applied with hexazinone to extend the period of weed control. Atrazine is applied on 40% of the crop for preemergence weed control and is nearly always used in combination with acetochlor, *S*-metolachlor, or tebuthiuron. Also, atrazine is an important herbicide option because some Mauritian sugarcane varieties are susceptible to diuron. Of the triazine herbicides, atrazine and hexazinone provide important preemergence and postemergence weed control. Ametryn is not used in Mauritius. Sometimes paraquat is applied with residual preemergence herbicides to control weeds that emerge before the crop tillers.

More than 80% of the plant and ratoon crops are treated with one or more postemergence herbicides. Asulam is used to control annual and perennial grasses. Ioxynil and 2,4-D esters are applied alone or in combination with residual herbicides to provide general weed control. Halosulfuron-methyl effectively controls purple nutsedge during sugarcane establishment or early in the ratoon crop. Minimum tillage is practiced on sloping land by applying glyphosate to kill the sugarcane stubble while the old interrows are being tilled to receive a new planting. Herbicides made it possible to control soil erosion and weeds and have enhanced environmental and economic sustainability.

Chemical weed control is sometimes augmented with manual weeding, a practice that is more commonly used by the small-scale growers who provide 30% of the total sugar tonnage. With the rising cost of labor and the gradual shift of workers to other industries, manual weeding is gradually being abandoned in favor of chemical weed control and mechanized tillage.

South Africa

South Africa is the major producer of sugar on the African continent, although sugarcane is also grown in many other African countries, often in small areas and mostly for local consumption. Starting about 1910, the industry established progressive research programs, including development of well-adapted varieties and cost-effective methods of weed control. Production is located north and south of Durban on the eastern coast, which is a region fed by moist trade winds from the Indian Ocean. Some production occurs at higher elevations in the interior. Historically, sugar production in South Africa has progressively increased to meet domestic needs and support regional exports. Today, sugarcane is produced in 16 regions on 312 000 ha.

Until two decades ago, most sugarcane weeding was achieved by hand labor and tillage. Since 1970 herbicide use has progressively increased, and today more than 80% of the crop receives one or more herbicide treatments. More than 2000 large-scale growers produce 70% of the crop, milling companies grow 17%, and 52 000 small-tract (1 ffl ha) operators grow the remaining 13%. Small-tract farmers rely on hand hoeing to control weeds, but programs are underway to provide education and herbicides to help improve the profitability of growing the crop.

Major weed problems include purple nutsedge, guineagrass, bermudagrass, and sorghums (Leibbrandt, 1995). Wild sorghums are pervasive annual weeds because eastern Africa is the center of genetic origin of these species, which are bred for grain production for other parts of the world. The perennial bermudagrass species are also indigenous to Africa. Johnsongrass was a major weed in past decades, but is less of a pest now because of better chemical control.

Numerous herbicides are available for the diversity of soils, climates, and environmental conditions, based on research by the South Africa Sugar Association Experiment Station. Major herbicides include ametryn, alachlor, atrazine, hexazinone, and metribuzin.

Ametryn was registered for use in sugarcane in South Africa in 1970 and is a leading herbicide. Ametryn is used in preemergence and postemergence applications, usually in combination with another herbicide – such as *S*-metolachlor, hexazinone, metribuzin, MCPA, diuron, and MSMA – to broaden the spectrum of weed control. Atrazine was introduced in 1972 and was commonly used for several years. A limited amount of atrazine is applied, usually in a tank mix with acetochlor or other herbicides for preemergence weed control. Hexazinone is applied in both preemergence and postemergence on at least 50% of the ratoon fields. Sugarcane varieties in South Africa exhibit good tolerance to triazine herbicides.

Diuron is used on 65% of the crop and is applied preemergence and/or post-directed. Paraquat and glyphosate are applied on 30% and 14% of the crop, respectively, as directed sprays under the sugarcane or as land cleanup treatments after harvest. MCPA is used on 15% of the crop for controlling broadleaf weeds and MSMA is applied on 20% of sugarcane for controlling grass weeds. Acetochlor, *S*-metolachlor, metribuzin, and tebuthiuron are each applied on 20% or less of the crop, depending on localized soil and weed conditions.

Postemergence treatments include paraquat, glyphosate, sulfosate, MCPA, and MSMA. Triclopyr is applied with diesel oil as a postemergence stem application to control exotic woody plants encroaching into sugarcane fields. Some herbicides labeled in South Africa are not available to US growers. For example, each year about 10% of the total crop is replanted, and a small portion of the old ratoons is sprayed with fluazifop-P to control perennial weeds and terminate crop growth. Nutsedge is a perennial problem, but halosulfuron-methyl and sulfentrazone give effective control. Sulcotrione and thiazopyr are also used to some extent in South Africa for sugarcane.

The South Africa Sugar Association maintains an industry-supported research program to develop new technologies to sustain sugar yields. Sugarcane growers are keenly interested in controlling costs while maintaining productivity. Mills are interested in increased sugar yields from both large tracts and small-scale growers.

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Chapter 16

Benefits of Triazine Herbicides and Other Weed Control Technology in Citrus Management

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Summary

Citrus is produced in subtropical climates with relatively high rainfall and in semiarid regions supplemented with irrigation. Abundant moisture, in addition to the frequent application of fertilizers and high temperature, encourages year-round luxuriant weed growth. Managing weeds is a necessity in citrus production. Weeds compete with citrus for moisture, nutrients, and sunlight and interfere with spraying, irrigation, and harvesting – causing 25–33% loss to citrus farmers. Weeds also reduce soil and air temperature, increasing the chance of frost damage to citrus trees during cold seasons. They are host to pathogens and harbor other pests. Thus, weeds cause considerable economic loss in citrus production (Jordan and Day, 1967; Tucker and Singh, 1983). For these and other reasons, chemical weed control has become an integral part of citrus production programs throughout the world.

Simazine was among the first herbicides registered for use in Florida citrus in 1962 and was recommended by the University of Florida as an excellent weed control tool for citrus (Kretchman and McCown, 1962). This early registration of simazine was for the control of annual weeds, while today's registration includes broadleaf weeds, annual vines, and annual grasses (Singh and Tucker, 1987).

Triazine herbicides have provided a significant contribution to the effective control of weeds in citrus around the world and still are major tools in citrus weed management strategies.

Introduction

The United States is currently the second largest citrus-producing country in the world. In the late 1970s, the United States ranked first in citrus production, with Florida alone producing more than any country outside the United States. However, increased planting in Brazil and the loss of much Florida acreage due to a series of freezes has resulted in Brazil becoming the number one citrus-producing nation (Jackson, 1991; FAO, 2003). Citrus fruits are second only to apple in world trade.

The common citrus fruits belong to three genera, *Citrus, Poncirus* (trifoliate orange), and *Fortunella* (in the *Rutacea* family) (Castle, 1987). Hereafter, 'citrus' will refer to any or all of these three genera. The citrus tree is a native of Southeast Asia (Woodhead, 1981).

This chapter will deal with citrus losses caused by weeds, major weeds infesting citrus groves around the globe, and controlling weeds in citrus with a special emphasis on triazine herbicides. A brief description of the control measures available in different parts of the world and their limitations are discussed.

Global Production of Citrus

Citrus trees are grown in all the continents of the world in areas where water and soil requirements can be met and temperatures do not generally fall below freezing. Citrus fruits are produced in more than 70 countries with tropical and subtropical climates, although the top 10 countries produce more than 70% of the world output. Citrus acreage and production have increased significantly from about 23 million metric tons in the early 1960s, to 48 million in 1975, to more than 75 million in 1991, and to almost 98 million metric tons in 2003 (Table 16.1). Effective weed management has contributed significantly to this increased production, and among the herbicides used for weed management in citrus groves, triazines have had a pivotal role.

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Table 16.1 Leading citrus-producing countries by cultivar in 2003[a]

Countries	Orange	Grapefruit Pomelos	Tangerine Clementine Mandarin Satsuma	Lemon Lime	Total
			x 1 000 000 metric tons		
Brazil	16.94	0.07	1.26	0.95	19.22
United States	10.47	1.87	0.49	0.94	13.77
China	1.65	0.36	9.00	0.60	11.61
Mexico	3.97	0.26	0.36	1.83	6.42
Spain	3.09	0.03	2.08	1.07	6.27
India	2.98	0.14	0.00	1.37	4.49
Iran	1.85	0.04	0.71	1.04	3.64
Italy	1.96	0.00	0.56	0.55	3.07
Egypt	1.73	0.00	0.50	0.30	2.53
Argentina	0.70	0.17	0.40	1.20	2.47
Turkey	1.22	0.13	0.53	0.50	2.38
Pakistan	1.40	0.00	0.50	0.10	2.00
South Africa	1.08	0.38	0.10	0.15	1.71
Greece	1.20	0.01	0.11	0.16	1.48
Morocco	0.82	0.00	0.48	0.01	1.31
Japan	0.11	0.00	1.15	0.00	1.26
Cuba	0.49	0.23	0.05	0.03	0.80
Israel	0.20	0.27	0.10	0.03	0.60
Australia	0.44	0.01	0.09	0.03	0.57
World	59.72	4.69	20.91	12.43	97.75

[a] *Source*: FAO (Food and Agriculture Organization of United Nations). 2003. FAO Yearbook of Production. Rome, Italy: FAO Statistics Division.

The United States produces 12–15 million metric tons of citrus annually. Although citrus is grown in six states, 96% is produced in Florida, California, and Texas. Florida alone produces 73% of the US total, with California and Texas accounting for 18% and 6%, respectively. However, citrus greening and citrus canker disease have the potential to dramatically change citrus production and agricultural practices in Florida and elsewhere while control technologies are being developed (USDA, 1999 and 2007; Gottwald, 2005; Graham *et al.*, 2006).

Over the past 20 years, the relative position of the major citrus-producing countries has changed. In 1975 the United States was the largest producer, followed by Brazil, Japan, Spain, Italy, Mexico, Israel, India, Argentina, and China. Currently, Brazil is the largest producer, followed by the United States (Table 16.1). Collectively these two countries produce about 33% of the world's citrus and nearly 45% of oranges. They also produce the most frozen concentrate juice for the market. Brazil has overtaken the United States in production because of strong government support of its citrus program and severe freezes during the 1980s in the United States, which greatly reduced US production (Jackson and Davies, 1999).

With the gradual shift toward free markets, citrus production in China has increased substantially. China is by far the largest producer of mandarin citrus, primarily Satsuma, and is also a major producer of orange. Mexico has moved into fourth position and is a major producer of orange and lime crops. Production in Spain has remained fairly steady over recent years; the country is a major producer of fresh orange, mandarin, and lemon crops, primarily for the European market. Citrus production also has increased in India and Iran, largely for local consumption. These countries primarily produce orange crops, although India is a major producer of acid citrus fruit. Notable decreases in production have occurred in Japan, Israel, and Cuba. Economic conditions in Japan and Cuba have forced a reduction in acreage; while in Israel the competition from its Mediterranean neighbors and a severe shortage of water have caused a decline in production (Jackson and Davies, 1999).

Weeds in Citrus Production

The warm climate in citrus-growing regions is favorable for weeds to germinate and to grow year-round. The importance of weed control in citrus is well recognized by growers throughout the world (Jordan and Day, 1973; Mersie and Singh, 1989). Weed competition is especially severe with young trees, since they produce very little shade and usually receive frequent overhead or microsprinkler irrigation and fertilization, encouraging luxuriant weed growth.

The reduction of soil moisture by weeds is critical in arid and semiarid regions and elsewhere at times when moisture is inadequate. Water use by weeds decreases its availability for crops, making production more costly. In mature

Table 16.2 Major weeds in citrus worldwide

Weed type	Common name	Botanical name
Grasses	Bahiagrass	*Paspalum notatum*
	Barnyardgrass	*Echinochloa crus-galli*
	Large crabgrass	*Digitaria sanquinalis*
	Southern sandbur	*Cenchrus echinatus*
	Yellow foxtail	*Setaria glauca*
	Bermudagrass	*Cynodon dactvlon*
	Guineagrass	*Panicum maxicum*
	Johnsongrass	*Sorghum halepense*
	Annual bluegrass	*Poa annua*
	Pangolagrass	*Digitaria decumbens*
	Paragrass	*Brachiaria mutica*
	Torpedograss	*Panicum repens*
	Vaseygrass	*Paspalum urvillei*
	Brome	*Bromus* spp.
Sedge	Purple nutsedge	*Cyperus rotundus*
Broadleaves	Black nightshade	*Solanum nigrum*
	Burning nettle	*Urtica urens*
	Common chickweed	*Stellaria media*
	Common lambsquarters	*Chenopodium album*
	Common mallow	*Malva neglecta*
	Common purslane	*Portulaca oleracea*
	Common ragweed	*Ambrosia artemisiifolia*
	Florida pusley	*Richardia scabra*
	Redroot pigweed	*Amaranthus retrofl exus*
	Spanishneedles	*Bidens bipinnata*
	Spotted spurge	*Euphorbia maculata*
	Tufted knotweed	*Polygonum caespitosum*
	Spotted burclover	*Medicago arabica*
	Dogfennel	*Eupatorium capillifolium*
	Field bindweed	*Convolvulus arvensis*
	Sweet broomweed (Goatweed)	*Scoparia dulcis*
	Largeleaf lantana	*Lantana camara*
	Mustard	*Brassica* spp.
	Catchweed bedstraw	*Galium aparine*
Vines (perennial broadleaves)	Air-potato	*Dioscorea bulbifera*
	Balsamapple	*Momordica charantia*
	Moonflower	*Ipomoea alba*
	Stranglervine	*Morrenia odorata*
	Virginia creeper	*Parthenocissus quinquefolia*

California trees, citrus tree trunk and canopy growth, leaf nitrogen level, fruit yield, and fruit quality were decreased by competition from annual weeds and bermudagrass (Jordan, 1981). Suzuki (1981) reported that in Japan, weeds in summer absorb and transpire large amounts of water from the soil and compete with citrus trees. Moisture and nitrogen levels in the soil decreased particularly where large crabgrass and tufted knotweed were present (Ito and Ukei, 1981).

Major Weeds of Citrus and Their Economic Impact

The most common or major weeds reported in citrus in different areas of the world are listed in Table 16.2 (Jordan and Day, 1967, 1970; Goren and Monselise, 1969; Milella and Deidda, 1973; DeBarreda, 1977; Giudice, 1981; Suzuki, 1981; Singh and Tucker, 1984b; Mersie and Singh, 1989). According to their life cycles, these weeds are classified as annual (e.g., crabgrass, common lambsquarters), biennial (e.g., wild carrot), or perennial (e.g., torpedograss, vines, field bindweed) (Ashton and Monaco, 1991). A second classification method is based on the cotyledons of weeds: monocotyledons or grasses and dicotyledons or broadleaf species. These classifications are useful when selective herbicides are used.

Annuals and summer-growing perennials dominate in arid, subtropical, humid, and tropical climates. In the tropical regions, the luxuriant growth of a large variety of perennial species makes the consequences of poor weed control even

more damaging to citrus production. Weeds compete with citrus for nutrients and water, harbor insect pests, host plant pathogens, and lower efficiency of orchard operations. Interference of weeds with citrus trees often results in reduced citrus yields and fruit quality (Jordan, 1981; Jordan and Russell, 1981). Soil and air temperatures are usually lower in orchards with weeds than in those without weeds; these lowered temperatures increase the chance for freeze damage to citrus trees during cold seasons. Dried weeds in the orchard can also be a fire hazard (Tucker and Singh, 1983).

Yield loss in fruit and nut crops caused by weeds that are not adequately controlled by available herbicides or weed management techniques was estimated at $450 million annually in the United States (Chandler *et al.*, 1984).

Methods of Weed Control and Management

Early attempts at citrus weed management dealt mainly with mechanical control of weeds by mule- or horse-drawn plows and cultivators and by hand hoeing. Today, due to the scarce supply of labor and/or its cost, the citrus industry relies on the use of herbicides, which can provide weed control not only under the tree canopy, but also within the row middle. In surveys conducted since 1993, up to 95% of the US citrus acres receive an herbicide treatment. Limited grower trials in Florida were begun in 1962; but it was not until 1964 that extensive use of herbicides began (Ryan, 1969). For more than 40 years, herbicides have been an economical method of weed control in citrus groves. Weed control accounts for 24% of the total production cost in Florida citrus, representing the largest single cost component. Futch (1997) estimated that the management of weeds costs the Florida citrus industry $146 million per year.

Citrus trees are a long-term investment, and growers cannot afford crop damage or yield loss from weed competition, cultural operations, or misapplication of chemicals. The most critical time to control weeds in trees is from planting to early establishment, which spans from 3 to 6 years depending upon soil fertility.

Weeds as ground cover in row middles, while somewhat competitive, can still play a positive role in grove management. Important decision-making factors when considering ground cover in row middles are: (1) the identification of native and introduced species; (2) knowledge of their relative level of competitiveness and interference with trees and cultural practices; (3) an understanding of their impact on insect and plant disease management strategies; and (4) an informed selection of efficacious, cost-effective and environmentally compatible management options (Futch and Singh, 2000).

Not all plant species are equally competitive with citrus trees. Grasses, especially sod-forming species, are more aggressive competitors than most broadleaf species. Vines in tree canopies can become very competitive for sunlight. Mowed grass can be very competitive due to the moisture demands for its regrowth. Relatively sparse weed growth on poor sandy soils may be more harmful to citrus than similar competition on heavier soils with greater moisture and nutrient reserves to be shared between trees and weeds. Many weed seeds of numerous species reside in the surface layers of soil, ensuring weed cover for most of the citrus growing season. By use of appropriate measures to suppress their germination, weed reservoirs can be greatly reduced. However, one season or year of reduced weed management can all but eliminate benefits accrued over time (Futch and Singh, 2000). In the United States, weeds in citrus groves are controlled principally through a combination of tillage, mowing, and herbicides (Reitz and Long, 1953; Lange, 1970; Tucker and Singh, 1983). Weed control methods in citrus are generally categorized as preventive, physical, cultural, biological, and chemical (Anderson, 1983).

Prevention Methods

There is a common saying that 1 year of seeding (allowing weeds to produce seeds) is equal to 7 years of weeding. Thus, prevention is the key in reducing future losses from weeds. Hall and Tucker (1987) recommended a prevention program for sweet broomweed in Florida citrus groves by detecting the weed before it becomes established. Identifying and controlling new weeds prior to widespread dissemination greatly reduce the cost of future weed control operations (Tucker and Singh, 1983).

Physical Methods

Cultivation: Tillage is the traditional method of weed control (Lange, 1970; Giudice, 1981) and is still used as a major weed management technique in citrus groves in many countries. Deep tillage, though, may also have harmful effects. Tillage can destroy the valuable layer of citrus feeder roots that absorb nutrients, water, and oxygen in the topsoil (Jordan and Day, 1973) and can contribute to soil erosion. Field cultivators may also increase the weed population by bringing buried seeds to the surface or by spreading rhizomes, tubers, or stolons throughout the grove.

Slashing, Hand Hoeing, and Mulching: Bredell (1973) compared several physical methods with chemical measures to control weeds in citrus. Herbicides were found to be the most effective tool in managing weeds. Depending

on the species, weed growth was controlled to a considerable extent by plastic or straw mulches, in combination with herbicides. Plastic mulch also saves moisture, prevents emergence of weeds (except bermudagrass), and stimulates growth and yield of citrus. Donadio *et al.* (1988) found a significant shift of weed species after several management treatments (e.g., hoeing, cover crop, tillage, and herbicides). The major weed species in hoed areas were Jamaican crabgrass, natalgrass, common purslane, and sida species. These results show the limitation of employing a single control method in citrus.

Burning: Burning is sometimes employed for land preparation before planting orchards and is common in the tropics. Burning can control not only weeds, but also other pests. However, burning may cause tree damage in established orchards if a large amount of dry weeds is available as fuel (Jordan and Day, 1970).

Weed Control with Hot Water: Hot water to control weeds in citrus orchards and in other crops has shown potential (Anonymous, 1993a, b). The results of such treatments were comparable to contact herbicides.

Cultural Methods

Tree Density: Planting density of an orchard can be used to manage weeds. As the density increases, particularly in the row, the orchard floor surface becomes shaded more rapidly by tree canopies, suppressing weed growth (Tucker and Singh, 1983).

Sod and Mowing: A common weed management practice in orchards, including citrus groves, is to keep a sod (sward or living mulch) on the entire orchard floor or between tree rows, especially on hillside orchards or in areas where soil erosion is a problem (Skroch and Shribbs, 1986). The sod can compete with trees for nutrients and soil moisture. Frequent mowing to maintain the sod between the rows is often combined with herbicide applications along the tree row over the root zone of the trees (Jordan and Day, 1970; Tucker and Singh, 1983). Continual mowing suppresses tall weeds, promotes the growth of dwarf weeds, and prevents seed production. Mowing, however, has a high energy demand and can spread weeds both by seed and by vegetation (Tucker and Singh, 1983). With the frequency of mechanical mowing required and its increasing cost, chemical mowing and low rates of postemergence herbicides in low-volume applications (e.g., wiping) have become increasingly popular (Tucker and Singh, 1983; Singh and Tucker, 1984a; Smith, 1993).

Cover Crops: Ideal cover crops should suppress weeds and provide little interference with the citrus crop. Jones and Embleton (1967) recommended using legumes in young citrus orchards before weeds become thoroughly established. However, in mature orchards, other alternatives such as mustard species are used as cover crops in citrus orchards.

Grazing: McLeod and Swezey (1980) reported that geese have been occasionally used for weed control in orchards and vineyards in California and Oregon. Grazing by geese is effective only against certain palatable weeds. Geese can be destructive to the trees and are difficult to manage properly (Day and Jordan, 1967).

Biological Control

Bioherbicide: Devine™, a suspension of chlamydospores of the fungus *Phytophthora palmivora*, is the most common biological control agent used in citrus. This pathogen was originally found on milkweed vine or stranglervine in citrus groves (Tucker and Singh, 1983; Watson, 1992). In Florida, Devine is applied postemergence between May and September after the vines have grown from seeds or from rhizomes following winter kill of the shoots. Though Devine is a useful mycoherbicide due to its efficacy on stranglervine and its safety to citrus, there are limitations on its widespread use. These include special handling and refrigeration requirements, specific environmental requirements (wet soil before and after application), and restrictions on mixing with wetting agents, fertilizers, or pesticides (Knapp *et al.*, 1987).

Insects: This method has received little attention as a means of combating weeds in citrus. Habeck (1977) showed that insects could be used against largeleaf lantana and stranglervine. A number of leaf-mining beetles have been used successfully in Australia and Hawaii to control largeleaf lantana, which is a major weed in Florida citrus orchards (Tucker and Singh, 1983).

Chemical Control Methods: In the past, clean cultivation throughout the year was advocated by some growers. This practice resulted in the disappearance of practically all organic matter from the soil, severely reducing its ability to hold water and nutrients. Over time, a new concept of clean cultivation emerged in which herbicides are utilized, eliminating mechanical cultivation and mowing. This system maintained very good tree growth since chemicals do not damage tree roots as repeated cultivation does (Jackson, 1991).

Chemical weed control practices in citrus have been thoroughly reviewed by various researchers (Ryan, 1969; Jordan *et al.*, 1977; Jordan, 1978; Tucker and Singh, 1983; Mersie and Singh, 1989; Singh *et al.*, 1990; Sharma and Singh, 1999). Herbicides used in citrus can be divided into two groups: soil-applied (preemergence) and

Table 16.3 Herbicides used in citrus in the United States

Herbicide	Preemergence	Herbicide	Postemergence
Bromacil	G[a] & BL[b]	Dalapon	G
Dichlobenil	BL & G	Clethodim	G
Diuron	BL & G	Fluazifop	G
EPTC	G & BL	Glufosinate	Nonselective
Napropamide	BL & G	Glyphosate	Nonselective
Norflurazon	G & BL	MSMA	G
Oryzalin	G & BL	Paraquat	Nonselective
Oxyfluorfen	BL & G	Sethoxydim	G
Pendimethalin	G & BL	Sulfosate	Nonselective
Simazine	BL & G	Terbacil	G & BL
Thiazopyr	G & BL	Trifluralin	G & BL
		2,4-D	BL

[a]G ffl Grass weeds.
[b]BL ffl Broadleaf weeds.

foliar-applied (postemergence). Herbicides for citrus in the United States are listed in Table 16.3 (updated from Mersie and Singh, 1989; Ashton and Monaco, 1991), and their use has been extensively researched (Leyden, 1969; Ryan, 1969; Milella and Deidda, 1973; Tucker and Phillips, 1973; Jordan *et al.*, 1977; Jordan, 1978; DeBarreda and DelBusto, 1981; Singh and Tucker, 1984a, b, 1988; Singh *et al.*, 1990).

Triazine and Other Preemergence Herbicides

In 1955 monuron was registered as the first preemergence herbicide in citrus (Day, 1955), followed by diuron and simazine. Simazine was recommended by the University of Florida as an excellent weed control tool for citrus beginning in 1962 (Kretchman and McCown, 1962).

Simazine controls an extensive range of grasses and broadleaf weeds when applied to the soil. The minimal leaching of simazine and the resulting protection to plant roots that this affords, adds to its selectivity in deep-rooted crops such as citrus. Simazine's persistence in the soil makes it valuable for long-term weed control (Brian, 1976). Simazine is widely used in the United States, treating approximately 50%, 62%, and 18%, respectively of the total area grown for orange, grapefruit, and lemon (Doane AgroTrak, 2005).

Early registration of simazine was for the control of annual weeds, while the present registration is for the control of broadleaf weeds, annual vines, and annual grasses (Singh and Tucker, 1987). The early recommended application rate was 7.2–10.8 kg a.i./ha once a year for both bearing and nonbearing trees at least 1 year of age (Kretchman and McCown, 1962). Early studies showed that simazine was very safe to trees and was tolerated at rates of up to 40 lb product/acre (45 kg/ha) by citrus trees (Kretchman, 1960). Experiments in a young grove showed that trees treated with diuron or simazine starting 1 year after planting made significantly more growth than trees manually cultivated by hoe (Ryan, 1965b). When the trees began bearing, treated trees yielded significantly more than nontreated control trees. In general, the treatments that resulted in the greatest tree growth and yield were those that provided the best weed control (Ryan, 1965b).

In 1971, the maximum rate per acre for simazine was reduced from 10.8 to 9.0 kg a.i./ha, with a note to use no more than 3.6 kg a.i./ha per application in bedded groves (Tucker *et al.*, 1971). There were few changes after 1971, but in 1991 the rates were reduced again to 2.25–4.5 kg a.i./ha per application. Application rates of up to 9.0 kg a.i./ha maximum annually, with lower rates for trees less than 1 year old (Tucker and Singh, 1991; Singh and Tucker, 1997), have remained constant since. Additional water stewardship directions were added to the simazine label in 2006, including a 50-foot setback from rural wells. Key weeds controlled in citrus by simazine include balsamapple and spanishneedles. Simazine provides partial control of honeyvine milkweed. In a study by DeBarreda (1977), herbicides penetrated the soil to different depths. Bromacil leached more rapidly than other compounds. The order of leaching of the compounds analyzed was: bromacil ffi atrazine ffi simazine and terbumeton ffi terbuthylazine ffi diuron and terbutryn ffi trifluralin.

Ametryn, another triazine herbicide, was previously used for weed control in citrus. This herbicide first appeared in recommendations in 1979 for control of broadleaf weeds, annual grasses, and some perennial grasses. Ametryn was recommended at use rates of 3.6–7.2 kg a.i./ha, with a maximum of 5.4 kg a.i./ha for both shallow, poorly drained flatwood soils (soils having more organic matter and clay) and bedded groves (trees planted on raised beds). It was recommended that ametryn should not be applied to trees less than 2 years old. Between 1984 and 1988 the application rates were increased to 7.2–10.8 kg a.i./ha, with the annual rate not to exceed 13.6 kg a.i./ha, and with lower rates

used on young trees (Knapp *et al.*, 1984, 1988). Due to better weed control alternatives, ametryn is not currently used in citrus in the United States.

Application of diuron at 3.3 kg a.i./ha twice a year resulted in mild foliar symptoms of diuron phytotoxicity on several trees in the third year (Ryan, 1965b). However, when diuron applications were discontinued for 2 years, the phytotoxicity symptoms gradually disappeared. No evidence of simazine phytotoxicity was observed in these experiments. Both diuron and simazine need rainfall or irrigation following application for satisfactory results, but simazine performance appears to be more dependent on moisture.

Terbacil and bromacil were introduced later to control perennial grasses. Bromacil was used initially for eradication of torpedograss, one of the most difficult grasses to control in Florida groves (Kretchman, 1962; Ryan and Kretchman. 1963; Ryan, 1965a), and it is the most effective herbicide for bermudagrass control in Israel (Goren and Monselise, 1969), Japan (Oohata, 1969), South Africa (Herholdt, 1969), California, and Texas (Jordan *et al.*, 1977). Where broadleaf weeds are a problem, a combination of bromacil and diuron is recommended (Lange *et al.*, 1975).

Norflurazon, a preemergence herbicide in citrus, is widely used for the control of annual grasses and some broadleaf weeds and provides excellent weed control around newly planted trees (Singh and Tucker, 1984c) and citrus nurseries (Singh and Tucker, 1983a). Norflurazon is not leached readily from the surface, as it has low water solubility of 28 ppm (Singh *et al.*, 1985). To be active, norflurazon must be absorbed by roots and translocated to leaves, since its primary mode of action is the inhibition of carotenoid biosynthesis (Bartels and Watson, 1978; Sandman *et al.*, 1980). In citrus, only limited translocation (0.6–2.5%) of absorbed norflurazon was reported (Achhireddy and Singh, 1986).

Oryzalin has been registered for use in citrus for several years, but its weed control spectrum of activity is not broad enough for it to be widely used (Singh and Tucker, 1985). Similarly, napropamide and EPTC had efficacy challenges at the recommended rates under Florida conditions (Singh and Tucker, 1984b). Oxyfluorfen, presently registered for nonbearing citrus, provided excellent weed control in citrus nurseries, but was phytotoxic to citrus rootstocks (Singh and Tucker, 1984c).

Pickett *et al.* (1992) reported that California citrus growers depend on herbicides for weed control. Simazine was rated as the most important herbicide used in citrus production. Abdel-Rehman *et al.* (1994) reported that control of annual broadleaf weeds was most effective with simazine plus fluazifop and least effective with fluazifop alone. Application of diuron or atrazine once at 1–4 kg a.i./ha or twice at 1 ┘ 1, 1 ┘ 2 or 2 ┘ 2 kg a.i./ha, significantly reduced grasses and broadleaf weeds. Broadleaf weeds were controlled by 2–4 kg a.i./ha of diuron and 2 ┘ 2 kg a.i./ha of simazine at the 60-day sampling period (Singh *et al.*, 1987). Perez (1976) reported that fluometuron, diuron, monuron, and simazine, all at 4.8 kg a.i./ha, and bromacil at 4 kg a.i./ha controlled weeds without injury to citrus on latosols, while simazine and diuron at 4.8 kg a.i./ha or bromacil at 4 kg a.i./ha caused no injury to grapefruit after two applications on sandy soil.

In citrus groves, 2.5–4.1 kg a.i./ha of glyphosate gave excellent control of torpedograss. Glyphosate plus diuron/bromacil, followed by glyphosate, showed some antagonism; however, with simazine, antagonism was overcome by increasing the glyphosate rate. Phosphonate chelating agents and ammonium sulfate partially overcame the antagonism of simazine and glyphosate against torpedograss (Baird *et al.*, 1983). DeBarreda and DelBusto (1978) reported that some 10 months after treatment when the numbers of tubers in soil samples were counted, the areas treated with the simazine mixture still showed 75% control. Bucsbaum and Gottleib (1979) reported that grasses were best controlled by simazine plus ametryn, followed by diuron plus terbutryn and then diuron plus ametryn.

Mijuskovic (1986) reported good results in groves 3 years old or older that were treated with preemergence applications of simazine, Casoron (dichlobenil), and Caragard combi (terbumeton plus terbuthylazine) in early spring – followed by glyphosate in late May or early June. None of the preemergence treatments damaged the crop, and glyphosate was safe to the tree as long as contact with the foliage was prevented. DelBosco *et al.* (1974) tested seven herbicides in citrus orchards. Autumn applications of formulation A 3611 (atrazine 25% plus ametryn 25%) provided excellent and long-lasting weed control at 15 kg/ha on clay and at 25 kg/ha on medium-textured soils. On sandy soils, autumn applications of all products tested controlled weeds until the beginning of spring. With spring applications, only Saminol 1089 (simazine 18% plus aminotriazole 34%) at 15 kg/ha controlled weeds, and only remained effective for 3 months.

Simazine (2.2 kg a.i./ha) plus alachlor at 4.5 and 9.0 kg a.i./ha gave good to excellent weed control with no phytotoxicity (Tucker and Phillips, 1977). Several herbicides were applied at different locations to determine their effectiveness against bermudagrass, bahiagrass, torpedograss, paragrass, guineagrass, and vaseygrass. Glyphosate was the most effective against all grasses, ametryn was moderately effective against bermudagrass, bahiagrass, and paragrass, but had poor activity against torpedograss, guineagrass, and vaseygrass (Phillips and Tucker, 1972). Lange *et al.* (1975) reported that diuron and simazine were safe to trees and ametryn appeared to be less injurious than the other herbicides tested.

In a leaching study, Futch and Singh (2000) recorded the mobility of bromacil, diuron, norflurazon, oryzalin, oxyfluorfen, simazine, and thiazopyr with different application rates of water. Based on their leaching rates, the herbicides were divided into three categories: low (oryzalin, thiazopyr, oxyfluorfen, and diuron), moderate (norflurazon and simazine), and high (bromacil). Additional water stewardship directions were added to the simazine label in 2006, including a 50 ft setback from rural wells.

Postemergence Herbicides

Herbicides in this category can be divided into two groups, contact and systemic herbicides, according to their translocation characteristics in plants (Tucker and Singh, 1983; Mersie and Singh, 1989). Foliar applied herbicides have little or no soil activity, and can be applied as directed sprays on weeds under the tree canopy without causing any injury to citrus trees. Monosodium methanearsonate, disodium methanearsonate, and dalapon were the first foliar herbicides used to control weeds in citrus and are effective against perennial grasses, especially johnsongrass and vaseygrass (Lange *et al.*, 1975). Dalapon spray runoff in sandy soils may cause tree injury if followed soon after with irrigation or rainfall (Herholdt, 1969).

Major contact herbicides used in citrus are paraquat and glufosinate, and systemic herbicides include 2,4-D, fluazifop, glyphosate, and sethoxydim. Contact herbicides are used mostly in conjunction with preemergence weed killers to burn down established annual weeds and avoid the need for cultivation. Contact herbicides may be used also when preemergence herbicides cannot be used due to inadequate selectivity. They are also used to control weeds that escape residual herbicides and when ground cover is desirable. For example, the steep slopes common in most Japanese citrus groves require a ground cover to protect soil from erosion by high precipitation (Suzuki, 1981).

Success Factors for Herbicides

Besides the type of herbicide, many other factors are important in determining the success of a weed control program with herbicides. These factors include: formulation of the herbicides, adjuvants, mixtures, equipment, spray volume, application rate, time, and frequency (Singh and Tucker, 1983b; Sharma and Singh, 1999, 2000); resistant weeds and citrus tolerance to herbicides (Jordan *et al.*, 1969; Tucker, 1977; Castle and Tucker, 1978; Suzuki, 1981; Singh and Achhireddy, 1984; Achhireddy and Singh, 1986); and environmental conditions, such as precipitation (Tucker and Singh, 1983). When considering chemical control, it is also important to evaluate herbicide persistence and leaching in soils (Jordan *et al.*, 1969).

The continuous use of herbicides for effective control of weeds on many farms and citrus orchards has led to concerns about groundwater contamination and other potential environmental impacts (Hallberg, 1988; Zhang *et al.*, 1997). This is especially true for bromacil, which is relatively water-soluble and is extensively used in citrus. There is ongoing progress in refining herbicide techniques to minimize impact of herbicides on the environment without sacrificing efficacy. A good example of a refined technique is the use of weed detector or navigation technology to spray herbicides only on those areas of a field with target weeds (Miller and Stafford, 1991; Barton, 1993).

Herbicide Application in Citrus

Use of Sprayers: Herbicides in citrus are mostly applied with conventional sprayers equipped with flat-fan nozzles using a 50–200-liter (L) per hectare carrier volume on citrus tree beds and between tree rows. In recent years, several application methods have been developed to supplement this conventional way of spraying herbicides. These include the distribution of certain herbicides through soil water rings and microsprinkler systems. Soil water rings are erected around trees during planting to hold irrigation water applied by tank trucks.

Conclusion

Citrus is a major crop in many countries of the world, with total annual production in the range of 98 million metric tons. The top five production countries are Brazil, United States, China, Mexico, and Spain. With production in the subtropical and semiarid regions having irrigation, weed control is a major factor in production cost. Historically, effective weed control was not possible until the registration of several herbicides in the 1950s–1960s. Simazine was introduced in many countries, including the United States, where it is used to control broadleaves, grasses, and problem vine species. Simazine's broad spectrum and economical cost led it to be one of the most used products for many years. Research has confirmed its selectivity to the different citrus species, and 2005 survey data show that simazine was used on approximately 50% of orange, 62% of grapefruit, and 18% of lemon acres grown in the United States. Simazine can be used alone, in combination with other residual herbicides, or in combination with burndown products

for postemergence and residual weed control. In addition to simazine, other residual products like diuron and norflurazon are commonly used. Glyphosate is widely used in combination with a residual product, or alone in multiple applications. Alternative methods of weed control would rely on hand weeding and multiple cultivations, both of which are prohibitively expensive and can result in greater soil erosion.

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Chapter 17

Triazine Herbicides for Weed Control in Fruit and Nut Crops

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Summary

Weed management in fruit, nut, and vine crops is an integral part of farm management. Each grower must manage vegetation around the crop plants to optimize growth and development of the crop and to obtain an economic yield.

Weed management has changed over the years. Initially weed control was accomplished by the use of hand weeding and cultivation. The principal method currently is an integrated weed management system using various in-row and between-row treatments. This involves cultivation, mowing, flaming, and mulching between the rows, and application of preemergence and postemergence herbicides in the rows.

Triazine herbicides, principally simazine, have been an integral part of this change in management practice. Simazine is used alone and in combination with other preemergence herbicides for weed control in the plant row. It has low water solubility, low volatility, long residual activity, and gives a broad spectrum of annual weed control.

Introduction

Deciduous fruit plants that lose their leaves each winter and become dormant include apple, pear, peach, prune, plum, cherry, apricot, fig, grape, bramble, and bush fruits. The deciduous nut crops include principally walnut, almond, pecan, pistachio, and hazelnut (filbert). Nearly 11 million tons (10 million metric tons) of fruit come from deciduous plants grown in 43 states in the United States. In 1998 in California alone, 8.9 million tons (8.1 metric tons) of fruits and nuts were harvested (Olds, 1998). Strawberry and pineapple, though not deciduous fruits, are included in this chapter because of triazine use on fruit crops. The major growing areas for the United States are shown in Table 17.1, and these same crops are grown in many countries throughout the world.

Table 17.1 Examples of fruit crops grown in different regions of the United States

Northwest	Southwest	Midwest	Northeast	Southeast
Apple	Almond	Apple	Apple	Apple
Caneberry[a]	Apple	Cherry	Caneberry[a]	Caneberry[a]
Cherry	Apricot	Grape	Grape	Grape
Filbert	Caneberry[a]	Peach	Strawberry	Peach
Grape	Cherry	Pecan	Blueberry	Pecan
Pear	Grape	Strawberry		Strawberry
Strawberry	Nectarine	Blueberry		Blueberry
Blueberry	Peach			
	Pear			
	Pecan			
	Pistachio			
	Plum			
	Prune			
	Strawberry			
	Walnut			

[a] Caneberry crops include blackberry, loganberry, boysenberry, ollalie berry, and raspberry.

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Approximately 3 million acres (A) (1.2 million hectares (ha)) of deciduous fruit, vine, and nut crops were grown in 2006 in the United States (USDA, 2007). In 1998, about 600 000 A (243 000 ha) were treated with preemergence herbicides and 1.5 million A (0.6 million ha) with postemergence herbicides (California Agricultural Resource Directory, 1999). These areas represent the size of orchards and vineyards, not the actual area treated. Typically the actual area treated is somewhere between 25% and 35% of the total orchard or vineyard area. Preemergence and postemergence herbicides are often used together in the same application, depending on timing and weeds present. Alternatively, preemergence herbicides may be followed later in the season with one or more applications of postemergence herbicide. In 1998, about 20% of the total crop acreage was treated with preemergence herbicides and 50% with postemergence herbicides.

Costs and Benefits of Weed Control

In the 1990s, weed control costs ranged from $45 to $122/A ($110 to $300/ha) per year during the time preceding the commercial productivity of the average crop (Elmore *et al.*, 1997). Weeding costs continue during the life of the orchard with labor, machinery, and chemical costs varying by the type of weed management chosen. Over a 3-year period, costs ranged from $96 to $138/A ($237 to $341/ha) in vineyards where the treatments consisted of strip herbicides and mowed resident cover crop between the vine rows and strip herbicide with annually planted cover crops that were mowed and disced. In the late 1960s in California alone, weeds cost deciduous fruit farmers more than $50 million annually (Lange, 1968). About half of this cost represented weed control expenses, while the other half was accounted for in loss of trees, tree growth, or yield. Today there are significantly greater weed control costs in fruits and nuts (approximately $200 million in California), but less tree growth and yield loss.

Weeds compete severely with newly planted trees until they are about 4 years old, depending on the species, growth of the trees, and the intensity of weed growth (Lange *et al.*, 1969a; Bould *et al.*, 1972; Robinson and O'Kennedy, 1978). In a study with no supplemental nitrogen fertilizer on apple and pear trees, tree growth and fruit production were greatly improved with nearly complete weed control using simazine and paraquat in the tree rows. Ries *et al.* (1963) researched the effect of simazine on nitrogen nutrition in peach and apple, and found that nitrogen concentration in the leaves and terminal growth was increased when simazine was used as a preemergence herbicide. Moderate to high rates of nitrogen fertilizer were required to overcome weed interference and to improve tree growth in plots with no herbicidal treatments (Raese, 1990). Neilsen and Hogue (1992) demonstrated over a 7-year period that herbicide control of orchard floor vegetation was required to maximize tree growth of a Delicious variety of apple.

Even in mature fruiting trees grown in association with sod, tree vigor can be reduced by weeds (Welker, 1984; Neilsen and Hogue, 1992). In apple, where dwarfing rootstocks are used, weeds compete during the life of the tree (Hogue and Peters, 1994). Perennial weeds such as field bindweed and johnsongrass compete with trees as long as the weeds are in the orchard. Field bindweed is particularly a troublesome weed during early establishment (Leonard and Lider, 1961), but also in mature trees and vines where it absorbs water and nutrients from the same rooting zone as the tree. Bermudagrass, nutsedge, and to a lesser extent, quackgrass, tend to be inhibited by shade as trees get larger. In pineapple culture, purple nutsedge has long been the number one perennial weed problem.

Controlling weeds has a number of benefits in addition to increased yields. Weed control helps to minimize habitat for insects that can cause direct damage and serve as vectors of virus (Kavanaugh, 1969; Duffus, 1971). Weed control also helps to reduce hosts of plant pathogens and viruses (Thresh, 1982; Norris, 1986), meadow or pine vole (Lord *et al.*, 1967; Byers, 1984; Sullivan and Hogue, 1987), pocket gopher (Sullivan and Hogue, 1987), and losses due to bark girdling (Byers, 1984; Merwin *et al.*, 1999). In the Southwest and in some South American countries, poisonous snakes may hide in tall grass at the base of trees, creating a worker safety issue in orchards and in pineapple fields. Controlling weeds minimizes the snake habitat.

Floor vegetation (weeds or cover crops) can be managed in orchards where its presence for all or part of the year is desirable, particularly if a clean strip down the tree row is maintained (Figure 17.1). Beneficial effects of the floor vegetation in these crops include the prevention of erosion, especially for orchards or vineyards on sloping terrain during periods of heavy rain. Weeds and vegetation also help improve water penetration in soils likely to surface seal or where subsurface impervious layers are a problem (Day *et al.*, 1968; Foshee *et al.*, 1997).

The Orchard, Vineyard, and Small Fruit Weed Problems

Both annual and perennial weeds are prevalent in orchards. Annuals often predominate after years of controlling weeds with tillage. In the 1960s there were few herbicides that were tolerated by orchard trees (i.e. did not cause phytotoxicity). After years of cultivation, 72% of the problem weeds in California orchards were annuals and 28% perennials (Lange, 1968). As more preemergence herbicides were developed and used, with or without reduced cultivation, a higher percentage of perennial weeds became common. The use of mechanical mowers to control the growth of weeds also promoted a shift to perennial weed species.

Figure 17.1 Annual weed control in mature almond orchard with preemergence herbicides in a strip down the tree row. (see Color Plate Section)

Important annual weeds in US orchards and vineyards include barnyardgrass, burdock, foxtail, large crabgrass, pigweed, common lambsquarters, nettle-leaf goosefoot, mustard, common purslane, puncture vine, little mallow, and sandbur. Important perennial weeds include poison ivy, orchardgrass, quackgrass, bermudagrass, johnsongrass, torpedograss, purple and yellow nutsedge, wild garlic, field bindweed, Canada thistle, Virginia creeper, trumpetcreeper, dandelion, horsetail rush, milkweed, volunteer asparagus, and horsenettle.

In some orchards where repeated applications of triazine herbicides have been used, there are isolated instances of triazine-resistant weeds. These include common groundsel in the United Kingdom (Holliday and Putwain, 1977) and common lambsquarters and pigweeds in Czechoslovakia, Poland, and Bavaria. Some studies indicated an increased prevalence of some tolerant weeds in orchards when triazine herbicides were used in certain crop weed systems of common vetch (Heeney *et al.*, 1981a), field bindweed (Meith and Connell, 1985), and quackgrass (Hertz and Wildung, 1978).

Annual and perennial weeds are very competitive in caneberry and strawberry. Annual grasses including large crabgrass, barnyardgrass, foxtails, and lovegrass are extremely competitive for small fruit crops. Broadleaf annuals, such as pigweed, nightshade, common lambsquarters, burdock, horseweed, fleabane, willowweed, little mallow, and many others, must also be controlled because of their competitive nature. Bristly oxtongue is a biennial weed that represents a problem. Perennial weeds, including bermudagrass, johnsongrass, and field bindweed, are yield limiting weeds in caneberry and grape, as are Canada thistle and quackgrass.

Weed Control in Orchards, Vineyards, and Small Fruit

In the late 1950s and early 1960s cultivation equipment was the principal method of weed control (Leefe and Longley, 1960; Leonard and Lider, 1961). At the same time, there were early reports showing that selective herbicides such as simazine and diuron could provide excellent weed control in vineyards and orchards in the United States and Europe (Bourdier, 1959; Doll, 1960; Huglin, 1960; Larson and Ries, 1960; Lulliard, 1961; Leonard *et al.*, 1964).

Simazine was the main product studied in early research on the triazines for weed control in tree fruits and vineyards (Doll, 1960; Larson and Ries, 1960). On mature grapevines in a deep, fine, and sandy loam soil, no differences in crop tolerance were observed between simazine and atrazine (Leonard and Lider, 1961). However, subsequent studies indicated that grapevines were more tolerant to simazine than to atrazine (Lange *et al.*, 1969a). Prometryn was intermediate between the two in terms of crop tolerance (Lange *et al.*, 1969a).

The effects of early weed control or vegetation management on fruit crops were extensively reviewed by Robinson (1974) and by Atkinson and Herbert (1979). Hogue and Neilsen (1987) also reviewed four major orchard vegetation management systems, one of them being herbicides. Weeds in orchards are controlled today in the United States principally by a combination of tillage, mowing, and herbicides.

Principal tillage tools include the hoe for removing weeds around the base of trees and the disc harrow for use between rows of trees. Power rototillers set for shallow cultivation are used occasionally between the tree rows. Tillage has the disadvantage of sometimes increasing disease and insect or mite problems (Yarwood, 1969), and in some soils it promotes the development of impervious layers, called plow or tillage pans (Meith and Connell, 1985).

However, the main disadvantages of tillage are undoubtedly soil degradation, increased erosion, increased breakdown of organic matter, and soil compaction (Foshee *et al.*, 1997). Tillage can also destroy or injure tree and vine roots at or below the cutting zone.

Mowing weeds using large mowers is a common practice. However, weeds adjacent to the trees are not removed, and often as many as 8 to 10 mowings per year are required (Meith and Connell, 1985). Unless the weeds next to trees are removed, as with hand labor, they continue to compete for soil moisture and nutrients.

Burning weeds require many trips through the orchard for perennial weed control. Propane gas jets are mounted on booms, with short booms for strip treatment and long booms for complete coverage. Burning easily reduces annual broadleaf weeds, but grasses (annual and perennial) are not easily controlled. Furthermore, burning can cause heat injury to the trees if weeds are allowed to get too large and dry before burning (Meith and Connell, 1985).

Chemicals such as simazine are used alone as herbicides, or more often in combination with other herbicides such as oxyfluorfen, oryzalin, or norflurazon, generally in four- to six-foot (1.2–2.0 meter (m)) strips down the tree row. The centers are either tilled or mowed parallel with the tree row. In order for strip chemical treatment to be successful, it is essential to eradicate perennial weeds by the use of repeated postemergence herbicide applications and tillage.

By far the most common floor vegetation management system in orchards and vineyards is a grassed alley and strip treatment using one or more preemergence herbicides in combination, followed by glyphosate, to keep the tree row clean throughout the growing season (Elmore and Donaldson, 2000). Simazine is often the base herbicide in these mixtures, primarily used for residual broadleaf weed control. In areas of low rainfall, resident weeds are allowed to grow as a cover, or a winter annual cover crop is planted. In areas of high rainfall, a perennial grass may be planted ('grassed alley'). Mowing the alley vegetation is common on contour plantings. In areas where frost damage to the crop is of concern, air temperature can be increased by using strip weed control where the soil is bare and where mowing or cultivating is used on vegetation between the tree or vine rows (Snyder and Connell, 1993; Donaldson *et al.*, 1997).

Some orchards have been treated with complete herbicide coverage similar to that used in noncultivated citrus (Robinson and O'Kennedy, 1978). Complete herbicide coverage is not used in deciduous orchards in most California soils as strip treatment has proved to be more flexible and inexpensive and has the added benefit of inter-row vegetation.

A variety of preemergence herbicides are used in orchards. They may be used alone or in combination, and are often applied with a low rate of simazine. These combinations are used to increase the weed control spectrum and to decrease the chance of weed resistance.

Deep-rooted perennials with underground storage organs tolerate simazine or diuron applied preemergence. Perennials such as bermudagrass, johnsongrass, and field bindweed should be controlled (often with a combination of frequent tillage and postemergence herbicides) before a program of annual weed control with preemergence herbicides is initiated.

Simazine has been widely used in raspberry, boysenberry, and blueberry production (Welker and Brogdon, 1968). When used alone, broadleaf weed control has been excellent, but annual grasses and perennial weeds were not controlled in blueberry (Hertz and Wildung, 1978). Spring-planted raspberry crops do not tolerate weed competition well (Lawson and Wiseman, 1976). Simazine and diuron have been used extensively in many states in new or established cane-planted raspberry.

Micropropagated raspberry is less tolerant to simazine, probably because of the shallow roots picking up more herbicide than the deeper rooting cuttings (Neal *et al.*, 1990). When simazine was used in a New York study, weed control was excellent. However, with rainfall plus supplemental irrigation, there was less plant establishment and growth in herbicide-treated vines compared to mulched plants (Trinka and Pritts, 1992). It was determined that mulching provided a more uniform microclimate (moisture) than the plots with no weeds or cover.

Producers attempt to eliminate perennial weeds in small fruits with soil fumigation and plastic mulches. In caneberry, low rates of simazine can be used in heavier soils. Caneberry has good crop tolerance to other preemergence herbicides such as oryzalin, napropamide, and pendimethalin, though their weed spectrum may be reduced compared to simazine.

Chemical Weed Control

There are important reasons for using herbicides to control weeds. Mechanical tillage, mowing, and burning are expensive, considering the number of times it is necessary to transverse the orchard, often 6 to 10 cultivations or 10 to 20 burnings per season to control weeds. Almonds require 12 to 18 cultivations (Meith and Connell, 1985). Hand hoeing around trees is expensive and labor is not always available. Cultivation also causes a significant degradation of the soil through loss of organic matter, erosion, and compaction. Thus, fewer orchards are tilled compared to 50 years ago when selective herbicides became available.

Timing and scheduling of cultivations for weed control are difficult, particularly on large farms with heavy clay soils. Tillage when the soil is wet often results in impervious layers and poor water penetration the following summer when soil moisture is limiting. Spraying equipment is lighter, less expensive to operate, requires less traffic through the orchard, and results in less soil compaction than tillage (Foshee *et al.*, 1997). In some soils, eliminating tillage increased water penetration (Meith and Connell, 1985). Under 'clean cultivation' where weeds were controlled between rows by tillage to promote heat absorption by bare soil, less frost injury was reported in almond, grape, and small fruits during bloom compared to cover-cropped areas (Donaldson *et al.*, 1997; Snyder and Connell, 1993).

Principles of Selective Chemical Weed Control in Trees, Vineyards, and Fruit

The principles of successful weed control in orchards include herbicide placement, movement, adsorption, absorption, translocation of herbicides, and their inherent biological activity. The residual characteristics of the herbicides are also important, as is the response of the trees and weeds.

Many triazines have been evaluated for weed control in orchards and vineyards, but primary emphasis will be placed on simazine as the herbicide of preference in most of these crops. In general, simazine is better tolerated by most tree fruits, nuts, and vines than high rates of atrazine, prometryn, propazine, terbutryn, terbuthylazine, or metribuzin.

Although much of the selectivity of herbicides to trees is due to herbicide placement, there is also physiological plant tolerance. Grape crops have shown differences in their varietal susceptibility to herbicides (Lider *et al.*, 1966). Rootstock-scion responses have been shown for almond (Lange and Elmore, 1967), peach, plum (Lourens and Lange, 1987), and apple (Karnatz, 1967; Lord *et al.*, 1970). The greater tolerance of peach to simazine compared to apricot is due to the physiological detoxification occurring in the scion of peach (Tweedy and Ries, 1966).

Placement of herbicides in the soil to optimize crop tolerance and weed control is dependent on many edaphic, climatic, and biotic factors. Weed seedlings are small and germinate close to the soil surface (Crafts and Robbins, 1962), whereas trees are large and established and their roots are deep. Proebsting (1943) found that root distribution of California orchard trees was largely in the top 2–4 ft (0.6–1.2 m) of the soil zone. Virtually no feeder roots were found in the zero to one-foot (0–0.3 m) soil depth. Atkinson and White (1980) found that the rooting structure of apple trees in England varied depending upon the orchard floor vegetation. Peach will exhibit different rooting patterns depending on the method of irrigation. Simazine is usually found in the top 3 in. (7.6 cm), even in light soil.

Triazine Movement and Irrigation

In perennial crops, preemergence herbicides are often applied and then moved into the soil by rainfall or by irrigation. Depending upon the characteristics of the herbicide, the timing from herbicide application to water application can be critical. Simazine can be applied to the soil surface and after rainfall it is moved into the soil without appreciable loss on the surface (Lange and Elmore, 1969). Simazine is very efficacious when irrigation water moves it into soil soon after application. It is also less susceptible to additional movement in soil with subsequent irrigation. Simazine is often applied and followed with a sprinkler irrigation of less than 1 in. (2.5 cm) of water. This is unlike other herbicides such as napropamide or trifluralin, and to a lesser extent pendimethalin or oryzalin.

Simazine is virtually nonvolatile and resists ultraviolet light degradation. These characteristics result in long residual activity. Simazine is relatively insoluble, remaining within the top few inches of most soils. The amount of simazine moving downward in the soil usually increased as the rate per acre was increased.

Controlled watering techniques (a variable continuous trickle or drip irrigation) are increasing throughout the world, particularly in California. Many of the hillsides formerly difficult to use for culture of trees and vines are now being developed with drip irrigation. With most of the plastic distribution systems above ground, mechanical cultivation for weed control is impossible, particularly in a perpendicular direction. Herbicides are a necessity with this form of irrigation.

Studies on preemergence herbicides in drip irrigation began about 1971. It was soon clear that while the overall annual weed control problem was less with drip irrigation, the weed problem in the continuously wet area of the emitter was greater. Most of the preemergence herbicides tested did not give residual weed control in the wet areas. Annual weed control often broke in late spring or early summer. Annual weeds, with a continuous supply of water in the areas of the emitter, were an even bigger problem and more difficult to control than under other less continuous irrigation. In addition, perennial weeds appeared more difficult to control, perhaps because of more favorable conditions for recovery.

There are three potential solutions for annual and perennial weed problems with drip irrigation. One is the use of preemergence herbicides that have residual weed control properties, particularly under continuous moisture. Another is the use of contact herbicides on the emerged weeds in the wet spot or persistent herbicides with contact activity. A third approach is the use of herbicides through the drip system when allowed by the product label (i.e., the use of herbicides capable of killing weeds as they germinate). With all three approaches it is essential that perennial weeds

be eliminated before converting to drip irrigation. Once perennial weeds are present, they must be eliminated as soon as possible by the best method available. The use of effective translocated herbicides, such as 2,4-D (for field bindweed), glyphosate (for field bindweed, johnsongrass, and bermudagrass), MSMA and glyphosate (for johnsongrass and nutsedge) are also effective chemical tools where they are registered for use.

Repeated fall, winter, and spring applications of a number of promising herbicide combinations were made for three consecutive years in a newly planted orchard under drip irrigation with excellent tree tolerance and weed control. An evaluation of the total sprayed plot area versus the wet spot immediately under the emitter showed a combination of simazine and oxadiazon to be consistently better than other treatments. Another useful combination was simazine and oryzalin. In a sandy soil (organic matter 0.13%, sand 72%, silt 22%, clay 6%) and with the irrigation and rainfall regime, most of the herbicides tended to be less efficacious on summer grasses and common lambsquarters in early June each year.

Soil Impact on Herbicide Activity

Triazine herbicide soil activity, movement, and residues depend primarily on content of organic matter, and to a lesser extent, clay colloids (Nearpass 1965; Day *et al.*, 1968; Weber *et al.*, 1969). Soils that are low in organic matter or clay usually require lower herbicide rates, but result in more potential phytotoxicity (Lange *et al.*, 1969a). Simazine (4.0 kg/ha) or diuron (5.0 kg/ha) gave season-long weed control in soils of the Northwest (Hogue and Neilsen, 1987).

Triazine Foliar Symptoms and Their Importance in Tree Fruit, Nuts, and Vines

Tree foliage symptoms represent a visual warning of the upper limit of herbicide selectivity under the conditions being tested. Years of university research and testing on crop tolerance and phytotoxicity have helped fruit and nut growers determine the best practices for weed control (Leonard and Lider, 1961; Lange and Crane, 1967; Lange and Fischer, 1969; Lange *et al.*, 1969b; Atkinson and White, 1980; Hogue, 2002). In addition, Lange conducted hundreds of tests on several triazines and on many California crops that contributed greatly to current herbicide use directions on labels.

Foliar symptoms reported in phytotoxicity tests will vary with the herbicide, rate, and method of application, and to some degree the tree species. Simazine is applied to soil and has no foliar effect if applied to leaves. Simazine must be taken up by plant roots from the soil water and is translocated to leaves. Symptoms from high rates of simazine used in phytotoxicity tests appear initially as marginal chlorosis or yellowing in mature leaves, followed by chlorosis. The leaf veins remain green unless extremely high rates are tested. Symptoms generally do not appear the following year and new foliage is not affected unless excessive rates are tested, causing new leaves to become necrotic.

Symptoms observed in phytotoxicity tests of soil-applied atrazine and metribuzin were similar to simazine, except they appeared more rapidly after application, progressed more quickly into the interveins, and caused the leaf margins to become necrotic. Symptoms observed in terbutryn and prometryn phytotoxicity tests were chlorosis in the leaf veins, rather than interveins, which is more typical of injury from diuron or terbacil.

Herbicide Residues in Soils

All herbicides degrade in soil, but at variable rates (Dawson *et al.*, 1968; Rouchard *et al.*, 2000). The rates of breakdown or deactivation of herbicides are related to a number of soil and environmental factors (Upchurch and Mason, 1962; Upchurch *et al.*, 1966). Surface-applied herbicides volatilize at varying rates, dependent on their vapor pressure (Kearney *et al.*, 1964). Some surface-applied herbicides also break down from ultraviolet light.

Once in the soil, deactivation is related to kind and quantity of clay and to organic matter content (Upchurch and Mason 1962; Day *et al.*, 1968; Weber *et al.*, 1969). Burnside *et al.* (1961) reported that simazine remains in the upper few centimeters of soil and breaks down readily at high temperatures and low pH. Holly and Roberts (1963) emphasized the variability in the breakdown of simazine. In one study, simazine residue in soil was evaluated 1 year after treatment, where the half-life was 59 days. However, after 12 years of treatment, the half-life was 46 days, a slight but significant enhanced degradation rate (Rouchard *et al.*, 2000).

Herbicides Used in Orchards, Vineyards, and Small Fruit

Simazine is one of the most widely used preemergence triazine herbicides in orchards. It has low water solubility, low volatility, long residual activity, and gives a broad spectrum of annual weed control. Most seedling broadleaf plants are controlled, though a few are tolerant. Many grasses are also controlled, but grasses are often the first weeds to establish after applications of simazine. Perennial weeds are not generally controlled and simazine has no contact activity on plant foliage.

Trees vary in their response to simazine (Lange *et al.*, 1969a). Walnut was the most tolerant of all deciduous fruit or nut tree species in this study, which used rates that exceeded the label use rates. Several scientists have reported increased nut quality (Larson and Ries, 1960; Neilsen and Hogue, 1992), tree growth, and leaf nitrogen from applications of simazine (Tweedy and Ries, 1966; Neilsen and Hogue, 1992). The extent of use and crops treated with simazine in California and in crops in the United States are covered in Tables 17.2 and 17.3.

A variety of herbicides have been used in combination with simazine and other triazines. These herbicides have preemergence and postemergence activity and are primarily effective on grass and perennial weeds. The preemergence herbicides used in combinations with simazine include oryzalin, pendimethalin, prodiamine, norflurazon, oxyfluorfen, and diuron. Glyphosate, oxyfluorfen, paraquat, and amino triazole (used in some parts of the world) are

Table 17.2 California use of simazine in 2004[a]

Crop	Pounds applied	Acres treated
Almond	73 985	143 206
Apple	1742	1598
Avocado	15 552	11 017
Blueberry	280	154
Boysenberry	78	57
Cherry	140	181
Grape	247 550	243 179
Nectarine	8778	11 856
Olive	10 566	6878
Peach	13 974	21 259
Pear	2365	1568
Pecan	119	152
Plum	787	916
Walnut	51 834	41 282
All citrus	206 469	102 567
Noncrops	93 000	–

[a] California Department of Pesticide Regulations. 2004 Annual Statewide Pesticide Use Report.

Table 17.3 Summary of simazine use on US fruit and nut crops, average for the years 2002–2005[a]

Crop	Total US acres grown	% Treated with Simazine[b]	Simazine rank among residual herbicides	Total simazine acres
Almond	676 885	25.0	2	169 221
Apple	412 067	26.3	1	108 374
Avocado	68 741	16.0	1	10 999
Cherry	125 291	13.0	2	16 288
Filbert	31 492	47.2	1	14 864
Grape, Raisin	303 915	51.8	1	157 428
Grape, Table	101 627	36.2	2	36 789
Grape, Wine	564 678	37.6	2	212 319
Grapefruit	118 554	62.5	2	74 096
Lemon	73 492	17.9	2	13 155
Orange	921 266	50.5	2	465 239
Peach	151 046	28.7	1	43 350
Pear	68 945	19.5	1	13 444
Pecan	462 796	4.0	1	18 512
Prune	132 700	1.1	4	1460
Strawberry	49 450	7.2[c]	2	3560
Walnut	271 402	30.6	2	83 049
Total simazine acres for crops in this table				**875 098**

[a] Doane Marketing Research.
[b] Note that actual % of acres treated may be lower, as the simazine label allows the herbicide to be applied twice per year in certain crops; Doane's survey methods for these specialty crops do not allow repeat-treated acres to be distinguished from single-treated acres, resulting in possible double counting of some acres treated.
[c] Simazine is registered for use in strawberry in Washington and Oregon only and not in other states. Gianessi and Reigner (2002) reported that simazine is used on 53% of the strawberry crops in states where it is registered for use on strawberry.

effective as postemergence herbicides, and when added to simazine, the mixture provides broad-spectrum, residual control.

Other triazine herbicides that have been studied include ametryn (Chaney *et al.*, 1966), atrazine (Leonard and Lider, 1961; Leonard *et al.*, 1964; Lange *et al.*, 1969a), cyanazine (Lange unpublished data), prometryn (Leonard *et al.*, 1964; Chaney *et al.*, 1966; Tweedy and Ries, 1966; Lange and Crane, 1967; Lange *et al.*, 1969a), terbutryn and terbuthylazine (Lange unpublished data), and metribuzin (Hogue and Peters, 1994). Although each of these herbicides has been effective for weed control, there generally has been lower crop tolerance in some orchard trees from these herbicides compared to simazine.

Nut Crops

Most of the nut crops, except pistachio, are grown as deep-rooted, widely spaced trees that are usually furrow, broad-basin, sprinkler, or in some cases, drip irrigated. Simazine is used in many nut orchards down the tree row, alone or in combination with another preemergence herbicide. It is often applied at rates of 1.1–4.5 kg/ha, but when used in combination with other herbicides, the rate is lowered to 1.1–2.2 kg/ha. Annual applications are made on light soils. With heavy soils and heavy weed population, walnut and pecan orchards may receive a split application – half in the late fall or winter and half in the spring, or simazine in the fall followed by diuron or other herbicides in the spring. Most applications are to strips down the tree rows, though some broadcast treatments are made. Walnut trees are the most tolerant (Lange *et al.*, 1967), though pecan is also very tolerant (Patterson and Goff, 1994). Jaynes (1969) discussed the successful use of simazine in filbert, pecan, hickory nut, chestnut, and walnut. Foshee *et al.* (1997) has conducted extensive work using herbicides to reduce soil compaction around young pecan trees.

Almond crops, like peach crops, are usually grafted on peach rootstocks. The cultural requirements of almond are similar to those of peach, except that they are usually grown on sandy soil, partially to facilitate soil preparation for harvest. Because of the sweeping procedure used in harvest, almond farmers must have a virtually weed-free orchard. Avoiding soil cultivation is important to reduce dust associated with mite infestation. Simazine has shown satisfactory weed control at low rates in the heavier soils. In the lighter sandy soils, simazine has been successfully applied to raised beds with furrow or flood irrigation. The Mission and related varieties of almond are more susceptible to herbicide injury than other varieties (Lange and Elmore, 1967). Low rates of simazine plus moderate rates of oryzalin and norflurazon have given adequate crop tolerance and season-long weed control.

In California approximately 126 000 pounds of simazine were applied to 184 000 A of nut crops in 2004. Doane Marketing Research conducts annual surveys of herbicide use on fruit and nut crops grown in major producing states in the United States. Survey results for the years 2002–2005 were averaged to provide more reliable data. Table 17.3 summarizes simazine use patterns on US fruit and nut crops, including total crop acres grown, % crop acres treated with simazine, and the rank of simazine among all residual herbicides used on the crop. Simazine was used on 25%, 47.2%, 4.0%, and 30.6% of almond, filbert, pecan, and walnut crops, respectively. Simazine was the most frequently used residual herbicide on filbert and pecan, and the second most frequently used herbicide on almond and walnut. Oxyfluorfen was the most frequently used residual herbicide on almond and walnut. Simazine was used on an average of 169 221 A of almond, 14 864 A of filbert, 18 512 A of pecan, and 83 049 A of walnut crops each year.

Apple and Pear

Apple crops are grown in most states in the United States and extensively in Canada, China, Europe, and South Africa. Considerable research has been conducted on apple (Benson and Degman, 1961; Skroch and Chambers, 1967; Skroch, 1970; Robinson and Lord, 1970; Heeney *et al.*, 1981a; Hogue and Neilsen, 1987; Hogue and Peters, 1994). Simazine is used extensively in apple and pear orchards. Generally, strip treatments down the tree row are used. Carlson and Ries (1967) and Ramírez and Nitsche (1987) found no effect of simazine on pear trees. Postemergence herbicides like glyphosate or paraquat are used with simazine to control standing weeds. Combinations of herbicides and herbicide rotations (Heeney *et al.*, 1981b) have resulted in good weed control and have increased yields. In California approximately 4100 pounds of simazine were applied to 3200 A of apple and pear crops in 2004.

For the years 2002–2005, simazine was applied to 26.3% of apple and 19.5% of pear crops grown in the United States (Table 17.3). Simazine was the most frequently used residual herbicide in both apple and pear, being applied to an average 108 374 A of apple and 13 444 A of pear.

Peach

Simazine is an important herbicide in peach orchards for the control of winter annuals. In a nursery, peach seedlings did not show any symptom of simazine injury at 4.5 kg/ha. There were also increases in trunk diameter and seedling height as compared to the untreated control (Arnold and Alrich, 1980). In another study, simazine and other herbicides

increased the cold hardiness of peach bark and wood compared to inadequate weed control (Marriage and Quamme, 1980). Where oryzalin can be used in combination with simazine, the combination gave excellent control of summer annuals in California peach.

Oxyfluorfen, oryzalin, napropamide, and norflurazon are additional weed control tools developed for peach crops. In California approximately 23 000 pounds of simazine were applied to 33 000 A of peaches and nectarines in 2004.

For the years 2002–2005, simazine was applied to 28.7% of peach crops grown in the United States (Table 17.3). Simazine was the most frequently applied residual herbicide on peach crops and is used on an average 43 350 A each year.

Prune and Plum

Prune and plum have less crop tolerance to simazine at high rates than the other stone fruit (Chaney *et al.*, 1966; Elmore *et al.*, 1970; Almoida *et al.*, 1987). In a field experiment in California where simazine was applied for 2 years on French prune, 'Marianna 2624' plum rootstock, or Imperial prune on 'Myrobalan 29C' plum rootstock, there was some phytotoxicity observed, though weed control was excellent and trunk diameter increased in most instances over an untreated check (Elmore *et al.*, 1970). Simazine has been used with good crop tolerance at low rates for winter weeds, particularly in some of California's heavier soils. In California approximately 800 pounds of simazine were applied to 900 A of plum in 2004.

For the years 2002–2005, simazine was applied to 1.1% of US prune acres (Table 17.3). Simazine ranked fourth among residual herbicides, being applied to an average 1460 A of prune annually.

Apricot and Cherry

Simazine is an important weed control tool in cherry (Gilbert *et al.*, 1965; Anderson, 1989) and in apricot. A combination of simazine and either oryzalin, norflurazon, or oxyfluorfen showed excellent weed control in sour cherry with no tree injury (Anderson, 1989). In California approximately 150 pounds of simazine were applied to 200 A of cherry and apricot crops in 2004.

For the years 2002–2005, simazine was applied to 13.0% of US cherry crops (Table 17.3). Simazine ranked second among residual herbicides used on cherry, being applied to an average 16 288 A. Oxyfluorfen was the most frequently used residual herbicide, treating an average of 16.3% of acres.

Grape

Simazine has been used as a preemergence herbicide in grape since the early 1960s (Doll, 1960; Huglin, 1960; Leonard and Lider, 1961; Lange *et al.*, 1969b; Chitkara *et al.*, 1979; UCIPM Manual, 1992). Though there are different tolerance levels to simazine among different grape varieties (Lider *et al.*, 1966; Lange *et al.*, 1970), most grape will tolerate simazine. Early research comparing simazine with other triazines and mixtures in young grape cuttings is shown in Table 17.4.

Table 17.4 A comparison of herbicides alone and in combinations on weed control in young grape cuttings and rootings in California[a,b]

Herbicide	Rate (kg/ha)	Weed control[c]
Simazine	2.2	10.0
Simazine	4.5	10.0
Terbutryn[d]	4.5	10.0
Simazineffiterbutryn[d]	2.2 ffi 2.2	10.0
Metribuzin	0.5	6.7
Metribuzin	2.2	8.7
Metribuzinffioryzalin	0.5ffi 2.2	10.0
Metribuzinffioryzalin	1.1ffi 4.5	10.0
Check	–	5.3

[a] From Lange unpublished data.
[b] Average of three replications on a Hanford sandy loam (OM 0.6%, sand 58%, silt 32%, clay 10%). Evaluated 5/14/73 for weed control and phytotoxicity.
[c] Weed control: 0 ffi none; 7 ffi commercially accepted; 10 ffi no weeds. Grasses included crabgrass, barnyardgrass, and witchgrass.
[d] Terbutryn is not currently registered for grape in the United States.

In vineyards in California, when horseweed or hairy fleabane is present, simazine is a critical component of preemergence herbicide combinations (Elmore and Donaldson, 2000). If left uncontrolled, these weeds interfere with hand or mechanical harvesting. In California approximately 248 000 pounds of simazine were applied to 243 000 A of grape in 2004.

Doane's surveys of US herbicide use (Table 17.3) distinguish between raisin grape, table grape, and wine grape crops. For the years 2002–2005, simazine was used on 51.8%, 36.2%, and 37.6% of raisin grape, table grape, and wine grape crops, respectively, ranking first among residual herbicides for use on raisin grapes and second for use on table and wine grapes. Oxyfluorfen was the most frequently used residual herbicide on table and wine grapes, being used on 56.2% and 61.4% of acres, respectively. Simazine was used on an average of 157 428 A of raisin grapes, 36 789 A of table grapes, and 212 319 A of wine grapes each year.

Berry

Simazine is an important preemergence herbicide in a variety of berry crops, including caneberry, cranberry, and blueberry (Wise *et al.*, 2007). Caneberry crops include blackberry, loganberry, boysenberry, ollalie berry, and raspberry. Some of these may also be referred to as brambles or bushberries. Because of the nature of their growth, mostly on trellis, bushberry crops need a weed-free strip down the trellis row – or around plants where they are grown as a bush. Simazine is registered for preemergence weed control in many caneberry crops and has been well tolerated in raspberry (Trinka and Pritts, 1992), blackberry, and related species. In California approximately 65 pounds of simazine were applied to 80 A of caneberry in 2004. NASS survey data show that 44% of raspberry crops in Washington were treated with simazine in 2003 (USDA NASS, 2003). Simazine also was used on 25% of blueberry crops in Oregon and on 20% in Georgia in 2003. In Michigan, 28% of blueberry crops received simazine in 2001 (Michigan, 2001). Welker and Brogdon (1968) found that the quality of blueberry was unaffected by 6 years of treatment with simazine and diuron. They also suggested rotation of chemicals for controlling a wide range of weed species. Simazine remains an important herbicide in berry production throughout the United States.

Pineapple

Weeds that reproduce by seeds in pineapple present a different problem from those that reproduce vegetatively. Plants that produce small seeds are generally easily controlled with a preemergence herbicide. Large-seeded weed species, whose seeds are capable of germinating deep in the soil, are not adequately controlled by preemergence herbicides. In addition to preemergence applications, large-seeded species such as field bindweed and balsam apple may require a more selective type of herbicide that can be applied by boom sprays to control weeds without damaging the pineapple plants. Weeds that reproduce by vegetative means – such as yellow and purple nutsedge, oxalis, bermudagrass, and field bindweed – are not usually controlled by preemergence herbicides. These weeds require vigorous preplant treatment, followed by selective systemic herbicides. While a selective herbicide is the ultimate answer to many of these problems, it may be necessary in specific cases to rely on nonselective systemic herbicides for eradication of local infestations and to prevent seeding and spreading of species that are difficult to control.

In the late 1950s, many herbicides were compared for preemergence weed control in pineapple. Many of these were triazines, which gave better weed control compared to monuron and diuron.

Ametryn readily penetrates plant foliage, so it has given striking results with postemergence control of grasses and broadleaves in pineapple, in addition to exhibiting considerable preemergence weed control in pineapple. When compared in three environmental conditions, it gave better weed control in wet than in dry areas and generally gave better weed control than monuron or diuron. Ametryn controlled mature crabgrass and goosegrass in two different experiments at rates of 5.6–11.2 kg/ha. Young weeds are controlled at rates of 2–4 kg/ha. Ametryn offers one of the best controls of large-seeded weed species that are not controlled by monuron. It is also efficacious under conditions of high rainfall and where difficulties have been encountered in timing of applications. Approximately 90% of pineapple grown is treated with ametryn.

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Chapter 18

Benefits of Triazine Herbicides in the Production of Ornamentals and Conifer Trees

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Summary

Ornamental plants contribute immensely to quality of life. Wood and wood fibers are important to human survival and comfort. The production of ornamentals and conifer trees is limited in unique ways by competition from both native and introduced wild vegetation. Over the course of more than four decades the triazine herbicides have provided safe, effective, and economical weed management in ornamental plants and forest conifers. This has greatly improved the efficient production and quality of these crops by: reducing labor requirements; improving plant growth, survival, vigor, and aesthetic value; and shortening crop rotation times. Triazine herbicides have been largely responsible for the abundant supply of high-quality Christmas trees in the United States, as well as the success and improvement of reforestation in Western coniferous forests.

Triazine herbicides have revolutionized reforestation practices on many forested areas. Plantation tree survival has risen dramatically, resulting from the combination of better weed control and improved seedling production technology. Neither component alone would have provided for the degree of success forest managers enjoy today. Whether the weed problem is exacerbated by summer drought as in the West, by rank overtopping by herbs as in the South and Northeast, or by persistent competing trees such as oaks in the South and aspens in the Lake States, triazine herbicides have proven effective and economical in bringing tree survival and growth to levels thought unachievable in 1960. They have not only improved reforestation practice, but also have permitted establishment of conifers on many sites considered too poor for either agriculture or forestry. Now many of those sites have grown up to timber and, having had little competition in their juvenile years, are proving to be highly productive forests.

The Importance of Weed Management in the Production of Ornamentals

Ornamental plants and conifers grown for forest products or Christmas trees differ from many annual and perennial herbaceous crops in that they are slow growing in their early stages of development. Many conifers, for example, may be 5 or 6 years old before attaining a height of 45 centimeters (cm) (1½ ft). Their slow growth makes them especially vulnerable to weed competition. In forests or in plantations, weed competition for light, nutrients, and water frequently results in high seedling or transplant mortality, greatly reduced growth, delayed harvest, markedly reduced plant quality, and ultimately reduced profitability. In ornamental plant production, heavy weed infestations are so destructive to plant growth and quality that the weeds cannot be tolerated and must be controlled for a successful crop.

Young ornamentals and conifer seedlings are especially vulnerable to weed competition in dry seasons and in nonirrigated situations (Ahrens *et al*., 1969; Brown, 1980; Jones, 1981; Balneaves, 1982; Newton and Preest, 1988). High mortality may occur and surviving plants may suffer reduced growth. In long-term nursery experiments, weeds growing for periods of only 4–6 weeks before removal reduced ornamental plant weights by 35–60% during the first two seasons (Ahrens, 1982). Selective chemical control of weeds invariably improves ornamental plant growth and reduces the time to market.

In Christmas tree plantings, poor weed management can delay harvest for 1–3 years and reduce plant quality and, therefore, market value (Brown *et al*., 1989; Townsend, 1995b). Extending a rotation from 9 to 12 years means a

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minimum of 33% reduction in gross income and three additional years of plant maintenance, including shearing, fertilizing, and applying crop protection chemicals. Shearing costs are greatly increased in plantations where weeds are poorly managed.

Conventional tillage and cultivation (often very effective methods of reducing weed competition in row crop culture) and mulching are not feasible in many types of ornamental plant or forest conifer cultures. Hand weeding or mechanical removal and selective chemical controls are the major methods for reducing weed competition in most ornamental plant and conifer tree production systems. Labor availability and the high costs of securing and maintaining labor limit the use of manual weed management in many ornamental and most conifer tree crops.

In one experiment in nursery plantings, two hand weedings required 1537 man-hours per hectare (h/ha) (622 hours per acre (h/A)) during the first season and 712 h/ha (288 h/A) during the second (Ahrens, 1982). Hand weeding conifer seedbeds required 74–99 person-days/ha (30–40 person-days/A) annually or 593–790 h/ha (240–320 h/A) (Ahrens *et al*., 1976). Five hand weedings in ground covers over two seasons required more than 3385 h/ha (1370 h/A) (Ahrens, 1979), and selective herbicides reduced hand weeding time by more than 90% the first year and 85% the second year.

Hand and mechanical weed removal methods also create certain hazards to crops and to workers (Ahrens *et al*., 1969; Ahrens, 1981). Mowing as a primary method of reducing weed competition in plantations frequently results in crop losses when weed-covered trees are mowed down by mistake. Weeds such as thistles, poison-ivy, and certain vines are hazardous to workers. Hand hoeing causes further losses because hoeing must be repeated several times during each season to be effective (Ahrens, 1961), and it greatly increases the risk of barking or destroying the crop plants. Bingham (1968) reported that ornamental plant growth was greater when chemicals (rather than hand hoeing or tillage) were used to control weeds. Elmore *et al*. (1990) reported that simazine and other herbicides markedly increased trunk diameters of ornamental trees. Trunk diameters were directly correlated with weed control ratings.

The use of chain saws for brush cutting in forestry is not only hazardous for workers, but also cutting deciduous woody plants merely encourages them to resprout, sometimes worsening the competition 1 or 2 years later (Bernstein, 1981; Griffith, 1981). Brush cutting and subsequent resprouting can have a major negative input on natural regeneration of conifers in forestry and in Christmas tree culture.

Additional reasons to manage weeds in ornamental plants and conifer trees include fire protection, frost protection, rodent and disease mitigation, nutrient management, and aesthetics. Uncontrolled weeds and brush become serious fire hazards in the fall or early spring when herbaceous weeds are dry. Plantations of conifers have been lost because weeds were not controlled and fires started. Fires are less destructive when herbaceous vegetation is controlled.

Young ornamentals and conifers are sensitive to late spring frosts in low areas. Bare ground around young plants absorbs daytime solar radiation and releases it during the night, often reducing frost injury to crop plants. Herbaceous vegetation around crop plants increases their susceptibility to frost damage in late spring.

Heavy weed growth around conifers increases humidity and impedes air movement, which encourages needlecast and rust diseases that attack the trees, especially in wet seasons. Furthermore, rust diseases in conifers are worsened when uncontrolled weeds, such as ferns and fireweed in the plantation, serve as alternate hosts for fungal pathogens (Allen *et al*., 1995).

Heavy weed growth creates a favorable habitat for rodents such as pine and meadow voles and pocket gophers, which chew and girdle both deciduous plants and conifers (Crouch, 1979; Cole *et al*., 1998). Providing weed-free conditions or low-growing vegetation around these plants allows predation by hawks, owls, foxes, and coyotes, which helps to keep the rodents in check.

Several studies have clearly shown that uncontrolled herbaceous weed growth may utilize more of the fertilizer than slower-growing woody ornamentals and conifer trees. The net result is that fertilization without weed management often is detrimental to crop productivity and profitability (Morgan and McCormack, 1973; Braekke *et al*., 1986; Wheeler *et al*., 1987; Townsend, 1995b; Roth and Newton, 1996).

Aesthetics may be irrelevant in forests managed exclusively for fiber production, where the goal is to achieve optimal site productivity. More frequently, and especially in public lands, aesthetics are important in gaining public acceptance of forest management practices. In ornamental plant production, however, the appearance of weedy fields may negatively impact salability of nursery stock. Nurseries that grow quality stock and establish good reputations do not allow their fields to become excessively weedy. Since many perennial weeds may travel in and spread from root balls and even in bare-root systems, buyers often are cautious about purchasing nursery stock produced in weedy environments. Prominent weed species examples are hedge and field bindweed, mugwort, yellow and purple nutsedge, quackgrass, and horsenettle. When transported to a residential environment in soil around roots of ornamentals, these weeds can be especially difficult to control. Transporting noxious weeds is also illegal.

The scope of weed pests that affect growth and productivity of deciduous ornamentals and conifers is vast and includes herbaceous annuals, biennials, and perennials as well as woody vines and many species of trees. More than

1900 weed species are listed as being important or potentially important in the United States and Canada alone. It is estimated that up to 100 weed species may inhabit any given site. In the absence of weed management practices, a complete ground cover of weeds can be expected every year on all agricultural and forested sites. In the case of natural forest regeneration, these plants can delay or even stop forest development for decades (Newton *et al.*, 1968; Tappeiner *et al.*, 1992).

Role of the Triazine Herbicides in the Production of Ornamental Plants

Simazine, atrazine, hexazinone, propazine, and prometryn all have been tested around the world for use in ornamental plants, including nurseries and Christmas trees. Only simazine, atrazine, and hexazinone are currently registered for these uses in the United States. Propazine is currently registered in the United States for container-grown ornamentals in greenhouses and for preemergence control in sorghum. In the past, prometryn was registered for conifer seedbeds.

Possibly because of its low water solubility of 6.2 milligrams/liter (mg/L) and lack of foliar activity, simazine has caused the least injury of any triazine herbicide to a broad range of ornamental plant species. Both prometryn and propazine have proven selective in conifer seedbeds and transplant beds, and atrazine and hexazinone have proven selective in certain conifer transplants and forest plantings (Kozlowski and Kuntz, 1963; Ahrens *et al.*, 1976; Valkova, 1989). Prometryn, atrazine, and hexazinone are absorbed by leaves as well as roots and have postemergence as well as preemergence activity on weeds. However, the foliage of actively growing woody plants also can be sensitive at certain rates to these herbicides, reducing their selectivity. Conifer injury is reduced or eliminated by herbicide application during conifer dormancy. Prometryn at rates as low as 0.52 kilograms/hectare (kg/ha), however, proved highly effective in controlling emerged seedling weeds and in providing residual control for several weeks in newly germinated conifer seedlings.

The versatility and general usefulness of simazine in ornamental nursery and conifer plantings cannot be overestimated. Simazine controls a wide range of annual and perennial weed seedlings and, depending on dosage and timing, also controls rosettes of winter annual weeds such as hawksbeard and horseweed (Ryan, 1968). Simazine also controls or suppresses perennial grasses such as quackgrass and timothy. Although 'normal' use rates of simazine are considered to be 2.2–3.3 kg/ha, it is widely known that rates as low as 0.56 kg/ha are effective in preemergence control of many broadleaf weeds, especially when simazine is combined with other herbicides (Bing, 1974; Busto *et al.*, 1977; Karhiniemi, 1977; Carter, 1979; Corell and Bing, 1983). In the United States, simazine rarely is used alone in ornamental plantings, but rather is combined at reduced rates with other preemergence herbicides. At rates of 1–2 kg/ha, it controls most broadleaf annual weeds for 2–3 months and controls annual grasses for shorter periods. Simazine has proven especially effective when combined with preemergence herbicides such as oryzalin, trifluralin, prodiamine, DCPA, napropamide, *S*-metolachlor (metolachlor), or pronamide to provide longer control of annual grasses (Ahrens, 1971; Ticknor, 1977; Bing, 1981; Bennett and Wood, 1985).

Although normally used as a preemergence herbicide during the growing season, simazine also controls emerged seedlings of overwintering annuals – provided it is applied during the dormant season, in the fall, or before the weeds start active growth in the spring (Weller and Carpenter, 1983). Such weeds include common chickweed, horseweed, shepherd's purse, common groundsel, annual bluegrass, and narrow-leaved hawksbeard (and fleabane species in California). In the United States, simazine is the most effective preemergence herbicide for fall use in ornamental nurseries. However, to control triazine-resistant forms of common groundsel, annual bluegrass, and other triazine-resistant weeds, it has been necessary to use alternative herbicides or combine simazine with other herbicides (Ryan, 1968). Where such combinations are used in ornamental and Christmas tree plantations, triazine-resistant weeds have not been serious problems.

Development of weed resistance to triazine herbicides has necessitated alternative weed control strategies. Rotation of triazines with other herbicides and combining triazines with other chemistries has been effective in ornamentals and Christmas tree plantations (Van Himme, 1989). Supplemental treatments in Christmas trees have been effective, using directed sprays of phenoxy herbicides or glyphosate before resistant weeds mature and produce seeds. Weed resistance from triazines is not a problem in most forest settings where the herbicide is applied only once or twice in a rotation, since 1 or 2 years of herbaceous weed control normally ensures survival and eventual dominance by conifers. When the conifer canopy closes, virtually all herbaceous vegetation is shaded out for some decades; hence resistant weeds, if present, fail to survive from one generation to the next.

The compatibility of simazine with most preemergence and postemergence herbicides has also made it extremely useful in ornamental plant and conifer systems. Successful postemergence combinations with simazine have included sethoxydim, fluazifop-P-butyl, clethodim, glyphosate, paraquat, diquat, and glufosinate (Ahrens, 1973a, 1981; Ahrens and Cubanski, 1985). Adding simazine to the postemergence herbicides usually does not reduce postemergence weed

control, nor does it affect ornamental tolerance to the postemergence herbicide. Simazine has had no significant effect on the rooting potential of cuttings taken from treated plants for propagation (Ahrens, 1973b).

The extremely low volatility of simazine, combined with its high stability on soil surfaces and low foliar uptake, are valuable attributes in spraying in areas containing sensitive plants. Because of its low foliar activity, drift onto nontarget crops from ground applications of simazine is extremely rare. Although greenhouse use of simazine is not registered in the United States, study results and many observations indicate that under normal circumstances there are no vapor hazards from the use of simazine around ornamental plants. The only instances of simazine volatility in greenhouse under-bench use have been where simazine was sprayed on heating pipes or was applied at extremely high rates (Whitcomb and Santelmann, 1977). In simulated greenhouse experiments, simazine at 4.48 or 13.44 kg/ha caused no injury to young tomato and forsythia plants exposed only to potential vapors for 2 weeks at daytime temperatures of 32–38ffiC (Ahrens, 1978, 1984). A normal rate of 3 kg/ha usually controls annual weeds for 2–4 months.

The low volatility and soil stability of simazine and its lack of foliar activity allow it to be applied for preemergence weed control in areas of adequate rainfall during most seasons of the year without soil incorporation. Although providing long residual weed control and leaving residues that can injure sensitive crops in rotations, studies indicate that simazine has little direct effect on soil microbes. In fact, simazine stimulates mycorrhizal development in some species (Smith and Ferry, 1979; Trappe *et al.*, 1984).

Simazine is widely used for preemergence control in field-grown nursery stock in North America. Selective use in container nurseries also has been demonstrated (Ahrens, 1972; Fretz, 1974; Wadsworth, 1975; Bing, 1983). It is less widely used in container-grown nursery production because of potential leaching (Elmore *et al.*, 1976), but is especially effective when applied in the fall or winter to control winter annual weeds in containers. Simazine currently is registered in the United States for 50 species of woody ornamental nursery stock and Christmas trees. Tables 18.1 and 18.2 list selected species of conifers and deciduous ornamental plants, respectively, and their observed tolerance to simazine at rates of 2.2–3.3 kg/ha. Information for these tables was obtained from personal observations and the literature, including those references by Ries *et al.* (1959); Ahrens (1961); Ticknor (1972); and Schubert *et al.* (1986).

Selective doses of simazine are tolerated by most woody plants (Robinson and Kelly, 1989). Smaller plants and newly transplanted plants of the same species are more sensitive than larger, established plants. Seedlings are the most sensitive, and deciduous shrubs are usually more sensitive than conifers. However, many exceptions exist. Large-seeded deciduous trees such as walnut and chestnut are very tolerant of simazine (Ahrens, 1969). Whereas many newly seeded conifers are sensitive, larger 1-year-old seedlings often tolerate rates of 0.6–1.1 kg/ha (Ahrens *et al.*, 1976).

Woody plant tolerance to the triazines is affected by both physiological and soil factors. The triazines are metabolized to nonphytotoxic hydroxytriazines more rapidly in tolerant plants (Raugh *et al.*, 1966; Lund-Hoie, 1969a; McNeil *et al.*, 1984; Gaskin and Fletcher, 1997), and plants with larger, deeper root systems can escape exposure to and absorption of these herbicides. Adsorption of the herbicides on organic matter and clay reduces their leaching to root zones. Of the triazines, hexazinone is the most water soluble and is the least bound to soils (Vencill, 2002).

Plant species are more tolerant to injury from the triazines as the clay and organic matter content of soils increases. Minor chlorosis of deciduous ornamental plants caused by simazine has little long-term effect on vigor and plant growth (Ahrens, 1966). The growth-promoting benefits provided by weed control often counterbalance and mask phytotoxic effects. There is also evidence that sublethal concentrations of simazine and hexazinone are beneficial to plant growth (Lund-Hoie, 1969b; Ries and Wert, 1972; Johnson and Stelzer, 1991). Elevated nitrogen and protein contents of plants have been obtained with simazine treatment. When simazine was used to control weeds in balsam fir, the trees had elevated protein levels and increased succulence, which resulted in increased deer browsing (Morgan and McCormack, 1973).

Because of its absorption by plant foliage as well as by roots, atrazine at agricultural use rates is not tolerated by most ornamental deciduous woody plants during active growth. Depending on dosage and plant species, atrazine can also injure actively growing conifers (Ahrens, 1985). Applying atrazine before bud break of fir and tolerant deciduous trees avoids the foliar injury. Conifers may also be treated before planting.

Hexazinone also is absorbed by plant foliage and is not tolerated at any time by actively growing foliage of most deciduous plants. Like atrazine, hexazinone is only safe on most conifer foliage during the dormant season – either before bud burst of tolerant firs, spruces, and Douglas-firs or after terminal growth has slowed in tolerant pines (Boyd 1984). Tolerances of selected conifers to atrazine and hexazinone are listed in Table 18.1. Preplanting use of hexazinone also reduces tree injury (Townsend, 1995a). Because of potential injury to conifers, hexazinone is registered for Christmas trees in the eastern United States at lower rates (0.29 to 0.51 Kg/ha) than in the Pacific Northwest or in the Maritime Provinces of Canada (Townsend, 1995a, 1995b).

The triazine herbicides simazine, atrazine, and hexazinone are commonly used in Christmas tree plantings in North America. Hexazinone is widely used in certain pines and on other conifer species in natural stands in regions where the organic matter content in soil is adequate to prevent excessive leaching to conifer root zones. Simazine and

Table 18.1 Observed conifer tolerance to triazine herbicides[a]

Common name	Latin name	Simazine 2.2–3.3 kg/ha[b]	Atrazine 2.2–3.3 kg/ha[b]	Hexazinone 1.1–2.2 kg/ha[b]
Arborvitae	*Thuja* spp.	T[c]		
Eastern arborvitae (white cedar)	*Thuja occidentalis*	T[c]	T	
Giant arborvitae	*Thuja plicata*	ST	T	
Cypress	*Champaecyparis* spp.	T		
Ellwood false cypress	*C. ellwoodi*	T	T	
Balsam fir	*Abies balsamea*	T[c]	T	
White fir	*A. concolor*	T[c]	T[c]	
Fraser fir	*A. fraseri*	T[c]	T	
Grand fir	*A. grandis*	T[c]	T[c]	T[c]
Nikko fir	*A. homolepsis*	ST		
Shasta red fir	*A. magnifi ca shastensis*	T		
Nordmann fir	*A. nordmanniana*	T		
Noble fir	*A. procera*	T	T[c]	T[c]
Douglas-fir	*Pseudotsuga menziesii*	T[c]	T[c]	T[c]
Eastern hemlock	*Tsuga canadensis*	T[c]	T	
Junipers	*Juniperus* spp.	T[c]		
Hetz juniper	*J. chinensis* 'Hetz'	T	T	
Creeping juniper	*J. horizontalis*	T	T	
Eastern red cedar	*J. virginiana*	T[c]		
Larch	*Larix* spp.	ST		S
Pines	*Pinus* spp.	T	T	T
Lodgepole pine	*P. contorta latifolia*	T[c]	T[c]	
Western white pine	*P. monticola*	ST		
Mugho pine	*P. mugo mughus*	T[c]		
Austrian pine	*P. nigra*	T[c]	T[c]	T[c]
Ponderosa pine	*P. ponderosa*	T	T[c]	T[c]
Red pine	*P. resinosa*	T[c]		T
Eastern white pine	*P. strobus*	T[c]	T	
Scots pine	*P. sylvestris*	T[c]	T[c]	T[c]
Loblolly pine	*P. taeda*		T	T[c]
Knobcone pine	*P. attenuata*	T[c]	T[c]	
Bishop pine	*P. muricata*	T[c]	T[c]	
Virginia pine	*P. virginiana*	T		
Monterey pine	*P. radiata*	T[c]	T[c]	ST
Jeffrey pine	*P. Jeffreyi*		T[c]	
Slash pine	*P. elliottii*		T[c]	
Colorado spruce	*Picea pungens*	T[c]	T[c]	
Norway spruce	*P. abies*	T[c]	T	
White spruce	*P. glauca*	T[c]	T	
Red spruce	*P. rubens*	T[c]		
Sitka spruce	*P. sitehensis*		T[c]	
Yew	*Taxus* spp.	T[c]		
Upright yew	*T. cuspidata capitata*	T	T	
Spreading yew	*T. cuspidata*	T	T	
Hybrid yew	*T. x media*	T	T	

[a]Response to herbicide applications by plants established in the field for one full year or more. In some cases, recently transplanted plants tolerated the herbicide; in other cases they did not. T: Tolerant, S: Susceptible, ST: Injury noted in some situations or species.
[b]Note that tolerances are influenced strongly by soil and climatic characteristics. Regional conditions may dictate higher use rates than shown. Simazine is labeled for use up to 4.4 kg/ha for many species or up to 3.3 kg/ha in tree nurseries.
[c]Registered in the United States for use on this species.

atrazine are primary herbicides in plantation-grown Christmas trees – applied alone or with other preemergence and postemergence herbicides. Atrazine provides improved control of established annual and perennial weeds, and hexazinone controls herbaceous and woody weeds. Because Christmas trees require several years from planting to harvest, the low cost of triazine herbicides is extremely important to the economics of Christmas tree production. The more recent herbicides registered for Christmas tree production cost 3–10 times as much as simazine or atrazine.

Table 18.2 Observed deciduous tree and ornamental plant tolerance to simazine[a]

Common name	Latin name	Simazine 2.2–3.3 kg/ha[b]	Common name	Latin name	Simazine 2.2–3.3 kg/ha[b]
Alder	*Alnus* spp.	T	Holly	*Ilex* spp.	ST[c]
Azaleas, rhododendrons	*Rhododendron* spp.	ST	Honey locust	*Gleditsia* spp.	T[c]
			Honeysuckle	*Lonicera* spp.	ST
Apple	*Malus*	T[d]	Hornbean, European	*Carpinus belalus*	T
Ash	*Fraxinus* spp.	T	Horse chestnut	*Aesculus hippocastunum*	T
Aucuba	*Aucuba japonica*	T			
Bamboo spp.	*Bamboo* spp.	T	Hydrangea	*Hydrangea* spp.	ST
Barberry	*Berberis* spp.	T[c]	Leucothoe	*Leucothoe catesbaei*	T
Beautyberry	*Callicarpa bodinieri geraldi*	S	Lilac	*Syringa* spp.	ST
			Linden	*Tilia* spp.	ST
Beech	*Fagus* spp.	T	Magnolia	*Magnolia* spp.	ST
Boxelder	*Acer negundo*	T[c]	Manzanita	*Artostaphylos* spp.	T
Broom	*Cytisus* spp.	T	Maple	*Acer* spp.	T
Butterfly bush	*Buddleia* spp.	T	Mockorange	*Philadelphus* spp.	ST
Camellia	*Camellia japonica*	S	Mountain laurel	*Kalmia latifolia*	T
Caragena	*Caragena* spp.	T[c]	Oak	*Quercus* spp.	T[c]
Ceanothus	*Ceanothus prostratus* and others	T	Oregon grape	*Mahonia* spp.	T[c]
			Pieris (andromeda)	*Pieris* spp.	ST
Cherry	*Prunus* spp.	T	Peach, plum	*Prunus* spp.	T[d]
Chestnut	*Castanea* spp.	T	Pear	*Pyrus communis*	T[d]
Cotoneaster	*Cotoneaster* spp.	ST[c]	Pistachio	*Pistacia* spp.	T
Necklace cotoneaster	*C. conspicua decora*	S	Poplar	*Populus* spp.	ST
Deutzia	*Deutzia* spp.	ST	Potentilla	*Potentilla* spp.	ST
Dogwood	*Cornus* spp.	T[c]	Privet	*Ligustrum* spp.	ST
Dogwood, flowering	*C. fl orida*	ST	Rose	*Rasa* spp.	T
Eucalyptus	*Eucalyptus* spp.	T[c]	Rose, Rugosa	*Rose rugosa*	S
Euonymus, winged	*Euonymus alatus*	S	Russian olive	*Eleagnus* spp.	T[c]
Euonymus, winter creeper	*E. fortunei*	T	Shadbush	*Amelanchier* spp.	T
			Snowberry	*Symphoricarpus* spp.	ST
Euonymus, evergreen	*E. japonicus*	T	Spirea	*Spirea* spp.	ST
Firethorn	*Pyracantha* spp.	T	Sumac	*Rhus* spp.	T
Flowering almond	*Prunus glandulosa*	ST	Sycamore	*Platanus* spp.	T
Flowering quince	*Chaenomeles* spp.	ST	Tamarisk	*Tamarix* spp.	T
Forsythia	*Forsythia* spp.	ST	Viburnum	*Viburnum* spp.	ST
Ginkgo	*Ginkgo biloba*	T	Walnut	*Juglans* spp.	T[d]
Hawthorn	*Crataegus* spp.	T	Weigela	*Weigela* spp.	ST
Heath	*Erica* spp.	T	Willow	*Salix* spp.	T
Heather	*Calluna* spp.	T		*Xylosma* spp.	ST

[a]Response to simazine applications by plants established in the field for one full year or more. In some cases, recently transplanted plants were injured; in other cases they were not. T: Tolerant, S: Susceptible, ST: Injury noted in some situations or species.
[b]Note that tolerances are influenced strongly by soil and climatic characteristics. Regional conditions may dictate higher use rates than shown. Simazine is labeled for use up to 4.4 kg/ha for many species or up to 3.3 kg/ha in tree nurseries.
[c]Registered in the United States for this species and included on ornamental label.
[d] Registered in the United States for this species and included on crop label.

Importance of Weed Management in Production of Forest Conifers

Weeds influence the net productivity of forest sites. Foresters rate land productivity in terms of 'site quality,' typically measured by the height reached by trees at a specified age. For example, if trees reach 27 m (90 feet) at 50 years, the 'site index' is described as SI_{50} ffl 27 m. With an increased site index, the economic productivity and value of forest land increase disproportionately because the yield increases. The time required to reach maximum yield decreases and trees become more cylindrical, hence more efficiently utilized.

Weeds decrease the apparent measures of site index (Hanson, 1997). Virtually every analysis of the influence of herbaceous or woody competition has shown that trees grow at a decreased rate under the influence of almost any degree of competitive cover. Wagner *et al*. (1989) illustrate a diagram in which it is apparent that decreases in growth occur with very little competition. Hanson (1997) displayed growth models indicating that ponderosa pine follows this classic curve for at least 15 years. Ortiz-Funez (1989) observed that Douglas-fir in the same plantation exhibited a similar tendency at 7 years of age, but when the competing shrubs grew larger, they caused mortality of nearly all the planted trees. Hanson (1997) showed that the level of competition leads to different degrees of response in the

two conifer species, and that the response might occur at different stages in the life of a tree. In both species, growth declined at a progressively slower rate with increased competition until trees were so stressed that they showed little additional response until mortality occurred. This phenomenon has been reported in the Southeast (Creighton *et al.*, 1986; Mitchell *et al.*, 1999), Northeast (Newton *et al.*, 1992), boreal forest (Cole and Newton, 1997), and elsewhere in the Pacific Northwest (Crouch, 1979; Pabst *et al.*, 1990). The same phenomenon has been observed in Christmas tree plantations for decades and is the reason foresters strive for weed-free culture in many areas.

Growth losses may occur at different times in the life of a plantation. Annual herb competition sometimes leads to delays in conifers reaching dominance, after which their growth will either parallel those grown without competition (Newton and Preest, 1988) or will continue to diverge (Hanson, 1997). In other reports, growth divergence continues beyond the tracking period (White and Newton, 1989; Newton *et al.*, 1992), and competition from shrubs and hardwoods are more typically (but not exclusively) the cause of long-term losses.

If growth loss due to competition is reflected in the time it takes for growth of weeded versus unweeded crops to become parallel (as in the case of annual herb effects on a good site), the loss may be calculated in terms of years of delay or years of lost production, plus the compound interest effect of delaying harvest at any level. If the growth rates continue to diverge (as with hardwoods or shrubs causing some overtopping), one can only indicate that at the end of the data coverage period, the loss has reached a certain level and is likely to increase according to the established trend. However, when 70-year-old Douglas-fir can be expected to have a harvest value of some $30 000/acre and conifers in the South may have value increments approaching this in shorter cycles, there is great uncertainty in making projections for growth loss – apart from the generalization that losses can be significant from both mortality and from decreased and delayed yields.

Herbaceous weeds can kill conifer seedlings by severely depleting soil water (Newton, 1964; Cleary, 1970), by providing a nutrient sink that leads to inadequate net nutrient availability for vigorous growth, and by causing mechanical damage when lodging crushes seedlings in a dark environment. Herbaceous vegetation responds quickly to removal of overstory trees. Conifer seedlings capable of growing only a few centimeters in their first year are at a serious disadvantage, regardless of how much potential growth they might produce if and when they become dominant. Obviously, once a tree is several meters tall, its dominance over herbs will reverse this pattern. However, the tree must survive long enough to dominate the herbs. Often it takes several years for conifer seedlings to out-compete the herbaceous plant layer, or even to survive.

Herbs have several features that render them difficult competitors for conifer seedlings. First, they tend to grow far more rapidly than conifers. Second, they have a very high ratio of transpirational surface to biomass, drawing substantial water from the soil in the process of completing their annual growth cycle. Third, they concentrate many of their roots in the most fertile soil of the A horizon (in which young conifer seedling roots are confined). Thus, these seedlings have to adapt to a new environment while herbs are quickly depleting soil water and tying up nutrients.

Role of Triazine Herbicides in Production of Forest Conifers

Triazine herbicides are primarily effective in controlling herbaceous weeds. A notable exception is the use of granular hexazinone for controlling woody invaders such as oaks (*Quercus* spp.) or aspen (*Populus tremuloides*) in conifer stands on medium- and fine-textured soils in the South or in boreal forests. Hexazinone also is used in the Maritime Provinces of Canada for controlling woody and herbaceous growth in natural Christmas tree stands (Townsend, 1995a). The impact of herbaceous plant cover is somewhat different from that of woody plant competitors, so control strategies on various forest conifer sites may differ.

Triazine herbicides are specifically adapted to controlling the kind of weeds most troublesome to conifers (Glover *et al.*, 1989). The symmetrical triazines – atrazine and simazine – quickly adsorb onto soil colloids and remain active for some months, preventing herbaceous weeds from occupying soil resources in the zone where conifer seedling roots are developing during their first year. Peterson (1976) showed that conifers have the ability to metabolize these triazines by conjugation with proteins, resulting in their deactivation. He also showed that low concentrations of atrazine in soil water may actually increase net photosynthesis in Douglas-fir. The typical clay loam soil of western Oregon adsorbs atrazine to an extent that the remainder in soil solution is within the range that is stimulatory to Douglas-fir. Thus, there are great advantages in having an herbicide with the properties of atrazine for herbaceous weed control.

Hexazinone is relatively mobile in coarse- to medium-textured forest soils under the influence of abundant moisture (Newton and Cole, 1997), and its penetration deeper in soil facilitates control of perennials, several shrubs, and hardwoods (Minogue *et al.*, 1988; Quicke *et al.*, 1996). It has replaced other triazines on sites where woody as well as herbaceous competitors are important.

Soil persistence of triazines is important in forest usages. Much literature confirms that significant gains are made by reducing herbaceous plant cover for more than 1 year following planting. The triazines (e.g., simazine, atrazine,

and hexazinone) show decreasing order of persistence in surface soil. In some instances, simazine can provide 2 years of freedom from annual weed invasions in northern climates. Atrazine, applied in spring, will provide 1 year with little competition and a second year with only partial reinvasion of weeds. Triazine rates range up to 4 lbs. a.i./acre (4.48 kg/ha) for reforestation. In some regions of the country such as western Oregon and Washington, this higher rate is needed to control germination of broadleaf weeds resistant to the sulfonylurea herbicides, such as sulfometuron. In moist areas, hexazinone is unlikely to provide more than 1 year of weed-free growth, but it controls more herbaceous and woody species than atrazine or simazine.

Detection in forest groundwater seldom occurs with the triazines due to infrequent use. In addition, forest soils are typically high in organic matter and tend to bind triazine molecules until they degrade in place. Very little nutrient leaching occurs from rich forest ecosystems due to the very active processes in place for retaining a variety of elements and compounds that would otherwise be moderately mobile (Miller and Newton, 1983).

Forestry uses of triazines involve only one or two applications on the same site during a rotation, which may span 30–70 years or more. Under these conditions, accumulation of herbicide residues and potential leaching and runoff hazards are greatly diminished.

Conclusions

Over the course of more than four decades, the triazine herbicides have provided safe, effective, and economical weed management in ornamental plants and forest conifers. This weed management has greatly improved the efficient production of these crops by reducing labor requirements, improving plant quality, and shortening times to harvest.

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Chapter 19

Benefits of Triazine Herbicides in Turf

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Summary

The triazine herbicides atrazine, simazine, and metribuzin have been used for effective weed control in warm-season turfgrasses for at least 40 years. This is especially true for symmetrical triazines (atrazine and simazine), which provide economical, broad-spectrum weed control in most warm-season turfgrasses. The triazines are especially important for winter weed control in lawns and sports turf, including use during turfgrass establishment and sod production and for control of weeds such as goosegrass. In general, the triazines and other herbicides improve the quality of lawns and add value to property. Weed control in golf courses and other sports turf improves the uniformity and playability of turf. Economical and effective weed control is also important in sod production; weed-infested sod may not be marketable. In addition, weed infestations in sod can reduce tensile strength and cause problems in harvesting and handling. The residual control provided by atrazine and simazine is important in Southern turf. Atrazine and simazine alone provide broad-spectrum, residual weed control in about 305 000 hectares (ha) of warm-season turfgrasses during the dormant period.

Atrazine and simazine are used in warm-season turfgrasses predominantly to control broadleaf weeds and annual bluegrass. Atrazine is used primarily by commercial lawn care operators in centipedegrass and St. Augustinegrass and in homeowner package products – in particular those formulated on fertilizer. Simazine, on the other hand, is often the symmetrical triazine of choice for weed control on golf courses, large commercial turf landscapes, and in sod production. Metribuzin, an asymmetrical triazine, is used on a limited basis in bermudagrass turf, mainly combined with either monosodium methanearsonate (MSMA) or disodium methanearsonate (DSMA) for postemergence control of goosegrass.

This chapter focuses on the importance of triazines for weed control in the establishment and maintenance of warm-season turfgrasses. While many other herbicides have been developed for use in warm-season turfgrasses, the triazines are still an integral and economically important family of herbicides for homeowners, lawncare experts, sports field managers, golf course superintendents, sod producers, and other turfgrass specialists.

Weed Control in Turf

While atrazine was first released for experiment station evaluation in 1957, the first research in turf was not published until 1962 (Klingman) when researchers evaluated weed control in zoysiagrass, tall fescue, and bermudagrass. Klingman found that 2.2 kg/ha simazine or atrazine provided excellent broadleaf weed control, while simazine controlled crabgrass somewhat better than atrazine at 6 months after treatment. He also reported effective preemergence control of horseweed, Carolina geranium, venuslookingglass, Virginia pepperweed, cutleaf eveningprimrose, annual lespedeza, hop clover, and rabbitfoot clover.

In that same year, Burt *et al.* (1962) observed only slight discoloration to sodded or sprigged St. Augustinegrass with 5 kg/ha atrazine applied as a wettable powder and no injury from granular formulations of atrazine or simazine at up to 10 kg/ha. In a separate experiment, Burt (1964) observed little to no injury with 5 or 10 kg/ha simazine applied to zoysiagrass, St. Augustinegrass, centipedegrass, or hybrid bermudagrass (Ormond cultivar). However, 10 kg/ha atrazine injured bahiagrass, centipedegrass, and two hybrid bermudagrasses (cultivars Tifgreen or Ormond), while good tolerance (25% or less injury at 1 month after treatment) was observed if the atrazine rate was reduced

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to 5 kg/ha. Engel *et al.* (1968) also investigated use of triazine herbicides in zoysiagrass establishment and observed that atrazine was more injurious than simazine. They also observed differential tolerance to the triazines between zoysiagrass cultivars. At that time, few herbicides were available for use on turfgrasses at establishment, so much of the research focused in this area. Throughout the 1970s and 1980s, a more concerted effort was made to refine the uses of triazine herbicides in turf. The spectrum of weeds controlled, application timings, and tank-mix combinations were evaluated. Today, triazine herbicides are an integral and economical tool for turfgrass professionals in managing weeds in warm-season turfgrasses.

Early research evaluating combinations of MSMA with the *s*-triazines (atrazine or simazine) was conducted in Hawaii where annual weeds often germinate and flourish throughout the year in the tropical environment. Murdoch and Ikeda (1974) reported poor goosegrass control with up to three applications of 2.2 kg/ha MSMA alone or with single applications of atrazine (1.7 kg/ha) or simazine (2.2 kg/ha). However, goosegrass control was increased to 88% or greater by applying MSMA in combination with atrazine or simazine, followed by a second MSMA treatment 1 week later.

Early weed control research in the southern United States indicated that two postemergence applications of metribuzin (1.1 kg/ha) were required to control large crabgrass consistently (Johnson, 1975b). However, goosegrass proved to be slightly more susceptible to metribuzin, requiring two applications of only 0.6 kg/ha to control the weed at least 99% (Johnson, 1975b, 1976c, 1977b). This was in stark contrast to the amount of MSMA needed to provide equivalent goosegrass control (two applications of 3.3 kg/ha). When applied preemergence, Lewis (1981) observed that a minimum rate of 0.8 kg/ha metribuzin controlled large and smooth crabgrass throughout the growing season with minimal bermudagrass injury. In 1985, Lewis reported complete control of smooth crabgrass as well as dallisgrass with two applications of 0.6 kg/ha metribuzin, whereas fenoxaprop-ethyl was ineffective in controlling dallisgrass.

Further research with MSMA plus metribuzin combinations (Johnson, 1980a) revealed that 2.2 kg/ha MSMA combined with 0.1 kg/ha metribuzin controlled 96–98% of goosegrass without the excessive bermudagrass injury found when higher rates of metribuzin alone were used to control goosegrass effectively. Similar results were also observed in Florida (Tucker, 1979). Tank-mix combinations of metribuzin plus DSMA also controlled goosegrass effectively in Virginia without excessive bermudagrass injury (Bingham, 1983). Metribuzin was an essential component in the tank-mix, since even numerous applications of DSMA alone failed to control goosegrass. These findings clearly indicated that triazine herbicides could be used to increase the effectiveness of organic arsenical herbicides while reducing the total amount of herbicide.

Perhaps the greatest use of atrazine and simazine in nonoverseeded, dormant bermudagrass turf is for winter annual weed control. Generally, these two herbicides provide broad-spectrum weed control without causing bermudagrass injury or delay in spring transition. Research in Georgia (Johnson, 1975a) indicated that 2.2 kg/ha simazine applied in October controlled annual bluegrass, hop clover, common chickweed, and corn speedwell consistently across two locations over a 3-year period. Researchers in Arkansas (Troutman *et al.*, 1977) reported greater than 80% control of a broadleaf weed complex including henbit, common chickweed, and corn speedwell with 1.7 or 3.4 kg/ha of simazine applied preemergence without delaying spring transition. Further research (Johnson, 1977a) revealed that 0.6 kg/ha metribuzin applied in January or February at two locations controlled lawn burweed, parsley-piert, corn speedwell, henbit, and common chickweed with a single application and caused no delay in spring transition. Metribuzin also improved appearance ratings. Centipedegrass is very tolerant to atrazine (Johnson, 1979), while St. Augustinegrass is tolerant to both simazine and atrazine (Johnson, 1976c). This information has given turf managers options for controlling grassy weeds in centipedegrass, where organic arsenical herbicides cannot be used. Johnson (1979) also observed that three monthly applications of 2.2 kg/ha atrazine controlled established bahiagrass in a centipedegrass turf, but a second treatment regime the following year was necessary for complete control.

Since turfgrass weed control research with the triazine herbicides began, numerous weed species have reportedly been controlled. Table 19.1 summarizes some of these reports.

Warm-Season Turfgrass Establishment

Sprigging (also known as plugging) is a common practice for establishing St. Augustinegrass, zoysiagrass, centipedegrass, and bermudagrass. Vegetative propagation is the most common way to establish St. Augustinegrass, zoysiagrass, and hybrid bermudagrasses. Competition from weeds or injury from herbicides during establishment often reduces the rate of growth during the first year (Johnson, 1973), but some injury from herbicides may be acceptable if weeds are controlled or suppressed enough to compensate for the injury. Atrazine is currently labeled for weed control in newly sprigged centipedegrass, St. Augustinegrass, and zoysiagrass. A side effect is the temporary slowing of growth, which may occur along with yellowing. Simazine is labeled for weed control in newly sprigged bermudagrass, centipedegrass,

Table 19.1 Reported weeds controlled with triazine herbicides

Herbicide	Weed species[a]	Reference
Simazine or atrazine	Annual bluegrass	Huffine (1965) and Johnson (1975a)
	Lawn burweed	Johnson (1977a)
	Large crabgrass	Klingman (1962)
	Horseweed	Klingman (1962)
	Venuslookingglass	Klingman (1962)
	Carolina geranium	Klingman (1962), Troutman *et al*. (1977)
	Virginia pepperweed	Klingman (1962)
	Cutleaf eveningprimrose	Klingman (1962)
	Common lespedeza	Klingman (1962)
	Hop clover	Klingman (1962) and Johnson (1975a)
	Rabbitfoot clover	Klingman (1962)
	Common chickweed	Huffine (1965), Johnson (1975a), and Troutman *et al*. (1977)
	Corn speedwell	Johnson (1975a) and Troutman *et al*. (1977)
	Henbit	Huffine (1965) and Troutman *et al*. (1977)
	Parsley-piert	Johnson (1975a)
	Purple deadnettle	Troutman *et al*. (1977)
Atrazine	Bahiagrass[b]	Johnson (1979)
	Bermudagrass[c]	McCarty (1996)
	Smutgrass	Nishimoto and Murdoch (1994)
	Common lespedeza	Johnson (1979)
Metribuzin	Dallisgrass	Lewis (1985)
	Goosegrass	Johnson (1975b, 1976c, 1977b, 1980a), Lewis (1981), Tucker (1979), and Bingham (1983)
	Large crabgrass	Johnson (1975b, 1976c) and Lewis (1981)
	Smooth crabgrass	Lewis (1981, 1985)
	Common lespedeza	Johnson (1979)
	Lawn burweed	Johnson (1977a) and King (1982)
	Corn speedwell	Johnson (1977a)
	Henbit	Johnson (1977a)
	Common chickweed	Johnson (1977a)
	Parsley-piert	Johnson (1977a)

[a] Only genera reported in some literature.
[b] Multiple applications over two seasons required.
[c] Sequential applications tank mixed with ethofumesate.

St. Augustinegrass, and zoysiagrass, but simazine may also cause temporary slowing of growth and yellowing. Metribuzin is not labeled for use during establishment of any turfgrasses.

Johnson (1973, 1976a, b) evaluated atrazine and simazine for weed control in newly sprigged St. Augustinegrass and centipedegrass. Johnson (1973) applied atrazine or simazine immediately after sprigging. He reported that average grassy weed control at the end of the growing season was 81% for 1.1 kg/ha simazine and 57% for 1.1 kg/ha of atrazine. By doubling the atrazine application (2.2 kg/ha), grassy weed control results were similar to those from an application of 1.1 kg/ha simazine. He observed a reduction in early turfgrass growth for centipedegrass and St. Augustinegrass when simazine was applied at 1.1 kg/ha or greater, and for St. Augustinegrass when atrazine was applied at rates of 3.4 kg/ha. However, by the end of the growing season, even the lowest application rate of 1.1 kg/ha simazine or atrazine significantly increased percent ground cover of both turfgrasses. Coats (1975) reported that 2.2 kg/ha simazine or atrazine reduced the survival and growth rates of vegetatively propagated centipedegrass when applied either prior to sprigging, immediately after sprigging, or either 7 or 14 days after sprigging. Johnson (1976b) found that if atrazine or simazine immediately followed centipedegrass sprigging, establishment improved if the sprigs were dipped in activated charcoal.

Engel *et al*. (1968) reported that zoysiagrass cultivars exhibited a differential response to simazine or atrazine when established from rhizomes. They found that Midwest zoysiagrass and the selection B21-15(22) were substantially less tolerant to simazine and atrazine than cultivar Meyer and the selections B52-22(24) and B21-15(4). In their trials, atrazine was generally more phytotoxic than simazine, especially on more susceptible cultivars. Simazine at 1.4 kg/ha did not significantly reduce zoysiagrass stolon survival. Fry *et al*. (1986) evaluated the influence of simazine on the establishment of Meyer zoysiagrass from plugs. Only during the first of 2 years at one of two locations did 2.2 kg/ha simazine reduce zoysiagrass establishment as compared to the untreated acreage. Tweedy (1975) reported

that 2.2 kg/ha simazine provided excellent weed control for up to 3 months and did not injure zoysiagrass cultivars Meyer and Midwest.

Although only labeled for established bermudagrass, 0.1 kg/ha metribuzin plus 2.2 kg/ha MSMA was shown to be safe to apply up to 1 week before sprigging bermudagrass (Johnson, 1980b); 2 and 4 weeks were required after treatment prior to planting from stolons and seeds, respectively. Bingham and Hall (1985) found that 0.3 kg/ha metribuzin alone – or tank-mixed preparations with 2.2 kg/ha MSMA – did not reduce the percent ground cover of Vamont, Midiron, or Tifway bermudagrass cultivars when applied 3 or 5 weeks after sprigging. Several researchers evaluated simazine during the establishment of bermudagrass from sprigs. Green (1985) reported that simazine at 1.7 kg/ha did not reduce cultivar U-3 bermudagrass cover through 12 weeks after sprigging. Simazine at 3.3 kg/ha reduced U-3 bermudagrass cover at 7 weeks after sprigging, but not at 12 weeks. Deal (1967) applied 2.8 kg/ha simazine either before or after sprigging the Tufcote cultivar of bermudagrass, and observed severe injury or death of most of the sprigs. Simazine at 2.2 kg/ha controlled broadleaves and grassy weeds for up to 3 months without injuring Tiffine and U-3 bermudagrass cultivars (Tweedy, 1975). Troutman *et al.* (1976) evaluated the effects of simazine on sprigged bermudagrass in soil previously sterilized with methyl bromide and found that 1.1 or 2.2 kg/ha simazine inhibited the spreading of bermudagrass but had no effect on pegging. Lewis and Lilly (1966) applied 2.2 kg/ha simazine or atrazine in April for 3 consecutive years. Vigor of germinating bermudagrass seedlings was not affected in soil taken in October from 0 to 5 cm or 5 to 10 cm depths from these herbicide-treated plots after the third year.

Availability of preemergence herbicides for sprigging turfgrasses is very limited. Oxadiazon is only labeled for bermudagrass, zoysiagrass, or seaside paspalum, but triazines – especially simazine – are still important for reducing competition from broadleaf and grassy weeds during establishment of warm-season turfgrasses.

Weed Resistance to Triazine Herbicides

Triazine-resistant annual bluegrass in the United States was first reported in California (Holt and LeBaron, 1990), but triazine resistance among common turfgrass weeds has not been widely reported in the southeastern United States. However, at least two populations of annual bluegrass in Mississippi golf courses have been identified as being resistant to both simazine and atrazine (Kelly and Coats, 1997a, b, 1999). These populations were determined to be 1000-fold more resistant to simazine than a triazine-susceptible biotype. Investigation into herbicide use patterns at these golf courses revealed that simazine had been used for at least 12 consecutive years at one location and for more than 15 years at the second location. These use patterns are similar to those observed where triazine-resistant weed populations have been confirmed (Ryan, 1970).

The mode of resistance for these two annual bluegrass populations was determined to be a modified binding site on the D1 protein in photosystem II (Kelly and Coats, 1999). A serine 264 to glycine mutation in the chloroplast *psb*A gene was responsible for this resistance and was the same as that observed in other cases of chloroplast-based triazine resistance in higher plants (Hirschberg and MacIntosh, 1983; Goloubinoff *et al.*, 1984; Hirschberg *et al.*, 1984; Schonfeld *et al.*, 1986; Bettini *et al.*, 1987; Eberlein *et al.*, 1992). Quite simply, continually using simazine on these two golf courses caused a shift in the native population from those plants having serine in position 264 of the chloroplast *psb*A gene to those plants having glycine in that position, preventing the binding of the triazine herbicides. As with cases of other triazine-resistant weeds, these two annual bluegrass populations were readily controlled by other herbicides with modes of action different from the triazines. Pronamide and ethofumesate were effective at controlling these populations postemergence, while prodiamine, oryzalin, oxadiazon, pendimethalin, or dithiopyr effectively controlled both populations preemergence (Kelly and Coats, 1997a, b). These findings show that widespread resistance may be prevented or delayed by alternating herbicides with differing modes of action in the program for controlling winter annual weeds in turfgrass.

Sod Production

In sod production, crops are typically reestablished from the strips or ribbons of undisturbed turfgrass left in the field after harvest, or from rhizomes remaining in the soil. After harvest, reestablishment may take 8 to 16 months with exposed soil susceptible to weed invasion (McCarty and Cisar, 1989). Quality and tensile strength of the sod can be reduced by weed infestations (Turner *et al.*, 1990).

Several papers have been published on the effects of atrazine or simazine in production of centipedegrass or St. Augustinegrass sod (Turner and Dickens, 1987; Turner *et al.*, 1990; McCarty *et al.*, 1995). Turner and Dickens (1987) evaluated the effects of atrazine applied at 0.6, 1.1, or 2.2 kg/ha every 2 or 4 weeks beginning on April 20 (ffi3 days) and continuing to August 5 (ffi3 days) on established centipedegrass. During the application season, there was no visible injury to centipedegrass from 0.6 kg/ha atrazine, with slight to no injury observed following an application of 1.1 kg/ha atrazine at 4-week intervals. Atrazine applied at 0.6 kg/ha every 2 weeks or 1.1 kg/ha every 4 weeks did

not reduce the tensile strength of centipedegrass sod when harvested in September. In another study, Turner *et al.* (1990) generally observed no effect on tensile strength, root number, or root length of centipedegrass sod treated with 2.2–3.4 kg/ha atrazine or simazine. Atrazine applied to previously harvested sod at 1.1 or 2.2 kg/ha had no effect on St. Augustinegrass injury, density, or tensile strength (McCarty *et al.*, 1995). In addition, no unrooted stolons were observed 2–8 weeks after treatment. Sharpe *et al.* (1987) reported that 3.3 kg/ha atrazine or simazine had no effect on tensile strength of bermudagrass or zoysiagrass sod harvested at 2, 4, or 8 weeks after treatment.

Both atrazine and simazine are very important herbicides in production of warm-season turfgrass sod. Many alternatives to the triazines used in established turf cannot be used in sod production due to root growth inhibition. Normal triazine use rates are 2.2 kg/ha followed by 1.1 kg/ha on established turfgrasses or sod, except 4.5 kg/ha followed by 2.2 kg/ha on Florida muck soils. See product labels for detailed instructions.

Alternatives to the Triazine Herbicides

Triazine herbicides have great utility in turfgrass establishment and weed control. Although other herbicides may be used to control weeds that are susceptible to the triazines, more than one herbicide is often needed, and most alternative herbicides are more expensive.

With the introduction of dinitroaniline herbicides for use in fine turfgrass, the primary use for atrazine and simazine became winter annual weed control. In golf course management and sod production, a single application of simazine provides excellent broad-spectrum control of numerous winter annual species.

Annual bluegrass has shown susceptibility to currently available preemergence herbicides such as oryzalin, pendimethalin, dithiopyr, prodiamine, or oxadiazon (Smith *et al.*, 1986; Webster *et al.*, 1986; Higgins *et al.*, 1991; Lewis, 1991; McCarty, 1991; Coats, 1992; Lewis, 1994; Murphy and Johnson, 1995). However, these herbicides do not provide broadleaf control equal to the triazines. Although annual bluegrass control is often very good with these herbicides, there are usually some broadleaf weeds that are not controlled, such as lawn burweed and purple cudweed (Murphy and Johnson, 1995).

Common broadleaf weeds in turfgrasses, such as those listed in Table 19.1, are also controlled by other herbicides such as 2,4-D, dicamba, dichloroprop, 2-methyl-4-chlorophenoxyacetic acid (MCPA), mecoprop, clopyralid, and triclopyr, or combinations of these products (Bingham and Shaffran, 1982; Bingham *et al.*, 1986; Coats *et al.*, 1994; Johnson and Murphy, 1995; Murphy and Johnson, 1995).

Preemergence control of lawn burweed (Grant *et al.*, 1990; Johnson and Murphy, 1995), henbit (Minner, 1994), short buttercup, hop clover, curly dock, and buckhorn plantain (Grant *et al.*, 1990; Chandran *et al.*, 1998) has been reported with isoxaben. However, control appears to be rate dependent with such species as curly dock and parsley-piert.

Table 19.2 Cost comparison between atrazine, simazine, and alternative herbicides for weed control in warm-season turfgrasses[a]

Use	Common name	Rate (kg/ha)	Cost ($/kg)	Cost ($/ha)
Annual bluegrass	Atrazine	1.1–2.2	6.14	6.75–13.50
	Simazine	1.1–2.2	9.09	10.00–20.00
	Bensulide	13.4–16.8	27.50	368.50–462.00
	Benefin	3.4	45.76	155.58
	Dithiopyr	0.56	341.00	190.96
	Oxadiazon	2.2–4.5	187.00	411.40–841.50
	Oryzalin	3.4	41.54	141.24
	Pendimethalin	3.4	22.77	77.42
	Prodiamine	0.84	158.95	133.52
	Glyphosate (dormant turf)	1.1	40.66	44.72
	Glufosinate (dormant turf)	0.84	154.40	129.70
	Pronamide	0.56–1.1	181.06	101.40–199.17
Winter broadleaf weeds	Atrazine	1.1–2.2	6.14	6.75–13.50
	Simazine	1.1–2.2	9.09	10.00–20.00
	Metribuzin	0.41–0.56	22.68	9.30–12.70
	2,4-D amine	1.1	7.99	8.79
	Dicamba	0.56	42.90	24.02
Goosegrass	MSMA ffl metribuzin	2.2 ffl 0.07	5.41 ffl 22.68	11.90 ffl 1.59
	Diclofop	0.84–1.12	125.95	105.80–141.60

[a] Rates are from 2002 Weed Control Guidelines for Mississippi and are based on 1998 retail averages.

Chandran *et al*. (1998) also suggest that isoxaben may provide up to 6 months of control of common winter annual weeds.

Metribuzin is an economical and effective postemergence option for controlling goosegrass. Although diclofop-methyl is very effective at controlling goosegrass (McCarty, 1989; Taylor and Coats, 1992), applications must be made in a timely manner and are limited to bermudagrass golf turf. Johnson (1994) reported somewhat inconsistent goosegrass control with diclofop-methyl, but consistency was improved by tank mixing with dithiopyr. Goosegrass can be controlled with preemergence applications of currently available herbicides such as oxadiazon, oryzalin, prodiamine, dithiopyr, or pendimethalin, but repeat applications at an interval of 6–8 weeks are necessary.

Recently sulfonylurea herbicides have been shown to control some of the weeds in turfgrasses that are controlled by the triazines. Rimsulfuron provides excellent control of annual bluegrass (Wehtje and Walker, 2002). Other sulfonylurea herbicides such as trifloxysulfuron, foramsulfuron, metsulfuron, chlorsulfuron, sulfosulfuron, and flazasulfuron in addition to rimsulfuron provide good to excellent control of annual bluegrass and (or) some common broadleaf weeds such as common chickweed, mouseear chickweed, henbit, lawn burweed, and white clover (Belcher and Walker, 2002; Tucker*et al*., 2004; Stephenson *et al*., 2005; Taylor *et al*., 2005; Warren *et al*., 2005).

While many winter annual weeds controlled by atrazine and simazine may also be controlled by other herbicides, these herbicides do not provide the broad-spectrum weed control obtained with atrazine or simazine. If simazine or atrazine were not available in fine turf, the use of two or more herbicides would be required to provide equal control. Table 19.2 provides a cost comparison between triazines and alternative herbicides for weed control in warm-season turfgrasses.

Conclusions

Atrazine and simazine have been widely used in warm-season turf for many years to control broadleaf weeds and certain grass weeds. They provide economical control at selective rates in commercial and homeowner turf and in sod production of certain species. Triazines continue to have a place in turf, even with the development of new turf herbicides.

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Chapter 20

Methods of Analysis for Triazine Herbicides and Their Metabolites

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Summary

The science and technology of analytical chemistry have made steady and remarkable advances over the last 50 years. Nowhere has this been more evident than for progress on methods to analyze organic chemicals developed as pesticides and, in particular, the triazine herbicides. Methods of triazine analysis traditionally involve an extraction step in which the analyte is removed from the matrix – such as soil, water, or crop. This extract is then subjected to a 'clean up' in various ways to isolate the analyte further from the other chemical components that are extracted. The next step is to concentrate the purified fraction to a smaller volume to allow the analyte to be detected. A small portion of this final fraction is then injected into an instrument capable of selectively detecting and quantifying the triazine in the sample.

The early analysis methods for the triazines involved complicated, labor-intensive extraction and cleanup procedures. The development of solid-phase extraction (SPE) – in which a sample or extract is passed through a small, disposable cartridge containing a sorbent – dramatically shortened and simplified the sample preparation procedures. Much of the emphasis in extraction methods has been on water samples, which can often be passed through an SPE cartridge directly. The triazine compounds simply bind to the SPE material and are washed out using a small quantity of elution solvent. Small portions of the resulting fraction can be directly injected into an instrument for quantitation. A related approach has been to expose fiber coated with SPE material to a sample to allow the triazines present to bind to it. The fiber is then inserted into a gas chromatograph (GC) and heated until the triazines desorb and enter the separation and detection system; alternatively the fiber can be introduced into the liquid stream of a liquid chromatograph (LC). Another method has been to prepare polymers that are molecularly imprinted for a specific compound, significantly improving specificity. Imprinted polymers are formed during precipitation polymerization using the target compound as template. These approaches have simplified extraction procedures and have opened the door to automation for triazine extraction and detection.

GC or, later, LC is employed both to separate the triazines and their metabolites from other compounds and to detect and quantify their presence. GC equipped with nitrogen–phosphorus (NPD) or electron-capture detectors still find use, but cost reductions in instruments for mass spectrometry (MS) have greatly increased the use of MS in routine analysis. MS provides confirmatory evidence of the identity of the compound. Different configurations of MS instruments allow the analyst to detect smaller quantities than previously possible. Detection limits are now several orders of magnitude lower than when the triazine herbicides were first introduced.

The use of LC also has increased in use in recent years, driven by greater sensitivities of the detectors. Traditional ultraviolet (UV) and photo diode-array detectors were frequently employed in triazine analysis, but advances in source designs have provided efficient coupling of MS with LC. The advantage of LC is the ability to analyze polar metabolites not amenable to analysis using GC. Recent progress in LC/MS/MS instrumentation has enabled the direct aqueous injection (DAI) of a water sample without prior cleanup.

Capillary electrophoresis (CE) provided an orthogonal separation technique. The retention mechanism can be manipulated with buffers or addition of surfactants to form micelles for the analysis where size and charge differences

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are insufficient. As with LC, the detector was typically a UV light detector, but interfaces with MS are now the most desired combination. Very high resolution is possible with such systems. However, sensitivity limitations based on injection volume restrictions and reproducibility still inhibit the technique for routine analysis.

Immunoassay methods use antibodies developed against one or more triazines and enzymes that create a colored signal. The result is a sensitive, simple method of detecting triazines in a wide variety of matrices. An immunoassay requires significant resources to develop, but once developed can provide rapid, inexpensive analyses that can be specific to a single triazine or sensitive to a variety of triazines.

Introduction

Analytical methodologies for the triazine herbicides have improved significantly since their original development. During the 50-year period in which the triazines have been used as herbicides, analytical equipment and extraction systems have evolved to be able to detect very small amounts of triazines in samples. More recently, methods have also been developed for extracting and isolating a wide array of metabolites.

Several books have included chapters on analytical methods for triazine herbicides: *Residue Reviews*, *Volume 32, The Triazine Herbicides* (Gunther and Gunther, 1970); *Analysis of Pesticides in Water, Volume III* (Chau and Afghan, 1982); and Triazine herbicide methodology (Yokley, 2003) in the *Handbook of Residue Analytical Methods for Agrochemicals* (Lee, 2003). In the mid-1990s, several books have been published on analytical methods for pesticides in water, including the triazines, (Stan, 1995a, b; Barceló and Hennion, 1997). Reviews of analytical procedures are also published periodically in the journals *Analytical Chemistry* (e.g., Sherma, 1995) and *Journal of Chromatography A* (e.g., Pacakova *et al.*, 1996). This chapter will cover the last 20 years of method development in triazine analysis with a focus on new developments. During this period, new approaches for the extraction, detection, and quantification were developed, replacing the previous methods in most laboratories.

The three main categories of new analytical methodology are: extraction technology, separation and detection technology, and immunoassay analysis. The majority of recent work has focused on the analysis of water samples for triazine herbicides and their metabolites, so this review is heavily weighted in that arena.

The most recent advancements in the analysis of the triazines and their metabolites have generated a great deal of literature on the occurrence of triazines in the environment. Technology has provided us with tools to find chemicals in minute quantities that may not have any measurable level of risk associated with them. Detection in itself does not confer any measure of biological activity or risk, so it is important that risks are put into scientific perspective in reports on chemical occurrence in the environment.

Extraction Technology

Water Extraction

The most widely adopted change in sample preparation for triazine analysis in water has been the use of SPE in place of liquid–liquid extraction (LLE). The main advantage is the elimination of large volumes of waste solvent and solvent-saturated water. In addition, large volumes of water can be passed through a cartridge, while traditional LLE is limited to the size of separation funnel. The number of articles on SPE has grown very rapidly since the mid-1980s (Berrueta *et al.*, 1995), and a wide variety of phases and formats have been tested to optimize extraction efficiency for each particular application.

Many studies on triazines in water have included SPE in the extraction step. The United States Environmental Protection Agency (USEPA) Method 525, intended for organic compound analysis in drinking water, is based on SPE and includes many triazines (USEPA, 1994). Wachob (1984) and Sherma (1986) may have been among the first to report the use of SPE for triazine extraction from water. Since then, many large surveys of surface or groundwater used simple C18 (18-carbon alkane-bonded silicone packing) cartridge methods to extract some triazines with good recoveries (Bagnati *et al.*, 1988; Nash, 1990; Schuette *et al.*, 1990; Thurman *et al.*, 1990; Schottler *et al.*, 1994; Watts *et al.*, 1994; Zahradnickova *et al.*, 1994; Sabik *et al.*, 1995; Novak and Watts, 1996). C18 cartridges are packed with a solid phase made up of a silica backbone with octadecyl (18 carbons) carbon chains bonded to it. The method typically involves passing up to 1 L of sample water through a cartridge, followed by a period of drying by pulling air through the cartridge. Analytes are eluted with a few milliliters of a solvent such as methanol, ethyl acetate, or methylene chloride, and the eluate volume is then reduced under a stream of nitrogen or rotary evaporation.

In a modification of the usual solvent extraction step, six triazines were successfully recovered using C18 cartridges and 250 mL samples, but terbutryn required a 0.25% NH_3 (ammonia) washing step prior to elution with acetonitrile (Vitali *et al.*, 1994). The authors ascribed this effect to secondary cation exchange sites in the solid phase.

One liter of water, pH 7.0, and ethyl acetate elution (Molto *et al.*, 1991) were determined to be optimal for the extraction of prometryn, propazine, and simazine from natural waters using C18-bonded silica. Wells *et al.* (1994) also found pH 7.0 to be optimal for metribuzin or atrazine, but methanol was a better elution solvent than ethyl acetate. Riley and Keese (1996) had comparable recoveries of simazine from laboratory water using C18 disks or cartridges.

Another SPE format is the disk, in which the solid phase is impregnated in or layered on a filter disk. This provides significant advantages in sample extraction because flow rates can be as high as 200 mL/min as compared to the 2–5 mL/min typical of cartridges. Albanis and Hela (1995) extracted a variety of water samples using 47 mm C18 disks and had good recoveries for relatively clean water – but lake, river, and sea water reduced recoveries to less than 40% for some triazines. Styrene–divinylbenzene is an alternative to C18 and works well for atrazine and simazine in both reagent-grade water and river samples (Crespo *et al.*, 1994; Pichon *et al.*, 1998). The styrene–divinylbenzene disks were less susceptible to breakthrough and matrix effects. Bulk C18 sorbent is another format shown to be very effective at removing cyanazine from river water and with much less sample preparation than for the disk format (Bengtsson *et al.*, 1994).

Laboratories often extract water samples soon after collection and store the cartridges or disks for later elution and analysis. Senseman (1995) tested the effects of desiccation and storage temperature on the stability of four triazines. Neither atrazine nor simazine were significantly affected by storage conditions, but cyanazine and metribuzin recoveries were improved by freeze-drying or frozen storage of the disks. Nash (1990) found atrazine to be stable on C18 cartridges for 150 days under refrigeration. Storage stability studies show that atrazine, its chlorotriazine metabolites, and several other triazine compounds are stable in ground and surface water when stored in the dark at refrigerator temperature (4°C) for 2 years. Atrazine, simazine, and their applicable chlorotriazine metabolites are also stable in ground and surface water for 14 months when stored at room temperature in dark-colored amber bottles.

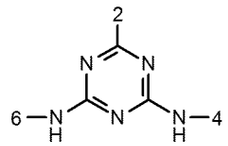
Psathaki *et al.* (1994) tested Amberlite XAD-2 resin and C18 cartridges for atrazine and simazine recovery from 1 L groundwater samples. Both phases worked well (74–85% recovery) for the triazines, but the C18 worked better for organophosphates. The XAD-2 resin required much more preparation, however, including multiple sonication and washing steps, and also required a relatively high volume (100 mL) for elution. This partially negates the advantage of SPE over LLE in reduced solvent usage and evaporation times.

One extraction phase developed for triazines is graphitized carbon black, which has been shown to be versatile for a wide range of pesticides (Di Corcia *et al.*, 1987; Di Corcia and Marchetti, 1992; Di Corcia *et al.*, 1993; Bucheli *et al.*, 1997; Crescenzi *et al.*, 1997). It has also been shown to be very effective for retaining the more polar metabolites (e.g., deisopropylatrazine (DIA), deethylatrazine (DEA), and hydroxyatrazine (ATOH); see Table 20.1, also Appendix, Table A3) (Berg *et al.*, 1995; Di Corcia *et al.*, 1997; Pichon *et al.*, 1995; Schulein *et al.*, 1995). The methods published require considerably more column preparation and elution steps than traditional C18 methods. Pichon *et al.* (1995) also included the polar triazine metabolites ammeline, ammelide, and cyanuric acid, with good recoveries using a graphitic carbon sorbent.

Recovery of triazine metabolites by SPE has been problematic due to their higher polarities and lower affinities for the adsorbents than the parent compound. DEA and DIA are poorly recovered from well-water samples using the C18 phase, while the method has excellent parent recovery (Benfenati *et al.*, 1990; Nash, 1990; Barceló *et al.*, 1993; Durand and Barceló, 1993). Others have had somewhat better results for DEA using the C18 phase, although recoveries often are below 70% (Thurman *et al.*, 1990; Meyer *et al.*, 1993; Cassada *et al.*, 1994; Schottler *et al.*, 1994; Sabik *et al.*, 1995; Novak and Watts, 1996). The affinity of the isopropyl side chain for the octadecyl phase is greater than the ethyl side chain. Once both side chains are removed, very little affinity remains, and didealkylatrazine (DDA) is not retained on C18 columns (Thurman *et al.*, 1990; Sabik *et al.*, 1995). Increasing the length of the side chains increases the adsorption to the C18 phase due to greater Van der Waals interactions (Mills and Thurman, 1992; Mills *et al.*, 1993). Metribuzin and several metabolites are retained by C18, but the diketo metabolite recoveries may be low (Lawrence *et al.*, 1993). Mean recoveries of 95–100% are obtained for atrazine, its three chlorotriazine metabolites, and seven other compounds in water using C-18 and SCX mode SPE and GC/MSD analysis (Huang *et al.*, 2003) at a lower limit of method validation of 0.10 ppb. Acceptable recoveries at 0.20 ppb are also obtained for atrazine, its chlorotriazine metabolites, and 13 other compounds using a styrene–divinylbenzene copolymer and two graphitized carbon black SPE cartridges for sample preparation and analysis using electrospray ionization-liquid chromatography (ESI-LC)/MS (Tanabe and Kawata, 2004).

Few studies include the hydroxytriazine metabolites because they are more difficult to extract and analyze than most parent or dealkylated triazines. C18 cartridges worked well for ATOH and hydroxyethylterbutyltriazine extraction from well water, but not for hydroxysimazine (Saez *et al.*, 1996). The elution step in this study was accomplished with a 70:30 v/v mix of acetonitrile and 0.005 M H_2KPO_4. A strong cation exchange cartridge (SCX), with a sulfonic acid moiety as the retention mechanism through cation exchange, works well with the hydroxytriazines (Lerch and Donald, 1994; Sabik *et al.*, 1995). The analytes are eluted with an acetonitrile/buffer mix with good

Table 20.1 Chemical structures of selected *s*-triazines



	Substitution at ring position		
Compound	**2**	**4**	**6**
Atrazine	ß Cl	ß C_2H_5	ß $CH(CH_3)_2$
Simazine	ß Cl	ß C_2H_5	ß C_2H_5
Propazine	ß Cl	ß $CH(CH_3)_2$	ß $CH(CH_3)_2$
Cyanazine	ß Cl	ß C_2H_5	ß $C(CH_3)_2CN$
Terbuthylazine	ß Cl	ß C_2H_5	ß $C(CH_3)_3$
Atraton	ß OCH_3	ß C_2H_5	ß $CH(CH_3)_2$
Prometon	ß OCH_3	ß $CH(CH_3)_2$	ß $CH(CH_3)_2$
Ametryn	ß SCH_3	ß C_2H_5	ß $CH(CH_3)_2$
Prometryn	ß SCH_3	ß $CH(CH_3)_2$	ß $CH(CH_3)_2$
Terbutryn	ß SCH_3	ß C_2H_5	ß $C(CH_3)_3$
Aziprotryn	ß SCH_3	ß N_3	ß $C(CH_3)_3$
Hydroxyatrazine	ß OH	ß C_2H_5	ß $CH(CH_3)_2$
Hydroxysimazine	ß OH	ß C_2H_5	ß C_2H_5
Hydroxydesmetryn	ß OH	ß CH_3	ß $CH(CH_3)_2$
Deethylatrazine	ß Cl	ß H	ß $CH(CH_3)_2$
Deisopropylatrazine	ß Cl	ß C_2H_5	ß H
Didealkylatrazine	ß Cl	ß H	ß H

recoveries from both water and sediment extracts. Parent triazines also can be retained on the SCX (Land, 1994). Another approach is to mix the phases together in one cartridge, although the retention of weakly held DEA and DIA is poor if cations were present in the water (Mills *et al.*, 1993). Mixing a phenyl-bonded phase with the octadecyl phase to improve DEA and DIA retention improved recoveries, but only marginally (Benfenati *et al.*, 1990). McLaughlin and Johnson (1997) reported good recoveries for both DEA and DIA, as well as atrazine and simazine, using a partially nonendcapped version of C18 cartridges.

Most of the extraction methods have involved 'off-line' SPE, but isolation of the analytes can occur 'on-line' immediately prior to chromatographic analysis (Bagheri *et al.*, 1992; Marce *et al.*, 1995; Prosen *et al.*, 1995; Slobodnik *et al.*, 1996). This approach involves passing a water sample through an adsorption column, then eluting it directly to an attached analytical column for high-performance liquid chromatography (HPLC) analysis. Slobodnik *et al.* (1996) also included an initial elution to a GC MS for analysis, while Grob and Li (1989) used a direct HPLC to GC connection with an evaporation precolumn. Coquart and Hennion (1991) used two precolumns of different adsorbents to accomplish both triazine adsorption from natural and drinking water and elimination of interferences prior to analysis. Overall, the off-line SPE methods are simple and flexible, while the on-line methods lend themselves to automation (Liska, 1993).

Another development has been the use of solid-phase microextraction (SPME) (Boyd-Boland and Pawliszyn, 1995; Choudhury *et al.*, 1996; Gorecki *et al.*, 1996; Graham *et al.*, 1996). The technique developed by Belardi and Pawliszyn (1989), Author and Pawliszyn (1990), Pawliszyn (1997), and Pawliszyn (2002), utilizes a fiber coated with an adsorbent that is introduced into a vial of sample water, which is stirred to bring the analytes into contact with the fiber. The analytes are then thermally desorbed in the injection port of a GC. This technique offers significant advantages in automation and simplicity, and it is likely to be widely adopted as its commercial availability increases. For example, the analysis of several parent triazines is accomplished using polydimethylsiloxane divinylbenzene (PDMS-DVB) SPME fibers in conjunction with GC/MS with limits of detection lower than 17 ppt(Frias *et al.*, 2003). SPME-GC-MS/MS is used as a multi-residue method for the analysis of ffi40 compounds, including a few triazines (Goncalves and Alpendurada, 2004) with detection limits in the low ng/L concentration. Other publications include the analysis of a few triazines and several other herbicides (Lambropoulou *et al.*, 2002a; Albanis *et al.*, 2003). Factors affecting the use of SPME are described (Lambropoulou *et al.*, 2002b) with simplicity, low cost, and sensitivity quoted as advantages. Hollow fiber liquid-phase microextraction (LPME) is reported as a less costly

alternative to SPME, especially for 'dirty' aqueous samples (presence of humic acids), and is applicable to the analysis of several parent triazines at concentrations ranging from 7 to 63 ng/L (Shen and Lee, 2002).

Traditional LLE is being used in many laboratories (Munch *et al.*, 1990; Pereira *et al.*, 1990). At least one laboratory relied on LLE primarily to quantitatively extract the polar atrazine metabolites (e.g., deethyldeisopropylatrazine), in addition to several parent triazines and numerous other analytes of varying polarity (Yokley and Cheung, 2000). This was prior to the development of SPE cartridges capable of retaining the more polar metabolites (Huang *et al.*, 2003). Variations on LLE are also being reported, including microextraction using a high ratio of sample water to solvent (Potter *et al.*, 1991; Molina *et al.*, 1995) and a unique membrane system in which the analyte is extracted across the membrane as the sample is passed by it (Trocewicz, 1996; Knutsson *et al.*, 1996).

Several approaches have been introduced for using highly selective sorbents to concentrate triazine residues from environmental waters. One system uses the antigen–antibody interactions now common in immunoassays, as described later in this chapter. However, in this case the antibodies are bonded to a silica backbone and the sample is passed through the sorbent material as with other SPE procedures. Many different triazines and metabolites have been concentrated using this approach (Pichon *et al.*, 1995, 1996). Another technology uses molecularly imprinted polymers (MIP) to retain triazines selectively (Muldoon and Stanker, 1995; Siemann *et al.*, 1996; Bjarnason *et al.*, 1999; Chapuis, 2003; Shoji, 2003). Polymers are formed in the presence of specific molecules of interest, such as atrazine, which are subsequently stripped using strong solvents. The resulting polymer can selectively bind the molecules from extracts. This system has the advantage of much greater tolerance to solvents as compared to immunosorbents, which in turn allows for analysis of typical extracts. Selective MIPs also have been reported for propazine (Cacho *et al.*, 2004), desmetryn (Kochkodan, 2002), and terbuthylazine (Pap, 2002). More recent work by Turiel *et al.* (2003) has improved the understanding of cross-reactivity and binding sites. However, immunosorbents appear to have greater binding capacity and selectivity.

Soil Extraction

Most soil extraction methods still involve either soxhlet extraction or shaking or sonicating the soil in a mixture of solvent and water as in earlier procedures (Yokley *et al.*, 2000), and several have noted improved extraction efficiencies at elevated temperatures (Cabras *et al.*, 1989; Huang, 1989; Huang and Pignatello, 1990; Wenheng *et al.*, 1991; Turin and Bowman, 1993; Watts *et al.*, 1994; Gan *et al.*, 1999). Derivatization of methanol extracts from soil and crops resulted in adequate chromatographic separation for simazine and atrazine to eliminate further cleanup procedures (Gong *et al.*, 1999). Microwave extraction has also been reported, using water or a weak acid solution followed by SPE of the supernatant with cyclohexyl-bonded silica cartridges for parent atrazine, DEA, and DIA (Steinheimer, 1993); investigators working with microwave extractions coupled with SPME noted improvements in specificity and sensitivity (Shen and Lee, 2002). Success with nonaqueous microwave extractions with mean recoveries ffi80% have also been reported (Vryzas and Papadopoulou-Mourkidou, 2002). Microextraction, in which the sample and extractant amounts are reduced to a fraction of traditional methods, has been demonstrated to work well for parent compounds and several metabolites (Steinwandter, 1991, 1992).

Liquid–liquid partitioning of the analytes from the soil extract is now being replaced by SPE in many cases to reduce solvent use (Wachob, 1984; Huang, 1989; Wenheng *et al.*, 1991; Redondo *et al.*, 1993; Weil and Haberer, 1991; Mills and Thurman, 1992; Turin and Bowman, 1993; Watts *et al.*, 1994; Ramos *et al.*, 1999). In these methods, the organic component of the extraction solvent is evaporated to leave an aqueous phase for SPE. A novel approach that eliminates the solvent extraction step altogether involves the use of a nonpolar resin placed in contact with water extracts from soil for 5 days, followed by elution. This approach compared well with C18 extraction (Basta and Olness, 1992).

Supercritical fluid extraction (SFE) is another approach to analyzing soil and sediment for triazines, and was widely applied when commercial equipment became available in the early 1990s. Optimizing the conditions for SFE is often the most difficult aspect of the method since pressure, temperature, and modifiers to the CO_2 extracting solvent have an impact on extraction efficiencies (Van der Velde *et al.*, 1994). The parent triazines are usually extracted with good recoveries (Janda, 1989; Ashraf *et al.*, 1991 and 1992; Alzaga *et al.*, 1994; Lopez-Avila *et al.*, 1994), but the metabolites are often more difficult to extract using SFE (Papilloud and Haerdi, 1994, 1995; Steinheimer *et al.*, 1994). Using up to 20% acetone as a modifier and increasing temperatures and pressures brought DIA and DEA recoveries up to acceptable levels (Robertson and Lester, 1994). The modifiers themselves have caused degradation of the parent triazine during SFE extraction, so adequate controls should be in place (Papilloud *et al.*, 1996). Heated water has also been used to extract triazine residues from soil, followed by SPE extraction (Steinheimer, 1993; Crescenzi *et al.*, 1999; Di Corcia *et al.*, 1999). When compared to traditional Soxhlet methodology, subcritical water extraction, followed by a partition with dichloromethane, has resulted in a 10-fold time savings, a decrease to less than 2% of the organic solvent consumed, and an improvement in precision (Richter *et al.*, 2003).

Food Extraction

The most common method for extracting triazines from foods involves some variation on the Luke method of solvent extraction, liquid–liquid partioning, and cleanup on various columns (AOAC, 1985; Mattern *et al.*, 1990, 1991; Holland *et al.*, 1995; Pardue, 1995). Graphitized carbon black, cation exchange, and C18 SPE columns have been used to extract triazine residues from food extracts (Battista *et al.*, 1989; Wittmann and Hock, 1993b; Pardue, 1995). The use of SFE has been demonstrated for atrazine analyses in meat products, although an additional filtration step to remove fat was required (Nam and King, 1994). The immunosorbent procedure mentioned in the previous section on water has also been successfully applied to fruit and vegetable extracts (Lawrence *et al.*, 1996). Both molecularly imprinted SPE (Cacho *et al.*, 2004) and SPME (Lord *et al.*, 2003) have been applied successfully to vegetables with improvements in analyte specificity and a decrease in analysis time and solvent consumption.

Detection Technology

Gas Chromatography

Column Technology

The most significant change in GC has been the wide use of fused silica capillary columns with typical diameters of 0.25, 0.32, and 0.50 mm (widebore), which have replaced most packed column applications over the past two decades. Improved instrumentation and column technology (i.e., greater number of theoretical plates, improved bonded stationary phases, and improved inertness) have led to the nearly universal acceptance of capillary columns. This high-resolution gas chromatography (HRGC) is the most widely used method for the analysis of triazines and is the standard that other less specific analytical methodology is judged against (e.g., enzyme-linked immunosorbent assay (ELISA)) (Newman *et al.*, 1996). A bonded polyethylene glycol stationary phase is typically required to separate the various homologues of parent alkylamino-*s*-triazines, but columns varying in polarity from bonded (5%-phenyl) methylpolysiloxane (Yokley and Cheung, 2000) to polyethylene glycol (Huang *et al.*, 2003) have been found to be applicable to the analysis of polar metabolites. Capillary columns coupled with element-specific detectors such as the NPD and electron-capture provide low limits of detection. Bardalaye and Wheeler (1985) report detection limits of 0.1μg/L–10μg/L when determining triazines by HRGC/NPD. Although the electron-capture detector is less sensitive than the NPD for most chlorinated triazines, it can be useful for multi-component analyses of halogenated pesticides (Garcia-Repetto *et al.*, 1996; Liska and Slobodnik, 1996).

Injection Systems

Automated split/splitless and cold on-column injection systems have been the primary systems for the past decade. The large volume on-column injection, a subsequent improvement, uses a retention gap to achieve reconcentration of the analyte as the solvent evaporates and escapes through the solvent vapor exit valve. Injections of up to 100 uL were previously applicable to HPLC, but not to GC. Large volume on-column injection (LVI) is used to improve detection limits and to decrease sample preparation time. Termonia and Termonia (1997) reported injections of 100 uL of hexane extract by this technique. Injections of this size allowed the use of full-scan MS, which is a less sensitive but information-rich technique, rather than selected ion monitoring. This application of LVI resulted in increased specificity for various pesticides, including atrazine in drinking water at detection limits of 100 ng/L. Acceptable recoveries were obtained for 11 of 13 compounds studied at the 0.1 to 0.8 ng/L concentration using SPE followed by LVI (40 μL) GC/MS (Sabik *et al.*, 2003). Recoveries for the two polar triazine metabolites, DEA and DIA, were 55–60% and 29%–46%, respectively. A novel approach to extraction uses membrane-assisted solvent extraction of parent triazines (in essence, in-vial LLE) followed by analysis using LVI-GC/MS (Hauser *et al.*, 2002). However, recovery data were reported only at 6.7 ppb, and simazine was not quantitatively recovered even at this high concentration level.

Typically splitless injection is used for trace analysis by capillary GC. Splitless injections can exhibit problems with carryover, poor repeatability, and labile analytes. Penton (1991) reports improved results with the temperature-programmable injector. With a temperature-programmable injector, samples are injected into a glass insert at an injector temperature below the boiling point of the analysis solvent; the injector temperature is then rapidly programmed to a higher value. Penton reported this technique offered greater ease of optimization and improved precision.

Atomic Emission Detector

The atomic emission detector is a tunable, element-specific detector that uses microwave-induced helium plasma to generate temperatures high enough to break molecular bonds. The generated free atomic species undergo electron excitation to higher energy states, followed by relaxation and photon emission at characteristic frequencies

for a given element; the detector response is directly proportional to the molar concentration of that element. Thus, compound independent calibration curves based on a particular element are possible. These can be useful when key standards are not available for verification of standard purity and for simplification of calibration curves. The tunable selectivity and compound independent calibration curves for nitrogen-containing pesticides have been examined by Olson and Carrell (1995). Although typically less sensitive by an order of magnitude than an NPD, the specificity of an atomic emission detector is greater than an NPD and allows detection and confirmation based on multiple elements by taking wavelength snapshots (e.g., atrazine gives a response for N and Cl) (Eisert *et al.*, 1994).

Mass Spectrometry, Mass Spectrometry/Mass Spectrometry, and Multiple Stages of Mass Spectrometry (MS)
During the past decade, the cost, complexity, and size of mass spectrometers have decreased. These factors, coupled with an increase in user-friendly software, have resulted in a proliferation of the use of HRGC/MS or HRGC/mass selective detector for quantitative work (Hernandex *et al.*, 1996). For environmental monitoring of multi-residues when accuracy and unequivocal identification are essential, the mass spectrometer is generally regarded as the most appropriate detector. Most triazine pesticides and their moderately polar metabolites, not including conjugates and hydroxylated metabolites, can readily be detected by electron ionization, with high abundance of molecular ion and fragment ions for confirmation. Huang (1989) reports limits of detection for atrazine and simazine in soil and water by stable, label isotopic dilution using HRGC/MS at 0.05 ppb for water and 0.5 ppb for soil. Cassada *et al.* (1994) also reported success with this technique for polar atrazine metabolites in water and sediment. Triazines can be quantitated by positive and negative chemical ionization, using various reagent gases such as methane (Bagheri *et al.*, 1992). Stan and Brockhorn (1991) report improved detection limits using ammonia as the reagent gas. Since the use of ammonia typically results in pseudomolecular ion generation only, the absence of confirming ions could lead to false positives. In this circumstance, multiple reaction monitoring can be used with tandem mass spectrometry equipment (Rostad *et al.*, 1989). Combined gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) at resolutions of 5 000–10 000 can play an important role when greater levels of specificity are required. For instance, Cai *et al.* (1993) reported detection limits of 0.2–0.5 ppb utilizing HRGC/HRMS.

MS/MS with a triple quadrupole mass spectrometer is an essential complement to those ionization techniques that produce predominately pseudomolecular ions (MffIH)ffI and (M⌋ H)⌋ . It is also essential for positive and negative ion application (MffISolvent or buffer adduct) – such as in ESI, chemical ionization, and fast atom bombardment. Early workers in the field of MS/MS proposed that MS/MS alone provided sufficient separation without a chromatography component. They soon became aware that chromatography is often essential for the development of rugged, reliable methods. Often, MS/MS can be used to reduce sample cleanup and to simplify the chromatography, but rarely can the chromatography be omitted. Various triazines and their moderately polar metabolites have been analyzed successfully by GC/positive chemical ionization/MS/MS (Rostad *et al.*, 1989) with detection limits of 200 picograms (pg), using a neutral loss regime to eliminate interferences found when using the electron ionization mode. In some circumstances, GC/electron ionization/MS/MS with multiple reaction monitoring can provide higher specificity than GC/HRMS (Gael *et al.*, 1992).

The ion trap mass spectrometer has several advantages over the more costly tandem mass spectrometer. The first attempts to introduce the ion trap mass spectrometer commercially as an HRGC/MS instrument met with some difficulty due to the finite limit of the number of ions that the trap can hold at any one time. Minimizing this problem with a quick pre-scan and automatic gain control has improved the functionality of this instrument for qualitative and quantitative analyses. The ion trap mass spectrometer has excellent sensitivity and fast scanning capabilities and has been studied for the unambiguous identification and quantitation of ultra-trace levels of triazines (Pereira *et al.*, 1990; Sandra *et al.*, 1995). The development of the ion trap mass spectrometer has provided MS/MS at a reasonable cost, although it is limited to product ion analysis only. Papadopoulou-Mourkidou *et al.* (1997) report 1–10 ppb detection limits in full scan mode for a variety of pesticides including triazines. Steen *et al.* (1997) combined LVI GC with ion trap MS/MS to achieve identification and quantitation at the 0.1–1 ppb level, a 10-fold increase over MS alone. Ion trap MS was utilized in conjunction with C-18 SPE and atrazine-d_5 isotope dilution analysis to monitor several triazines and degradation products in river water (Cai *et al.*, 2004). Detection limits in the ng/L concentration range were obtainable, but recoveries were poor for DEA and DIA. With the ion trap mass spectrometer, the multiple stages of collision-induced dissociation (MS/MS) show promise for the qualitative mass spectrometry of complex molecules.

The time-of-flight mass spectrometer has been around for many years; however improvements in the technique, including delayed extraction, multiple reflections to increase the effective flight-path length, and higher digitization rates, have resulted in increased utility of this type of instrumentation. Current commercial instrumentation, capable of 7 000 resolution (50% valley definition), can provide a 20 millidalton (mda) selection window for quantitation to improve specificity equivalent to that of a typical magnetic instrument. For qualitative work, accurate mass determinations of 5 ppm can be achieved (Green and Newton, 1999).

High-Performance Liquid Chromatography

Column Technology

Modern columns are generally stable, reproducible, and readily available for a wide range of compound classes. Reverse phase columns packed with 3–5 μm, bonded phases are the most popular columns used with modern HPLC. The popularity of this column results from the versatility of C18 and the particle size, which represent a good compromise between column back pressure and high efficiency. This packing has replaced the 10 μm particles used during the 1980s, such as those reported for the simultaneous analysis of atrazine and ATOH without derivatization (Solinas *et al.*, 1982). Stahl *et al.* (1989) used the more efficient 5 μm column for the analysis of several triazines in drinking and groundwater samples in conjunction with SPE cleanup and UV detection to achieve low part-per-trillion (ppt) detection. Part of the functionality of HPLC is the ability to handle large injection volumes (hundreds of microliters), as long as the injection solvent has low elution properties when compared to the mobile phase (Schroyer and Capel, 1996). Other bonded phases – NH_2 for normal phase or reverse phase separations and C8, the eight carbon version of the C18 phase – are also very useful to manipulate selectivity (Carbras *et al.*, 1989).

The analyst has a choice of whether to use HRGC or HPLC for most triazine herbicides, such as atrazine or simazine. Some of the newer classes of chemistry, including sulfonyl ureas with triazine functionality, are only amenable to HPLC. Chlorsulfuron and its metabolites are successfully analyzed using a C8 reversed phase column (Pomeschikov *et al.*, 1990). Medium to high polarity triazines are best analyzed by HPLC (Aguilar *et al.* 1996). ATOH has historically been difficult to analyze at trace levels. ATOH can be analyzed without derivitization by HPLC with a limit of quantitation of 0.1 ppb using a C8 column (Lerch and Donald, 1994). Berg *et al.* (1995) later reported the simultaneous detection of atrazine, ATOH, and other major polar metabolites utilizing the optimization power of a diode-array detector with absorbance measured continuously in the range of 200–356 nm with routine quantitation at less than 0.02 ppb.

Through improvements in instrument and column technology, better efficiency and significantly lower solvent consumption can be achieved with the use of 1 and 2 mm and even smaller columns. Some instrumentation is compatible with 2 mm columns, which have optimum flows at 0.2 mL/min and a 5-fold increase in efficiency (Sanchez-Rascero and Dios, 1988; Andrievskii *et al.*, 1989).

The recent use of 1.7 μm size particles in ultra pressure liquid chromatography (UPLC) has considerably increased resolution and sensitivity. Analysis times of many minutes have been reduced to 1–2 min, which allows many more sample analyses to be completed per unit time. The number of applications is growing for a wide range of analytes using UPLC interfaced to MS and/or MS/MS due to the concurrent chromatographic and spectrometric increases in sensitivity. The continued addition of columns with new and varying functionality will serve to increase the number of applications further.

UV Detectors

UV absorbance with a fixed wavelength detector (254 and 280 nm) was the standard detector for HPLC for many years (Parker *et al.*, 1983). This detector offered high sensitivity, but not universal detection. A significant improvement was the grating, variable wavelength detector, which offers the ability to 'tune' detection based on spectral characteristics of the compound of interest (Qiao *et al.*, 1991). Later units offer improved sensitivities and expanded the usefulness of UV as a detector. The diode-array detector is the most flexible, although slightly less sensitive. It readily offers optimization of detection wavelength and bandwidth for maximum detectability (Schussler, 1989) and can offer collection of the complete UV spectrum for identification purposes (Slobodnik *et al.*, 1992; Aguilar *et al.*, 1996; Barceló *et al.*, 1996).

Mass Spectrometry, Mass Spectrometry/Mass Spectrometry, and Multiple Stages of Mass Spectrometry

Just a few years ago, it seemed unlikely that the combined capabilities of HPLC and MS could be comparable to GC/MS. Many attempts met with varying degrees of success. A few of these techniques, including the moving belt and direct liquid introduction, are of historical interest. Improvements in HPLC/MS technology with the development of particle beam, flow fast atom bombardment, and thermospray (e.g., Voyksner *et al.*, 1987; Barceló *et al.*, 1993) represented a significant advancement. Initial challenges included poor sensitivity, lack of user friendliness, and a variety of other problems. For example, quantitation by particle beam was problematic due to the matrix or carrier effect (increases in signal when coeluting components are present). Marce *et al.* (1995) determined that the matrix effect and linearity could be improved for particle beam experiments by using the standard addition technique. Particle beam is unique in that it is the only modern technique that can provide HPLC/electron ionization spectra for qualitative and quantitative applications. Thermospray exhibits excellent pseudomolecular ion generation in the form of $(MffIH)^{ffl}$ and $(M^{J}\ H)^{J}$, or for positive and negative ion application (Mffl mobile phase or buffer) (Fischer and Michael, 1995; Barceló *et al.*, 1996; Papilloud *et al.*, 1996). For qualitative use, background noise with thermospray limits the detection of the peak of interest, requiring the use of another detector (e.g., UV) or the use of extracted ion current

profile searching. Thermospray was a useful qualitative and quantitative technique for triazines but has been replaced by atmospheric pressure ionization (Pozzebon *et al.*, 2003; Borba da Cunha *et al.*, 2004). The atmospheric pressure ionization interface, which includes both ESI and atmospheric pressure chemical ionization (APCI), represented a quantum leap in ease of use and sensitivity for polar and ionic molecules with sensitivity approaching and sometimes exceeding that possible with GC/MS. Barceló *et al.* (1995) reported for a variety of triazines that thermospray allowed a detection limit of 1–10 ng, whereas ESI permitted 10–100 pg. Cai *et al.* (1996) reached the ppt level using microbore ESI. Adduct formation is also possible with atmospheric pressure ionization, but with proper optimization the $(M+H)^+$ and the $(M-H)^-$ are typically the base peaks. As with other soft ionization techniques, MS/MS is required to obtain fragmentation data both for qualitative determinations and for quantitative confirmation to overcome the lack of information in the spectra obtained by soft ionization techniques like thermospray, ESI, or APCI (Abian and Barceló, 1993; Banoub *et al.*, 1995) (Borba da Cunha *et al.*, 2004). Although ESI is often the mode of choice, APCI may be equally useful for triazines, and some researchers have found it easier to optimize and reproduce. For example, the on-line coupling of turbulent flow SPE columns with APcI-LC/MS/MS (Asperger, 2002; Koal, 2003) resulted in fast and low detection level (0.050 ppb) analyses of some triazines, in addition to analytes from several other classes of compounds.

Advances in source/interface designs have greatly increased ion formation and transfer into the MS. When coupled with higher ion transmission efficiencies due to improved pre-analyzer focusing optics and more sensitive electron- and/or photo-multipliers, has resulted in significant gains in sensitivity for LC/MS/MS instrumentation. This increase in sensitivity and the logical next step of injecting portions of water samples directly into a reverse-phase LC (highly aqueous mobile phase) has led to DAI analysis. Several applications of DAI LC-ESI/MS/MS have been reported for the analysis of various pesticides in water (Carpenter, 1997; Ingelse, 2001; Pozo, 2001; Fuhrman, 2003; Yu, 2003), demonstrating that analyses can be performed without a sample concentration step. For example, atrazine, simazine, and their respective chlorotriazine metabolites can be easily and reliably quantified at the 0.10 ppb concentration level in water (Huang, 2006) using DAI. This method was further refined to include several thiomethyl-triazine compounds, their metabolites, and other compounds and is more cost effective than immunoassay procedures due to the collection of multi-analyte data with no pre-injection sample preparation. Plus, the analysis is confirmatory and does not require a second, confirmatory analysis of samples with positive detections. Interestingly, the percent relative standard deviation (%RSD) calculated for data obtained from recovery experiments when using DAI tends to be much smaller than the %RSD calculated from data obtained when sample preparation is a requirement of the analysis. The absence of sample preparation removes the major factors responsible for analyte loss during the analysis.

The quadrupole/time-of-flight analyzer (QToF) has become a key option in the qualitative and quantitative analytical arena. Instruments with resolving power of 20 000 (50% valley definition) can provide $\pm$ 5 ppm mass accuracy for parent and product ion identification and for 20 mda mass selection windows quantitation. While the triple quadrupole retains the lead in sensitivity for quantification, the QToF has a decided edge on specificity (Micromass, 1999) and qualitative analysis.

Thin-Layer Chromatography

Classic thin-layer chromatography is an extremely useful, inexpensive technique for low-resolution separations, often with simple detection systems such as chlorine gas exposure followed by starch, potassium iodide (KI) spray (Madejski *et al.*, 1984). Developments including high-performance thin-layer chromatography and scanning densitometers have resulted in notable improvements in efficiency, reproducibility, and limits of detection. Multiple pesticides in soil and water samples have been analyzed by high-performance thin-layer chromatography using sample automation and a densitometer scanning several different wavelengths. Recoveries were acceptable at the low μg/L or ng/g level (Vigne *et al.*, 1991).

Capillary Electrophoresis

CE provides an orthogonal mode of separation to other forms of chromatography and is capable of handling not only charged species, but neutral species through micellar electrokinetic chromatography (MEKC) (Komarova and Kartsova, 2002). The use of CE with UV detection for pesticides has been demonstrated to provide high efficiency, speed, and low solvent consumption (Penmetsa *et al.*, 1996). Susse and Muller (1995) reported detection limits of 0.1 ppm using a fast scanning UV detector; this high detection limit minimizes the use of this technique for trace levels. Electrokinetic chromatography, coupled with ESI mass spectrometry, provided a powerful qualitative tool but was problematic due to the introduction of surfactant into the mass spectrometer. Yan *et al.* (1997) solved this problem with the use of an anodically migrating micelle moving away from the electrospray interface, whereas Nelson *et al.* (1996) used partial filling MEKC. With the latter technique, a small portion of the capillary is filled with surfactant

to achieve separation. The triazine analytes first migrate into the micellar plug where the separation occurs, and then into the electrophoresis buffer, which is free of surfactant before entering the mass spectrometer. After SPE sample preparation, micellar electrokinetic capillary chromatography (MEKC) and nonaqueous capillary zone electrophoresis (NA-CZE) were compared by analyzing various parent chloro- and methylthio-triazines at the 0.1 and 0.5 μg/L concentration levels in water (Carabias-Martinez *et al.*, 2002). The baselines obtained using NA-CZE were more stable and less susceptible to interferences than those obtained using MEKC. Good recoveries were obtained for all analytes using NA-CZE, but five of the six analytes have RSDs ffi20% for river water at the 0.10 ppb concentration. Good resolution, high efficiency, and fast analysis time were obtained during the analysis of 10 parent triazines and one metabolite at the 0.05 μg/L concentration after being subjected to SPE and MEKC (Frias *et al.*, 2004). Recoveries were acceptable for all analytes except DEA (52%). Detection limits, a weakness of CE due to injection volume limitations and detector cell size, have been significantly improved with various stacking techniques. Da Silva *et al.* (2003) realized detection limits of 2–46 ppb for four parent triazines and five other compounds in complex sample matrices such as carrots, utilizing a modified sweeping and stacking technique where a momentary positive voltage is applied to the inlet vial. Applications of stacking combined with off-line SPE have resulted in detection limits of 0.10 ppb in water.

Hyphenated Chromatographic Techniques

Hyphenated chromatographic techniques of various types (e.g., HRGC/HRGC and HPLC/HRGC) are being used in conjunction with a variety of detectors to improve detectability, increase specificity, and reduce cleanup costs for routine analysis. Vuruls *et al.* (1992) reported ng/L quantitation for aqueous atrazine samples by LC/GC/MS.

Comprehensive GC/GC using a thermal modulator and flame ionization detection allows for separation of 15 pesticides with a wide range in polarity in human serum – in less than 4 min. Limits of detection are in the low picogram range. A greater number of theoretical plates can be generated by a two-dimensional separation process as compared to a one-dimensional approach, with improved specificity resulting from simultaneous generation of two retention time sets (Liu *et al.*, 1994).

Immunoassay

Immunoassay technology evolved from Landsteiner's observation (1945) that antibodies were capable of discriminating between benzene rings derivatized at different positions. Yalow and Berson (1960) developed the first modern immunoassay as a means to monitor insulin in diabetics. This work set off such a flurry of activity in the development of assays for clinically important compounds that Yalow was awarded the 1967 Nobel Prize in Physiology and Medicine. The potential for measuring pesticides by similar means was first described by Ercegovich (1971). Early efforts to apply immunochemical technology in pesticide residue chemistry faced many hurdles, including adoption of the techniques used to develop antibodies and using them to achieve sensitive assays. Hammock and Mumma (1980) presented the first thorough review of these methods. Their work made an historic and seminal contribution to the literature of pesticide residue analysis by immunological methods.

Background

The application of immunoassays to environmental analyses has been the subject of numerous reviews (Hammock and Mumma, 1980; Newsome, 1986; Hammock *et al.*, 1987; Vanderlann *et al.*, 1988; Jung *et al.*, 1989; Hall *et al.*, 1990; Kaufman and Clower, 1991; Aston *et al.*, 1992; Nugent, 1992; Sherry, 1992; Van Emon and Lopez-Avila, 1992; Gee *et al.*, 1994; Hock and Niessner, 1995; Marco and Hammock, 1995; Meulenberg *et al.*, 1995; Pfeifer-Fukumura *et al.*, 1999).

The foundation of any immunoassay is an antibody. Most immunoassays use immunoglobulin G antibodies, the predominant serum antibody in man (Rose *et al.*, 1979). Individual immunoglobulin G antibodies possess two sites capable of binding to molecular configurations complementary in size, shape, and electrical charge. Antibodies serve *in vivo* as defensive proteins generated in response to molecular configurations perceived as foreign by a host organism. Antibodies generally are produced in response to substances having a molecular weight of at least 10 000. Smaller compounds, such as triazines, may be rendered immunogenic by conjugation to a large, antigenic carrier protein such as bovine serum albumin or keyhole limpet hemocyanin. Compounds that are too small to initiate antibody production, but can bind to antibodies produced in response to an immunogenic conjugate, are called haptens. Inoculation of an appropriate host with a carrier protein-hapten conjugate will result in the generation of antibodies with many specificities, some of which are reactive to the haptenic moiety. Antibodies produced from animal hosts – such as rabbits, mice, or sheep – are referred to as polyclonal because multiple cell lines produce antisera characterized by a range of specificity. Alternatively, antibodies produced in cell culture generate monospecific or monoclonal antibodies (Kohler and Milstein,

1975; Goding, 1983). Subsequent work extended production of whole antibodies or functional antibody fragments to bacterial and plant systems (Bryne *et al.*, 1996; Longstaff *et al.*, 1998; Strachan *et al.*, 1998; Grant *et al.*, 1999).

The specificity of an antibody to a small molecule can be controlled by the manner in which the immunogenic conjugate is designed. The orientation of the molecule relative to the carrier protein is critical. When an antibody specific to a given compound is desired, the portion of the molecule possessing the most distinctive structural features should be directed away from the protein's surface. Such a conjugate will produce antibodies reactive to those features and exhibit selective binding to molecules possessing them. If a structure common to a class of compounds is oriented in this manner, antibodies capable of recognizing many chemicals sharing this structure will be produced.

It is precisely the latter situation that is encountered by immunochemists working with triazine herbicides. Due to the large variety of compounds based on the common triazine theme (Table 20.1), it has generally not been possible to obtain antibodies specific to a given triazine, even with monoclonal technology. Of 42 triazine antibodies examined, only cyanazine (Bruun *et al.*, 2001) and terbuthylazine antibodies (Giersch *et al.*, 1993a) appeared to be specific to a single analyte (Table 20.2). Most triazine antibodies strongly recognize at least two compounds. However, broadly reactive antibodies also have utility. Multi-residue immunoassays that detect a variety of triazines can be useful screening tools. In any case, analysts should be familiar with the reactivity of the antibodies in use because interpretation of immunoassay data is dependent, in part, on the selective or nonselective nature of the antibodies (Baker *et al.*, 1993; Brady, 1995).

Types of Immunoassays

Immunoassays use antibody binding of specific molecular configurations and typically involve several steps. The antibody, a sample containing an analyte, and an analogue of the analyte of interest are combined and permitted to interact. Binding sites on the antibodies are occupied by the analyte or its analogue during this step; this incubation is often referred to as the inhibition step. After a predetermined time, this interaction is stopped by removal of test components not bound to the antibody. The amount of binding specific to the analyte of interest and, hence, the amount of analyte in the sample are determined by the difference in the binding of the analyte compared to the analogue. When a high concentration of analyte is present in a sample, little of the analogue can bind and a low signal will be produced. Conversely, if a sample has little or no analyte, higher amounts of the analogue will be bound and a high signal will be generated. Thus, the test signal is inversely proportional to the amount of analyte in the sample (Figure 20.1).

There are two primary types of immunoassays in use: enzyme immunoassay (EIA) (Maggio, 1980) and ELISA (Voller *et al.*, 1978). As their names indicate, both of these assays utilize enzymes as signal generators. The assays can be distinguished by how the enzyme tracer is utilized. If the enzyme is covalently coupled to an antibody, the test is an ELISA; if the enzyme is conjugated to an analogue of the compound being measured, the assay is an EIA. Enzymes such as horseradish peroxidase, or less frequently, alkaline phosphatase, are used to generate colorimetric signals that can be quantified by visible wavelength spectrophotometers. Dedicated instruments are available to accommodate polystyrene microtiter plates or culture tubes, the assay formats of choice. EIAs (Figure 20.2) are the simpler of the two assays to run. They involve a minimal number of steps and have relatively brief analysis times. This is especially true of some commercial products that complete analyses in less than 45 min. ELISAs (Figure 20.3), on the other hand, are more complex, have longer analysis times (often 5–6 h), and more liquid handling steps. Most commercial assays utilize some adaptation of the EIA methodology.

Water Analyses with Immunoassays

The first application of immunoassay methodology for residue chemistry was in the analysis of water. Much of this effort was devoted to the analysis of *s*-triazine herbicides, primarily atrazine. Many researchers studied the feasibility of immunoassays and restricted their analyses to field samples fortified in the laboratory (Bushway *et al.*, 1988; Wittmann and Hock, 1989; Rubio *et al.*, 1991; Giersch *et al.*, 1993b; Lawruk *et al.*, 1993; Dinelli *et al.*, 1995; Rodolico *et al.*, 1997) or to reagent water fortified in the laboratory (Lentza-Rizos, 1996).

Routine application of immunoassay to real-world samples, however, required demonstrating that the methodology could achieve results with untreated field samples that were similar to results obtained by accepted analytical techniques, such as GC or HPLC. Consequently, a series of investigations compared immunoassay to chromatographic methods for atrazine in water. Schlaeppi *et al.* (1989) analyzed 28 samples by immunoassay and HPLC. Thurman *et al.* (1990) analyzed ground and surface waters from the central United States (US) by immunoassay and GC with mass spectrometric detection (GC/MS). Fleeker and Cook (1991) used immunoassay and GC with thermionic specific detection to analyze samples from Minnesota, Iowa, and Nebraska. Bushway *et al.* (1991) used immunoassay and HPLC to analyze water samples collected in an agricultural region in the Czech Republic. Wüst and Hock (1992) analyzed water from the Rhine River using immunoassay and GC/MS. Bushway *et al.* (1992a) concluded immunoassay was a cost-effective

Table 20.2 Specificities of selected triazine antibodies

Primary analyte	Secondary analyte(s)[a]	Polyclonal (P) or monoclonal (M)	Reference
Atrazine	Propazine, Simazine, Ametryn, Prometryn	P	Huber (1985)
Atrazine	Propazine, Prometryn, Ametryn, Prometon, Atraton, Simazine	M	Bushway *et al.* (1988)
Atrazine	Propazine	M	Schlaeppi *et al.* (1989)
Atrazine	Propazine	P	Wittmann and Hock (1989)
Atrazine	Propazine	P	Dunbar *et al.* (1990)
Atrazine	Propazine, Cyanazine, Simazine, Prometryn, Terbuthylazine, Terbutryn	M	Karu *et al.* (1991)
Atrazine	Propazine, Cyanazine, Simazine, Terbuthylazine	M	Karu *et al.* (1991)
Atrazine	Ametryn, Propazine, Prometryn, Prometon	P	Rubio *et al.* (1991)
Atrazine	Propazine, Terbuthylazine, Cyanazine	M	Giersch (1993)
Atrazine[b]	Propazine, Cyanazine, Simazine	M	Muldoon *et al.* (1993)
Atrazine	Propazine	M	Muldoon *et al.* (1993)
Atrazine	Simazine, Propazine	M	Muldoon *et al.* (1993)
Atrazine	Propazine, Deethylatrazine	M	Franck *et al.* (1995)
Atrazine	–[c]	P	Dzantiev *et al.* (1996)
Atrazine	Propazine	P	Gascón *et al.* (1997)
Atrazine[b]	Ametryne, Cyanazine, Simetryne	M	Choi *et al.* (1999)
Aziprotryn	Atrazine, Propazine, Ametryn, Prometryn	M	Giersch and Hock (1990)
Cyanazine	Terbuthylazine, Terbutryn	P	Lawruk *et al.* (1993)
Cyanazine	—[d]	M	Bruun *et al.* (2001)
Deethylatrazine	Deisopropylatrazine	P	Wittmann and Hock (1991)
Deisopropylatrazine	Deethylatrazine	P	Lucas *et al.* (1995)
Didealkylatrazine	Deethylatrazine	P	Del Valle *et al.* (1996)
Hydroxyatrazine	Hydroxypropazine	M	Schlaeppi *et al.* (1989)
Hydroxyatrazine	Hydroxysimazine, Hydroxypropazine, Hydroxydesmetryn	M	Schlaeppi *et al.* (1989)
Hydroxyatrazine	–[e]	M	Mangler *et al.* (1994)
Hydroxypropazine	Hydroxyatrazine, Hydroxysimazine	P	Kido *et al.* (1997)
Hydroxysimazine	Hydroxyatrazine	P	Lucas *et al.* (1993a)
Prometryn	Atrazine, Ametryn, Terbutryn	M	Giersch and Hock (1990)
Prometryn	Ametryn, Prometon, Atrazine, Propazine	P	McConnell *et al.* (1994)
Propazine	Atrazine, Terbuthylazine	M	Mangler *et al.* (1994)
Propazine	Terbuthylazine, Atrazine	M	Winklmair *et al.* (1997)
Simazine	Atrazine, Ametryn, Terbutryn	P	Franck *et al.* (1995)
Simazine	Atrazine	P	Wortberg *et al.* (1996)
Simazine	—[c]	P	Dzantiev *et al.* (1996)
Simazine	Atrazine, Ametryn, Propazine, Terbutylazine, Deisopropylatrazine	P	Lawruk *et al.* (1996)
Terbuthylazine	–[d]	M	Giersch *et al.* (1993a)
Terbuthylazine	Propazine, Atrazine, Simazine	M	Winklmair *et al.* (1997)
Terbutryn	Ametryn, Atrazine, Terbuthylazine, Hydroxyterbutryn	P	Huber and Hock (1985)
Terbutryn	Atrazine, Propazine, Terbuthylazine, Prometryn	M	Giersch and Hock (1990)
Terbutryn	Terbuthylazine, Prometryn, Propazine	M	Giersch *et al.* (1993a)

[a] Compounds listed have been determined to be at least 20% cross-reactive. Refer to the reference noted for a more detailed evaluation.
[b] Two antibodies with similar reactivities were produced.
[c] Not analyzed.
[d] Antibody is specific to target analyte.
[e] Cross reactivity evaluation did not include other hydroxytriazines.

screening method compared to HPLC for analysis of Maine well-water samples. Goolsby *et al.* (1991) and Thurman *et al.* (1992) analyzed 127 surface-water samples from corn and soybean production areas in the midwestern United States using immunoassay and GC/MS. Brady *et al.*, (1995) analyzed 2 177 rural well-water samples from Wisconsin farming areas in government and industrial laboratories. Franck *et al.* (1995) obtained a favorable comparison between immunoassay and HPLC data for the analysis of 31 groundwater and surface-water samples. Gruessner *et al.* (1995) analyzed 224 samples from Vermont streams by immunoassay and GC/MS. Tasli *et al.* (1996) used immunoassay and GC/NPD to analyze soil water from a corn plot under irrigation. Del Valle *et al.* (1996) analyzed groundwater for DDA by immunoassay and HPLC and results of procedural recoveries between the methods correlated well. Hottenstein *et al.* (1996)

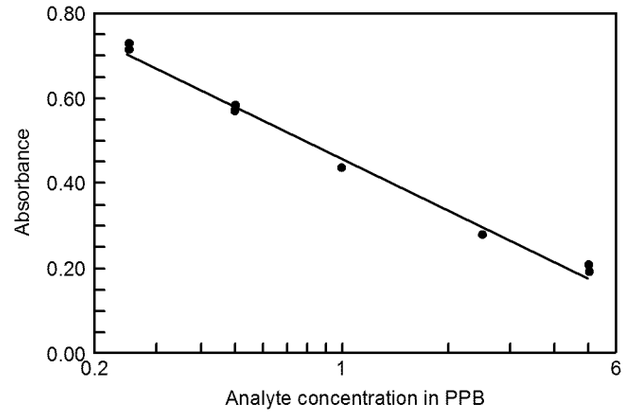


Figure 20.1 Typical immunoassay standard curve.

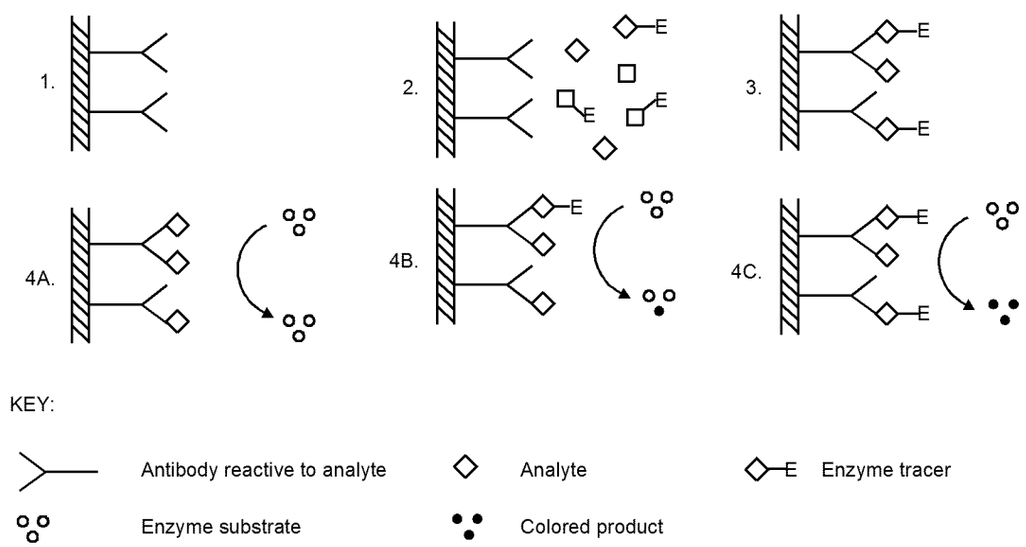


Figure 20.2 Schematic of enzyme immunoassay.

compared a magnetic particle-based immunoassay for atrazine with GC/MS. LaFrance *et al.* (1996) compared ELISA and GC procedures for determining atrazine in runoff water. Lydy *et al.* (1996) analyzed 149 surface water samples by EIA and GC/MS. They attributed a slight positive bias of EIA results to cross-reactivity, matrix interference, or underestimation of residues by GC. Newman *et al.* (1996) used an EIA to assess the stability of atrazine in water in a pesticide degradation study. Pomes *et al.* (1996) analyzed 1 725 storm runoff samples by EIA and confirmed 363 of these results by GC/MS. Dankwardt *et al.* (1997) used EIAs for atrazine and terbuthylazine to screen rainwater and surface-water samples from southern Germany. Dombrowski *et al.* (1997) used an EIA to screen for atrazine in groundwater at a military site near Denver, Colorado. Due to the broad cross-reactivity of the triazine kit used, they also detected cyanazine, which was not previously found at the site. Watts *et al.* (1997) analyzed stream water from South Carolina for metribuzin by EIA and GC/MS. Goolsby *et al.* (1997) analyzed 6 230 rainfall samples collected from 81 sampling sites in 23 states for atrazine by EIA and GC/MS. Pomes *et al.* (1998) analyzed 2 072 rainfall samples collected from the same sampling locations using EIA and GC/MS methods. Finally, Schneider *et al.* (1998) used a monoclonal antibody developed by Giersch (1993) to screen surface water samples for terbuthylazine.

Researchers have also used immunoassay techniques for analysis of triazines in soil leachate. Tasli *et al.* (1996), for example, analyzed soil water from an irrigated corn plot by immunoassay and GC/NPD. Amistadi *et al.* (1997) analyzed leachate collected from different tillage systems for atrazine by EIA and GC/NPD. Guillard *et al.* (1999) used an EIA to compare the concentration of atrazine in leachate from plots receiving band or broadcast applications.

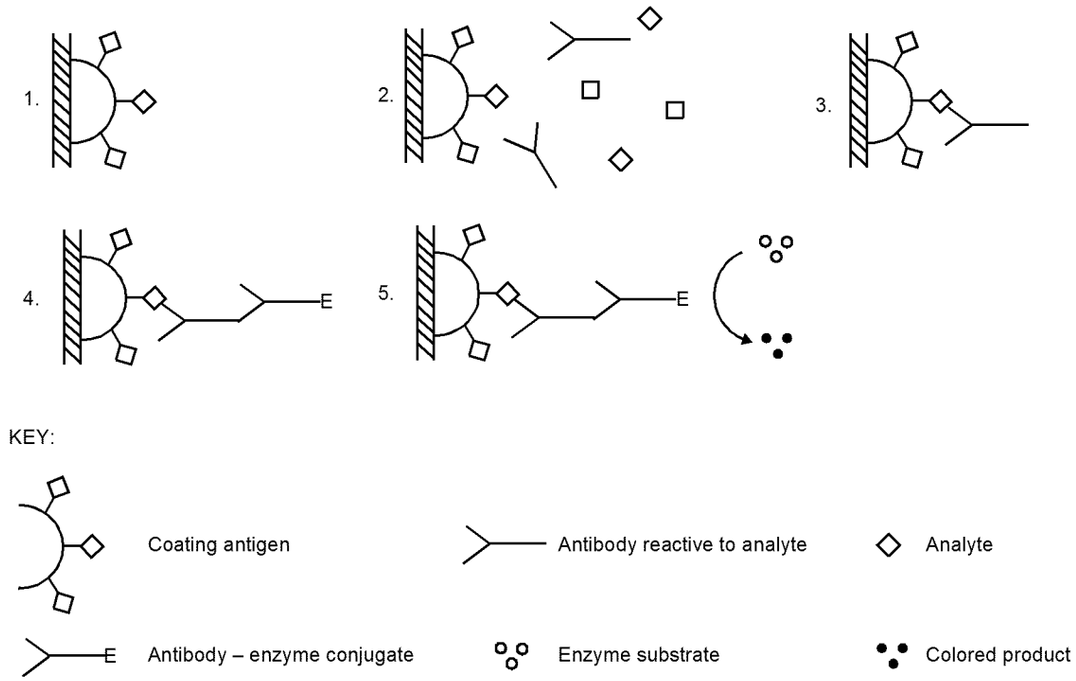


Figure 20.3 Schematic of enzyme-linked immunosorbent assay.

Several observations were common to investigators comparing immunoassays with other methods of analysis. The slope of the best fit line comparing results between methods was often slightly biased toward immunoassay results, reflecting the tendency of immunoassay to overestimate analyte concentrations. This tendency, in turn, produced a small percentage of false positive results, but only one false negative. Consequently, immunoassay was found to be an inexpensive, reliable screening method. Moreover, the technique is sufficiently sensitive to measure environmentally significant concentrations of analyte without sample extraction or pretreatment.

Some researchers have combined immunoassays for water analysis with conventional sample preparation techniques. Lucas *et al.* (1991) prepared 75 well-water samples by SPE and found good agreement with GC/thermionic specific detection results. Aga and Thurman (1993) concentrated samples 100-fold by SPE to obtain picogram sensitivity, while removing potentially interfering inorganic and organic substances. Gascón *et al.* (1995a, b) extracted brackish water samples by LLE with methylene chloride, achieving a 10- to 20-fold concentration of atrazine residues. Gascón and Barceló (1994) treated lyophilized samples in a similar manner after reconstitution to their original volumes.

Soil Analyses with Immunoassays

Although the utility of immunoassay was demonstrated for water analyses, few investigators have adapted immunoassay to the analysis of triazine residues in soil. Schlaeppi *et al.* (1989) extracted soils fortified with atrazine and ATOH by soxhlet extraction for 4 h. They found similar recoveries by ELISA and GC or HPLC for atrazine and the hydroxy metabolite. Leavitt *et al.* (1991) analyzed soils from 30 Michigan sites for atrazine using GC/NPD and immunoassay. The GC samples were exhaustively extracted by soxhlet for 23 h, in contrast to immunoassay samples that were extracted by a 15-min shake in 90% acetonitrile/water. The researchers found a reasonable correlation between results of each method, but noted a 20% decrease in the amount of weathered residues measured by immunoassay. These losses were attributed to the different extraction procedures. Goh *et al.* (1992a) compared immunoassay data to GC results obtained after two different soil extraction techniques. GC data gathered subsequent to a silica SPE cleanup of the original organic extract produced the best correlation. Del Valle and Nelson (1994) compared a variety of extraction and cleanup techniques for atrazine in soil using GC and immunoassay as the determinative steps. These scientists concluded that a 1-h shake in methanol/water followed by a C18 SPE cleanup yielded the best results. Del Valle *et al.* (1996) assayed a methanolic extract by immunoassay and HPLC for DDA. They observed an overestimation of residues of 12% when using immunoassay, as compared to the results obtained using HPLC. Schewes *et al.* (1994)

evaluated an atrazine immunoassay for determination of weathered residues in soil. Samples were extracted with acetone in a hot extractor. They found the EIA produced results that were in good agreement with an HPLC analysis of acetone extracts cleaned up by SCX SPE.

Most immunochemists have adopted a simplified approach for dealing with soil analyses. Soils are usually extracted by shaking in an organic or organic/aqueous solvent system at ambient temperature (Bushway *et al.*, 1988; Goh *et al.*, 1990, 1991, 1992a, b; Lucas *et al.*, 1991, 1993a, 1995; Stearman and Adams, 1992; Lawruk *et al.*, 1993; Stearman and Wells, 1993; Tasli *et al.*, 1996; Deng *et al.*, 1999; Wittmann and Schreiter, 1999). Sample cleanup consists of filtering the extract and diluting it into water to reduce the percentage of organic solvent. The dilute extract is analyzed in the same fashion as water samples. Since no effort is devoted to removing potential interfering substances interferences can arise due to the organic solvent added to the antibody-coated surface (Schlaeppi *et al.*, 1989; Stearman and Wells, 1993; Lucas *et al.*, 1993a) or to coextractives that nonspecifically effect antibody or enzyme marker performance (Goh *et al.*, 1990).

Previous studies indicated that triazines in soils require rigorous extraction conditions and a need for water in the extraction solvent. Mattson *et al.* (1970) found that minimally a 1-h reflux was required for extraction of weathered atrazine residues. Huang and Pignatello (1990) concluded that shaking samples for 2–4 h in hot (95°C) solvent was required to achieve extraction of atrazine residues, whereas shaking at room temperature yielded about 40% fewer residues. Increasing unheated shaking time to 24 h realized only minor improvement.

Food Analyses with Immunoassays

Application of immunoassay techniques to the analysis of triazine residues in foodstuffs must rely on freshly fortified samples because parent triazines are rarely found in food commodities. Bushway *et al.* (1989) analyzed atrazine-fortified liquid and solid foods by immunoassay and HPLC. Results between the methods could be successfully correlated when the immunoassay analytical standards were dissolved in control extract to compensate for commodity-specific interferences. This work was continued (Bushway *et al.*, 1992b; Ferguson *et al.*, 1993) using atrazine standards made up in seven milk matrices spanning a range of fat content. Milk products containing higher amounts of fat were found to be unsuitable for use in the standards, perhaps due to lipid sequestration of the analyte. Wittmann and Hock (1993a, b), on the other hand, used atrazine standards dissolved in distilled water or in milk (1.5% fat) and in fruit and vegetable juices. Extensive cleanup steps for extracts of atrazine-fortified milk, juices, and canned corn prior to immunoanalysis were ineffective because they recovered less than 60% of the amounts added for testing. Wigfield and Grant (1993) achieved mixed results analyzing fortified cornmeal. When immunoassay standards were dissolved into extract derived from triazine-free cornmeal, nearly quantitative recoveries were obtained for atrazine, while only 55% of the simazine applied was recovered. Two groups combined immunoassay with SFE. Nam and King (1994) found coextractives from beef tissues produced high background signals. Interferences, thought to be fat globules, were successfully removed by filtration. Lopez-Avila *et al.* (1996) amended five prepared baby foods with atrazine and cyanazine. Samples were mixed with diatomaceous earth to disperse the matrix and increase surface area prior to SFE. Overall, recoveries were higher for atrazine than for cyanazine, which averaged 59% recovered. Low recoveries were attributed to irreversible binding to the dispersal agent. Coupling pesticide residue techniques with immunoassay as the determinative step may be the key to success.

Pesticide Applicators

Reed *et al.* (1990) examined pesticide applicator exposure to atrazine during mixing–loading, boom application, and spray gun operation. Analysis of atrazine on patches of protective clothing taken from the chest, thighs, and forearms indicated that personnel were exposed to very low concentrations of atrazine. Lucas *et al.* (1993b) used an immunoassay for atrazine mercapturate in urine based on a monoclonal antibody developed by Karu *et al.* (1991). Samples collected from field workers were prepared for immunoanalysis by dilution into buffer. The researchers also used a polyclonal antibody to develop an immunoaffinity isolation technique for selective extraction of urinary metabolites. Lucas *et al.* (1995) evaluated the potential for screening urine for traces of DEA and DIA. Matrix effects required fortified samples to be diluted at least 10-fold into buffer before assay. Brady *et al.* (1998) adapted a commercially available triazine immunoassay to the detection of atrazine mercapturate in urine of pesticide applicators and mixer–loaders. Samples were extracted with an organic solvent system. The organic extract underwent separation by SPE and the eluate was transferred into buffer for analysis.

Other Applications

Muldoon and coworkers applied immunoassay to the analysis of *s*-triazines in pesticide waste and rinsate. They demonstrated accurate estimation of atrazine, simazine, and cyanazine as total *s*-triazines (Muldoon *et al.*, 1993). This was

accomplished using a group of antibodies, each possessing a different reactivity toward the analytes in the complex mixture. This work was extended to the triazine degradate, DDA (Muldoon *et al.*, 1994). Two assays were developed for the metabolite in the micromolar range. Pesticide waste and rinsate samples subjected to degradation by ozonolysis were fortified with DDA and analyzed by immunoassay and HPLC. Researchers also investigated the effect on immunoassay performance of agricultural materials that may be present in pesticide waste and rinsate samples (Muldoon and Nelson, 1994). A variety of formulating agents, surfactants, inorganic fertilizers, and nutrients were assessed for their effect on the immunoassay measurement of atrazine, simazine, and cyanazine. At high concentrations, these materials were generally found to suppress assay response. An SPE cleanup incorporated into the method easily separated the nonpolar analytes from polar interferences. An overview of the work is included in Muldoon and Nelson (1995).

Innovations in Assay Operation and Detection Systems

Several investigators developed flow injection systems in efforts to automate immunoassays. Kramer and Schmid (1991a, b) built a system around a membrane coated with atrazine antibodies. This structure supported an immunoassay analogous to colorimetric EIAs, except that flourescence detection was used. Sensitivities of 0.02–0.03 ppb were achieved. One sample took approximately 15 min to pass through the system. Wittmann and Schmid (1994) constructed a system using antibody-coated beads packed into a small column. Similar cycle times were noted, but sensitivity was increased to about 0.001 ppb. Alternatively, Wortberg *et al.* (1994) based their system on a column containing beads coated with atrazine haptens. These beads were equilibrated with atrazine antibodies coupled to a europium label. Free atrazine displaced the labeled antibodies, which were monitored by time-resolved flourescence detection. This system could detect 1.0 ppb of atrazine. Approximately 1 h was required to analyze a sample by this process. Kramer *et al.* (1997) used a column of protein A on polymethacrylate beads. Atrazine antibodies, samples, enzyme tracer, and fluorometric enzyme substrate were sequentially injected onto the column. Time of analysis was 50 min per sample. Researchers attained a detection limit of 0.02 ppb with this system. Bjamason *et al.* (1997) filled a 10 µL Plexiglas®[1] column with rabbit atrazine antibodies coupled to epoxy-activated Poros beads. The sample and a horseradish peroxidase tracer were injected simultaneously. Bound tracer was detected spectrophotometrically at 405 nm. The column could be reused for up to 120 cycles. This system detected 0.5 ppb of atrazine with an analysis time of 15 min. Gascón *et al.* (1997) developed a similar system using an Affi-Gel Hz column as the solid support. Bound enzyme was determined spectrofluorometrically. A complete cycle took approximately 20 min. A limit of detection of 0.075 ppb of atrazine was achieved. González-Martínez *et al.* (1998) examined three solid supports for antibody immobilization. They concluded recombinant protein A/G produced more reproducible results and greater sensitivity than controlled-pore glass beads or Affi-Gel Hz. This column was applied to the development of a fluorometric flow-through immunoassay (González-Martínez *et al.*, 1999). Analysis of water samples took approximately 23 min. Detection limits of about 0.01 ppb were reached. The column proved to be extremely rugged and could be reused more than 400 times. Lopez *et al.* (1999) utilized hapten-coated beads. Samples preincubated with atrazine antibodies were passed through the column. Antibodies bound to the column were in turn bound by an anti-rabbit antibody-horseradish peroxidase conjugate. This system had a detection limit of 0.15 ppb of atrazine.

Yazynina *et al.* (1999) introduced novel reagents into the inhibition step with the goal of decreasing the long analysis times typically required for ELISA measurements. A polyanion-protein A conjugate was introduced into the atrazine antibody-enzyme conjugate solution. Antibody bound by protein A was in turn bound to the walls of a microplate coated with a polycation. This innovation reduced overall time of analysis to 40 min and achieved a detection limit of 0.03 ppb of atrazine.

Research on replacing colorimetric determination with other detection systems was conducted by Hardcastle *et al.* (1988) using a luminescent substrate for peroxidase to increase the sensitivity of a tube assay for atrazine and simazine down to 0.01 ppb. Their substrate solution consisted of isoluminol, peroxide, and *p*-iodophenol. Ulrich and Niessner (1992) compared chromogenic and flourescence detection for use in an immunoassay for terbuthylazine. Similar results were obtained substituting the fluorescent substrate, 4-methylumbelliferyl phosphate, for the frequently used *p*-nitrophenyl phosphate. Both systems yielded detection limits of around 0.06 ppb. Wortberg and Cammann (1993) developed several time-resolved fluorescence immunoassays based on europium (III)-chelate labels for atrazine and terbutryn. They found using biotin-antibody and europium-streptavidin conjugates increased the number of labels bound, resulting in detection limits of 0.05 and 0.10 ppb of terbutryn and atrazine, respectively. Eremin (1995) developed a liquid-phase assay that quantitated simazine to 5 ppb by monitoring changes in fluorescence polarization. The signal from a fluorophore-labeled hapten increased as more of the label was bound by the antibody. No washing step

[1] Plexiglass is a registered trademark of ATOFINA Chemicals, Inc.

was needed to remove unbound substances. The author postulated that enhancement might arise from energy transfer to the bound label from aromatic amino acids in the antibodies' binding sites. Reimer *et al.* (1998) also used a europium label. A biotinylated hapten was incubated with an atrazine monoclonal antibody (Karu *et al.*, 1991) in the inhibition step. A europium-streptavidin conjugate permitted detection of 0.05 ppb of atrazine.

Research on adaptation of immunoassays to a dipstick format has simplified triazine analyses. Dipsticks consist of antibody-coated surfaces mounted on inert supports. The dipstick itself is inserted into sample solutions. After washing, the stick is transferred into substrate solution to generate the assay signal. Giersch (1993) coated nitrocelluose membranes with an atrazine monoclonal antibody. The test strips were dipped into test tubes containing samples and a horseradish peroxidase tracer. This test used a colorimetric substrate and detected 0.5 ppb of atrazine in approximately 20 min. Mosiello *et al.* (1998) developed strips for atrazine and terbuthylazine achieving detection limits of 0.10 and 1.2 ppb, respectively. Wittmann *et al.* (1996) evaluated nitrocelluouse, poly(vinylidene fluoride) and nylon membranes for antibody immobilization and selected the latter. They were able to detect 0.3 ppb of atrazine in 25 min measuring the colored reaction product with a portable reflectometer. Wittmann and Schreiter (1999) applied a terbuthylazine antibody (Giersch, 1993) to dipsticks utilizing colorimetric and luminescent substrates. The luminescent substrate enabled detection of 0.05 ppb compared to 3 ppb by the colorimetric substrate.

Biosensors require a biologically active surface that interacts with analyte, a transducer that converts the interactive event into an electrical signal, and electronics capable of receiving and reporting the signal. All of the sensors described below utilize antibodies and, hence, can be classified as immunosensors. Schmid *et al.* (1990) developed the first piezoelectric sensor for atrazine. A quartz crystal coated with an atrazine antibody underwent an increase in mass upon exposure to samples containing atrazine. The change in mass was accompanied by a decrease in resonant frequency. The concentration of atrazine was directly proportional to the change in frequency. This device could detect parts per million (ppm) amounts of atrazine. Guilbault *et al.* (1992) used a piezoelectric crystal coated with protein A and atrazine antibody. This system was capable of detecting 0.03 ppb of atrazine. Each crystal was used up to nine times, and crystals could be recycled by removal of the protein and recoating. Minunni and Mascini (1993) adapted a commercially available apparatus (BIAcore, Pharmacia Biosensors) that monitors changes in Surface Plasmon Resonance to the analysis of atrazine in water. Samples and a fixed amount of atrazine antibodies were introduced to a glass slide coated with gold and carboxymethylated dextran to which an atrazine ligand was covalently coupled. Antibodies bound to the ligand produced a change in the sensor's refractive index that was monitored by surface plasmon resonance. This sensor could measure 0.05 ppb of atrazine with an analysis time of 15 min per sample. Tom-Moy *et al.* (1995) developed a chemical sensor that operated in a similar manner, based on a surface transverse wave device. Atrazine hapten immobilized to the surface of the sensor competed for antibody introduced to the sensor with each sample. Antibodies bound to the fixed hapten brought about an increase in the signal. Each analysis was carried out in about 3 min. Less than 1 ppb of atrazine could be detected. Sandberg *et al.* (1992) developed a biosensor based on a polyacetylene film coated with atrazine antibodies, which bound a glucose oxidase enzyme marker. Addition of glucose and lactoperoxidase generated iodine ion, which interacted with the polyacetylene film. The glucose oxidase marker was exchanged for analyte and reduced the amount of iodine ion. The concentration of analyte was determined by monitoring changes in the conductivity of the film. Thus, the material that served as the physical support for the antibodies was also the source of the output signal. O'Daly *et al.* (1995) utilized peroxidase by coating the surface of an electrode sequentially with colliodial gold, a layer of protein, and atrazine antibody. An atrazine-horseradish peroxidase enzyme tracer bound to antibody generated a measurable current in the presence of peroxide. Atrazine in a sample displaced the enzyme and caused a proportional decrease in current. Brecht *et al.* (1995) developed an immunosensor for atrazine employing reflectometer interference spectroscopy. Antibodies preincubated with samples were exposed to glass surfaces derivatized with an atrazine hapten. Antibody bound to the surface altered the reflectance spectrum; changes in signal were monitored by a diode array simultaneous spectrometer. Each analysis took approximately 30 min to complete. This system could detect 0.25 ppb of atrazine. A similar system was developed by Schipper *et al.* (1997) using a Mach-Zehnder interferometer. The surface of a planar waveguide was derivatized with an atrazine-protein conjugate. Samples premixed with antibodies were introduced to the surface. Antibody binding to the surface altered the interference pattern of a split laser beam passed over the waveguide. This system had a time of analysis of less than 10 min per sample and a detection limit of 0.10 ppb of atrazine. Baumner and Schmid (1998) developed an amperometric sensor using atrazine and terbuthylazine antibodies adsorbed on polyvinyl chloride sheets. The sheets were added to solutions containing free analyte and hapten-derivatized liposomes that entrapped ascorbic acid. Liposomes not bound by antibody migrated along the strip until lysed by surfactant. Released ascorbic acid was detected electrochemically. This sensor could detect less than 1.0 ppb of atrazine and terbuthylazine with a time of analysis of 20–30 min. Skládal *et al.* (1999) developed an optical immunosensor for atrazine based on a commercially available resonant mirror affinity sensor (IAsys, Affinity Sensors, UK). The sensor monitored the kinetics of antibody binding to an atrazine-albumin conjugate. An analysis

took approximately 30 min to complete. The detection limit for atrazine in soil samples was found to be 1.0 ppb. Each sensor could be reused more than 100 times.

Immunoassay Validation Studies for Atrazine in Water

In early validation tests, Hock (1991) and an Immunoassay Study Group conducted two studies to evaluate the measurement of atrazine in water by immunoassay. The first study found that only a few of the 13 laboratories successfully completed the analyses of 10 samples. A second study was run under tighter constraints in that fewer participants used only one of three assays. Data from these measurements were more promising. An important conclusion from this work was the strong emphasis placed on the need to train analysts, despite the apparent simplicity of immunoassays. Mouvet *et al.* (1995) carried out comparative trials on five triazine test kits available to European users. Kits were evaluated for cross reactivity, lowest detectable dose, and reproducibility. Most kits allowed determination below 0.10 ppb, and the interassay coefficient of variation for all products was less than 20%. Hayes *et al.* (1996) used a magnetic particle-based assay (Rubio *et al.*, 1991) to characterize performance of 14 laboratories in terms of recovery, precision, and the effect of water source. The mean recovery of all fortified samples was 104%, with a RSD of 15.7%, and the assay performed equally well for all six water sources. Meulenberg *et al.* (1999) evaluated a commercially available triazine assay for application in water quality control in the Netherlands. Three laboratories analyzed fortified surface water to assess the detection limit, precision, linearity, accuracy, and matrix effects. They found the assay was useful for discriminating between positive and negative samples, but felt analysts must receive specialized training to conduct the analysis properly. Brady *et al.* (2001) conducted an interlaboratory study for the validation of an atrazine immunoassay under the Safe Drinking Water Act, involving 19 laboratories certified to conduct compliance-monitoring analyses, including water treatment plants and government and private laboratories. During later work, this method was found to overestimate concentrations due to interferences by the oxidizing agents (e.g., chlorine dioxide) used in water treatment facilities. The effects of these confounders were essentially eliminated by adding reducing agents to immunoassay kits used for analyses. The validation of immunochemically based residue methods under USEPA guidelines is reviewed by Brady (2003).

Advantages and Disadvantages of Immunoassays

Immunoassays offer the residue chemist some tremendous advantages, but it is also important to understand the limitations of the technology. Foremost among the benefits are speed of analysis, low cost on a per sample basis, and relatively low cost of instrumentation. Analysis time ranges from about 45 min to 2½ h. Within these timeframes, up to 42 samples may be analyzed. Because water samples are analyzed directly without extraction or sample cleanup, it is not unusual for a single chemist using 96 well microtiter plates to assay more samples in 1 day than can be analyzed by a team employing conventional analytical methods in 1 week (Brady *et al.*, 1995). Even when antibody-coated tubes are used instead of microtiter plates, data on 8 to 10 samples can be in hand in less than 1½ h (Rubio *et al.*, 1991), generally before the same samples could be extracted for GC analysis.

The advantage of immunoassays is that they default to false positives (Baker *et al.*, 1993), allowing the elimination of negative samples from the confirmation process. Thus, using immunoassays to screen samples in single-analyte studies may greatly reduce the analytical costs of large studies, given that the majority of samples usually do not contain detectable residues.

The primary disadvantages of the immunoassay technique are the initial development costs for a detection kit and the inability to handle more than one analyte per analysis.

Continued Technological Advances

Continued advances in source/interface designs for the coupling of LC and MS/MS have increased ion formation and transfer into the mass spectrometer. Improved optics (focusing lenses, etc.) have increased ion transmission through the MS and to the detector. These improvements have resulted in a significant increase in sensitivity, and as a result, quantification at the parts per trillion concentration level is routine today and was not possible a decade ago. Advances in source/interface design to increase the abundance of ion formation, transfer, and transmission through the MS may lead to even more sensitive instruments. In addition, the use of smaller particle sizes in LC (e.g., UPLC) also increases resolution, selectivity, and sensitivity.

Further enhancements in instrumental sensitivity will depend on concurrent improvements in the quality of HPLC grade solvents and the ability to maintain a contaminant-free laboratory environment.

Conclusions

Column technology for both GC and LC has greatly advanced over the last two decades. Greater resolution, sensitivity, diverse functionality, and improvements in column manufacturing processes to increase reproducibility have made significant impacts not only on the analysis of triazines but on all chemical analyses. Mass spectrometry, especially MS/MS, has become the detection system of choice for GC and LC due to its sensitivity, selectivity, and confirmatory capabilities. Sample preparation for water is no longer required when analyzing samples at the sub-ppb concentration level using DAI-LC/MS/MS. The analysis of triazines and many other compounds in water are now analyzed using LC/MS/MS rather than GC/MS (or GC/MS/MS) since GC requires the transfer of the desired analytes from the water matrix into a suitable organic solvent prior to injection. Simultaneously obtaining quantitative and confirmatory data for several analytes per injection makes DAI-LC/MS/MS less costly per analyte per sample than immunoassay. Immunoassay still has an important niche in analyzing water samples. However, when multi-analyte information is required, LC/MS/MS and GC/MS (for nonaqueous samples) become the preferred techniques.

The analysis of triazines has dramatically changed over the last two decades. The range of molecular weights amendable to analysis has increased and the limits of detection have been vastly lowered. The development of new methods for detecting the triazines also has resulted in improved chemical isolation and detection techniques for many other chemicals unrelated to the triazines.

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Glossary of Terms, Acronymes, and Abbreviations Used

APcI	Atmospheric pressure chemical ionization
ATOH	Hydroxyatrazine
C18	18-carbon (octadecyl) alkane-bonded silica packing
C8	8-carbon (octyl) alkane-bonded silica packing
CE	Capillary electrophoresis
CI	Chemical ionization
DAI	Direct aqueous injection
DDA	Didealkylatrazine
DEA	Deethylatrazine
DIA	Deisopropylatrazine
EI	Electron ionization
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
GC	Gas chromatography
HPLC	High-performance liquid chromatography
HRGC	High-resolution gas chromatography
HRMS	High-resolution mass spectrometry
KI	Potassium iodide
LC	Liquid chromatography
LLE	Liquid–liquid extraction
LPME	Liquid-phase microextraction
LVI	Large-volume on-column injections
MEKC	Micellar electrokinetic chromatography
(MfflH)ffl	Positive pseudomolecular ion
(M⌡ H)⌡	Negative pseudomolecular ion
MS	Mass spectrometry
MSD	Mass selective detector
MS^n	Multiple stages (n) of mass spectrometry in sequence
NA-CZE	Nonaqueous capillary zone electrophoresis
NPD	Nitrogen–phosphorus detector
QToF	Quadrupole/time-of-flight analyzer
RSD	Relative standard deviation

SCX	Strong cation exchange column
SFE	Supercritical fluid extraction
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
Tof	Time-of-flight mass spectrometer
UPLC	Ultra pressure liquid chromatography
UV	Ultraviolet detection

Chapter 21

Triazine Soil Interactions

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Summary

The fate of triazine herbicides in soils is controlled by three basic processes: transformation, retention, and transport. This chapter focuses primarily on soil properties and processes that influence retention. While transformation includes both biological and abiotic decomposition, only abiotic processes are covered in this chapter.

Sorption of triazines on surfaces of soil particles is the primary means by which triazines are retained in soils. Soils are very complex mixtures of living organisms, various types of organic matter, and mineral particles. These soil constituents have many different types of surfaces. On a very general level, the various surface sites in soils may be classified as ionic, polar, and nonpolar. The ionic and polar sites interact with polar functional groups on triazine molecules. However, these sites also have a high affinity for water, and triazines must compete with water for these sites. Water is very competitive, and generally outcompetes the chlorotriazines for ionic and polar soil surface sites. The methoxy-, methylthio- and hydroxytriazines are somewhat more competitive against water for the ionic and polar soil surface sites than the chlorotriazines. The nonpolar sites on soil surfaces have a low affinity for water and therefore readily interact with nonpolar portions of triazine molecules (the alkyl side chains). Triazines interact with soil most strongly when the different functional groups on the triazine molecules are closely matched with active sites on the soil surface.

Transformation of triazines is primarily the result of degradation caused by microorganisms. However, triazines are also subject to a slow chemical degradation process known as hydrolysis. Chemical hydrolysis of atrazine, for example, is a process where the chlorine atom is removed from the atrazine molecule and replaced with a hydroxyl (OH) group. Chemical hydrolysis is relatively fast in acidic and alkaline soils, but it is relatively slow in neutral soils. In neutral soils, the rate of chemical hydrolysis of triazines increases when the triazine is adsorbed on the surfaces of soil particles. Hydroxytriazines, the products of chemical hydrolysis, are very strongly held by soil surfaces and hence move very slowly in soils. The hydroxytriazines have no biological activity.

The amount of a triazine sorbed on a soil can range from 0% to 100%, but typically ranges from 50% to 80% of the amount applied. Sorption of triazines by soils generally shows a moderate to weak correlation with the organic matter content of soils. The percent clay in soils is generally only weakly correlated with triazine sorption. However, the amount of clay surfaces and the nature of those surfaces are far more important than the percent clay in determining sorption of triazines by soil clays. The pH of a soil has a big influence on sorption of triazines. As a general rule, sorption of triazines increases with decreasing soil pH. The length of time a triazine has been in a soil (aging) also has a big influence on sorption. Generally, the longer the triazine has been in the soil the more difficult it is to desorb. The cause of this aging effect is only partially understood. Other factors – such as levels of dissolved organic carbon, triazine concentration, soil water content, and temperature – may influence sorption of triazines by soils. Unfortunately, many solute leaching models rely solely on the organic matter content of soils to account for variability in triazine sorption among soils. Such models, while better than assuming all soils are the same, miss much of the variability in triazine sorption among soils.

Introduction

Three basic processes – degradation, retention, and transport – determine the fate of triazines in soil environments (Gunther and Gunther, 1970). Both the direction and rate of these processes depend on the chemical nature of the

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triazines, and the chemical, biological, and hydraulic properties of the soil. This chapter focuses on chemical processes that influence the retention and abiotic degradation of triazines in soil environments. The chapter is divided into two main sections. The first section is a discussion of basic chemical mechanisms that govern triazine interactions with soil materials. The second half of the chapter is a review of literature related to triazine interactions with soils and focuses on articles published through 1997, covering the first 40 years of triazine use. There have been more than 700 papers published since 1997 on triazine–soil interactions. The majority of these papers confirm the fundamental processes controlling sorption and abiotic degradation described in the chapter. Certain new research areas of expanded interest have been included in the review, that is recognition that soils contain substantial amounts of black carbon, which is a major sorbent of triazines, and the bioavailability of aged triazine residues in soils.

Mechanisms for Triazine Interactions with Soil Materials

Chemistry of Triazines

The chemistry and physical properties of various *s*-triazines are summarized in Tables A1 and A2 of the Appendix. The triazines are Lewis bases as the ring nitrogen (N) atoms may donate electron pairs for the formation of covalent bonds. In aqueous systems, triazines exist as either neutral or protonated (cationic) forms depending on the pKa of the compound and the pH of the system. The most basic ring N and the most likely site of protonation are located in the 5 position between the electron-rich alkylamino side chains (Figure 21.1).

The pKa of an organic base is the pH in an aqueous system, where half of the compound is present in the neutral form and half in the protonated form. Substitutions in the 2 position greatly influence the basicity of the *s*-triazines (Weber, 1967). The chloro-*s*-triazines are very weak bases with pKa values between 1.6 and 1.9. The methoxy-*s*-triazines and methylthio-*s*-triazines have pKa values between 4.0 and 4.8, and the hydroxy-*s*-triazines have pKa values greater than 5.0. In soil solutions (pH 4.5 to 8.0), the chloro-*s*-triazines are overwhelmingly present in their neutral forms. The methoxy-*s*-triazines, methylthio-*s*-triazines, and hydroxy-*s*-triazines, however, are present as neutral species in neutral and alkaline soil solutions, but may be present as both neutral and protonated species in acidic soil solutions. This chapter also includes data on metribuzin, which is an asymmetrical triazine herbicide having somewhat different soil properties.

Water solubility is a macroscopic property indicating the average hydrophilic or hydrophobic character of a compound. The solubility of *s*-triazines in neutral water at 20°C ranges from less than 5 mg/L to more than 3000 mg/L, depending on the nature and properties of the substituents in the 2, 4, and 6 positions (Ward and Weber, 1968). On a molecular scale, the hydrophobic and hydrophilic functionalities of triazines are spatially separated. The lone pair electrons on the ring N-atoms readily form hydrogen bonds with water molecules, and thus triazine rings are hydrophilic. On the other hand, the alkylamino side chains in the 4 and 6 positions are hydrophobic. Because triazines have both hydrophilic and hydrophobic functionalities, they exhibit dual solubility behavior analogous to that exhibited by detergents and phospholipids. Upon sorption, free energy is minimized when triazine molecules are positioned so that the hydrophobic moieties interact with hydrophobic surfaces and the hydrophilic moieties interact with water or other polar molecules. The solubility data presented in Table A2 of the Appendix are for molecular species present in neutral aqueous systems. As the pH of an aqueous system approaches the pKa of the triazine, the triazines become increasingly protonated and their solubility increases sharply.

Triazine Interactions with Organic Matter in Soil

Soil organic matter and both humic and fulvic acids have been shown to have a high affinity for triazines (Walker and Crawford, 1968; Weber *et al.*, 1969; Hayes, 1970; Stevenson, 1972; Senesi and Testini, 1982; Borggaard and Streibig, 1988; Laird *et al.*, 1994; Senesi *et al.*, 1995). Although it is widely recognized that the organic matter in soil is important in sorption of triazines, the mechanisms governing triazine–organic matter interactions are only partially understood.

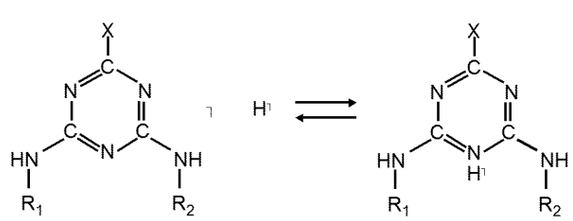

Figure 21.1 Protonation of *s*-triazine.

A thorough discussion on the nature and properties of soil organic matter is beyond the scope of this chapter, however simplistically soil organic matter may be viewed as a mixture of humic and nonhumic materials. The nonhumic materials are living organisms, the still recognizable remains of plant, animal, and microbial tissues, and black carbon. Nonhumic materials include all of the various classes of biomolecules, such as carbohydrates, proteins, and lipids. The majority of the nonhumic organic materials in soils are carbohydrates. These materials include cellulose, starch, glycogen, and the simpler mono- and disaccharides. Triazines may form H-bonds with electropositive groups on carbohydrates; however, the interaction is minimal due to competition from water molecules.

Lipids are a large class of compounds that have hydrophobic straight alkyl chains with polar linkages to broader structures. The triglycerols (fats), for example, have three straight carbon chains with carboxylate ester linkages to glycerol. The phospholipids have two alkyl chains with carboxylate ester linkages and a phosphate group with an ester linkage. The phosphate group is acidic and typically exists as an anion in aqueous solutions. Though soil lipids provide a good environment for sorption of triazines because they have both polar and nonpolar regions that interact with triazine rings and alkyl side chains, respectively, sorption is limited by the very low concentrations of these compounds.

Only a few studies have investigated the relative contribution of nonhumic organic materials in soils to the sorption of triazines. Dunigan and McIntosh (1971), using selective sequential extraction, reported that fats, oils, waxes, and resins in a Walla Walla silt loam had a negligible capacity for sorption of atrazine, while polysaccharides exhibited a small sorption capacity. In general, the quantity of nonliving biomolecules in well-aerated mineral soils is small as these are the primary food of living organisms; thus, nonliving biomolecules are believed to make only a small contribution to sorption of triazines by mineral soils. On the other hand, nonliving biomolecules are a major component of peats and mucks and may provide the dominant sites for sorption of triazines in these soils.

Black carbon (also termed char, bio-char, pyrogenic C, or simply charcoal) includes a wide range of materials derived from incomplete combustion of bio-organic materials, and includes everything from slightly carbonized biological tissue to charcoal to soot to graphite (Schmidt and Noack, 2000; Preston and Schmidt, 2006). Properties and chemical composition of bio-char depend on the properties of the original bio-material from which the bio-char was derived, as well as the degree of thermal alteration. In general, soil black carbon is dominated by aromatic carbon (ffi70%) and has low H/C (ffi0.7) and O/C (ffi0.3) ratios (Hammes *et al.*, 2006). On aging in-soil environments exposed surfaces of black carbon are oxidized forming carboxylic groups. Estimates of the amount of black carbon in soils range from 1% to 50% of the total C, but most estimates range from 5% to 30% of soil organic carbon (SOC) (Skjemstad *et al.*, 2002; Brodowski *et al.*, 2005).

Black carbon has a high affinity for many organic molecules and may dominate sorption of triazines if present in significant quantities in soils (Yang and Sheng, 2003; Ahmad *et al.*, 2006). Laird *et al.* (1994) found that 'organic matter associated with the coarse clay fraction' contributed disproportionately to the adsorption of atrazine and was the dominant phase responsible for hysteresis during desorption. We now know that the 'organic matter associated with the coarse clay fraction' was black carbon.

The majority of organic materials in mineral soils are humic substances. Humic substances are a highly heterogeneous group of acidic macromolecules (molecular weight 1000 to more than 300 000) that bear no physical resemblance to the organic compounds of living organisms. Humic materials are believed to form through oxidative degradation of organic tissues to relatively recalcitrant monomers, followed by the polymerization of these monomeric substances into high molecular weight compounds (Stevenson, 1972; Hayes, 1991). Various substituted polyphenols, quinones, and amino acids are thought to dominate the humic monomers, but many other compounds including mono- and disaccharides are also likely included. The joining of these monomers to form macromolecules is probably an abiotic process (largely a condensation reaction), which is mediated by inorganic surfaces (Wang and Huang, 1989; Wang, 1991). This leads to the formation of ˆ O ˆ , ˆ NH ˆ , ˆ N ¨ , and ˆ S ˆ linkages. Evidence from ^{13}C-NMR shows that 35–40% of humic structures are single-ring aromatic units. Fused aromatic structures are thought to be an insignificant component of humic substances (Hayes, 1991). Stability of humic substances is due to the large size of the macromolecules which inhibits microbial ingestion, the recalcitrant nature of many of the units, the diversity of the humic molecules, and the association of the humic substances with clay minerals that sequester humics, protecting the humics from biodegradation.

Humic materials are highly acidic due principally to carboxylic and phenolic groups, with lesser contributions from the aliphatic- and enolic-OH groups. Total acidity of humic materials may range from 1 mol/kg to more than 14 mol/kg. The pKa for most acidic functional groups is between 5 and 7. Other functional groups in humic substances include quinone and ketonic carbonyl, amino, and sulfhydryl groups. Charged and highly polar functional groups form strong hydrogen bonds with water molecules, and the high density of these groups imparts a hydrophilic character to humic substances on a macroscopic scale. On a molecular scale, aliphatic groups may associate, forming localized hydrophobic regions.

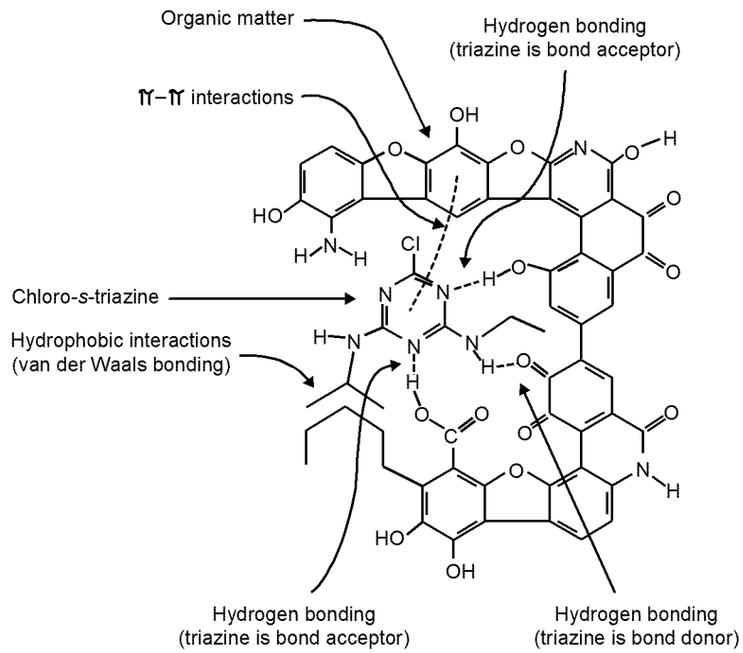

Figure 21.2 Possible interactions between the organic matter in soil and a chloro-*s*-triazine.

Bonding of triazines with soil organic matter appears to include several mechanisms. Under pH conditions found in soils (pH 4–8), relatively strong H-bonds form between triazine ring N atoms (triazine is the bond acceptor) and acidic carboxylic, phenolic, and the amide organic functional groups in the soil organic matter (Kalouskova, 1989). Protons of the triazine amine groups form H-bonds with electronegative centers in the organic matter, principally the quinone, ketonic, and aldehyde groups. Such H-bonding is favored when the system pH is above the pKa of the triazine and below the pKa of the acidic functional group. Atrazine, for example, forms complexes through hydrogen bonds with amide and carboxylic acid functional groups found in organic matter (Welhouse and Bleam, 1993a, b) and possibly with phenol- and quinone-like functional groups. Ionic bonding occurs when protonated triazines interact with anionic organic groups; it is favored when the system pH is below the pKa of the triazine and above the pKa of the acidic organic functional group. In soil environments, such situations are rare for the chloro-*s*-triazines (pKa $\leq$ 2.0), but are somewhat more common for the methoxy- and methylthio-*s*-triazines (pKa 4.0–4.8). However, ionic bonding likely accounts for both the high affinity of hydroxy-*s*-triazines (pKa 5.0–6.0) for soil surfaces and the substantial nonsingularity observed for desorption of hydroxy-*s*-triazines from soils (Clay and Koskinen, 1990b; Clay *et al.*, 1996).

In aqueous environments, high-energy envelopes form around the hydrophobic alkyl side chains of triazine molecules due to disruption of the H-bond network of water. Upon sorption, free energy is minimized when triazines are positioned so that the alkyl side chains are solvated by aliphatic moieties of the organic matter in soil. As described above, soil humic substances are hydrophilic on a macroscopic scale. On a molecular scale, however, they have numerous aliphatic groups that associate with each other to create molecular-scale hydrophobic regions. The hydrophobic alkyl side chains of triazines interact readily with these sites. Finally, charge transfer interactions involving the electron donor triazine ring and the electron acceptor aromatic moieties of humic substances may contribute substantially to the overall retention of the methoxy-, methylthio- and hydroxy-*s*-triazines by humic substances (Piccolo *et al.*, 1992; Senesi, 1992; Senesi *et al.*, 1995). Charge transfer interactions are apparently less important for the binding of chloro-*s*-triazines to humic substances (Martin-Neto *et al.*, 1994). The presence of a Cl atom at the 2 position withdraws electron density, inhibiting the triazine ring from donating electrons.

A precise description of bonding between triazines and humic substances is complicated by the extreme heterogeneity of humic substances. However, it is clear that all of the above mechanisms contribute to the sorption of triazines and that two or more mechanisms may contribute to the interaction energy for a given molecule. The stereo chemistry of each potential binding site determines which mechanisms are involved. Figures 21.2 and 21.3 summarize the types of interactions that may contribute to the retention of chloro-*s* -triazines and protonated-keto-triazines, respectively.

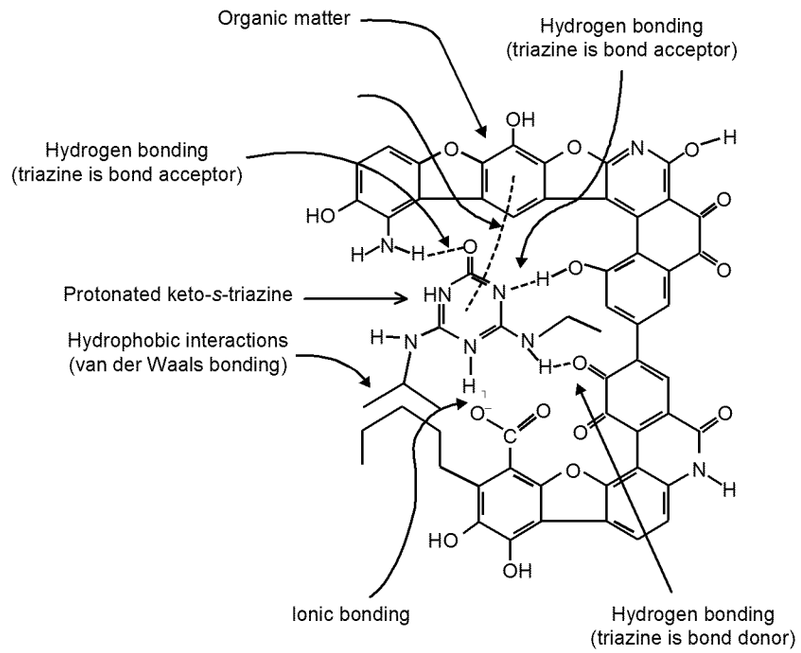

Figure 21.3 Possible interactions between the organic matter in soil and a protonated keto-*s*-triazine.

Triazine Interactions with Inorganic Soil Materials

Triazines interact with surfaces of inorganic materials in soil environments. The nature and the extent of these interactions depend on both the properties of the inorganic surfaces and the chemistry of the soil solution. Inorganic surfaces in soil environments may be grouped as uncharged, variable charge, and permanent charge surfaces.

Uncharged Surfaces

The three general classes of uncharged inorganic surfaces in soils are: surfaces terminated by bridging oxygens (┘M ˆ O ˆ M└) (┘M represents any metal (e.g., silicon, aluminum, etc.) in octahedral or tetrahedral coordination with structural oxygens or hydroxyls); surfaces terminated by bridging hydroxyls (┘M ˆ OH ˆ M└); and surfaces terminated by nonbridging, valence-satisfied hydroxyls (┘M ˆ OH).

Uncharged surfaces having bridging oxygens generally terminate with oxygens that are shared between two silicon (Si) tetrahedra (siloxane surfaces). Examples of uncharged siloxane surfaces are the basal surfaces of talc and pyrophylite and the siloxane surfaces of kaolinite. Uncharged surfaces terminated by bridging hydroxyls are referred to as gibbsitic surfaces. The terminal OH groups on gibbsitic surfaces are shared by three adjacent octahedra. Two of the octahedra are occupied by aluminum (Al), and the third is vacant on basal surfaces of gibbsite and on the OH surface of kaolinite. With serpentine minerals, all three of the octahedra are occupied by divalent cations – typically magnesium (Mg). Silanol surfaces are the only significant valence-satisfied, nonbridging hydroxyl surfaces found in soils. Silanol groups are predominantly neutral, but they may be negatively charged or positively charged depending on pH. Hence, their classification as uncharged or variable charged surfaces is problematic. In most soil environments, silanol surfaces carry a very small net negative charge.

Valence-satisfied surfaces of soil minerals are relatively inert (Sposito, 1984). Indeed, interaction energies between a water molecule and the siloxane (1.49×10^{-20} J) and gibbsitic (1.23×10^{-20} J) surfaces of kaolinite are substantially below the condensation enthalpy for two water molecules (7.3×10^{-20} J) at 298 K (Sposito and Babcock, 1966). As a result, water molecules do not form hydrogen bonds with these surfaces, a factor which has led several authors (Chen, 1976; Skipper *et al.*, 1989; Bleam, 1990; Jaynes and Boyd, 1991; Güven, 1992) to conclude that valence-satisfied surfaces are hydrophobic. Triazines are readily sorbed on valence-satisfied surfaces through a combination of hydrophobic bonding and dispersive forces (van der Waals). The hydrophobic alkyl side chains bond directly with the surfaces, while the polar triazine rings interact with water in the soil solution. The net effect is that the triazine acts as a surfactant, lowering the surface free energy associated with the surface–water interface.

Variable Charge Surfaces

Variable charge surfaces in soil environments are terminated by nonbridging hydroxyls (┘ M ˆ OH) that are coordinated with iron (Fe), Al, manganese (Mn), titanium (Ti), or Si. Nonbridging hydroxyls carry a partial negative charge, and on protonation they become surface-water molecules (┘ M ˆ OH_2) with a partial positive charge:

$$┘ M ˆ OH^{-1/2} ┐ H┐ \bullet ┘ M ˆ OH_2┐^{1/2}$$

The point of zero net charge (PZNC) is the pH value where the positive and negative charges are equal in magnitude. Both the PZNC and the magnitude of the partial charge carried by the nonbridging hydroxyl groups will vary inversely with the ratio of the valance to the coordination number of the underlying metal cation (Table 21.1).

Nonbridging hydroxyls coordinated with Fe and Al are common and contribute most of the variable charge surfaces in soils. These hydroxyls occur on surfaces of oxide and hydroxide minerals, on poorly crystalline oxyhydroxide minerals, and on the lateral or 'broken' edges of phyllosilicates. Nonbridging surface hydroxyls coordinated with Ti and Mn occur on amorphous and poorly crystalline minerals and on the oxyhydroxide coatings of other minerals. The Ti and Mn minerals generally contribute little to the total surface area of soils (with the exception of some Oxisols), but where present, these minerals have nonbridging ┘ M ˆ OH groups. Surfaces of tectosilicates (e.g., quartz, feldspars, etc.) terminate with nonbridging ┘ Si ˆ O^{-1}, ┘ Si ˆ OH, and ┘ Si ˆ $OH_2┐^{1}$ groups. However, as mentioned previously, the neutral ┘ Si ˆ OH groups are stable over a wide pH range (2–8) and dominate tectosilicate surfaces in soil environments. Variable charge surfaces readily form hydrogen bonds with water molecules and are, therefore, strongly hydrophilic. Surface hydroxyls interact with the protons on water molecules, and bound water sites interact with lone pair electrons on the oxygen atoms of water molecules.

In the absence of water, triazines are readily sorbed on variable charge surfaces. Triazines may solvate metal cations retained on these surfaces or interact directly with the surfaces through hydrogen bonding. The ring N-atoms of triazines form hydrogen bonds with surface water molecules, while the protons of the amino groups form hydrogen bonds with surface hydroxyls (Figure 21.4).

In aqueous systems, however, molecular triazines are not competitive with water molecules and, therefore, are not retained on variable charge surfaces. Although protonated triazines may form ionic bonds with negative charge sites on variable charge surfaces, such bonding is generally limited to rare systems in which the pH is below the pKa of the triazine and above the PZNC of the surface. The lack of favorable sites on variable charge surfaces for the hydrophobic alkyl side chains is perhaps the most significant factor limiting sorption of triazines on variable charge surfaces in aqueous systems. Consistent with the above interpretations, negligible sorption of atrazine on amorphous iron oxides and on goethite in aqueous systems was reported by Borggaard and Streibig (1988), and neither atrazine nor simazine were found to be sorbed on ferrihydrite (Celis *et al.*, 1997). Furthermore, Fe- and Al-oxyhydroxide coatings on soil clays have been shown to decrease substantially the affinity of mineral surfaces for atrazine (Laird *et al.*, 1994).

Table 21.1 Influence of valence and coordination number of underlying metal cations on properties of variable charge surfaces

Metal	Valence	Coordination No.	Charge on ┘ M ˆ OH[a]	PZNC[b]
Si	4	4	0.0	└ 3
Mn	4	6	− 0.33	3–4
Ti	4	6	− 0.33	5–7
Al	3	6	− 0.5	6–8
Fe	3	6	− 0.5	7–9

[a]┘ M ˆ OH • nonbridging hydroxyls.
[b]PZNC • point of zero net charge.

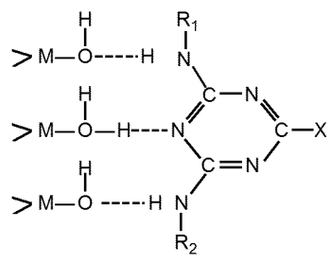

Figure 21.4 Hydrogen bonding between *s*-triazine and active sites on variable charge surfaces in the absence of water.

Permanent Charge Surfaces

Permanent charge surfaces are the dominant type of inorganic surface in temperate region soils. Examples of permanent charge surfaces include the basal surfaces of smectites, vermiculites, and illites. Permanent charge surfaces terminate with bridging oxygens shared between two Si tetrahedra (Si ^ O ^ Si) or between one Si and one Al tetrahedra (Si ^ O ^ Al). Negative charge is not uniformly distributed on permanent charge surfaces; rather it is localized in basal oxygens proximal to sites of isomorphous substitution. With tetrahedrally charged clays, the negative charge is carried by the three basal oxygens of the aluminate tetrahedra (Farmer and Russell, 1971; Bleam, 1990). The negative charge is spread over as many as 10 basal oxygens with octahedrally substituted 2:1 phyllosilicates.

The primary interaction between water and permanent charge surfaces is through hydration of adsorbed metal cations (Russell and Farmer, 1964; Farmer and Russell, 1971; Sposito and Prost, 1982; Johnston *et al.*, 1992). Small multivalent cations (e.g., Al^{3+}, Mg^{2+}, and Ca^{2+}) retain both inner and outer hydration shells, whereas large monovalent cations (e.g., K^+, NH_4^+, Rb^+, and Cs^+) retain only inner hydration shells. To a lesser extent, water molecules may also interact with basal oxygens proximal to sites of isomorphous substitution. Basal oxygens bridging Si and Al tetrahedra are strong Lewis bases and readily form hydrogen bonds with water molecules (Sposito and Prost, 1982; Bleam, 1990). The Lewis basicity of Si ^ O ^ Si basal oxygens proximal to sites of octahedral substitution appears to be insufficient for the formation of hydrogen bonds. Nonetheless, these basal oxygens carry a partial negative charge that interacts with the positive dipoles of water molecules. On the other hand, basal oxygens distal from sites of isomorphous substitution are valence-satisfied and are considered hydrophobic (Jaynes and Boyd, 1991; Laird, 1996). Thus, the relative hydrophilic or hydrophobic character of permanent charge surfaces varies dramatically on a molecular scale. Basal oxygens close to charge sites are hydrophilic, while basal oxygens only a few angstroms away are hydrophobic (hydrophobic nanosites). With low charge-density clays, the charge sites are widely separated and a substantial portion of the total surface area is hydrophobic. In contrast, the hydrophilic regions associated with charge sites on high charge-density surfaces coalesce, leaving little or no hydrophobic surface.

Triazines interact with permanent charge surfaces through a variety of mechanisms, including hydrophobic bonding, hydrogen bonding, van der Waals bonding, and ionic bonding (Figures 21.5 and 21.6). Because of the heterogeneous nature of permanent charge surfaces on the molecular scale, several of these mechanisms may contribute simultaneously to the interaction energy of a single triazine molecule. Hydrophobic bonding occurs between the alkyl

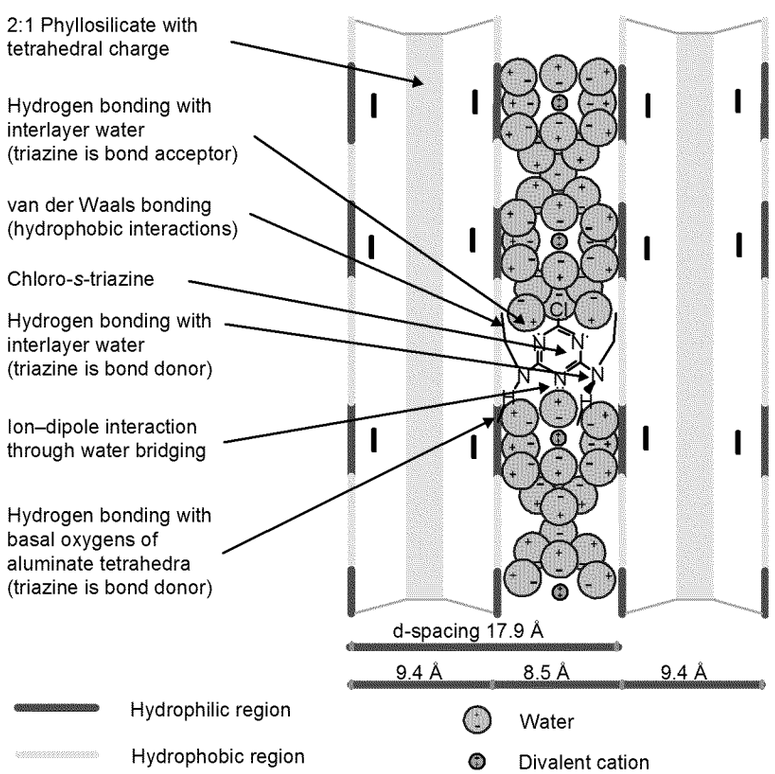

Figure 21.5 Possible interactions between hydrated smectite surfaces and a chloro-*s*-triazine. (See Color Plate Section.)

side chains of triazines and the hydrophobic nanosites on permanent charge surfaces. The ring N-atoms may form hydrogen bonds with nearby surface water molecules – typically water molecules solvating metal cations adsorbed on permanent charge sites. Ionic bonding occurs between protonated triazines and negative, permanent charge sites.

The strength of the interactions between triazines and permanent charge surfaces depends on which bonding mechanism dominates and on the steric fit between the various hydrophobic and hydrophilic groups on the triazine molecule and the various nanosites on the permanent charge surfaces. Water is ubiquitous on the surfaces of minerals in soil environments. Therefore, the strength of interactions between triazines and permanently charged surfaces also depends on the competitiveness of the various organic functional groups relative to water for the surface nanosites.

The solution pH, the nature of adsorbed metal cations, and the surface charge density of permanent charge surfaces greatly influence not only the extent of triazine sorption, but also the strength of the interaction between triazine and the permanent charge surface. Bailey *et al.* (1968) reported that a highly acidic H-smectite sorbed 100% of six chloro- and methoxy-*s*-triazines in their systems, whereas only simeton was completely sorbed on Na-smectite. Laird *et al.* (1992) demonstrated that Ca-smectites sorb anywhere from 0% to 100% of added atrazine from neutral aqueous (0.01 M $CaCl_2$) systems (Figure 21.7) and that the affinity for atrazine increases as the surface charge density of smectites decreases. Celis *et al.* (1997) found nearly total sorption of atrazine and simazine on Fe(H)-smectite (pH • 2.9) and much less sorption on Ca-smectite (pH • 7.9). Sawhney and Singh (1997) also report much greater sorption of atrazine on Al(H)-smectite relative to Ca-smectite. The electronegativity of the adsorbed metal cations determines

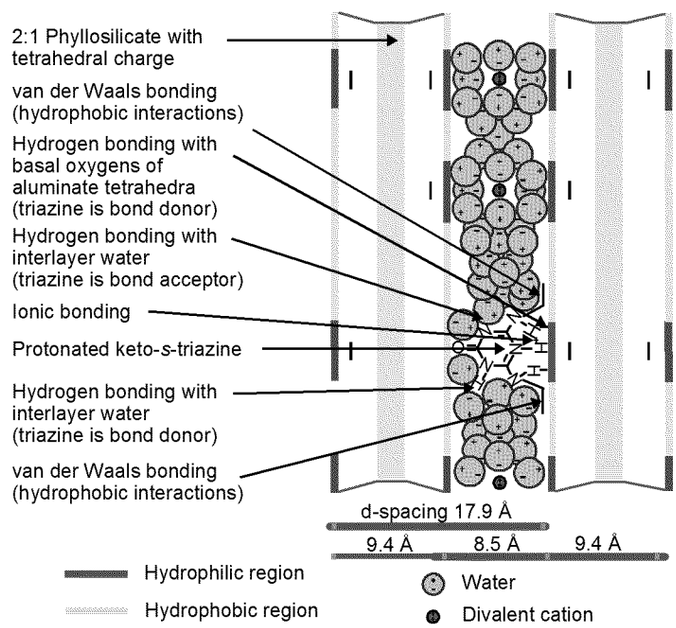

Figure 21.6 Possible interactions between hydrated smectite surfaces and protonated keto-*s*-triazine. (See Color Plate Section.)

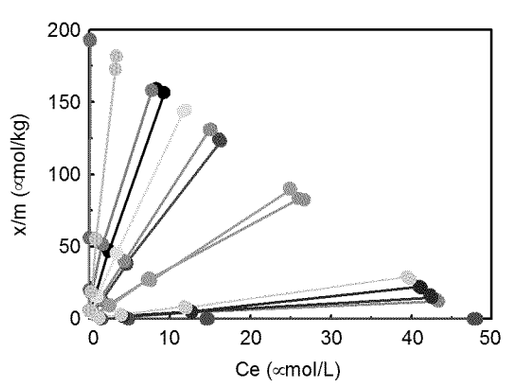

Figure 21.7 Isotherms for sorption of atrazine on Ca-smectites in 0.01 M $CaCl_2$ (data from Laird *et al.*, 1992). (See Color Plate Section.)

the extent of polarization of water molecules in the primary hydration shell around the metal ion, and the extent of polarization largely determines the strength of interactions between those water molecules and the lone pair electrons on ring nitrogen atoms of triazines. As mentioned previously, hydrophobic nanosites are larger and more common on low charge-density surfaces and smaller and less common on high charge-density surfaces (Laird, 1996). Hence surface charge density is inversely related to the contribution of hydrophobic bonding to the total interaction energy between triazines and permanently charged surfaces.

Hydrolysis of Triazines

The abiotic hydrolysis of triazines in soil environments is catalyzed by acidic sites on the surfaces of both organic and inorganic soil constituents. The surfaces of soil constituents have both Lewis acid sites (which accept electron pairs) and Brönsted acid sites (which donate protons). However, triazines are not competitive with water and OH groups for complexation with Lewis acid sites, so in soil environments hydrolysis is catalyzed primarily by Brönsted acid sites. Four types of Brönsted acid sites are found on soil surfaces (Mortland, 1970):

1. Acidic organic functional groups, such as carboxyls and phenols, may disassociate with the release of a proton.
2. Hydronium ions electrostatically retained on negative surface-charge sites may be released through cation exchange.
3. Nonbridging water molecules coordinated with exposed structural metal ions on variable charge surfaces may donate protons.
4. Water molecules associated with the hydration shells of metal cations adsorbed on ion exchange sites may hydrolyze and release protons.

The strength of surface acidity increases with decreasing water content and increasing electronegativity of the exchangeable metal cations (Mortland *et al.*, 1963; Farmer and Mortland, 1966; Mortland, 1968; Mortland and Raman, 1968). Electronegative cations withdraw electrons from oxygens of solvating water molecules, facilitating proton release. Due to this effect, the surface acidity increases for soil materials saturated with $K^{+} < Na^{2+} < Ca^{2+} < Mg^{2+} < Al^{3+} < Fe^{3+}$. The effect of water content on the strength of surface acidity is less well understood, but it is likely due to increasing polarization of surface water molecules with decreasing water content (Mortland and Raman, 1968). Although surface acidity is diminished in aqueous systems, Ca-smectites suspended in distilled water have been shown to promote significant protonation of weak bases by as much as two pH units above the pKa of the base (Feldkamp and White, 1979; Laird and Fleming, 1999).

The surface acidity of soil constituents catalyzes both the protonation and hydrolysis of triazines. For example, Armstrong *et al.* (1967) found the rate of atrazine hydrolysis to be an order of magnitude higher in aqueous systems containing sterilized soil than in aqueous systems without soil at the same pH. The half-life for hydrolysis of atrazine in the aqueous system containing sterilized soil was 22 days. However, abiotic hydrolysis rates are greatly influenced by pH (increase with decreasing pH) and by the nature of the substituents in the 2 position. Hydrolysis rates are influenced to a lesser extent by the surface properties of the soil constituents, the polarizing power of adsorbed metal cations, and the moisture status of the soil.

Surface catalyzed hydrolysis of chloro-*s*-triazines occurs in three basic steps:

1. The chloro-*s*-triazine is sorbed as a neutral molecule on a soil surface through hydrophobic interactions involving the alkyl side chains and through polar interactions involving one of the ring N-atoms and a Brönsted acid site.
2. A proton is then transferred from the Brönsted acid site to one of the ring N-atoms.
3. Protonation withdraws electron density from and facilitates nucleophilic attack on the C atom in the 2 position (Figure 21.8).

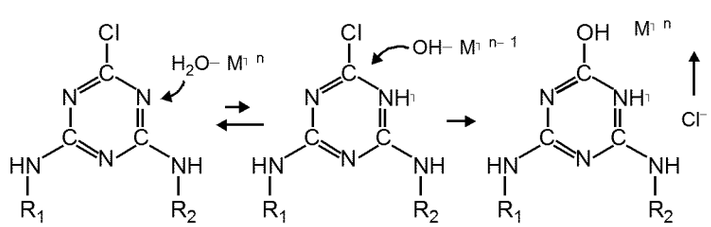

Figure 21.8 Surface catalyzed protonation and hydrolysis of a chloro-*s*-triazine.

As stated previously, the ring N in the 5 position is the most basic, and hence the most likely, site of protonation (Figure 21.1); however, hydrolysis is more likely when one of the N-atoms ortho to the C atom in the 2 position is protonated.

The net products of this reaction are a protonated hydroxy-*s*-triazine and Cl^-. The Cl^- is electrostatically repelled from the surface due to the dominance of the negative charge on soil surfaces. On the other hand, the protonated hydroxy-*s*-triazine is held on the surface substantially more tightly than the original chloro-*s*-triazine due to the added contribution of the electrostatic interaction. The pKa values of the hydroxy-*s*-triazines are all greater than 5 (Weber, 1970a, b); hence with the help of surface acidity, adsorbed protonated hydroxy-*s*-triazines tend to remain protonated. Protonated hydroxy-*s*-triazines are further stabilized by both tautomerism and resonance (Russell *et al.*, 1968; Laird, 1996).

Quantification of Triazine Interactions with Soils

Because sorption directly or indirectly controls transformation and transport of triazines, there is continued interest in characterizing both mechanistically and quantitatively how triazines are sorbed in soil. Sorption mechanisms and the relative contributions of individual soil constituents to triazine sorption by soils have been evaluated using a variety of analytical techniques (Table 21.2). Such studies typically involve quantification of sorption on individual soil constituents or fractions (i.e., humic acid, fulvic acid, water soluble soil organic matter, clay) (Tables 21.3 and 21.4), or quantification of changes in sorption after selectively removing soil constituents, such as iron oxides or organic matter (Huang *et al.*, 1984; Laird *et al.*, 1994), or after determining sorption on model sorbents (Table 21.5).

Studies on sorption of triazines by individual soil constituents and by model sorbents have been very helpful in evaluating sorption mechanisms and in assessing the potential contribution of various constituents to triazine sorption by soils. However, intimate associations between organic substances, silicate clays, and oxyhydroxide materials modify the sorptive properties of the individual constituents. Associations between soil constituents influence soil properties – such as pH, specific surface area, and functional group availability – which in turn influence triazine sorption behavior. For instance, atrazine and simazine sorption behavior is different for synthetic mixtures of model soil

Table 21.2 References on the characterization of triazine sorption on soil constituents

Triazine	Sorbent	Analytical technique	Reference
Ametryn	HA[b]	IR[e], ESR[f], DTA[g]	Senesi and Testini (1982)
Ametryn	Clay	IR	Brown and White (1969)
Atraton	Clay	IR	Brown and White (1969)
Atrazine	HA	Gel chromatography, UV[h], IR, FTIR[i], ESR, ultrafiltration	Kalouskova (1989); Martin-Neto *et al.* (1994); Senesi *et al.* (1995); Sullivan and Felbeck (1968); Wang *et al.* (1991); Wang *et al.* (1992)
Atrazine	Organics	NMR[j]	Brown and Flagg (1981); Welhouse and Bleam (1993a, b)
Atrazine	Organic colloids	Gel chromatography	Wijayaratne and Means (1984)
Atrazine	FA[c]	Ultrafiltration	Gamble *et al.* (1986a, b); Wang *et al.* (1990)
Atrazine	Clay	IR	Brown and White (1969)
Chlorazine	Clay	IR	Brown and White (1969)
Desmetryn	HA	IR, ESR, DTA	Senesi and Testini (1982)
GS 18183	Clay	IR, X-ray	Hermosin *et al.* (1982)
Ipaton	Clay	IR	Brown and White (1969)
Ipazine	Clay	IR	Brown and White (1969)
Methoprotryn	HA	IR, ESR, DTA	Senesi and Testini (1980, 1982)
Metribuzin[a]	WSSOM[d]	gel filtration	Pennington *et al.*, (1991)
Prometon	HA	IR, ESR, DTA	Senesi and Testini (1980, 1982)
Prometon	Clay	IR	Brown and White (1969)
Prometryn	Clay	IR, X-ray	Brown and White (1969); Hermosin *et al.* (1982)
Prometryn	HA	IR	Sullivan and Felbeck (1968)
Propazine	Clay	IR	Brown and White (1969)
Secbuthylazine	Clay	IR, X-ray	Hermosin *et al.* (1982)
Simazine	HA	FTIR, ESR, IR	Senesi *et al.* (1995); Sullivan and Felbeck (1968)
Simazine	Clay	IR	Brown and White (1969)
Simeton	Clay	IR	Brown and White (1969)
Terbuthylazine	Clay	IR, Xray	Hermosin *et al.* (1982)
Trietazine	HA	IR	Sullivan and Felbeck (1968)
Trietazine	Clay	IR	Brown and White (1969)

[a] An asymmetric triazine; [b] HA: humic acid; [c] FA: fulvic acid; [d] WSSOM: water soluble soil organic matter; [e] IR: infrared; [f] ESR: electron spin resonance; [g] DTA: differential thermal analysis; [h] UV: ultraviolet; [i] FTIR: Fourier transform IR; [j] NMR: Nuclear-Magnetic Resonance.

Table 21.3 References on the quantification of triazine sorption K_d or K_f, on humic materials

Chemical	No. of humic materials	Reference
Atraton	2	Gilmour and Coleman (1971)
Atrazine	22	Almendros(1995); Borggaard and Streibig (1988); Dunigan and McIntosh (1971); Gamble and Khan (1990); Gilmour and Coleman (1971); Hance (1967); Hance (1971); Harris and Warren (1964); Hayes *et al*. (1968); Li and Felbeck (1972); McGlamery and Slife (1966); Moyer *et al*. (1972); Saint-Fort and Visser (1988); Schiavon*et al*. (1992); Weber (1993); Wijayaratne (1982)
Cyanazine	12	Dios Cancela *et al*. (1990)
Desmetryn	2	Hayes *et al*. (1968)
Hydroxyatrazine	1	Gamble and Khan (1990)
Metribuzin[a]	2	Sharom and Stephenson (1976)
Prometon	3	Gilmour and Coleman (1971); Nearpass (1971)
Prometryn	7	Almendros (1995); Carringer*et al*. (1975); Doherty and Warren (1969); Gilmour and Coleman (1971); Kozak *et al*. (1983); LaFleur (1979b); Lee and Farmer (1989); Moyer *et al*. (1972)
Simazine	2	Doherty and Warren (1969)
Terbutryn	8	Gaillardon *et al*. (1981)

[a] An asymmetric triazine.

Table 21.4 References on the quantification of triazine sorption, K_d or K_f, on clays

Chemical	No. of clays	Reference
Ametryn	3	Hance (1969a, c); Yamane and Green (1972); Weber (1970a, b)
Atraton	4	Bailey *et al*. (1968); Hance (1969a, c); Weber (1970a, b)
Atrazine	41	Bailey *et al*. (1968); Barriuso *et al*. (1994); Borggaard and Streibig (1988); Celis *et al*. (1997); Fruhstorfer *et al*. (1993); Gilchrist *et al*. (1993); Gilmour and Coleman (1971); Hance (1969b, 1971); Harris and Hurle (1979); Harris and Warren (1964); Laird (1996); Laird *et al*. (1992, 1994); Moyer *et al*. (1972); Scott and Lutz (1971); Terce and Calvet (1978); Weber (1970a, b, 1993); Yamane and Green (1972)
Chlorazine	2	Frissel and Bolt (1962)
Desmeton	1	Weber (1966)
Hydroxypropazine	1	Weber (1966); Weber *et al*. (1969)
Hydroxyipazine	1	Weber (1966)
Ipaton	1	Weber (1966)
Ipatryn	1	Weber (1966)
Ipazine	1	Weber (1966)
Prometon	7	Bailey *et al*. (1968); Weber (1970a, b); Weber *et al*. (1965, 1969); Weber and Weed (1968)
Prometryn	5	Carringer *et al*. (1975); Doherty and Warren (1969); Hance (1969a, c); Moyer*et al*. (1972); Weber *et al*. (1969)
Propazine	3	Bailey *et al*. (1968); Doherty and Warren (1969)
Simazine	5	Bailey *et al*. (1968); Doherty and Warren (1969); Harris and Hurle (1979); Scott and Lutz (1971)
Simeton	3	Bailey *et al*. (1968); Weber *et al*. (1969)
Simetryn	1	Hance (1969a, c)
Terbutryn	3	Terce and Calvet (1978)
Tetraetatone	1	Weber *et al*. (1969)
Trietaton	1	Weber *et al*. (1969)
Trietanine	2	Bailey *et al*. (1968)

Table 21.5 References on the quantification of triazine sorption, K_d or K_f, on model sorbents

Chemical	No. of sorbents	Reference
Ametryn	1	Yamane and Green (1972)
Atrazine	30	Borggaardand Streibig (1988); Celis *et al*. (1997); Dunigan and McIntosh (1971); Hance (1969b, 1971); Harris and Warren (1964); Schiavon *et al*. (1992); Seta and Karathanasis (1997); Stehouwer *et al*. (1994); Yamane and Green (1972)
Metribuzin[a]	8	Dao (1991); LaFleur (1979a)
Prometon	4	Weber *et al*. (1968)
s-ethyl metribuzin[a]	2	Dao (1991)
Terbutryn	7	Gaillardon *et al*. (1983)

[a]An asymmetric triazine.

colloids (e.g., montmorillonite, ferrihydrite, and humic acid) compared to that observed for the individual constituents (Celis *et al.*, 1997). Humic acid coatings on Ca-montmorillonite increased triazine sorption relative to the untreated Ca-montmorillonite, whereas associations between humic acid and ferrihydrite and between montmorillonite and ferrihydrite decreased triazine sorption. Replacing exchangeable cations on montmorillonite with Fe promotes triazine sorption by the clay, whereas Fe saturation of humic acids reduces triazine sorption by decreasing the availability of ionizable humic acid functional groups. Analyses of desorption show that in all cases triazine sorption by model soil colloid associations was reversible.

Sorption is most commonly quantified using distribution coefficients (K_d), which simplistically model the sorption process as a partitioning of the chemical between homogeneous solid and solution phases. Sorption is also commonly quantified using sorption isotherms, which allow variation in sorption intensity with triazine concentration in solution. Sorption isotherms are generally modeled using the empirical Freundlich equation, $S = K_f C^{1/n}$, in which S is the sorbed concentration after equilibration, C is the solution concentration after equilibration, and K_f and $1/n$ are empirical constants. K_d and K_f are used to compare sorption of different chemicals on one soil or sorbent, or of one chemical on several sorbents. K_d and K_f are also commonly used in solute leaching models to predict triazine interactions with soils under various environmental conditions.

The amount of a triazine retained or sorbed by soil can range from 0% to 100% of the amount applied, but typically sorption on silt loam, loam, or clay loam surface soils ranges from 50% to 80% of the amount applied. Although sorption of triazines (particularly atrazine) by soils has been studied for more than 40 years, there continue to be numerous studies each year to quantify sorption by different soils and to characterize the factors that affect triazine sorption. For instance, in a review of literature for 1964–1984, Koskinen and Moorman (1985) found 343 published K_d values for sorption of atrazine on 148 soils. These published K_d values averaged 4.0 ± 4.0. From 1985 through 1995, 35 additional references reported K_d or K_f values for atrazine alone (Table 21.6). Average reported K_d values are 2.4 ± 7.3 for 109 surface and subsurface soils (Paya-Perez*et al.*, 1992) and 4.9 ± 1.9 for 117 surface soils (Jaynes *et al.*, 1995).

Effect of Organic Matter in Soil

Measured K_d values for sorption of triazines by soils are often observed to be correlated with the organic carbon content in soil. For instance, atrazine sorption was correlated with organic carbon in studies involving 25 Missouri agricultural soils ($r^2 = 0.82$) (Talbert and Fletchall, 1965), 9 surface and subsurface Nebraska soils (Stolpe and Shea, 1995), 36 Wisconsin surface and subsurface soils ($r^2 = 0.84$) (Seybold *et al.*, 1994), 51 Belgian soils ($r^2 = 0.85$) (Van Bladel and Moreale, 1982), and 109 surface and subsurface Spanish soils ($r^2 = 0.82$) (Paya-Perez *et al.*, 1992). Also, sorption was greater on the earthworm burrow linings than on bulk soil. These linings are enriched in organic carbon and soluble organic carbon relative to bulk soil, (Stehouwer *et al.*, 1993, 1994). Sorption for other triazines has also been correlated with the organic carbon content of soils. For example, Van Bladel and Moreale (1982) found a correlation (i.e., $r^2 = 0.77$) between K_d values for sorption of cyanazine and the organic carbon content of 51 soils.

Statistical correlation between K_d values and organic carbon content is evidence that soil organic matter has a particularly high affinity for triazines; however, such correlations should not be inferred as evidence of a partition mechanism. As previously discussed, sorption is a complex synergism of many mechanisms. Furthermore, several reports have found little or no correlation between triazine sorption and the organic carbon content of soil. For instance, Koskinen and Moorman (1985) in their review of 1964–1984 literature found that for soils with less than 10% organic carbon, there was only a weak correlation ($r^2 = 0.64$) between atrazine K_d values and SOC content, using single variable regression (Figure 21.9). More recently, little correlation was found between atrazine sorption and the organic carbon content of 26 surface and subsoil samples of 6 soils ($r^2 = 0.61$) (Johnson and Sims, 1993), and no correlation between K_d and organic carbon content was found for 15 soil samples from surface and subsurface horizons of 5 soils (Sonon and Schwab, 1995). Johnson and Sims (1993) also found no correlation between cyanazine K_d values and the organic carbon content ($r^2 = 0.38$) of 26 surface and subsoil samples from 6 soils.

Solute leaching models often require that sorption be predicted for soils on which no prior measured sorption data is available. A common means of extending limited sorption data has been to express sorption on an organic carbon (OC) basis, ($K_{oc} = K_d$/% OC), where % OC is the percent organic carbon in the soil. With pesticides for which sorption is strongly correlated with organic carbon content, K_{oc} values have much less soil-to-soil variability than K_d values. With triazines the efficacy of expressing sorption on an organic carbon basis to reduce soil-to-soil variability of sorption coefficients is mixed. For instance, Koskinen and Moorman (1985) found greater variability among K_{oc} values than K_d values for both atrazine ($K_d = 4.0 \pm 4.0$, $K_{oc} = 190 \pm 279$) and simazine ($K_d = 5.0 \pm 12$, $K_{oc} = 225 \pm 338$).

Effect of Clay Content

Although triazines are dominantly sorbed by soil organic matter, the clay minerals also make a substantial contribution to triazine sorption by soils. For instance, the organic and inorganic components comprise 11% and 89%

Table 21.6 References on the quantification of triazine sorption, K_d or K_f, on surface and subsurface soils

Chemical	Sorption	No. of soils	References
Ametryn	K_d	38	Liu *et al*. (1970); Liu and Qian (1995)
	K_f	2	Yamane and Green (1972)
Atrazine	K_d	485	Barriuso and Calvet (1992); Barriuso *et al*. (1992a); Clay *et al*.(1988b); Dunigan and McIntosh (1971); Green and Yamane (1970); Hermosin *et al*. (1982); Hilton and Yuen (1963); Huggenberger *et al*. (1973); Hurle and Freed (1972); Jaynes *et al*. (1995); Johnson and Sims (1993); Liu and Qian (1995); Moreale and Van Bladel (1981); Nearpass (1967); O'Bien and Green (1969); Paya-Perez *et al*. (1992); Persicani *et al*. (1995); Pestemer and Auspurg (1987); Pignatello and Huang (1991); Roy and Krapac (1994); Selim and Ma (1995); Seybold *et al*. (1994); Shelton *et al*. (1995); Stehouwer*et al*. (1994); Talbert and Fletchall (1965);Van Bladel and Moreale (1982); Weber (1993); Wehtje *et al*. (1983)
	K_f	152	Barriuso *et al*. (1992a); Brouwer *et al*. (1989); Burkhard and Guth (1981); Businelli *et al*. (1992); Chen and Wagenet (1997); Clay and Koskinen (1990a, b); Clay *et al*. (1988a); Dao and Lavy (1978); Dousset *et al*. (1997); Embling *et al*. (1983); Graber *et al*. (1995); Green and Corey (1971); Grover and Hance (1970); Guo *et al*. (1991a, b); Hance (1967); Harris (1966); Hayes *et al*. (1968); Huang *et al*. (1984); Iglesias-Jiménez *et al*. (1996); Jones and Estes (1984); Liu *et al*. (1995); Ma *et al*. (1993); Mersie and Seybold (1996); Moreau and Mouvet (1997); Moyer*et al*. (1972); Pignatello and Huang (1991); Raman *et al*. (1988); Rao *et al*. (1979); Rao and Davidson (1979); Rochette and Koskinen (1996); Seybold and Mersie (1996); Singh *et al*. (1990); Sonon and Schwab (1995); Stehouwer *et al*.(1993); Swanson and Dutt (1973); Walker and Blacklow (1994); Wietersen *et al*. (1993); Wijayaratne (1982); Yamane and Green (1972)
Cyanazine	K_d	81	Johnson and Sims (1993); Liu and Qian (1995); Moreale and Van Bladel (1981); Van Bladel and Moreale (1982)
	K_f	38	Boesten and van der Pas (1988); Businelli *et al*. (1992); Clay *et al*. (1988a); Dios *et al*. (1991); Gamerdinger *et al*. (1991); Majka and Lavy (1977)
Deethylatrazine	K_d	10	Roy and Krapac (1994)
	K_f	10	Brouwer *et al*. (1989); Mersie and Seybold (1996); Moreau and Mouvet (1997); Seybold and Mersie (1996)
Deisopropylatrazine	K_f	7	Brouwer *et al*. (1989); Mersie and Seybold (1996); Seybold and Mersie (1996)
Dipropetryn	K_d	4	Liu and Qian (1995)
	K_f	6	Murray *et al*. (1975)
Hydroxyatrazine	K_f	10	Clay and Koskinen (1990b); Mersie and Seybold (1996); Moreau and Mouvet (1997); Seybold and Mersie (1996)
Hydroxypropazine	K_f	2	Weber *et al*. (1969)
Metribuzin[a]	K_d	38	Ladlie *et al*. (1976); LaFleur (1979a); Mallawatantri and Mulla (1992); Peter and Weber (1985); Savage (1976); Scott and Phillips (1972); Sharom and Stephenson (1976)
	K_f	29	Boesten and van der Pas (1988); Bouchard *et al*. (1982); Dao (1991); Graham and Conn (1992); Hance (1976); Harper (1988); Mallawatantri *et al*. (1996)
Prometon	K_d	30	Scott and Phillips (1972); Talbert and Fletchall (1965); Liu and Qian (1995)
Prometon	K_f	8	Harris (1966); Singh*et al*. (1990); Weber *et al*. (1969)
Prometryn	K_d	47	Doherty and Warren (1969); LaFleur (1976); Liu and Qian (1995); Talbert *et al*. (1971); Talbert and Fletchall (1965)
	K_f	15	Davidsonand McDougal (1973); Harris (1966); Hayes *et al*. (1968); Hermosin *et al*. (1982); Murray*et al*. (1975); Weber *et al*. (1969)
Propazine	K_d	25	Talbert and Fletchall (1965)
Propazine	K_f	8	Burkhard and Guth (1981); Harris (1966)
s-ethyl metribuzin[a]	K_f	4	Dao (1991)
S-glutathione atrazine	K_f	4	Clay and Koskinen (1990b)
Simazine	K_d	168	Alva and Singh (1990, 1991); Day *et al*. (1968); Frissel and Bolt (1962); Hermosin *et al*. (1982); Hilton and Yuen (1963); Hodges and Talbert (1990); Hurle and Freed (1972); Liu and Qian (1995); Nearpass (1965); Nicholls *et al*. (1984); Reddy *et al*. (1992); Talbert and Fletchall (1965); Williams (1968)
	K_f	52	Burkhard and Guth (1981); Businelli *et al*. (1992); Gamerdinger *et al*. (1991); Hance (1976); Harris (1966); Kookana *et al*. (1990–1993); Singh *et al*. (1989); Spurlock and Biggar (1990); Streck *et al*. (1995); Walker and Blacklow (1994)
Simetryn	K_d	2	Kanazawa (1989)
	K_f	2	Kanazawa (1989)
Terbuthylazine	K_d	3	Zsolnay *et al*. (1994)
	K_f	31	Businelli *et al*. (1992); Dousset *et al*. (1997)
Terbutryn	K_d	49	Barriuso and Calvet (1992); Barriuso *et al*. (1992b); Gaillardon *et al*. (1978); Liu and Qian (1995)
	K_f	10	Barriuso and Calvet (1992)
Trietazine	K_d	1	Frissel and Bolt (1962)

[a] An asymmetric triazine.

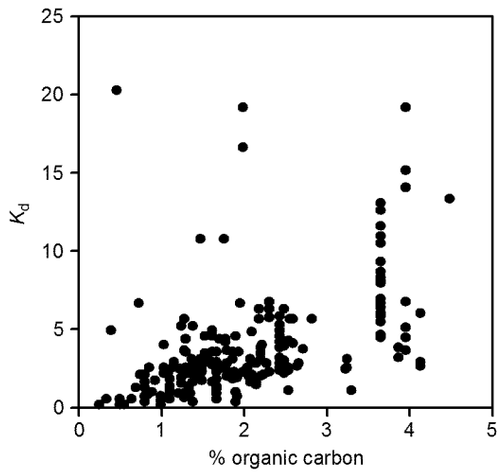

Figure 21.9 Relationship between K_d for sorption of atrazine on soils and the percent organic carbon content of the soils (data from Koskinen and Moorman, 1985).

respectively of the clay size fraction of a Webster soil and were found to contribute 68% and 32% respectively of the affinity of the soil clay for atrazine (Laird *et al.*, 1994). On a mass basis, clay-size particles have substantially more surface area and more active sites than either silt or sand-size particles. Smectites, in particular, have a large potential for influencing triazine sorption because they contribute much of the inorganic surface area of soils. It has been suggested that sorption should be expressed on the basis of soil surface area rather than on a mass basis (Pionke and DeAngelis, 1980); however, surface area calculations do not consider the chemical nature of soil surfaces, which is equally as important as the total amount of surface in determining sorption capacities and, in particular, sorption mechanisms.

The type of clay present in a soil influences triazine sorption (Brown and White, 1969). Furthermore, variations in surface properties among different samples of the same clay type greatly influence sorption. For instance, sorption of atrazine on 13 clay samples, of which smectite was the dominant mineral, ranged from 0% to 100% of added atrazine (Figure 21.7), and was inversely correlated to the surface charge density of the smectites (Laird *et al.*, 1992). Such data illustrate the complexity of sorption processes and the reason why simple predictive models relying on % OC, % clay, or surface area normalizations may fail to predict accurately the sorption of triazine by a particular soil.

Effect of pH

Numerous reports have shown that soil pH affects triazine sorption (Table 21.7). As a general rule, sorption of triazines by soils increases with decreasing pH. For instance, with soil pH in the range of 4–6, more atrazine is sorbed by soil than with a pH of 7 or greater (McGlamery and Slife, 1966; Goetz *et al.*, 1988; Clay *et al.*, 1988b; Clay and Koskinen, 1990b; Liu *et al.*, 1995). At low soil pH levels, cation exchange may be the dominant binding mechanism; at high pH, hydrogen bonding (Welhouse and Bleam, 1993a, b) and hydrophobic attraction increase in importance.

Increasing soil pH by adding ammonia-based fertilizer has been shown to decrease atrazine sorption by 50% and to increase atrazine desorption from soil (Liu *et al.*, 1995). It is unclear if the effects were due only to changes in pH. Increasing the soil pH with ammonia also increased the dissolved organic carbon content from 60 to 700 ppm in the soil solution, which may have affected the atrazine sorption–desorption characteristics. Changing the base from ammonia to KOH or NaOH also influenced the dissolved organic carbon content and affected atrazine sorption (Clay *et al.*, 1996). Furthermore, high solution pH promotes hydrolysis of atrazine to hydroxyatrazine, which may influence sorption.

Effect of Dissolved Organic Carbon

Dissolved organic carbon in soil solution has been shown to increase, decrease, or have little or no measurable effect on the initial binding of triazines to soil. No relationship was found between dissolved organic carbon and sorption of terbuthylazine on two soils; however, in a third soil, sorption K_d was inversely correlated to dissolved organic carbon (Zsolnay *et al.*, 1994). Similarly, dissolved organic content from some sources increased atrazine sorption, but from other sources had no effect (Barriuso *et al.*, 1992a).

Dissolved organic carbon content does, however, appear to influence the release of atrazine from soil (Clay and Koskinen, 1990a; Liu *et al.*, 1995), with more released in the presence of dissolved organic content. Fulvic acid in

Table 21.7 References for the effect of selected factors on triazine sorption to soil

Factor	Triazine	References
pH	Ametryn	Yamane and Green (1972)
	Atrazine	Barriuso and Calvet (1992); Clay*et al*. (1988b); Clay and Koskinen (1990b); Huang *et al*. (1984); Liu *et al*.(1995); McGlamery and Slife (1966); Nearpass (1967); Walker and Blacklow (1994); Weber (1993); Wijayaratne (1982); Yamane and Green (1972)
	S-glutathione atrazine	Clay and Koskinen (1990b)
	Hydroxyatrazine	Clay and Koskinen (1990b)
	Hydroxypropazine	Weber *et al*. (1969)
	Metribuzin[a]	Ladlie *et al*. (1976)
	Prometon	Weber *et al*. (1969)
	prometryn	Weber *et al*. (1969)
	Simazine	Nearpass (1965, 1967)
	Terbutryn	Barriuso and Calvet (1992)
Organic amendments	atrazine	Guo *et al*. (1991a, b); Shelton *et al*. (1995); Stehouwer *et al*. (1993)
	Terbutryn	Gaillardon *et al*. (1978)
Aging	Atrazine	Barriuso *et al*. (1992a, b); Barriuso and Koskinen (1996); Capriel *et al*. (1985); Khan (1982a, c); Khan and Behki (1990); Koskinen and Rochette (1996); Koskinen *et al*. (1995); O'Bien and Green (1969); Pignatello and Huang (1991); Pignatello *et al*. (1993); Rao and Davidson (1980)
	Cyanazine	Boesten and van der Pas (1983)
	Metribuzin[a]	Boesten and van der Pas (1983)
Temperature	Ametryn	Yamane and Green (1972)
	Atrazine	Dao and Lavy (1978); Hurle and Freed (1972); McGlamery and Slife (1966); Rochette and Koskinen (1996); Talbert and Fletchall (1965); Yamane and Green (1972)
	Metribuzin[a]	Graham and Conn (1992)
	Prometon	Weber *et al*. (1965)
	Propazine	Talbert and Fletchall (1965)
	Simazine	Hurle and Freed (1972); Talbert and Fletchall (1965)
Electrical conductivity	Atrazine	Dao and Lavy (1978); Hurle and Freed (1972)
	Simazine	Alva and Singh (1991)
Cation saturation	Atrazine	Nearpass (1967); Swanson and Dutt (1973)
	Prometon	Weber *et al*. (1965)
Moisture content (soil : solution ratio)	Atrazine	Green and Yamane (1970); Grover and Hance (1970); Hance and Embling (1979); Nearpass (1967); Rochette and Koskinen (1996)
	Simazine	Nearpass (1967)
Component removal	Atrazine	Huang *et al*. (1984); Laird *et al*. (1994)
Surfactants	Atrazine	Huggenberger *et al*. (1973); Iglesias-Jiménez *et al*. (1996)

[a] An asymmetric triazine.

solution may form a complex with atrazine (Haniff *et al*., 1985; Gamble *et al*., 1986a, b) or catalyze its hydrolysis to the hydroxy species (Gamble and Khan, 1990). Wang *et al*. (1990) reported that small molecular weight fractions of fulvic acid compete with atrazine for binding sites on larger molecules.

Effect of Concentration

The amount of a triazine applied to soils has been shown to influence their retention by soils, and in most cases, the percentage sorbed to soil decreases as the triazine concentration increases. This trend is indicated by the slopes of Freundlich isotherms ($1/n$ └ 1.0). For instance, the average value for $1/n$ for atrazine sorption isotherms in 43 soils from references cited in this section is 0.85 (Clay *et al*., 1988a; Brouwer *et al*., 1989; Clay and Koskinen 1990a, b; Pignatello and Huang 1991; Stehouwer *et al*., 1993; Gaber *et al*., 1995; Liu *et al*., 1995; Sonon and Schwab 1995; Stolpe and Shea 1995; Rochette and Koskinen 1996). On the other hand, atrazine sorption has also been shown to be concentration independent in a number of soils (Gamerdinger *et al*., 1991; Guo *et al*., 1991a, b; Roy and Krapac, 1994; Sonon and Schwab, 1995).

The cause of the concentration dependence is not known. Using the data from 62 isotherms from the above references, there is no correlation between $1/n$ for atrazine sorption and organic carbon content, clay content, or pH. It appears the heterogeneity of soils results in a continuum of sorption sites with differing amounts of low- and high-energy sites in different soils.

Effect of Aging

The incubation time or aging of triazines in soil influences retention (Capriel *et al*., 1985; Pignatello and Huang, 1991; Barriuso *et al*., 1992a, b). Early in the aging process, most added triazines are relatively easily desorbed. However, over time larger portions of the amount applied become very slowly desorbable, nondesorbable, or bound to soil (Rao and Davidson, 1980; Khan, 1982a, c; Pignatello and Huang, 1991; Winkelmann and Klaine 1991). Aging effects on sorption–desorption processes have been characterized by the calculation of apparent sorption coefficients, $K_{d,app}$, for triazine remaining after a given incubation period (Pignatello and Huang 1991; Barriuso *et al*., 2004; Regitano *et al*., 2006). $K_{d,app}$ values have generally increased with incubation time by a factor of 2–42 in aged samples as compared to $K_{d,app}$ for 'fresh' samples, and is directly related to the age of the residue.

It is generally accepted that the increase in sorption resulting from aging decreases the availability of the triazine for transport, plant uptake, and microbial degradation, resulting in the pesticide becoming increasingly recalcitrant. A variety of studies have suggested that only the pesticide in solution, or pesticide that is readily desorbable from soil, is available for either transport or degradation. Availability would be directly related to the pesticide's ability to be desorbed from soil. Therefore, weakly sorbed and easily desorbed pesticides would be readily available for transport and biodegradation. In contrast, pesticides that are strongly sorbed and hysteretic during desorption would be slowly available over time, and extremely strongly sorbed pesticides would be unavailable, since they tend to form bound residues. In column elution studies, aged atrazine residues were less mobile than freshly injected atrazine (Pignatello *et al*., 1993). Triazines that persist in soils also become increasingly less bioavailable, as indicated by markedly declining rates of biodegradation with aging (Radosevich *et al*., 1995; Kristensen *et al*., 2001; Park *et al*., 2003; Barriuso *et al*., 2004; Regitano *et al*., 2006). In some instances, the sorbed fraction of the pesticide is totally resistant to microbial attack, whereas in others sorption only reduces its release rate, but does not eliminate biodegradation.

Pesticide-degrading microorganisms have been recently used to initially characterize the bioavailability of aged pesticide residues. For instance, the bioavailability of aged atrazine residues to *Pseudomonas* sp. strain ADP (Mandelbaum *et al*., 1995; Jacobsen *et al*., 2001), *Ralstonia* sp. strain M91-3 (Radosevich *et al*., 1995), and *Agrobacterium radiobacter* strain J14a (Struthers *et al*., 1995) has been determined. A recent study suggested that bacteria can access specific regions where the herbicide is sorbed, which was supported by higher atrazine mineralization rates than would be predicted by solution concentrations (Park *et al*., 2003). In other studies, it was found that in spite of increased $K_{d,app}$ values with aging, *Pseudomonas* sp. strain ADP could mineralize both solution phase (aqueous extractable) and sorbed phase (methanol extractable) atrazine (Barriuso *et al*., 2004) and simazine (Regitano *et al*., 2006).

In many soils, even freshly added triazines are not reversibly desorbed; that is, the desorption isotherm does not match the sorption isotherm (Table 21.8). This phenomena, known as hysteresis (1/*n*-sorption 1/*n*-desorption), has been observed for atrazine in several studies (Clay *et al*. 1988a; Clay and Koskinen, 1990a, b). There are several proposed explanations for hysteresis. Physical and chemical changes in soil solution may influence triazine retention (Clay *et al*., 1988b; Clay and Koskinen, 1990a; Gamerdinger *et al*., 1991). Triazines may become incorporated into soil organic matter complexes (Wang *et al*., 1991, 1992) or become chemically or microbially degraded, with the metabolites differentially bound to soil (Capriel *et al*., 1985; Clay and Koskinen, 1990a). Freundlich desorption coefficients

Table 21.8 References on the quantification of triazine desorption from soil

Triazine	Sorption coefficient	No. of soils	References
Atrazine	K_f	16	Clay and Koskinen (1990a, b); Clay *et al*. (1988a); Gaber *et al*. (1995); Liu *et al*. (1995); Ma *et al*. (1993); Mersie and Seybold (1996); Moreau and Mouvet (1997); Raman *et al*. (1988); Rochette and Koskinen (1996); Seybold and Mersie (1996); Stehouwer *et al*. (1993); Swanson and Dutt (1973)
Cyanazine	K_f	1	Clay *et al*. (1988a)
Deethylatrazine	K_f	6	Mersie and Seybold (1996); Moreau and Mouvet (1997); Seybold and Mersie (1996)
Deisopropylatrazine	K_f	3	Mersie and Seybold (1996); Seybold and Mersie (1996)
S-glutathione atrazine	K_f	4	Clay and Koskinen (1990b)
Hydroxyatrazine	K_f	10	Clay and Koskinen (1990b); Mersie and Seybold (1996); Moreau and Mouvet (1997); Seybold and Mersie (1996)
Metribuzin[a]	K_f	4	Mersie and Seybold (1996)
Prometon	K_d	7	LaFleur (1976); Weber *et al*. (1969)
Simazine	K_d	23	Williams (1968)

[a]An asymmetric triazine.

can be dependent on the sorbed atrazine concentration, with low concentrations retained to a greater extent and more difficult to desorb than higher concentrations (Barriuso *et al.*, 1992). Also, in aged field residues desorption K_f values increase slightly with aging.

It appears that triazines bind to soil by several mechanisms, and the mechanisms or binding strengths change with time. For example, supercritical fluid (SF)-CO_2 extracted 48% of applied atrazine 35 days after application, but only 31% after 138 days. Extraction efficiency using SF-CO_2/5% methanol was 66% of the atrazine present in the soil after 35 days compared to 50% at 138 days (Koskinen *et al.*, 1995). These data indicate that either binding mechanism(s) become stronger with time or that there are multiple binding sites with different binding energies. In the latter case, atrazine on labile sites may have been desorbed and degraded, leaving only the atrazine bound to high-energy sites.

As triazines age in the field they form bound residues (Table 21.9). The most stable bound residues are associated with humified organic matter – especially when that humified organic matter is associated with the coarse clay size fraction (0.2–2 μm). The largest proportion of total bound atrazine residues in whole soil was in the coarse clay size fraction, which also contained 50% of the total organic carbon (Barriuso and Koskinen, 1996). The ratio of bound residues to organic carbon content decreased with particle size and was highest in the fraction ⌋ 50 μm, which is rich in nonhumified organic matter.

Bound triazine residues are very resistant to decomposition. Nine years after application of ^{14}C-atrazine to soil, about 50% of the ^{14}C was still present in the bound form in humic materials (Capriel *et al.*, 1985). Of this bound ^{14}C-atrazine, hydroxyatrazine, deethylhydroxyatrazine, and deisopropylhydroxyatrazine could be detected in measurable quantities. Khan and Behki (1990) conducted a laboratory study showing that a *Pseudomonas* spp. could release bound ^{14}C residues from soil treated with ^{14}C-atrazine.

Effect of Water Content

Methods commonly used to obtain sorption coefficients require the moisture in the soils to be above field capacity so that the aqueous phase containing the test pesticide can be separated from the soil. Using the slurry technique, most research has focused on the effects of the soil:solution ratio on sorption (Table 21.7). There has been little research on the effect of soil moisture.

Rochette and Koskinen (1996) developed a system that uses supercritical CO_2 to remove pesticides from the aqueous phase of an unsaturated soil without first requiring the separation of the solution from soil. Using this technique, sorption coefficients were found to increase with increasing organic carbon and clay contents for three field-moist soils. Moreover, sorption significantly increased in sand as gravimetric moisture content increased from 4% to 16%, and in a silt loam as moisture increased from 9.6% to 27%.

Effect of Temperature

A variety of studies have shown that as temperature increases, sorption may increase, decrease, or remain the same, with isosteric heats of sorption being very low (Table 21.7). These studies have used the batch slurry technique, so the impact of temperature on water–triazine interactions may mask surface–triazine interactions. In contrast, at 10% soil moisture isosteric heats of atrazine sorption ranged from − 10 to − 12 kcal/mol determined with the SF technique (Koskinen and Rochette, 1996). Sorption coefficients in field-moist soils were much greater than are typically obtained with the batch slurry system, while heats of sorption were much more negative, indicating greater sorption at low moisture contents.

Table 21.9 References on triazine-bound residues in soil

Triazine	Parameter-investigated	References
Atrazine	Localization	Barriuso *et al.* (1991)
	Elution	Pignatello *et al.* (1993)
	Desorption	Barriuso *et al.* (1992c)
	Distribution	Barriuso and Koskinen (1996); Capriel *et al.* (1985)
Prometryn	Extractability	Dupont and Khan (1992); Khan (1982b, c); Khan and Hamilton (1980)
Metribuzin[a]	Extractability	Dupont and Khan (1992)
Simazine	Desorption	Scribner *et al.* (1992)

[a]An asymmetric triazine.

Conclusions

Triazine interactions with soils and soil materials are extremely complex and involve numerous bonding mechanisms and synergistic effects. Bonding mechanisms include covalent, ionic, and H-bonding, as well as π–π, van der Waals, and hydrophobic interactions. The electro- and stereo-chemistry of each active site is unique, affording an opportunity for several of the above bonding mechanisms to interact simultaneously with triazine molecules. Thus, the organic and inorganic constituents of soils may be visualized as having a nearly unlimited number of active sites with a continuum of potential bonding energies. Although macroscopic soil properties – such as % OC, % clay, pH, % moisture, and temperature – are statistically correlated with triazine sorption by soils, such correlations offer only vague hints as to the nature of bonding mechanisms. Simplistic sorption prediction models, such as sorption coefficients normalized by the organic carbon content of soil, often work reasonably well – but may fail spectacularly for specific soils or specific conditions due to the complexity of the sorption process.

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Chapter 22

Microbial Degradation of *s*-Triazine Herbicides

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Summary

The ways in which microorganisms affect the fate of *s*-triazine herbicides and, in a larger sense, all organic pesticides in the environment, are the subject of intense investigation. Among the pesticides, atrazine represents one of the most extensively investigated compounds.

Recently there have been several advances in our understanding of the cellular and molecular mechanism(s) by which microorganisms transform *s*-triazines. Enzymes and genes for the dealkylation, dechlorination, and subsequent mineralization of *s*-triazine herbicides to harmless compounds – such as carbon dioxide (CO_2) and ammonia (NH_3) – have been reported in great detail. Moreover, sequencing of these genes has led to the finding of homologous genes on transferable elements (plasmids) among various bacterial species. Some microorganisms have the ability to remove *s*-triazines completely from the environment. Current research is focused on the application of these fundamental results in order to develop better tools to understand microbial degradation and aid in environmental remediation.

Introduction

Symmetrical triazines, like other anthropogenic or natural organic chemicals introduced into the environment, are subjected to microbial transformation processes. Environmental sites of most interest are agricultural fields, lakes, rivers, sediments, potable water, and groundwater. These sites also play a key role in the degradation of *s*-triazines and the eventual complete mineralization (e.g., CO_2 and NH_3) of these compounds.

Beginning in the 1950s, when triazines such as simazine, atrazine, prometryn, and ametryn were first synthesized and tested as selective herbicides in the Geigy laboratories in Basel, Switzerland (Gast *et al*., 1955), massive research efforts have focused on the transformation and use of these compounds in the environment. The *s*-triazines represent one of the most widely used and probably the most extensively studied family of herbicides. One of the driving forces for this research was the outstanding performance of triazines with respect to their selective herbicidal effects and crop tolerance.

With the advent of improved analytical procedures, it became apparent that the large volume of *s*-triazine herbicides used, led to seasonal presence of triazines in water bodies. Nevertheless, in spite of their frequent detections, it was recognized that *s*-triazines are susceptible to degradation and did not accumulate in the environment (Brown, 1978; Cook, 1987).

Due to the large amount of information regarding the degradation of *s*-triazines and their interactions and fate in soils covered extensively in other chapters, this chapter will focus on the basic concepts and mechanisms involved in microbial degradation of these compounds.

The difficulty of elucidating mechanisms and pathways for the degradation of *s*-triazine compounds is illustrated by the continuous effort over more than 40 years to define the respective roles of biotic versus abiotic degradation pathways. As early as the 1960s it was evident that the capacity of soil microbial populations to release CO_2 from *s*-triazines was variable. Degradation depended on the microbial composition of the soil (diversity and biomass) and on soil conditions (i.e., soil type, temperature, humidity, pH, additional energy sources, etc.) (Knusli *et al*., 1969; Walker, 1987).

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Table 22.1 Microorganisms capable of degrading *s*-triazine herbicides in pure culture

Bacteria and actinomycetes	Target *s*-triazine	Additional substrates	Moiety removed	Reference
Acinetobacter junii	Simazine		Side chain	Feakin *et al*. (1995)
Agrobacterium radiobacter	Atrazine	Ametryn, cyanazine, prometon, simazine	Ring and side chain	Moscinski *et al*. (1996)
Bacillus cereus	Prometryn	Simetryn	Side chain	Mizrachi (1994)
Bacillus spp.	Atrazine		Ethyl side chain	Korpraditskul *et al*. (1993)
Klebsiella pneumonia	Deethylatrazine	Deisopropylatrazine	Chlorine and side chain	Cook and Hutter (1981)
Nocardia	Atrazine		Side chain	Giardina *et al*. (1982)
Pseudomonas ADP	Atrazine	Propazine, simazine	Mineralization	Mandelbaum and Wackett (1996)
Pseudomonas strain *26*	Prometryn		Methylthio, side chain	Grossenbacher (1986)
Pseudomonas strain *A*	Atrazine		Side chain	Grossenbacher *et al*. (1984)
Pseudomonas spp. strains	Atrazine		Side chain	Behki and Khan (1986)
Pseudomonas spp. strain DSM 93-99 (YAYA6)	Atrazine		Mineralization	Yanze-Kontchou and Gschwind (1995)
Pseudomonas fl uorescens strains LMG 10141 and 10140	Atrazine		Side chain	Vandepitte *et al*. (1994)
Rhodococcus TE1	Atrazine	Propazine, simazine, cyanazine	Side chain	Behki (1995)
Rhodococcus rhodochrous	Atrazine		Side chain	Feakin *et al*. (1995)
Rhodococcus B-30	Atrazine	Propazine, simazine	Side chain	Behki and Khan (1994)
Rhodococcus TE1	Atrazine		Side chain	Shao and Behki (1996)
Rhodococcus corallinus NRRLB-15444R	Deethylsimazine	Deethylatrazine	Chlorine and amine groups	Cook and Hutter (1984)
Rhodococcus corallinus strain 11	Atrazine	Atrazine metabolites	Ring and amino groups	Cook and Hutter (1984)
Rhodococcus NI86/21	Atrazine		Side chain	Cook and Hutter (1984)
Rhodococcus corallinus	Atrazine			Behki *et al*. (1993)
Soil bacterium	Atrazine		Side chain	Giardina *et al*. (1980)
Streptomyces strain PS1/5	Atrazine	Cyanazine, metribuzin, prometryn	Side chain	Shelton *et al*. (1996)
Fungi				
Aspergillus fumigatus	Simazine		Side chain	Kearney *et al*. (1965)
Pleurotus pulmonarius	Atrazine		Side chain	Masaphy *et al*. (1996)
Phanerochaete chrysosporium	Atrazine	Simazine, propazine, Terbuthylazine		Mougin *et al*. (1997)
Hymenoscyphus ericae 1318	Atrazine			Donnelly *et al*. (1993)

Obtaining a more complete understanding of microbial genes and enzymes involved in the mineralization of the *s*-triazines required isolation of pure microbial cultures capable of their transformation (Table 22.1). This task was achieved in the 1990s after more than three decades of worldwide research (Yanze-Kontchou and Gschwind, 1994; Mandelbaum *et al*., 1995; Radosevich *et al*., 1995a, b; Mandelbaum and Wackett, 1996; Moscinski *et al*., 1996; Boundy-Mills *et al*., 1997; Bouquard *et al*., 1997; Struthers *et al*., 1998). The study of pure cultures and their genes and enzymes helped delineate the basic mechanisms by which microorganisms interact with *s*-triazines in the complex and variable soil environment.

A sequence of important review publications covered much of the information on the biodegradation of *s*-triazines from the 1960s until the end of the 1980s. Knusli and Gysin (1960) described the pioneering work on the biological properties of triazine compounds and first reviewed the chemistry and herbicidal properties of triazine derivatives. Kaufman and Kearney (1970), Knusli *et al*. (1969), and Esser *et al*. (1975) summarized a large number of investigations from the 1960s to early 1970s. Results presented in these reviews indicated that three major degradative pathways co-existed: hydrolysis at C atom 2, *N*-dealkylation at C atoms 4 and 6, and splitting of the *s* -triazine ring (Figure 22.1).

Since no correlation could be observed between herbicide dissipation and $^{14}CO_2$ evolution from ring-labeled atrazine in the early days of *s*-triazine research, it was postulated by several researchers that complete ring cleavage was not the main route of triazine degradation in soils (Knusli and Gysin, 1960). Moreover, the microbiologically mediated evolution of $^{14}CO_2$ from ^{14}C-*s*-triazines applied to soils was only regarded as a significant degradation route when side-chain labeled triazines were used (Couch *et al*., 1965; Kaufman and Blake, 1970; Esser *et al*., 1975). In a review published in 1989, Erickson and Lee concluded that results of many years of research indicated the genetic systems required for microbial biodegradation of atrazine and simazine had developed in fields where atrazine had

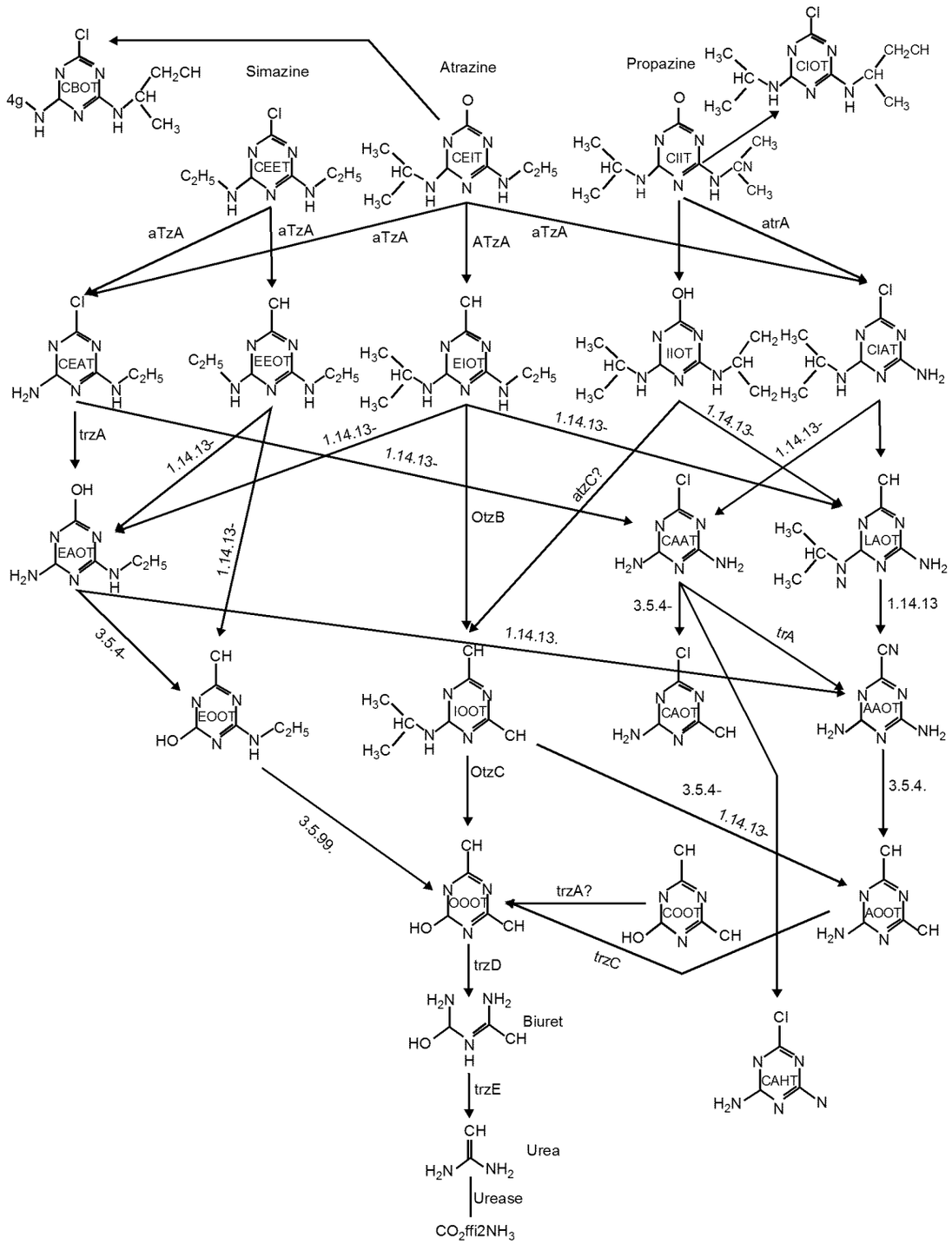

Figure 22.1 Possible microbial degradation pathways of simazine, atrazine, and propazine (Cook *et al*., 1985)[a].

[a] Numbers adjacent to arrows correspond to a possible enzyme class involved with the specific transformation. When a specific gene is known, its abbreviated name is shown next to the arrow. The informal nomenclature of Cook *et al*. (1985) is given inside the rings and is based on the substitution on the ring: A, amino; B, butylamino; C, chloro; E, ethylamino; H, hydro; I, isopropylamino; M, methylamino; O, hydroxy (or the keto tautomeric form); P, cyclopropylamino; S, methylthio; T, triazine ring; X, methoxy. The sequence of the letters for substituents is usually given in order of descending mass, except for C, S, and X, which always have priority because they dominate the properties of the compound. The system allows the structure to be deducted from the abbreviations and the abbreviation to be deducted from the structure, and it is widely used in the literature on microbial degradation of triazines.

been used for many years. This genetic adaptation appeared to be an evolving process, as organisms with the capacity to biodegrade atrazine had not been readily found throughout the environment (Geller, 1980).

Over the last 40 years, it has been shown that microbial biodegradation pathways for *s*-triazine herbicides involve a series of hydrolytic cleavage reactions of chloro, amino, and alkylamino groups from the *s*-triazine ring (Cook, 1987) and/or oxidative dealkylation of the side chains (Erickson and Lee, 1989). While biodegradation under nitrogen-limited conditions occurred for deisopropylatrazine, deethyldeisopropylatrazine, ammeline, *N*-isopropylammelide, ammelide, melamine, and cyanuric acid, it did not occur for the most widely used herbicides – atrazine and simazine (Cook, 1987). In studies in the 1980s, Cook (1987) and Erickson and Lee (1989) identified microorganisms that degraded the side chains of chlorinated triazines, but failed to dechlorinate atrazine before the molecule was dealkylated (Figure 22.1). Moreover, since the enzymatic hydrolysis of chlorinated *s*-triazine had not been demonstrated (Cook and Hutter, 1981, 1986; Cook *et al*., 1983; Behki and Khan, 1986; Hogrefe *et al*., 1986), it was suggested that the presence of both alkyl side chains inhibits dechlorination.

Results from many studies have indicated that pathways for the degradation of several *s*-triazine compounds generally converge to cyanuric acid, which is further subjected to hydrolytic ring cleavage to produce CO_2 and NH_4^+ (via hydrolysis to biuret) and urea. However, the complete understanding of the molecular and biochemical basis for the breakdown of cyanuric acid was largely due to the work of Eaton and Karns (1991a, b). Although the convergence of pathways to cyanuric acid may indicate that bacteria mastered cyanuric acid biodegradation before the compound was introduced into nature by man, this has not been proven. The possible intrinsic production of cyanuric acid in nature by forest fires or volcanic action was suspected (Cook, 1987), but its actual presence in nature from nonanthropogenic sources has not been demonstrated.

Considerable work since the 1980s has focused on the utilization of microorganisms as agents for the degradation of *s*-triazines in the environment. Cook (1987) provides an excellent review of the steps necessary to biodegrade high concentrations of *s*-triazines at spill sites.

Our review differs from previous reviews, largely by the fact that in the 1990s a considerable amount of new information was generated regarding the biodegradation of *s*-triazines at the molecular and enzymatic levels. These new insights may help explain some previously misinterpreted data regarding the microbial metabolism of *s*-triazines and may serve as a model for understanding broader questions on the evolution of new pathways in microorganisms for coping with current-day xenobiotic compounds (Shapir *et al*., 2007). More recent studies on atrazine have shown that bacteria most likely evolved mechanisms for the dechlorination of chlorinated *s*-triazines, as a first step to yield hydroxymetabolites, while the side chains were still present on the ring (Mulbry, 1994; de Souza *et al*., 1995, 1996b; Boundy-Mills *et al*., 1997). This process was previously considered by many researchers to be mainly due to chemical, rather than biological mechanisms and was thought to be catalyzed by pH, clay, and soil organic matter (Mandelbaum *et al*., 1993b). On a broader scope, today we have molecular tools, including those allowing whole genome analyses that are necessary to study the distribution and evolution of enzymes as a natural, hydrolytic mineralization pathway for chlorinated *s*-triazines. Moreover, this basic knowledge may help us better manage and remediate sites impacted by *s*-triazine herbicides (Strong *et al*., 2000).

Early Research on *s*-Triazine Interactions with Microorganisms

The *s*-triazine compounds emerged as an important group of herbicides in an era in which all chlorinated pesticides were the targets of intensive research. Some environmental studies were published prior to 1965 on *s*-triazines and other pesticides (Chandra *et al*., 1960; Guillmat, 1960; Voderberg, 1961; Eno, 1962; Kaufman, 1964; Koltcheva and Markova, 1964; Millikan and Fields, 1964; Farmer and Benoit, 1965; Sikka *et al*., 1965). However, the reported effects of *s*-triazine herbicides on soil microorganisms have been often variable and contradictory, with many records of stimulation, inhibition, and indifference in microbial activities. For example, Kaiser *et al*. (1970) concluded that activity was less disturbed in soil rich in organic matter and clay, and that the direct action on microflora was reversible.

Shortly after the introduction of *s*-triazine herbicides into agricultural markets, evidence of the biodegradability of these compounds started to accumulate. Early studies on microbial metabolism of pesticides lagged far behind comparable studies in mammalian species. The dealkylated-chlorometabolites formed through the patterns of microbial metabolism were similar to those found in rodent studies. However, as knowledge of microbial degradation advanced, it became apparent that in many cases the patterns of degradation in these two groups of organisms were often very different (Matsumura, 1982). This may be because transformations in mammals are aimed at removing the pesticide from the body, whereas microorganisms tend to break down pesticides for energy and anabolic purposes.

The involvement of microorganisms in the breakdown of *s*-triazine herbicides was initially detected because repeated applications of *s*-triazines in soil (or in artificial media inoculated with soil) caused an increase in microflora that decomposed the herbicides. One of the earliest direct observations of the ability of microorganisms to degrade

s-triazines was made by Knusli and Gysin (1960). Guillemat (1960) suggested the fungi (i.e., *Stachybotris*) that degrade organic matter in soils are able to break down simazine as a nitrogen (N) source. Reid (1960) isolated *Corynebacterium* and *Streptomycetes* spp. strains that grew slowly in the presence of triazines, and Charpentier and Pochon (1962) noted that enrichments containing members of the genera *Empedobacter, Achromobacter*, and *Microbacterium* decomposed simazine. Bryant (1963) studied the decomposition of several *s*-triazine herbicides from the 6-isopropylamine series that were differentiated by substitution in the second and fourth positions on the ring. He determined that substitutions on the fourth position determined the dominant flora and that simazine (ethylamine) enriched for *Arthrobacter* sp., ipazine (diethylamine) enriched for *Pseudomonas* sp., and propazine (isopropylamine) enriched for species of both genera. Following similar principles, Duke (1964) isolated microorganisms capable of decomposing atrazine, atraton, and ametryn. He noted that microorganisms selected from soils treated with triazines evolved more CO_2 when reseeded in soils with the herbicides than in soils without the herbicides. Kaufman (1964) showed that the microbial populations enriched from a soil containing simazine were different from that enriched from soil without simazine. He isolated primarily fungi, streptomycetes, and *Arthrobacter* strains.

Evolution of Microbial Abilities to Degrade Chlorinated *s*-Triazines

Microorganisms, through their diverse metabolic abilities, are largely responsible for the chemical balance of the biosphere and for the degradation of a majority of the known 8–10 million organic compounds (Wackett, 1996).

The role of microorganisms in the dissipation of pesticides in the soil has long been recognized (Audus, 1949). A few *as*-triazines (asymmetrical triazines) are known to occur naturally (e.g., the antibiotic Fervenulin) (Laskin and Lechavalier, 1984) and might have been previously challenged by microorganisms, the *s*-triazines are generally regarded as xenobiotics developed specifically to help control weeds (Esser *et al*., 1975). Relative to the extended evolutionary period of microorganisms in nature, agriculture has only been around for about 10 000 years, and the introduction of organic herbicides (Hartley and West, 1969) is only a half-century old. Therefore, on an evolutionary scale, the time for microbial adaptation for degrading the influx of new xenobiotic compounds is exceptionally short, and it is an ongoing process. It is striking that despite the apparent novelty of the structure of herbicides based on the *s*-triazine ring, and despite the fact that ring carbons of *s*-triazines are in the oxidation state of CO_2 (and do not serve as an energy source), microorganisms evolved catabolic pathways for their complete mineralization.

There is much evidence from observational and molecular research that indicates microbial adaptation for the mineralization of *s*-triazine herbicides has occurred since their first introduction into agriculture in the mid-1950s:

1. In most cases the half-lives of *s*-triazines in soils with a history of *s*-triazine application are considerably shorter than in soils without application history (Ostrofsky *et al*., 1997; Vanderheyden *et al*., 1997).
2. Most microorganisms isolated for their ability to degrade *s*-triazines were obtained from soils with extensive exposure to *s*-triazines.
3. The *s*-triazines that were considered nonbiodegradable became biodegradable after a number of years. For example, melamine (triamine *s*-triazine) was considered nonbiodegradable in the 1930s (Scholl *et al*., 1937), but by the 1960s it was considered moderately biodegradable (Hauck and Stephenson, 1964), and in 1981 was reported completely biodegradable and registered as a slow release N fertilizer (Allan, 1981; Cook *et al*., 1983).
4. Of the more than 200 bacterial colonies isolated from an atrazine-mineralizing mixed culture, none were found to individually degrade atrazine; however, when mixed together, their degradation ability was restored (Mandelbaum *et al*., 1993a).
5. For many years the complete mineralization of atrazine by bacteria was considered to be possible only through the combined efforts of two or more bacteria (a bacterial consortium). For example, Behki and Khan (1986) indicated that the removal of the isopropyl group from atrazine by a *Pseudomonas* sp. results in a substrate (deisopropylatrazine) that a *Rhodococcus* strain is able to mineralize completely (Cook and Hutter, 1984).
6. Despite extensive efforts over 40 years, it was not until 1993 that a pure culture of an atrazine-mineralizing bacterium was isolated, patented (Mandelbaum and Wackett, 1996), and deposited in the American Type Culture Collection (ATCC #55464). Interestingly, within a short time several other pure bacterial cultures that could mineralize atrazine were also described (Yanze-Kontchou and Gschwind, 1994; Mandelbaum *et al*., 1995; Radosevich *et al*., 1995a; Mandelbaum and Wackett, 1996; Moscinski *et al*. 1996; Boundy-Mills *et al*., 1997; Bouquard *et al*., 1997; Struthers *et al*., 1998).
7. While the *N*-alkyl side chain of atrazine was previously considered to hinder bacterial dechlorination (Behki and Khan, 1986), both mixed and pure bacterial cultures have been isolated that can rapidly dechlorinate atrazine (Mandelbaum *et al*., 1993b; Radosevich *et al*., 1995a; Mandelbaum and Wackett, 1996; Moscinski *et al*., 1996;

Bouquard *et al*., 1997; Struthers *et al*., 1998). Moreover, the bacterial *atzA* gene responsible for atrazine dechlorination (de Souza *et al*., 1995) and the atrazine chlorohydrolase enzyme have both been isolated and characterized (de Souza *etal*., 1996b).

8. Several microorganisms share identical genes encoding the enzymes ammelide aminohydrolase and cyanuric acid amidohydrolase (Eaton and Karns, 1991a, b; Karns and Eaton, 1997). This indicates that gene transfer between species has played an important role in the evolution and spread of *s*-triazine degradative capabilities within the soil microbial community (Eaton and Karns, 1991b; Karns and Eaton, 1997).
9. The *atzA*, *atzB*, and *atzC* genes – encoding enzymes necessary for the first three steps in atrazine biodegradation by *Pseudomonas* strain ADP – reside on a large, self-transmissible plasmid (de Souza *et al*., 1998a, b).
10. A *Rhizobium* strain isolated in France can dechlorinate atrazine to yield hydroxyatrazine. The dechlorinating enzyme shares 92% homology with the *atzA* enzyme previously purified from *Pseudomonas* strain ADP (Bouquard *et al*., 1997).
11. Nearly identical*atzA*, *atzB*, and *atzC* genes have been found in five phylogenetically and geographically distinct atrazine-degrading bacteria (de Souza *et al*., 1998b).

Taken together, these lines of evidence support the idea that evolution of metabolic pathways occurred after the introduction of atrazine (Shapir *et al*., 2007). Moreover, they may also indicate that certain use patterns of atrazine could potentially increase the ability of soil microorganisms to degrade the herbicide rapidly (Entry *et al*., 1995a; Vanderheyden *et al*., 1997; Shaner and Henry, 2007).

Genetics of *s*-Triazine Degradation

Despite many years of intensive research, it was not until the mid-1990s that significant genetic information about atrazine biodegradation started to accumulate. However, information concerning the genes involved in the metabolism of *s*-triazines laid some of the groundwork for more recent discoveries with atrazine (Table 22.2). For example, an inducible set of genes that encode the enzymes for 1,3,5-triazine-2,4,6-triamine (melamine) metabolism were isolated from *Pseudomonas* strain NRRLB-12227 (Eaton and Karns, 1991a). While NRRLB-12227 did not degrade atrazine, it degraded melamine and used the intermediates as a sole N source. Strain NRRLB-12227 also degraded *N*-isopropylammeline, *N*-ethylammeline, ammelide, and cyanuric acid. Three of the genes involved in the melamine degradation pathway – *trzB*, *trzC*, and *trzD* – have been cloned. Similar degradative genes have been isolated from *Pseudomonas* strain NRRLB-12228 and *Klebsiella pneumonia* strain 99 (Eaton and Karns, 1991a, b). The encoding ammelide aminohydrolase (*trzC*) and cyanuric acid amidohydrolase (*trzD*) from strain NRRLB-12227 are located on a large IncI plasmid in *Klebsiella pneumonia* strain 99 (Karns and Eaton, 1997).

Genes encoding atrazine degradation activity from *Rhodococcus* strains have also been reported by Nagy *et al*. (1995a) and by Shao and Behki (1996). In *Rhodococcus* strain TE1, an *atrA*, gene-mediating *N*-dealkylation of atrazine has been cloned (Shao and Behki, 1995).

The *atrA* gene was not expressed in *Escherichia coli*. *R. corallinus* NRRLB-15544R has the ability to dechlorinate the *s*-triazines deethylsimazine and deethylatrazine (Mulbry, 1994). The strain, however, did not degrade atrazine or simazine. The gene responsible for the dechlorination or deamination has been sequenced and is termed *trzA* (Shao *et al*., 1995). Recombinant *Rhodococcus* strains expressing both the *atrA* and *trzA* genes have been shown to transform atrazine to *N*-isopropylammelide and *N*-ethylammelide (Shao *et al*., 1995).

Table 22.2 Microbial genes involved in degradation of *s*-triazines

Gene	Enzyme	Source	Reference
trzA	*s*-triazine hydrolase	*Rhodococcus corallinus*	Shao *et al*. (1995)
trzC	Ammelide aminohydrolase	*Klebsiella pneumonia* strain 99	Karns and Eaton (1997)
trzD	Cyanuric acid hydrolase	*Klebsiella pneumonia* strain 99	Karns and Eaton (1997)
trzE	Biuret aminohydrolase	*Klebsiella pneumonia* strain 99	Karns and Eaton (1997)
atzA	Atrazine chlorohydrolase	*Pseudomonas* strain ADP	de Souza *et al*. (1996b)
atzB	Hydroxyatrazine aminoethylhydrolase	*Pseudomonas* strain ADP	Boundy-Mills *et al*. (1997)
atzC	*N*-isopropylammelide isopropylamino hydrolase	*Pseudomonas* strain ADP	Sadowsky *et al*. (1998)
atrA	Cytochrome P-450	*Rhodococcus* strain NI86/21	Shao and Behki (1995)
thcBCD	Monooxygenase	*Rhodococcus* strain NI86/21	Shao and Behki (1996)

A cytochrome P-450 has also been shown to be involved in atrazine degradation in *Rhodococcus* strains (Nagy *et al.*, 1995a). During atrazine degradation by *Rhodococcus* strain NI86/21, *N*-dealkylated metabolites and an isopropyl alcohol derivative were produced. The cytochrome P-450 monooxygenase system involved in atrazine degradation by strain NI86/21 was originally found to be involved in the degradation of thiocarbamate herbicides (Nagy *et al.*, 1995a). The *thcBCD* genes in *Rhodococcus* strains (Shao and Behki, 1996) encode the cytochrome P-450 system. Analysis of a *thcB-lacZ* fusion showed that expression of *thcB* was induced 10-fold in the presence of the herbicide EPTC. However, atrazine, simazine, or carbofuran – although metabolized by the system –had no effect on *thcB* induction. A regulatory protein encoded by the *thcR* gene is transcribed divergently from *thcB* and is essential for *thcB-lacZ* expression. Moreover, results from *thcR-lacZ* fusion studies showed that *thcR* is expressed constitutively (Shao and Behki, 1996). Another atrazine and thiocarbamate inducible gene, *thcE*, has also been isolated from *Rhodococcus* strain NI86/21 and shown to have strong amino acid homology to N,N[1]-dimethyl-4-nitrosoaniline oxidoreductase (Nagy *et al.*, 1995c).

The study of the dechlorination and downstream mineralization of chlorinated *s*-triazines has been greatly facilitated by the isolation of a pure bacterial culture, *Pseudomonas* strain ADP (Mandelbaum *et al.*, 1995) (Figure 22.2). *Pseudomonas* strain ADP uses atrazine as a sole source of N for growth, and the organism completely mineralizes the *s*-triazine ring of atrazine under aerobic and anoxic growth and nongrowth conditions. Complementation and transposon *Tn*5 mutagenesis approaches were used to isolate and to characterize gene regions that encode atrazine degradation activity from *Pseudomonas* strain ADP (de Souza *et al.*, 1995, 1996b; Boundy-Mills *et al.*, 1997; Sadowsky *et al.*, 1998).

Our overall approach has been to clone and express atrazine-metabolizing genes from *Pseudomonas* strain ADP in *E. coli* and then to delineate the genes and gene products. The first step was the construction and screening of a *Pseudomonas* strain ADP total genomic library. It was discovered that *E. coli* clones containing the gene that encoded the first metabolic step (and potentially subsequent steps) made clearing zones on agar plates containing 500 ppm atrazine (de Souza *et al.*, 1995). Previously, plates containing atrazine at a concentration exceeding its solubility limit facilitated the isolation of *Pseudomonas* strain ADP in pure culture (Figure 22.2).

A 21.5-kb *Eco*RI genomic DNA fragment from *Pseudomonas* strain ADP, designated pMD1, was shown to encode atrazine degradation activity in *E. coli* (Figure 22.3). Atrazine degradation was demonstrated by a zone-clearing assay on agar medium containing crystalline atrazine (Figure 22.2) and by chromatographic methods. A gene conferring the atrazine clearing phenotype was subsequently subcloned as a 1.9-kb *Ava*I fragment in pACYC184, designated pMD4 (Figure 22.3), and was expressed in *E. coli*. Cloning and random *Tn*5 mutagenesis showed that the 1.9-kb *Ava*I

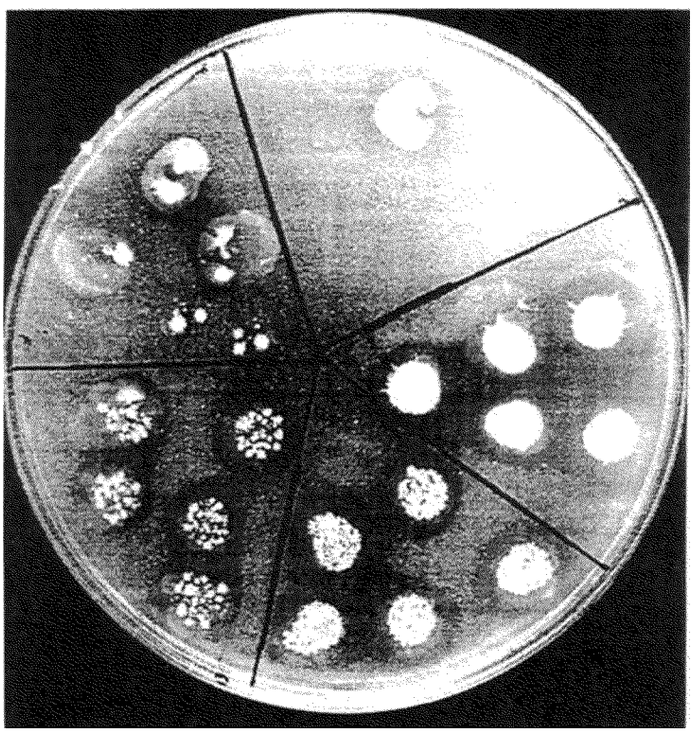

Figure 22.2 Plate assay for examining the biodegradation of atrazine.[a]
[a] Serial dilutions (10^{-4} to 10^{-7}) of *Pseudomonas* strain ADP were added to the surface of a petri dish containing minimal growth medium supplemented with 700-ppm atrazine. Plates were incubated at 35°C for 48 h. Colonies surrounded by clearing-zone show atrazine degradation.

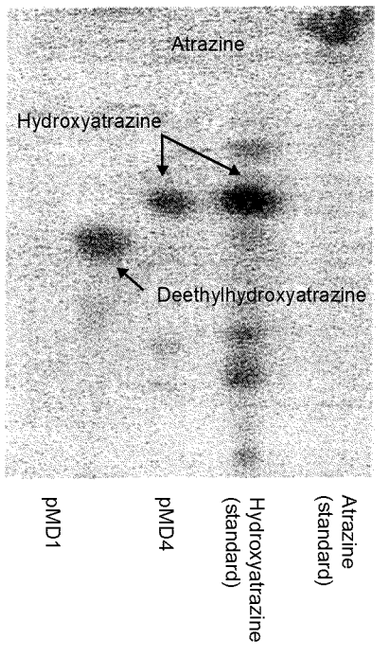

Figure 22.3 Thin layer chromatograph of (U-ring ^{14}C) atrazine degradation products of pMD1 and pMD4.[a]
[a] The silica gel plate was developed in chloroform: methanol:formic acid:water (74:20:4:2 v/v). Metabolites were visualized by phosphor imaging.

fragment was essential for atrazine dechlorination. High-pressure liquid and thin-layer chromatographic (TLC) analyses (Figure 22.3) established that the *E. coli* containing pMD4 both degraded atrazine and accumulated hydroxyatrazine. Hydroxyatrazine was detected only transiently in *E. coli* containing pMD1. A 0.6-kb *Apa*I-*Pst*I fragment from pMD4 (Figure 22.4), containing the atrazine chlorohydrolase gene, hybridized to DNA from atrazine-degrading bacteria isolated in the United States and Europe (de Souza *et al*., 1996b, 1998a). The *atzA*, *atzB*, and *atzC* genes encoding enzymes for atrazine catabolism are globally distributed and located on a self-transmissible plasmid in *Pseudomonas* strain ADP (de Souza *et al*., 1998a). Sequence data for the pMD4 gene region that encodes atrazine transformation ability indicated that a single, open reading frame of 1419 nucleotides *atzA* encodes atrazine dechlorination activity. The enzyme atrazine chlorohydrolase (*atzA*), a polypeptide of 473 amino acids, had significant amino acid identity (41%) with *trzA* – a dechlorinating enzyme from *Rhodococcus corallinus* – which has melamine as its preferred substrate (Shao *et al*., 1995; de Souza *et al*., 1996b). Our results indicate that *atzA* is a relatively small gene that produces a protein product (atrazine chlorohydrolase) with the ability to transform atrazine to hydroxyatrazine. Consequently, *atzA* is an ideal candidate for use in engineering bacteria or plants to metabolize atrazine to hydroxyatrazine, thereby remediating atrazine-containing soils at spill sites. Improvements in methods have facilitated continued progress in purification and characterization of genes and enzymes involved in microbial degradation of triazines (Martinez *et al*., 2001; Raillard *et al*., 2001; Seffernick *et al*., 2001, 2002; Shapir *et al*., 2002, 2005a, b; Sajjaphan *et al*., 2002, 2004; Wackett *et al*., 2002).

Researchers subsequently discovered that hydroxyatrazine could be incorporated into plates above its solubility limit to screen for its metabolism by other *E. coli* clones. This facilitated the isolation of *atzB*, the second step in the atrazine metabolic pathway (Boundy-Mills *et al*., 1997). Transposon *Tn*5 mutagenesis localized *atzB* to the same 21.5-kb genomic DNA fragment (pMD1) as *atzA*. The *atzB* gene encodes a 481 amino acid polypeptide that transforms hydroxyatrazine to *N*-isopropylammelide [2,4-dihydroxy-6-(isopropylamino)-*s*-triazine] (Boundy-Mills *et al*., 1997). This is due to the apparent hydrolytic removal of the *N*-ethyl group of hydroxyatrazine. Interestingly, *atzB* also shows significant substrate specificity. This enzyme will cleave the ethylamine side chain from the triazine ring, but will not remove the bulkier isopropylamine group. In addition, *atzB* showed 24.9% amino acid identity to *trzA*, an enzyme from *R. corallinus* strain NRRLB-15544R, which catalyzes a hydrolytic deamination of melamine that is similar to *atzA*. Comparison of the DNA sequences of *atzA* and *atzB* indicated that more than 600 nucleotides of upstream sequence were virtually identical.

N-isopropylammelide was subsequently used as the starting compound to screen the *Pseudomonas* strain ADP gene library for the third gene in the pathway. This gene, *atzC*, catalyzes the degradation of *N*-isopropylammelide to cyanuric acid. The higher solubility of this compound led to a combinatorial screening assay using microtiter plates and

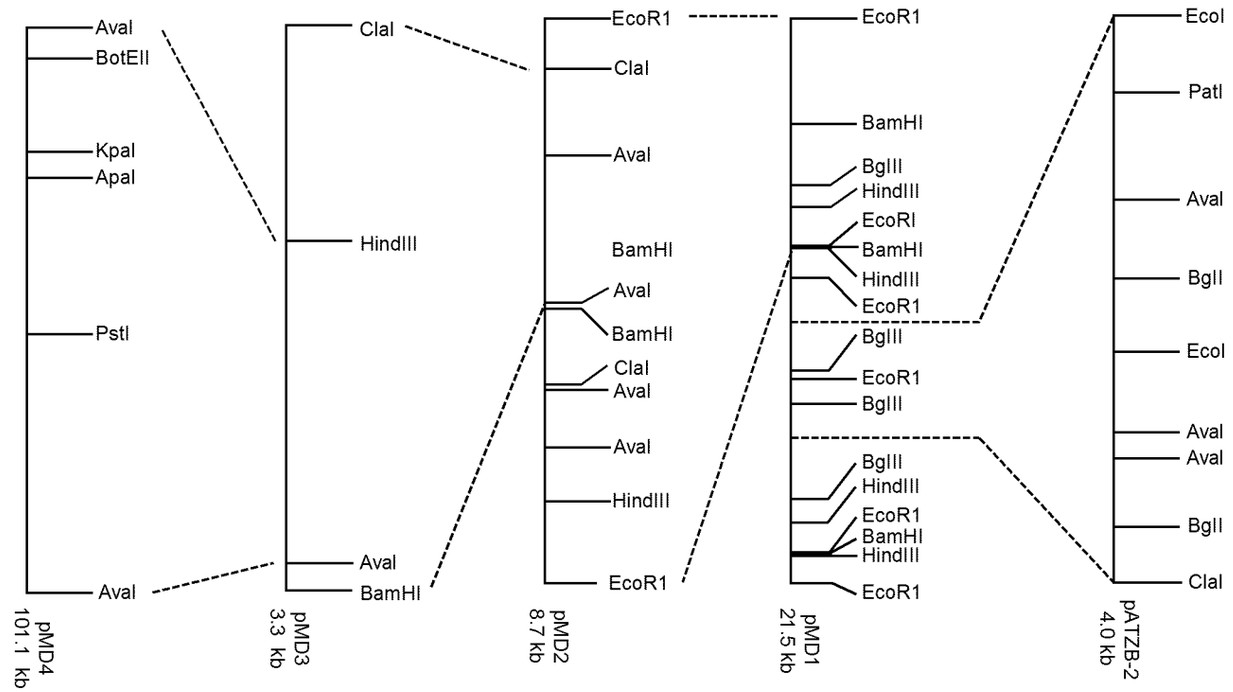

Figure 22.4 Restriction enzyme map of a 21.5 kilobase (kb) DNA fragment from *Pseudomonas* strain ADP (adapted from de Souza *et al.*, 1995).[a]

[a] The DNA fragment was successively subcloned into plasmid pACYC184 to produce plasmids pMD2, pMD3, pMD4, and pATZB-2. Plasmid pMD4 contains the *atzA* gene, while the fragment in pATZB-2 contains the *atzB* gene. Letters above the lines refer to restriction enzyme sites for the enzymes EcoRI, PstI, AvaI, BglIII, ClaI, BamHI, and HindIII. The pMD1 clone was constructed using cosmid pLAFR3.

mixtures of putative clones in a liquid medium. High-pressure liquid chromatography was used to analyze for substrate disappearance. Several *E. coli* clones expressing this activity were identified, and high-pressure liquid chromatography and GC-mass spectrometry analyses confirmed that the product produced was cyanuric acid (Figure 22.1). The *atzC* from *Pseudomonas* strain ADP removes the isopropylamine side chain of hydroxyatrazine after the ethylamine side chain is removed by the action of *atzB*. The *atzC* encodes a protein of 403 amino acids, and subsequent sequence analysis indicates that *atzC* shows modest amino acid sequence identity of 29% and 25% to cytosine deaminase and dihydroorotase, respectively (Sadowsky *et al.*, 1998).

The *atzA, atzB* and *atzC* genes are localized to a 96-kb self-transmissible plasmid, pADP-1, in *Pseudomonas* strain ADP (de Souza *et al.*, 1998a). The *atzABC* genes encoding atrazine catabolism are globally distributed and located on a self-transmissible plasmid in *Pseudomonas* strain ADP (de Souza *et al.*, 1998b). The *atzA* gene was flanked by DNA showing greater than 95% sequence identity to insertion sequence IS1071 from *Alcaligenes* strain BR60. The ability of this region of DNA to move selectively was observed in a derivative strain of *Pseudomonas* strain ADP that showed a spontaneous loss of the *atzA* gene. A gene probe for the insertion element hybridized to genomic DNA from other atrazine-degrading bacteria. Taken together, these data indicate atrazine catabolism through hydroxyatrazine is widespread, and they suggest potential molecular mechanisms for the global dispersion of the *atzABC* genes (de Souza *et al.*, 1998b).

The identification of the first three genes and their respective metabolic products reveals the overall metabolic logic of atrazine metabolism by *Pseudomonas* strain ADP. Previously, proposed pathways for bacterial atrazine metabolism described alkyl group removal, which leaves amino and chloro-substituent groups on the ring. In *Pseudomonas* strain ADP, all three substituent groups are replaced with hydroxyl groups to produce cyanuric acid, an intermediate setup for ring cleavage and subsequent partitioning by amide hydrolysis reactions (Figure 22.1). Many bacteria can metabolize cyanuric acid, suggesting that acquisition of the *atzA, atzB*, and *atzC* gene suite could confer on many bacteria the ability to grow on atrazine as the sole N source. More recently, a genomics approach was used to define the genes and enzymes involved in *s*-triazine catabolism in *Arthrobacter aurescens* (Mongodin *et al.*, 2006), a metabolically versatile bacterium that may have the ability to degrade more than 500 different *s*-trazine compounds (Shapir *et al.*, 2007).

Enzymology of *s*-Triazine Degradation

The genes and enzymes involved in *s*-triazine metabolism have only recently been identified (Table 22.2 and Figure 22.1). Prior to their discovery, the metabolism of atrazine in the environment was attributed mainly to 'co-metabolic pathways.' Since this metabolism was observed to be slow, one microorganism was thought to carry out only partial metabolism of the herbicide, and microorganisms failed to grow on atrazine as a sole source of carbon (C) and energy. In the early 1980s, three bacterial catabolic pathways for *s*-triazine compounds were shown to converge at cyanuric acid (Cook and Hutter, 1981, 1984; Grossenbacher *et al.*, 1984; Cook *et al.*, 1985) (Figure 22.1). The enzymatic transformation of cyanuric acid was initially studied in *Pseudomonas* strain D, which sequentially catabolized cyanuric acid to produce CO_2 and NH_4^{ffi} as final products. The identified intermediates are biuret and urea (Cook *et al.*, 1985), and each reaction involves the hydrolysis of C ß N bonds. Although this pathway was first identified in a *Pseudomonas* strain, it is likely that the pathway is widespread among microorganisms that can utilize cyanuric acid. In addition to bacteria, fungi have also been shown to degrade *s*-triazines. For example, the fungus *Sporothrix schenckii* JZ 6.2 can use cyanuric acid, biuret, and urea as sole N sources (Zeyer *et al.*, 1981). The degradation of all three substrates was also shown to occur in cell-free extracts (Beilstein *et al.*, 1981). These studies conclusively show that the ring moiety of the *s*-triazine herbicides is broadly susceptible to complete mineralization once the substituents on the ring are removed. Since the side chains of *s*-triazine herbicides contain the only available sources of energy that microorganisms can effectively obtain when degrading those herbicides, the bioenergetic incentive for degradation of the *s*-triazine herbicides lies with the degradation of the side chains (Cook, 1987). Indeed *s*-triazine dealkylation has been found to be a major degradation pathway in many fungi and bacteria (Kaufman and Blake, 1970; Cook, 1987).

The isolation of pure cultures of bacteria capable of complete mineralization of atrazine (Mandelbaum *et al.*, 1995; Radosevich *et al.*, 1995a; Yanze-Kontchou and Gschwind, 1995; Mandelbaum and Wackett, 1996; Moscinski *et al.*, 1996; Boundy-Mills *et al.*, 1997; Bouquard *et al.*, 1997; Struthers *et al.*, 1998) has greatly facilitated our understanding of the enzymes required for *s*-triazine degradation. Moreover, a close examination of well-characterized enzymatic and bacterial systems reveals a high degree of specificity between *s*-triazine substrates and the enzymes involved in their degradation, as follows:

1. Both alkyl side chains are necessary for activity of *s*-triazine dealkylating enzymes from *Rhodococcus* strain B-30 (Behki and Khan, 1994).
2. The chlorine–carbon bond seems to inhibit dealkylation reactions. Cook and Hutter (1984) reported dechlorination and deamination of deethylatrazine by *Rhodococcus* sp. However, their isolate failed to degrade atrazine without prior removal of the chlorine from C 2 on the ring.
3. Nagy *et al.* (1995a, c) identified an enzyme in *R. corallinus* NRRLB-15444 that can dechlorinate atrazine, but only after the prior removal of either the isopropyl or ethyl side-chains. The enzyme, however, catalyzes deamination of several less substituted *s*-triazines.
4. Substitution of the chlorine atom on ring C 2 of atrazine with a methylthio group impaired the ability of *Pseudomonas* strain ADP (Mandelbaum *et al.*, 1995) or *Pseudomonas* strain YAYA6 (Yanze-Kontchou and Gschwind, 1995) to mineralize herbicides such as ametryn.
5. A mixed culture able to mineralize atrazine could also degrade cyanazine and simazine, but cyanazine was not completely mineralized (Grigg *et al.*, 1997).
6. Purified atrazine chlorohydrolase from *Pseudomonas* strain ADP can catalyze hydrolysis of an atrazine analog substituted at the chlorine substituent by fluorine, but not analogs containing the pseudohalide azido, methoxy, and cyano groups, or the thiomethyl and amino groups. Moreover, atrazine analogs ranging in size from methyl to *t*-butyl with a chlorine substituent at C 2 and *N*-alkyl groups all underwent dechlorination by atrazine chlorohydrolase (Seffernick *et al.*, 2000).
7. Atrazine dealkylation has also been studied at the molecular and enzyme levels. During atrazine degradation by *Rhodococcus* strain NI86/21, *N*-dealkylated metabolites and an isopropyl alcohol derivative are produced. The cytochrome P-450 monooxygenase system that is involved in degradation of thiocarbamate herbicides by strain NI86/21 (Nagy *et al.*, 1995a, b) was also found to be required for atrazine dealkylation.

The *atzA* enzyme from *Pseudomonas* strain ADP has been purified from cell-free extracts of *E. coli* (pMD4) (de Souza *et al.*, 1996b). The *atzA* precipitates from solution with 20% (w/v) NH_4SO_4, making it relatively easy to purify. By gel filtration chromatography, the molecular weight of the *atzA* holoenzyme is estimated to be 245 000; when combined with the deduced subunit molecular weight of 52 421 obtained through gene sequencing, the results are consistent with either an $\alpha 4$ or $\alpha 5$ subunit structure. The protein, as isolated, does not contain significant quantities of metal. Activation by the addition of $CoSO_4$, $MnSO_4$, or $FeSO_4$ to assay mixtures, however, has been shown to

Table 22.3 Substrate range of atrazine chlorohydrolase (*atzA*) from *Pseudomonas* strain ADP[a]

Substrate degraded	Substrate not degraded
Atrazine	Deethyldeisopropylatrazine
Deethylatrazine	Melamine
Deisopropylatrazine	
Simazine	
Terbuthylazine	

[a] Determined using purified enzyme *in vitro*.

Table 22.4 Amino acid sequence identity between atrazine chlorohydrolase (*atzA*) and other entries in protein databases

Accession designator	Enzyme name	Microorganism	% Amino acid identity to *atzA*
Swiss Protein database			
P18314	Urea amidohydrolase	*Klebsiella aerogenes*	20
P25524	Cytosine deaminase	*E. coli*	22
GenBank/EMBL database			
RERTRZA	*N*-ethylammeline chlorohydrolase	*Rhodococcus corallinus*	41
S69145	Urease alpha subunit	*Rhizobium meliloti*	23
X63656	Cytosine deaminase	*E. coli*	22
D31856	Imidazolone-5-proprionate hydrolase	*Bacillus subtilus*	22

contain higher metal quantities (de Souza, 2002). Work is currently ongoing to determine if a specific coordination environment exists for metals and, if so, what the role of such a putative site might be in catalysis.

The reactions catalyzed by *atzA* are now understood in some detail. First, the conversion of atrazine to hydroxyatrazine is a hydrolytic reaction as demonstrated by showing the incorporation of ^{18}O from ^{18}O-H_2O into the hydroxyl group of the product (de Souza *et al*., 1996b). Substrate specificity studies are summarized in Table 22.3 and show that only substrates containing a chlorine atom and an alkylamino side chain were hydrolyzed. Purified *atzA* can catalyze hydrolysis of an atrazine analog substituted at the chlorine substituent by fluorine, but not analogs containing the pseudohalide azido, methoxy, and cyano groups or the thiomethyl and amino groups. Moreover, atrazine analogs ranging in size from methyl to *t*-butyl with a chlorine substituent at C 2 and *N*-alkyl groups all underwent dechlorination by atrazine chlorohydrolase (Seffernick *et al*., 2000). Melamine is not a suitable substrate for *atzA*. The K_m of *atzA* was calculated to be 150 M, and the V_{max} was 2.6 mmol/min/mg protein. Based on a holoenzyme molecular weight of 245 000, the equivalent K_{cat} is 11 s^{ffl1}. The K_{cat}/K_m value of *atzA* for atrazine is 7⌋ 10^4. While this indicates that the activity of the natural enzyme for the herbicide is reasonably high, it possibly can be further improved by protein engineering. Such improvement might be significant for applications such as enzymatic wastewater treatment or groundwater remediation.

Several other proteins show a low, but significant amino acid identity with *atzA* (Table 22.4). All of these, urease-alpha subunit (urea amidohydrolase), cytosine deaminase, and imidazolone-5-propionate hydrolase, catalyze hydrolytic reactions with substrates involved in the metabolism of nitrogenous compounds (Sadowsky *et al*., 1998). A *Rhizobium* strain capable of atrazine dechlorination has been isolated from a soil that was previously treated with atrazine (Bouqard *et al*., 1997). This bacterium could not mineralize atrazine, and it accumulated hydroxyatrazine as the sole metabolite after long-term incubations. Interestingly, 22 of the 24 identified amino acids at the N-terminus of the atrazine halidohydrolase from *Rhizobium* were identical with *atzA* from *Pseudomonas* strain ADP.

Studies on the properties of *atzA* are relevant for potential applications of this enzyme in removing atrazine from water and soil that require remediation. Atrazine is an effective herbicide, while hydroxyatrazine is nonphytotoxic and binds rapidly to soil. In this context, the first metabolic step carried out by *Pseudomonas* strain ADP represents the best possible step from an environmental standpoint. While the intact organism catalyzes atrazine hydrolysis and subsequent reactions, the enzyme could prove more efficacious for the following reasons:

1. *Pseudomonas* strain ADP makes only a low level of *atzA* because cell N needs are modest.
2. *atzA* expression is down-regulated by inorganic N sources that are present in most contaminated waters.
3. The enzyme can be produced cheaply and in large quantities by recombinant bacteria.

Biodegradation of *s*-Triazines in the Environment

The biological degradation of *s*-triazines in soil and water depends on the presence and activity of indigenous bacteria and fungi that possess enzymatic machinery to transform the *s*-triazine molecule. Several factors are thought to influence the competitiveness of indigenous and applied microorganisms in soils and aquifers. These can be divided into biotic and abiotic factors, including: nutrient limitation and specificity, moisture requirement, electron acceptors, pH, temperature, soil texture and porosity, organic matter content, antibiotic and bacteriocin production, solute types and concentrations, numbers and type of other indigenous microorganisms, selective predation by protozoa, residence time in soil, and ability to form resting structures. All of these factors, either singly or in combination, play major roles in the selective activity of various members of the microbial community. Depending on the microbial population that occupies the soil, biodegradation of *s*-triazines may yield various metabolites.

Biotic versus Abiotic Degradation

Assessment of the biotic and abiotic mechanisms for the breakdown of *s*-triazines is necessary in order to identify the major degradation pathways and to understand the soil conditions necessary for these mechanisms to occur. Until relatively recently, it was widely accepted that the dechlorination of chloro-*s*-triazines in soils is catalyzed by chemical processes, while *N*-dealkylation reactions are biologically mediated (Armstrong and Chesters, 1968; Obien and Green, 1969; Kaufman and Blake, 1970; Skipper and Volk, 1972; Cook and Hutter, 1984; Erickson and Lee, 1989; Adams and Randtke, 1992). Only *s*-triazine compounds with less bulky side chain substituents were believed to undergo bacterially mediated dechlorination (Cook and Hutter, 1984). This paradigm changed when rapid bacterial dechlorination of atrazine was observed in both mixed (Casper and Landsmann, 1992; Mandelbaum *et al*., 1993a; Weber, 1995) and pure (Mandelbaum *et al*., 1995) bacterial cultures and in soils inoculated with mixed or pure cultures (Mandelbaum *et al*., 1993a, b, 1995, 1997; Mandelbaum and Wackett, 1996; Shapir and Mandelbaum, 1997). This paradigm, however, remains rooted relatively deeply. Despite the fact that bacterial dechlorination of atrazine for mixed bacterial cultures was first published by Mandelbaum *et al*. (1993a) and is now understood at the pure culture (Mandelbaum *et al*., 1995), enzymatic, and molecular levels (de Souza *et al*., 1996a, b, 1998b), some publications still ascribe dechlorination of chlorinated *s*-triazines in soils as being solely due to chemical catalysis (Aislabie and Lloydjones, 1995; Ma and Selim, 1996).

Many authors cite the work of Armstrong and Harris (1967) in support of a chemical mechanism for soil hydroxyatrazine formation (Armstrong and Chesters, 1968; Zimdahl *et al*., 1970; Li and Felbeck, 1972; Skipper and Volk, 1972; Erickson and Lee, 1989; Adams and Randtke, 1992; Blumhorst and Weber, 1994). It should be noted, however, that Skipper and Volk (1972) and Skipper *et al*. (1967) suggested that both chemical and slow microbiological reactions contributed to atrazine degradation in some Oregon soils. In contrast, the isolation of *Pseudomonas* strain ADP and its atrazine chlorohydrolase enzyme, and the discovery of other bacteria carrying homologous enzymes and genes (Yanze-Kontchou and Gschwind, 1994; Mandelbaum *et al*., 1995; Radosevich *et al*., 1995a; Mandelbaum and Wackett, 1996; Moscinski *et al*., 1996; Bouquard *et al*., 1997; Boundy-Mills *et al*., 1997; Struthers *et al*., 1998; Martinez *et al*., 1999), suggest that microbial-mediated dechlorination of atrazine may be significant.

Coupled with the fact that microbial systems have been conclusively shown to dechlorinate chlorinated *s*-triazines, it is likely that the role of microbial dechlorination was largely underestimated. For example, in a comparison of sterile and nonsterile soils from the vadose and saturated zones of soil profiles, it was concluded that microbial mechanisms, more than any other, contributed to the formation of hydroxyatrazine in the unsaturated surface soil (Kruger *et al*., 1997).

Biodegradation in Soil

As with all organic chemicals, the eventual mineralization of herbicides in soils may be attributed almost entirely to microbial degradation in the soil system (Alexander, 1994). Even with postemergence applications, when the herbicide is primarily received by plant foliage, the soil surface is usually the major end-recipient. The degradation of herbicides applied to soil is of great practical importance because distinct soils exhibit differences in their ability to degrade herbicides such as atrazine (Sparling and Aislabie, 1996).

The soil is a complex structure with close interrelationship among factors that influence biodegradation of pesticides, such as the structure of the pesticide, presence of an effective, active microbial community capable of degradation, and bioavailability of the compound in space and time (sorption, moisture content, temperature, nutrients, and soil pH) to enzymes or to whole cells (Aislabie and Lloydjones, 1995).

The *s*-triazines are applied to soil as pre- or postemergence herbicides and are used to control weeds during the full crop growing season. Due to their relatively low aqueous solubility and adsorption to organic matter and clays, the major biodegradation occurs in the organic or A layers of the soil, while usually slower degradation rates are recorded in the subsoils (Fomsgaard, 1995). The degradation of *s*-triazines in soils occurs through several metabolic

pathways, involving a complex set of reactions comprising mainly oxidative or hydrolytic dealkylation and dechlorination reactions before ring cleavage (Figure 22.1). Atrazine dealkylation proceeds with preference for removal of the ethyl side chain over the isopropyl side chain (Sirons *et al.*, 1973). Typically this degradation process is assumed to follow first-order kinetics with estimated half-lives varying from a few weeks to many months.

The degradation pathway for *s*-triazine herbicides in soils and water has been studied extensively since their introduction to agriculture in the 1950s. For example, the relative roles of chemical versus biological degradation have been scientifically debated (Mandelbaum *et al.*, 1993b; Blumhorst and Weber, 1994; Lai *et al.*, 1995). Over the years, it has been presumed that biological dealkylation reactions prevail in soils (Byast and Hance, 1981), while dechlorination was previously considered to occur primarily through chemically catalyzed reactions (Erickson and Lee, 1989; Ma and Selim, 1996).

New data on genes encoding the dechlorination of atrazine in soils (de Souza *et al.*, 1998a), coupled with inherent difficulties in the extraction and analysis of hydroxylated metabolites, might have led researchers to underestimate the role of microbial dechlorination as a first step in the degradation of chlorinated *s*-triazines (Barrett, 1996). Hydroxytriazines bind strongly to soil organic matter and their extraction from soil is difficult (Sirons *et al.*, 1973). This, along with inadequate chromatographic protocols, might have biased extraction results and water monitoring toward favoring detection of dealkylated products over hydroxylated metabolites. For example, the extensive use of gas chromatography for the detection of both *s*-triazine parent compounds and their dealkylated metabolites is inadequate for the detection of hydroxylated compounds without the use of derivatization (Cai *et al.*, 1996). The strong binding of hydroxylated metabolites is caused by multiple modes of binding (e.g., mainly by cation exchange, but also by hydrogen bonding and charge transfer) (Senesi and Chen, 1989). Therefore, the efficiency of their extraction depends on the use of solvents that primarily disrupt cation exchange bonds (Lerch *et al.*, 1997). Indeed, mixed-mode extraction recovered 42.8% of bound atrazine residues from aged soil, and 88% of this fraction were identified as hydroxylated products (Lerch *et al.*, 1997).

Studies using adequate extraction and analytical procedures have indicated that hydroxylated metabolites are widely formed in soils through the activity of microorganisms (Winkelmann and Klaine, 1991; Demon *et al.*, 1994; Sorenson *et al.*, 1994; Chung *et al.*, 1995; Lerch *et al.*, 1995; Nakagawa *et al.*, 1995, 1996; Kruger *et al.*, 1996; Topp *et al.*, 1996; Vanderheyden *et al.*, 1997). In addition, since hydroxylated metabolites have much higher Koc values than their corresponding parent compounds, even when they exist in soils in high concentrations they leach to a much lesser extent (Seybold and Mersie, 1996). Moreover, deethylatrazine and deisopropylatrazine have Koc values of 35 and 51, respectively, which can result in faster leaching than their corresponding parent compounds (Barrett, 1996) or hydroxyatrazine. Information about the *s*-triazine degradation pathways in soils is necessary in order to produce accurate risk assessment models and in order to achieve scientifically informed regulatory policy.

Presence of *s*-Triazine-Degrading Microorganisms

The *in situ* biodegradation of *s*-triazine herbicides is dependent on the presence, number, and activity of microorganisms that possess the appropriate enzymatic capability. For example, reduced degradation rates that normally occur with increased soil depth have been attributed to reduced total bacterial activity and to the absence of specific atrazine-degrading bacteria (Shapir and Mandelbaum, 1997; Vanderheyden *et al.*, 1997). Since in most soils the population of *s*-triazine-degrading bacteria is low or nonexistent, inoculation with pure (Mandelbaum *et al.*, 1995; Yanze-Kontchou and Gschwind, 1995; Radosevich *et al.*, 1996, 1997; Masaphy and Mandelbaum, 1997) or mixed cultures (Mandelbaum *et al.*, 1993a; Finklea and Fontenot, 1996) of *s*-triazine-degrading bacteria could show that slow rates of degradation are caused by the absence of an appropriate microbial agent and by soil conditions. For example, when *Pseudomonas* strain YAYA6was inoculated into an atrazine-containing soil, the herbicide was rapidly mineralized to concentrations below 0.1 mg/kg, and the results showed that concentrations below 0.01 mg/kg could be reached (Yanze-Kontchou and Gschwind, 1995). However, under suboptimal conditions, slower degradation occurred.

Atrazine biodegradation in soil was less efficient when the water content of the soil was reduced, when the oxygen supply was limited, and when soil pH was below 7. Moreover, in a soil with high organic matter content and in a soil preincubated with atrazine prior to the addition of the bacteria, the lower bioavailability limited the rate of atrazine biodegradation. *Pseudomonas* strain ADP can also significantly accelerate the degradation of aged atrazine in soils with high clay and organic matter content (Mandelbaum *et al.*, 1995), but degradation is much more rapid when bacteria are inoculated into soils immediately after atrazine application (Masaphy and Mandelbaum, 1997).

Radosevich *et al.* (1995a) studied atrazine mineralization by indigenous microbial communities inoculated with a *Ralstonia pickettii* (previously strain M91-3 (Stamper *et al.*, 1997)) in surface soil and subsurface zones. The authors found that the mineralization rate constants (k) and overall mineralization (P-max) were higher in microcosms that were not sterilized prior to inoculation, indicating that the native microbial populations in the sediments were contributing to the overall release of $^{14}CO_2$ from (U-^{14}C -ring)-atrazine and (^{14}C -ethyl)-atrazine. They also concluded

that under vadose zone and subsurface aquifer conditions, low temperatures and the lack of degrading organisms are likely to be the primary factors limiting the biodegradation of atrazine.

In a different study, riparian forest soils with low atrazine-degrading activity were inoculated with the fungi *Phanerochaete chrysosporium* or *Trappea darkeri* on wood-chip formulations. A significant increase in the rate of atrazine degradation was recorded over noninoculated controls (Entry *et al*., 1996). More recently, Strong *et al*. (2000) showed that combined bioaugmentation and biostimulation strategies significantly increased atrazine degradation in a spill-site field soil. From these and other studies, it is obvious that the absence of an actively degrading population of microorganisms is a primary limiting factor that can be overcome by augmentation if other parameters are not limiting.

Soil Organic Matter and pH Level

In the presence of an active biodegrading microbial population, soil organic matter may support microbial activity, but its content greatly affects the sorption and bioavailability of basic hydrophobic compounds such as *s*-triazine herbicides (Ma and Selim, 1996). Organic matter content of soil positively correlates with sorption of *s*-triazines and has been shown to have the greatest adsorption affinity of all soil constituents (Brouer *et al*., 1990; Laird *et al*., 1994). The decrease in bioavailability of sorbed *s*-triazines is pH and time dependent. The longer the herbicides remain sorbed to organic matter, the more time and energy is required to remove them fully (Loehr and Webster, 1996; Pignatello and Xing, 1996; Xing *et al*., 1996).

The influence of organic matter content on bioavailability of *s*-triazines is impacted by soil pH. Ionization, water solubility (K_{sp}), and sorption to soil organic matter (K_{oc}) are all pH dependent. The *s*-triazines are basic herbicides with pk_A values of 1–5. Low soil pH values will increase both their solubility and sorption through a cation exchange mechanism. In organic soils, the bioavailability of chloro-*s*-triazines (such as atrazine, simazine, and propazine) should be the lowest in the lower range (below pH 2.0), while methoxy-*s*-triazines (such as prometon) and methylthio-*s*-triazines (such as ametryn, prometryn, and terbutryn) are moderately basic (Weber, 1994). Since low pH values also increase the solubility of the *s*-triazines, retention of the herbicides in the upper soil level (where most of the biodegradation occurs) will be highly dependent on the organic matter content of the soil.

Microorganisms both influence and are themselves influenced by the alkalinity of soil. Microorganisms have a pH range for optimum growth and activity. Most agricultural soils have a pH between 5 and 9, and microorganisms with optimum performance in this range are the most common. Fungi, as a group, tend to be more acid tolerant than bacteria (Brock *et al*., 1994). Bacteria capable of atrazine degradation have been reported to perform better when the pH values are neutral or basic (Korpraditskul *et al*., 1993; Feakin *et al*., 1995; Yanze-Kontchou and Gschwind, 1995). The activity of microorganisms in soil may change the pH of their *milieu*, with consequent change in the bioavailability of sorbed *s*-triazines and changes in the activity of degradative enzymes. Increases in soil pH can occur during proteolysis or under denitrifying conditions, while acidification can occur during the metabolism of carbohydrates, oxidation of organic N to nitrite or nitrate, sulfide to elemental sulfur or sulfate, ferrous to ferric ion, or other metal oxidations.

Exogenous Organic Matter and Fertilizer Amendments

Organic matter and fertilizer amendments may change the bioavailability of applied herbicides and may have a direct effect on the microorganisms involved (Alvey and Crowly, 1995; Yanze-Kontchou and Gschwind, 1995). Exogenic organic matter not only influences the sorption of *s*-triazine parent compounds and their metabolites (Barriuso and Houot, 1996; Beck and Jones, 1996), but it also can modify the amount, composition, and activity of the microbial biomass involved with *s*-triazine degradation or influence the *s*-triazine degraders present in the soil (Kruglov *et al*., 1996; Lima *et al*., 1996).

The complexity of interactions between pesticides and soil organic amendments under various environmental conditions (Scow and Johnson, 1997) has contributed to large differences in reports of their effects on the fate of *s*-triazine herbicides. For example, McCormick and Hiltbold (1966) observed that the rate of inactivation of atrazine was directly related to metabolism by microorganisms, and that the addition of a C source (e.g., glucose) increased degradation. Addition of inorganic salts or straw also increased atrazine degradation (Hand, 1973), and cornmeal, ryegrass, and poultry litter stimulated the degradation of cyanazine in a Dundee silt loam. However, the amendments affected patterns of metabolite accumulation differentially. After 42 days, 45% of the ^{14}C was recovered as dechlorinated (hydroxy cyanazine) metabolites in ryegrass-amended soil, as compared to $\leq$ 16% in other treatments. Significantly less ^{14}C was extracted from cornmeal-amended soil than from the other treatments, indicating a relationship between cyanazine dissipation and incorporation into nonextractable bound residues. All three amendments stimulated fluorescein diacetate hydrolysis, indicating enhanced microbial activity (Wagner and Zablotowicz, 1997).

The influence on bacterial and fungal biomass and atrazine mineralization of manure applied to pasture soils has been assessed. Greater amounts of atrazine were mineralized when manure was applied than when soil received no manure or fertilizer, and these results coincided with an increase in bacterial and fungal biomass (Entry and Emmingham, 1995). Variable effects of organic amendments have been observed by Kruglov *et al.* (1996); straw and sludge accelerated the degradation of prometryn, while lupine and corn slowed degradation. However, decreased degradation rates have also been reported in many other cases. The transformations of atrazine, simazine, and terbutryn in soil after compost addition were monitored during long-term laboratory incubations (Barriuso *et al.*, 1997). Compost addition to soil generally decreased herbicide mineralization and favored the stabilization of herbicide residues. However, most of the residues were unextractable and were bound. A reduction in terbuthylazine metabolism was also reported in several soils from sites that received organic amendments over a period of 31 years (Gerstl *et al.*, 1997).

Masaphy and Mandelbaum (1997) indicated that long-term irrigation with treated wastewater increased organic matter in soil but decreased the overall mineralization rate of atrazine. The authors indicated that initial atrazine mineralization was greater in soils irrigated with treated wastewater, but after a few days of interaction between the soil and the applied herbicide, mineralization rates decreased as a result of decrease in bioavailability.

Carbon and N dynamics may be particularly important for selective enrichment of microorganisms that are capable of using xenobiotic compounds as a source of N for growth. Alvey and Crowley (1995) reported that in the absence of organic amendments, 73% of the atrazine was mineralized after 11 weeks when soil was spiked with 100 mg/kg of atrazine. Soils amended with rice hulls, starch, and compost yielded mineralization rates of 88%, 75%, and 59%, respectively, in the same period. In contrast, | 10% of the atrazine was mineralized in soils amended with glucose, Sudan hay, or sodium citrate. All treatments that received supplemental inorganic N had a considerably slower rate of atrazine mineralization than corresponding treatments without N addition. However, the different effects of the organic matter supplements indicated that there was no relationship between the C/N ratio of the soil and atrazine mineralization. These results demonstrate that while atrazine mineralization is suppressed under high N conditions in this soil, the mineralization rate is also influenced by poorly understood population dynamics related to the nutrient composition and complexity of specific organic amendments.

Cook (1987) suggested that the N atoms of *s*-triazines can be used as an N source for microorganisms. Therefore, stimulation of biodegradation may be achieved by creating N-limiting conditions so that microorganisms are forced to utilize the *s*-triazine molecule as an N source. This idea may prove to be useful under aerobic conditions – but not under oxygen-limited conditions or when organic N sources are used. However, both increases and decreases in atrazine degradation have been reported in soils fertilized with nitrogenous compounds. Mandelbaum *et al.* (1995) reported that under aerobic conditions, high levels of ammonium nitrate inhibited the degradation of atrazine by *Pseudomonas* strain ADP. However, Katz *et al.* (1999) observed that nitrates did not interfere with atrazine degradation when the same bacterium used nitrate as an electron acceptor.

While atrazine degradation to hydroxyatrazine was enhanced by the addition of ammonium sulfate in anaerobic wetland sediments (Chung *et al.*, 1995), the addition of 2.0 g/L of ammonium nitrate into aerobic wetland water sample reactors clearly inhibited atrazine degradation (Ro and Chung, 1995). In ^{15}N tracer studies done with *Pseudomonas* strain ADP (which can use all five N atoms of atrazine as a sole N source), Bichat *et al.* (1997) indicated that while organic N sources had little effect on atrazine degradation, nitrate and ammonium delayed atrazine degradation.

Leita *et al.* (1996) investigated the fate of terbuthylazine in cultivated drainage lysimeters filled with soil in order to investigate its disappearance in relation to the N fertilization level. The disappearance rate of terbuthylazine was faster in the N fertilized soils. Addition of a dairy manure amendment increased the rate of atrazine mineralization, while NH_2HPO_4 amendments prevented mineralization (Gan *et al.*, 1996). When NH_4NO_3 was added to three grassland soils at a level of up to 500 kg/ha of NH_4NO_3, atrazine mineralization was suppressed relative to the unamended control (Entry *et al.*, 1995b). The authors suggested that the added N stimulated fungal biomass that responded in an opposite manner to herbicide mineralization. From studies with lignolytic enzyme systems, it was observed that lignolytic activity is stimulated by N limitation in several, but not all, species of fungi (Kirk and Farrel, 1987; Reid, 1991). In addition, Entry *et al.* (1996) reported that atrazine degradation by pure cultures of *Phanerochaete chrysosporium* was inhibited by additional N source.

Degradation of Mixtures of Herbicides

Radosevich *et al.* (1995b) studied the biodegradation of binary and ternary mixtures of atrazine, simazine, and cyanazine using a pure culture of the atrazine-degrading bacterium *Ralstonia pickettii* (previously strain M91-3). While the bacterium used atrazine and simazine indiscriminately, cyanazine degradation was slow and delayed until the depletion of the two other herbicides. There was no apparent effect of other commonly used herbicides on the rate of atrazine

degradation by this bacterium. Yanze-Kontchou and Gschwind (1994) reported that *Pseudomonas* strain YAYA6 degraded atrazine in mixtures with other triazine herbicides. The culture degraded atrazine and simazine at comparable rates, whereas the other triazines were at best only partially degraded.

The degradation of several triazine herbicides was also studied by Grigg *et al.* (1997) using a mixed culture of microorganisms. They reported that while atrazine, cyanazine, and simazine were degraded in 6 days in liquid culture, metribuzin, which can be degraded by the fungus *Cunninghamella echinulata Thaxter* ATCC #38447, was not (Schilling *et al.*, 1985). Atrazine was mineralized in the presence of other single pesticides, but the degradation rate was reduced when co-contaminants were present (Grigg *et al.*, 1997). In an interesting study by Arnold *et al.* (1996), Fenton's reagent and the catabolic activity of *Rhodococcus corallinus* and *Pseudomonas* strain D were combined to detoxify *s*-triazines in pure solutions and mixed wastes. In solutions containing only atrazine, complete atrazine decomposition was accomplished with 2.69-mM Fenton's reagent. Moreover, while *Rhodococcus corallinus* degraded these products in less than 10 minutes, combining *R. corallinus* with *Pseudomonas* strain D increased mineralization. When applied to a pesticide rinse water containing atrazine, cyanazine, alachlor, metolachlor, and EPTC, greater than 99% of the pesticides were degraded with 12.2-mM Fenton's reagent. Subsequent treatment with *R. corallinus* and *Pseudomonas* strain D degraded all chlorinated *s*-triazine intermediates and released 70% of tracer (U-ring-^{14}C) atrazine in 10 days. Since the use of *R. corallinus* obviated the need for additional chemical pretreatment (e.g., acidification or base hydrolysis has been used in previous studies prior to microbial incubations), this method may prove promising for the on-site treatment of pesticide rinse water (Arnold *et al.*, 1996).

Ozone treatment, followed by biological degradation, has also been investigated as a means to remove residual contaminants in pesticide waste and rinsates. Somich *et al.* (1990) performed on-site treatment of pesticide waste and rinsates with ozone (for 18 h) and then circulated the material through a biologically active soil column (for 48 h). Concentrations of atrazine, cyanazine, and metolachlor were decreased from 17, 30, and 82 ppm, respectively, to | 5 ppm, and ozonolysis yielded products that were much more amenable to biological degradation than parent material.

Temperature and Moisture

Temperature and moisture are two of the most important environmental variables that affect microbial growth, survival, and activity. At optimal temperature and moisture conditions, chemical and enzymatic reactions in the cell will occur the most rapidly and growth and activity will be the highest. However, below and above these optimal conditions, microbial activity decreases. The microbial degradation of *s*-triazines appears to follow the same pattern. The effect of soil moisture and temperature on the degradation of terbutryn was evaluated by Chu-Huang *et al.* (1975). They reported that after 20 weeks of incubation above 10°C and at 14% soil moisture, phytotoxic levels of terbutryn to wheat were not detected in Teller sandy loam soil.

To verify experimentally the interaction between soil type, moisture content, and temperature, it is necessary to design a multivariable experiment. Reinhardt and Nel (1993) conducted a study to evaluate the influence of temperature and soil water content on the persistence of atrazine in a clay soil and loamy sand; the latter represented soils in which atrazine carryover is not expected. By 30 days after application, distinct but not significant degradation of atrazine occurred in the light soil only. However, subsequent determination at 60 days after application revealed that soil watered to field capacity significantly increased degradation in both soils. Virtually no degradation of atrazine occurred in air-dry soil. The lowest temperature regime (30°C day/8°C night) significantly reduced the rate of degradation in the light soil only. It was suggested that the higher pH and higher adsorptive capacity of the clay caused atrazine to persist for a longer time in the clay soil.

The influence of temperature and moisture content of a Regina heavy clay soil on the persistence of cyanazine, metribuzin, and atrazine has also been evaluated (Smith and Walker, 1989). Degradation of the herbicides approximated first-order kinetics at temperatures in the range 5–30°C and at moistures above 20%. The breakdown of cyanazine was faster than metribuzin, which in turn was more rapid than atrazine. McCormick and Hiltbold (1966) investigated the microbial degradation of atrazine in soils at several different temperatures. The degradation rate approximately doubled with each 10°C increase from 10°C to 30°C. Frank and Sirons (1985) showed that breakdown of atrazine in the field was slower under winter than summer conditions, and Mandelbaum *et al.* (1993b) reported that the mineralization of atrazine by *Pseudomonas* strain ADP was much higher at 40°C than at 15°C, but minimal at 7°C.

Soil Depth in the Vadose (Unsaturated) Zone

Most published degradation studies on *s*-triazine herbicides have focused on the upper soil layers (Frank and Sirons, 1985; Winkelmann and Klaine, 1991; Topp *et al.*, 1994; Mandelbaum *et al.*, 1995). Nevertheless, the occurrence of *s*-triazine herbicides and their metabolites in subsurfaces (Harper *et al.*, 1990; Fomsgaard, 1995; Lavy *et al.*, 1996; Ng

et al., 1995; Persicani *et al.*, 1995; Stolpe and Shea, 1995; Vanderheyden*et al.*, 1997) and groundwater (Harper *et al.*, 1990; Ritter, 1990; Maas *et al.*, 1995; Skark and Obermann, 1995; Bottoni *et al.*, 1996; Kolpin *et al.*, 1996; Liu *et al.*, 1996;) has increased the importance of elucidating degradation rates of these compounds in subsurface environments.

The vadose zone beneath the cultivated soil horizon is the last barrier that can effectively detoxify, adsorb, or immobilize leaching herbicides before they reach aquifers. In most cases, a decrease in degradation rates is observed with increasing depth (Harper *et al.*, 1990; Adams and Thurman, 1991; Fomsgaard, 1995; Kruger *et al.*, 1997; Miller *et al.*, 1997; Vanderheyden *et al.*, 1997).

Metribuzin was used by Harper *et al.* (1990) to demonstrate the kinetics and factors limiting pesticide biodegradation in the vadose zone. Reduced rates of degradation were found immediately below the surface soil. Using an agricultural site in Ohio, Radosevich *et al.* (1996) studied the mineralization of atrazine by indigenous microbial communities and by the constraints associated with atrazine biodegradation in environmental samples collected from surface soil and subsurface zones. Atrazine mineralization in soil and sediment samples was monitored and mineralization at 10°C was slow and linear. The authors concluded that in vadose zone and subsurface aquifer conditions, low temperatures and the lack of degrading organisms were likely to be the primary factors limiting the biodegradation of atrazine. Sinclair and Lee (1992) compared degradation rates of atrazine in active (nonsterile) and sterile (autoclaved) subsoil samples. The reason for the lack of degradation in the active soil was attributed to lack of bacterial populations. Similarly, Shapir and Mandelbaum (1997) concluded that the limited atrazine degradation in subsurface soils in Israel was due to a lack of degrading organisms and was not directly related to the lack of a C source. Kruger *et al.* (1997) concluded that the half-life of atrazine was greater in subsurface soils than in surface soil. However, when the subsoil was saturated, the half-life of atrazine decreased 4-fold due to microbial degradation.

Some observations on oxygen limitation at deeper soil depths indicate that atrazine transformation and mineralization are retarded as the soil environment becomes more anoxic (Nair and Schnoor, 1992; Vink and Vanderzee, 1997). Interestingly, the study of the genes and enzymes involved in atrazine mineralization by *Pseudomonas* strain ADP revealed that the first three enzymatic steps consist of a series of hydrolytic reactions that can proceed in the absence of free oxygen (Figure 22.1). Indeed, Shapir *et al.* (1998) reported on the ability to mineralize atrazine under denitrifying conditions in authentic aquifer sediments underlying a corn field. Also, Katz *et al.* (1999) reported that continuous culture of *Pseudomonas* strain ADP could completely mineralize atrazine in the presence of other denitrifying bacteria and under continuous denitrifying conditions. Sparling and Aislabie (1996) detected greater atrazine mineralization in Twyford subsoil at lower depths than at the surface of the same soil. They attributed atrazine breakdown to the presence of a larger population of atrazine-degrading bacteria, but not to an overall increase in biomass or microbial counts. Vanderhyden *et al.* (1997) reported that there is a large variability in the dissipation rates observed within various subsoil samples of some cores. They concluded that subsoil samples containing stones and iron compounds were more active with respect to atrazine degradation and had greater microbial counts. Lavy *et al.* (1996) reported that 3-year-old simazine was still biodegradable in subsoil samples.

Research has previously shown that bacteria are not uniformly distributed in soil, reflecting soil structure and available nutrients (Richaume *et al.*, 1993). The distribution of microorganisms throughout the soil can also be considered from the applied ecological perspective of 'patch dynamics,' where patch formation is a reflection of intrinsic and extrinsic forces (Rao *et al.*, 1986). The same authors also showed spatial variability in the degradation of pesticides applied to a soil system.

Aging in Soil

The measure of the total chemical concentration present in a soil does not adequately indicate the availability of chemicals for biodegradation or release, nor does it indicate the potential for chemical transport (Loehr and Webster, 1996). In most soils, 'aged' pesticides are less amenable to biodegradation. An atrazine mineralizing bacterial isolate was used as a biological probe to investigate the effects of atrazine residence time on subsequent bioavailability and biodegradation (Radosevich *et al.*, 1997). The results indicated that a significant fraction of the solution-phase atrazine was sequestered from microbial attack and that the unavailable fraction increased with soil residence time. Indeed, existing data indicated that chemicals freshly added to soils are more amenable to losses, including biodegradation, than chemicals that have been in contact with soils for extended periods of time. The sorption and desorption behavior of field-aged residues from a corn field under 20 years of continuous application were compared to that of ^{14}C-simazine added to the same soil (Scribner *et al.*, 1992). The apparent sorption coefficients of the aged residues, determined from 24- and 48-h desorption experiments, were approximately 15 times higher than the sorption coefficients of freshly added simazine. Aged simazine residues were also shown to be biologically unavailable to sugar beet and microbial degraders, whereas newly added simazine showed herbicidal damage to sugar beet and was substantially (about 48%) degraded during 34-day incubation in soil.

An interesting experimental approach was taken by Lavy *et al.* (1996) for a study of the degradation and leaching properties of aged pesticides in the subsoil. Formulations of atrazine and metribuzin were applied separately to two moist Arkansas subsoils and buried for up to 3 years. The treated subsoils were placed in one of two storage container types, buried *in situ* at 30, 90, or 150 cm with the soil profile, and retrieved at 6-month intervals. Statistical evaluation of these data found that in spite of the long weathering time, both degradation and leaching of aged pesticides still occurred. No aging effect was shown in a different study where the decomposition rates of aged versus fresh simazine were measured under laboratory conditions in soils containing old residues and in those previously untreated (Byast and Hance, 1981). Simazine disappeared at about the same rate whether it was freshly added or added as a residue. There was no evidence that any of the compounds had induced a soil microflora adapted to degrade the herbicide.

Rhizosphere Soil

The rhizosphere is the soil zone around the roots in which microbial biomass is impacted by the presence of plant roots (Rovira and Davey, 1973). The ability of the rhizosphere to stimulate microbial activity has been long known. With the increased awareness of the role that microorganisms play in the degradation of *s*-triazine herbicides, research began to focus on possible enhanced degradation in the rhizosphere. The rhizosphere contains significant amounts of plant-released carbonaceous substances, which in turn increase and diversify the microbial population around the root zone (Schortemeyer *et al.*, 1997). Up to 50% of plant assimilates that are translocated into the roots can be excreted as root exudes (Sauerbeck and Johnen, 1974), and these serve as a food base for microorganisms.

Gallaher and Mueller (1966) studied the effect of the plant rhizosphere on pesticides by examining the persistence of atrazine and metribuzin in the presence or absence of a corn or soybean crop. In the first year of the study, dissipation was not affected by the crops. In a second season, herbicide degradation was slower in the presence of the crops, indicating no enhancement of degradation due to rhizosphere effects. Seibert *et al.* (1995) found no significant influence of corn plants on atrazine mineralization in the rhizosphere, despite the enhanced microbial activity. Only after the harvest of the corn did the residues remaining in the soil increase the C/N ratio and increase atrazine degradation. Similarly, Alvey and Crowley (1996) reported that corn seedlings had no significant effect on the rate of atrazine mineralization, either by an indigenous microflora or in soil inoculated with atrazine-mineralizing bacteria. However, numbers of atrazine-mineralizing bacteria at the end of the incubation period were greater in the planted soil. The enhanced survival of atrazine-degrading bacteria in the rhizosphere might have an important role in sustaining atrazine-degrading bacteria in the soil. Herbicide-resistant plants, such as kochia, have been demonstrated to enhance atrazine degradation in soils with elevated concentrations of herbicides, including -10^{J} typical field application rates (Perkovich *et al.*, 1996).

Jordahl *et al.* (1997) reported that hybrid poplar root exudates stimulated soil microbial activity involved in chemical degradation. A significant increase occurred in populations involved in the degradation of several chemicals, but only a minor increase occurred in populations that degrade atrazine. Rhizosphere effects on atrazine degradation are unclear and remain under investigation.

Electron Acceptors

Oxygen deficiencies under field conditions have been reported to retard *s*-triazine degradation. For example, Ro and Chung (1995) reported that in wetland sediments amended with nutrients, 10 ppm of atrazine reduced to less than 10 ppb within 3 weeks under aerobic conditions. Under anaerobic conditions, less than 50% degradation was reported in 38 weeks. However, Kruger *et al.* (1996) reported an opposite observation for a saturated soil where a 4-fold increase in degradation was reported.

The behavior of simazine (among other pesticides) has been investigated under simulated redox conditions that mirror those occurring at the terrestrial–aqueous interface (Vink and Vanderzee, 1997). Under low oxygen conditions, both reductive and oxidative metabolites were formed, and the simazine transformation rate decreased with decreasing O_2 concentrations. Redox conditions appear to be an important screening parameter to assess environmental risks. Nair and Schnoor (1992) also reported that the atrazine ring and its isopropyl side chain were mineralized much more slowly under denitrifying conditions than under aerobic conditions.

Second-order rate constants have been developed for the biotransformation of atrazine under aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions (Aislabie and Lloydjones, 1995). Acetate-fed batch-reactor techniques were used with seed cultures taken from acclimated biofilm columns. Results of these studies showed that the four electron acceptor conditions tested resulted in some biotransformation of atrazine, although differences among the four electron acceptor conditions were not statistically significant. It is obvious that these relatively slow rates can dramatically change with different microorganisms. For example, *Pseudomonas* strain ADP grew in the

presence of atrazine and mineralized the herbicide under denitrification conditions (Shapir *et al.*, 1998; Katz *et al.*, 1999), and the degradation rate constant was at least three orders of magnitude greater than that reported by Nair and Schnoor (1992).

Agricultural Practices

Crop management practices alter the soil environment, which in turn affect microbial growth and biodegradation processes that transform plant residues and applied pesticides in the soil. Agricultural management practices can have an important effect on the microbial attenuation of *s*-triazines, however these effects have been only occasionally addressed.

Soils Repeatedly Treated with s-Triazines

Scudder (1963) observed that in soil repeatedly treated with simazine, degradation rates improved over time. Subsequently, extremely high mineralization rates have been observed in several geographically distinct agricultural soils. Mineralization of the atrazine ring has been studied in soils from experimental plots in Grignon, France that experienced different crop rotations but had similar physicochemical properties (Barriuso and Houot, 1996). Rapid mineralization rates were found in plots under continuous corn receiving atrazine every year. The rapid mineralization of the atrazine ring was observed without any previous microbial enrichment. This mineralization was also related to the presence of a chloro-substitution on the ring. Rapid mineralization was also observed with simazine, but not with terbutryn – a thiomethyltriazine. However, low mineralization rates were measured in plots under continuous wheat or permanent grass rotations that also had never received atrazine. Vanderheyden *et al.* (1997) reported rapid degradation of atrazine in soil and subsoil samples taken from six Belgian corn fields. Rapid degradation occurred in samples taken from surface, and in some cases, from subsurface soils. Experiments with ring-labeled atrazine showed that the microorganisms responsible for the rapid degradation cleaved the triazine ring and extensively mineralized the molecule.

The ability of acclimated soils to degrade atrazine can be maintained for a long time, even in the absence of repeated applications. Horswell *et al.* (1997) reported little or no lag in mineralization of atrazine in a soil that had not been exposed to atrazine for 15 years. Sediments from southern Ontario repeatedly exposed to atrazine surges were tested for their ability to degrade atrazine (Nakagawa *et al.*, 1996). Atrazine-mineralizing bacteria were found in alluvial surface sediments distributed throughout the study area. The authors reported a very fast atrazine degradation rate with a half-life of about 1 day in activated sediments, comparable to the most rapid atrazine degradation rates obtained by enrichment cultures. The bacterial cultures from their sediments could be used in constructed wetlands for the degradation of atrazine in runoff.

Crop Rotation

Atrazine mineralization rates were measured under two management practices – a continuous corn plot receiving annual application of atrazine and a crop-rotation plot with corn–soybean–wheat and hairy vetch, with reduced use of atrazine during corn years (Ostrofsky *et al.*, 1997). The agricultural site received atrazine applications for about 25 years before the study. (^{14}C-U-ring) atrazine was added to the soil samples in biometer flasks, and $^{14}CO_2$ evolution was measured. Sterile soil controls showed no evolution of $^{14}CO_2$. Within 30 days of incubation, about 80% of the initial radioactivity was evolved as $^{14}CO_2$ in the continuous corn soils collected during different seasons of the year. Parallel samples from crop rotations showed 15% to 30% atrazine mineralization, while samples from the control soils showed 3% to 7% $^{14}CO_2$ evolution within 80 days of incubation. Prior amendment of soil samples from the three sites with 1 ppm atrazine accelerated subsequent mineralization after 90 days of incubation, indicating enhanced activity of indigenous microorganisms. The continuous corn mineralization data suggest that under certain conditions a single annual atrazine application sustains an active microbial community throughout the year.

Forest Rotation

Studies done in riparian areas have shown that microbial communities are capable of degrading herbicides faster in forest soils than in pasture soils. Entry *et al.* (1995a) tested the influence of riparian forest age (at three riparian sites) on herbicide degradation in the soil. Active and total fungal and bacterial biomass and mineralization of atrazine were measured in the litter and in the top 10 cm of mineral soil in forests on the three sites that were 20–40, 60–90, and 120–300 years old. Results indicated that microbial communities in old-growth riparian areas have a greater capacity to degrade herbicides than do such communities in young-growth forests. Management of riparian forests to long rotations may tend to increase herbicide degradation and thus decrease levels of pesticides in lakes and streams.

Bioremediation

The use of microorganisms or other biological agents to reclaim soils and water has been termed 'bioremediation' (Atlas and Parmer, 1990). Bioremediation of contaminated soils and water involves the interaction between the contaminant, microorganisms, and the environment. To use bioremediation processes to their full potential, all three components must be simultaneously managed (Madsen, 1991; Sadowsky and Turco, 1999). Composting, land farming, above–ground bioreactors, and several *in situ* treatment methods are current techniques used for the bioremediation of contaminated soils and water (Sadowsky and Turco, 1999). *In situ* processes are advantageous when the contamination covers a relatively large surface area or is situated deep into the soil profile. To remove pollutants at the site of contamination, either natural or engineered microorganisms are added or indigenous soil microorganisms are stimulated to degrade contaminants (Gibson and Sayler, 1992; Bollag and Bollag, 1995).

Chemical handling areas (such as those found at dealerships), mixing areas, and loading areas are particularly vulnerable to accidental spills and are often characterized by co-contamination with various agricultural chemicals (Krapac *et al*., 1995). This 'point-source' contamination of chemicals usually arises from accidental spills, rinsate disposal into evaporation pits, accidental back-siphoning into wells, or discharge of industrial effluents. Point-source contamination of groundwater with atrazine can potentially cause detections at levels exceeding the regulated drinking water MCL.

Although bioremediation processes have been used for decades in wastewater treatment, their application to contaminated soils, groundwater, and industrial effluents is fairly new (Sayler *et al*., 1991) and still undergoing intensive development. A reduction in clean-up costs is one of the principle factors driving the heightened interest in the use of bioremediation processes at herbicide spill sites. Reported and projected costs have been as low as \$75 to \$200 per yard^3, as compared to the more conventional technologies such as incineration or secure landfilling, where costs may be in the range of \$200 to \$800 per yard^3 (Gabriel, 1991).

Pretreatment of contaminants with various reagents to produce degradates more amenable to microbial mineralization (Leeson *et al*., 1993) and treatment of soil slurries with microbial enzymes (Nelson and Jones, 1994) have been successful in remediation.

Land farming has been one of the major methods used for the bioremediation of herbicide-contaminated soils, and it usually involves biostimulation of existing microorganisms (Felsot *et al*., 1992). This can be achieved by modifying the existing environmental conditions – such as temperature, pH, moisture, carbon, nitrogen, and/or electron acceptors – so the indigenous microbial population is permitted to flourish and to degrade herbicides. However, the major barrier for land farming of *s*-triazine-contaminated soils is the lack of appropriate indigenous microbial strains on site that can degrade *s*-triazine herbicides. This problem may be alleviated by bioaugmentation with laboratory strains adopted for *s*-triazine degradation (Table 22.l) under conditions similar to those prevailing at the bioremediation site.

The best documented bioremediation effort to date, conducted on a large scale and brought to successful completion, was carried out by Ciba-Geigy Corporation at its St. Gabriel, Louisiana atrazine production plant (Finklea and Fontenot, 1996). The on-site remediation of 19 000 m^3 of atrazine-contaminated soil was achieved using land farming and bioaugmentation techniques. The operation lasted about 6 months, with degradation of total *s*-triazines from an initial range of 100 to 300 ppm in some soil parcels to | 2ppm.

Phytoremediation approaches (the use of green plants and their attendant bacteria to remove contaminants from soil and water, either directly by plant uptake or by stimulating microbial activity in the rhizosphere) have attracted interest as a possible low-cost remediation technology (Sadowsky and Smith, 1996). To date the application of phytoremediation strategies to clean up environmental contamination due to *s*-triazine herbicides has been limited. However, the uptake and degradation of atrazine by poplar trees has been reported (Burken and Schnoor, 1996, 1997).

In another novel approach, Strong *et al*. (2000) used cross-linked and killed *Escherichia coli* expressing the *Pseudomonas* spp. ADP *atzA* gene to bioremediate 26 m^3 of soil in South Dakota that was heavily contaminated by an atrazine spill (up to 29 000 ppm). Bioremediation was successful in reducing the atrazine concentration to less than 100 ppm, and the soil was eventually land farmed following treatment. This was the first field-scale atrazine remediation study done in the United States using killed, recombinant microorganisms.

Pseudomonas strain ADP was also shown to be useful to bioremediate atrazine-contaminated sediments taken from a shallow aquifer underlying a cornfield continuously receiving atrazine and terbuthylazine (Shapir *et al*., 1998). When atrazine was present at low concentrations (similar to those from nonpoint-source contamination), under denitrifying conditions the bacterium mineralized 75% of the atrazine in 4 days; under high concentrations (as in spill sites), 78% of the atrazine was mineralized in 15 days. This study indicated that bioaugmentation with an effective atrazine-mineralizing bacterium, even under oxygen-limited conditions, could have a significant impact in the bioremediation of atrazine in water and soil.

The use of other isolated pure (Table 22.1) and mixed microbial cultures (Grigg *et al*., 1997) that rapidly mineralize *s*-triazines may further improve the prospects for bioremediation. Moreover, novel approaches to enhance the

activity of native bacteria can be exercised. For example, the atrazine-degrading activity of wild-type bacteria can be facilitated by rapid growth on additional nutrients or by the use of artificial electron acceptors in anaerobic environments. This coupling can create problems for *in situ* bioremediation as it necessitates the addition of large amounts of nutrients to contaminated environments, such as aquifers. This approach, termed biostimulation, can be technically difficult due to the *in situ* production of bacterial biomass. In an attempt to minimize coupling between expression of biodegradative activity and growth, Matin *et al.* (1995) used *Escherichia coli* starvation promoters to control toluene monooxygenase synthesis. The use of these starvation-induced promoters to drive expression of atrazine-degradation genes holds great promise for future bioremediation efforts.

Conclusions

The interactions between *s*-triazines and microorganisms have been studied over nearly 50 years and new research has led to important discoveries. The isolation of pure cultures that are able to modify or completely mineralize *s*-triazines has led to the discovery of new genes and enzymes that are involved in the degradation and mineralization of *s*-triazines by soil bacteria. Studies carried out in soils with a history of repeated *s*-triazine applications indicate that rapid degradation and mineralization of atrazine developed in various soils (Barriuso and Houot, 1996; Bradley *et al.*, 1997; Pussemier *et al.*, 1997).

The advent of new molecular tools facilitates unprecedented opportunities for environmental microbiologists to quantify further the factors influencing the activity of *s*-triazine-degrading microorganisms. Reporter gene systems, polymerase chain reaction-based techniques, and hybridization with specific gene probes are among the tools that will provide quantitative data on the expression and transfer of the *s*-triazine-degrading genes that play such an important role in the overall fate of this important group of herbicides.

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Chapter 23

Nonbiological Degradation of Triazine Herbicides: Photolysis and Hydrolysis

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Summary

Triazine herbicides absorb sunlight weakly at wavelengths ffi290 nanometers (nm), thus, dissipation of the triazine herbicides in the atmosphere and in surface waters via photodegradation occurs mainly by indirect photolysis or photosensitized reactions.

Current information on the photochemical dissipation of the triazine herbicides in the atmosphere is very limited. No studies concerning the vapor-phase photolysis of these herbicides have been reported, and only two studies have investigated the phototransformation of triazine herbicides when associated with atmospheric aerosols. Photodegradation of atrazine and terbuthylazine was observed in these studies, but the significance of photodegradation in the dissipation of atmospheric concentrations of these herbicides has yet to be established.

In contrast, the photodegradation of aqueous solutions of the triazine herbicides has been much more thoroughly studied. These studies have investigated the effects of sensitizers that are present in natural waters – such as dissolved organic carbon, acetone, nitrate, hydrogen peroxide, and semiconductor metal oxide particulates (like titanium dioxide). Photolysis of the 2-chloro-*s*-triazine herbicides has been studied most frequently and, of these, atrazine has been studied in greatest detail. Using sunlight or simulated sunlight (ffi290 nm), photolysis proceeds by dechlorination and hydroxylation to form the corresponding hydroxytriazine. Other reactions include dealkylation and eventually deamination to form cyanuric acid.

The increased rates of photodegradation of the triazine herbicides observed in the presence of naturally occurring sensitizers indicate that photodegradation plays a significant role in the dissipation of these herbicides in natural waters. With most of the sensitizers studied thus far, cyanuric acid was the stable end product, rather than complete mineralization of the triazine herbicide.

Several studies have investigated the use of photosensitized reactions to remove triazines from water. For example, complete mineralization of several triazine herbicides was observed when aqueous solutions of these herbicides were photolyzed in the presence of titanium dioxide immobilized in a photocatalytic membrane.

Although hydrolysis of the triazine herbicides is temperature and pH dependent, these herbicides are considered to be hydrolytically stable under the pH and temperature conditions encountered in natural waters. However, the relatively slow hydrolysis rates in natural waters may be enhanced somewhat by the presence of dissolved organic carbon (DOC) (in the form of fulvic acids and a variety of low-molecular-weight carboxylic acids and phenols) that has been shown to catalyze the hydrolysis of several triazine herbicides. Although microbial degradation is probably the most important mechanism of dissipation of the triazine herbicides in soils, abiotic hydrolysis of these herbicides also occurs. Hydrolysis in soils is affected by the pH, organic matter (humic acid) content, and the type and content of clay in the soil.

Introduction

Several processes may play a role in the environmental dissipation of *s*-triazine herbicides. Dissipation processes can include microbial or chemical degradation in soil; metabolism or conjugation in plants; photodegradation in air, water, and on soil and plant surfaces; and volatilization and transport mechanisms. This chapter will address photolytic degradation and abiotic hydrolysis of the currently used triazine herbicides, the triazinone herbicides (metribuzin and metamitron), and the triazinedione herbicide hexazinone.

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Table 23.1 Water solubilities, vapor pressures, Henry's Law constants, and pK_a values of currently used triazine herbicides

Triazine herbicide	Water solubility[a] at 20–25fflC; pH ffi 7 mg/L[b]	Vapor pressure[a] 20–25fflC mPa ffl 10^{3}[c]	Henry's Law constants[a] Pa m^3/mol ffl 10^{4}[d]	pK_a values[a]
2-chloro				
Atrazine	33	38.5	1.5	1.7
Cyanazine	171	20	–[e]	0.63
Propazine	5.0	3.9	1.79	1.7
Simazine	6.2	2.94	0.56	1.62
Terbuthylazine	8.5	150	40.5	2.0
2-methylthio				
Ametryn	200	365	4.1	4.1
Desmetryn[f]	580	133	0.48	4.0
Dimethametryn	50	186	9.5	4.1
Prometryn	33	165	12	4.1
Simetryn	400	95	0.507	4.0
Terbutryn	22	225	15	4.3
2-methoxy				
Prometon	750	306	–	4.3
Triazinone				
Metamitron	1700	0.86	0.001	–
Metribuzin	1050	58	0.1	1.1[g]
Triazinedione				
Hexazinone	29,800	30	–	2.2

[a]Unless otherwise noted, all values are from Pesticide Manual 2006.
[b]Milligrams per liter.
[c]MilliPascals.
[d]Pascal cubic meter per mole.
[e]Data not available.
[f]Values are from Pesticide Manual 1997.
[g]Weber (1980).

Triazine Herbicides

The triazine herbicides currently used are mostly 4,6-alkylamino-*s*-triazine compounds with either a 2-chloro, 2-methylthio, or 2-methoxy substituent (Table 23.1). The *N*-alkyl groups may be methyl, ethyl, 1-methylethyl (isopropyl), 1,1-dimethylethyl (*tertiary*-butyl), 1,2-dimethylpropyl, or 2-methylpropanenitrile. Absorbed by roots or leaves of plants, these herbicides are applied either preemergence or postemergence to control annual broadleaf weeds and annual grasses in a wide variety of crops. The triazine herbicides listed in Table 23.1 have the same mechanism of action in plants, as all are photosynthetic electron transport inhibitors.

Based on the volatility classes suggested by Wania and MacKay (1996), the triazine herbicides, which have vapor pressures ranging from approximately 0.001 to 0.365 milliPascals (mPa) (Table 23.1), have relatively low vapor pressures and would tend to evaporate very slowly under ambient conditions in the global environment. Evaporation from water surfaces would also be slow as indicated by relatively low Henry's Law constants (Table 23.1). Water solubilities of the triazine herbicides vary by more than two orders of magnitude at pH 7 (negative logarithm of the hydrogen ion concentration) (Table 23.1). The 2-methoxy- and the 2-methylthio-*s*-triazine herbicides are generally much more basic than the 2-chloro-substituted compounds, and this order of basicity is reflected by their respective pK_a values (negative logarithm of the acid–base dissociation constant) (Table 23.1). Thus, in natural waters in which pH values generally range from pH 5 to 9, the 2-chloro-*s*-triazine herbicides will be present only in their molecular forms, whereas the 2-methoxy- and 2-methylthio-substituted analogues will be partially protonated at pH values less than 6.0–6.5.

Only sunlight at wavelengths longer than 290 nm is available for environmental photoreactions. Aqueous solutions of the molecular forms of the 2-chloro-*s*-triazine herbicides absorb light only weakly at wavelengths greater than 290 nm (Ward and Weber, 1969). In contrast, aqueous solutions of the molecular forms of the 2-methylthio- and 2-methoxy-*s*-triazine herbicides do not absorb sunlight directly (Weber, 1967, 1980; Ward and Weber, 1969). However, in waters at pH values less than pH 6.0 to 6.5, protonation of the 2-methylthio- and 2-methoxy-*s*-triazine herbicides results in a bathochromic shift in the absorption maxima of these compounds. However, this shift to longer wavelengths only permits the 2-methylthio-substituted triazine herbicides to absorb light weakly at wavelengths

greater than 290 nm (Weber, 1967, 1980; Ward and Weber, 1969). Thus, both the 2-substituents (Ruzo *et al.*, 1973; Burkhard and Guth, 1976; Rejto *et al.*, 1983; Pacáková *et al.*, 1988) and, to a lesser extent, the *N*-alkyl groups (Ruzo *et al.*, 1973; Rejto *et al.*, 1983; Chen *et al.*, 1984) play a major role in determining photolysis rates of the triazine herbicides.

Photolysis

Environmental photoreactions require that the compound of interest absorb solar light energy either directly or indirectly. Because the ozone (O_3) layer in the upper atmosphere absorbs practically all of the sun's emitted radiation below 290 nm (Koller, 1966), it is generally accepted that only light at wavelengths longer than 290 nm is available for environmental photoreactions. However, the energy available in the near ultraviolet (UV) portion of the spectrum [399 kilojoules per mole (kJ/mol) at 300 nm, 297 kJ/mol at 400 nm] (Watkins, 1974; Woodrow *et al.*, 1983) is adequate to break various covalent bonds in organic molecules homolytically. Light-absorbing entities are referred to as chromophores. The energy (E) of a quantum of light, expressed in kJ, is related to its wavelength (λ) by the equation:

$$E = Nhc/\lambda$$

In this equation, N = Avogadro's number (6.022×10^{23} molecules/mol), h = Planck's constant [6.626×10^{-34} joule second (J sec)], and c = the speed of light [3×10^{10} centimeters per second (cm/sec)].

Direct Photolysis

Reactions undergone by chromophores as a direct consequence of absorbing photons are referred to as 'direct photolysis.' Absorption of a photon of light produces an electronically excited state of the chromophore. The initial energy absorption leads to an excited singlet state that can give rise to an excited triplet state through the nonradiative transition of intersystem crossing. When the excitation energy of either excited state is dissipated by chemical reaction, the chromophore is said to undergo a direct photochemical reaction. Such reactions may involve radical formation by homolysis, or isomerization of double bonds. The excitation energy of the excited states may also be dissipated by other competing pathways. The singlet and triplet excited states may fluoresce or phosphoresce, respectively, or excitation energy may be transferred to other molecules; for example, solvent molecules.

Direct photolysis of chromophores at the low concentrations found in environmental waters obeys a first-order rate expression (Hedlund and Youngson, 1972):

$$-d[C]/dt = \theta k_a[C] = k_p[C]$$

In this expression, $[C]$ = molar concentration of the chromophore, θ = the quantum yield, k_a = rate constant for light absorption and is the sum of the k_a values for all wavelengths of sunlight absorbed by the herbicide, and the product θk_a = the photolysis rate constant k_p.

The quantum yield (θ) is a measure of the efficiency of the photochemical excitation process, which may result in herbicide degradation and indicates the number of herbicide molecules degraded per photon absorbed. A value of 0 indicates that no chemical reaction occurred, while a value of 1 indicates that all molecules excited due to photon absorption were converted to products. Chain reactions, which can lead to quantum values greater than unity, are unlikely at the very low concentrations found in the aquatic environment.

Indirect Photolysis

Compounds that exhibit no UV spectrum above 290 nm would be expected to be photochemically stable when irradiated with sunlight. However, in water containing one or more chromophores, it is possible for a transparent substrate to absorb solar light energy indirectly. A reaction involving such substrates is initiated through light absorption by a chromophore referred to as a sensitizer. Excitation of the sensitizer may result in an excited singlet state, or an excited triplet state via intersystem crossing. This is followed either by energy transfer to the substrate or by transfer of electrons or hydrogen atoms to or from the substrate. These subsequent reactions are said to be photosensitized and are referred to as indirect photolysis. A second component of indirect photolysis occurs when the absorption of light energy by the sensitizer leads to the formation of a reactive species (such as hydroxy radical or singlet oxygen) that enters into a chemical reaction with the substrate. Examples of sensitizers occurring in natural waters include humic materials, nitrate (NO_3^-), and semiconductor metal oxide particulates.

Photolytic Degradation of the Triazine Herbicides

Most photolysis studies involving triazine herbicides have been carried out in aqueous solutions of these compounds. These studies have also been carried out in greatest detail with respect to identification of photolysis products, delineation of photolysis mechanisms, and rates of photolysis. The photolysis of thin films of the triazine herbicides has been studied less frequently and in much less detail. There have been no reports of vapor-phase photolysis studies; however, there have been two studies investigating the photolysis of a triazine herbicide sorbed to an aerosol. Only photolysis studies reported in 1970 or later have been included in the following discussion. Earlier photolysis studies of the triazine herbicides have been reviewed by Jordan *et al.* (1970).

Aqueous Solutions

In many of the early photolysis studies of aqueous solutions of the triazine herbicides, the experimental conditions employed were not reflective of sunlight or natural waters, but rather were employed to facilitate the isolation and the identification of photoproducts. For example, high concentrations of herbicide [10^{-4} to 10^{-2} molar (M)] (Pape and Zabik, 1970, 1972; Burkhard *et al.*, 1975) were sometimes used, and lamps that emitted light at wavelengths shorter than 290 nm were sometimes employed as light sources (usually low-pressure mercury vapor lamps from which the major irradiation is 254 nm) (Pape and Zabik, 1970; Khan and Gamble, 1983). In some studies, direct photolysis was the only mechanism available for photochemical transformation because distilled water was used as the photolysis medium (Pape and Zabik, 1970; Burkhard *et al.*, 1975), or photosensitizers not normally present in natural waters were used, such as benzophenone and organic dyes (Rejto *et al.*, 1983).

In more recent studies, experimental conditions have more closely approximated those encountered in the environment. Sunlight or lamps simulating natural sunlight have been employed as light sources. Herbicide concentrations have been more representative of those observed in natural waters. The isolation and identification of photoproducts at these concentrations have also been facilitated by the use of mass spectrometric instrumentation, which is highly sensitive and well suited to confirmation of target analytes and the identification of unknown compounds (Rejto *et al.*, 1983; Durand and Barceló, 1990; Pelizzetti *et al.*, 1990b, 1992; Durand *et al.*, 1991; Abián *et al.*, 1993; Barceló *et al.*, 1993; Sanlaville *et al.*, 1996; Mansour *et al.*, 1997), and by the use of radiolabeled triazine herbicides (Burkhard *et al.*, 1975; Burkhard and Guth, 1976). Naturally occurring photosensitizers, which might be expected to be present in natural waters, have been used to sensitize the photodecomposition of several triazine herbicides. These include acetone (which has been found in almost every natural aquatic environment) (Khan and Gamble, 1983), DOC such as fulvic and humic acids (Khan and Schnitzer, 1978; Khan and Gamble, 1983; Mansour *et al.*, 1988; Durand *et al.*, 1991; Sanlaville *et al.*, 1996), and DOC mimics such as oxalic acid, quinone, and salicylic acid (Hapeman *et al.*, 1998). Although the triazine herbicides are relatively unreactive to hydroxyl radicals compared to several other classes of pesticides (e.g., carbamates, amides and chlorophenoxyalkanoic acids) (Mabury and Crosby, 1996), the photolysis of aqueous solutions of triazine herbicides has been studied in the presence of several naturally occurring sensitizers that readily generate hydroxy radicals when irradiated with UV light at wavelengths ffi290 nm. These include nitrate (Torrents *et al.*, 1997; Hapeman *et al.*, 1998), hydrogen peroxide (H_2O_2) (Mansour *et al.*, 1988, 1997; Chan *et al.*, 1992; Sanlaville *et al.*, 1996), soil components including semiconductor metal oxide particulates like titanium oxide (TiO_2) (Pelizzetti *et al.*, 1990a, 1992; Sanlaville *et al.*, 1996; Mansour *et al.*, 1997; Texier *et al.*, 1999a, b, c; Peñuela and Barceló, 2000; Lackhoff and Niessner, 2002) or zinc oxide (Pelizzetti *et al.*, 1990a; Lackhoff and Niessner, 2002), ferric ions (Larson *et al.*, 1991; Sun and Pignatello, 1993; Balmer and Sulzberger, 1999; Peñuela and Barceló, 2000; Lackhoff and Niessner, 2002; McMartin *et al.*, 2003) and polyoxometalates (Texier *et al.*, 1999a, b, c; Hiskia *et al.*, 2001; Lackhoff and Niessner, 2002). The photolysis of atrazine has also been studied in aqueous suspensions of natural and anthropogenic particles (sand, soot, fly ash, dust, and volcanic ash) (Lackhoff and Niessner, 2002). Natural waters have also been used as photolysis media (Mansour *et al.*, 1988, 1997; Mansour, 1996; Konstantinou *et al.*, 2001; McMartin *et al.*, 2003; Navarro *et al.*, 2004), and the effect of surfactants and other formulation components on the photolysis of triazine herbicides in aqueous solutions has been investigated (Tanaka *et al.*, 1981; Pugh *et al.*, 1995).

Thin Films

Nearly all thin film photolysis studies involving triazine herbicides have utilized model surfaces such as filter paper (Jordan *et al.*, 1964; Morita *et al.*, 1988), aluminum (Jordan *et al.*, 1965), glass (Pape and Zabik, 1972; Chen *et al.*, 1984; Hubbs and Lavy, 1990), and silica gel (Lotz *et al.*, 1983). A shortcoming of the use of model surfaces is that herbicide dissipation due to volatility losses is often not accounted for (Hubbs and Lavy, 1990). Konstantinou *et al.* (2001) studied the sunlight photolysis of atrazine, propazine, and prometryn on soil (sandy clay loam, clay loam, and

sandy loam) thin-layer chromatographic plates, and in a study involving the sorption of atrazine to soil (loamy sand), Graebing *et al*. (2003) reported that the photolysis of atrazine was faster when sorbed to moist soil compared to dry soil. Since only percent photodegradation of the triazine herbicides was reported in these studies, they will not be subsequently discussed in greater detail. The photolysis of thin films of triazine herbicides on plant surfaces has not been studied.

Aerosols

Pesticides are known to be associated with atmospheric aerosols and thus may be subject to sunlight photolysis in the sorbed state. The photodegradation of triazine herbicides has been investigated when sorbed to suspended aerosols of kaolin and fly ash (Bossan *et al*., 1995), and silicon dioxide (Palm *et al*., 1997a).

Photolysis of the Triazine Herbicides in Aqueous Solution

The photolysis (ffi290 nm) of atrazine, ametryn, and atraton in water with acetone (Burkhard and Guth, 1976) and without acetone (Burkhard and Guth, 1976; Pacáková *et al*., 1988) indicated that the photolability of the 2-substituents was methylthio ffi chloro ffi methoxy. When riboflavin was used as the sensitizer, though, a different order of reactivity was reported (methylthio ffi methoxy ffi chloro) (Rejto *et al*., 1983). These studies (Burkhard and Guth, 1976; Rejto *et al*., 1983) have also established that dealkylation of the *N*-ethyl substituent occurred more readily than that of the *N*-(1-methylethyl) substituent. Other photolysis studies (Ruzo *et al*., 1973; Chen *et al*., 1984; Korte *et al*., 1997) also support this order of reactivity, although in a study involving direct photolysis (250 nm) of 2-methylthio-*s*-triazine herbicides (ametryn, prometryn, and desmetryn) in water, the rate of photolysis was found to be essentially independent of the *N*-alkyl group (Pacáková *et al*., 1988). *N*-dialkyl substituents tend to be more photolabile than *N*-alkyl substituents (Pelizzetti *et al*., 1990b; Korte *et al*., 1997).

Relative to the 2-methylthio- and 2-methoxy-*s*-triazine herbicides, the photolysis of aqueous solutions of the 2-chloro analogs has been studied most frequently (Table 23.2). Of the 2-chloro-*s*-triazine herbicides, atrazine has been studied in greatest detail. The nomenclature used in Table 23.2 and subsequently throughout the text for the triazine herbicides and their photoproducts is as follows: A | amino, B | *tert*-butylamino (1,1-dimethylethylamino), C | chloro, D | acetamido, E | ethylamino, F | formylamino, H | hydrogen, I | isopropylamino (1-methylethylamino), M | methoxy, O | hydroxy, P | 2-aminopropanol, S | methylthio, T | triazine ring, and Y | 2-aminopropionaldehyde. Note that the alkylamino side chains are indicated alphabetically in the acronyms for the triazine herbicides – for example, CEIT for atrazine and CBET for terbuthylazine. To indicate photodegradation of an alkylamino side chain, the position of the modified side chain is retained within the acronym. For example, CEAT is used to indicate the dealkylation of the isopropylamino side chain of atrazine. When photodegradation of an alkylamino side chain of simazine (CEET), propazine (CIIT), or terbuthylazine (CBET) results in the same photoproduct as that obtained with atrazine, the acronym for the photoproduct of atrazine is used. The same nomenclature protocol is used for the 2-methylthio-*s*-triazine herbicides.

Direct Photolysis in Aqueous Solution

Direct photolysis of aqueous solutions of the 2-chloro-*s*-triazine herbicides (atrazine, simazine, propazine) proceeds via excitation of the triazine molecule, followed mainly by dechlorination and hydroxylation to form the corresponding hydroxytriazine (Pape and Zabik, 1970; Khan and Schnitzer, 1978; Chan *et al*., 1992; Lai *et al*., 1995; Schmitt *et al*., 1995; Sanlaville *et al*., 1997; Torrents *et al*., 1997; Texier *et al*., 1999b; Héquet *et al*., 2001). This observation – plus the fact that when 2-chloro-*s*-triazine herbicides are photolyzed in methanol, ethanol, and *n*-butanol, the respective 2-alkoxy derivatives are formed – indicates a mechanism involving photochemical solvolysis rather than the involvement of hydroxyl radicals. This conclusion is supported by the fact that the rate of oxidation of atrazine was unaffected by the presence of either bicarbonate ion (Beltrán *et al*., 1993) or *tert*-butanol (Torrents *et al*., 1997), both strong hydroxyl radical scavengers.

Torrents *et al*. (1997), Texier *et al*. (1999b), and Héquet *et al*. (2001) studied the direct photolysis of atrazine (CEIT) under various experimental conditions (Table 23.2). In all the three studies, hydroxyatrazine (OEIT or G-34048) and dealkylated atrazine (CEAT or G-28279 and CAIT or G-30033) were detected (Figure 23.1). When atrazine was photolyzed (ffi290 nm) in ultrapure water (Torrents *et al*., 1997), the intermediary acetamido products (CEDT and CDIT) were also formed in detectable amounts. When irradiated (ffi290 nm) in distilled water, photoproducts of atrazine also included the didealkylated product (CAAT or G-28273) and the dealkylated products of hydroxyatrazine (OEAT or GS-17792, OAIT or GS-17794, and OAAT or GS-17791) (Texier *et al*., 1999b). Using UV-VIS radiation (quartz filter), Héquet *et al*. (2001) observed the same photoproducts (with the exception of

Table 23.2 Identified photolysis products[a] resulting from photolysis of aqueous solutions of several triazine herbicides

Reference	Photolysis products																						Sensitizer/light source
	OEIT	OEAT	ODIT	OAIT	OAAT	OOAT	COAT	COOT	OOOT	CDIT	CEPT	CEYT	CFIT	CEDT	CDDT	CAIT	CEAT	CADT	CAAT	HEIT	HAIT	HEAT	HAAT	
	Atrazine (CEIT)																							
Pape and Zabik (1970)	X																							254 nm
Burkhard *et al.* (1975)	X	X		X	X											X	X		X					acetone, sunlight
Burkhard and Guth (1976)	X	X		X	X											X	X		X					acetone, • 290 nm
Khan and Schnitzer (1978)	X																							254 nm
	X	X		X	X																			fulvic acid, 254 nm
Rejto *et al.* (1983)										X						X			X					riboflavin/sunlight
Carlin *et al.* (1990)	X				X	X	X	X	X										X					TiO_2, Cl_2, ffi340 nm
Pelizzetti *et al.* (1990b)	X				X	X	X	X	X	X						X	X		X					TiO_2 suspension, ffi340 nm
Durand *et al.* (1991)	X																							humic acid, ffi300 nm
Pelizzetti *et al.* (1992)	X	X		X	X	X	X	X	X	X	X	X	X			X	X		X					TiO_2 suspension, ffi340 nm
Chan *et al.* (1992)	X	X																						254 nm
	X	X	X	X	X	X	X	X	X	X						X	X		X					H_2O_2, 254 nm
Hessler *et al.* (1993)	X	X		X												X	X							H_2O_2, 254 nm
Pugh *et al.* (1995)	X			X	X	X			X							X	X		X					TiO_2 on fiberglass mesh, ffi280 nm
Torrents *et al.* (1997)	X									X				X		X	X							Ultrapure water, ffi290 nm
	X									X				X		X	X							DOC, ffi290 nm
	X	X		X						X				X	X	X	X	X	X					NO_3^-, ffi290 nm
	X	X		X						X				X	X	X	X	X	X					NO_3^-, DOC, ffi290 nm
DeLaat *et al.* (1997)	X															X	X		X					H_2O_2, 254 nm
Texier *et al.* (1999a)	X	X		X	X				X							X	X		X					TiO_2, sunlight
		X		X	X											X	X		X					$Na_4W_{10}O_{32}$, sunlight
Texier *et al.* (1999b)	X	X		X	X											X	X		X					ffi290 nm
	X	X		X	X					X				X		X	X		X					TiO_2, ffi290 nm
		X		X	X											X	X		X					$Na_4W_{10}O_{32}$, ffi290 nm, pH ≤ 2.4
Balmer and Sulzberger (1999)										X				X		X	X		X					Fe^{3+}/oxalate, pH ≤ 3.2–5.6, ffi290 nm
Héquet *et al.* (2001)	X	X		X	X				X							X	X							UV-VIS, quartz filter
	X	X		X	X				X							X	X		X					ffi290 nm, humic materials
Hiskia *et al.* (2001)	X				X	X			X							X	X		X					$K_4SiW_{12}O_{40}$, ffi320 nm
Konstantinou *et al.* (2001)	X															X	X							natural waters, distilled water, sunlight
	OEET	OEAT			OAAT	OOAT	COAT	COOT	OOOT	CEDT	CFET			CDFT	CDDT		CEAT		CAAT	HEET		HEAT	HAAT	
	Simazine (CEET)																							
Pape and Zabik (1970)	X																							254 nm
Pelizzetti *et al.* (1992)	X	X			X	X	X	X		X	X			X			X		X					TiO_2 suspension, ffi340 nm
Lai *et al.* (1995)	X	X																						254 nm
					X	X	X										X		X					O_3, 254 nm

	O	O		O	O	C	C	O	C	C	C	C		C	C	C		C	H	H		H	
	I	A		A	O	O	O	O	D	P	Y	F		D	A	E		A	I	A		A	
	I	I		A	A	A	O	O	I	I	I	I		D	I	I		A	I	I		A	
	T	T		T	T	T	T	T	T	T	T	T		T	T	T		T	T	T		T	
	Propazine (CIIT)																						
Pape and Zabik (1970)	X																						254 nm
Pelizzetti *et al*. (1992)	X	X		X	X	X	X	X	X	X	X	X			X	X		X					TiO_2 suspension, ffi340 nm
Barceló *et al*. (1993)	X														X				X	X			humic acids, ffi300 nm
Konstantinou *et al*. (2001)	X														X								natural waters, distilled water, sunlight
	O	O	O	O	O	C	C	O	C				C	C	C	C	C	C			H	H	
	B	B	E	A	O	O	O	O	B				E	D	B	E	A	A			E	A	
	E	A	A	A	A	A	O	O	D				D	D	A	A	D	A			A	A	
	T	T	T	T	T	T	T	T	T				T	T	T	T	T	T			T	T	
	Terbuthylazine (CBET)																						
Sanlaville *et al*. (1996)	X	X	X	X					X						X	X		X					H_2O_2, 254 nm
									X						X								TiO_2, ffi290 nm
Sanlaville *et al*. (1997)	X	X	X	X											X	X							254 nm
Mansour *et al*. (1997)				X					X						X	X		X					humic acid, H_2O_2, acetone, ffi290 nm
	O	O	O	O	O			O	S				S	S	S	S	S	S	H	H	H	H	
	E	E	A	A	O			O	D				E	D	A	E	A	A	E	A	E	A	
	I	A	I	A	A			O	I				D	D	I	A	D	A	I	I	A	A	
	T	T	T	T	T			T	T				T	T	T	T	T	T	T	T	T	T	
	Ametryn (SEIT)																						
Pape and Zabik (1970)																			X				254 nm
Burkhard and Guth (1976)	X	X	X	X											X	X		X	X	X	X	X	acetone, ffi290 nm
Rejto *et al*. (1983)									X						X			X					riboflavin/sunlight
	O	O		O	O				S					S	S		S	S	H		H	H	
	E	E		A	O				E					D	E		A	A	E		E	A	
	E	A		A	A				D					D	A		D	A	E		A	A	
	T	T		T	T				T					T	T		T	T	T		T	T	
	Simetryn (SEET)																						
Pape and Zabik (1970)																			X				254 nm
	O	O		O	O			O	S					S	S		S	S	H		H	H	
	I	A		A	O			O	D					D	A		A	A	I		A	A	
	I	I		A	A			O	I					D	I		D	A	I		I	A	
	T	T		T	T			T	T					T	T		T	T	T		T	T	
	Prometryn (SIIT)																						
Pape and Zabik (1970)																			X				254 nm
Khan and Gamble (1983)	X																		X				254 nm
	X																		X		X		humic or fulvic acid, 254 nm
Konstantinou *et al*. (2001)	X														X								natural waters, distilled water, sunlight
	O	O	O	O	O			O	M				M	M	M	M	M	M	H	H		H	
	E	E	A	A	O			O	D				E	D	A	E	A	A	E	E		A	
	I	A	I	A	A			O	I				D	D	I	A	D	A	I	A		A	
	T	T	T	T	T			T	T				T	T	T	T	T	T	T	T		T	
	Atraton (MEIT)																						
Pape and Zabik (1970)																							254 nm, no reaction
Burkhard and Guth (1976)	X	X	X	X											X	X		X					acetone, ffi290 nm
Rejto *et al*. (1983)									X						X			X					riboflavin/sunlightt

[a]A: amino, B: *tert*-butylamino (1,1-dimethylethylamino), C: chloro, D: acetamido, E: ethylamino, F: formylamino, H: hydrogen, I: isopropylamino (1-methylethylamino), M: methoxy, O: hydroxy, P: 2-aminopropanol, S: methylthio, T: triazine ring, Y: 2-aminopropionaldehyde. For further identification of these coded products and structures, see Appendix, Table 3.

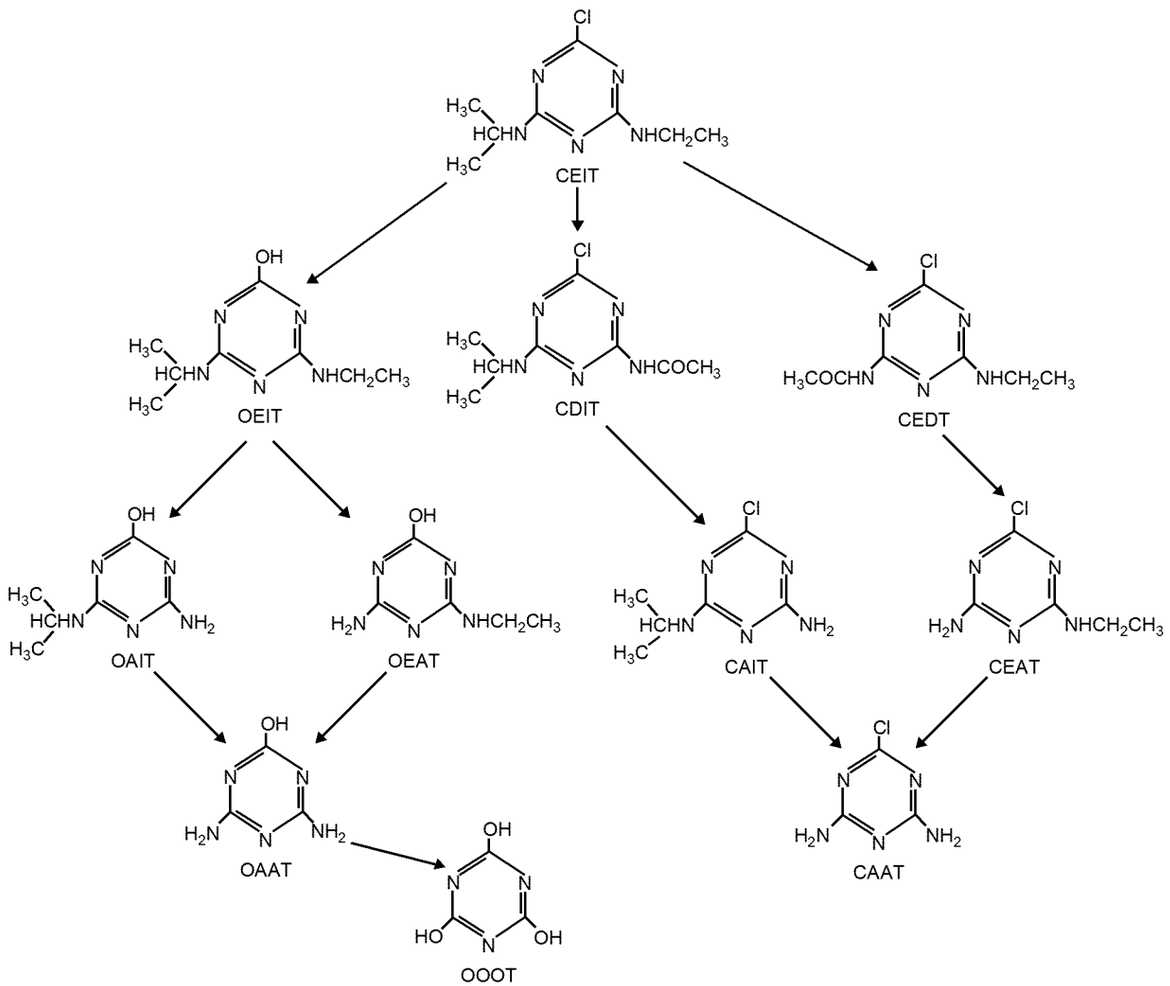

Figure 23.1 Direct photolysis of atrazine in aqueous solution.

CAAT), as well as the formation of cyanuric acid (OOOT or G-28251). Analogous products (CBAT or G-26379, CAET, OBET or GS-23158, OBAT, OAET, OAAT) were formed when an aqueous solution of terbuthylazine (CBET) was irradiated at 254 nm (Sanlaville *et al.*, 1997). Direct photolysis (254 nm) of an aqueous solution of simazine (CEET) resulted in the formation of hydroxysimazine (OEET or G-30414), as well as its dealkylated analog (OEAT) (Lai *et al.*, 1995) (Table 23.2).

In contrast, photolysis (254 nm) of aqueous solutions of the 2-methylthio-*s*-triazine herbicides (ametryn, simetryn, and prometryn) resulted in the substitution of the methylthio group with a hydrogen (H) atom and formation of the corresponding 2-H analogs (Pape and Zabik, 1970; Khan and Gamble, 1983) (Table 23.2). Thus, it is evident that photoreaction involving the 2-chloro and 2-methylthio substituents occurs by distinctly different mechanisms. Since the 2-H analogs are also formed in methanol, ethanol, *n*-butanol, and benzene (Pape and Zabik, 1970), solvent participation seems unlikely. Instead, the mechanism appears to involve a concerted rearrangement with an intramolecular hydrogen shift (Pape and Zabik, 1970). Photolysis (254 nm) of an aqueous solution of prometryn (SIIT) resulted in the formation of the 2-H (HIIT) and 2-hydroxy (OIIT or GS-11526) analogs (Khan and Gamble, 1983), whereas sunlight photolysis produced the dealkylated product (SAIT) (Konstantinou *et al.*, 2001).

Indirect Photolysis in Aqueous Solution Involving Energy Transfer

Rates of photolysis for atrazine (Mansour *et al.*, 1988) and other pesticides (Mansour *et al.*, 1997) have been reported to be greater in natural river waters than in distilled water. These enhanced photolysis rates in natural waters are most

likely due to the presence of naturally occurring sensitizers. Some natural sensitizers, such as acetone, may initiate photoreaction via energy transfer. The photolysis (ffi290 nm) of aqueous solutions of atrazine (Burkhard *et al.*, 1975; Burkhard and Guth, 1976), ametryn, and atraton (Burkhard and Guth, 1976) in the presence of acetone resulted in a much more extensive photolysis of these compounds. Photoproducts of atrazine and atraton included not only the corresponding 2-hydroxytriazine compounds formed by direct photolysis, but also the dealkylated and didealkylated products and their corresponding hydroxy compounds (Table 23.2). Analogous products resulted from the photolysis of ametryn along with the corresponding 2-H analogs. Photolysis of an aqueous solution of terbuthylazine in the presence of acetone was markedly enhanced (Sanlaville *et al.*, 1996). When riboflavin was used as a sensitizer for the photolysis (by sunlight) of aqueous solutions of the above three triazine herbicides, no loss of the 2-substituent was observed in the isolated photoproducts, which included the acetamido, deethylated, and didealkylated analogs (Rejto *et al.*, 1983). Humic acids in artificial sea water were observed to enhance the photolysis (ffi290 nm) of both atrazine and its primary photoproduct, 2-hydroxyatrazine (Durand *et al.*, 1990, 1991).

Indirect Photolysis in Aqueous Solution Involving Hydroxyl Radical Formation

Other sensitizers – including, for example, hydrogen peroxide, humic materials, nitrate, semiconductor particulates such as TiO_2, and polyoxometalates such as decatungstate ($W_{10}O_{32}^{4-}$) – generate reactive species that react with the substrate. When irradiated with near UV light at wavelengths ffi290 nm, dilute aqueous solutions of H_2O_2 (Draper and Crosby, 1984) and nitrate (Zafiriou, 1974; Zepp *et al.*, 1987), and suspensions of TiO_2 (Pelizzetti *et al.*, 1990b), generate hydroxyl radicals that act as a photooxidant. In contrast, aqueous solutions of humic materials in the presence of sunlight produce H_2O_2, which in turn generates hydroxyl radicals (Draper and Crosby, 1983). These photosensitizers may play important roles in photodegradation of triazine herbicides – not only in natural waters – but also in moist surface soil (Konstantinou *et al.*, 2001; McMartin *et al.*, 2003) where photochemical reactions may involve substrate in the adsorbed phase, as well as dissolved in the soil solution.

Effect of Nitrate

Sunlight irradiation of nitrate, which is present in many natural waters, has been shown to result in the formation of hydroxyl radicals (Zafiriou, 1974; Zepp *et al.*, 1987). Thus, during irradiation (ffi290 nm) of an aqueous solution of atrazine in the presence of nitrate (Torrents *et al.*, 1997), more rapid (by a factor of 7) and more extensive photodegradation occurred than during direct photolysis (Table 23.2). Photoproducts included the dealkylated and didealkylated products, plus their intermediary acetamido analogs (Figure 23.2). Dealkylation was favored over alkyl oxidation by a factor of 1.4, and the ethyl group was 1.7 times more reactive than the isopropyl group. In addition, hydroxyatrazine (OEIT) and the 2-hydroxy derivatives of the dealkylated products (OEAT and OAIT) were also detected. OEIT was shown to result only from the direct photolysis of atrazine, whereas OEAT and OAIT resulted from both the direct and indirect photolysis of CEAT and CAIT, respectively.

Effect of Semiconductor Metal Oxides

The photocatalytic degradation of the triazine herbicides (atrazine, simazine, propazine) using titanium oxide has been studied in greatest detail by Pelizzetti *et al.* (1990a, b, 1992) (Table 23.2). Using relatively mild photolysis conditions (ffi340 nm), the photocatalytic degradation of atrazine was much more rapid than under conditions of direct photolysis, where no degradation of atrazine was observed after 2 h of irradiation. Under these conditions, dechlorination followed by hydroxylation took place only to a limited extent. Alkyl chain oxidation and subsequent dealkylation represented the major degradation pathway. This was followed by successive formation of hydroxy derivatives (involving dechlorination and deamination) until cyanuric acid (OOOT), the photostable final product, was formed (Figure 23.3). The more extensive degradation of atrazine in the presence of TiO_2, compared to that obtained using radiation of less energy in the presence of nitrate, was most likely due to photocatalysis of the substrate in the adsorbed phase. Texier *et al.* (1999a, b) also reported many of the same photoproducts during photocatalysis (ffi290 nm) by TiO_2 of an aqueous solution of atrazine. Photocatalysis (ffi295 nm) of an aqueous solution of atrazine was slower in the presence of zinc oxide (ZnO) or ferric oxide (Fe_2O_3) compared to TiO_2 (Lackhoff and Niessner, 2002). Titanium oxide (Macounová *et al.*, 2001) and ferric oxide accelerated the photodegradation (ffi290 nm) of an aqueous solution of metamitron (Cox *et al.*, 1996); however, radiation was not required for the surface-catalyzed *N*-dealkylation of atrazine by manganese oxide (MnO_2), in which CEAT and CAIT were formed in equal amounts (Cheney *et al.*, 1998).

The proposed mechanism for photocatalytic degradation of the *N*-(1-methylethyl) group involves reaction of an hydroxyl radical with either a methinyl or the methyl carbon, which via the corresponding hydroperoxide subsequently leads to formation of the acetamido and formylamino (via aminopropionaldehyde) products, respectively

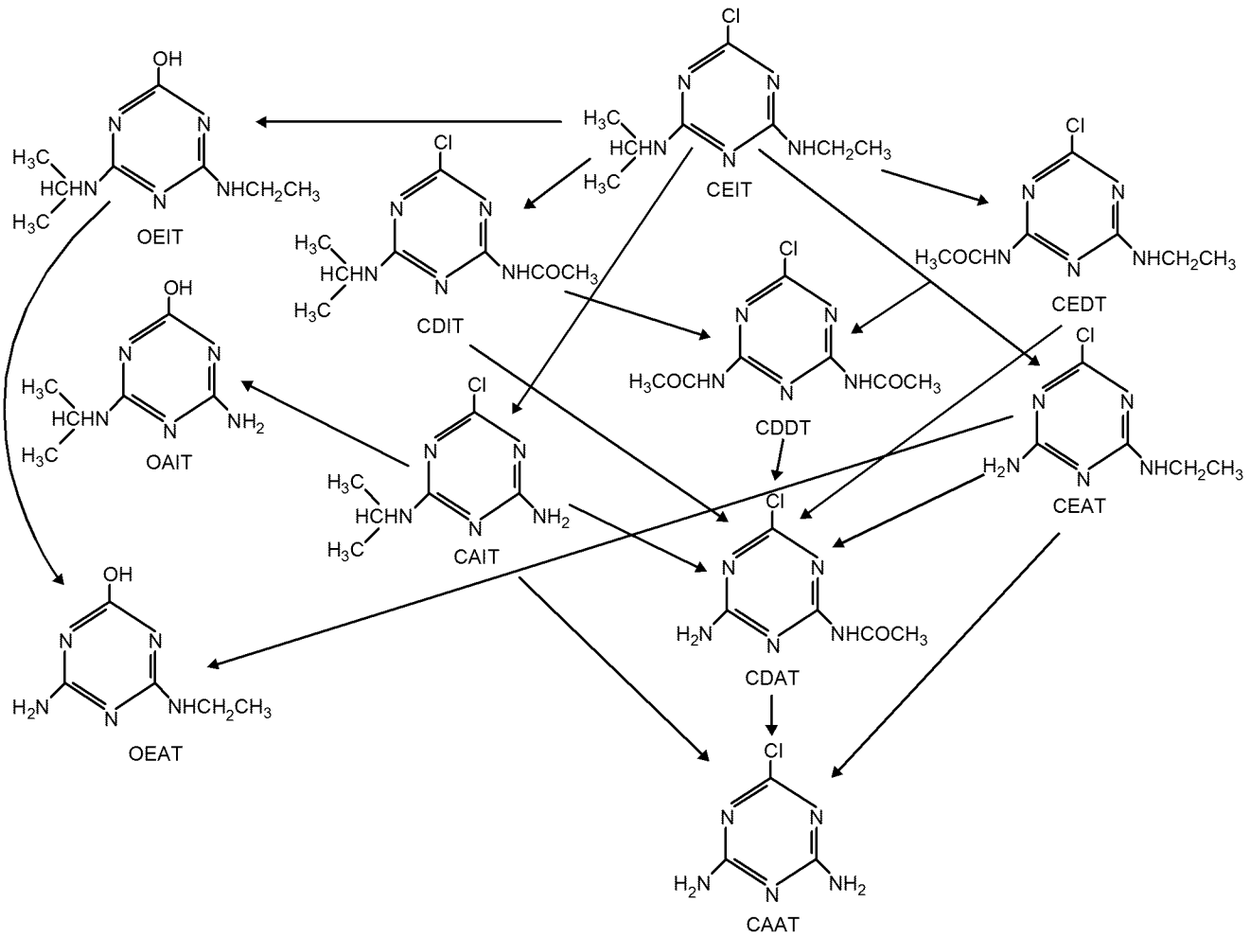

Figure 23.2 Photoproducts resulting from the photolysis (ffi290 nm) of an aqueous solution of atrazine in the presence of nitrate.

(Pelizzetti *et al.*, 1992) (Figure 23.4). Reaction of the *N*-ethyl group similarly leads to the formation of acetamido and formylamino products (Pelizzetti *et al.*, 1992) (Figure 23.5, Table 23.2). Further oxidation results in carboxyl group formation, with subsequent photocatalytic decarboxylation leading to the corresponding dealkylated products.

The presence of chlorine, which is frequently used to disinfect drinking water, did not markedly affect the photocatalytic degradation of an aqueous solution of atrazine in the presence of a suspension of TiO_2 (Carlin *et al.*, 1990). Many of the same photodegradation products formed using a suspension of TiO_2 (Figure 23.3) were also observed when an aqueous solution of atrazine was photolyzed (ffi280 nm or sunlight) in the presence of TiO bound to fiberglass mesh (Pugh *et al.*, 1995) (Table 23.2). Photocatalysis of aqueous solutions of terbuthylazine (Sanlaville *et al.*, 1996), simazine, and cyanazine (Hustert *et al.*, 1991) in the presence of a suspension of TiO_2 produced analogous dealkylated and didealkylated products. When TiO_2 was immobilized in a photocatalytic membrane, essentially complete mineralization of aqueous solutions of atrazine, simazine, propazine, terbuthylazine, ametryn, and prometryn was realized during photodegradation (Bellobono *et al.*, 1998). When trialkylvandanates were co-immobilized with TiO_2 in the membrane, photocatalytic degradation (mineralization) of these triazine herbicides was greatly enhanced (Bellobono *et al.*, 1998).

Effect of Ferric Ion

When irradiated with UV light in aqueous solution, hydrated ferric ions are photoreduced to ferrous ions with the production of hydroxyl radicals. Thus, the photolysis (ffi290 nm) of aqueous solutions of atrazine, ametryn, prometryn, and prometon in the presence of ferric perchlorate or ferric sulfate was greatly enhanced in comparison to direct photolysis (Larson *et al.*, 1991). In the absence of oxygen or in stream water, photoreaction rates were

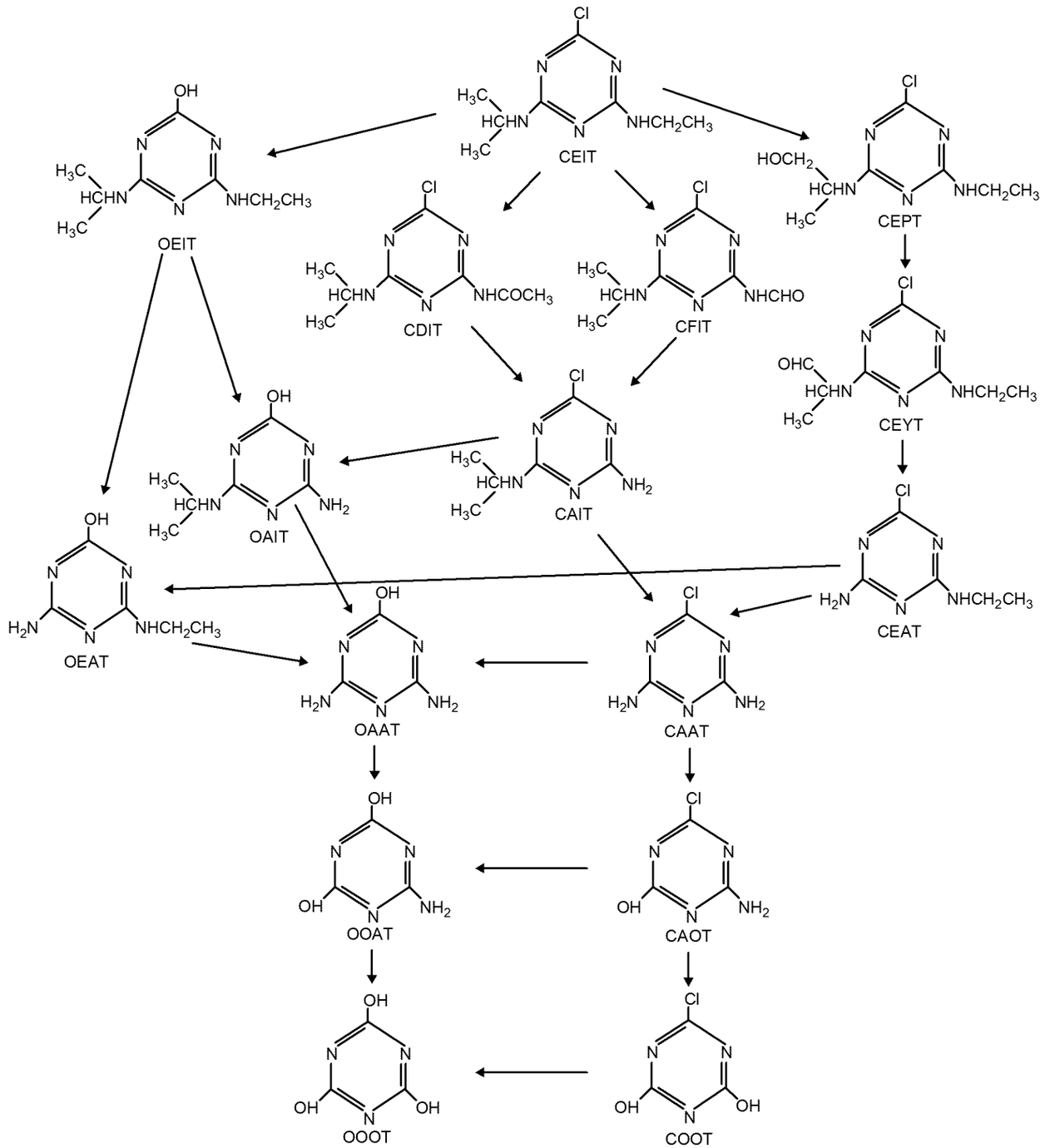

Figure 23.3 Products resulting from the photocatalytic (ffi340 nm) degradation of an aqueous solution of atrazine in the presence of TiO_2 particulates.

decreased. Photoproducts tentatively identified for photolysis of atrazine included the dealkylated products (CAIT, CEAT) and the intermediary acetamido analog, CDIT (Table 23.2). In a later study, Balmer and Sulzberger (1999) studied the photolysis (ffi290 nm) of an aqueous solution of atrazine in the presence of ferric ion and oxalate over a range of pH values. They identified the acetamido products (CEDT and CDIT), the dealkylated compounds, and

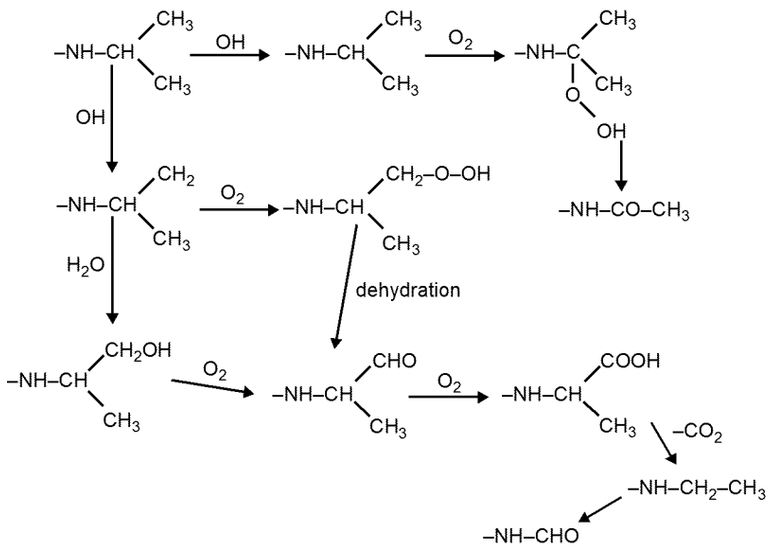

Figure 23.4 Proposed mechanism for the photocatalytic degradation of the *N*-(1-methylethyl) group in the presence of TiO_2.

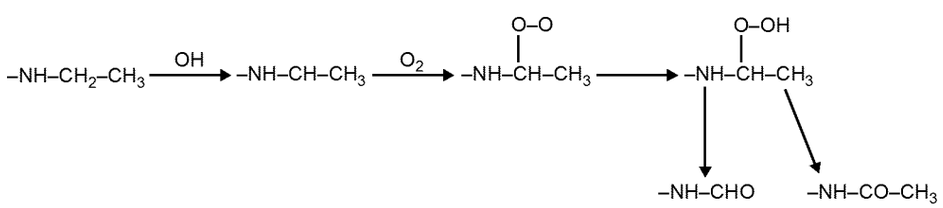

Figure 23.5 Proposed mechanism for the photocatalytic degradation of the *N*-ethyl group in the presence of TiO_2.

CAAT, the didealkylated product. No hydroxyl analogs were formed. Peñuela and Barceló (2000) reported that the rates of photolysis (sunlight, ffi286 nm) of aqueous solutions of atrazine or desethylatrazine (CAIT) were greater in the presence of ferric ion compared to TiO_2.

When ferric ion is in the presence of aqueous H_2O_2 and under acidic conditions, it produces hydroxyl radicals in Fenton-type reactions. When irradiated with UV light, hydroxyl radical production is enhanced because the resulting ferrous ion reacts with H_2O_2 to produce hydroxyl radicals as well. When an aqueous solution of (*ring*-UL-^{14}C) atrazine was irradiated (ffi300 nm) in the presence of Fe(III) chelates and H_2O_2, degradation of atrazine was more rapid than in the absence of UV light (Sun and Pignatello, 1993). There was no evolution of $^{14}CO_2$, indicating that no mineralization of the triazine ring occurred, as observed previously (Pelizzetti *et al.*, 1990a).

Effect of Polyoxometalates

Mylonas *et al.* (1996) and Mylonas and Papaconstantinou (1996) reported the photocatalysis (ffi320 nm) of 4-chlorophenol in the presence of three polyoxometalates (tungstates) relative to titanium oxide. Although catalysis is thought to proceed via an excited state of the polyoxometalate following light absorption, the mechanism is not completely understood (Texier *et al.*, 1999a and references therein). In kinetic studies of the photolysis of aqueous solutions of atrazine, Texier *et al.* (1999c) reported the photocatalysis by decatungstate ($W_{10}O_{32}^{4-}$) to be slower than that by TiO_2. In more detailed studies, Texier *et al.* (1999a, c) observed the formation of the dealkylated products (CEAT and CAIT), the didealkylated product (CAAT), and their hydroxylated analogs (OEAT, OAIT, and OAAT). The formation of the intermediary acetamido products (CEDT and CDIT) and hydroxyatrazine (OEIT) was not observed. When $SiW_{12}O_{40}^{4-}$ was used to effect photocatalysis (ffi320 nm), similar products (with the exception of OEAT) were formed, along with the hydroxylated products of OAAT (OOAT or G-25713 and OOOT) (Hiskia *et al.*, 2001).

Effect of Hydrogen Peroxide

The photolysis of aqueous H_2O_2 by UV light produces hydroxyl radicals, which in turn can react with organic substrates in water. Photooxidation products of triazine herbicides that result from reaction with UV/H_2O_2 are generally

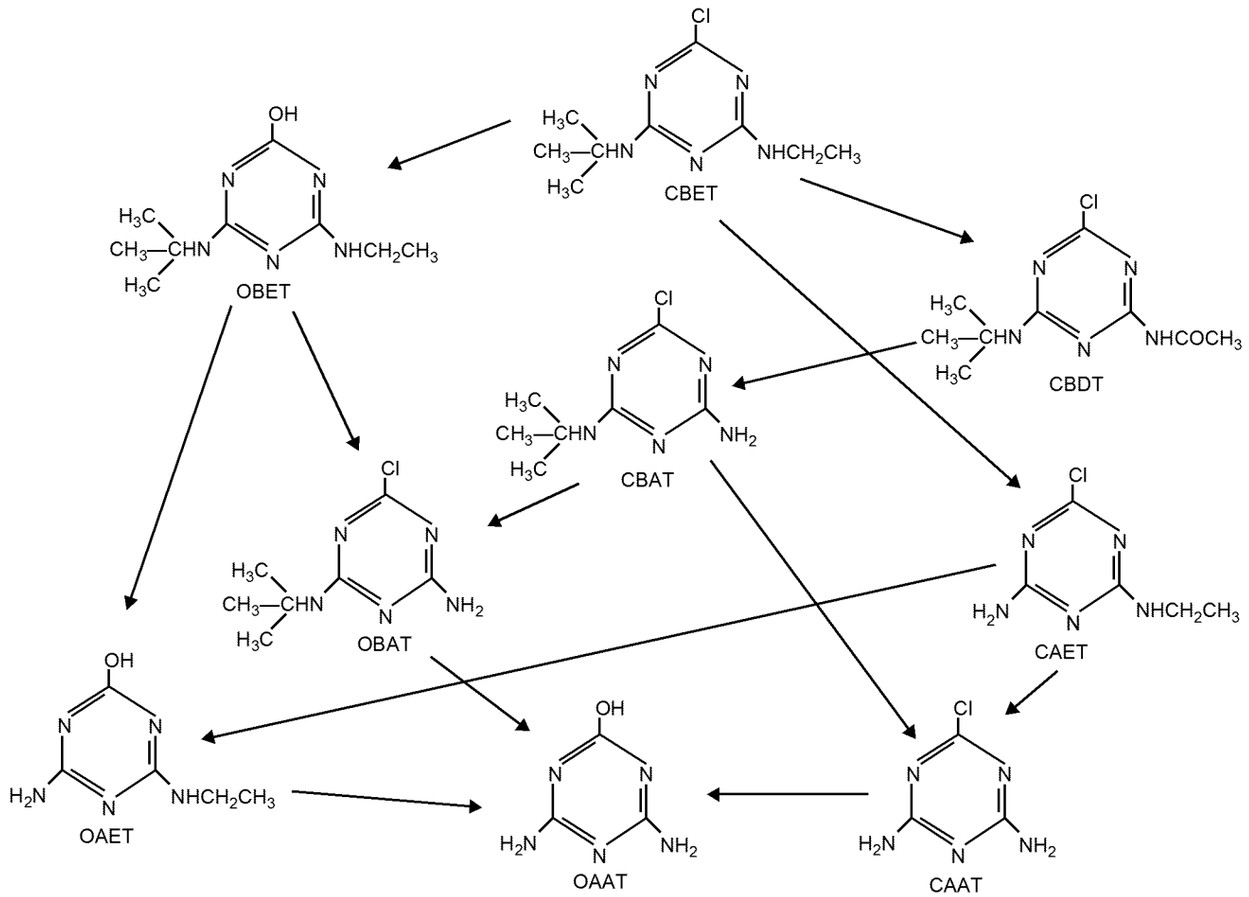

Figure 23.6 Photooxidation products resulting from the photolysis (ffi290 nm) of an aqueous solution of terbuthylazine in the presence of UV/H_2O_2.

the same as those that result from photodegradation sensitized by nitrate (Figure 23.2). For example, the photolysis (ffi290 nm) of an aqueous solution of terbuthylazine in the presence of UV/H_2O_2 resulted in the formation of hydroxy-terbuthylazine, dealkylated products, and the corresponding 2-hydroxy analogs (Sanlaville *et al.*, 1996) (Figure 23.6). Analogous photooxidation (254 nm) products were also observed for aqueous solutions of atrazine (Chan *et al.*, 1992; Hessler *et al.*, 1993; DeLaat *et al.*, 1997); however, in one of these studies (Chan *et al.*, 1992), deaminated photoproducts (COAT, OOAT, OOOT) were also identified (Table 23.2).

Effect of DOC

Natural waters and soil solutions contain naturally occurring dissolved organic materials, including humic substances. Soil humic substances are 'amorphous, dark-colored, hydrophilic, acidic, partly aromatic, chemically complex organic substances that range in molecular weight from a few hundred to several thousand' (Schnitzer, 1982). Humic substances are soluble in dilute base and are generally extracted from soil using alkaline extractants such as dilute sodium hydroxide. Humic substances consist primarily of humic and fulvic acids whose characterization is based on their solubility in base and acid. Humic acid is defined as that component of humic substances which is soluble in dilute base but is precipitated by acidification of the alkaline soil extract. Fulvic acid is defined as that component of humic substances that remains soluble in the alkaline soil extract after acidification; that is, it is soluble in both dilute base and acid. Fulvic acid has a lower molecular weight and a higher content of oxygen-containing functional groups (carboxyl, hydroxyl, and carbonyl) than humic acid. Humic and fulvic acids have a relatively high resistance to microbial degradation.

Both humic and fulvic acids can form complexes with the *s*-triazine herbicides (Senesi, 1992). Formation of these complexes has been reported with the 2-chloro- [atrazine (Haniff *et al.*, 1985; Martin-Neto *et al.*, 1994, 2001; Senesi

et al., 1995; Sposito *et al.*, 1996), simazine (Senesi *et al.*, 1995)], 2-methylthio- [ametryn, desmetryn (Senesi and Testini, 1982; Senesi *et al.*, 1987)], and 2-methoxy-*s*-triazine herbicides [prometon (Senesi and Testini, 1982; Senesi *et al.*, 1987)]. The mechanism by which such complexes are formed depends largely upon the acidic functional group content of the humic material, the basicity of the triazine herbicide, and the pH of the water. For example, for humic acids of high acidic functional group content and *s*-triazine herbicides of low basicity, the complexes tend to form by proton transfer and hydrogen bonding (Martin-Neto *et al.*, 1994; Sposito *et al.*, 1996). Conversely, for humic acids of low acidic functional group content and *s*-triazine herbicides of high basicity, electron transfer mechanisms are favored (Senesi *et al.*, 1995; Sposito *et al.*, 1996).

In the presence of sunlight, the formation of complexes may affect the rate and extent of photolysis of the triazine herbicides. Using DOC mimics, Hapeman *et al.* (1998) showed that the photochemical role of DOC is largely a function of its structure. DOC, including humic substances, may also absorb UV light at wavelengths greater than 290 nm. Thus, DOC could enhance the rate and extent of photodegradation of a triazine herbicide by sensitizing direct photolysis or, alternatively, by indirect photolysis when acting as a source of hydroxyl radicals. Conversely, photolysis is not always enhanced by the presence of dissolved organic matter in natural waters (Konstantinou *et al.*, 2001; McMartin *et al.*, 2003; Navarro *et al.*, 2004). In these situations, the DOC may not be an effective photosensitizer, or it may slow photoreactions by competing for incident UV light or acting as a hydroxyl radical scavenger (Mabury and Crosby, 1996; Hapeman *et al.*, 1998).

In the presence of fulvic acid, the photodegradation of an aqueous solution of atrazine, which produced only hydroxyatrazine (OEIT) during direct photolysis, was slower but more extensive with the dealkylated products (OAIT, OEAT, OAAT) also being formed (Khan and Schnitzer, 1978) (Table 23.2). Similarly, humic and fulvic acids slowed the photodegradation of prometryn, but caused more extensive degradation (Khan and Gamble, 1983) (Table 23.2). The formation of the dealkylated compounds that is characteristic of H_2O_2-mediated photoreactions of the triazine herbicides (Hessler *et al.*, 1993; DeLaat *et al.*, 1997) indicates that the fulvic and humic acids used in these studies were ultimately sources of hydroxyl radicals. The degradation of propazine was also slower in artificial sea water containing humic acids (Barceló *et al.*, 1993).

The photodegradation of an aqueous solution of terbuthylazine was not only accelerated, but was also more extensive in the presence of humic acids isolated from soil (Mansour *et al.*, 1997). In the absence of humic acids, only hydroxyterbuthylazine (OBET) was formed (Sanlaville *et al.*, 1996), whereas in the presence of humic acids, dealkylated products (CBAT, CBDT, CEAT, CAAT, OAAT) were formed (Table 23.2) (Sanlaville *et al.*, 1996; Mansour *et al.*, 1997). In contrast, fulvic acids isolated from stream water slowed the photolysis of terbuthylazine, most likely reflecting differences in structure between the soil- and stream-derived materials. The photodegradation of atrazine and its initial photoproduct OEIT (Table 23.2) in artificial sea water containing humic acids was also accelerated compared to photolysis in distilled water (Durand *et al.*, 1990, 1991).

Effect of Surfactants

Surfactants may enhance herbicide photolysis due to micellar solubilization of the herbicide and/or photosensitization if the surfactant contains a chromophore, which absorbs in the near UV wavelengths ffi290 nm. In one such study investigating the effects of surfactants on the photodegradation of triazine herbicides (Tanaka *et al.*, 1981), the surfactant Triton X-100, which contains the chromophoric aryl group, appeared to sensitize the photolysis of aqueous solutions of atrazine, ametryn, and prometon when irradiated with light at wavelengths ffi300 nm. However, in another study, the photocatalytic degradation of an aqueous solution of formulated atrazine in the presence of TiO_2, immobilized in a photocatalytic membrane, was slower than that for unformulated atrazine (Pugh *et al.*, 1995). Similar results were reported by Texier *et al.* (1999a) for photocatalysis of atrazine using particulate TiO_2. Pugh *et al.* (1995) suggested that this decreased rate of photocatalytic degradation was due to formulation components interfering with the catalyst, either through adsorption to the catalyst or by competing with atrazine for oxidation by the catalyst. In contrast, the photocatalysis by decatungstate ($W_{10}O_{32}^{4-}$), which is water soluble, was unaffected when an aqueous solution of formulated atrazine was photolyzed by sunlight (Texier *et al.*, 1999a).

Photolysis of Triazine Herbicides Associated with Aerosols

Two studies have been carried out to investigate phototransformation of triazine herbicides when associated with atmospheric aerosols. The photodegradation (ffi290 nm) of atrazine and terbuthylazine was studied when sorbed to kaolin (terrigenic) or fly ash (anthropogenic) aerosols that were constantly suspended in a reactor (Bossan *et al.*, 1995). Photodegradation of both herbicides was observed, but terbuthylazine appeared to degrade more rapidly than atrazine. In the other study, the reaction of terbuthylazine with hydroxyl radicals was investigated in an aerosol smog chamber (Palm *et al.*, 1997a). A silicon dioxide aerosol, to which terbuthylazine was sorbed, was irradiated with UV

light (ffi290 nm) in the presence of a hydroxyl radical precursor [H_2O_2, O_3, or nitrogen dioxide (NO_2)]. Degradation of terbuthylazine occurred, and the deethylated (CBAT) and intermediary acetamido (CBDT) products were formed.

Photolysis of the Triazinone Herbicides

The *as*-triazinone class of herbicides includes metribuzin and metamitron. In contrast to the *N*-alkyl groups of the *s*-triazine herbicides, these herbicides have a single *N*-amino group in the 4-position of the ring. Both metribuzin (Muszkat *et al*., 1998) and metamitron (Palm *et al*., 1997b) absorb UV light at wavelengths greater than 290 nm and thus are susceptible to degradation by direct photolysis.

Metribuzin

The photolysis of metribuzin has been studied in greatest detail by Raschke and coworkers (Raschke *et al*., 1998a, b). The photolysis (254 nm) of an aqueous solution of metribuzin was initially characterized by side-chain deamination to form deaminometribuzin (DA-metribuzin) and sulfoxidation and dealkylation of the 3-methylthio side chain to form diketometribuzin (DK-metribuzin) (Figure 23.7). Both of these processes resulted in the formation of

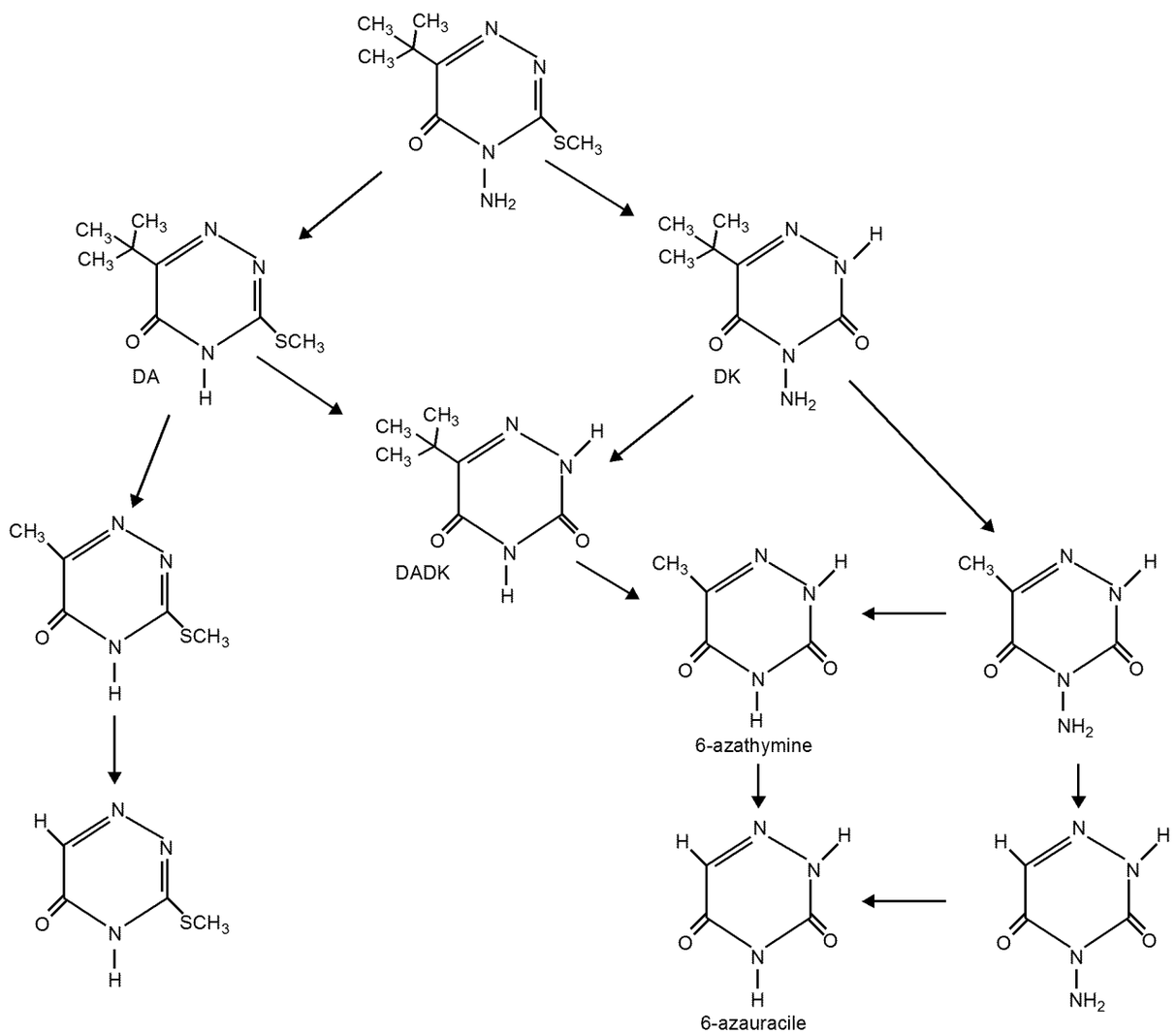

Figure 23.7 Photoproducts resulting from the photolysis (254 nm) of an aqueous solution of metribuzin.

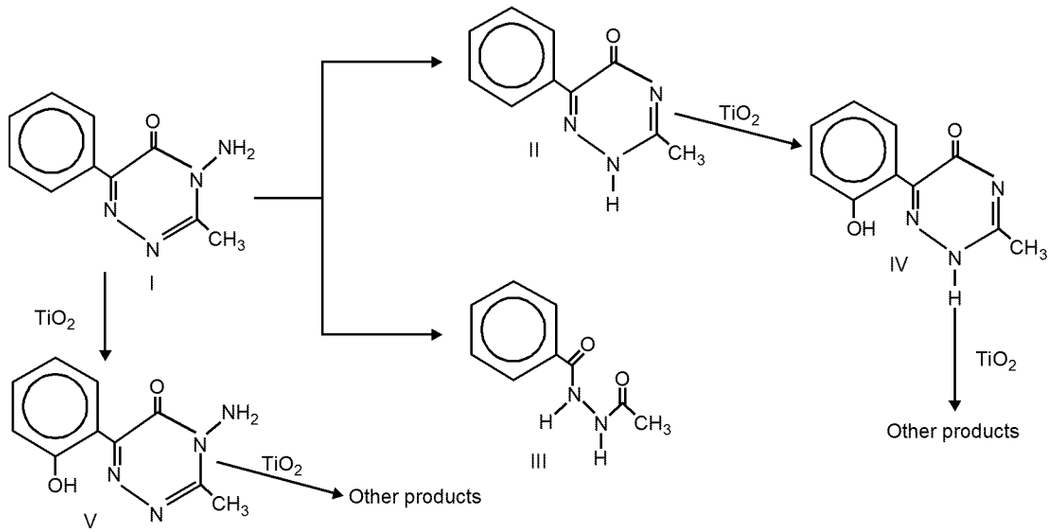

Figure 23.8 The major photoproducts resulting from the photolysis (ffi290 nm) of an aqueous solution of metamitron.

deaminodiketometribuzin (DADK-metribuzin). Continued photolysis resulted in degradation of these initial photoproducts, much of which involved the 1,1-dimethylethyl side chain, and eventually led to the formation of 6-azauracile and 6-azathymine, both of which are very stable photochemically. Long irradiation times were required to detect cleavage of the heterocyclic ring. Rapid photodegradation of metribuzin required the presence of oxygen (or hydrogen peroxide), and the rate of photodegradation was slowed by more than an order of magnitude in the absence of oxygen (Muszkat *et al.*, 1998).

These studies (Raschke *et al.*, 1998a, b) confirmed the formation of DA-metribuzin as the major initial photoproduct in the photolysis of aqueous solutions of metribuzin, as had been suggested earlier when light at wavelengths ffi290 nm had been used (Rosen and Siewierski, 1971; Pape and Zabik, 1972; Parlar and Korte, 1979). Metribuzin also underwent photodegradation when irradiated as a thin film on glass surfaces with UV light [sunlight (Devlin *et al.*, 1987; Peek and Appleby, 1989), ffi290 nm (Pape and Zabik, 1972)], with the formation of DA-metribuzin as the initial photoproduct (Pape and Zabik, 1972).

Metamitron

Although the photolysis of metamitron has not been reported in as great detail as that for metribuzin, side-chain deamination has also been identified as the major initial photoreaction when metamitron was irradiated with sunlight or simulated sunlight (ffi290 nm). Rosen and Siewierski (1971) reported that the sunlight photodegradation of an aqueous solution of metamitron (I) was rapid (ffi90% after 4 h of irradiation) and resulted in the formation of desaminometamitron (II) as the major photoproduct. Subsequently, others have reported similar results (Olmedo *et al.*, 1994; Cox *et al.*, 1996; Sancho *et al.*, 1997), although Palm *et al.* (1997b) have also tentatively identified a second photodegradation product that was assumed to result from cleavage of the triazinone ring (III) (Figure 23.8). Photolysis (365 nm) of an aqueous solution of metamitron was approximately six times more rapid in the presence of TiO_2 immobilized on a glass plate and resulted in the formation of two additional photoproducts, deaminohydroxymetamitron (IV) and hydroxymetamitron (V) (Macounová *et al.*, 2001). Both deaminohydroxymetamitron and hydroxymetamitron underwent further photodecomposition to unidentified products.

The photolysis of metamitron requires both oxygen and water. No photoreaction of metamitron was observed in methanol, acetonitrile, hexane, or oxygen-free water (Palm *et al.*, 1997b). Soil humic acids photosensitized the photolysis of metamitron in water (Cox *et al.*, 1996). However, a slower rate of sunlight photolysis of the herbicide was observed when metamitron was present as a complex with copper (II) (Sancho *et al.*, 1997).

Photolysis of the Triazinedione Herbicides

The triazinedione herbicides are characterized by an *s*-triazine ring with carbonyl groups in the 4- and 6-positions of the ring. Hexazinone is the only triazinedione herbicide currently in widespread use.

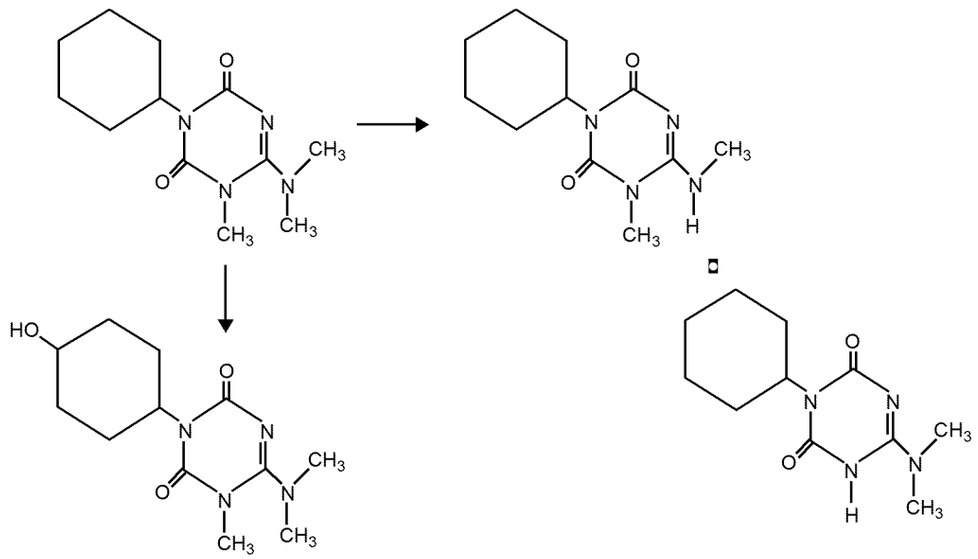

Figure 23.9 Simulated sunlight (ffi290 nm) photolysis of an aqueous solution of hexazinone.

Hexazinone

Simulated sunlight (ffi290 nm) photodegradation of an aqueous solution of hexazinone occurred very slowly [approximately 10% in 5 weeks], but increased in natural river water and in the presence of the sensitizers riboflavin and anthroquinone (Rhodes, 1980). The major routes of photodegradation included *N*-demethylation and hydroxylation of the cyclohexane ring (Figure 23.9). Mabury and Crosby (1996) also reported that the photolysis of hexazinone in water was slow. An aqueous solution of hexazinone in filtered field water from a flooded rice field showed no measurable degradation of the herbicide after 50 h of irradiation with September sunlight.

Removal of Triazines from Water by Photolysis

Strategies to effect the elimination or removal of triazine herbicides from water have included adsorption, conversion to less toxic products, and complete mineralization. Thus, there have been investigations of adsorption by granular activated carbon (Battaglia, 1989), microbial biodegradation (Cook, 1987), oxidation with chlorine dioxide (Miltner *et al.*, 1989), ozonation (Legube *et al.*, 1987; Adams *et al.*, 1990), ozonation followed by biodegradation (Kearney *et al.*, 1988), ozonation combined with hydrogen peroxide (Trancart, 1990), and ferric ion in the presence of hydrogen peroxide (Sun and Pignatello, 1993). The triazine herbicides absorb light in the UV spectral range, and the photolytic methods studied have included the use of high-intensity UV light (Peterson *et al.*, 1988), UV light in the presence of ozone (Kearney *et al.*, 1987; Benitez *et al.*, 1994; Lai *et al.*, 1995; Zwiener *et al.*, 1995) or hydrogen peroxide (Peterson *et al.*, 1988; Chan *et al.*, 1992; Beltrán *et al.*, 1993, 1996; Hessler *et al.*, 1993; Bourgine *et al.*, 1995; DeLaat *et al.*, 1997), vacuum–ultraviolet photolysis (Gonzalez *et al.*, 1994), photocatalytic oxidation using TiO_2 semiconductor particles (Pelizzetti *et al.*, 1990b; Hustert *et al.*, 1991; Pugh *et al.*, 1995; Bellobono *et al.*, 1998; Héquet *et al.*, 2001), UV light in the presence of ferric ion or ferric ion plus hydrogen peroxide (Larson *et al.*, 1991; Sun and Pignatello, 1993) and photocatalysis using polyoxometalates (Texier*et al.*, 1999a, b; Hiskia *et al.*, 2001).

Oxidative degradation of dissolved chemicals in water through catalytic or photochemical methods are generally referred to as Advanced Oxidation Procedures. These procedures are mainly light-induced oxidation processes in which highly reactive intermediates, such as hydroxyl radicals, are generated to oxidize dissolved organic compounds. Photo-induced methods that effect complete mineralization are preferred. However, the degree of mineralization of the substances versus formation of other products varies according to the method employed.

Use of UV/TiO_2

The effectiveness of photocatalytic membranes – in which TiO_2 alone or together with tri-(*tert*-butyl)- and tri-(isopropyl)vanadate were co-immobilized – were compared (Gianturco *et al.*, 1997; Bellobono *et al.*, 1998) for the

mineralization of dilute (0.5–10 μM) aqueous solutions of atrazine, terbuthylazine, simazine, prometryn, propazine, and ametryn saturated with ozone (O_3). Almost quantitative mineralization of the triazine herbicides was obtained using either membrane, but the rate of mineralization was approximately an order of magnitude greater with the membrane in which TiO_2 and the trialkyl vanadates were co-immobilized. The efficiency of photocatalytic membranes relative to TiO_2 suspensions (Hustert *et al.*, 1991) and to TiO_2 bound to a solid substrate such as fiberglass mesh (Pugh *et al.*, 1995; Macounová *et al.*, 2001) is attributed to their permeability; that is, active sites of the catalysts are easily and efficiently reached by permeation. When the TiO_2/trialkyl vanadate membrane was utilized with H_2O_2 instead of O_3, mineralization was dramatically decreased. As expected from the results of earlier studies, the process resulted in substantial formation of 2,4,6-trihydroxy-*s*-triazine (cyanuric acid or OOOT), which is stable to further reaction with hydroxyl radicals (Pelizzetti *et al.*, 1990b).

The feasibility of TiO_2-mediated solar photocatalysis of triazine herbicides has been investigated using aqueous TiO_2 suspensions (Pelizzetti *et al.*, 1990b; Hustert *et al.*, 1991), as well as a solar photocatalytic reactor utilizing TiO_2 bound to fiberglass mesh and designed for possible degradation of pesticides in rinse or wastewater (Pugh *et al.*, 1995). In these studies, no mineralization was observed of the triazine herbicides collectively studied (atrazine, simazine, cyanazine, prometryn, and prometon). Intermediate photoproducts were identified, characteristic of reactions with H_2O_2 (Pelizzetti *et al.*, 1990b), and in all cases the end product of the overall degradation process was cyanuric acid (Table 23.2). Chlorine, used for disinfection of water, has been shown to have little effect on the photocatalytic degradation of atrazine using TiO_2 (Carlin *et al.*, 1990).

Use of Short-Wavelength UV Light

Mineralization of aqueous solutions of the triazine herbicides has also been attempted by irradiation with short-wavelength UV light. Vacuum-ultraviolet photolysis (• 190 nm) of water produces hydrogen atoms and hydroxyl radicals as reactive intermediates. Using this method, mineralization was maximized (ffi90%) when an aqueous solution of atrazine was saturated with argon during photolysis (Gonzalez *et al.*, 1994). Purging the reaction mixture of oxygen with argon facilitated the participation of hydrogen atoms in the mineralization reactions, thus minimizing the production of cyanuric acid, which is stable under these conditions. High-intensity UV light (190–400 nm) has been used to study the degradation of an aqueous solution of propazine (Peterson *et al.*, 1988). No intermediate photolysis products were detected, indicating that extensive mineralization occurred. The rate of direct photolysis (254 nm) of an aqueous solution of cyanazine has also been determined (Benitez *et al.*, 1994).

Use of UV/O_3

UV light irradiation in the presence of O_3 has also been used to investigate the photodegradation of aqueous solutions of triazine herbicides. The combination of UV light with O_3 dissolved in water produces hydroxyl radicals, which results in the formation of photoproducts that are characteristic of the reaction of hydroxyl radicals with triazine herbicides (Pelizzetti *et al.*, 1990b). Oxidation of simazine by UV light (254 nm) in the presence of O_3 resulted in the formation of dechlorination, dealkylation, and deamination by-products (Lai *et al.*, 1995) (Table 23.2). The final product was cyanuric acid, which was stable to further oxidation by hydroxyl radicals. The oxidation of atrazine by UV/O_3 has also been studied in a pilot plant for drinking water treatment and, at flows of 30 m^3/h, concentrations of both atrazine and deethylatrazine could be decreased to less than 0.1 μg/L (Zwiener *et al.*, 1995).

Use of UV/H_2O_2

Photodegradation of triazine herbicides in water has been studied by several workers using UV light and hydrogen peroxide to produce hydroxyl radicals. A kinetic model was developed for the oxidation of atrazine by UV/H_2O_2 in dilute aqueous solution; it assumes that the direct photolysis and oxidation by hydroxyl radicals are the main reactions (DeLaat *et al.*, 1997). The model can be used to predict the effects of parameters such as H_2O_2 concentration, pH, and concentrations of hydroxyl radical scavengers – such as the bicarbonate ion and humic materials present in natural waters. Beltrán and coworkers (1993) investigated the UV/H_2O_2 oxidation of aqueous solutions of atrazine and its degradation products deethylatrazine and deisopropylatrazine (Beltrán *et al.*, 1996), as well as the effects of humic substances and bicarbonate ion on the rate of oxidation. The use of H_2O_2 (10^{-3} M) greatly increased the rate of degradation of these compounds compared to the use of UV light alone. This enhanced rate of atrazine photodegradation by use of H_2O_2 was also observed by other workers (Chan *et al.*, 1992; Hessler *et al.*, 1993; Bourgine *et al.*, 1995) and was used in an industrial-scale reactor to study the decrease in the concentration of atrazine in water at flows of 10–50 m^3/h (Bourgine *et al.*, 1995). As with other photooxidation systems involving the production of hydroxyl radicals as the reactive intermediate, no mineralization was evident and major photoproducts included

hydroxyatrazine, dealkylated atrazine, and the corresponding hydroxylated derivatives (Chan *et al.*, 1992; Hessler *et al.*, 1993; DeLaat *et al.*, 1997) (Table 23.2).

Use of UV/Ferric Ion and UV/Ferric Ion/H_2O_2

The use of ferric ions to sensitize the photolysis of triazine herbicides in water has been investigated as a means to detoxify pesticides. Ferric perchlorate or ferric sulfate was found to enhance the rates of photolysis (sunlight) of aqueous solutions of atrazine, ametryn, prometryn, and prometon by two to three orders of magnitude (Larson *et al.*, 1991). However, photolysis rates were lower in natural waters, implying that dissolved constituents in the natural waters competed effectively for the hydroxyl radicals. Enhanced photolysis (ffi300 nm) of an aqueous solution of atrazine was also observed in the presence of iron (III) chelates and hydrogen peroxide (Sun and Pignatello, 1993) due to Fenton-type reactions. However, mineralization of the triazine ring was not observed. Balmer and Sulzberger (1999) reported higher rates of photolysis (ffi290 nm) of an aqueous solution of atrazine in the presence of ferric oxalate and also studied the effect of oxalate concentration and pH on the rate of photolysis. Photolysis (ffi286 nm) of an aqueous solution of atrazine or deethylatrazine was more rapid in the presence of ferric ion/H_2O_2 compared to TiO_2/H_2O_2 (Peñuela and Barceló, 2000).

Use of Polyoxometalates

Photocatalysis of aqueous solutions of atrazine in the presence of the polyoxometalates $W_{10}O_{32}^{4-}$ (Texier *et al.*, 1999a, b, c; ffi290 nm) and $SiW_{12}O_{40}^{4-}$ (Hiskia *et al.*, 2001; ffi300 nm) has been reported. However, the photocatalysis of atrazine in presence of $W_{10}O_{32}^{4-}$ was slower than that by TiO_2 (Texier *et al.*, 1999a, b, c). In addition, as with TiO_2, photocatalysis with $W_{10}O_{32}^{4-}$ did not result in mineralization of the triazine ring, and decomposition of atrazine proceeded only to the formation of hydroxydidealkylated atrazine (OAAT).

Abiotic Hydrolysis of the Triazine Herbicides

The persistence of the triazine herbicides in surface and groundwater and in soil is dependent to some extent on their susceptibility to chemical hydrolysis. The environmental stability of the triazine herbicides to hydrolysis is dependent upon environmental parameters such as temperature, pH of the water or soil solution, and the presence of dissolved constituents that may catalyze hydrolysis.

Hydrolysis in Aqueous Solution

Natural waters generally tend to have pH values that range from about pH 5 to 9, and maximum temperatures of these waters would seldom exceed 30ffl C. Natural waters also contain inorganic dissolved anions (such as nitrate, sulfate, phosphate, carbonate, and bicarbonate) and associated cations (such as sodium, calcium, and magnesium). In addition, natural waters generally contain DOC in the form of humic or fulvic materials. DOC concentrations in natural waters are generally in the order of 10 mg/L or less [from 3.7 and 6.5 mg/L in creek water (Noblet *et al.*, 1996), 5.8 mg/L in well water (Héquet *et al.*, 1997); up to 5.3 mg/L in Chesapeake Bay (Torrents *et al.*, 1997); • 5 mg/L in the Rhone River (Héquet *et al.*, 1997)]; those in runoff from agricultural land tend to be somewhat higher [34.5 mg/L in effl uent from an agricultural drain (Widmer *et al.*, 1993); 7.5 to 26.3 mg/L in manmade farm ponds or dugouts (Corkal *et al.*, 1998); 9 to 43 mg/L in municipal reservoirs that derive their water from surface runoff (Peterson *et al.*, 1993)].

Effect of pH and Temperature

Under the pH and temperature conditions of natural waters, the various 2-chloro-, 2-methylthio-, and 2-methoxy-*s*-triazine herbicides are generally considered to be 'stable in solution between pH 5 and pH 9,' 'stable in neutral, weakly acidic and weakly alkaline media,' and 'stable to hydrolysis at 20ffl C in neutral, weakly acidic, and weakly alkaline media' (*Pesticide Manual*, 1997). Metribuzin, metamitron, and hexazinone are also considered to be stable under these conditions (*Pesticide Manual*, 1997). Such hydrolytic stability of the triazine herbicides is supported by hydrolysis studies (Rhodes, 1980; Widmer *et al.*, 1993; Noblet *et al.*, 1996; Héquet *et al.*, 1997) that indicate either very slow rates of hydrolysis or no measurable hydrolysis (Table 23.3) under these conditions.

Although hydrolytically stable under environmental conditions, the hydrolysis of the triazine herbicides (including metribuzin) in aqueous solution is temperature and pH dependent (Pesticide Manual, 1997). Hydrolysis of atrazine to hydroxyatrazine increased with increasing temperature (Plust *et al.*, 1981; Chan *et al.*, 1992; Héquet *et al.*, 1997) and decreased as pH values near 1 or 13 were changed toward values of 7 or neutrality (Armstrong *et al.*, 1967; Gamble

Table 23.3 Hydrolysis rate constants (k) for triazine herbicides under environmental conditions

pH	DOC mg/L	Temperature °C	K /week	Reference
Atrazine				
7.7	65	4	⌋ 3.1 − 10⌋ 3	Widmer *et al.* (1993)
7.7	65	30	⌋ 2.4 − 10⌋ 3	Widmer *et al.* (1993)
7.8	6	4	⌋ 3.1 − 10⌋ 3	Widmer *et al.* (1993)
7.8	6	30	⌋ 3.7 − 10⌋ 3	Widmer *et al.* (1993)
6	–	20	⌋ 1.1 − 10⌋ 3	Héquet *et al.* (1997)
8	–	20	⌋ 0.9 − 10⌋ 3	Héquet *et al.* (1997)
6	–	30	⌋ 1.3 − 10⌋ 3	Héquet *et al.* (1997)
8	–	30	⌋ 1.2 − 10⌋ 3	Héquet *et al.* (1997)
8	3.7–34.5	40	No detectable decrease in conc. after 43 days	Noblet *et al.* (1996)
Simazine				
8	3.7–34.5	40	No detectable decrease in conc. after 43 days	Noblet *et al.* (1996)
Hexazinone				
4, 7, 9	–	15	• 1% decrease in concentration after 5–8 week	Rhodes (1980)
7	–	25, 37	• 1% decrease in concentration after 5–8 week	Rhodes (1980)

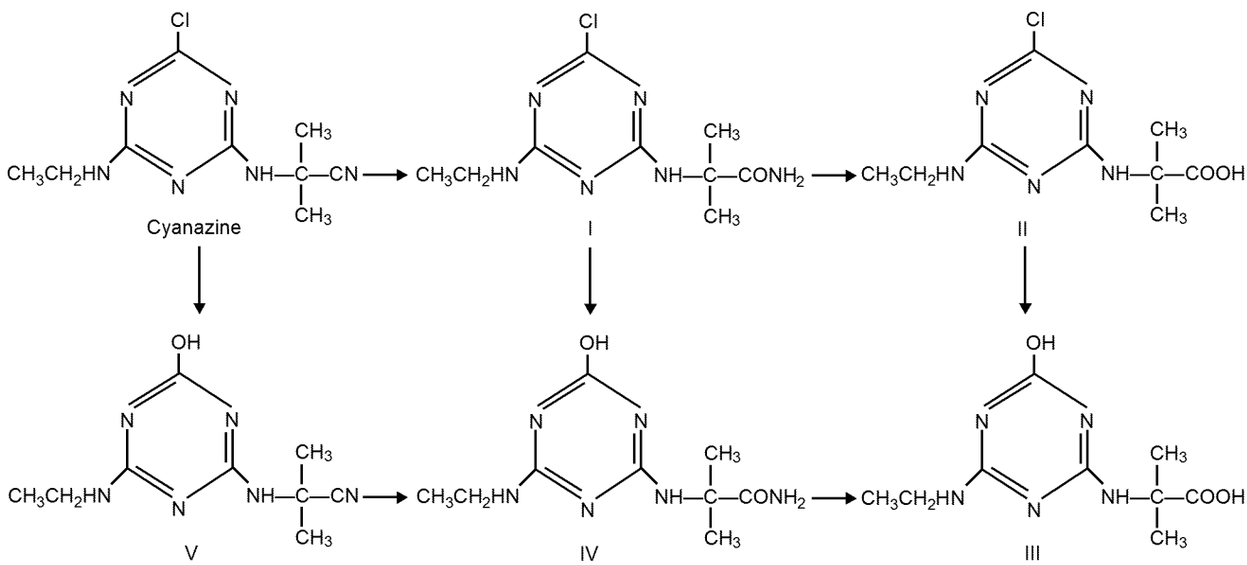

Figure 23.10 Hydrolysis products of cyanazine resulting from hydrolysis in the presence of mineral acids or bases, or in the presence of carboxylic acids or phenols.

et al., 1983; Chan *et al.*, 1992; Héquet *et al.*, 1997; Pesticide Manual, 1997). When cyanazine was hydrolyzed in dilute sulfuric acid, hydrolysis of the cyano group proceeded at a rate approximately an order of magnitude faster than hydrolysis of the 2-chloro group (Grayson, 1980). Thus, in the presence of a strong mineral acid, cyanazine was hydrolyzed mainly via the amide (I) to the carboxylic acid (II), with the subsequent formation of III and possibly IV as the 2-chloro group hydrolyzed (Figure 23.10). Hexazinone decomposes in strong acids and alkalis, and metamitron, although stable in acidic media, also decomposes in strong alkali (Pesticide Manual, 1997).

Effect of DOC

DOC in ground or surface water or in the soil solution may include humic and fulvic acids as well as low-molecular-weight carboxylic acids and phenols. The triazine herbicides form complexes with such humic materials through proton transfer, hydrogen bonding, or by electron-transfer reactions. (For a more complete discussion of the complexation of triazine herbicides with humic materials, see *Indirect Photolysis in Aqueous Solution Involving Hydroxyl Radical Formation – Effect of DOC* in this chapter.) The formation of these complexes can enhance the hydrolysis of the triazine herbicides (Sposito *et al.*, 1996). For example, the hydrolysis of atrazine was catalyzed by the presence of fulvic acid (Khan, 1978; Gamble and Khan, 1985) due to complexing between atrazine and protonated carboxyl groups of the fulvic acid through hydrogen bonding (Haniff *et al.*, 1985). Atrazine was similarly hydrolyzed in the presence of humic acid at pH values less than three (Martin-Neto *et al.*, 1994; Sposito *et al.*, 1996). Aqueous solutions of low-molecular-weight carboxylic acids or phenols catalyzed the hydrolysis of cyanazine (Grayson, 1986). Catalysis by carboxylic acids, such as crotonic acid and acetic acid, resulted in the 2-chloro group becoming the primary site of hydrolysis, rather than the cyano group as was the case when hydrolysis was catalyzed by a mineral acid or base (Grayson, 1980). Thus, hydrolysis proceeded via hydroxycyanazine (V) to the amido intermediate (IV), and then to the carboxylic acid (III) (Figure 23.10).

Hydrolysis in the Presence of Soil

The hydrolysis of atrazine to hydroxyatrazine in aqueous solution was greatly accelerated when soil, as a suspension, was added to the solution (Armstrong *et al.*, 1967). Both organic matter content and pH of the soils affected the rate of hydrolysis. Higher hydrolysis rates occurred with more acidic soils (lower pH) and with soils that had a higher organic matter content. Catalysis of atrazine hydrolysis by humic acid suspensions also proceeded by sorption or by complexing between atrazine and the humic acid (Gamble and Khan, 1988, 1990). The sorption or complexing of triazine herbicides (such as atrazine and terbutryn) to fulvic or humic acids was also affected by the nature of cations present in the soil (Raman *et al.*, 1984; Haniff *et al.*, 1985).

The clay content and the type of clay in soils also affected the hydrolysis of atrazine (Solinas *et al.*, 1983). Hydrolysis occurred following adsorption of atrazine to the surface of the clay. After several months, a portion of the resulting hydroxyatrazine became irreversibly bound (that is, unextractable) to the clay. Fly ash, added to aqueous suspensions of soil, increased atrazine hydrolysis (Albanis *et al.*, 1989).

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Chapter 24

Soil Movement and Persistence of Triazine Herbicides

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Summary

Although the majority of an applied triazine herbicide remains in the surface soil where it controls weeds while degrading, soil movement and persistence has been well studied and documented. A variety of factors affect triazine runoff, including method of application, soil properties, type of tillage, and environmental conditions. Estimates of triazine amounts in runoff from agricultural fields vary widely, with the highest concentrations occurring in the first 2 months after application.

In most field-leaching studies, which are typically limited to depths ffi2 m, triazines are retained and degraded in the top 50 cm of surface soil. Amounts of triazines detected in subsurface drainage water are typically low – about 0.1% of the triazine applied to crops.

Triazine persistence is usually characterized in terms of the time it takes for 50% of the triazine to either degrade ($t_{1/2}$) or dissipate (DT_{50}). Values of $t_{1/2}$ or DT_{50} range from 14 to 112 days, with a mean $t_{1/2}$ or DT_{50} of 36 ffl 25 days. In many cases, triazine dissipation has been shown to be biphasic, not first order. For instance, when applied in spring, initial rapid degradation occurs during the first 2 months after application, followed by slower degradation in the dry summer and cold fall and winter. Triazines can persist at low concentrations for long periods after application; however, they do not accumulate in soil after long-term use.

Thus, triazine movement and persistence are influenced by many factors, the interactions of which are not always easy to predict. Several models have been used as tools to estimate losses and to identify variables that will impact the rate and magnitude of loss. Considering the broad range in soil properties and climatic conditions used, some models performed well. However, modeling results and predictions are only estimates, and the fate and transport of triazines in the soil environment has been shown to be affected by many factors, including concentration, soil texture, variation in climate, and differences in tillage practices.

Introduction

The fate of an organic chemical applied for pest control is the net result of a variety of processes, such as transformation, retention, and transport, all acting in concert on the pesticide.

Transport of triazines away from the application site depends on the chemical properties of the triazine, soil chemical and physical properties and conditions, environmental variables, and their interactions with transformation and retention processes. Transformation and retention (sorption) processes affect the amount of triazine present and available for transport through the soil profile. Biotic and abiotic transformation processes reduce or eliminate the amount of the triazine available for transport away from the site of application. Triazine herbicides can be degraded to intermediate products (often retaining the triazine ring) or degraded completely to inorganic products by microbial, chemical, and photochemical means. Plants can remove some of the triazine from soil, that is poplar trees took up ┘ 11% of atrazine applied to silt loam soil (Nair *et al.*, 1993). In addition, tolerant plants have the ability to transform triazines by *N*-dealkylation, hydroxylation, conjugation with glutathione, and other pathways, all of which reduce triazines in soil.

Triazine biodegradation in soil is dependent on a number of factors, most importantly the presence, population, and activity of triazine-degrading microorganisms. Therefore, factors that affect soil microbial populations and activity

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influence atrazine degradation. Soil temperature, oxygen and water status, previous soil management, crop practices, and their interactions all affect rates of triazine biodegradation. Generally, triazine degradation is optimized as soil temperature and oxygen content increase, and as water content nears field capacity. Factors that affect triazine degradation also affect metabolite degradation.

Unlike the transformation processes that reduce the total amount of triazine present in soil, retention only decreases the amount available for weed control, microbial transformations, or transport. The amount retained or sorbed by soil can range from 0% to 100% of the amount applied, but sorption on silt loam, loam, or clay loam soils typically ranges from 50% to 80%. Triazine retention in soil is influenced primarily by organic carbon content, soil clay content and type, and soil pH. Other factors influencing retention include the amount of triazine applied, the amount of dissolved organic carbon (DOC) in soil solution, soil water content, and triazine to soil contact time (aging).

Knowledge of pesticide retention, transport, and transformation processes has practical significance for the farmer, public, researchers, and regulatory agencies. For instance, the United States Environmental Protection Agency (USEPA) has the option to classify a pesticide as 'restricted use' if it is mobile and persistent or if it has been found in groundwater in at least three counties (Cohen, 1991). A pesticide would be considered mobile if it has been detected below 75-cm deep in the soil profile. A pesticide would be considered persistent if its $t_{1/2}$ in any field exceeds 3 weeks. This chapter will review triazine mobility (transport processes) and persistence (longevity in soil or environment), focusing primarily on literature from 1970 to the mid 1990s. Since then, hundreds of articles have been published that confirm the basic information presented here. For reviews of research prior to 1970 on movement and persistence, see Helling (1970); LeBaron (1970); and Sheets (1970).

Transport

Although the downward transport of triazines by water is the most important route in evaluating the potential for presence in groundwater, other modes of transport away from the site of application should also be taken into consideration. These include plant uptake, upward transport to the soil surface by water, transport in surface runoff water and sediment, volatilization from the soil surface, spray drift during application, and movement on wind-eroded particles. This chapter will cover triazine transport across the soil surface and through the soil profile.

Movement to Surface Runoff Water

Many studies have shown triazine movement from the point of application to surface water (Nelson and Jones, 1994). In monitoring studies ranging from small streams to large agricultural river systems covering the entire Midwest, a variety of triazines and their metabolites have been detected, with the vast majority of detections well below the health advisory or maximum contaminant levels for the respective triazine (Wu *et al.*, 1983; Bouchard *et al.*, 1985; Miltner *et al.*, 1989; Frank *et al.*, 1990b; Periera and Rostad, 1990; Duncan *et al.*, 1991; Thurman *et al.*, 1991, 1992, 1994; Ciba-Geigy, 1992a, b; Sudo and Kunimatsu, 1992; Kolpin and Kalkhoff, 1993; Michael and Neary, 1993; Neary *et al.*, 1993; Periera and Hostettler, 1993; Richards and Baker, 1993; Squillace *et al.*, 1993; Davies *et al.*, 1994; McMahon *et al.*, 1994; Schottler *et al*,, 1994; Stamer *et al.*, 1994; Goolsby and Battaglia, 1995; Gruessner and Watzin, 1995; Lemieux *et al.*, 1995; Garmouma *et al.*, 1997; Ghidey *et al.*, 1997; Ma and Spalding, 1997; Gilliom and Hamilton, 2006; Gilliom *et al.*, 2006). There are also numerous reports of triazine monitoring in lakes and ponds (Buser, 1990; Morioka and Cho, 1992; Spalding *et al.*, 1994; Donald and Syrgiannis, 1995), tailwater pits (Kadoum and Mock, 1978), irrigation canals (Anderson *et al.*, 1978), marshes (Fletcher *et al.*, 1994), and estuaries (Kucklick and Bidleman, 1994).

Soil research studies show that there is a wide range in the amounts of triazine surface runoff. Of the research runoff studies summarized in Table 24.1, 57% had losses of ffi2% of applied chemical, and 77% had losses of ffi4%. Less than 7% of the studies had estimated losses of ┘ 10% of the applied chemical. The cumulative effects of many small runoff events have been modeled or estimated for a variety of surface water bodies (Pereira and Rostad, 1990; Albanis 1992; Pereira and Hostettler, 1993; Schottler *et al.*, 1994).

The highest-triazine concentrations in surface water have been detected in the first 2 months after application (in early summer), with decreasing concentrations during the fall and winter months (Frank *et al.*, 1990b; Thurman *et al.*, 1991, 1992). Losses are greatest when severe rainstorms occur soon after application. For seven small watersheds studied, regardless of tillage practice ┘ 70% of atrazine or metribuzin loss was due to the five largest transport events during a 9-year period (Shipitalo and Owens, 2006). Prior to rate reductions and setbacks on the current label, high triazine residue levels had been observed after fall applications (Frank *et al.*, 1990b). Triazines are sometimes transported to surface waters, riparian zones (Paterson and Schnoor, 1992), or into noncrop areas by flooding (Roy *et al.*, 1995) as free molecules and as molecules associated with DOC in surface runoff and on sediments (Triplett *et al.*, 1978; Wu, 1980; Wu *et al.*, 1983; Sauer and Daniel, 1987; Hall *et al.*, 1991; Gaynor *et al.*, 1992; Pantone *et al.*, 1992; Kolpin and Kalkhoff, 1993).

Table 24.1 Surface runoff of triazine herbicides, from research studies worldwide

Herbicide	Site[a]	Soil type[b]	Slope (%)	Variable[c]	Rate (kg/ha)	Time (year)	Maximum loss (g/ha)	Maximum loss % of applied	Reference
Atrazine	GA	SL	4		3.3	3	63	1.9	Smith *et al*. (1978)
	GA	SL	3	CC	3.3	3	27	0.8	Smith *et al*. (1978)
	GA	SL	4		3.4	3	65	1.9	Leonard *et al*. (1979)
	GA	SL	6.5		3.3	1	7	0.2	White *et al*. (1967)
	GA	LS	3		2.2	2	9	0.4	Rohde *et al*. (1981)
	GA	LS	3		4.5	2	110	2.4	Rohde *et al*. (1981)
	GA	L	1.3	F-EC	2.7	1	138	5.1	Wauchope (1987)
	GA	L	1.3	F-4L	3.2	1	269	8.4	Wauchope (1987)
	GA	L	1.3	F-9–0	3.2	1	371	11.6	Wauchope (1987)
	GA	L	1.3	F-80W	3.4	1	296	8.7	Wauchope (1987)
	GA	L	2.0	LE	2.0	1	34	1.7	Davis-Carter and Burgoa (1993)
	GA	L	2.0	LE	2.0	1	314	15.7	Davis-Carter and Burgoa (1993)
	IA	SL	5	NR	1.9	1	108	5.7	Baker *et al*. (1982)
	IA	SL	5	HR	1.9	1	17	0.9	Baker *et al*. (1982)
	IA	SL	7.1	SA	2.5	1	110	4.4	Baker and Laflen (1979)
	IA	SL	7.1	I	2.4	1	40	1.7	Baker and Laflen (1979)
	IA	SiL	10–15	RT	2.2	2	89	4.0	Ritter *et al*. (1974)
	IA	SiL	10–15	SC	2.2	2	528	24	Ritter *et al*. (1974)
	IL	SiL	3–5		0.4	1	0.6 ppb		Felsot *et al*. (1992)
	IL	SiL	3–5		0.4	1	3.8 ppb		Felsot *et al*. (1992)
	IN	SiL	5	RI	2.2			6.1	Zhang *et al*. (1997)
	IN	SL	5	RI	2.2			15.1	Zhang *et al*. (1997)
	KY	SiL	9	CT	2.2	1	41	1.9	Seta *et al*. (1993)
	KY	SiL	9	CH	2.2	1	30	1.4	Seta *et al*. (1993)
	KY	SiL	9	NT	2.2	1	17	0.8	Seta *et al*. (1993)
	KY	SiL	9	NT	2.2	2	15	0.7	Blevins *et al*. (1990)
	KY	SiL	9	CH	2.2	2	5	0.2	Blevins *et al*. (1990)
	KY	SiL	9	MB	2.2	2	17	0.8	Blevins *et al*. (1990)
	LA	CL	0.1	D	1.6	1	23	1.4	Southwick *et al*. (1990a)
	LA	CL	0.1	U	1.6	1	52	3.2	Southwick *et al*. (1990a)
	MD	SL	5.4		1.7	1	17	1.0	Wu (1980)
	MD	SiL	ffi2		1.7	1	51	3.0	Glotfelty *et al*. (1984)
	MD	SiL	3–5	NT	1.3	2	20	1.5	Isensee and Sadeghi (1993)
	MD	SiL	3–5	CT	1.3	2	13	1.0	Isensee and Sadeghi (1993)
	MD	SiCL	14	R	0.1	2	10	1.7	Hall *et al*. (1972)
	MD	SiCL	14	R	1.1	2	41	3.7	Hall *et al*. (1972)
	MD	SiCL	14	R	2.2	2	54	2.5	Hall *et al*. (1972)
	MD	SiCL	14	R	4.5	2	98	2.2	Hall *et al*. (1972)
	MD	SiCL	14	R	6.7	2	155	2.3	Hall *et al*. (1972)
	MD	SiCL	14	R	9.0	2	265	3.0	Hall *et al*. (1972)
	MN	L	6	Pr	1.7	1	32	1.9	Pantone *et al*. (1992)
	MN	L	6	Po	1.7	1	16	1.0	Pantone *et al*. (1992)
	NC	SCL	2–6	CT	1.6	2	32	2.0	Myers *et al*. (1995)
	NC	SCL	2–6	NT	1.6	2	138	8.6	Myers *et al*. (1995)
	OH	SiC	ffi1	NT-C	2.2	4	7	0.3	Logan *et al*. (1994)
	OH	SiC	ffi1	NT-S	2.2	4	10	0.4	Logan *et al*. (1994)
	OH	SiC	ffi1	MB-C	2.2	4	4	0.2	Logan *et al*. (1994)
	OH	SiC	ffi1	MB-S	2.2	4	1	0.1	Logan *et al*. (1994)
	OH		8–22	T	1.1	2	64	5.7	Triplett *et al*. (1978)
	OH	SiL	13	CH	2.2	9		ffi0.1	Shipitalo and Owens (2006)
	OH	SiL	7	CH	2.2	9		2.5	Shipitalo and Owens (2006)
	OH	SiL	11	NT	2.2	9		0.7	Shipitalo and Owens (2006)
	OH	SiL	10	NT	2.2	9		4.7	Shipitalo and Owens (2006)
	OH	SiL	6	CT	2.2	9		1.2	Shipitalo and Owens (2006)
	OH	SiL	7	CT	2.2	9		0.5	Shipitalo and Owens (2006)
	OH	SiL	9	CT	2.2	9		4.3	Shipitalo and Owens (2006)
	Ont	CL	0.5	B	1.1	1	25	2.3	Gaynor and van Wesenbeeck (1995)
	Ont	CL	0.5	B	1.1	1	19	1.7	Gaynor and van Wesenbeeck (1995)

(*continued*)

Table 24.1 (Continued)

Herbicide	Site[a]	Soil type[b]	Slope (%)	Variable[c]	Rate (kg/ha)	Time (year)	Maximum loss (g/ha)	Maximum loss % of applied	Reference
Atrazine	Ont	CL	–		1.7	4	51	3.0	Gaynor *et al.* (1995)
	Ont	CL	ffi1		1.8	3	98	5.4	Gaynor *et al.* (1992)
	Ont	CL	0.2		2.0	4	ffi1	ffi0.1	Frank *et al.* (1991a)
	Ont	CL		RT	1.1	1	7	0.6	Tan *et al.* (1993)
	OR	SiL	3–5		3.6	1	20	0.6	Gaynor and Volk (1981)
	Ont	SiL	ffi5	W	2.1	1	38	1.8	Ng *et al.* (1995)
	Ont	CL	ffi1		1.1	1	13	1.2	Ng *et al.* (1995)
	Ont	SiL	5		3.7	2	170	4.6	Bowman *et al.* (1994)
	Ont	CL		CT	1.1	1	7	0.6	Tan *et al.* (1993)
	PA	SiCL	3–4	CT	1.7	4	6	0.4	Hall *et al.* (1991)
	PA	SiCL	3–5	NT	1.7	4	2	0.1	Hall *et al.* (1991)
	TN	SiL		W	0.9	1	14	1.5	Klaine *et al.* (1988)
	TX	CL	5	NT	3.4	3	2	0.1	Jones *et al.* (1995)
	TX	C	ffi1	CH	2.0	1	38	1.9	Pantone *et al.* (1996)
	TX	C	ffi1	NT	2.0	1	22	1.1	Pantone *et al.* (1996)
	VA		5–13	CT	4.5	1	77	1.7	Foy and Hiranpradit (1989)
	VT		2–7	CT	0.9	2	14	14.9	Clausen *et al.* (1996)
	VT		2–7	RT	0.9	2	ffi1	0.3	Clausen *et al.* (1996)
	WI	SiL	6	RT	2.8	2	254	9.1	Sauer and Daniel (1987)
	WI	SiL	6	NT	2.8	2	214	7.6	Sauer and Daniel (1987)
Cyanazine	GA	SL	4		1.6	2	16	1.0	Leonard *et al.* (1979)
	GA	LS	2.5	CC	4.5	1	90	2.0	Wauchope *et al.* (1990)
	GA	LS	2.5	CC	4.5	1	81	1.8	Wauchope *et al.* (1990)
	GA	LS	2.5	F	4.5	1	153	3.4	Wauchope *et al.* (1990)
	GA	LS	2.5	F	4.5	1	167	3.7	Wauchope *et al.* (1990)
	GA	SCL	2		4.4	1	990	22.5	Hubbard *et al.* (1989)
	GA	S	2		4.4	1	695	15.8	Hubbard *et al.* (1989)
	GA	LS	2		4.4	1	79	1.8	Hubbard *et al.* (1989)
	MD	SiL	3–5	NT	0.34	2	6	1.9	Isensee and Sadeghi (1993)
	MD	SiL	3–5	CT	0.34	2	3	0.8	Isensee and Sadeghi (1993)
	PA	SiCL	3–4	CT	2.2	4	7	0.3	Hall *et al.* (1991)
	PA	SiCL	3–5	NT	2.2	4	1	0.1	Hall *et al.* (1991)
	PA	SiCL	14	CT	4.5	1	257	5.7	Hall *et al.* (1984)
	PA	SiCL	14	NT	4.5	1	34	0.8	Hall *et al.* (1984)
	PA	SiCL	14	NT	4.5	1	7	0.2	Hall *et al.* (1984)
	PA	SiCL	14	NT	4.5	1	8	0.2	Hall *et al.* (1984)
	VT		2–7	CT	3.0		20	6.5	Clausen *et al.* (1996)
	VT		2–7	RT	1.2		4	3.1	Clausen *et al.* (1996)
Hexazinone	GA	SL			1.7	1		0.5	Neary *et al.* (1986)
Metribuzin	KY	SiL	10	V	0.82	1	12	0.8	Malone *et al.* (1996)
	KY	SiL	10	NT	0.82	1	ffi1	0.01	Malone *et al.* (1996)
	KY	SiL	10	CT	0.82	1	10	0.6	Malone *et al.* (1996)
	MS	SiL	4	CT	0.42	1	97	23	Smith *et al.* (1995)
	MS	SiL	4	NT	0.42	1	84	20	Smith *et al.* (1995)
	MS	SiC	3	NT-V	0.4	3	29		Webster and Shaw (1996a)
	MS	SiC	3	NT	0.4	3	6		Webster and Shaw (1996a)
	MS	SiC	3	CT-V	0.4	3	9		Webster and Shaw (1996a)
	MS	SiC	3	CT	0.4	3	3		Webster and Shaw (1996a)
	MS	SiC	3	CT	0.4	3	9	0.2	Webster and Shaw (1996b)
	MS	SiC	3	NT	0.4	3	9	0.2	Webster and Shaw (1996b)
	OH	SiC	ffi1	NT-C	0.6	4	ffi1	ffi0.1	Logan *et al.* (1994)
	OH	SiC	ffi1	NT-S	0.6	4	16	2.6	Logan *et al.* (1994)
	OH	SiC	ffi1	MB-C	0.6	4	ffi1	ffi0.1	Logan *et al.* (1994)
	OH	SiC	ffi1	MB-S	0.6	4	ffi1	0.1	Logan *et al.* (1994)
	OH	SiL	13	CH	2.2	9		ffi 0.1	Shipitalo and Owens (2006)
	OH	SiL	7	CH	2.2	9		0.2	Shipitalo and Owens (2006)
	OH	SiL	11	NT	2.2	9		2.2	Shipitalo and Owens (2006)
	OH	SiL	10	NT	2.2	9		1.8	Shipitalo and Owens (2006)
	OH	SiL	6	CT	2.2	9		2.3	Shipitalo and Owens (2006)
	OH	SiL	7	CT	2.2	9		0.2	Shipitalo and Owens (2006)
	OH	SiL	9	CT	2.2	9		2.0	Shipitalo and Owens (2006)

Table 24.1 (Continued)

Herbicide	Site[a]	Soil type[b]	Slope (%)	Variable[c]	Rate (kg/ha)	Time (year)	Maximum loss (g/ha)	Maximum loss % of applied	Reference
Metribuzin	Ont	CL	0.5	B	0.5	1	9	1.8	Gaynor and van Wesenbeeck (1995)
	Ont	CL	0.5	B	0.5	1	7	1.4	Gaynor and van Wesenbeeck (1995)
	Ont	CL		CT	0.5	1	1	0.2	Tan *et al*. (1993)
	Ont	CL		RT	0.5	1	1	0.2	Tan *et al*. (1993)
	WA	SiL	20–26	CT	0.5	3	24	4.7	Brown *et al*. (1985)
	WA	SiL	20–26	NT	0.5	3	14	2.7	Brown *et al*. (1985)
Prometryn	OK	SL	1	RI	2.8	1	11	0.4	Baldwin *et al*. (1975)
	OK	SL	1	SM	2.8	1	90	3.2	Baldwin *et al*. (1975)
	TX	CL	5	NT	2.2	2	17	0.8	Jones *et al*. (1995)
	TX	CL	5	StMu	1.3	2	19	1.5	Jones *et al*. (1995)
	PA	SiCL	3–5	NT	1.7	4	4	0.2	Hall *et al*. (1991)
Simazine	Fra	SiL	5-15	CH	0.3			0.8	Lennartz *et al*. (1997)
	Fra	SiL	5-15	NT	0.6			1.3	Lennartz *et al*. (1997)
	Fra		6	CT	1.0	1	2	0.5	Louchart *et al*. (2001)
	Fra		10	NT	1.0	1	30	3.0	Louchart *et al*. (2001)
	Fra		6–10	W	1.0	1	ffi1	0.2	Louchart *et al*. (2001)
	OH		8–22	T	2.2	3	123	5.4	Triplett *et al*. (1978)
	TN	SiL	5		4.5			6.9	Stearman and Wells (1997)
Simazine/ atrazine	VA	SiL	10.7	NT	3.6	1	80	2.2	Foy *et al*. (1989)
	VA	SiL	10.7	NT-LS	3.6	1	35	1.0	Foy *et al*. (1989)
	VA	SiL	10.7	NT-HS	3.6	1	69	1.9	Foy *et al*. (1989)
	VA	SiL	10.7	CT	3.6	1	182	5.1	Foy *et al*. (1989)
	VA	SiL	10.7	CT-LS	3.6	1	135	3.8	Foy *et al*. (1989)
	VA	SiL	10.7	CT-HS	3.6	1	98	2.7	Foy *et al*. (1989)
Terbutryn	OR	SiL	3–5		3.6	1	204	5.7	Gaynor and Volk (1981)

[a]Two-letter names are US state abbreviations, Fra: France, Ont: Ontario, Canada.
[b]C: Clay, CL: Clay loam, L: Loam, LS: Loamy sand, S: Sand, SCL: Sandy clay loam, SiC: Silty clay, SiCL: Silty clay loam, SiL: Silt loam, SL: Sandy loam.
[c]B: Band, C: Corn, CH: Chisel plow, CT: Conventional tillage, LS: Low sludge, HR: High residue, HS: High sludge, I: Incorporated, LE: Lagoon effluent, MB: Moldboard plow, NR: No residue, NT: No-till, RI: Rainfall intensity, RT: Ridge-till, S: Soybean, SA: Surface applied, SM: Soil moisture, StMu: Stubble mulch, T: multiple tillage types, V: Vegetation, W: Watershed.

Deethylatrazine (DEA) and deisopropylatrazine (DIA) also have been detected in shallow, unsaturated surface-water runoff from a Eudora silt loam soil with DEA present at higher concentrations (Mills and Thurman, 1994a). Dissolved atrazine, DEA, and DIA concentrations in water samples from two closely spaced lakes indicated large differences in input from watershed nonpoint sources. Levels of these chemicals increased in response to spring and early summer runoff events (Spalding *et al*., 1994). In studies conducted by Gaynor *et al*. (1992, 1995), DEA was found in surface runoff samples that contained atrazine. Hydroxyatrazine (HA), deethyl hydroxyatrazine (DEHA), and deisopropyl hydroxyatrazine (DIHA) have also been identified in surface water (Lerch *et al*., 1995).

Movement to Groundwater

Triazines and metabolites can move through soil and have been detected in groundwater in monitoring studies in the United States (Gilliom and Hamilton, 2006; Gilliom *et al*., 2006). The majority of studies reporting triazine detections in groundwater are associated with the corn- and sorghum-producing areas in the midwestern United States, but monitoring results in other regions and countries have also been reported (Wilson *et al*., 1987; Pionke *et al*., 1988; Grandet *et al*., 1989; USEPA, 1990, 1992; Clark *et al*., 1991; Bushway *et al*., 1992; Domagalski and Dubrovsky, 1992; Felding, 1992a; Koterba *et al*., 1993; Masse *et al*., 1994; Brady *et al*., 1995; Skark and Zullei-Seibert, 1995; Kolpin *et al*., 1996, 1997a, 1997b; Richards *et al*., 1996; Garmouma *et al*., 1997). Monitoring results show that levels of triazines in groundwater are generally below water limit standards (Richards *et al*., 1996); in a study of 2227 sites sampled in 20 major hydrologic basins across the US from 1993 to 1995, concentrations of atrazine, cyanazine, simazine, and promaton were ffi1 μg/L at 98% of the sites with detections (Barbash *et al*., 2001). In some areas median levels of atrazine concentrations are declining (Kolpin *et al*., 1997b).

Direct downward leaching has been theorized to be the major source for low-level triazine concentrations in groundwater. For instance, throughout the irrigated corn production areas of the Platte River valley of central

Nebraska, contamination levels seem to reflect a steady state between the amount of new contaminant that enters the aquifer each year and the amount that is degraded (Wehtje *et al.*, 1983). However, contamination of groundwater can be the result of point source pollution and/or nonpoint source pollution. Atrazine in water from the Platte River was shown to have moved via induced recharge into nearby well-field groundwater (Duncan *et al.*, 1991). Also, in a series of studies monitoring farm wells in Ontario, Canada over 18 years, it was found that presence of atrazine was also attributed to runoff and spills occurring during mixing and loading operations (Frank *et al.*, 1979, 1987, 1990a). The only detection of atrazine during a 3-year monitoring study in Arkansas was attributed to a localized spill or handling error (Cavalier *et al.*, 1989).

In a monitoring study of 303 wells, the frequency of triazine detections in groundwater was inversely related to aquifer depth (Burkart and Kolpin, 1993) and to depth in the alluvial aquifer (Kalkhoff *et al.*, 1992). For instance, atrazine was detected in 18% of the samples from the upper 1.6 m of the alluvial aquifer, but was not detected below 3.4 m. Atrazine detection in 171 rural wells was not correlated to well depth, although it was rarely detected in wells ┘ 30-m deep (Maas *et al.*, 1995). Pionke *et al.* (1988) found that aquifer rock type and depth to the water table were not important factors in groundwater contamination with atrazine. Most of the atrazine detections in Nebraska were in areas with permeable soils and a depth to water of ffi15 m (Spalding *et al.*, 1989). As expected, triazine concentrations tend to be greater in shallow groundwater as compared to deep groundwater (Junk *et al.*, 1980; Isensee *et al.*, 1988, 1990; Pickett *et al.*, 1992; Koterba *et al.*, 1993; Isensee and Sadeghi, 1995;). Triazine levels in shallow groundwater appear to be controlled by seasonal fluctuations, indicating that atrazine dissipation does occur (Wehtje *et al.*, 1983). However, it has been shown that frequency of herbicide detections and the range and distribution of atrazine occurrences are dependent upon both landscape position and temporal inputs of recharge water and rainfall (Steinheimer and Scoggin, 2001). In the most definitive survey of ┘ 12 000 samples from wells throughout the midwestern United States, it was found that triazine concentrations are greater in wells that are shallow, older, dug, or driven; located close to cropland, feedlots, or chemical-mixing sites; or located in sandy soils (Richards *et al.*, 1996).

Triazines in groundwater are subject to continuing transport, retention, and transformation. By monitoring pesticide concentration profiles in groundwater, retention and persistence can be determined (Widmer and Spalding, 1995; Widmer *et al.*, 1995). Triazines are generally sorbed less and degraded more slowly in aquifer materials as compared to vadose-zone soils (Skark and Obermann, 1995). For instance, little retention and no transformation of atrazine were observed in an aerobic sand and gravel aquifer in a 2- to 3-month period (Agertved *et al.*, 1992). There also was only slight retention of atrazine, DEA, DIA, and cyanazine in an aerobic sand and gravel aquifer, and there were no detectable losses over a 2-month period (Widmer and Spalding, 1995; Widmer *et al.*, 1995). Low sorption of atrazine and DEA has also been reported for subsurface sediments (Roy and Krapac, 1994).

Downward movement of triazines may occur from percolating water carrying them to lower soil depths. Within well-structured soils with abundant macropores, triazines have been reported to move to deeper depths than in nonstructured soils with fewer pores. Increased permeability, percolation, and solute movement can result from increased porosity – especially in no-tillage systems where there is pore connectivity at the soil surface. Triazines can move to shallow groundwater by macropore flow in sandy soil if sufficient rainfall occurs shortly after they are applied (Ritter *et al.*, 1994a, b).

Plant roots are important in the creation and stabilization of soil macropores. Preferential flow through root-mediated soil pores has been demonstrated using inorganic ions, which are not sorbed onto soil organic matter and clays. Triazine movement through soil columns has been shown to be influenced by roots. Atrazine was present at higher concentrations and greater depths in soil with roots, presumably due to greater movement through channels created as the roots decayed (Zins *et al.*, 1991).

Earthworm burrows can function as preferential flow conduits as well. However, it is unclear if earthworm burrows actually increase triazine leaching since several factors influence these potential routes. First, the total organic carbon in the burrow lining is two to three times greater than in bulk soil and has been shown to increase atrazine sorption to the burrow lining (Stehouwer *et al.*, 1993, 1994). In fact, water and atrazine mixtures poured through earthworm burrows showed these linings greatly reduced the leaching or concentration of atrazine in solution exiting the pores (Edwards *et al.*, 1992).

The characteristics of each rainfall or irrigation event and the antecedent soil water content are also important considerations in determining if earthworm burrows or root-mediated macropores contribute to triazine movement. High-intensity rains shortly after atrazine application on relatively dry no-till soils produced the greatest amounts of preferential flow and movement of atrazine (Edwards *et al.*, 1993). However, leaching was reduced if rainfall was delayed or if low-intensity rains occurred prior to events that produce high percolates (Edwards *et al.*, 1993). After the first rainfall, surface-applied atrazine leached less in subsequent high-percolate, high-volume storms, regardless of intensity or volume of percolate produced by the first storm. Shipitalo *et al.* (1990) reported that the first storm after application moved atrazine into the soil matrix, thereby reducing the potential for transport in macropores during subsequent rainfalls. The relative contribution of macropores to chemical transport and water movement appears

to be greatest when the soil is dry, and it decreases as the soil becomes wetter (Shipitalo and Edwards, 1996). Along with movement through earthworm burrows and root macropores, triazines can also be vertically transported via irrigation return flows (Junk *et al.*, 1980).

Movement of triazines through the zone where plant roots appear in soil is a function of water availability. Crop canopy plays a significant role in the distribution of incoming precipitation reaching the soil surface, causing a differential movement of triazines. For instance, the least precipitation throughfall occurs within 20 cm of the row in corn and soybean after establishment of the canopy (Dowdy *et al.*, 1995). Atrazine movement was reduced when the herbicide was applied as a band over the row and corn foliage sheltered incoming precipitation. Essentially all atrazine remained in the top 7 cm of a loamy sand soil during the first 22 days after application, with very little lateral movement beyond the spray band. Dowdy *et al.* (1995) also found that some atrazine moved from the soil surface into the top 30 cm of soil, but did not move deeper. Furrow or drip irrigation may also influence water distribution in soil, producing an asymmetric atrazine-leaching pattern (Wang *et al.*, 1997).

Differences in triazine leaching among soils have been attributed to differences in physical and chemical properties of the soils. These properties affect retention and transformation, leaching volumes and velocities, presence of macropores, and field management – including crop residues, fertilizer, and herbicide-use practices. The influence of these variables on triazine movement through the profile can be illustrated by the numerous studies that have been conducted using disturbed and undisturbed soil columns. While column studies can be useful to distinguish the effects of varying soil properties or treatments on soils, we need to emphasize that it is not useful to extrapolate column-leaching results to the field. Experimental conditions greatly affect data from small soil columns and make it difficult to compare studies. Also, studies using disturbed soil columns may significantly underestimate leaching. For instance, under unsaturated conditions almost no atrazine (0.05% of that applied) was leached through large disturbed soil columns, compared to 12% for undisturbed columns of the same soil (Azevedo *et al.*, 1996). Soil columns may also overestimate leaching if a high flow rate is used or wall effects are not eliminated. Table 24.2 lists only studies using columns with a minimum size of 10 | 50 cm.

In most column-leaching studies, the bulk of triazines remain near the soil surface. For instance, Kruger *et al.* (1993) found that in a 60-cm column of Iowa soil taken from a field with no previous pesticide history, approximately 1.2% of the ^{14}C-atrazine was recovered in leachate over a 12-week period. By the end of the experiment, 77% of the ^{14}C applied remained in the upper 10 cm of soil, and bound residue was the primary component. Both atrazine and degradation products (DIA ┘ HA ┘ DEA ┘ DEHA ┘ DIHA) were found in the top 10 cm of surface soil.

Triazines and metabolites remain in the surface soil as a result of sorption processes. In intact soil cores containing a silt loam soil, atrazine leaching was primarily influenced by sorption-related nonequilibrium at low pore water velocities and by a combination of both transport- and sorption-related nonequilibrium at high pore water velocities (Gaber *et al.*, 1995).

The depth and amount of triazine movement depends on soil texture, amount and timing of water application, soil horizons, crop residues, and fertilizer placement. For instance, small amounts (approximately 3% of that applied) of ^{14}C from ^{14}C-atrazine leached to a depth of 60 to 100 cm within 35 weeks in sand and silt loam columns, with most remaining in the top 15 cm (Alhajjar *et al.*, 1990). Mobility of ^{14}C-atrazine in a Sparta sand was greater than in a Plainfield sand due to higher hydraulic conductivity, lower water holding capacity, and less sorption due to lower organic C and clay contents (Wietersen *et al.*, 1993b). In lysimeters packed with Plainfield sand, the maximum movement of atrazine after 21 weeks under natural rainfall was 30 cm, compared to 70 cm when supplemental irrigation was supplied (Bowman, 1989, 1991). Atrazine dissipation was faster under rainfall (DT_{50} − 2.5 weeks) than under supplementary watering (DT_{50} − 3.5 weeks). The difference in dissipation between the two treatments was attributed to greater movement away from the surface soil where faster degradation occurs. In undisturbed soil columns where 100% of the surface was covered with residue, greater amounts of atrazine were recovered in the first 5 cm of leachate than from zero-residue columns with high- and medium-saturated hydraulic conductivities (Green *et al.*, 1995). The time for peak atrazine concentration in leachate was reduced as residue levels increased for columns with high-saturated conductivities. In contrast, soil cores covered with 200 kg/ha or 2000 kg/ha of crop residue reduced leaching by 26% and 37%, respectively, as compared to soil cores without crop residue (Sigua *et al.*, 1993). The authors also reported that the age of the residue may influence its movement. Soil cores covered with recently harvested vegetation reduced leaching by 39% as compared with cores covered with aged residue, presumably due to a combination of greater hydrophobicity and higher sorptive capacity of the fresh residue.

In undisturbed soil columns, higher atrazine concentrations occurred in the leachates of plow-tillage columns than in no-tillage columns (Levanon *et al.*, 1993). However, more atrazine has been reported to leach through untilled cores than tilled cores. Increasing the number of earthworms in soil cores increased the amount of atrazine leached through both untilled cores and tilled cores (Sigua *et al.*, 1995). Changing soil surface pH with fertilizer also may influence atrazine's leaching potential. For instance, application of NH_4OH increased surface soil and leachate pH,

Table 24.2 Triazine leaching in soil from research studies worldwide

				Soil solution			Soil			
Herbicide	**Site[a]**	**Soil type[b]**	**Study type[c]**	**Time (week)**	**Depth (m)**	**Conc. (μg/L)**	**Time (week)**	**Depth (cm)**	**Conc. μg/kg or (%)**	**Reference**
Ametryn	Tai	L	F				4	3	0.01	Wang *et al* (1996)
Atraton	FL	S	C_1				1	46	tr	Rodgers (1968)
Atrazine	Eng	C	C_5	15h	1.1	0.02%				Beck *et al*. (1995)
	FL	S	C_1		0.6	tr				Rodgers (1968)
	FL	S	C_1				1	30	tr	Rodgers (1968)
	Fra	LC	C_1	23	0.7	9	40	36	tr	Dousset *et al*. (1995)
	Fra	C	C_1	22	0.7	24	40	54	tr	Dousset *et al*. (1995)
	Fra	C	C_1	24	0.7	19	40	36	tr	Dousset *et al*. (1995)
	Fra		C_1	12	0.6	1.9%				Schiavon (1988b)
	IA	SCL	C_2				15	┘50	0.2%	Kruger *et al*. (1993)
	IA	L	C_3		0.6	12%				Azevedo *et al*. (1996)
	IA	L	C_3		0.6	0.05%				Azevedo *et al*. (1996)
	IL	S	C_1	4	0.6	62%	4	53	0.6%	Guo *et al*. (1991)
	IL	S	C_1	4	0.6	38%	4	45	0.1%	Guo *et al*. (1991)
	MN	SiL	C_3		0.6	10%		45	1.0%	Zins *et al*. (1991)
	MN	SCL	C_4	78	1.1	0	78	40	0.3%	Sorenson *et al*. (1993)
	MN	SiCL	C_4	78	0.8	ffi0.04%	78	80	0.1%	Sorenson*et al*. (1994)
	MN	CL	C_4	78	0.8	ffi0.01%	78	80	0.1%	Sorenson*et al*. (1995)
	NC	LS	C_3				13	88	6.1%	Lee and Weber (1993)
	Ont	S	C_2				1	60	tr	Bowman (1991)
	Ont	S	C_2	21	0.7	tr	21	┘20	10%	Bowman (1993)
	Ont	S	C_2	12	0.7	tr	21	70	tr	Bowman (1989)
	Ont	S	C_2	21	0.8	0	12	50	tr	Bowman (1990)
	Ont	SiL	C_2	21	0.8	200	1	50	ffi2%	Bowman (1990)
	Que	SL	C_3	3	0.8	11	7	60	5	Smith *et al*. (1992)
	WA	S	C_5	3	2.4	2%				Melancon *et al*. (1986)
	WI	S	C_3				156	9.3%	9.3	Wietersen *et al*. (1993a)
	WI	S	C_3	22		0.1%	22	┘40	3%	Wietersen *et al*. (1993b)
	WI	S	C_3	22		5.6%	22	┘40	9%	Wietersen *et al*. (1993b)
	WI	S	C_4	35	1.0	0.12%	35	┘60	3.3%	Alhajjar *et al*. (1990)
	WI	SiL	C_4	35	1.0	0.08%	35	┘60	3.3%	Alhajjar *et al*. (1990)
	WI	SiL	C_3	8	0.8	0.6				Hanson *et al*. (1997)
	WI	SiL	C_3	8	0.8	1.8				Hanson *et al*. (1997)
	Zim	SL	C_2				6	40		Chivinge and Mpofu (1990)
	Aus	SL	F				7	40	10	Stork (1997)
	Aus	S	F				8	┘15	3%	Walker and Blacklow (1995)
	CA	SL	F				1	┘20	12%	Clendening *et al*. (1990)
	CA	LS	F				6	300	tr	Troiano *et al*. (1993)
	CA	LS	F					┘30	18.8%	Ghodrati and Jury (1992)
	CT	LS	F					┘189	1	Huang and Frink (1989)
	CT	SL	F					┘180	9	Huang and Frink (1989)
	Den	SL	F		1.2	8				Felding (1992b)
	Den	SL	F		1.2	4				Felding (1992b)
	Fra		F	6	0.8	6				Tasli *et al*. (1996)
	GA	LS	F				2	┘20	3%	Rohde *et al*. (1981)
	Gre	C	F	1	1.0	13				Albanis *et al*. (1988)
	Gre	L	F	1	1.0	16				Albanis *et al*. (1988)
	Gre	SiL	F	1	1.0	12				Albanis *et al*. (1988)
	IA	SiL	F					┘15	tr	Workman *et al*. (1995)
	Isr	SiCL	F				104	350	7	Graber *et al*. (1995)
	Ita		F				19	281	5	Bacci *et al*. (1989)
	Ita	L	F				7	┘25	10	Di Muccio *et al*. (1990)
	Ita	LS	F				4	┘25	50	Di Muccio *et al*. (1990)
	KS	SiL	F	14	0.3	5	24	137	1	Adams and Thurman (1991)
	KS	SiCL	F	20	4.6	4	24	168	7	Adams and Thurman (1991)
	KS	SiL	F	3	0.3	7	8	45	12	Mills and Thurman (1994b)
	KS	SiL	F				12	30	90	Sophocleous *et al*. (1990)
	KS		F					┘180	ffi1	Juracek and Thurman (1997)

Table 24.2 (Continued)

				Soil solution			Soil			
Herbicide	**Site[a]**	**Soil type[b]**	**Study type[c]**	**Time (week)**	**Depth (m)**	**Conc. (μg/L)**	**Time (week)**	**Depth (cm)**	**Conc. μg/kg or (%)**	**Reference**
Atrazine	MD	SiL	F				20	┘60	ffi20	Gish *et al.* (1991b)
	MD	SiL	F-NT				31	┘100	5	Helling *et al.* (1988)
	MD	SL	F				1	┘24	tr	Wu (1980)
	MD	SL	F-CT	3	1.8	35				Gish *et al.* (1995)
	MD	SiL	F-CT				2	┘20	24	Sadeghi and Isensee (1992)
	MD	SiL	F-CT					90	tr	Starr and Glotfelty (1990)
	MD	SiL	F-CT				6	┘40	8	Isensee and Sadeghi (1994)
	MD	SL	F-NT	56	1.8	7				Gish *et al.* (1995)
	MD	SiL	F-NT				2	┘20	183	Sadeghi and Isensee (1992)
	MD	SiL	F-NT					90	tr	Starr and Glotfelty (1990)
	MD	SiL	F-NT				6	┘40	14	Isensee and Sadeghi (1994)
	MD	SiL	F-CT				2	┘40	17	Sadeghi and Isensee (1996)
	MD	SiL	F-NT				2	┘40	3	Sadeghi and Isensee (1996)
	MN	SL	F				22	┘30	16	Khakural *et al.* (1995)
	MN	SiL	F				19	┘40	33	Khakural *et al.* (1995)
	MN	CL	F				8	┘60	13	Khakural *et al.* (1995)
	MN	SL	F				3	┘21	11	Koskinen *et al.* (1993)
	MN	SiL	F				3	┘21	12	Koskinen *et al.* (1993)
	MN	CL	F				3	┘21	7	Koskinen *et al.* (1993)
	MO	SiL	F	13	1.3	280				Tindall and Vencill (1995)
	NC	LS	F				4	┘24	ffi0.1%	Keller and Weber (1995)
	NE	SiCL	F				112	┘46	200	Burnside *et al.* (1963)
	NE	L	F				112	┘46	300	Burnside *et al.* (1963)
	NE	L	F				112	┘46	800	Burnside *et al.* (1963)
	NE	L	F				63	40	ffi50	Ghadiri *et al.* (1984)
	NE	SL	F	8	1.5	2.2	26	┘150	0.2	Wehtje *et al.* (1984)
	NY	SiL	F				4	┘21	3	Chammas *et al.* (1997)
	OH	SiL	F	1	0.4	10 000				Edwards *et al.* (1993)
	Ont	L	F				9	┘15	2.6%	Birk and Roadhouse (1964)
	Ont	CL	F				53	┘30	10	Frank *et al.* (1991a)
	Ont	CL	F				80	┘10	40	Frank and Sirons (1985)
	OR	SiL	FL				2	┘16	300	Gaynor and Volk (1981)
	OR	SiL	FU				2	┘16	ffi100	Gaynor and Volk (1981)
	PA	SiCL	F				26	┘76	80	Hall and Hartwig (1990)
	PA	SL	F				26	┘61	30	Hall and Hartwig (1990)
	PA	SiCL	F	1	1.2	10	8	┘30	30	Hall and Hartwig (1978)
	PA	CL	F	1	1.2	20	8	┘107	60	Hall and Hartwig (1978)
	PA	SiCL	F-CT		1.2	5	11	┘91	2	Hall *et al.* (1989)
	PA	SiCL	F-CT		1.2	21				Hall *et al.* (1991)
	PA	SiCL	F-NT		1.2	22	11	┘91	6	Hall *et al.* (1989)
	PA	SiCL	F-NT		1.2	46				Hall *et al.* (1991)
	Sas		F				156	┘68	30	Smith *et al.* (1975)
	SD	SiCL	F		0.8	0.7%				Clay *et al.* (1994)
	Spa	SL	F				17	┘20	200	Gómez de Barreda *et al.* (1996)
	Swi	SiL	F					100	3%	Flury *et al.* (1995)
	Swi	LS	F					┘10	tr	Flury *et al.* (1995)
	TN		F				4	┘10	40	Klaine *et al.* (1988)
	VA	SiL	F-NT				20	┘61	12	Foy and Hiranpradit (1989)
	VA	SiL	F-CT				20	┘61	33	Foy and Hiranpradit (1989)
	WI	S	F-MB					┘60	tr	Sauer *et al.* (1990)
	WI	S	F-NT					┘15	tr	Sauer *et al.* (1990)
	WI	S	FI		1.4	ffi4				Fermanich *et al.* (1996)
Cyanazine	GA	SCL	C_5		0.8	1.6%				Hubbard *et al.* (1989)
	GA	LS	C_5		0.8	78%				Hubbard *et al.* (1989)
	GA	S	C_5		0.8	60%				Hubbard *et al.* (1989)
	MD	SiL	F				31	┘20	tr	Helling *et al.* (1988)
	MD	SiL	F				20	┘60	ffi30	Gish *et al.* (1991b)
	NE	SiCL	F				58	5	tr	Majka and Lavy (1977)

(*continued*)

Table 24.2 (Continued)

				Soil solution			Soil			
Herbicide	**Site[a]**	**Soil type[b]**	**Study type[c]**	**Time (week)**	**Depth (m)**	**Conc. (μg/L)**	**Time (week)**	**Depth (cm)**	**Conc. μg/kg or (%)**	**Reference**
Cyanazine	NE	LS	F				58	5	tr	Majka and Lavy (1977)
	Net	LS	F				17	┘10	tr	Boesten *et al.* (1989)
	Net	LS	F				8	┘15	25	Ahuja *et al.* (1996)
	Ont	CL	F				30	┘7	690	Frank *et al.* (1991b)
	Ont	SL	F				2	┘40	5	Yoo *et al.* (1981)
	PA	SiCL	F				26	┘76	30	Hall and Hartwig (1990)
	PA	SL	F				26	┘46	20	Hall and Hartwig (1990)
	PA	SiCL	F-CT		1.2	13	11	┘30	1	Hall *et al.* (1989)
	PA	SiCL	F-CT		1.2	14				Hall *et al.* (1991)
	PA	SiCL	F-NT		1.2	21	11	┘30	3	Hall *et al.* (1989)
	PA	SiCL	F-NT		1.2	61				Hall *et al.* (1991)
	Tai	L	F				4	5	0.02%	Wang *et al.* (1996)
Hexazinone	MN	S	C_2	14	1.5	30				Stone *et al.* (1993)
	MN	S	C_2	14	1.5	37				Stone *et al.* (1993)
	MN	S	C_2	14	1.5	65				Stone *et al.* (1993)
	MN	S	C_2	14	1.5	57				Stone *et al.* (1993)
	MN	S	C_2	14	1.5	46				Stone *et al.* (1993)
	MN	S	C_2	14	1.5	37				Stone *et al.* (1993)
	Alb	L	F	32	0.8	100	52	┘20	2%	Feng *et al.* (1989, 1992)
	Aus	SCL	F					┘15	270	Allender (1991)
	Den	SL	F		1.2	2				Felding (1992b)
	Den	SL	F			43				Felding (1992b)
Ipazine	FL	S	C_2				1	10	tr	Rodgers (1968)
Metribuzin	Ont	S	C_2				1	60	tr	Bowman (1991)
	Aus	S	F					┘20	1%	Kookana *et al.* (1995)
	GA	SL	FB				1	┘30	0.2	Jones *et al.* (1990)
	GA	SL	FC				1	┘30	0.3	Jones *et al.* (1990)
	GA	SL	F-CT				2	6	tr	Jones *et al.* (1990)
	GA	SL	F-NT				2	23	tr	Jones *et al.* (1990)
	IL	S	F				2	┘76	2	Fleming *et al.* (1992a)
	MN	LS	F	16	1.5	1				Burgard *et al.* (1994)
	NS	SL	F				8	┘15	10	Jensen *et al.* (1989)
	Net	LS	F				17	┘10	tr	Boesten *et al.* (1989)
	OK	SiL	F-CT				5	42		Dao (1995)
	OK	SiL	F-NT				5	20		Dao (1995)
	WA	SiL	F				26	30	tr	Brown *et al.* (1985)
Procyazine	Ont	SL	F				1	┘40	9	Yoo *et al.* (1981)
	PA	SiCL	F				26	┘76	200	Hall and Hartwig (1990)
	PA	SL	F				26	┘61	20	Hall and Hartwig (1990)
Prometon	CA	SL	F				1	┘20	6%	Clendening *et al.* (1990)
Prometryn	FL	S	C_2				1	10	tr	Rodgers (1968)
	Aus	S	F					┘15	tr	Kookana *et al.* (1995)
	CA	L	F				17	┘90	70	Miller *et al.* (1978)
	CA	LS	F					┘30	9.4%	Ghodrati and Jury (1992)
	OK	SL	F					┘5	0	Baldwin *et al.* (1975)
Propazine	FL	S	C_2				1	46	tr	Rodgers (1968)
	KS	SiL	F	25	0.3	8	8	45	5	Mills and Thurman (1994b)
	TX	CL	F-NT				41	┘30	6	Jones *et al.* (1995)
	TX	CL	F-SM				41	┘15	9	Jones *et al.* (1995)
Simazine	FL	S	C_2				1	30	tr	Rodgers (1968)
	Isr	S	C_5				2d	┘14	tr	Koren and Shlevin (1977)
	Aus	S	F				8	┘15	3%	Walker and Blacklow (1995)
	Aus	S	F					┘15	tr	Kookana *et al.* (1995)
	CA		F				1d	145	20	Goh *et al.* (1992)
	CT	SL	F					┘180	10	Huang and Frink (1989)
	Eng	SL	F				27	┘3	tr	Hance *et al.* (1981)
	Eng	CL	F				27	┘3	tr	Hance *et al.* (1981)
	Eng	L	F-5.6				182	25	50	Clay and Stott (1973)

Table 24.2 (Continued)

				Soil solution			Soil			
Herbicide	**Site[a]**	**Soil type[b]**	**Study type[c]**	**Time (week)**	**Depth (m)**	**Conc. (μg/L)**	**Time (week)**	**Depth (cm)**	**Conc. μg/kg or (%)**	**Reference**
Simazine	Eng	L	F-22.4				182	61	40	Clay and Stott (1973)
	Isr	S	F				1	┘ 30	tr	Koren and Shlevin (1977)
	KS	SiL	F				8	45	5	Mills and Thurman (1994b)
	NE	SiCL	F				112	┘ 46	200	Burnside *et al.* (1963)
	NE	L	F				112	┘ 30	100	Burnside *et al.* (1963)
	NE	L	F				112	┘ 46	500	Burnside *et al.* (1963)
	PA	SiCL	F				26	┘ 76	30	Hall and Hartwig (1990)
	PA	SL	F				26	┘ 107	60	Hall and Hartwig (1990)
	PA	SiCL	F-CT		1.2	4	11	┘ 91	1	Hall *et al.* (1989)
	PA	SiCL	F-CT		1.2	43				Hall *et al.* (1991)
	PA	SiCL	F-NT		1.2	22	11	┘ 91	2	Hall *et al.* (1989)
	PA	SiCL	F-NT		1.2	48				Hall *et al.* (1991)
	Sas		F				156	┘ 68	60	Smith *et al.* (1975)
	Spa	SL	F				17	┘ 20	50	Gómez de Barreda *et al.* (1996)
	Tai	L	F				4	4	0.09%	Wang *et al.* (1996)
Terbumetone	Spa	SL	F				17	┘ 20	80	Gómez de Barreda *et al.* (1996)
Terbuthy-lazine	Ont	S	C_2	2d	0.7	1.1%	21	50	tr	Bowman (1989)
	Spa	SL	F				17	┘ 20	100	Gómez de Barreda *et al.* (1996)
	Swi	SiL	F					┘ 50	ffi0.1%	Flury *et al.* (1995)
	Swi	LS	F					┘ 10	tr	Flury *et al.* (1995)
Terbutryn	OR	SiL	FL				2	┘ 16	200	Gaynor and Volk (1981)
	OR	SiL	FU				2	┘ 6	100	Gaynor and Volk (1981)
	Spa	SL	F				17	┘ 20	10	Gómez de Barreda *et al.* (1996)

[a]Two-letter names are US state abbreviations, Alb: Albania, Aus: Australia, Den: Denmark, Eng: England, Fra: France, Gre: Greece, Isr: Israel, Ita: Italy, Net: Netherlands, Ont: Ontario, Canada, Que: Quebec, Canada, Sas: Saskatoon, Canada, Spa: Spain, Swi: Switzerland, Tai: Taiwan, Zim: Zimbabwe.
[b]C: Clay, CL: Clay loam, L: Loam, LS: Loamy sand, S: Sand, SCL: Sandy clay loam, SiC: Silty clay, SiCL: Silty clay loam, SiL: Silt loam, SL: Sandy loam.
[c]C: Column studies, C_1: 10 │ 50–75 cm, C_2: 15 │ 70–150 cm, C_3: 20 │ 60–90 cm, C_4: 30 │ 90–120 cm, C_5: 50–80 │ 60–240 cm, CH: Chisel plow, CT: Conventional tillage, F: Field, FB: Field bare, FC: Field cover, FI: Field irrigated, FL: Field limited, FU: Field unlimited, F22.4: Field 22.4 kg, F5.6: field 5.6 km, MB: Moldboard plow, NT: No-till, RT: Ridge-till.

resulting in increased atrazine amounts in leachates from columns containing silty clay loam and clay loam soils (Liu *et al.*, 1995). In a study using soil column field lysimeters in three different soil types, atrazine's mobility was inversely related to the mean % organic matter content of the soil profiles and was directly related to soil pH and soil-leaching potential indices (Weber *et al.*, 2007).

Triazine metabolites have been found throughout soil-leaching columns. They may leach from upper layers or be formed at lower soil depths. DEA and DIA are the metabolites most likely to leach as a result of their lower retention by soil (Barriuso *et al.*, 1992; Bowman, 1990; Muir and Baker, 1978; Schiavon, 1988a, b). For instance, in a study by Kruger *et al.* (1993), atrazine, DEA, and DIA were found at all depths; other metabolites leached to a lesser extent. In a 13-week leaching study with 60-cm intact soil columns, the percentage of ^{14}C-DEA recovered was greatest in the first leaching event (1.3% of the amount of ^{14}C applied), indicating preferential flow (Kruger *et al.*, 1996), while the total amount of DEA lost due to leaching was 3.6%.

DEA also has been found in soil water at greater depths than atrazine or DIA (Adams and Thurman, 1991), and it is often detected in groundwater at higher concentrations than either atrazine or DIA (Kolpin *et al.*, 1996). HA does not readily leach through soil (Schiavon, 1988a, b) because it is tightly bound. Therefore, HA detected deep in the soil profile is attributed to degradation or hydrolysis *in situ* at these depths (Sorensen *et al.*, 1993, 1994, 1995). Relative mobilities in a Honeywood silt loam were greater for DEA than for atrazine, but relative mobilities were about the same in Plainfield sand (Bowman, 1990). Maximum movement of DEA and atrazine in Plainfield sand was to about 40 cm after 8 weeks (Bowman, 1990, 1993). In field lysimeters receiving supplemental water, DEA was more mobile than atrazine. Metribuzin and its metabolites DA (diamino metribuzin metabolite), DK (diketo

metribuzin metabolite), and DADK (desaminodiketo metribuzin metabolite) were considerably more mobile than either atrazine or DEA in the same soil (Bowman, 1991).

There are numerous field studies showing that triazines leach into the vadose zone (Table 24.2; Wehtje *et al.*, 1984; Helling *et al.*, 1988; Adams and Thurman, 1991; Clay *et al.*, 1994), into subsurface drains (Table 24.3), and into groundwater (previous references, also Frank *et al.*, 1991a; Isensee *et al.*, 1988; Isensee *et al.*, 1990; Pionke and Glotfelty, 1990; Starr and Glotfelty, 1990; Verstraeten *et al.*, 1995). Most of the field-leaching studies are limited to depths ffi2 m. In most studies, the majority of atrazine is bound and degraded in the top 50 cm of surface soil, similar

Table 24.3 Triazine transport to subsurface drains from research studies worldwide

Herbicide	Site[a]	Soil type[b]	Drain depth (m)	Treatment[c]	Time (year)	Applied rate (kg/ha)	Maximum concentration (μg/L)	Maximum loss (g/ha)	Maximum loss % of applied	Reference
Atrazine	IA	L	1.2		2	1.7	8.2	9.8	0.6	Jayachandran *et al.* (1994)
	IA	L	1.2	T	3	2.8			0.4	Weed *et al.* (1995)
	IA	L	1.2	T	3	2.9		11		Kanwar *et al.* (1997)
	IN	SiL	0.7		5	1.1		0.8	0.1	Sichani *et al.* (1991)
	IN	SiL	0.7		3	1.1	10	0.7	0.1	Kladivko *et al.* (1991)
	Ita	SL	0.4		1	1.4	510			Persicani *et al.* (1995)
	LA	CL	1.0		0.3	1.6		0.6	ffi0.1	Southwick *et al.* (1990a, 1990b)
	LA	CL	1.0		1	1.6		2.8	0.2	Bengston *et al.* (1990)
	LA	C	1.0		2	4.5	403	120	2.7	Southwick *et al.* (1992)
	LA	C	1.0	AT	0.3	2.2	144	46	2.1	Southwick *et al.* (1995)
	MN	CL	1.2	T	5	3.3	1.5	4.4	0.1	Buhler *et al.* (1993)
	New	SiL	0.8		3	1.5	4.6	8.3	0.6	Milburn *et al.* (1995)
	NY	SCL	0.8	T	1	4.5	0.4			Steenhuis *et al.* (1990)
	NY	SCL	0.8	T	1	4.5	0.1			Steenhuis *et al.* (1988)
	OH	SiC	1.0	T	4	2.2	59	31	1.4	Logan *et al.* (1994)
	Ont		1.0		2		6	0.8		Thooko *et al.* (1994)
	Ont	CL	0.6		1	0.55	ffi16			Ng *et al.* (1995)
	Ont	C	0.7	CS	4	1.2	3.5	8.7	0.7	Von Stryk and Bolton (1977)
	Ont	CL	1.0		4	2.4	22	15.3	0.6	Frank *et al.* (1991a)
	Ont	CL	1.0		3	1.8		40.1	2.2	Gaynor *et al.* (1992)
	Ont	CL		T	4.0	1.7		61	3.6	Gaynor and van Wesenbeeck (1995)
	Ont	CL	0.6	T-CT	1	1.1	104	2	0.2	Tan *et al.* (1993)
	Ont	CL	0.6	T-RT	1	1.1	104	2	0.2	Tan *et al.* (1993)
	Que	SL	1.2–1.6		2	2.8	11	2.1	0.1	Muir and Baker (1976)
Cyanazine	IA	SiCL	1.2		2	2.2	570		0.2	Czapar *et al.* (1994)
	IA	L	1.2	T	3	2.8		2		Kanwar *et al.* (1997)
	IN	SiL	0.7		5	2.3		0.7	ffi0.1	Sichani *et al.* (1991)
	IN	SiL	0.7		3	2.3	10	0.8	ffi0.1	Kladivko *et al.* (1991)
	Ont	CL	1.0–1.2		3	2.4	0	0	0	Frank *et al.* (1991b)
	Ont	SL			1	2.0		ffi0.1	ffi0.1	Yoo *et al.* (1981)
	Que	SL	1.2–1.6		2	3.4	1.1	ffi0.1	ffi0.1	Muir and Baker (1976)
	Que	SL	1.2–1.6		2	1.1	4.1	1.2	0.1	Muir and Baker (1976)
Metribuzin	IA	L	1.2	T	3	0.45			0.9	Weed *et al.* (1995)
	IA	L	1.2	T	3	0.5		3		Kanwar *et al.* (1997)
	LA	C	1.0		0.3	1.1	94	20	1.8	Southwick *et al.* (1995)
	New	SiL	0.8		1	0.75	1.5		0.3	Milburn *et al.* (1991)
	OH	SiC	1.0	T	4	0.6		0.17	ffi0.1	Logan *et al.* (1994)
	Ont	CL	0.6	T-CT	1	0.5	49	0.7	ffi0.1	Tan *et al.* (1993)
	Ont	CL	0.6	T-RT	1	0.5	49	0.7	ffi0.1	Tan *et al.* (1993)
	Que	SL	1.2–1.6		2	0.6	1.7	0.07	ffi0.1	Muir and Baker (1976)
Procyazine	Ont	SL			1	1.6		0.02	ffi0.1	Yoo *et al.* (1981)
Simazine	Eng	SiCL	1.0		1	1.2	1.4			Brooke and Matthiessen (1991)
Terbuthylazine	Ger		0.7–1.1		1	0.84	1.4			Traub-Eberhard *et al.* (1995)

[a]Two-letter names are US state abbreviations, Eng: England, Ger: Germany, Ita: Italy, New: New Brunswick, Canada, Ont: Ontario, Canada, Que: Quebec, Canada.
[b]C: Clay, CL: Clay loam, L: Loam, SCL: Sandy clay loam, SiC: Silty clay SiCL: Silty clay loam, SiL: Silt loam, SL: Sandy loam.
[c]AT: Application time, CS: Cropping system, CT: Conventional tillage, RT: Ridge-till, T: Tillage.

to what has been observed in column-leaching studies. Atrazine and its degraded derivatives are consistently found in small amounts at lower depths in the soil profile. In a field column-leaching study, atrazine was detected in a silty clay loam and a clay loam to a depth of 76 cm approximately 2 months after application, but the majority remained in the top 30 cm of surface soil (Hall and Hartwig, 1978). Hall *et al.* (1989) detected atrazine at all soil depths down to 122 cm. Differences in the yearly extent and magnitude of leaching losses were strongly correlated to rainfall distribution and to the number of leaching events proximal to application.

Two months after application of ^{14}C-atrazine to a sandy loam soil, ^{14}C-labeled atrazine, DEA, DIA, and HA were detected at the 30- to 40-cm depth, but were not found in deeper soil layers (Sorenson *et al.*, 1993). Twelve months after application of ^{14}C-atrazine to a silt loam soil, ^{14}C-labeled atrazine, DEA, DIA, and HA were detected at the 70- to 80-cm depth (Sorenson *et al.*, 1995). One month after application of ^{14}C-atrazine to a clay loam soil, ^{14}C-labeled atrazine and HA were detected at the 70- to 80-cm depth (Sorenson *et al.*, 1994). No DEA was observed at this depth until 16 months after application, and DIA was never detected.

Atrazine levels in a loamy soil were similar for a given depth and sampling time after application, regardless of whether the site was conventionally tilled or received a no-tillage treatment. The most atrazine was present in the upper 5 cm of the soil profile, and only a trace was found at the 40-cm depth (Ghadiri *et al.*, 1984). Distribution patterns through field soil under plow- and conservation-tilled corn were quite similar. The bulk of the atrazine was in the surface soil, but small amounts were found at the deepest sampling depth of 90 cm (Starr and Glotfelty, 1990). Atrazine appeared to move by both one-dimensional movement through the soil matrix and by rapid downward movement through macropores, bypassing most of the soil matrix. However, Gish *et al.* (1995) obtained slightly different results when water samples were analyzed. Atrazine movement was reduced under no-till as compared to tilled conditions. Under no-tillage, atrazine was detected in ffi28% of the water samples obtained from suction lysimeters at 1.5- and 1.8-m depths (Gish *et al.*, 1995). In contrast, under tilled conditions, atrazine was detected in 53% of the soil water samples obtained from suction lysimeters at 1.5- and 1.8-m depths.

Subsurface drains placed 1–2 m below the soil surface to help drain wet areas are effective tools in evaluating leaching of pesticides or nutrients in the field. Monitoring the drainage outflow for triazine concentration can determine both the timing of fluxes and the cumulative triazine loss through leaching over a large area (Table 24.3). Water discharge into surface waters from subsurface drains normally occurs in spring and early summer, both before and after spring herbicide applications.

Amounts of triazines detected in subsurface drainage water are typically low. For instance, in 68% of the atrazine studies summarized in Table 24.3, only about 0.1% of applied atrazine was found in subsurface drains. In one study, DEA was detected in concentrations equal to or greater than atrazine (Muir and Baker, 1976). In Ontario, atrazine and DEA were found in all samples of subsurface drainage water collected from a 1-m depth, with about 1.9% in subsurface drainage following a fall application and up to 0.2% following a spring application (Frank *et al.*, 1991a). The maximum amount of atrazine in subsurface drains was 2.7–3.6% of the amount applied (Southwick *et al.*, 1992; Gaynor *et al.*, 1995), compared to 0.1% for cyanazine (Muir and Baker, 1976), 1.8% for metribuzin (Southwick *et al.*, 1995), and ffi0.1% for procyazine (Yoo *et al.*, 1981).

In Indiana, small amounts of atrazine were detected in subsurface drain flow within 3 weeks of application – after less than 2-cm net subsurface drain flow from a poorly structured silt loam soil with low organic matter (Kladivko *et al.*, 1991). The rapid appearance of atrazine indicated the possibility of preferential flow. Atrazine, DEA, and DIA were also found in subsurface drainage water in Iowa; the order of concentration was atrazine ┘ DEA ┘ DIA (Jayachandran *et al.*, 1994). Levels of triazines in subsurface drains ranged from 0.1 to 29 μg/L, with concentrations declining with time (Milburn *et al.*, 1995). A spill on one of these plots, followed by 71 mm of rainfall within a few days, resulted in subsurface drain concentrations of 150 μg/L. This concentration decreased to ffi3.0 μg/L within 6 days of the initiation of subsurface drain flow. Atrazine has also been found in subsurface drainage in a clay soil with an average concentration of 0.4μg/L for 24–30 months after the last application (Buhler *et al.*, 1993).

Management Effects on Transport

Specific management practices influence triazine runoff and leaching, including fertilizer type, tillage crop residues, and previous crop history, as well as triazine application, formulation, and placement (Baker and Mickelson, 1994). Tillage systems affect various soil properties, such as soil moisture, temperature, pH, organic matter, water flow, and microbial populations, especially at and near the soil surface. These factors can affect transformation, retention, and transport of herbicides in soil. Interactions of and compensations between these processes can influence our prediction of triazine transport in soil. Therefore, triazine movement is usually studied under one management practice at a time.

Application methods can affect triazine movement. When applied in 50-cm bands, triazine runoff was reduced by 69% as compared with a broadcast application, with the reduction mainly attributed to movement into the soil profile (Gaynor and van Wesenbeeck, 1995). Smaller amounts of atrazine were lost in runoff after being applied postemergence as compared to preemergence due to the plants reducing the runoff flow rate. The greatest amount in runoff was ffi2% of the atrazine applied (Pantone *et al.*, 1992). The injection slot created by fertilizer application increased atrazine movement when the application and the fertilizer injection slot overlapped, due to the physical disturbance of the soil and the effect of the fertilizer on reducing atrazine sorption to soil (Clay *et al.*, 1994). Irrigation with secondary sewage effluent leached atrazine to 115 cm as compared to 63 cm using very pure water. This difference can be attributed to increased atrazine partitioning into the aqueous phase and into the DOC load of the effluent, resulting in less sorption onto the soil (Graber *et al.*, 1995).

Triazines are primarily transported in soil in the aqueous phase. The amount of water infiltration or runoff that can carry pesticides varies greatly due to spatial and temporal variation in soil properties and processes that impact pesticides. In addition, tillage systems affect the amount of water moving over and through the soil. Conservation tillage systems have often reduced runoff and increased infiltration. Some studies have shown less triazine runoff from no-till than from conventional-tillage plots (Triplett *et al.*, 1978; Glenn and Angle, 1987; Hall *et al.*, 1984, 1991). Also, in some studies there are greater losses of triazines in root zone leachates under no-till compared to conventional-tillage plots (Hall *et al.*, 1989, 1991; Isensee *et al.*, 1990; Isensee and Sadeghi, 1994, 1996). The greater triazine losses in some no-till systems may be due to more structured soils with fewer exposed sorption sites and more macropores. When conditions favoring preferential flow occurred, the groundwater (at 1-m depth) underlying no-till sites had three to four times higher concentrations of atrazine and cyanazine than sites under conventional tillage (Gish *et al.*, 1991c).

Water management is a technique that has been used to reduce herbicide surface runoff. Tile drains can reduce surface herbicide loss by channeling surface water runoff to tile drainage, which increases herbicide sorption to soil and subsequent degradation. For instance, atrazine runoff losses were reduced in tile drained fields, as compared to fields without tile drains, because of a significant decrease in surface runoff volume (Southwick *et al.*, 1990a). In a study of water management of tile drains, it was found that less atrazine and metribuzin was lost by surface runoff from free or controlled drainage systems than from a controlled drainage with subsurface irrigation system (Gaynor *et al.*, 2002). Application of sufficient irrigation water to incorporate simazine into the soil after application is another water management technique that has shown promise in reducing herbicide runoff (Liu and O'Connell, 2002)

In contrast, Blevins *et al.* (1990) found that while there was a third as much runoff volume from no-till plots over a 2-year period as compared to conventional-tillage plots, atrazine loss in runoff was about the same in both systems (ffi1% of applied atrazine). Most of the loss occurred between application and corn canopy closure. Also, Fermanich *et al.* (1996) found that although cumulative drainage under no-till plots was always greater than under moldboard-plow plots, there was no consistent effect of tillage on atrazine loading.

Use of controlled-release herbicide formulations has been proposed as a method to reduce herbicide mobility in soil. Ideally, controlled-release formulations would only release the amount of chemical into soil solution necessary to control weeds, with the remaining herbicide unavailable for leaching. Since 1964, no atrazine granules or other controlled-release triazine formulations have been commercially available, mainly due to weed efficacy issues; however, research continues on the subject. In laboratory studies, triazine leaching has been reduced by starch-encapsulated (Gish *et al.*, 1991a; Boydston, 1992; Fleming *et al.*, 1992a, b, c; Schreiber *et al.*, 1993; Mervosh *et al.*, 1995), alginate (Johnson and Pepperman, 1995a, b), and lignin (Riggle and Penner, 1988) controlled-release formulations. Acrylic polymers added to triazine formulations have also retarded the movement of both atrazine (Lee and Weber, 1993; Narayanan *et al.*, 1993; Wietersen *et al.*, 1993a) and simazine (Alva and Singh, 1991; Reddy and Singh, 1993) in sand columns. Organoclay formulations of hexazinone have displayed slow release properties in water and have been shown to retard hexazinone leaching through soil, while maintaining an herbicidal efficacy similar to that of a commercially available wettable powder formulation (Celis *et al.*, 2005). However, for the most part, the effectiveness of controlled-release formulations in reducing triazine leaching in the field (Fleming *et al.*, 1992a; Lee and Weber, 1993; Wietersen *et al.*, 1993a; Gish *et al.*, 1994, 1995), and the effectiveness of these formulations in controlling weeds have not justified their commercial use (Buhler *et al.*, 1994a). There could be alternative markets for these products in the future. For instance, an organoclay-base formulation of simazine displayed similar herbicidal efficacy and slower vertical movement of the herbicide compared to a standard commercial formulation in field plots of a typical sport turfgrass surface (Cornejo *et al.*, 2007).

Addition of organic wastes to agricultural soils is becoming a common practice as a waste disposal strategy and to improve the physical and chemical soil properties. However, the use of organic wastes as soil amendments can affect movement of herbicides. The effects on herbicide movement depend on the source and amount of added amendment and the physical and chemical properties of the soil. For instance, although pig manure slurry and cow manure

added to soil did not affect leaching (Rouchaud *et al.*, 1994), amendments applied at 2.1 tons total carbon per hectare reduced atrazine leaching in a sandy, coarse-textured soil in the following the order: waste activated carbon ⌋ digested municipal sewage sludge ⌋ animal manure (Guo *et al.*, 1991). Simazine sorption was greater on soil amended with solid olive-mill organic waste as compared to unamended soils, reducing the amount of herbicide available for leaching, thereby resulting in retarded vertical movement in soil columns (Albarran *et al.*, 2004). In a study of the effects of solid urban organic waste on sorption, persistence, and leaching potential of simazine, it was found that in a sandy soil, reduction in large-size conducting pores upon amendment resulted in a greater reduction of leaching than that suggested from small differences between simazine sorption and degradation in unamended and amended soils (Cox *et al.*, 2001).

Vegetative filter strips (VFS) appear to hold promise for protecting surface water supplies by reducing herbicide runoff. A 6-m strip of oats at the slope base reduced atrazine loss in runoff by greater than 64% when atrazine was applied either preemergence or incorporated before planting in corn (Hall *et al.*, 1983). Rye and fescue VFS reduced metribuzin losses by 50% to 85% (Webster and Shaw, 1996a; Tingle *et al.*, 1998), and reduced atrazine, DEA, and DIA losses by 44% (Patty *et al.*, 1997). Wetlands have been shown to attenuate runoff peaks for transient triazine (Alvord and Kadlec, 1996). Deep-rooted poplar trees also appear to hold promise for protecting water supplies (Paterson and Schnoor, 1992; Burken and Schnoor, 1996). A 6-m wide VFS composed of trees, shrubs, and grass almost completely removed terbuthylazine from runoff (Vianello *et al.*, 2005). An excellent review (Krutz *et al.*, 2005) includes efficacy of VFS for abatement of herbicide runoff and a discussion of parameters (i.e., VFS width, vegetation type, area ratio, etc.), which affect herbicide retention in VFS. This review also discusses the need to evaluate herbicide retention at the field and watershed scales using a systems approach, which incorporates various in-field BMPs with VFS, vegetated ditches, and wetlands.

Herbicide Persistence

Persistence is interpreted as how long a particular amount of herbicide remains in a given volume of soil and is measured as the herbicide left in soil after being subjected to transport and to degradation and decomposition. Triazine persistence is the time it takes for 50% of the triazine to either degrade (a microbial process), decompose (a chemical process), or dissipate (the net effect of degradation, decomposition, and transport). By assuming first-order kinetics, a degradation and decomposition half-life ($t_{1/2}$) or 50% dissipation time (DT_{50}) can be calculated. Values of $t_{1/2}$ and DT_{50} for atrazine range from 14 to 112 days, with a mean $t_{1/2}$ and DT_{50} of 36 ffl 25 days. Since both $t_{1/2}$ and DT_{50} are commonly reported in the literature, no attempt was made to distinguish between them (Table 24.4).

The wide range in $t_{1/2}$ and DT_{50} can be attributed to many factors. The results of individual studies used are highly specific to the experimental conditions. In the 1970s detection limits were mg/kg versus μg/kg in the 1990s. Dissipation studies vary with depth of soil sampled, and the concentration at a particular sampling time is influenced by the depth of the sample analyzed. The lack of uniformity in the application of herbicides makes it difficult to determine the initial soil concentration (Walker and Blacklow, 1995). In most studies, only two to four samples are analyzed to obtain the initial concentration, and there are too few sampling times for calculation of accurate dissipation kinetics (Helling *et al.*, 1988, Clendening *et al.*, 1990).

Triazine dissipation in surface soil is not always first-order (Koskinen *et al.*, 1993), and triazine persistence in the field may be biphasic. For instance, triazines applied in spring in the Midwest experience an initial rapid degradation during the first two months after application, followed by slower degradation in the dry summer and cold fall and winter. In one experiment, the $t_{1/2}$ was calculated as 55 days if only growing season data were used, compared to 134 days if data for the entire year were used (Weed *et al.*, 1995). Calculations of the DT_{50} of atrazine in surface soil after spring applications at a number of sites in Ontario, Canada ranged from 2.4 to 3.0 months. A relatively rapid breakdown occurred during the first five months after application, followed by slow dissipation during fall, winter, and early spring (Frank and Sirons, 1985). Atrazine applied in the spring declined rapidly over the summer months ($t_{1/2}$ – 37 and 64 days for two consecutive years); however, $t_{1/2}$ was 125 days when data from the winter months were included. A fall application of atrazine resulted in a 198-day half-life (Frank *et al.*, 1991a). In another study, atrazine dissipation was zero-order during the first few months following a spring application, but first-order over the 320-day sampling period (Brejda *et al.*, 1988).

Cyanazine dissipation is also biphasic, with a rapid drop in concentration from 3 to 15 days following application, followed by a slower period of disappearance from 15 to 100 days (Yoo *et al.*, 1981). Similarly, only 12% of applied metribuzin remained 115 days after application; 2% still remained 1095 days after application (Conn and Cameron 1988). However, it appears that triazines do not accumulate in soil after long-term use. For instance, after 14 years of annual applications of 5 kg/ha, the soil concentration of atrazine was 1.1 mg/kg, while the concentration of simazine was 0.6 mg/kg (Damanakis and Daris, 1981). There also was no accumulation of atrazine after 8 years of repeated

Table 24.4 Triazine persistence in soils from research studies worldwide

Herbicide	Site[a]	Soil type[b]	Rate (kg/ha)	Variable[c]	$t_{1/2}$, DT_{50} (days)	Carryover % (days)	Reference
Ametryn	Tai	L			6		Wang *et al*. (1995)
Atrazine	AL	SL	1.1	pH5	15		Hiltbold and Buchanan (1977)
	AL	SL	1.1	pH7	23		Hiltbold and Buchanan (1977)
	AL	SL	3.4	pH5	22		Hiltbold and Buchanan (1977)
	AL	SL	3.4	pH7	28		Hiltbold and Buchanan (1977)
	AL	SiL	1.1	pH5	18		Hiltbold and Buchanan (1977)
	AL	SiL	1.1	pH7	36		Hiltbold and Buchanan (1977)
	AL	SiL	3.4	pH5	25		Hiltbold and Buchanan (1977)
	AL	SiL	3.4	pH7	37		Hiltbold and Buchanan (1977)
	AL	SL	1.1	pH5	6		Hiltbold and Buchanan (1977)
	AL	SL	1.1	pH7	10		Hiltbold and Buchanan (1977)
	AL	SL	3.4	pH5	8		Hiltbold and Buchanan (1977)
	AL	SL	3.4	pH7	11		Hiltbold and Buchanan (1977)
	Aus	SL	0.7		62	3 (330)	Stork (1997)
	Aus	C	4.1		53–63		Bowmer (1991)
	Aus	C			68–75		Swain (1981)
	Aus	S	1.0		28 (65%)[d]		Walker and Blacklow (1995)
	Bar	C			16–22		Wood *et al*. (2005)
	CO	L	2.0	I	60 (57%)		Walker and Zimdahl (1981)
	CO	L	2.0	D	60 (53%)		Walker and Zimdahl (1981)
	CO	CL	0.8	Y	6–7		Shaner and Henry (2007)
	CO	CL	1.5	Y	4–18		Shaner and Henry (2007)
	CO	LS	0.8		5		Shaner and Henry (2007)
	Eng	SL	2.0		58		Nicholls *et al*. (1982)
	Eng	C	1.0		125 (32%)		Smith and Walker (1989)
	GA	LS	2.24	R	10		Rohde *et al*. (1981)
	GA	LS	4.48	R	7		Rohde *et al*. (1981)
	IA	L	2.8	T	55	20 (365)	Weed *et al*. (1995)
	Ita				48		Bacci *et al*. (1989)
	Ita	L	2.0		21 (64%)		Di Muccio *et al*. (1990)
	Ita	LS	2.0		29 (61%)		Di Muccio *et al*. (1990)
	KS	SiL	4.5		15		Sophocleous *et al*. (1990)
	LA	CL	1.6	ND	35		Southwick *et al*. (1990b)
	LA	CL	1.6	D	36		Southwick *et al*. (1990b)
	MD	SiL	2.8	NT-Co	31		Gish *et al*. (1991b)
	MD	SiL	2.8	CT-Fa	44		Gish *et al*. (1991b)
	MD	SL	1.7	CR-F	110	11 (336)	Gish *et al*. (1994)
	MD	SL	1.7	F	36	ffi1 (336)	Gish *et al*. (1994)
	MD	SiL	2.8	Y	60		Helling *et al*. (1988)
	MD	SiL	2.8	Y	72		Helling *et al*. (1988)
	MD	SiL	1.34	CT	26–35		Isensee and Sadeghi (1994)
	MD	SiL	1.34	NT	26–35		Isensee and Sadeghi (1994)
	MD	SL	1.7			5–13 (365)	Wu (1980)
	MN			W	8–14		Detenbeck *et al*. (1996)
	MN	SL	2.2	Y	21 (0%)		Koskinen *et al*. (1993)
	MN	SL	2.2	Y	21 (13%)		Koskinen *et al*. (1993)
	MN	SiL	2.2	Y	21 (29%)		Koskinen *et al*. (1993)
	MN	SiL	2.2	Y	21 (58%)		Koskinen *et al*. (1993)
	MN	CL	2.2	Y	21 (27%)		Koskinen *et al*. (1993)
	MN	CL	2.2	Y	21 (0%)		Koskinen *et al*. (1993)
	MN	S			21		Clay *et al*. (2000)
	MO	C	2.2		12		Ghidey *et al*. (1997)
	MS	SiL		C/Y	9–17		Krutz *et al*. (2007)
	ND	SL			45		Clay *et al*. (2000)
	NE	SL	2.2		46	ffi2 (375)	Brejda *et al*. (1988)
	NE	L	1.1	CT	42		Ghadiri *et al*. (1984)
	NE	L	1.1	NT	50		Ghadiri *et al*. (1984)
	NS	SiL	3.0		60 (67%)		Walker and Zimdahl (1981)
	NY	SL	3.0		60 (62%)		Walker and Zimdahl (1981)
	OH	SiL	3.4	C/Y	31–38		Workman *et al*. (1995)
	OH	SiL	1.7	C/Y	36–54		Workman *et al*. (1995)

Table 24.4 (Continued)

Herbicide	Site[a]	Soil type[b]	Rate (kg/ha)	Variable[c]	$t_{1/2}$, DT_{50} (days)	Carryover % (days)	Reference
Atrazine	Ont	L	2.2	Co	76 (40%)	7 (365)	Birk and Roadhouse (1964)
	Ont	L	2.2	Fa	76 (81%)	5 (365)	Birk and Roadhouse (1964)
	Ont	S	2.25	Ra	30		Bowman (1991)
	Ont	S	2.25	Ra•	34		Bowman (1991)
	Ont	S	2.25	Ra	28		Bowman (1990)
	Ont	S	2.25	Ra•	28		Bowman (1990)
	Ont	SiL	2.25	Ra	28		Bowman (1990)
	Ont	SiL	2.25	Ra•	29		Bowman (1990)
	Ont	S	2.25	F-EC	26		Bowman (1993)
	Ont	S	2.25	F-25G	28		Bowman (1993)
	Ont	CL	2.4	Y	37	5 (500)	Frank *et al*. (1991a)
	Ont	CL	2.4	Y	64	17 (372)	Frank *et al*. (1991a)
	Ont	CL	1.1	R	101	5 (365)	Frank and Sirons (1985)
	Ont	CL	2.2	R	90	4 (365)	Frank and Sirons (1985)
	Ont	CL	3.3	R	98	6 (365)	Frank and Sirons (1985)
	Ont	CL	1.8	CT	53–69		Gaynor *et al*. (1992)
	Ont	CL	1.8	NT	63–69		Gaynor *et al*. (1992)
	Ont	CL	1.8	RT	53–99		Gaynor *et al*. (1992)
	Ont	CL	1.8	RV	50–53		Gaynor *et al*. (1992)
	Ont	CL	1.7	CT-Y	33–62		Gaynor *et al*. (1998)
	Ont	CL	1.7	RT-Y	33–75		Gaynor *et al*. (1998)
	Ont	CL	1.7	RV-Y	31–53		Gaynor *et al*. (1998)
	Ont	CL	1.7	NT-Y	3556		Gaynor *et al*. (1998)
	Ont	CL	3.3		60	10 (365)	Sirons *et al*. (1973)
	Ont	CL	1.1		150 (92%)		Gaynor *et al*. (1987)
	OR	SiL	3.6	UL	12 (75%)		Gaynor and Volk (1981)
	OR	SiL	3.6	L	10 (26%)		Gaynor and Volk (1981)
	Rum	SiCL	1.0		68		Pestemer *et al*. (1984)
	Rum	SiL	1.0		36		Pestemer *et al*. (1984)
	SD	SiCL			45		Clay *et al*. (2000)
	Spa	SiL			30	8 (365)	Durand and Barceló (1992)
	Spa	SCL		Y	29 (62%)		Obrador *et al*. (1993)
	Spa	SCL		Y	37 (69%)		Obrador *et al*. (1993)
	Spa	SL		Y	32 (83%)		Obrador *et al*. (1993)
	Spa	SL		Y	37 (66%)		Obrador *et al*. (1993)
	Spa	L		Y	31 (50%)		Obrador *et al*. (1993)
	Spa	L		Y	30 (60%)		Obrador *et al*. (1993)
	Tai	L			16		Wang *et al*. (1995)
	TN	L	2.2	C	33		Gallaher and Mueller (1996)
	TN	L	2.2	NC	17		Gallaher and Mueller (1996)
	WI	S				21	Clay *et al*. (2000)
	Zim	L	1.75			3 (365)	Chivinge and Mpofu (1990)
Cyanazine	Eng	C	1.0		30		Smith and Walker (1989)
	MD	SiL	2.24	NT-Co	12		Gish *et al*. (1991b)
	MD	SiL	2.24	CT-Fa	13		Gish *et al*. (1991b)
	MD	SiL	2.24	Y	31		Helling *et al*. (1988)
	MD	SiL	2.24	Y	11		Helling *et al*. (1988)
	MD	SiL	1.3	CT-Y	14 (83%)		Sadeghi and Isensee (1997)
	MD	SiL	1.3	NT-Y	14 (6%)		Sadeghi and Isensee (1997)
	Ont	CL	2.4	Y	27	0 (365)	Frank *et al*. (1991b)
	Ont	CL	2.4	Y	12	0 (365)	Frank *et al*. (1991b)
	Ont	CL	3.3		30		Sirons *et al*. (1973)
	Que	SL	2.0		6		Yoo *et al*. (1981)
Hexazinone	Alb	L	4.1	R		10 (365)	Feng *et al*. (1992)
	Alb	L	2.3	R		9 (365)	Feng *et al*. (1992)
	Alb	SiL	1.4		104 (66%)		Feng (1987)
	Ont	SL	1.6	SS		1 (365)	Prasad and Feng (1990)
	Spa				35		Fernandez *et al*. (2001)
Metribuzin	AK	SiL	0.6		115 (88%)	2 (1095)	Conn and Cameron (1988)
	Aus	S	0.53		27		Kookana *et al*. (1995)

(*continued*)

Table 24.4 (Continued)

Herbicide	Site[a]	Soil type[b]	Rate (kg/ha)	Variable[c]	$t_{1/2}$, DT_{50} (days)	Carryover % (days)	Reference
Metribuzin	CO	SL	1.1	R	44		Hyzak and Zimdahl (1974)
	CO	SL	2.2	R	43		Hyzak and Zimdahl (1974)
	Den	SL			32		Henriksen *et al.* (2004)
	Eng	SL	2		29		Nicholls *et al.* (1982)
	Eng	C	1.0		60		Smith and Walker (1989)
	Eng	SCL	1.0		9–12		Walker and Welch (1992)
	Fin	SL	1.4			8 (365)	Junnila *et al.* (1993)
	IA	L	0.45	T	32	2 (365)	Weed *et al.* (1995)
	Leb	SL	1.1		5		Khoury *et al.* (2003)
	Leb	C	2.0		6		Khoury *et al.* (2003)
	MN	LS	1.1	Y	28	17 (365)	Burgard *et al.* (1994)
	MN	LS	0.6	Y	31	18 (365)	Burgard *et al.* (1994)
	NE	SiCL	0.56	CT	5		Sorenson *et al.* (1991)
	NE	SiCL	0.56	NT	12		Sorenson *et al.* (1991)
	NE	SiCL	0.56	CT	13		Sorenson *et al.* (1991)
	NE	SiCL	0.56	NT	15		Sorenson *et al.* (1991)
	Ont	S	2.25	Ra	22		Bowman, (1991)
	Ont	S	2.25	Ra•	14		Bowman (1991)
	Ont	SL	2.0			ffi10 (365)	Webster and Reimer (1976)
	PEI	SiL	0.5	SA	6		Jensen *et al.* (1989)
	PEI	SiL	0.5	In	16		Jensen *et al.* (1989)
	PEI	SL	0.5	SA	5		Jensen *et al.* (1989)
	PEI	SL	0.5	In	14		Jensen *et al.* (1989)
	TN	L	0.56	C	43		Gallaher and Mueller (1996)
	TN	L	0.56	NC	16		Gallaher and Mueller (1996)
	WA	SiL	0.45		102–112		Brown *et al.* (1985)
Procyazine	Que	SL	1.6		13		Yoo *et al.* (1981)
Prometon	Zim	L	2.0			6 (365)	Chivinge and Mpofu (1990)
Prometryn	Aus	S	1.1		58		Kookana *et al.* (1995)
	Spa	L	2		59–63		Redondo *et al.* (1994)
Simazine	Aus	S	2.0		28		Kookana *et al.* (1995)
	Aus	S	1.0		28 (53%)		Walker and Blacklow (1995)
	Eng	L	2.8			2 (365)	Clay and Stott (1973)
	Eng	L	22.4			8 (365)	Clay and Stott (1973)
Simazine	Rum	SiCL	3		70		Pestemer *et al.* (1984)
	Rum	SiL	4		48		Pestemer *et al.* (1984)
	Spa				44		Fernandez *et al.* (2001)
	Tai	L			14		Wang *et al.* (1995)
Terbuthylazine	Zim	L	1.75			1 (365)	Chivinge and Mpofu (1990)
Terbutryn	Ger	LS			20		Auspurg *et al.* (1989)
	Ger	SL			35		Auspurg *et al.* (1989)
	OR	SiL	3.6	UL	10 (23%)		Gaynor and Volk (1981)
	OR	SiL	3.6	L	10 (4%)		Gaynor and Volk (1981)

[a]Two-letter names are US state abbreviations, Alb: Albania, Aus: Australia, Bar: Barbados, Den: Demark, Eng: England, Fin: Finland, Ger: Germany, Ita: Italy, Leb: Lebanon, Ont: Ontario, Canada, PEI: Prince Edward Island, Canada, Que: Quebec, Canada, Rum: Rumania, Spa: Spain, Tai: Taiwan, Zim: Zimbabwe.
[b]C: Clay, CL: Clay loam, L: Loam, LS: Loamy sand, S: Sand, SCL: Sandy clay loam, SiC: Silty clay, SiCL: Silty clay loam, SiL: Silt loam, SL: Sandy loam.
[c]C: Crop, CH: Chisel plow, Co: Corn, CR: Controlled release, CT: Conventional tillage, C/Y: Crop/year, D: Dryland, D: Surface drainage, F: Formulation, Fa: Fallow, I: Irrigated, In: Incorporated, L: Limed, MB: Moldboard plow, ND: No drainage, NT: No-till, R: Rate, Ra: Rain, Ra• : Rain• extra water, RT: Ridge-till, RV: Ridge valley, SA: Surface applied, SS: Spot spray, T: Tillage, UL: Unlimed, W: Wetlands, Y: Year.
[d]Number in parentheses is the % dissipated during the time listed.

applications of 3.0 kg/ha (Rahman *et al.*, 1986), or after 20 years of repeated application of 1.4–2.2 kg/ha (Khan and Saidak, 1981).

The soil texture, initial and seasonal water content, biological activity, and other variables influence the rate of degradation in both the rapid and slow phases. For instance, persistence is affected by soil texture and climatic variation from year to year. In a silt loam two months after application, only 32% of the atrazine applied to the site remained, while 45% remained in a clay loam and 35% remained in a sandy loam (Sorenson *et al.*, 1993, 1994, 1995).

However, degradation then slowed, with 16% still remaining in the silt loam 16 months after application, as compared to 20% in the clay loam and 22% in the sandy loam. In a 2-year study on the same three soils, dissipation was much slower in year two than in year one. Averaged over both years, 21 days after application 93%, 56%, and 85% of the atrazine applied still remained in the sandy loam, silt loam, and clay loam soils, respectively, with ┘ 95% of the amount present remaining in the surface soil (Koskinen *et al.*, 1993). In a third study on the same sandy loam soil a year later, after 30 days the majority of the atrazine remaining (161 μg/kg) was in the top 15 cm of soil (Buhler *et al.*, 1994b). About 2 μg/kg was present at 45–60 cm. By 60 days after application, only 5 μg/kg remained in the top 15 cm of surface soil.

Similar variability in atrazine persistence was observed in Nebraska soils. By 61 days after application, levels of atrazine in a loam soil decreased to 75% of the application level in both conventional-tillage and no-tillage plots (Ghadiri *et al.*, 1984). In a similar study the following year, only trace amounts remained 80 days after application. In many cases, effects of climatic conditions on initial triazine dissipation may not influence triazine persistence by the end of the growing season. For instance, atrazine residues in a number of Michigan soils at the end of the growing season after a severe drought were similar to those at the end of a normal growing season (Leavitt*et al.*, 1991).

Initial soil triazine concentration has been shown to affect persistence, with higher rates having slower dissipation (Davidson *et al.*, 1980). In clay loam and sandy loam soils, atrazine persistence in the field was greater for high-rate treatments than for low-rate treatments during the first six months (Gan *et al.*, 1996). On an absolute basis, however, the amount of atrazine dissipated from the high-rate treatment was greater than the low-rate treatment.

Tillage impacts many soil properties, including the amount and distribution of organic matter, temperature, moisture, soil structure, bulk density, pH, and microbial biomass and activity, which have been shown to affect persistence. Changes in soil properties in turn affect pesticide retention, transport, and transformation. No-till farming commonly leads to higher organic carbon content, lower pH, greater microbial biomass and activity, higher moisture content, and cooler temperature in surface soil compared to conventional tillage, with the possible net result being decreased persistence in no-till soils. For instance, atrazine carryover in soil was less of a problem under reduced-tillage systems than in conventional-tillage systems (Burnside and Wicks, 1980). The upper 45 cm of clay loam soil contained more than twice as much residual atrazine in moldboard-plow plots than in no-tillage plots 12 months after the final atrazine application (Buhler *et al.*, 1993). More atrazine was recovered in the top 10 cm of surface soil under conventional till than under no-till (Sauer *et al.*, 1990; Sadeghi and Isensee, 1992, 1994, 1996; Isensee and Sadeghi, 1994, 1996). Cyanazine persistence was twice as long in conventional tillage as in no-till (Sadeghi and Isensee, 1997).

Some studies have shown that the compensating effects of tillage on soil properties and processes result in no net effect on triazine persistence. Although moldboard-plow plots usually had the largest atrazine concentrations at any given sampling time, tillage had little significant effect on the overall distribution and dissipation of atrazine in soil (Weed *et al.*, 1995). In a sandy loam soil, atrazine persistence was not significantly different in fields under no-till or conventional-tillage management (Gish *et al.*, 1994). In another study, similar dissipation of atrazine in both no-till and conventional-tillage systems was attributed to the low pH of the soil that resulted from long-term application of NH_4NO_3, which catalyzed hydrolysis to HA (Ghadiri *et al.*, 1984). Hall *et al.* (1989) found no differences in persistence of three triazines in soil under conventional as compared to no-till systems. However, the next year less triazine mass was recovered in no-till soils than in conventional-till soils two months after application.

Any initial impact of tillage on persistence during the first months after application becomes insignificant by the end of the growing season. For example, at the end of the growing season ffi2% of the spring-applied atrazine remained in a clay loam soil under ridge, conventional, and zero tillage systems (Gaynor *et al.*, 1987). Greater metribuzin persistence in no-till plots as compared to tilled plots was attributed to lower temperature and to reduced microbial activity in the spring; however, there was little residual metribuzin in any treatment late in the growing season (Sorenson *et al.*, 1991).

The interactions of the factors that affect triazine persistence and transport are difficult to estimate. For this reason, several models have been used as tools to estimate losses and identify variables that may impact the magnitude of loss, including Leaching Estimation and Chemistry Model-Pesticide (LEACHP) (Wagenet and Hutson, 1989). The LEACHP model was evaluated to predict atrazine movement in sandy loam, silt loam, and clay loam soils in Minnesota during three consecutive years (two dry and one wet) (Khakural *et al.*, 1995). Considering the broad range in soil properties and climatic conditions used in testing, the model performed well. However, model predictions are only estimates, and triazine persistence and transport in the soil environment are highly variable and dependent on site-specific conditions.

It is fair to say that much is known about the movement and persistence of triazine herbicides in soil. One only has to peruse the more than 300 citations from this chapter, which does not include the hundreds of articles published prior to 1970 nor the articles still being published every month or so, to realize that more is known about the behavior of triazines in soils than any other herbicide family. This is good from the perspective that it allows the users and

regulators of the triazines a sound basis upon which to make decisions. The widespread interest in the triazines is directly linked to their phenomenal success as effective herbicides in the major crops of the world. Few herbicides have provided economical weed management over such a long period of time. Based on the movement and persistence data cited here, it is possible to predict with a level of confidence where, when, and how much of a specific triazine will occur following an application. Of course, this can lead to a false sense of security since the single factor that influences this predictable behavior more than anything else is the highly unpredictable behavior of specific weather events, especially events of intense rainfall that produce runoff or field flooding shortly after the application of triazines. It is, however, comforting to know that when the triazine finally gets to its new location, we can then again predict how it will behave. The type of data that has been produced by the hundreds of researchers cited in this chapter is important in weighing risks to nontarget organisms due to off-target movement of triazines.

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Chapter 25

Hazard Assessment for Selected Symmetrical and Asymmetrical Triazine Herbicides

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Summary

The triazine herbicides constitute a class of crop protection chemicals of great agronomic importance around the world. This class of herbicides includes the asymmetrical triazines metribuzin and metamitron, the symmetrical triazine herbicides, and hexazinone. The major symmetrical triazines are further divided into chloro-*s*-triazines: including simazine, atrazine, terbuthylazine, propazine, cyanazine; the thiomethyl-*s*-triazines (also referred to as methylthiotriazines): ametryn, prometryn, terbutryn; and the methoxy-*s*-triazine: prometon. Triazine herbicides inhibit photosynthesis in certain broadleaf and grassy weeds, and they generally have low toxicity to animals. Evaluation of hazard profiles of the triazine herbicides reveal that these products are generally not acutely toxic, are well-tolerated when administered to animals over a long period of time, and do not cause birth defects or affect reproduction. Further, the triazines do not produce cancer in mice or male rats. The chloro-*s*-triazines produce an earlier onset or an excess of mammary tumors in female Sprague–Dawley (SD) rats at high doses, but not in the Fischer 344 (F-344) rats. Because of the unique nature of reproductive aging in female SD rats, the carcinogenic response in this strain is not considered relevant to humans.

Introduction

Triazines have been used as selective herbicides in agriculture in the United States and other parts of the world for 50 years (Stevens and Sumner, 1991). The triazines exert their phytotoxic effects through inhibition of the Hill reaction in photosynthesis (Gysin and Knüsli, 1960). Even after five decades of use, certain of these triazine herbicides remain agronomically and commercially important, especially for the preemergent control of broadleaf weeds. They have become important 'mixing partners' for many of the newer herbicides since they offer a broad spectrum of weed control (Gressel *et al.*, 1982). The safety of this class of chemistry has been reassessed by regulatory authorities around the world. There are ongoing reviews and changes in regulatory positions, especially as new studies become available. In this review, the tabulations of the lowest observed effect levels (LOELs) and no observed effect levels (NOELs) were based upon published regulatory decisions, such as US Environmental Protection Agency (USEPA) Registration Eligibility Decisions (REDs) and European Union reviews, when available. When such assessments were not available, individual studies were consulted, if available to the reviewers, and the LOELs and NOELs reported by the study director were utilized. Throughout this document, doses are specified on a mg/kg/day basis, and standard conversion factors have been used to convert feeding levels typically expressed as ppm concentrations in feed to mg/kg/day dose units.

The symmetrical triazines (*s*-triazines) have a chlorine, thiomethyl, or methoxy group at the 2-position of the ring and are usually substituted in the 4- and 6-positions with alkylamino group. Cyanazine contains a 2-cyano-isopropylamino-substituent at the 4-position on the ring. The asymmetrical triazine metribuzin retains the triazine ring, but since the nitrogen atoms are unevenly spaced, aromaticity is maintained by the presence of the carbonyl group. Metribuzin also has a thiomethyl-substituent on the ring.

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Table 25.1 USEPA acute toxicology classification scheme

Toxicology category	Signal word	Oral LD_{50} (mg/kg)	Dermal LD_{50} (mg/kg)	Inhalation LC_{50} (mg/L)	Eye irritation	Skin irritation
I	Danger[a]	Up to 50	Up to 200	Up to 0.2	Corrosive. Corneal opacity not reversed in 7 days	Corrosive
II	Warning	From 50 to 500	From 200 to 2000	From 0.2 to 2.0	Corneal opacity reversed in 7 days; irritation persisting 7 days	Severe irritation at 72 h
III	Caution	From 500 to 5000	From 2000 to 5000	From 2.0 to 20	No corneal opacity; irritation reversed within 7 days	Moderate irritation at 72 h
IV	Caution	Greater than 5000	Greater than 5000	Greater than 20	No irritation	Mild or slight irritation at 72 h

[a] The word 'Poison' is used on the label if the 'Danger' category is based on oral, dermal, or inhalation toxicity.

Animal metabolism is similar for the chloro- and thiomethyl-substituted triazines, with both undergoing conjugations with glutathione (Bakke *et al.*, 1972). Prometon (methoxy substituted) is not readily conjugated, but all of the triazine herbicides show side-chain dealkylation of the amino groups.

The objective of this chapter is to summarize the hazard profile for several of the commercially important herbicides in this class.

Acute Toxicity Studies

Acute toxicity generally applies to effects that result from a single dose or single exposure of a chemical. Acute toxicity studies are conducted by administering the chemical orally, dermally, or by inhalation to determine the dose that causes 50% mortality. The values calculated through the oral and dermal routes of exposure are referred to as Lethal Dose 50 (LD_{50}). The LD_{50} is defined as that amount of chemical required to kill 50% of the test animals in a group within the first 14 days following exposure; Lethal Concentration 50 (LC_{50}) is equivalent to the concentration of chemical administered (usually as an aerosol) to kill 50% of the animals by inhalation exposure. Acute studies are conducted to evaluate the irritation potential of the chemical after application to skin and eyes. Studies conducted for the USEPA must strictly comply with 'Good Laboratory Practices' (USEPA, 1979). These tests are used to establish the product labels for all crop protection chemicals (Sumner *et al.*, 1995). The criteria used are presented in Table 25.1.

The commercially important triazine herbicides are relatively nontoxic, and they are not generally irritating to the skin or eye (Table 25.2).

Toxicity After Repeat Exposure

Dermal toxicity is evaluated by applying the chemical to the skin for 6 h a day for 21 days in rat studies, or 28 days in rabbit studies. Feeding studies are used to evaluate the toxicological effects of the chemical when a known dose is administered orally.

The oral feeding studies involve giving rats, mice, or dogs diets containing the chemical for various lengths of time. Rat and mouse feeding studies are conducted for intervals of 28 days, 90 days, 1 year, and for the lifetime of the animals (24 months for rats, 18 months for mice). When dogs are used as the test animal, studies are usually conducted for 28 days, 90 days, 1 year, or 2 years. In all cases, animals are divided into test groups of 10–50 rats or mice and four to six dogs. At least four test groups are used in each study, one control group receiving no chemical and three groups receiving low, medium, or high concentrations of the chemical in their diets. In these studies, urinalysis, hematology, and clinical chemistry parameters are evaluated, and gross and microscopic pathological examinations are performed on up to 50 tissue samples. Maximally tolerated doses are tested in order to demonstrate toxicity (up to 1000 mg/kg/day in the diet). In this fashion, it is possible to determine whether a chemical damages or alters any organ or tissue. In addition, it is possible to establish levels of the chemical that produce the NOEL, and the lowest level at which effects are noted (LOEL). The response of repeated exposure of rats and dogs to the selected triazine herbicides are presented in Table 25.3.

Table 25.2 USEPA acute hazard classification of the technical grade for selected triazine herbicides

Triazine technical[a]							
Group	**Herbicide**	**Eye irritation**	**Skin irritation**	**Oral LD_{50} mg/kg**	**Dermal LD_{50} mg/kg**	**Inhalation LC_{50} mg/L**	**Signal word**
s-Cl	Atrazine	Nonirritating	Nonirritating	1869–3090	ffi2000	ffi5.8	Caution
	Simazine	Slight irritation	Mild	ffi5000	ffi2000	ffi1.7	Caution
	Propazine	Slight irritation	Nonirritating	ffi5050	ffi5050	ffi1.22	Caution
	Terbuthylazine	Moderate irritation	Mild	1000–1590	ffi2000	ffi5.3	Caution
	Cyanazine	Nonirritating	Nonirritating	182–334	ffi2000	0.81	Warning
s-SCH3	Ametryn	Mild irritation	Nonirritating	1009–1356	ffi2020	ffi5.2	Caution
	Prometryn	Slight irritation	Mild irritation	1802–2076	ffi3170	4.96	Caution
	Terbutryn[b]	Nonirritating	Nonirritating	2450	ffi2000	ffi2.2	Caution
s-OCH3	Prometon	Slight irritation	Nonirritating	1520–4350	ffi2020	ffi3.2	Caution
Asym[c]	Metribuzin	Nonirritating	Nonirritating	2200–2300	ffi20 000	ffi0.65	Caution

[a] Table 25.2 lists only technical products; formulated products used for agriculture may have more restrictive labeling due to the formulants used. Commercial formulations of prometon (Danger, Corrosive) and the 4L formulation of prometryn (Warning) carry more restrictive signal words due to formulants.
[b] Studies were conducted on an 80% formulation of active ingredient (80W).
[c] *Asym*. ffl asymmetrical.

Table 25.3 Hazard assessment for repeat exposure to selected triazine herbicides

Triazine			mg/kg/day		
Group	**Herbicide**	**Species/study**	**NOEL[a]**	**LOEL[b]**	**Target organ, Tissue, or system**
s-Cl	Atrazine[c]	Rat/90-day oral	3.3	34.5	Body weight
		Dog/52-week oral	4.97	33.7	Heart/myocardium
	Simazine[c]	Rat/90-day oral	┘ 14.3	14.3	Body weight
		Dog/90-day oral	6.9	64	Body weight
	Propazine[c]	Rat/90-day oral	13	50	Body weight
		Dog/90-day oral	7	25	Body weight
	Terbuthylazine	Rat/28-day oral	┘ 2.3	2.3	Body weight, organ weights
		Rat/90-day oral study 1[d]	2.1	7.1	Body weight, hematological and clinical chemistry
		Rat/90-day oral, study 2[e]	4.0	8.0	Body weight
		Dog/52-week oral	0.4–1.25	1.25–7.8	Body weight
	Cyanazine	Rat/90-day oral	2.5	5.0	Body weight
		Dog/52-week oral	0.7	3.0	Hematological effects
s-SCH_3	Ametryn	Rat/90-day oral	7.4	36	Hematological effects
		Dog/52-week oral	7.2	70	Liver
	Prometryn	Rat/90-day oral	2.5	25	Body weight
		Dog/104-week oral	3.7	37.5	Liver, kidney, bone marrow
	Terbutryn[f]	Rat/90-day oral	50	140	Body weight
		Dog/26-week oral	10	25	Stomach
s-OCH_3	Prometon	Rat/90-day oral	5	15	Body weight
		Dog/52-week oral	5	20	Body weight
Asym.[g]	Metribuzin	Rat/104-week oral	1.3	13.8	Body weight, liver, thyroid
		Dog/104-week oral	3.4	55.7	Body weight, liver, kidney

[a] No observable effect level.
[b] Lowest observable effect level.
[c] USEPA has utilized the atrazine chronic NOEL of 1.8 mg/kg/day for cumulative risk assessment.
[d] UK 2007 draft review submitted to the European Commission.
[e] USEPA 1995 Registration Eligibility Decision.
[f] Studies were conducted on an 80% formulation of active ingredient (80W).
[g] *Asym*. ffl asymmetrical.

With the exception of rats and dogs fed terbuthylazine and cyanazine and rats fed metribuzin, the NOEL values were 2.5 mg/kg/day or higher, and LOEL values were 15 mg/kg/day or higher. Furthermore, the most common observation was not a specific organ or tissue effect, but a reduction in body weight gain. Microscopically, the liver was the most common target organ.

Developmental and Reproductive Toxicity

Hazard testing also includes the examination of the potential of a chemical to affect the development of offspring and the determination of whether it induces birth defects in either rats or rabbits. These tests have been described as teratology studies, but are now usually referred to as developmental toxicity studies. In addition to developmental toxicity studies, a reproduction study is conducted in rats. This involves feeding diets containing the chemical to young adult male and female rats for approximately 3 months prior to mating. The females are allowed to produce a litter of offspring that are then reared to adulthood. The animals are fed diets containing the chemical during this entire period of time. After reaching sexual maturity, the second-generation animals are allowed to mate. The third generation is then examined. The results of such studies conducted with the selected triazine herbicides are presented in Table 25.4.

The triazine herbicides, with the exception of cyanazine, did not produce developmental or reproductive effects at maximally tolerated doses. Cyanazine produced developmental effects in rats and rabbits at the highest doses tested. Effects noted at doses that were toxic to the mothers were cyclopia and diaphragmatic hernia in rabbits and an apparent increase in the incidence of skeletal variations (i.e., anomalies) in rats (USEPA, 1994).

Mutagenicity

Weisburger (1975) noted that certain chemical carcinogens are capable of interacting directly with genetic material such as DNA. Based upon this association, several short-term tests were introduced into hazard testing for crop protection chemicals to identify the alteration of genetic material or mutation. These include tests to examine the possible interaction with: genes (gene mutation tests); chromosomes (clastogenic tests); and DNA (classified as other tests). The results for selected triazine herbicides are presented in Table 25.5.

All of the triazines were found to be negative in the specific tests listed in Table 25.5. The overall mutagenic potential of atrazine, simazine, and cyanazine has been reviewed (Brusick, 1994; Hauswirth and Wetzel, 1998; Bogdanffy *et al.*, 2000; USEPA, 2003a), and the weight of the evidence indicates that they are not mutagenic. Cyanazine showed only limited evidence of mutagenicity (USEPA, 1994).

Carcinogenicity Bioassays

Since individuals may be exposed to low levels of chemicals over a portion of their lifespan, studies to evaluate lifetime exposures are conducted in animal bioassays. An important aspect of these studies is the evaluation of the potential of a compound to cause cancer and the number of tumors and time of onset of tumors is analyzed. For the laboratory studies, mice and rats are divided into at least three treatment groups and a control group, with a minimum of 50 animals of each sex in each group. These groups of mice and rats are fed selected concentrations of the test chemical in their diet for 18 months and 24 months, respectively. The levels of the test chemical administered in the diet are generally selected from repeat dose feeding studies that are at least 90 days in duration and are normally used to establish the NOEL, LOEL, and Maximum Tolerated Dose (MTD) (Farber, 1987). The MTD is defined as the highest concentration of test chemical that can be administered without causing the death of the animal; often a 10% reduction in body weight gain has been used as a default criterion for establishing the MTD.

Following lifetime feeding studies at the prescribed treatment levels, veterinary pathologists examine approximately 50 tissues from each animal for the presence of tumors or other evidence of tissue damage. The results of such oncogenicity studies in mice, conducted with the selected triazine herbicides, are presented in Table 25.6.

None of the selected triazines showed any evidence of inducing tumors in mice, despite high feeding levels; doses ranged from 87 to 1140 mg/kg/day and were equal to or exceeded the MTD. The chloro-*s*-triazines (e.g., atrazine, cyanazine, propazine, and simazine) resulted in either an increased incidence or an earlier onset of mammary tumors when administered to female SD rats at high feeding levels, as presented in Table 25.7.

The thiomethyl- and methoxy-*s*-triazines, as well as the asymmetrical triazine metribuzin, were not carcinogenic – some even in the SD rat – and at feeding levels exceeding the MTD; the exception was terbutryn, where an increased incidence of mammary, thyroid, and liver tumors were observed in female SD rats at feeding levels that exceeded the MTD.

The SD rat is a commonly used laboratory animal. However, it has limitations when used to evaluate the effects of chemicals on the endocrine system, including the pituitary and mammary gland, because of a high spontaneous tumor incidence in these organs. At about 9–12 months of age, the SD rat begins to experience prolonged periods

Table 25.4 Summary of the results of rat and rabbit developmental studies and of a two-generation rat reproduction study with triazine herbicides

Triazine					mg/kg/day		
Group	**Herbicide**	**Study/species**	**Developmental/ reproduction**	**Toxicity observed**	**HDT[a]**	**LOEL[b]**	**NOEL[c]**
s-Cl	Atrazine	Developmental/rat	None	↓Body weight gain	700	70	10
		Developmental/rabbit	None	↓Body weight gain	75	75	5
		Reproductive/rat	None	↓Body weight gain	35	39	3.7
	Simazine	Developmental/rat	None	↓Body weight gain	600	300	30
		Developmental/rabbit	None	↓Body weight gain	200	75	5
		Reproductive/rat	None	↓Body weight gain	29–35	6	0.6
	Propazine	Developmental/rat	None	↓Body weight gain	600	100	10
		Developmental/rabbit	None	↓Body weight gain	50	10	2
		Reproductive/rat	None	↓Body weight gain	50	50	5
	Terbuthylazine	Developmental/rat[d]	None	↓Body weight gain	30	30	5
		Developmental/rabbit[d]	None	↓Body weight gain	4.5	4.5	ffi4.5
		Reproductive/rat[e] study 1	Reduced fertility	↓Body weight gain	20–26	4.5–26	0.4[f]–4.5[g]
		Reproductive/rat[e] study 2	None	↓Body weight gain	25–36	7.3–10.4	3.6–5.2[h]
		Reproductive/rat[e] study 3	None	↓Body weight gain	14.6–18.1	7.1–11.4	3.5–4.5[h]
	Cyanazine	Developmental/rat	Positive	↑Malformations	75	5	ffi5
		Developmental/rabbit	Positive	↑Malformations	4	2	1
		Reproductive/rat	None	↓Body weight gain	15	5	1.5
s-SCH_3	Ametryn	Developmental/rat	None	↓Body weight gain	250	50	5
		Developmental/rabbit	None	↓Body weight gain	60	60	10
		Reproductive/rat	None	↓Body weight gain	131	131	13
	Prometryn	Developmental/rat	None	↓Body weight gain	250	250	ffi50
		Developmental/rabbit	None	↓Body weight gain	72	72	12
		Reproductive/rat	None	↓Body weight gain	96.7	47.8	0.6
	Terbutryn[i]	Developmental/rat	None	↓Body weight gain	500	500	50
		Developmental/rabbit	None	↓Ossified sternabra	75	75	10
		Reproductive/rat	None	↓Body weight gain	150	150	15
s-OCH_3	Prometon	Developmental/rat	None	↓Body weight gain	360	120	36
		Developmental/rabbit	None	↓Body weight gain	24.5	24.5	3.5
		Reproductive/rat	None	↓Body weight gain	75	25	1
Asym.[j]	Metribuzin	Developmental/rat	None	↓Body weight gain	200	200	70[k]
		Developmental/rabbit (New Zealand)	None	↓Body weight gain	135	45	15[f]
		Developmental/rabbit (Dutch)	None	↓Body weight gain	85	30–85[f,k]	10–30[f,k]
		Reproductive/rat	None	↓Body weight gain	37.5	7.5	1.5

[a] Highest dose tested.
[b] Lowest observed effect level.
[c] No observed effect level.
[d] USEPA 1995 Registration Eligibility Decision.
[e] UK 2007 Draft Review submitted to the European Commission.
[f] Maternal NOEL.
[g] Reproductive NOEL.
[h] Defined by the European Commission as a NOAEL based upon parental toxicity.
[i] Studies were conducted on an 80% formulation of active ingredient (80W).
[j] Asym. ffl asymmetrical.
[k] Developmental NOEL.

of estrus (Eldridge *et al.*, 1996; Simpkins *et al.*, 1998). Most laboratory rats (SD rats prior to 9–12 months) spend about 20–25% of their time in estrus. The SD rat spends increasing amounts of time in estrus after this period, often 40% by 12 months of age, and in some cases achieves persistent estrus at senescence (Eldridge *et al.*, 1998). This unique physiology is related to a deficiency in the neuroendocrine control of the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. With decreasing release of GnRH, the pituitary secretion of luteinizing

Table 25.5 Results of mutagenicity studies with selected triazine herbicides

Triazine		Gene mutation			Clastogenic	Other	
Group	**Herbicide**	**Ames**	***E. coli* REC**	**Mouse lymphoma**	**Micronucleus**	**DNA repair**	**Dominant lethal**
s-Cl	Atrazine	Negative	Negative	Negative	Negative	Negative	Negative
	Simazine	Negative	–	Negative	Negative	Negative	-
	Propazine	Negative	Negative	Negative	Negative	Negative	–
	Terbuthylazine	Negative[b]	Negative	Negative	Negative[c]	Negative	–
	Terbuthylazine	Negative	–	Negative	Negative	Negative	–
s-SCH3	Ametryn	Negative	–	Negative	Negative	Negative	Negative
	Prometryn	Negative	Negative	Negative	Negative	Negative	–
	Terbutryn	Negative	–	–	Negative	Negative	Negative
s-OCH3	Prometon	Negative	–	–	Negative	Negative	–
Asym.[a]	Metribuzin	Negative[b]	–	–	Negative	Negative	Negative

[a] *Asym.* ffl asymmetrical.
[b] Also negative in Chinese hamster ovary/HGPTR assay.
[c] Also negative in the Chinese hamster ovary.

Table 25.6 Results of carcinogenicity studies in mice

Triazine		Cancer potential	Feeding level mg/kg/day			Other effects	Reference
Group	**Herbicide[a]**		**HDT[b]**	**NOEL[c]**	**LOEL[d]**		
s-Cl	Atrazine	Negative	386[e]	43	194–247	↓ Body weight gain and thrombi in both sexes	Hazelette and Green (1987)
	Simazine	Negative	542–652[e]	5.3	132	↓ Body weight gain in both sexes	Hazelette (1988)
	Propazine	Negative	450[e]	15	450	Cardiac fibrosis and focal degeneration	IRIS[f] 1997a
	Terbuthylazine	Negative[f] study 1	87–89[e]	17[g]	87–89	↓ Body weight gain in both sexes; Hematological changes in males	USEPA (1995)
		Negative[h] study 2	99–118	14.6–15.5[g]	37–40	↓ Body weight gain	UK (2007)
	Cyanazine	Negative	143	1.4	3.6	↓ Body weight gain in both sexes	USEPA (1994)
s-SCH3	Ametryn	Negative	300[e]	ffi300	ffi300	↓ Body weight gain in both sexes	USEPA (2004)
	Prometryn	Negative	429	143	429	↓ Body weight gain in both sexes	USEPA (1996)
	Terbutryn[i]	Negative	429[e]	429	ffi429	No effects observed	Jessup (1980)
s-OCH3	Prometon	Negative	1140[e]	60	570	↓ Survival, kidney necrosis, hepatocellular hypertrophy, splenic atrophy	Osheroff (1988)
Asym.[j]	Metribuzin	Negative	438–567	111–139	438–567	↓ Hematocrit, ↑ liver weight; Hematological changes	USEPA (1998)

[a] CD1 mice were tested for all chemicals except terbuthylazine, where Tif: MAGf was used.
[b] Highest dose tested.
[c] No observable effect.
[d] Lowest observable effect level.
[e] Maximum tolerated dose exceeded.
[f] USEPA 1995 Registration Eligibility Decision.
[g] Draft European Commission review defined this dose as a NOAEL.
[h] UK 2007 draft review submitted to the European Commission.
[i] Studies were conducted on an 80% formulation of active ingredient (80W).
[j] *Asym.* ffl asymmetrical.

hormone (LH) gradually decreases until it is inadequate to stimulate ovulation. Thus, the female SD rats become 'stuck in estrus.' As a result of these prolonged periods of estrus, the rats experience prolonged exposure to estrogen and prolactin produced by the ovary and pituitary, respectively (Simpkins *et al.*, 1998). Both of these hormones are known to produce mammary tumors in rats (Cutts and Noble, 1964).

Table 25.7 Results of carcinogenicity studies in rats

Triazine		Tumor	Feeding level (mg/kg/day)				
Group	**Herbicide[a]**	**Response**	**HDT[b]**	**NOEL[c]**	**LOEL[d]**	**Other effects**	**Reference**
s-Cl	Atrazine	Mammary (SD)[a]	50[e]	2.5	20	↓Body weight gain in both sexes	USEPA (2003a, b)
		Negative (F-344)[a]	20	4.8	20	↓Body weight gain in both sexes	USEPA (2003a, b)
	Simazine	Mammary (SD)	45.8–63.1[e]	0.5	4.2–5.3	↓Body weight gain in both sexes; Hematologic effects and ↑mortality in females	USEPA (2006)
	Propazine	Mammary (SD)	50[e]	5.8	50	↓Body weight gain in both sexes	Stevens *et al.* (1994)
	Terbuthylazine	Mammary, Leydig[f] study 1 (Tif:RAI)[a]	42–53[e]	1.2	1.2	↓Body weight gain in both sexes	USEPA (1995)
		Negative[g] study 2 (Wistar)	1.6	0.35	1.6	↓Body weight gain	UK (2007)
		Mammary[g] study 3 (Wistar)	5.5–7.6	0.4	1.7	↓Body weight gain	UK (2007)
	Cyanazine	Mammary (SD)	2.5[e]	0.2	1.0	↓Body weight gain in females; ↑Hyper-activity in the males	USEPA (1994)
s-SCH3	Ametryn	Negative (SD)	145-176[e]	21	145	↓Body weight gain in both sexes; Hematological effects in females	USEPA (2004)
	Prometryn	Negative (SD)	75[e]	29	61	↓Body weight gain in both sexes	USEPA (1996)
	Terbutryn[h]	Mammary, Thyroid, Liver (SD)	150[e]	0.1	15	↓Body weight gain in both sexes	Stevens *et al.* (1994)
s-OCH3	Prometon	Negative (SD)	75	1	25	↓Body weight gain in both sexes	Stevens *et al.* (1994)
Asym.[i]	Metribuzin	Negative (Fischer)	42–54	1.3	14–18	↓Body weight gain; ↑liver and thyroidweights	USEPA (1998)

[a] Strain of rat tested (SD ffl Sprague–Dawley, Tif:RAI ffl Sprague–Dawley derived strain of rat, F-344 ffl Fischer 344).
[b] Highest dose tested.
[c] Lowest observable effect level.
[d] No observable effect level.
[e] Maximum tolerated dose exceeded.
[f] USEPA 1995 Registration Eligibility Decision. Elevated incidence at doses that exceed the MTD. Inadequate evidence. Classified as Category D.
[g] UK 2007 draft review submitted to the European Commission.
[h] Studies were conducted on an 80% formulation of active ingredient (80W).
[i] *Asym.* ffl asymmetrical.

The reproductive aging process observed in the female SD rat is unique and is apparently specific to certain outbred strains and species. Thus, other strains of rats, like the inbred F-344, do not demonstrate this deficiency and do not have a high spontaneous incidence of mammary or pituitary tumors (Eldridge *et al.*, 1998).

Detailed studies on atrazine have shown that F-344 rats administered high doses of atrazine do not develop either an increased incidence or an early onset of mammary tumors (Wetzel *et al.*, 1994; Thakur *et al.*, 1998), unlike the findings noted in similarly treated female SD rats (Stevens *et al.*, 1994; Wetzel *et al.*, 1994; Hauswirth and Wetzel, 1998). Furthermore, when ovarian estrogen was eliminated from the female SD rats by surgical removal of the ovaries, no mammary tumors were found (Stevens *et al.*, 1999). Likewise, atrazine is not carcinogenic in mice or male SD rats (Hauswirth and Wetzel, 1998).

Examination of the reproductive cycles of intact female SD rats fed high doses of atrazine over their lifetimes showed that prolonged periods of estrus occurred earlier in the treated group than in the control group (Hauswirth and Wetzel, 1998). Subsequent studies showed that high doses of atrazine administered to female SD rats reduced the magnitude of the LH, resulting in ovulation failing to occur (Simpkins *et al.*, 1998). However, lower doses of atrazine had no effect on the LH surge, the estrous cycle, or the earlier appearance of mammary tumors (Simpkins *et al.*, 1998) – indicating that even in female SD rats there is a threshold dose below which there are no adverse effects on reproductive processes. Finally, when atrazine-treated animals were given a supplemental dose of GnRH, the hormone that is responsible for triggering the LH surge, that surge was restored, indicating that the LH-releasing mechanisms function normally in atrazine-treated animals (Cooper *et al.*, 1995).

Epidemiology

Several reviews of the epidemiological evidence relating to atrazine exposure and the occurrence of cancer have been conducted by Loosli (1995), Neuberger (1996), and Sathiakumar and Delzell (1997). The weight of the evidence indicates there is no basis for concluding that there is a causal association between exposure to atrazine and cancer in humans. Cohort studies conducted at a production facility over a long period of follow-up have not identified any increased cancer risk, including the risk of non-Hodgkin's lymphoma (MacLennan *et al.*, 2002). The number of prostate cancer cases at a production facility is fully accounted for by the prostate cancer screening bias operative at the plant as a result of an advanced medical surveillance program (Hessel *et al.*, 2004). The null results reported for prostate cancer in the large cohort of licensed pesticide applicators who are members of the US government-sponsored Agricultural Health Study support this conclusion (Alavanja *et al.*, 2003; Rusiecki *et al.*, 2004; Engel *et al.*, 2005). In fact, Blair *et al.* (2005) stated that, 'No exposure and response gradient was noted for any cancer among farmers exposed to atrazine, including prostate.'

A review of the case-control studies conducted by the National Cancer Institute, principally on non-Hodgkin's lymphomas, has not established a causal association between atrazine use and the occurrence of this disease (De Roos *et al.*, 2003). This conclusion has also been reached in numerous authoritative reviews (USEPA, 2003a, b; International Agency for Research on Cancer (IARC), 1999; United Kingdom (UK), 1996, 2000; Australian Pesticides and Veterinary Medicines Authority (APVMA, 2004).

The ecological epidemiology studies, which do not measure exposure or disease at the level of the individual, have generated null relationships, inverse relationships (Van Leeuwen *et al.*, 1999), and a few positive associations that have not been supported by the results from cohort studies (Mills, 1998, 2003; Muir *et al.*, 2004). The results from some of the studies have been contradictory (e.g., Kettles *et al.*, 1997 versus Hopenhayn *et al.*, 2002) or implausible (Van Leeuwen *et al.*, 1999). A more definitive cohort study in Iowa and North Carolina failed to show any association between atrazine exposure and breast cancer among the wives of Agricultural Health Study workers (Engel *et al.*, 2005).

Chlorotriazine Cancer Classification

A review of the mode of action data underlying the mammary tumor response observed in female SD rats treated with high doses of chlorotriazines has been reported elsewhere (Wetzel *et al.*, 1994; Eldridge *et al.*, 1996; Eldridge *et al.*, 1999; Stevens *et al.*, 1999; Eldridge and Wetzel Chapter 26). Based upon a weight of the evidence analysis, USEPA concluded that this mode of action is not relevant to humans. Alternate modes of action have also been considered and discounted by USEPA. Thus the weight of the evidence indicates that atrazine and the chlorotriazines are not genotoxic (Brusick, 1994) or estrogenic (Eldridge *et al.*, 1999; Eldridge and Breckenridge, 2007). Furthermore, recent studies indicate that atrazine does not increase the expression or activity of aromatase in intact rodents (Modic, 2004; Modic *et al.*, 2004; Rayner *et al.*, 2003), fish (Kazeto *et al.*, 2003, or frogs (Hecker *et al.*, 2004, 2005a, b; Murphy *et al.*, 2006; Park *et al.*, 2006), as has been proposed by Fan *et al.* (2007a, b).

Reviews from the USEPA (2003a), the European Union (UK, 2000), the APVMA (2004), and IARC (1999) concluded that the mechanism underlying the occurrence of atrazine-induced mammary tumors in female SD rats is not relevant to humans. Atrazine is classified as not likely to be a human carcinogen by the USEPA.

Based upon these results, it is concluded that:

1. The chloro-*s*-triazines accelerate the onset timing of mammary tumors in the female SD rat, a strain of rat that is already prone to developing mammary tumors spontaneously because of an inherent age-dependent deficiency in the regulation of the estrous cycle in the SD rat.
2. The earlier appearance of mammary tumors in female SD rats treated with high doses of atrazine is attributed to an increased exposure to endogenous estrogen and prolactin, secondary to the lengthening of the estrous cycle.
3. Removal of endogenous estrogen in female SD rats by ovariectomy prevents the appearance of mammary tumors, even in animals that have received high doses of atrazine.
4. In the SD female there is a lifetime dose of atrazine (ffi2.5 mg/kg) that has no effect on the estrous cycle or mammary tumor incidence and/or onset.
5. The mammary tumor response to high doses of atrazine is unique to the female SD rat and is not observed in male SD rats, three strains of mice, or in the F-344 rat.
6. The effects of atrazine on the reproductive aging processes observed in female SD rats are not relevant to humans. In women, reproductive senescence is characterized principally as an ovarian failure with a decrease in endogenous estrogen exposure at menopause, not the increase that is characteristic of the female SD rat in a state of persistent estrus.

7. The International Agency for Research on Cancer (IARC, 1999) concluded there is strong evidence that the mechanism by which atrazine increases the incidence of mammary gland tumors in SD rats is not relevant to humans and that atrazine is *not classifi able as to its carcinogenicity to humans*.
8. After review by its Scientific Advisory Panel (SAP), the USEPA (2000, 2002, 2003a) has concurred with IARC (1999) that the mammary tumor response observed in the SD female rat is not considered relevant to humans and USEPA classified atrazine as *not likely to be a human carcinogen*.
9. In addition, other regulatory bodies around the world have reviewed the data on atrazine and arrived at the same conclusion as IARC and USEPA. The APVMA (October 2004) concludes that 'published epidemiological data provides support for the absence of carcinogenicity potential for atrazine,' and maintained an earlier (1997) conclusion that animal data on the carcinogenicity of atrazine has *no* relevance to humans (National Registration Authority for Agricultural and Veterinary Chemicals Existing Chemicals Review). The UK's Rapporteur Monograph on Atrazine (2000) conducted for the European Union concluded that the 'classification of atrazine as a carcinogen is not appropriate.'
10. The USEPA's Office of Pesticide Programs concluded again in the October 31, 2003 Interim Reregistration Eligibility Decision (IRED) that 'considering the animal data and the human epidemiological data, atrazine is "not likely to be carcinogenic in humans"' (USEPA, 2003b).
11. After a second USEPA Scientific Advisory Panel reviewed additional data on prostrate cancer in July 2003, USEPA's revised IRED (October 31, 2003) concludes that 'the Agency did not find convincing evidence of an association between triazines or atrazine and cancer' (USEPA, 2003b).
12. The lack of relevance of these data to humans is supported by 50 years of manufacturing and use history for atrazine and other triazine herbicides. To date there is no evidence linking atrazine exposure to any human health effects (Sathiakumar *et al.*, 1992; Loosli, 1995; Neuberger, 1996).
13. In addition, publications in 2003, 2004, and 2005 from a recent large-scale government study show no association of atrazine and cancer (Alavanja *et al.*, 2003; Blair *et al.*, 2005; Engel *et al.*, 2005; Rusiecki *et al.*, 2004).

Overall Hazard Assessment

Evaluation of hazard profiles for the triazine herbicides reveals that these products are generally not acutely toxic, are well-tolerated when administered to animals over a long duration of time, are generally not developmental or reproductive toxins, and are not mutagenic or carcinogenic in mice or male rats. The chloro-*s*-triazines appear to produce an earlier onset or an increased evidence of mammary tumors in female SD rats at high doses. Because of the unique nature of reproductive aging in female SD rats, the carcinogenic response in this strain is not relevant for human risk assessment. Furthermore, a review of the epidemiological studies on atrazine does not support an association of exposure to atrazine and the occurrence of cancer.

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Chapter 26

Mode of Action of Atrazine for Mammary Tumor Formation in the Female Sprague-Dawley Rat

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Summary

Atrazine is a widely used agricultural herbicide. It inhibits plant photosynthesis but has very low toxicity in animals and humans. However, long-term high-dose studies using Sprague-Dawley (SD) female rats revealed an increased incidence or earlier appearance of mammary tumors. The mammary tumor response was unique to the SD female rat and was not seen in the male SD rat, three strains of mice, or the Fischer 344 (F-344) rat, which more closely models the human female than the SD rat. A high spontaneous incidence of mammary tumors occur naturally in aged SD female rats, and it was of interest to determine if the response noted with atrazine could be related to the mechanism underlying their normal occurrence.

Because mammary tumors in rats are usually promoted by estrogens and/or prolactin, the possibility that atrazine impacts hormonal activity has been investigated in a large number of published studies; however, no evidence of estrogenicity or prolactin-stimulating activity has been reported. It has been observed, however, that estrous cycles can be disrupted by very high doses of atrazine in the SD rat, which can lead to an increased percentage of lifetime days with elevated estrogen levels. The estrogen is present as a secretion from ovaries, which appear not to ovulate.

Additional research has found that high doses of atrazine administered to female SD rats – acutely or chronically – reduce the output of luteinizing hormone (LH), a pituitary hormone that is necessary for timely ovulation and normal estrous cycling. This specific inhibitory mechanism of atrazine action is a key step in the mode of action responsible for mammary tumors in the SD rat.

Reduced LH secretion, disrupted estrous cycling, and an internal environment supportive of mammary tumor growth are all normal occurrences in aging SD rats, and it appears that administering atrazine enhances these normal, age-related events. The F-344 rat that is not as disposed to these age-related events was also unresponsive to atrazine for the disruption of estrous cycles, in addition to being unresponsive to atrazine for the development of mammary tumors. Because the reproductive decline as women age toward menopause does not progress in the same manner as in SD female rats, it is highly unlikely that atrazine could have a similar effect in women. Furthermore, the no-effect levels for this set of responses in the SD rat are thousands of times greater than potential human exposure. Data support the conclusion that current exposure levels to atrazine would not pose a threat to human reproductive health from the mechanisms observed in test rodents. These data also support the conclusion that atrazine is not likely to cause cancers in humans.

Introduction

Atrazine is used in agriculture to control growth of annual grass and broadleaf weeds and is the most widely used herbicide in corn and sorghum crops. Atrazine specifically inhibits photosynthesis in plants by preventing electron

[a] Dr. Larry Wetzel has passed away. He was the pioneer and lead researcher on the mode of action of atrazine in the SD rat, as well as a gifted colleague who is sorely missed.

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transfer at the reducing site of chloroplast complex II (Good, 1961) and correspondingly demonstrates a low-level toxicity to nonphotosynthetic organisms. For example, the oral LD_{50} of atrazine in rodents is 3000 mg/kg, and the maximum tolerated dose (MTD) in chronically fed rats is about 40 mg/kg (Wetzel *et al.*, 1994). Both are more than 10 000 times the concentration required to cause a 50% activity reduction (IC_{50}) in chloroplasts (Tischer and Strotmann, 1977).

Atrazine has been extensively examined in a variety of chronic toxicologic studies, with high no observed effect levels (NOEL) for chronic toxicity (┐ 70 ppm) in rats, mice, and dogs (Hauswirth and Wetzel, 1998). Assessment of reproductive and developmental toxicity has identified a NOEL of 5 mg/kg/day in New Zealand white rabbits and 25 mg/kg/day in SD rats (Hauswirth and Wetzel, 1998). A two-generation feeding study in SD rats identified a NOEL of 5 mg/kg/day (Hauswirth and Wetzel, 1998). Atrazine has also been assessed in more than 40 mutagenicity–genotoxicity tests using *in vivo* markers, as well as prokaryotic and eukaryotic cells (Plewa *et al.*, 1984; Franekic *et al.*, 1990; Brusick, 1994), and a complete weight-of-evidence analysis concluded that the herbicide is not genotoxic or mutagenic [Brusick, 1994; International Agency for Research on Cancer (IARC), 1999; US Environmental Protection Agency (USEPA), 2003].

Animal Bioassay Data

In chronic feeding studies with SD female rats, an increased incidence or earlier appearance of mammary tumors was associated with atrazine dosing at levels near the MTD (Table 26.1). This finding was first discovered in a 24-month study, as significant increases in mammary adenocarcinomas (but not fibroadenomas) appeared with 70, 500, and 1000 ppm atrazine (Hauswirth and Wetzel, 1998). Many additional studies were subsequently conducted in the SD rat (Table 26.1). There was no effect on mammary tumor incidence or time to response when equally high doses of atrazine were administered to F-344 female rats, or to male rats of either the F-344 or SD strains (Wetzel *et al.*, 1994). Four studies of orally administered atrazine have also been conducted on three strains of mice: (C57BL/6X3CH/Anf)F_1, (C57BL/6XAKR)F_1, and CD-1. All treatments produced negative results at chronic feeding doses up to 3000 ppm (Innes *et al.*, 1969; Stevens *et al.*, 1998).

Several conclusions were drawn from these rodent study results. First, the increased incidence or earlier onset of mammary tumors occurred only in SD rats, an animal with a normally high spontaneous background incidence of the tumors (44% grand mean in the studies illustrated in Table 26.1). Second, tumor histology showed qualitatively identical pathology in treated and untreated rats. Third, tumor incidence in either control or treated groups never reached

Table 26.1 Mammary tumor incidence rates in five 2-year dosing studies with atrazine in female Sprague-Dawley rats[a]

Tumor type	**Feeding level (ppm)**								
Data source	**0**	**10**	**25**	**50**	**70**	**100**	**400**	**500**	**1000**
Adenocarcinoma									
S-1994, Table 5, #1	11/54	8/52				12/54			13/49
S-1999, Table 2, p.76	15/88	16/69			27/69[b]			27/70[b]	43/89[b]
S-1999, Table 4	17/60				13/59		22/60		
S-1994, Table 5, #3	8/30	4/40		5/40				6/29	
S-1999, Table 6	12/80		18/80	20/78[b]	14/80		27/80[b]		
Overall percent	**20.2**	**17.4**	**22.5**	**21.2**	**26.0**	**22.2**	**35.0**	**33.3**	**40.6**
Fibroadenoma									
	11/54	20/52				14/54			22/49[b]
	29/88	29/69			36/69			39/70	45/89[b]
	39/60				30/59		41/60		
	4/30	6/40		10/40				8/29	
	16/80		25/80	33/78[b]	29/80[b]		25/80[b]		
Overall percent	**31.7**	**34.2**	**31.3**	**36.4**	**45.7**	**25.9**	**47.1**	**47.5**	**48.6**
Mammary tumor									
	22/54	28/52				26/54			35/49
	35/88	40/69			48/69			48/70[b]	65/89[b]
	46/60				34/59		49/60		
	11/30	10/40		13/40				11/29	
	24/80		34/80	44/78	38/80		43/80		
Overall percent	**44.2**	**48.4**	**42.5**	**48.3**	**57.7**	**48.2**	**65.7**	**59.6**	**72.5**

[a] Compiled from Stevens *et al.* 1994 (S-1994), Stevens *et al.*1999 (S-1999).
[b] Significantly different from 0 ppm incidence, p └ 0.05.

100%. A graph of cumulative incidence from one of the studies (Wetzel *et al.*, 1994) is shown in Figure 26.1. The principal effect of treatment in this study was to cause an earlier onset of tumor appearance, which was essentially matched by the control group after 24 months. Note also in Figure 26.1 the absence of tumor response in F-344 female rats, both in the control and treated groups.

Because the treatment-associated tumor responses occurred only in one sex of one strain of a species highly prone to develop these tumors spontaneously, a hypothesis was developed that the treatment-associated results may be related to normally occurring endocrine-mediated events in the SD strain. If normal aging in the SD strain predisposes an animal to mammary tumor development, then high-dose atrazine treatment might modify (i.e., enhance) the *rate* of developing pathology, rather than create a new pathology. This effect would also help explain the negative mammary tumor results in F-344 female rats (Haseman *et al.*, 1990; Wetzel *et al.*, 1994), in male rats, and in male or female mice, which do not have the same high incidence of spontaneous tumors related to endocrine levels.

Tests for Estrogen-Related Activity

In addition to determining if high doses of atrazine may act to modify the appearance of normal, age-associated pathology in female rats, it was also necessary to evaluate the possibility that atrazine might possess significant intrinsic hormonal activity. Rodent mammary tumors are typically hormone-dependent, and the presence of estrogens and/or prolactin has been shown to promote tumor growth (Noble and Cutts, 1959; Cutts and Noble, 1964; Welsch *et al.*, 1970; Welsch, 1985, 1987; Thompson and Ronan, 1987; Russo and Russo, 1996). Further, a number of other chlorinated hydrocarbons, including some active pesticides, are known to stimulate estrogen-mediated responses (Bulger *et al.*, 1979; Ousterhout *et al.*, 1981; Uphouse, 1985; McLachlan and Newbold, 1987).

Early evidence that atrazine is not estrogenic can be found in the negative results from the teratology and reproductive toxicology tests in rats and rabbits (Hauswirth and Wetzel, 1998). However, a large number of specific *in vitro* and *in vivo* tests have now been conducted to assess directly the potential estrogenicity of atrazine, and a summary of findings is shown in Table 26.2. Results from these studies clearly indicate no evidence of estrogenicity. Some weak antiestrogenic effects have been observed, but only at doses approaching the limit of solubility or toxicity.

Lack of triazine estrogenicity was also revealed by histomorphological examination of the mammary gland from the SD rats of a 2-year feeding study at levels of 0, 70, or 400 ppm. Changes were noted that were indicative of early senescence, for example secretory activity with duct ectasia, increased acinar/lobular development, and galactocele formation (Eldridge *et al.*, 1998). An exogenous estrogen would have exaggerated ductal epithelial hyperplasia in the treated groups, but this was not seen to any greater extent in treated animals than in the controls. Furthermore, there were no reproductive tract changes indicative of exogenous estrogen exposure (e.g., epithelial thickening, increased mitotic activity, and increased cornification in the vagina; cystic endometrial hyperplasia and squamous metaplasia of endometrial glands in the uterus; and reduced numbers of or absence of corpora lutea in the ovary). Instead, the results were consistent with a prolonged (i.e., earlier in time) exposure of the 400 ppm animals to *endogenous* estrogens with an equalization of effects over time relative to the 70 ppm and control animals. Equalization would certainly

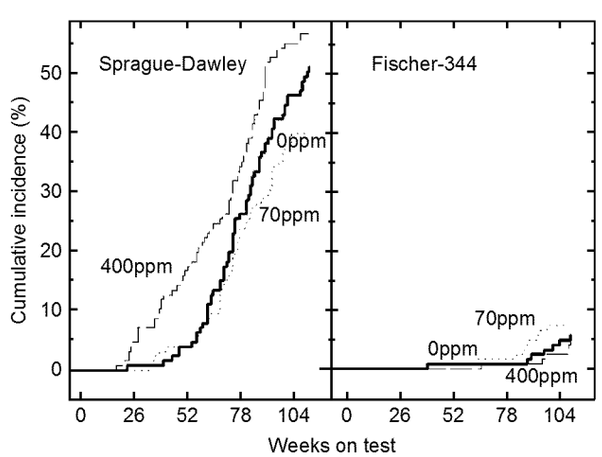

Figure 26.1 Cumulative incidence of mammary tumors in Sprague-Dawley and Fischer-344 female rats fed atrazine in the diet for 104 weeks. (Only palpated masses proven at necropsy to be mammary tumors were included. The incidence plot of 400 ppm in the SD study was significantly different from the 0 ppm plot (p 0.05), but the final incidence percentages were not different (N 60 per dose)).

Table 26.2 Summary from the literature of estrogen-related responses of rats to atrazine exposure

Test	Stimulated[a]	Inhibited	Author
In Vitro			
MCF-7 cells, ER-mediated proliferation	No	No	Connor *et al.* (1996)
MCF-7 cells, ER-mediated proliferation	No	NA[b]	Soto *et al.* (1995)
MCF-7 cells, nuclear DNA-PgR complex	No	No	Connor *et al.* (1996)
MCF-7 cells, ER-mediated genetic expression	No	No	Connor *et al.* (1996)
HeLa cells, ER-mediated genetic expression	No	No	Balaguer *et al.* (1996)
Yeast cells, ER-mediated proliferation	No	No	Connor *et al.* (1996)
Yeast cells, ER-mediated genetic expression	No	Weak[c]	Tran *et al.* (1996)
ER binding:			
rat uterus, *in vitro*		Weak	Tennant *et al.* (1994b)
rat uterus, *in vitro*		No	Danzo (1997)
rat uterus, *ex vivo*		Weak	Tezak *et al.* (1992)
transfected yeast		Weak	Tran *et al.* (1996)
In Vivo			
Rat, OVX[d], mammary tumor growth	No	NA	Stevens *et al.* (1998)
Rat, OVX, uterine weight	No	Weak	Tennant *et al.* (1994a)
Rat, OVX, uterine weight	No	Weak	Connor *et al.* (1996)
Rat, OVX, progesterone receptor expression	No	Weak	Tennant *et al.* (1994a)
Rat, OVX, progesterone receptor expression	No	Weak	Connor *et al.* (1996)
Rat, OVX, uterine thymidine incorporation	No	No	Tennant *et al.* (1994a)
Rat, OVX, uterine peroxidase reaction	No	Weak	Connor *et al.* (1996)
Rat, intact, reproductive tract histology	No	NA	Eldridge *et al.* (1998)

[a]No indicates a negative response to atrazine was observed, even at doses that were ┐ 10^6 times the doses of estradiol that induced a response.
[b]NA indicates that the assessment was not done.
[c]Weak indicates a response to atrazine at doses ┐ 10^5 times the effective dose of estradiol.
[d]OVX: Ovariectomized.

not have occurred with an exogenous estrogen source. Additional related evidence for the lack of estrogenicity of atrazine was noted in another 2-year feeding study in SD rats that resulted in no mammary tumors in ovariectomized animals treated with feeding levels │ 400 ppm (Stevens *et al.*, 1998).

Direct tests of triazines in the standard ovariectomized rat model have repeatedly failed to show any sign of estrogenicity, using such established estrogen-responsive parameters as progesterone receptor expression, tissue peroxidase, thymidine incorporation into DNA, and uterine weight (Figure 26.2). Some of the tests revealed a weak inhibition by atrazine, when administered along with estrogen to ovariectomized rats. *In vitro* tests of estrogenicity using cultured mammary tumor cells or yeast cells also failed to demonstrate any estrogen-like response to atrazine, even when the cells were transvected with known molecular markers that responded easily to natural estrogen. Finally, only when extremely high concentrations were used did atrazine manifest any interacting with the estrogen receptor (Table 26.2).

Therefore, when it became clearly understood that atrazine is neither estrogenic nor a genotoxic, direct-acting carcinogen – and that the atrazine-associated tumor responses appeared only in female SD rats, a strain with a high, normally occurring incidence of mammary tumors – it became important to study the effect of high doses of atrazine on the SD animal model's own endocrine system and hormonal milieu.

It is well-established that control of the SD female estrous cycle begins to decline at a relatively young age (Meites *et al.*, 1977; Finch, 1978), and it does so in a manner that leads to episodes of persistent estrus and to prolonged secretion of estrogen from unovulated ovarian follicles. The hypothesis was that if atrazine treatment in the SD rat were able to disrupt estrous cycling earlier than normal (but in the same direction as the spontaneously occurring disruption), a mode of action linking atrazine treatment to earlier mammary tumor growth in the female SD rat could be established.

Effects on Estrous Cycling

In women, the passage from normal menstrual cycles into reproductive senescence results from exhaustion of ovarian follicles, with a concomitant decline in estrogen secretion (Nicosia, 1986; Carr, 1992). After menopause, the hypothalamus retains the capacity to regulate anterior pituitary hormone secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and the mechanism for transduction of the estrogen signal to gonadotropin feedback control appears to be intact. Serum levels of LH and FSH increase markedly and can respond to negative feedback

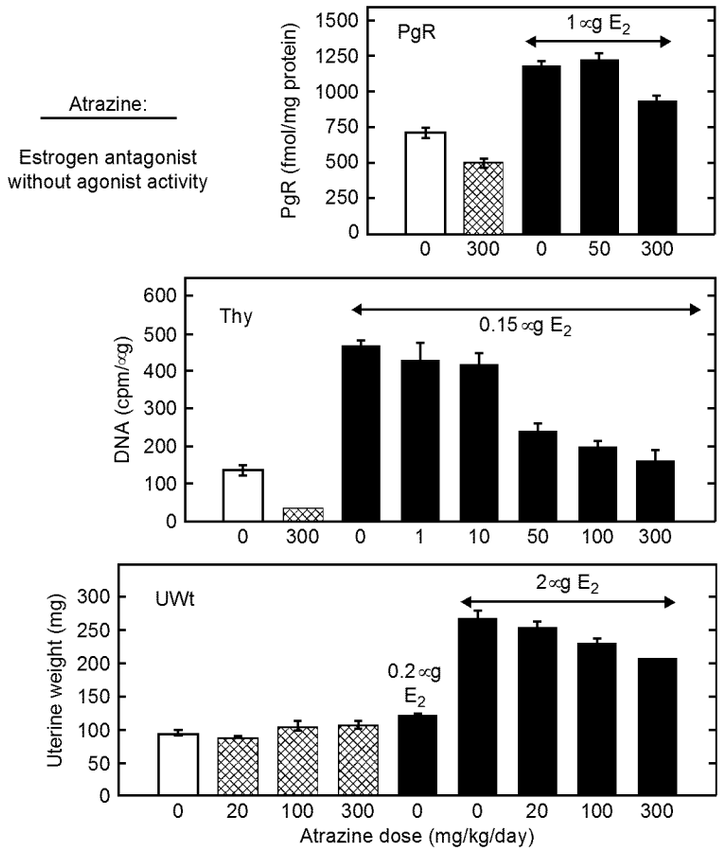

Figure 26.2 Lack of estrogenic activity in rat uterus by atrazine in three *in vivo* tests. (Solid bars represent means − S.E. for OVX rats treated with estradiol s.c. and atrazine by gavage (doses, in mg/kg/day on abscissa). Cross-hatched bars are responses in rats administered atrazine without estradiol. Top panel shows progesterone receptor expression. Middle panel shows incorporation of thymidine into uterine DNA. Bottom panel shows wet uterine weight. See Tennant *et al.* (1994a) for additional details.)

effects of hormone replacement therapy (Nicosia, 1986). Appropriate estrogen treatment can induce LH surges in postmenopausal women (Tanaka and Katayama, 1982).

By contrast, in some rodent strains (including SD rats), the ovary retains a substantial population of primordial follicles (Meites *et al.*, 1977; Simpkins, 1983). Reproductive senescence in these animals appears to result from a decline in the capacity of the hypothalamus to convert the estrogen priming signal into an LH surge sufficient to induce ovulation (Meites *et al.*, 1977; Wise, 1982, 1984; Simpkins, 1983). The normal 4- to 5-day estrous cycle begins to lengthen due to the unovulated follicles, finally reaching a state of continuous estrus (Huang *et al.*, 1978; Lu *et al.*, 1979) that can continue for the remainder of the animal's life. This state is characterized by a moderate but continuous secretion of estrogens, low serum progesterone, and cornified vaginal epithelium (Lu *et al.*, 1979). Serum prolactin is also elevated as a result of estrogen stimulation of pituitary prolactin synthesis and secretion (Maurer, 1982).

The F-344 rat exhibits a very different reproductive pattern in senescence. Through the first 1.5 years of age, the majority of animals maintain normal 4- to 5-day estrous cycles (Estes and Simpkins, 1984). Later, the strain displays normal cycles interspersed with periods of extended maintenance of ovarian corpora lutea and a hypersecretion of progesterone (Lu *et al.*, 1979; Estes and Simpkins, 1984). This condition is appropriately called repeated pseudopregnancy. Serum LH concentrations are only slightly reduced (Estes and Simpkins, 1984). More importantly, the hypothalamic–pituitary axis maintains the capacity to mediate the estrogen-induced hypersecretion of LH and the normal ovulation that is common in aged F-344 rats (Lu *et al.*, 1980; Estes *et al.*, 1982b; Estes and Simpkins, 1984). The only recognized neuroendocrine deficit in F-344 rats is the inability to reduce the episodic diurnal and nocturnal prolactin surges (Estes and Simpkins, 1982, 1984; Estes *et al.*, 1982) that maintain the corpora lutea during pseudopregnancy episodes (Beach *et al.*, 1975).

Table 26.3 Distribution of abnormal estrous cycle episodes in Sprague-Dawley female rats treated with atrazine[a]

Data from:	Interval (weeks)	0 mg/kg 0 ppm	2.5 mg/kg 25 ppm	5 mg/kg 50 ppm	40 mg/kg 400 ppm	200 mg/kg
Number of animals with normal estrous cycles	***2–4[a]***	***67***	***67***	***65***	***50***	***33***
	1–2	64	71	69	50	
	5–6	72	80	75	78	
	9–10	81	80	82	74	
	13–14	76	73	69	48	
	17–18	64	64	60	45	
	21–22	56	50	49	29	
	25–26	41	38	32	16	
No. of animals with a diestrous block • 8 days	***2–4[a]***	***9***	***8***	***7***	***16***	***30***
	1–2	6	6	3	16	
	5–6	4	1	3	3	
	9–10	2	1	0	4	
	13–14	0	4	2	2	
	17–18	2	3	1	5	
	21–22	7	4	3	9	
	25–26	5	9	5	6	
No of animals with an estrous block • 7 days	***2–4[a]***	***9***	***8***	***7***	***18***	***30***
	1–2	0	0	0	0	
	5–6	0	0	1	1	
	9–10	1	0	1	2	
	13–14	10	5	5	20	
	17–18	13	12	12	27	
	21–22	13	21	26	34	
	25–26	26	32	34	50	

[a] ***Data from the 4-week gavage study are underlined and in bold-face italic font.*** Other data are from the 26-week feeding study.
Number ⌋ animals/treatment group.

Because rodent mammary tumors are promoted by estrogen, as discussed earlier, it was hypothesized that a principal reason for the spontaneous, naturally occurring mammary tumors in SD senescent rats was the exposure to prolonged episodes of their own estrogen secretion, while the failure of tumor responses in aged F-344 rats is due to low spontaneous estrogen levels (Figure 26.1). The hypothesis was then extended to include an atrazine mode of action that leads to advanced prolonged estrus episodes in treated SD rats, thus promoting the same normally occurring mammary tumors that result from endogenous estrogens generated by the animals' own ovarian follicles.

To test these hypotheses, two studies were conducted in young SD female rats administered atrazine – one 4-week study using daily gavage, and one 26-week study using dietary feeding (*cf.* Eldridge *et al.*, 1999a). Results of vaginal cytology monitoring are shown in Table 26.3. In the 4-week gavage study, significant effects were observed with doses of 40 and 200 mg/kg, but not at 2.5 and 5 mg/kg. The number of animals displaying normal estrous cycles fell, and most abnormalities appeared as extended periods of diestrus. This result was consistent with earlier reports from Cooper and coworkers, who examined early estrous cycling responses in SD and Long-Evans rat strains administered 300 mg/kg atrazine (Cooper *et al.*, 1996).

When atrazine was administered in the diet, the early diestrous response initially appeared, again at 400 ppm (Table 26.3). However, after 5–6 weeks, these diestrous episodes ceased, and by 13–14 weeks, estrus, not diestrus, was the predominant estrous cycle abnormality. As before, the response was significantly evident only for the animal group fed 400 ppm (40 mg/kg/day). Also important to note is that during the 26-week sampling period, the degree of estrous cycle abnormality increased spontaneously in the control groups, and also as episodes of repeated estrus.

In a separate 6-month study, female SD rats were fed atrazine in their diet, and estrous cycling patterns were examined. In each 2-week block of examination, results were expressed as the percentage of days in estrus, diestrus, or proestrus. An estrus designation indicated that high blood–estrogen levels had occurred. Results (Figure 26.3) showed that all dose groups, including the controls, increased their percentage of days in estrus, beginning at 13–14 weeks, and that the effect of a 400 ppm level was significantly greater than that of 0, 25, 50, or 70 ppm.

These results supported the hypothesis in that: (1) SD female rats developed persistent estrous episodes spontaneously, signaling prolonged endogenous estrogen exposure, and (2) animals exposed to 400 ppm atrazine displayed an even greater degree of the same abnormal estrous cycle pattern as the controls, and they did so earlier in the study.

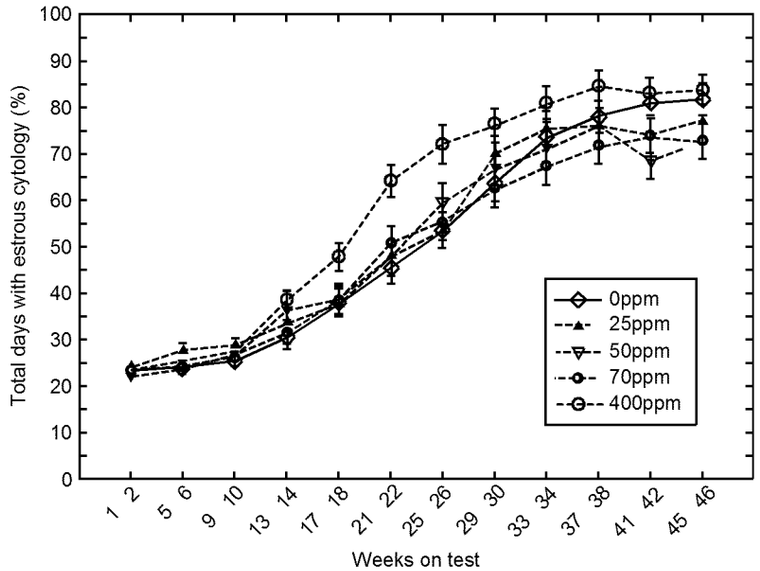

Figure 26.3 Percent of total days with estrous cytology in SD female rats fed atrazine in the diet. (Vaginal cytology was monitored daily for 14-day intervals, followed by 14 days of rest. Estrous cytology was defined by the presence of a majority of keratinized (cornified) cells in the lavage. Each point represents the mean ± S.E. of 60 animals per dose group. The mean responses to 400 ppm at 13–14, 17–18, 21–22, and 25–26 weeks were significantly different from controls (ANOVA, $p < .05$); other mean values were not significantly different.)

This is clear evidence that high-dose atrazine exposure caused female SD rats to have more endogenous estrogen, which alone could support an earlier onset of mammary tumors in this strain of rats.

Atrazine Effect on the Pituitary LH Surge

In female SD rats, regular ovulation is controlled by secretion of pituitary FSH and LH, culminating in a surge of LH on the afternoon of proestrus, inducing ovulation 12 h later. With advancing age in the female SD rat, LH surge magnitude is reduced, leading to irregular estrous cycling patterns from ovulatory failure (Meites *et al.*, 1977; Everett and Tyrey, 1982; Wise, 1982, 1984; Simpkins, 1983). The finding of a greater incidence of irregular estrous cycling among animals treated with atrazine led to a hypothesis that high-level atrazine treatment in female SD rats could block or reduce LH surges. This was tested by administration of atrazine to ovariectomized SD rats primed with estrogen. Some of the data presented below were previously published (Eldridge, *et al.*, 1999b).

Short-term effects of atrazine on the LH surge were evaluated in female rats that were ovariectomized and simultaneously implanted with a sustained-release capsule containing estradiol in oil (4 mg/mL). This produced estrogen levels comparable to those seen during normal preovulatory surges of LH and those that also produce daily surges of LH in young rats (Beach *et al.*, 1975; Wise, 1984). Results showed that 300 mg/kg atrazine administered orally for 3 days (Figure 26.4), or 40 mg/kg and 200 mg/kg atrazine administered orally for 28 days (Figure 26.5), significantly suppressed the expected LH surge. Because aging SD rats display a spontaneous reduction of LH surges (Beach *et al.*, 1975), this effect of atrazine is consistent with the hypothesis that the triazine can reduce the age at which persistent estrus occurs in the SD rat.

To study the effect of chronic dietary exposure to atrazine on the quality of LH surges, groups of 10 SD female rats were placed on an ad libitum diet containing 0, 25, 50, or 400 ppm atrazine. After 6 months of feeding, all animals were ovariectomized and implanted with an estrogen-containing silastic capsule. On the fourth day following the estrogen capsule implantation, each animal was bled from the jugular vein at 2-h intervals. LH was measured and results were plotted with each animal's peak value placed at time 0.

Figure 26.6 shows that the animals fed 400 ppm atrazine for 6 months had a significantly diminished LH peak, compared to the 0 ppm control group. There was no effect of atrazine treatment at feeding levels of 25 or 50 ppm. Thus, exposure of young SD female rats to atrazine at the same long-term feeding level (400 ppm) that was observed previously to enhance mammary tumor formation also significantly diminished the surge of LH that is necessary for ovulation. Furthermore, treatment with 25 and 50 ppm, which was not previously associated with mammary cancer in rats, also did not suppress the LH surge.

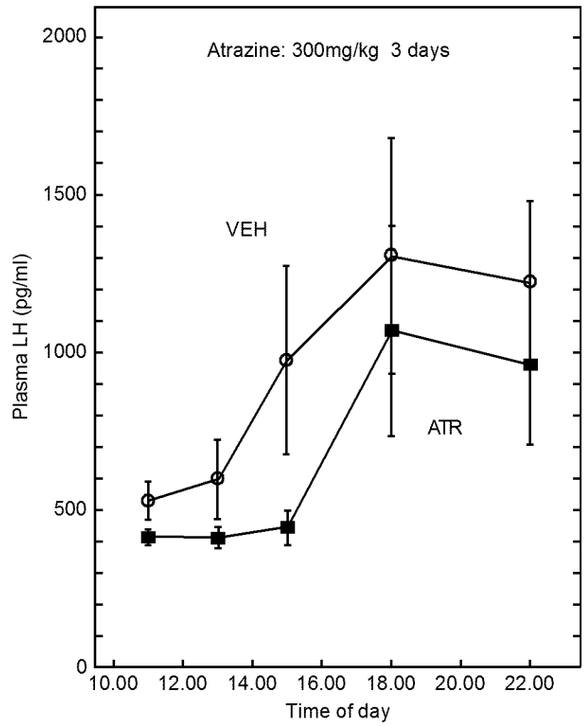

Figure 26.4 LH surges in SD rats administered atrazine for 3 days and then ovariectomized and implanted with a silastic capsule containing estradiol. (Points represent means ± S.E. of 10 animals sampled from the jugular vein at the indicated times, 3 days after capsule implantation.)

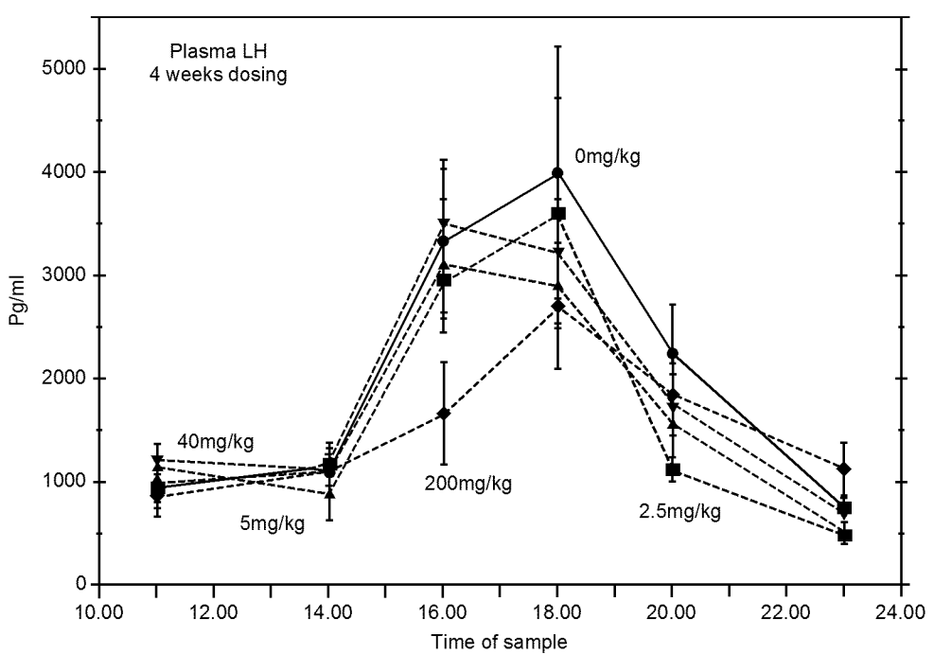

Figure 26.5 LH surges in SD rats administered atrazine for 28 days and then ovariectomized and implanted with a silastic capsule containing estradiol. (Points represent means ± S.E. of 15 animals sacrificed at each time interval, for each dose, 3 days after capsule implantation.)

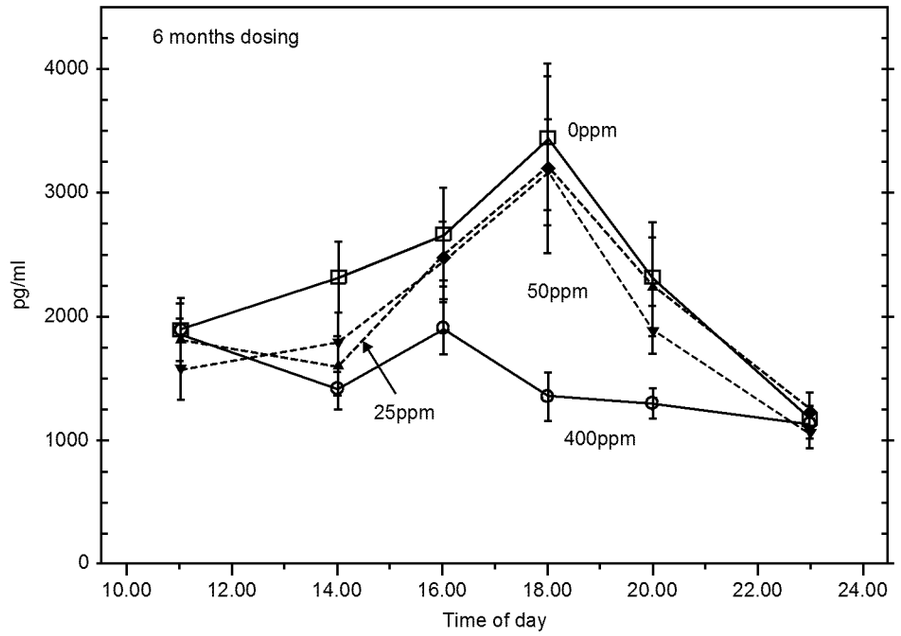

Figure 26.6 LH surges in SD rats administered atrazine for 26 weeks and then ovariectomized and implanted with a silastic capsule containing estradiol. (Points represent means ± S.E. of 15 animals sacrificed at each time interval, for each dose, 3 days after capsule implantation.)

The results showing suppression of LH surges in certain strains of rat have been confirmed by studies of Cooper *et al.* (2000) in intact cycling rats and McMullin *et al.* (2004) in ovariectomized rats treated with estrogen plus progesterone. Additional studies have reported that atrazine administration to immature male rats (Stoker *et al.*, 2000) or female (Laws *et al.*, 2000; Ashby *et al.*, 2002; Rayner *et al.*, 2004) delayed the onset of puberty, again due to a suppression of LH secretion. Trentacoste *et al.* (2001) and Friedmann (2002) have reported that chronic administration of atrazine suppressed LH secretion in peripubertal male rats. As was observed with our own studies, all of these reports described effects occurring only at very high levels of dosing (i.e., in excess of the MTD at 40 mg/kg).

Correlation of Estrous Cycling Patterns and Mammary Tumors

As reported previously, the dose-related effect of chronic atrazine treatment on the control of estrous cycling in female SD rats occurs principally in the first 6 months on test, yet mammary tumors typically appear during the second year. Estrous cycling records during the first year of a study also were evaluated as the test animals were maintained and examined for tumor development over 2 years. There was a search for certain estrous cycling patterns that might predict mammary tumor outcomes. Senescent female SD rats develop two types of mammary tumors, generally designated as adenocarcinoma and fibroadenoma. Some develop both types; many develop only one (or none).

Sielken *et al.* (2005) have conducted a statistical analysis of factors that might predict tumor development in the female SD rat treated with atrazine. They found that the number of days in estrus (i.e., a surrogate for endogenous estrogen exposure) was a far better predictor of the occurrence of adenocarcinomas than was the atrazine dose. Likewise, the development of galactoceles and the finding of mammary secretory activity (surrogate for endogenous prolactin exposure) was a better predictor of fibroadenoma development than was the atrazine dose. These results are consistent with an interpretation that atrazine alters the estrous cycle of susceptible female SD rats and thereby enhances endogenous estrogen exposure leading to adenocarcinoma development. Estrogenic stimulation may also promote pituitary release of prolactin, as evidenced by the formation of galactoceles, which would concurrently enhance the development of fibroadenomas in the mammary gland.

Conclusions

After several years of research on the development of mammary tumors in aging Sprague-Dawley rats administered atrazine, the results clearly point to a strain-related mode of action. The SD rat has a strain-related peculiar predisposition to

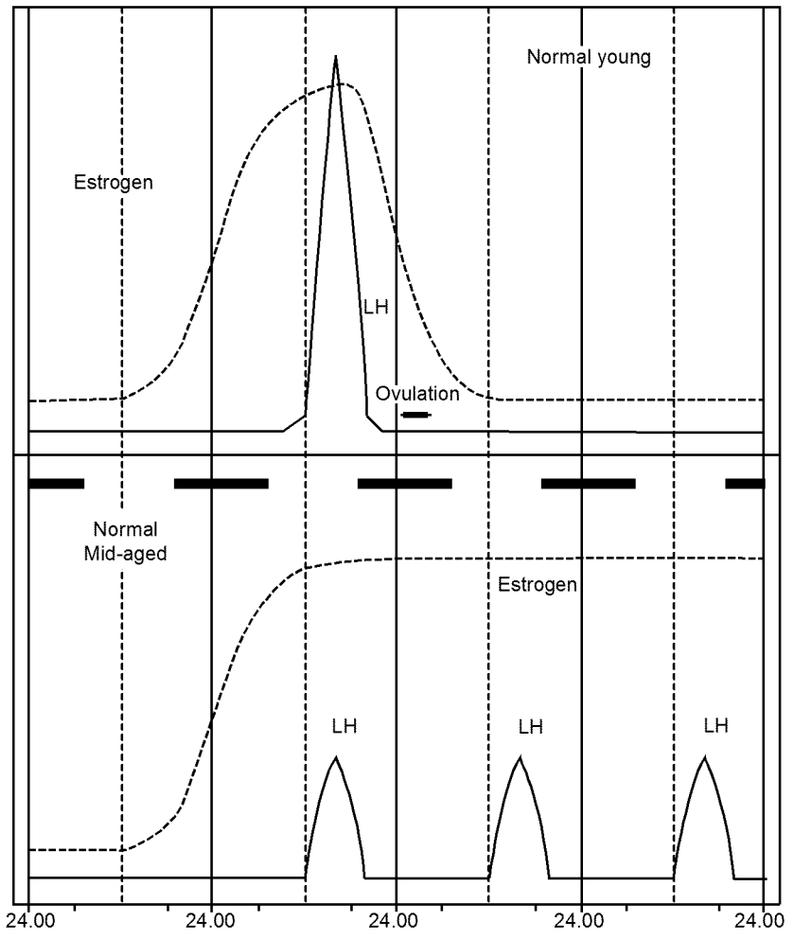

Figure 26.7 Schematic comparison of LH surges and endogenous estrogen levels in young and middle-aged SD female rats. (In young animals, rising estrogen from ovarian follicles triggers a massive LH surge once every 4th or 5th day. In middle-age, neuroendocrine defiits result in an LH surge insufficient for ovulation. Thus, estrogen secretion is maintained from unovulated follicles, as suboptimal surges are repeated.)

premature hypothalamic senescence that reduces its capacity to generate surges of pituitary LH. Failure of sufficient LH secretion during reproductive proestrus leaves these animals in an anovulatory state, with persistent secretion of ovarian estrogens (Figure 26.7). These studies have demonstrated that the process begins at under 6 months of age (Figure 26.3). Because mammary tumors are easily promoted by elevated estrogen levels, the tumors are an expected outcome in the second year of life (controls in Figure 26.1 and Table 26.1). Failure of F-344 females or of SD male rats to develop mammary tumors from the same mechanism is predictable since these animals have low-estrogen levels throughout life.

High-dose exposure to atrazine (usually in excess of the MTD) suppresses the capacity of female SD rats to mount an LH surge, acutely and chronically (Figures 26.4 to 26.6). This treatment-related effect produces the same outcome as normal aging in the SD rat, that is the animals cannot ovulate regularly, estrogen levels persist, and mammary tumor growth is promoted. The estrous cycling effect of atrazine is not immediate, but it does appear sooner than the normal senescence-related change. Thus the tumor response appears to be shifted earlier in time, but to the same degree and type as control animals or animals treated with ineffective doses of atrazine. In essence, atrazine has advanced a result of aging *in an animal model that is so disposed.* In a different animal model (e.g., the F–344 female rat) that maintains strong LH surge capacity into old age, atrazine is without effect, even at doses in excess of the MTD.

These responses in SD rats are not relevant to mechanisms of menopause in humans. Reproductive senescence in women is a low-estrogen environment, as it is in F-344 rats. Although not completely similar to the human in its pattern of reproductive senescence, the F-344 female rat does share with the human female the following features: both have a late-life reproductive senescence, both experience low-estrogen levels during late life, and both retain the ability

to control LH secretion during reproductive senescence. As such, the F-344 rat more closely models the human female than does the SD rat, and the SD strain would appear to be a poor surrogate model for reproductive senescence in the human female. Atrazine did not cause either an earlier onset or increase in mammary tumors in the F-344 rat.

Comprehensive mode of action studies support the conclusion that atrazine's effects on mammary tumors in the female SD rat are not relevant to humans. Furthermore, the chronic treatment no-effect levels determined in studies of the estrous cycle and mammary tumor response in the female SD rat are thousands of times higher than potential human exposure levels.

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Chapter 27

Dietary Exposure Assessment of the Triazine Herbicides

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Summary

Since implementation of the Food Quality Protection Act (FQPA) by the United States Congress in 1996, combining exposures to pesticides via food, water, and residential uses has been a primary focus of the United States Environmental Protection Agency (USEPA). Dietary exposure to the triazine herbicides is measured as the triazine residue level in or on a given food commodity, multiplied by the amount of that commodity consumed. The resulting exposure value is then expressed relative to a toxicity endpoint (usually a no-effect level) in the most sensitive mammal species tested. An assessment of dietary exposure is performed using a tiered process, starting with a Tier I conservative, unrealistic worst-case estimate and followed by a series of refinements to provide the most realistic risk assessment possible. In general, major refinements have not been necessary for the triazine class of chemicals since dietary exposure to the triazines has been determined to be minimal, even with conservative, upper-bound estimates. In this discussion, an overview of the exposure assessment process is presented and exposure values (Tier I and refined) for several representative triazine herbicides are compared. In addition, an overview of the various state and federal monitoring programs is presented, as well as the actual occurrence of triazine residues in various stages of food commerce.

The principles of dietary risk assessment have been described as a tiered approach. Tier I assessments were provided for five major domestic triazines using conservative tolerance values and a 100% crop treated assumption. Subsequent refinements were described for atrazine and simazine by using appropriate field trial data, realistic animal commodity residues, and estimated percentage crop treated (Tier III). Significant exposure reductions were demonstrated for atrazine and simazine in progressing from a conservative and unrealistic Tier I estimate to a more refined Tier III assessment. Exposure reductions can also be shown for all other triazines using Tier III estimates. In general, the triazine chemical class demonstrates a wide margin of safety with minimal refinement. Sources of triazine monitoring data from government and other surveys have been described, and results confirm the lack of detections in food at various points in United States commerce. The low exposure to triazines reflected by conservative estimates and the lack of detections in various monitoring programs demonstrate the safety of the triazine herbicides. From these results it can be concluded that the triazine class poses no dietary health risk to the general population or to sensitive subpopulations.

Background

Worldwide there have been a total of 20 commercialized triazine herbicides. Of the 20 triazines, 7 are currently registered for land use within the United States: ametryn, atrazine, metribuzin, prometryn, simazine, terbutryn, and prometon. For purposes of this discussion, only dietary estimates for the 5 most widely used domestic triazines are presented; since the USEPA revoked cyanazine tolerances in 2004, prometon is not used for food crops and terbutryn has very limited use. Additionally, propazine was used under USEPA Section 18 registrations in the 1990s, and in 2007 was registered for weed control in sorghum (USEPA, 2007).

Dietary exposure to pesticides (or to xenobiotics in general) is determined by calculating the product of the amount of chemical in or on the food and the total quantity of food consumed. The quantity of chemical potentially consumed in foods can be estimated from data obtained from residue field trials, metabolism studies, and/or monitoring data. Information from these sources is then analyzed with one of several available models containing food consumption factors from surveys conducted by the United States Department of Agriculture (USDA). For calculation of

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dietary exposure and corresponding risk, three USEPA acceptable food consumption surveys have been conducted to date: the Nationwide Food Consumption Survey (NFCS) conducted from 1977 to 1978; the Continuing Survey of Food Intake by Individuals (CSFII) conducted from 1989 to 1991; and again from 1994 to 1996. In addition, a supplemental CSFII survey was conducted in 1998 in which the nutritional intakes of approximately 5300 children younger than 10 years of age were recorded to facilitate better exposure estimates for children. The USDA collected demographically representative food intake information over a period of time to reflect temporal variations in intake by the United States population. Respondents were surveyed for either 3 days (NFCS and CSFII, 1989–1992) or for 2 nonconsecutive days (CSFII, 1994–1996, in addition to the Children's Supplemental Survey in 1998) and provided extensive details – including the type, brand, and quantity of food consumed at each eating occasion. The actual number of respondents ranged from about 16 000 (1994–1996) to 30 000 (1977–1978), corresponding to about 32 000 to 90 000 days of food consumption information, respectively, depending on the survey. This food consumption information was in turn used to derive the average actual amounts of raw agricultural commodities consumed per person for the overall United States population. It was also used to derive information by population subgroups, delineated by season, geographical region, sex, age, and ethnic background.

In the eighties and early nineties, the USEPA evaluated dietary risk with an analysis method known as the Dietary Risk Evaluation System (DRES) (USEPA, 1991), which was based on the USDA's 1977 to 1978 National Food Consumption Survey. Consequently, dietary exposure assessments became generically referred to as 'DRES analyses.' Currently, the USEPA is using the Dietary Exposure Evaluation Model (DEEM™, Version 7.87) (Exponent, 2000), which allows exposure to be calculated from 1994 to 1996 CSFII along with the 1998 supplemental children's survey information.

All of the previously mentioned exposure methods can be used to estimate either chronic exposure (over a period of years) or acute exposure (single day) for the United States population and population subgroups. Both chronic and acute assessments are usually based on a no observed adverse effect level (NOAEL) in an animal species. Acute exposure is defined relative to an acute (single dose) toxicological endpoint (usually a NOAEL) and may be expressed as a margin of exposure (MOE) or as a percentage of an acute reference dose that is based on a NOAEL and an uncertainty factor (see below).

Chronic dietary risk can be expressed as a percent of the chronic reference dose (%cRfD) or as a MOE. The reference dose (RfD) is defined as the NOAEL (usually obtained from a long-term feeding study with rodents or dogs) divided by an uncertainty factor. Using atrazine as an example, the cRfD is 0.0018 mg/kg body weight/day, which is based on a NOAEL of 1.8 mg/kg body weight/day from a 6-month luteinizing hormone (LH) surge study in the rat and a 1000-fold uncertainty factor. The 1000-fold uncertainty factor includes a 100-fold safety factor for intra- and inter-species variations, plus an additional 10-fold FQPA safety factor.

Due to the high doses necessary for acute effects as observed in short-term toxicity tests and to the lack of effects seen at earlier time-points in long-term studies, only chronic reference doses are used in conjunction with exposure for the calculation of triazine dietary risk. Therefore, the remainder of this discussion is limited to chronic exposure and risk.

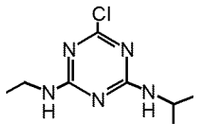
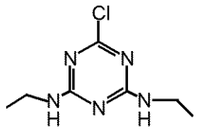
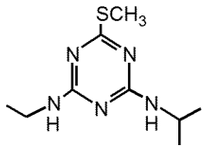
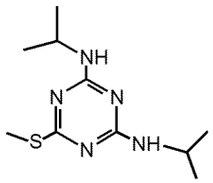
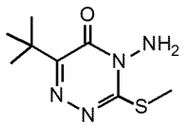
The Risk Assessment Process

A majority of the triazine herbicides with food uses are grouped into three chemical classes: chloro-*s*-triazines, methylthio-*s*-triazines, and asymmetric triazines. A fourth chemical class, methoxy-*s*-triazines, has been commercialized, but no food crop uses are registered within the United States. The NOAEL and corresponding reference dose for some of the chloro-*s*-triazines, methylthio-*s*-triazines, and an asymmetric triazine are presented in Table 27.1.

Definition of the moieties of toxicological concern is the first step in conducting a dietary exposure assessment for any chemical. The residues of concern for the chloro-*s*-triazines include the sum of the parent triazine and the dealkylated metabolites bearing the unchanged chloro-moiety. All chloro-*s*-triazines, including the corresponding dealkylated chloro-metabolites, are considered to be of equal importance with respect to toxicological effects (residues of concern). Similarly, for methylthio-*s*-triazines, parent plus dealkylated metabolites bearing the methylthio group are summed for each raw agricultural commodity; this in turn is entered into the exposure assessment. Metribuzin is in the asymmetric triazine class and bears a methylthio substituent. The residues of concern for metribuzin are the parent plus three triazinone (keto) metabolites.

A maximum allowable legal limit of triazine residues, or tolerance, is established for the residues of concern. Tolerances became mandatory with the passage of the Federal Food, Drug, and Cosmetic Act (1958). Tolerances are established from field trial data generated by using the maximum label rate and minimum preharvest interval on each commodity for which the triazine is registered. The highest residue quantity obtained from these field trials is used as the basis for establishing the tolerance. Tolerance values are used in a very conservative, less refined estimate of residue levels in the risk assessment process.

Table 27.1 Triazine structures, toxicological endpoints and safety factors

Triazine	Structure	NOAEL (mg/kg/day)	Safety factor	RfD (mg/kg/day)
Chloro-*s*-triazines				
Atrazine		1.8	1000	0.0018[a]
Simazine		1.8	300	0.006[b]
Methylthio-*s*-triazines				
Ametryn		7.2	100	0.072[c]
Prometryn		3.75	100	0.04[d]
Asymmetric triazines				
Metribuzin		1.3	100	0.013[e]

[a] USEPA Atrazine Interim Reregistration Eligibility Decision (IRED), January 2003.
[b] USEPA Simazine Reregistration Eligibility Decision (RED), April 2006.
[c] USEPA Ametryn Reregistration Eligibility Decision (RED), September 2005.
[d] USEPA Prometryn Reregistration Eligibility Decision (RED), February 1996.
[e] USEPA Metribuzin Reregistration Eligibility Decision (RED), February 1998.

In evaluating dietary exposure, the USEPA takes a tiered approach by first making a conservative, screening-level, worst-case estimate (Tier I). If the resulting data warrants, subsequent refinements are made to obtain more realistic exposure estimates (Tier II–IV). The tiered approach for assessing chronic dietary exposure is presented below.

Tolerance levels with 100% crop treated assumptions (Tier I)
↓
Tolerance levels with actual percent of crop treated assumptions (Tier II)
↓
Anticipated (average) field trial residue levels with actual percent of crop treated assumptions, average processing factors from processing studies, or average residues from monitoring data (percent of crop treated inherent) (Tier III)
↓
Average residue levels obtained from special studies (grocery store collection, cooking studies) (Tier IV)

Table 27.2 Tolerance level dietary exposure assessments (Tier I) for the most widely used triazines[a]

Triazine	Chronic exposure (mg/kg body weight/day)	Tier I (percent RfD)
Atrazine		
United States population, 48 states	0.00113	62.72
Simazine		
United States population, 48 states	0.00212	35.27
Ametryn		
United States population, 48 states	0.00068	0.94
Prometryn		
United States population, 48 states	0.00051	1.27
Metribuzin[b]		
United States population, 48 states	0.00738	56.79

[a] Tier I assessments are screening level assessments assuming tolerance levels, 100% of crops treated, and unrealistic use scenarios, see Tables 27.3 and 27.4 for refned assessments.
[b] USEPA's estimate in the Metribuzin Eligibility Reregistration Document (February 1998) was 36% for the United States population.

Tier I

The worst-case estimate (Tier I) utilizes tolerance values for each raw agricultural commodity, including animal-based foods (meat, milk, poultry, and eggs). The tolerance values for all applicable commodities are published in the USEPA's *Code of Federal Regulations.* A Tier I assessment also includes the assumption that 100% of the United States crops planted for a particular food are treated with the triazine in question. Multiplying the tolerance value by the corresponding food consumption factor (obtained from food survey information) yields the maximum theoretical exposure. Tier I exposure assessments for the five most widely used domestic triazines are shown in Table 27.2. These Tier I assessments were made using methodology provided using the DEEM software by Exponent and USDA's 1994–1996 CSFII with the 1998 Supplemental Children's Survey database.

Exposures are calculated for numerous population subgroups delineated by geography, ethnic origin, and age. In general, non-nursing infants (ffi1 year) and children 1- to 6-years old have the highest theoretical dietary exposures as compared to the overall United States population. Often these two groups are estimated to be the most sensitive population subgroups in any dietary exposure assessment due to their higher consumption of food relative to body size. This population sensitivity is fairly consistent within all triazine exposure assessments.

Tier I exposure assessments represent an unrealistic, worst-case scenario since maximum use rates and minimum preharvest intervals are rarely used in normal agricultural practice. In addition, all triazine herbicides are not used on 100% of all planted acres. Further refinements lead to a more realistic assessment and to a significant reduction in the exposure estimate.

Tier II

Tier I assessments can be refined by adjusting for the actual percent of crop treated. The USEPA considers the percent of crop treated of tolerance residue levels a Tier II exposure assessment. This adjustment is made by mathematically decreasing the tolerance value by a fraction equivalent to the percent of crop treated in the United States. Although the percent of crop treated adjustment can reduce exposure dramatically if the triazine is used only on a small percent of the total crops planted, the use of tolerance values still provides a very conservative exposure estimate. In a Tier II assessment, livestock and poultry feed tolerances can be used to estimate the maximum amount of triazine-treated feed ingested by livestock and poultry and to predict the transfer of residues to edible animal products.

Tier III

Dietary exposure calculations can be further refined by using more realistic field trial, metabolism, or monitoring data to generate Tier III assessments. The refinements in chronic Tier III assessments include the utilization of average field trial residues and adjustment of these residues with the percent of crop treated for commodities ingested by humans. If the triazine is registered on a livestock or poultry feed item, the potential exists for transfer of triazine residues from the feed to ingested animal commodities. Tier III assessments include calculations that result in an estimate of these potentially transferred residues. In dietary risk assessments, nondetected residues are assigned a value corresponding to the limit of quantitation (or ½ LOQ) instead of zero, and therefore the lower bounded exposure estimate

Table 27.3 Estimated dietary exposure to atrazine and corresponding chloro-metabolite residues (Tier I versus Tier III)[a]

Population subgroup	Percent RfD (RfD ffi 0.0018 mg/kg/day)	
	Tier I	Tier III
United States population, 48 states, all seasons	62.72	0.22
Non-nursing infants (ffi1 year old)	99.17	0.44
Children (1 to 6 years)	159.67	0.72

[a] Although the methodology provides risk characterizations for many population subgroups, the comparison between Tier I and III assessments was made for only the most sensitive subpopulations.

Table 27.4 Estimated dietary exposure to simazine and corresponding chloro-metabolite residues (Tier I versus Tier III)[a]

Population subgroup	Percent RfD (RfD ffi 0.006 mg/kg/day)	
	Tier I	Tier III
United States population, 48 states, all seasons	35.27	0.03
Non-nursing infants (ffi1-year old)	80.3	0.06
Children (1 to 6 years)	110.12	0.09

[a] Although the methodology provides risk characterizations for many population subgroups, the comparison between Tier I and III assessments was made for only the most sensitive subpopulations.

from Tier III/IV analyses are determined by the sensitivity of the analytical method, rather than by the levels of actual detected residues. The two examples for atrazine (Table 27.3) and simazine (Table 27.4) show the significance of using average field trial residues, realistic estimates of residues in animals, and data for actual percent of crop treated to assess exposure. A significant reduction in the theoretical exposure to atrazine is achieved after application of Tier III approaches, as seen in Table 27.3.

In the case of atrazine, the most widely used triazine, refinement of the conservative Tier I estimate for all populations results in at least a 200-fold reduction in exposure and risk. In addition, the total exposure and risk for atrazine using a Tier III approach is approximately 138 000-fold to 450 000-fold less than the chronic NOAEL obtained from a rodent study. A 600-fold to 1600-fold difference exists between the NOAEL and maximum theoretical exposure and risk when tolerance values are utilized. Further refinement of the estimate using Tier IV methodology would result in even lower exposure and risk, since this Tier III analysis used data generated from structured field trials (maximum label rate and minimum preharvest interval).

The most recent USEPA dietary assessment for atrazine used 1.8 mg/kg (chronic NOAEL from a 6-month rat study) with a 1000-fold safety factor (cRfD ffl 0.0018 mg/kg/day). This analysis also confirmed that potential dietary exposure for all exposed population subgroups was less than 1% of the cRfD (USEPA, 2003).

Another example of exposure and corresponding risk reduction using Tier III methodology is provided in Table 27.4 for simazine (the triazine with the most registered uses in the United States). The Tier III analysis – using average field trial residues, realistic residue estimates in animal commodities, and percent of crop treated – showed at least a 1000-fold reduction in exposure for the United States population and for the most sensitive population subgroups as compared to the tolerance-based Tier I assessment.

Estimation of Residues in Animal Commodities

A tiered approach is also used for calculating estimated residues in animal commodities (meat, milk, and eggs), and higher-tier calculations can have a significant impact in decreasing estimates of dietary exposure and risk. The Tier III assessment for atrazine and simazine (Tables 27.3 and 27.4) is based on calculations of the estimated theoretical residue in animal commodities, whereas the Tier I assessments use tolerance values. These theoretical residues are often referred to as 'secondary residues.' Calculations for estimating secondary residues in animal commodities are performed by constructing livestock (beef, dairy, and poultry) diets comprised of treated feed items to obtain a

theoretical 'dietary burden.' In order to calculate secondary residues, the dietary burden is multiplied by the percent of transfer from feed to tissues and milk, obtained from animal feeding studies. Animal feeding studies are required by the USEPA whenever a pesticide is applied directly to livestock, to crops, or to crop parts used for livestock feed. In general, separate metabolism studies are required for poultry and ruminants if the triazine in question is registered on crops likely to be fed to them. In certain cases metabolism results are used to estimate residue transfer from feed to animal commodities.

Tier I

Tier I assessments utilize tolerance-level residues in meat, milk, poultry, and eggs. Tolerances for animal commodities are often established using a worst-case livestock or poultry diet. This is created by selecting, if possible, only treated feeds and then maximizing the percent used. Often these calculations result in a diet construct that is nutritionally inadequate. In addition, the highest residues (or an average of the highest average field trial residues) for treated feed items are used in the assessment, creating a very conservative dietary burden. The superficially high dietary burden is then coupled with transfer information from ^{14}C-metabolism studies or from multiple-level feeding studies to estimate worst-case residues in animal commodities. If worst-case calculations result in residues that are below the analytical LOQ, the tolerance is often established at LOQ. In some cases, tolerances are not required if feeding studies demonstrate negligible transfer (below what could be detected with available analytical methodology) at these exaggerated feeding levels.

Tolerances for animal food commodities are required only if the pesticide is registered for use on crops that can be constituents of livestock or poultry diets. Allowable feed items are published in the USEPA'sResidue Chemistry Test Guidelines (USEPA, 1996). Examples of crops not used for animal feed are strawberries, lettuce, grapes, and pecans. Examples of crops that can be included in livestock and poultry diets are wheat, corn, citrus, apples, and almonds.

Tier II

Some Tier II assessments use tolerance values for animal commodities. Alternatively, secondary residues in animal commodities may be calculated from a diet construct made from treated feed items containing tolerance-level residues. It should be noted that using tolerance-level crop residues in a hypothetical cattle or poultry diet, in which the number and proportion of treated feed items have been maximized, results in a conservative exposure assessment. First, tolerance-level residues represent the upper boundary maximum of residues expected in fed commodities. In addition, hypothetical diets that maximize treated items may be unrealistic and do not contain adequate nutrition to sustain livestock (lactating or otherwise) and poultry.

Tier III

For chronic Tier III assessments, the average field trial values for treated feed items are used to construct a nutritionally adequate livestock or poultry diet. A realistic diet (containing adequate amounts of fiber, protein, etc.), coupled with average residues in crop feed items, provides for the most accurate assessment of residues in animal commodities. A representative cattle diet determined to be nutritionally sound was used in the chronic Tier III assessment for atrazine (Table 27.5).

Table 27.5 Representative cattle diet used in the Tier III assessment for atrazine and its corresponding chloro-metabolites

Cattle feed commodity	Maximum percent of diet[a]	Estimated percent of diet	Percent dry weight[a]	Percent of crop treated	Anticipated residue (ppm)	Diet contribution (ppm)
Corn silage	50	35	40	68.95	0.04573	0.02759
Corn grain	40	25	88	68.95	0.00032	6.2E-05
Wheat middlings	60	15	88	1.2	0.002	4.1E-06
Sorghum forage	50	5	35	63.25	0.0779	0.00704
Alfalfa hay	60	10	89	0	0	0
Soybean meal	15	10	92	0	0	0
Dairy supplement	NA	NA				
Total		100%				0.03469

[a] Maximum percent of diet and percent dry weight taken from USEPA's OPPTS 860.1000 Residue Chemistry Test Guidelines (Table 1).

In the cattle diet example above, the actual mean residue value measured from analysis of field trial samples is multiplied by the estimated percent of diet and crop treated. The resulting value is then adjusted for moisture content by dividing by percent dry weight. After summing each of the adjusted feed components, the total diet contribution (or dietary burden) is obtained. The calculated dietary burden is then multiplied by a transfer factor (obtained from metabolism or livestock feeding studies) to yield anticipated residues in meat and milk. The resulting residues in meat and milk are significantly less than tolerance-level residues in animal commodities (see below) when nutritionally adequate diets and average field trial residues are used to calculate the dietary burden (Table 27.6).

The anticipated residues for poultry commodities are calculated in the same manner, although the poultry calculations do not require a moisture (dry weight) correction since poultry feed consists mostly of grains and seeds (containing a high percentage of dry matter). Conversely, cattle feed contains a higher percentage of water, so the adjustment factor is warranted. Animal food commodity residues, along with residue values from directly ingested triazine-treated crops, are entered into the exposure model. These residue levels are multiplied by average consumption values to estimate average exposures. These resulting exposures (and associated risks) are summed across all applicable foods and compared to the appropriate toxicological endpoint.

Triazine Monitoring Data

Tier III and IV assessments have not been necessary for the triazine class of chemicals in general, since exposure has been determined to be minimal with Tier I and II assessments. Based on various state and federal residue monitoring programs, more realistic (average) anticipated residues could be calculated.

As indicated above, tolerances represent the maximum legal amount of pesticide allowed on a food item. Tolerance enforcement analyses are performed by the USDA and the United States Food and Drug Administration (USFDA). The results of these analyses are incorporated into various databases, which can be used to estimate dietary exposure. In addition, residue testing of commodities is conducted by state agencies, grower groups, processors, and food purveyors. These testing efforts ensure that residue levels are either far below tolerance values or are nondetectable. The presence of triazines in the various monitoring databases is discussed below.

USFDA Monitoring

Through its Pesticide Program, the USFDA samples individual lots of domestically produced and imported foods and analyzes them for pesticide residues to enforce the tolerances set by the USEPA (USFDA, 1989–2005). Domestic samples are collected as close as possible to the point of production in the distribution system ('farm gate'). Imported samples are collected at the point of entry into United States commerce. The emphasis is on the raw agricultural product, which is analyzed in an unwashed, whole (unpeeled), unprocessed state. Processed foods are also included.

USFDA monitoring data are available on all 19 triazines registered for use on crops worldwide (prometon does not have a crop use). Triazines not registered in the United States are monitored by the USFDA's analyses of imported foods; 13 additional triazine herbicides that are either not registered in the United States or are no longer used have been monitored, along with the 7 domestic triazines mentioned previously. These additional triazines include cyanazine, cyprazine, desmetryn, dimethametryn, dipropetryn, methoprotryne, procyazine, propazine, secbumeton, simetryn, terbumeton, terbuthylazine, and trietazine. Monitoring of desmetryn, dipropetryn, procyazine, and secbumeton was discontinued for 1 year in 1998. Monitoring for all 19 triazines resumed for years 1999 through 2001. In 2002, monitoring of 10 triazines was discontinued (the four mentioned for 1998 above plus dimethametryn, simetryn, terbumeton, terbutryn, trietazine, and methoprotryne). In 2003, 13 triazines were included in the monitoring.

As indicated above, USFDA collects surveillance data on numerous crops; some may not have registered triazine uses. In the 16-year period of 1988 through 2003, the number of triazines monitored ranged from 8 in 1988 to 19 in 1997 and in 1999 through 2001; 13 triazines were monitored in 2003. During this time, 197 519 samples were analyzed

Table 27.6 Anticipated residues in livestock commodities and associated tolerances

Food item	Feed level (ppm)	Transfer factor (slope)	Anticipated residue (ppm)	Tolerance (ppm)
Milk	0.0346919	0.01090	0.0003781	0.02
Meat	0.0346919	0.00331	0.0001148	0.02
Liver	0.0346919	0.00259	0.0000899	0.02
Kidney	0.0346919	0.00195	0.0000676	0.02
Fat	0.0346919	0.00043	0.0000149	0.02

in the USFDA Pesticide Program. This total includes the Total Diet Survey (TDS), which accounts for approximately 1030 samples per year of foods prepared for consumption. Targeted monitoring of select triazines in specific commodities was conducted in 1990, 1991, 1992, 1993, and 1994. No residues were found. A selected survey of 19 triazines and 4 metabolites in various commodities was conducted in 1995 and 1996. The only triazines detected in the program including the targeted analyses were atrazine and simazine. Simazine was detected in or on approximately 12 samples of oranges taken in 1989, 1995, 1996, and 1997. The residues ranged from 'trace' to 0.08 ppm, all well below the tolerance of 0.25 ppm. Atrazine was detected in 1989 and 1997 in approximately 6 samples of escarole and lettuce as an inadvertent residue. Atrazine residues ranged from 'trace' to 0.045 ppm. An atrazine tolerance petition is currently pending at the USEPA to address these inadvertent residues. Atrazine and simazine were not detected in the 1998 USFDA monitoring program. Significant quantifiable residues of atrazine and simazine were not detected in the 1999–2003 programs. The only other triazine detects found were in 2002 for cyprazine and cyanazine and in 2003 for ametryn, prometryn, and simazine. These detectable residues were not listed as major, frequent, or violative.

In addition, USFDA analyzed 8731 animal feed samples from 1988 through 2003. No triazine detections were reported in any of the feed samples. Milk was also analyzed in the FDA monitoring program, and out of a total of 866 milk samples surveyed in 1991 and 1992, no triazine residues were detected (USFDA, 1991, 1992).

Results of the USFDA pesticide programs (compliance, surveillance, special) demonstrate the lack of significant numbers of triazine detects and verifies that exposures to triazines are minimal.

USDA Monitoring

The most refined available residue data come from the USDA's Pesticide Data Program (PDP). Composite samples are taken from grocery distribution centers immediately prior to delivery to the grocery store, and residues (if any) are most representative of intake at the dinner table ('table gate'). By collecting samples from grocery distribution centers, residue information is significantly upgraded with respect to statistical reliability and realistic evaluation of exposure. State and federal laboratories perform analyses for the USDA on more than 100 pesticides, using refined USFDA multiresidue (tolerance enforcement) methods to obtain greater levels of sensitivity. In 2005, 251 pesticides, metabolites, degradates, and isomers were monitored.

The USDA monitors fruits and vegetables for up to six triazine herbicides, including atrazine and simazine (the most widely used triazines). Additionally, atrazine has been monitored in wheat, and both atrazine and simazine have been monitored in milk, heavy cream, and butter. From 1991 through 2005, atrazine was detected in a total of 12 of 85 175 fruit and vegetable samples analyzed. Inadvertent residues for which there was no tolerance established at the time were detected in 6 spinach samples and ranged from 0.028 to 0.04 ppm. Inadvertent residues ranging from 0.003 to 0.017 ppm were found in 6 lettuce samples. Atrazine was detected in 28 of 3548 wheat samples. Residues ranged from 0.003 to 0.031 ppm. Since the atrazine tolerance on wheat is 0.25 ppm, the maximum detect was 1/8th of the tolerance. Atrazine was not detected (limits of detection (LOD) ranged from ffi0.001 to ffi0.012 ppm) in 4478 whole milk, heavy cream, or butter samples analyzed from 1996 to 2005. Corn syrup was added to the screened commodities in 1998 and 1999; atrazine was not detected (ffi0.002 ppm) in the 454 samples analyzed.

PDP monitoring for simazine began in 1996. Two orange samples out of 57 533 fruit and vegetable samples analyzed from 1996 to 2005 contained detectable residues of 0.02 ppm (well below the tolerance of 0.25 ppm). There were no simazine detects (LODs ranged from ffi0.001 to ffi0.005 ppm) in the 4477 milk, heavy cream, or butter samples analyzed from 1996 to 2005. Simazine also was not detected (ffi0.004 ppm) in 454 corn syrup samples analyzed in 1998 and 1999. Four additional triazines (ametryn, cyanazine, metribuzin, and prometryn) were included in the corn syrup monitoring. No residues (ffi0.002 to ffi0.015 ppm) of these triazines were detected.

Since 2000, ametryn, metribuzin, and prometryn have been monitored in fruits and vegetables. Through 2005, a total of 9633 samples have been screened for ametryn with no detects. A total of 26 487 samples have been screened for metribuzin with 13 detects. A single residue of 0.050 ppm was detected in sweet bell peppers, for which there is no tolerance. A single detect of 0.05 ppm was found in potatoes, well within the 0.6 ppm tolerance; 11 detects were found in asparagus, with only one (0.4 ppm) exceeding the 0.1 ppm tolerance. Prometryn monitoring covered 19 321 samples with 4 detects being recorded; 3 detects were in celery (0.013–0.017 ppm), well within the 0.5 ppm tolerance. A single residue of 0.017 ppm was found in asparagus, for which there is no tolerance. Cyanazine monitoring was added in 2001, and no detects were found in the 519 samples analyzed from 2001 through 2005.

Metribuzin was monitored in 2586 milk, heavy cream, and butter samples from 2003 through 2005. No residues (LODs ranged from 0.3 to 6.0 ppb) were detected.

Several special grain monitoring programs have also been conducted. From 2002 through 2005, 4362 samples of barley grain, soybeans, and wheat flour were analyzed for metribuzin residues with no detects (ffi0.010 ppm). Similarly, 1945 wheat flour samples were analyzed for cyanazine with only a single 8 ppb detect (tolerance 100 ppb).

Livestock tissues were added to PDP monitoring in 2000. Poultry tissue screening was conducted in 2000 and 2001. There were no detects in 930 samples of adipose and muscle tissues analyzed for atrazine and simazine residues (LODs ranged from 1.1 to 13.4 ppb). There were also no detects in 1564 samples of adipose tissue, muscle, and liver analyzed for metribuzin residues (LODs ranged from 1.5 to 2.6 ppb). Beef tissue screening was conducted in 2001 and 2002. A total of 1238 muscle tissue samples were analyzed for atrazine and simazine residues, with no detected residues recorded (LODs ranged from 1.1 to 1.5 ppb). A total of 1835 adipose tissue, muscle, and liver samples were analyzed for metribuzin residues. No detected residues were recorded (LODs ranged from 0.6 to 4.5 ppb). Pork (swine) tissue screening was conducted in 2005. A total of 704 adipose and muscle samples were analyzed for atrazine, simazine, and metribuzin. No detectable residues were observed (LODs ranged from 0.3 to 12 ppb).

Thus, 15 years of annual PDP monitoring confirms the general lack of triazine residues and indicates that exposures to triazines are minimal. Although the LOQ/LOD may vary among laboratories participating in the PDP, all LOQ and LOD values are significantly lower than tolerance levels.

Processor Monitoring

The National Food Processors Association (NFPA) maintains a database on processed foods, or foods 'ready to eat.' As described by Elkins *et al.* (1998), NFPA'sProtective Screen Program was developed in 1960 and has been used to prevent illegal or unnecessary residues in processed foods. Four triazines have been included in the NFPA monitoring program: atrazine, cyanazine, simazine, and ametryn. Through 1997, the NFPA database contained 6563 analyses for these four herbicides, and only 2 detects were found. One positive detect was observed for simazine in corn at 0.04 ppm, and one atrazine detect was observed in wheat at 0.05 ppm. Both were well within the tolerance for these compounds in their respective commodities. This industry monitoring indicates that anticipated residues in processed foods are well below existing tolerances for both compounds.

State Monitoring

Individual states frequently generate specific monitoring programs to address areas of concern. In 1993, the State of Wisconsin (WDATCP, 1993) initiated a special program to monitor groundwater, corn silage, and dairy cow milk from farms using atrazine on corn at the maximum state label rate (2.0 lb a.i./A (1.8 kg a.i./ha)). Only one water sample contained a detectable atrazine residue (0.76 ppb), well below the enforcement standard of 3 ppb. Analyses by the state showed no residues at 0.1 ppm (LOQ) in the corn silage or at 0.01 ppm (LOQ) in whole milk. No atrazine or dealkylated chloro-*s*-triazine residues were found in the silage at 0.05 ppm or in the milk samples at 0.002 ppm (10-fold lower than the atrazine tolerance in milk).

European Monitoring

Survey or monitoring data are also available from programs conducted outside the United States. Published results of monitoring in the United Kingdom in 1996 indicate that two triazines were monitored in that year: simazine and cyanazine (MAFF, 1997). Cyanazine residues were not detected in commercial leek samples at a screening level of 0.05 ppm. Simazine residues were not detected in commercial gooseberries at 0.05 ppm, or in 'pick your own' crops of gooseberries, black currants, red currants, loganberries, raspberries, or strawberries at screening levels ranging from 0.05 to 0.1 ppm. Although these results are applicable to UK dietary exposure and not United States dietary exposure, they confirm data from United States monitoring programs where no or minimal detects of triazines in food were observed.

Conclusion

A recent review by the USEPA of atrazine (USEPA, 2006) concluded that dietary exposure to atrazine and its chlorinated metabolites is low. The extremely low frequency and magnitude of detectable triazine residues in monitoring surveys of more than 250 000 commodity samples confirm that human exposure to triazines through the diet is minimal.

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Chapter 28

Probabilistic Assessment of Laboratory-Derived Acute Toxicity Data for the Triazine Herbicides to Aquatic Organisms

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Summary

The basic framework for ecotoxicological risk assessment is the integration of exposure and effects profiles into a risk estimate. The first step in this tiered approach has traditionally been a simple screening-level 'worst-case' estimate or measurement of environmental concentration that is compared with the effect level for the most sensitive species as a ratio, the hazard quotient (HQ). Higher tiers in the risk assessment process now make use of probabilistic approaches for assessing both exposure and effects. This process was used to characterize the toxicity of the triazines – hexazinone, prometryn, cyanazine, ametryn, metribuzin, atrazine, simazine, prometon, and terbutryn – using distributions based on log-probability transformed data. Several of the triazines had limited data for the sensitivity of aquatic plants. The slopes of the distributions were similar except for hexazinone (with a small data set) and prometryn, indicating a similar range of sensitivity in the plants tested. Algae were the most sensitive plants, although with the exception of atrazine, there was generally a paucity of data for macrophytes. The range of sensitivity to triazines in aquatic plants was about 80, with prometryn having the lowest 10th centile[1] (0.9 μg/L) and prometon the greatest (70 μg/L). Aquatic animals were less sensitive to the triazines than were the plants; however, the range of toxicity between the least and most sensitive animal (200) was wider for animals than for plants. Toxicity to fish and arthropods varied, with fish or crustaceans sharing the position of most sensitive aquatic animal, depending on the herbicide. The 10th centile for atrazine in aquatic arthropods was 405 μg/L and in fish 3592 μg/L. For aquatic animals, terbutryn gave a 10th centile of 1379 μg/L, and hexazinone was the least toxic with a 10th centile of 81 109 μg/L. These lower centiles derived from the toxicity distributions of the triazine herbicides could be used to assess the relevance of environmental concentrations and to refine monitoring programs.

Introduction

Ecological risk assessment of pesticides and other substances evolved in the 1990s with the development of new approaches [United States Environmental Protection Agency (USEPA), 1992, 1998; NRC, 1993; Environment Canada, 1997; Hart, 2001; EUFRAM, 2005]. These approaches all have the same basic framework, where exposure and effects profiles are integrated into a risk estimate (USEPA, 1992, 1998). In addition to this similarity, several approaches to risk assessment have required the use of tiers, where the lower tiers are conservative screening-level tools, while the upper tiers are more realistic in their assessment of both exposure and effects data (SETAC, 1994; ECOFRAM, 1999). Tier I has traditionally been a simple 'worst-case' estimation of environmental concentration that is compared with the effect level for the most sensitive species as a ratio (HQ). This ratio is in turn compared to a set of criteria to determine if a level of concern (LOC) has been exceeded (Urban and Cook, 1986). If the HQ shows a potential hazard, further tiers of risk assessment with more realistic and more complete exposure and effects data can be used for refining the assessment. The report of the Aquatic Risk Assessment and Dialogue Group (SETAC, 1994) indicated that the higher tiers in the process should make use of probabilistic approaches to assessing both exposure and effects. This approach has been used in the assessment of ecological risks from a number of pesticides and other substances (Klaine *et al.*, 1996; Solomon *et al.*, 1996; Solomon and Chappel, 1998; Cardwell *et al.*, 1999; Giesy *et al.*, 1999; Hall *et al.*, 1999; Giddings *et al.*, 2000,

[1] Centile is a rank on a scale of 0 to 100 and is equivalent to the percentile.

ED_001334_00003007-00442 EPA-HQ-2017-008866

2001; Hendley *et al.*, 2001; Maund *et al.*, 2001; Solomon and Takacs, 2001; Travis and Hendley, 2001) and has been put forward as a procedure applicable to risk assessment of pesticides for regulatory purposes (USEPA, 1998; ECOFRAM, 1999).

In the case of some substances, particularly newly registered pesticides, toxicity data are often limited to required studies and would normally be too few for use in a higher tier risk assessment. For older substances, such as many of the triazines, considerable data may be available (Solomon *et al.*, 1996) and can be usefully applied to assessing risks from measured concentrations in the environment. Even in the absence of large data sets of exposure concentrations, these toxicity data may be useful for assessing the need for more extensive monitoring or for focusing monitoring programs on regions or locations where the risks are judged to be greatest (Solomon, 1999). Thus, these distributional analyses of toxicity can be used as benchmarks for comparative risk assessment, for assessing additive toxicity of mixtures for compounds with the same mode of action, and for prioritization of environmental sampling. It is in this context that this chapter presents an analysis of the acute toxicity of the several triazine herbicides to aquatic organisms.

Triazine residues have been detected in surface and ground waters (Solomon *et al.*, 1996; Solomon and Chappel, 1998), and the question of the significance of these reported concentrations is often raised. The assessment of toxicity data can provide useful benchmark concentrations related to the sensitivity of various organisms to the substance under discussion. A number of jurisdictions have developed water quality guidelines that can be used to judge environmental concentrations of pesticides. These guidelines are usually developed from the toxicity value for the most sensitive organism tested, often with the additional application of a 'safety' or uncertainty factor (Canadian Water Quality Guidelines, CWQG, 1999). These guidelines are designed to be protective in a wide variety of environments in the jurisdiction to which they apply. However, the guidelines may be unnecessarily conservative, and other assessment criteria such as those derived from probabilistic analyses are likely to be more realistic.

Pesticides frequently have very specific mechanisms of action, and this may confound the analysis of toxicity distributions if organisms of very different physiological and biochemical sensitivity are lumped together (Solomon, 1996; ECOFRAM, 1999). The primary mechanism of action of the triazine herbicides on plants is inhibition of photosynthesis. The triazines were developed specifically as herbicides and have a mechanism of action that is unique to plants. Thus, the potency of the triazines would be expected to be much greater in the more sensitive plants than in animals. The triazines inhibit photosynthesis via competition with plastoquinone II at its binding site in the process of electron transport in photosystem II (Devine *et al.*, 1993). This inhibition results in the cessation of carbohydrate synthesis, leading to a subsequent reduction in the carbon pool and a buildup of CO_2 within the plant cell (Solomon *et al.*, 1996). The binding of atrazine and other triazines to the plastoquinone II binding site is reversible. When exposure of plants to atrazine ceases, photosynthetic activity increases, leading to recovery of energy production and growth potential (Jensen *et al.*, 1977; Brockway *et al.*, 1984; Hamala and Kollig, 1985; Hoagland *et al.*, 1993), provided that energy reserves have not been used up or that chlorophyll has not been destroyed during the period of inhibition.

Because animals lack a photosynthetic mechanism, they are less sensitive to the triazines and other photosynthetically active herbicides than plants. Acute toxicity to mammals and birds is low, and these substances are not generally regarded as being hazardous to the applicator, to terrestrial organisms, or to the general public. A review of the toxicity values for birds and honeybees reported in tests conducted for the purposes of registration confirms this general observation (Montague, 2000). For this reason, the focus of this assessment has been on toxicity to aquatic organisms.

Methods

The process used to characterize the toxicity of each of the triazines was to compile all of the available data for aquatic species into a cumulative frequency distribution. The distribution was described by a linear regression of the log-probability transformed data. Toxicity data for some of the triazines have been analyzed before (Solomon *et al.*, 1996; Solomon and Chappel, 1998), but this analysis represents an expansion of the data sets to include more values. Toxicity data for the triazines were obtained from the USEPA Pesticide Toxicity Database (Montague, 2000) and from the open scientific literature. For the USEPA Pesticide Toxicity Database, only data from core (C) and supplementary studies (S) were used. For other studies reported in the open literature, those judged unsatisfactory by the criteria used in the AQUIRE database (AScI, 1994) were omitted from the analyses. The omitted studies generally lacked adequate control data. As the triazines do not bioconcentrate to a great extent, data from static, static-renewal, and flow-through bioassays were all used. The acute toxicity data consisted of measures of lethal concentrations causing death in 50% of the population tested (LC_{50}) and of effective concentrations causing a specified effect in 50% of the population tested (EC_{50}). For the purposes of this analysis, EC and LC were treated similarly. A number of exposure time periods are commonly used for laboratory toxicity testing of aquatic organisms. The data used in this analysis were derived from acute assays conducted over periods from 48 to 96 h for animals and up to 240 h for plants. These toxicity values are listed in Table 28.1. In addition to the triazines listed in Table 28.1, toxicity data for atrazine

Table 28.1 Listing of acute toxicity values to aquatic organisms for some triazine herbicides[a]

Herbicides and species studied	Common name[b]	Duration hours	Concentration[c] (μg/L)	Geometric mean	Reference
Ametryn					
Achnanthes brevipes	Algae[P]	72	19[E]		Montague, 2000
Chlorella spp.	Algae[P]	72	320[E]		Montague, 2000
Chlorococcum spp.	Algae[P]	240	10[E]		Montague, 2000
Cyclotella nana	Algae[P]	72	55[E]		Montague, 2000
Dunaliella tertiolecta	Algae[P]	240	20[E]		Montague, 2000
Isochrysis galbana	Algae[P]	240	10[E]		Montague, 2000
Monochrysis lutheri	Algae[P]	72	14[E]		Montague, 2000
Navicula inserta	Algae[P]	72	97[E]		Montague, 2000
Neochloris spp.	Algae[P]	72	36[E]		Montague, 2000
Nitzschia closterium	Algae[P]	72	62[E]		Montague, 2000
Phoedactylum tricornutum	Algae[P]	240	50[E]		Montague, 2000
Porphyridium cruentum	Algae[P]	72	36[E]		Montague, 2000
Selenastrum capricornutum	Algae[P]	168	4[E]		Montague, 2000
Stauroneis amphoroides	Algae[P]	72	26[E]		Montague, 2000
T. fl aviatilis	Algae[P]	72	58[E]		Montague, 2000
Anguilla japonica	Eel[F]	48	1500[L]		Yokoyama, *et al.*, 1988
Carassius auratus	Goldfish[F]	96	14 000[L]		Montague, 2000
Carassius carassius	Crucian carp[F]	96	27 000[L]		Bathe *et al.*, 1973
Carassius carassius	Crucian carp[F]	48	30 000[L]	28 460	Bathe *et al.*, 1973
Cyprinodon variegatus	Sheepshead minnow[F]	96	5800[L]		Montague 2000
Ictalurus sp	Catfish[F]	96	25 000[L]		Bathe *et al.*, 1973
Ictalurus sp	Catfish[F]	96	25 000[L]	25 000	Bathe *et al.*, 1973
Leiostomus xanthurus	Spot[F]	48	1000[L]		Butler 1965
Lepomis macrochirus	Bluegill sunfish[F]	96	3700[L]		Johnson and Finley, 1980
Lepomis macrochirus	Bluegill sunfish[F]	96	4100[L]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	96	19 000[L]		Bathe *et al.*, 1973
Lepomis macrochirus	Bluegill sunfish[F]	48	25 000[L]	9213	Bathe *et al.*, 1973
Oncorhynchus mykiss	Rainbow trout[F]	96	3200[L]		Johnson and Finley, 1980
Oncorhynchus mykiss	Rainbow trout[F]	96	3400[L]		Bathe *et al.*, 1973
Oncorhynchus mykiss	Rainbow trout[F]	48	7000[L]		Bathe *et al.*, 1973
Oncorhynchus mykiss	Rainbow trout[F]	96	13 500[L]	5663	Tscheu-Schluter and Skibba, 1986
Oryzias latipes	Medaka[F]	48	5000[L]		Nishiuchi and Hashimoto, 1967
Pimephales promelas	Fathead minnow[F]	96	5700[L]		Montague 2000
Poecilia reticulata	Guppy[F]	96	300[L]		Bathe *et al.*, 1973
Poecilia reticulata	Guppy[F]	48	500[L]		Bathe *et al.*, 1973
Poecilia reticulata	Guppy[F]	96	7000[L]		Tscheu-Schluter and Skibba, 1986
Poecilia reticulata	Guppy[F]	96	8000[L]		Tscheu-Schluter and Skibba, 1986
Poecilia reticulata	Guppy[F]	72	8500[L]		Bathe *et al.*, 1973
Poecilia reticulata	Guppy[F]	96	17 000[L]	3266	Tscheu-Schluter and Skibba, 1986
Daphnia magna	Water flea[C]	48	28 000[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	40 000[E]	33 466	Marchini *et al.*, 1988
Crangon crangon	Shrimp[C]	48	33 000[E]		Portmann and Wilson, 1971
Crangon crangon	Brown shrimp[C]	48	1000[E]	5745	Butler, 1965
Physa acuta	Snail[M]	48	6200[E]		Nishiuchi and Yoshida, 1972
Cyanazine					
Anabaena fl os-aquae	Algae[P]	120	24[E]		Montague, 2000
Anabaena fl os-aquae	Algae[P]	120	24[E]	24	Montague, 2000
Lemna gibba	Duckweed[P]	336	64[E]		Montague, 2000
Lemna gibba	Duckweed[P]	336	64[E]	64	Montague, 2000
Navicula pelliculosa	Algae[P]	120	5[E]		Montague, 2000
Navicula pelliculosa	Algae[P]	120	5[E]	5	Montague, 2000
Selenastrum capricornutum	Algae[P]	96	20[E]		Montague, 2000
Selenastrum capricornutum	Algae[P]	120	6[E]		Montague, 2000
Selenastrum capricornutum	Algae[P]	120	6[E]	8	Montague, 2000
Skeletonema costatum	Marine diatom[P]	120	18[E]		Montague, 2000
Skeletonema costatum	Marine diatom[P]	120	18[E]	18	Montague, 2000
Cirrhinus mrigala	Carp, hawk fish[F]	96	6300[L]		Rao and Dad, 1979
Ictalurus punctatus	Channel catfish[F]	96	10 400[L]		Montague, 2000
Ictalurus punctatus	Channel catfish[F]	96	11 300[L]		Montague, 2000
Ictalurus punctatus	Channel catfish[F]	96	17 400[L]	12 693	Montague, 2000

(*Continued*)

Table 28.1 (Continued)

Herbicides and species studied	Common name[b]	Duration hours	Concentration[c] (μg/L)	Geometric mean	Reference
Labeo rohita	Rohu[F]	96	4800[L]		Dad and Tripathi, 1980
Labeo rohita	Rohu[F]	48	8600[L]	6425	Dad and Tripathi, 1980
Lepomis macrochirus	Bluegill sunfish[F]	96	10 400[L]		Mayer and Ellersieck, 1986
Lepomis macrochirus	Bluegill sunfish[F]	96	20 300[L]		Mayer and Ellersieck, 1986
Lepomis macrochirus	Bluegill sunfish[F]	96	22 500[L]	16810	Montague, 2000
Mystus vittatus	Catfish[F]	96	30800[L]		Dad and Tripathi 1980
Mystus vittatus	Catfish[F]	48	45700[L]	37 517	Dad and Tripathi, 1980
Oncorhynchus mykiss	Rainbow trout[F]	96	900[L]		Mayer and Ellersieck 1986
Oncorhynchus mykiss	Rainbow trout[F]	96	4000[L]		Davies *et al.*, 1994
Oncorhynchus mykiss	Rainbow trout[F]	96	9000[L]	3188	Montague, 2000
Pimephales promelas	Fathead minnow[F]	96	16300[L]		Montague, 2000
Pimephales promelas	Fathead minnow[F]	96	17500[L]		Mayer and Ellersieck, 1986
Pimephales promelas	Fathead minnow[F]	96	19400[L]		Mayer and Ellersieck, 1986
Pimephales promelas	Fathead minnow[F]	96	21300[L]	18529	Montague, 2000
Rasbora heteromorpha	Harlequinfish[F]	96	7600[L]		Tooby *et al.*, 1980
Tilapia mossambica	Tilapia[F]	96	11300[L]		Rao and Dad, 1979
Ceriodaphnia dubia	Water flea[C]	48	32990[L]		Ort *et al.*, 1994
Chironomus tentans	Midge[I]	48	6630[L]		Dad and Tripathi, 1980
Daphnia magna	Water flea[C]	48	35500[E]		Marchini *et al.*, 1988
Daphnia magna	Water flea[C]	48	42000[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	49000[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	53000[E]		Nebeker *et al.*, 1986
Daphnia magna	Water flea[C]	48	84000[E]		Nebeker *et al.*, 1986
Daphnia magna	Water flea[C]	48	86000[E]		Nebeker *et al.*, 1986
Daphnia magna	Water flea[C]	48	93000[E]		Nebeker *et al.*, 1986
Daphnia magna	Water flea[C]	48	95000[E]		Nebeker *et al.*, 1986
Daphnia magna	Water flea[C]	48	106000[E]	66719	Nebeker *et al.*, 1986
Gammarus fasciatus	Scud	96	2000[L]		Montague, 2000
Palaemonetes pugio	Grass shrimp[C]	48	56000[L]		Montague, 2000
Hexazinone					
Selenastrum capricornutum	Algae[P]	120	7[E]		Montague, 2000
Selenastrum capricornutum	Algae[P]	168	126[E]	29	Montague, 2000
Anabaena fl osaquae	Bluegreen[P]	72	2014[E]		Abou-Waly *et al.*, 1991
Anabaena fl osaquae	Bluegreen[P]	120	2375[E]		Abou-Waly *et al.*, 1991
Anabaena fl osaquae	Bluegreen[P]	168	2752[E]	2361	Abou-Waly *et al.*, 1991
Anguilla japonica	Eel[F]	48	75000[L]		Yokoyama *et al.*, 1988
Lepomis macrochirus	Bluegill sunfish[F]	96	238000[L]		Montague, 2000
Oncorhynchus gorbuscha	Pink salmon[F]	96	236000[L]		Wan *et al.*, 1988
Oncorhynchus gorbuscha	Pink salmon[F]	72	280000[L]		Wan *et al.*, 1988
Oncorhynchus gorbuscha	Pink salmon[F]	96	676000[L]		Wan *et al.*, 1988
Oncorhynchus gorbuscha	Pink salmon[F]	72	728000[L]		Wan *et al.*, 1988
Oncorhynchus gorbuscha	Pink salmon[F]	48	839000[L]		Wan *et al.*, 1988
Oncorhynchus gorbuscha	Pink salmon[F]	96	1408000[L]		Wan *et al.*, 1988
Oncorhynchus gorbuscha	Pink salmon[F]	72	1559000[L]		Wan *et al.*, 1988
Oncorhynchus gorbuscha	Pink salmon[F]	48	1621000[L]	747124	Wan *et al.*, 1988
Oncorhynchus keta	Chum[F]	96	285000[L]		Wan *et al.*, 1988
Oncorhynchus keta	Chum[F]	72	934000[L]	4583	Wan *et al.*, 1988
Oncorhynchus kisutch	Coho[F]	96	246000[L]		Wan *et al.*, 1988
Oncorhynchus kisutch	Coho[F]	96	923000[L]	476506	Wan *et al.*, 1988
Oncorhynchus mykiss	Rainbow trout[F]	96	146700[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	257000[L]		Wan *et al.*, 1988
Oncorhynchus mykiss	Rainbow trout[F]	48	286000[L]		Wan *et al.*, 1988
Oncorhynchus mykiss	Rainbow trout[F]	96	872000[L]		Wan *et al.*, 1988
Oncorhynchus mykiss	Rainbow trout[F]	96	1964000[L]	450067	Wan *et al.*, 1988
Oncorhynchus nerka	Sockeye[F]	96	317000[L]		Wan *et al.*, 1988
Oncorhynchus nerka	Sockeye[F]	48	318000[L]		Wan *et al.*, 1988
Oncorhynchus nerka	Sockeye[F]	72	318000[L]		Wan *et al.*, 1988
Oncorhynchus nerka	Sockeye[F]	96	925000[L]		Wan *et al.*, 1988
Oncorhynchus nerka	Sockeye[F]	72	927000[L]		Wan *et al.*, 1988
Oncorhynchus nerka	Sockeye[F]	48	974000[L]	546952	Wan *et al.*, 1988
Oncorhynchus tshawytscha	Chinook[F]	96	317000[L]		Wan *et al.*, 1988
Oncorhynchus tshawytscha	Chinook[F]	72	1096000[L]	589434	Wan *et al.*, 1988

Table 28.1 (Continued)

Herbicides and species studied	Common name[b]	Duration hours	Concentration[c] (μg/L)	Geometric mean	Reference
Pimephales promelas	Fathead minnow[F]	96	274 000[L]		Montague, 2000
Tilapia mossambica	Tilapia[F]	96	380 000[L]		Liong *et al.*, 1988
Daphnia magna	Water flea[C]	48	151 600[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	85 000[E]	113 517	Montague, 2000
Palaemonetes pugio	Grass shrimp[C]	96	78 000[E]		Montague, 2000
Metribuzin					
Selenastrum capricornutum	Duckweed[P]	144	21[E]		Montague, 2000
Egeria densa	American frog's-bit[P]	336	22[E]		Davis, 1981
Myriophyllum spicatum	Millfoil[P]	672	64[E]		Davis, 1981
Ictalurus punctatus	Catfish[F]	96	3400[L]		Clemens and Sneed, 1959
Ictalurus punctatus	Catfish[F]	72	3800[L]		Clemens and Sneed, 1959
Ictalurus punctatus	Catfish[F]	48	5000[L]	4012	Clemens and Sneed, 1959
Lepomis macrochirus	Bluegill sunfish[F]	96	75 960[L]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	96	92 000[L]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	96	131 300[L]	97 173	Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	42 000[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	64 000[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	76 770[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	99 000[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	147 000[L]	78 617	Montague, 2000
Rasbora heteromorpha	Harlequinfish[F]	96	140 000[L]		Tooby *et al.*, 1975
Ceriodaphnia dubia	Water flea[C]	48	26 500[L]		Ort *et al.*, 1994
Chironomus riparius	Midge[I]	48	175 000[E]		Buhl and Faerber, 1989
Crassostrea virginica	Eastern oyster[M]	96	40700[E]		Montague, 2000
Crassostrea virginica	Eastern oyster[M]	96	42000[E]		Montague, 2000
Crassostrea virginica	Eastern oyster[M]	96	49800[E]	43 990	Montague, 2000
Daphnia magna	Water flea[C]	48	4200[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	41 800[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	98 500[E]	25 860	Montague, 2000
Penaeus duorarum	Pink shrimp[C]	96	48 270[L]		Montague, 2000
Prometon					
Chlorococcum spp.	Green algae[P]	240	500[E]		Montague, 2000
Chlorococcum spp.	Green algae[P]	240	1500[E]	866	Montague, 2000
Dunaliella tertiolecta	Green algae[P]	240	5000[E]		Montague, 2000
Dunaliella tertiolecta	Green algae[P]	240	1500[E]	2739	Montague, 2000
Isochrysis galbana	Marine haptophyte[P]	240	1000[E]		Montague, 2000
Isochrysis galbana	Marine haptophyte[P]	240	500[E]	707	Montague, 2000
Phaeodactylum tricornutum	Marine diatom[P]	240	2000[E]		Montague, 2000
Phaeodactylum tricornutum	Marine diatom[P]	240	250[E]	707	Montague, 2000
Selenastrum capricornutum	Green algae[P]	120	98[E]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	96	41 500[L]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	96	32 000[L]	36 442	Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	16 000[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	19 600[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	20 000[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	48	12 000[L]		Bathe *et al.*, 1973
Oncorhynchus mykiss	Rainbow trout[F]	96	12 000[L]	15 529	Bathe *et al.*, 1973
Cyprinodon variegatus	Sheepshead minnow[F]	96	47 300[L]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	96	15 500[L]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	96	40 000[L]	24 900	Bathe *et al.*, 1973
Carassius carassius	Crucian carp[F]	48	70 000[L]		Bathe *et al.*, 1973
Carassius carassius	Crucian carp[F]	96	70 000[L]	70 000	Bathe *et al.*, 1973
Ictalurus sp	Bullhead[F]	48	30 000[L]		Bathe *et al.*, 1973
Ictalurus sp	Bullhead[F]	96	20 000[L]	24 495	Bathe *et al.*, 1973
Poecilia reticulata	Guppy[F]	48	14 000[L]		Bathe *et al.*, 1973
Poecilia reticulata	Guppy[F]	96	12 000[L]	12 961	Bathe *et al.*, 1973
Daphnia magna	Water flea[C]	48	59 800[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	25 700[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	38 000[E]	38 798	Marchini *et al.*, 1988
Mysidopsis bahia	Mysid[C]	96	17 700[L]		Montague, 2000
Crassostrea virginica	Eastern oyster[M]	96	27500[E]		Montague, 2000

(*Continued*)

Table 28.1 (Continued)

Herbicides and species studied	Common name[b]	Duration hours	Concentration[c] (μg/L)	Geometric mean	Reference
Prometryn					
Anabaena fl os-aquae	Bluegreen algae[P]	120	40[E]		Montague, 2000
Anabaena spp.	Green algae[P]	336	500*		Yee *et al.*, 1985
Chlamydomonas segnis	Algae[P]	336	750*		Yee *et al.*, 1985
Lemna gibba	Duckweed[P]	336	12[E]		Montague, 2000
Navicula pelliculosa	Marine diatom[P]	120	1[E]		Montague, 2000
Selenastrum capricornutum	Green algae[P]	96	12[E]		Montague, 2000
Skeletonema costatum	Marine diatom[P]	120	8[E]		Montague, 2000
Spirodela polyrhiza	Large duckweed[P]	168	52[E]		Liu and Cendeno-Maldonado, 1974
Anguilla japonica	Eel[F]	48	1500[L]		Yokoyama *et al.*, 1988
Carassius auratus	Goldfish[F]	96	4000[L]		Montague, 2000
Carassius carassius	Crucian carp[F]	48	30 000[L]		Bathe *et al.*, 1973
Carassius carassius	Crucian carp[F]	96	27 000[L]	28 460	Bathe *et al.*, 1973
Ictalurus sp	Catfish[F]	48	25 000[L]		Bathe *et al.*, 1973
Ictalurus sp	Catfish[F]	96	25 000[L]	25 000	Bathe *et al.*, 1973
Lepomis macrochirus	Bluegill sunfish[F]	96	10 000[L]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	48	25 000[L]		Bathe *et al.*, 1973
Lepomis macrochirus	Bluegill sunfish[F]	96	3700[L]		Johnson and Finley, 1980
Lepomis macrochirus	Bluegill sunfish[F]	96	19 000[L]		Bathe *et al.*, 1973
Lepomis macrochirus	Bluegill sunfish[F]	96	10 000[L]	11 194	Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	48	7000[L]		Bathe *et al.*, 1973
Oncorhynchus mykiss	Rainbow trout[F]	96	3400[L]		Bathe *et al.*, 1973
Oncorhynchus mykiss	Rainbow trout[F]	96	3200[L]		Johnson and Finley, 1980
Oncorhynchus mykiss	Rainbow trout[F]	96	2900[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	7200[L]	4368	Montague, 2000
Oryzias latipes	Medaka, high-eyes[F]	48	5000[L]		Nishiuchi and Hashimoto, 1967
Poecilia reticulata	Guppy[F]	48	500[L]		Bathe *et al.*, 1973
Poecilia reticulata	Guppy[F]	72	8500[L]		Tscheu-Schluter, 1976
Poecilia reticulata	Guppy[F]	96	300[L]		Bathe *et al.*, 1973
Poecilia reticulata	Guppy[F]	96	7000[L]		Tscheu-Schluter, 1976
Poecilia reticulata	Guppy[F]	96	17 000[L]	2730	Tscheu-Schluter, 1976
Crangon crangon	Common shrimp[C]	48	33 000[L]		Portmann and Wilson, 1971
Daphnia magna	Water flea[C]	48	18 590[E]		Montague, 2000
Daphnia magna	Water flea[C]	48	40 000[L]	27 269	Marchini *et al.*, 1988
Mercenaria mercenaria	Quahog clam[M]	48	21 000[E]		Montague, 2000
Mysidopsis bahia	Mysid shrimp[C]	96	1700[L]		Montague, 2000
Physa acuta	Bladder snail[M]	48	6200[L]		Nishiuchi and Yoshida, 1972
Semisulcospira libertina	Marsh snail[M]	48	6000[L]		Nishiuchi and Yoshida, 1972
Simazine					
Anabaena fl os-aquae	Bluegreen algae[P]	120	36[E]		Montague, 2000
Chlamydomonas noctigama	Algae[P]	72	450[E]		Kallqvist and Romstad, 1994
Chloroccocum spp.	Algae[P]	240	200[E]		Montague, 2000
Dunaliella tertiolecta	Algae[P]	240	500[E]		Montague, 2000
Isochrysis galbana	Algae[P]	240	500[E]		Montague, 2000
Lemna gibba	Duckweed[P]	336	140[E]		Montague, 2000
Navicula pelliculosa	Algae[P]	120	90[E]		Montague, 2000
Phaeodactylum tricornutum	Algae[P]	240	500[E]		Montague, 2000
Selenastrum capricornutum	Algae[P]	120	100[E]		Montague, 2000
Selenastrum capricornutum	Algae[P]	72	200[E]	141	Kallqvist and Romstad, 1994
Skeletonema costatum	Algae[P]	120	600[E]		Montague, 2000
Barbus ticto	Barbus[F]	96	24 500[L]		Rao and Dad, 1979
Cirrhinus mrigala	Carp[F]	96	2500[L]		Dad and Tripathi, 1980
Danio sp	Danio[F]	96	12 600[L]		Rao and Dad, 1979
Ictalurus natalis	Yellow bullhead[F]	96	110000[L]		Montague, 2000
Ictalurus punctatus	Channel catfish[F]	96	85 000[L]		Montague, 2000
Ictalurus sp	Catfish[F]	96	65 000[L]		Bathe *et al.*, 1973
Ictalurus sp	Catfish[F]	96	80 000[L]	72 111	Bathe *et al.*, 1973
Labeo rohita	Rohu[F]	96	2500[L]		Dad and Tripathi, 1980
Lepomis gibbosus	Pumpkinseed sunfish[F]	96	27 000[L]		Montague, 2000
Lepomis macrochirus	Bluegill sunfish[F]	96	90 000[L]		Bathe *et al.*, 1973
Lepomis macrochirus	Bluegill sunfish[F]	96	100 000[L]		Mayer and Ellersieck, 1986
Lepomis macrochirus	Bluegill sunfish[F]	96	118 000[L]		Cope, 1965
Lepomis macrochirus	Bluegill sunfish[F]	96	8 100 000[L]	304 546	Watkins *et al.*, 1985

Table 28.1 (Continued)

Herbicides and species studied	Common name[b]	Duration hours	Concentration[c] (μg/L)	Geometric mean	Reference
Lepomis macrolopus	Redear sunfish[F]	96	54000[L]		Montague, 2000
Micropterus salmoides	Largemouth bass[F]	96	46 000[L]		Montague, 2000
Morone saxatilis	Striped bass[F]	96	250[L]		Wellborn, 1969
Morone saxatilis	Striped bass[F]	48	440[L]	332	Wellborn, 1969
Morone saxatilis	Striped bass[F]	48	ffi180 00ð		McCann and Hitch, 1980
Mystus vittatus	Catfish[F]	96	28 600[L]		Dad and Tripathi, 1980
Oncorhynchus mykiss	Rainbow trout[F]	96	40 500[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	44 600[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	56 000[L]		Cope, 1965
Oncorhynchus mykiss	Rainbow trout[F]	96	60 000[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	70 500[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	70 500[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	96	70 500[L]		Montague, 2000
Oncorhynchus mykiss	Rainbow trout[F]	48	85 000[L]	60 554	Alabaster, 1969
Oncorhynchus tshawytscha	Chinook[F]	96	6600[L]		Bond *et al.*, 1959
Oncorhynchus tshawytscha	Chinook[F]	96	7000[L]	6797	Bond *et al.*, 1959
Oryzias latipes	Medaka[F]	96	ffi40 00ð		Nishiuchi and Hashimoto, 1967
Pimephales notatus	Bluntnose minnow[F]	96	66 000[L]		Montague, 2000
Pimephales promelas	Fathead minnow[F]	96	5000[L]		Mayer and Ellersieck, 1986
Pimephales promelas	Fathead minnow[F]	96	10 000[L]		Mayer and Ellersieck, 1986
Pimephales promelas	Fathead minnow[F]	96	510 000[L]	29 434	Mayer and Ellersieck, 1986
Poecilia reticulata	Guppy[F]	72	3000[L]		Tscheu-Schluter, 1976
Poecilia reticulata	Guppy[F]	48	3900[L]		Tscheu-Schluter, 1976
Poecilia reticulata	Guppy[F]	96	49 000[L]	8307	Bathe *et al.*, 1973
Asellus brevicaudus	Sowbug[C]	48	ffi100 00ð		Sanders, 1970
Chironomus tentans	Midge[I]	48	3580[L]		Dad and Tripathi, 1980
Cypridopsis vidua	Seed shrimp[C]	48	3200[L]		Sanders, 1970
Cypridopsis vidua	Seed shrimp[C]	48	3700[L]	3441	Montague, 2000
Daphnia magna	Water flea[C]	48	1000[E]		Sanders, 1970
Daphnia magna	Water flea[C]	48	1100[E]	1049	Montague, 2000
Daphnia pulex	Water flea[C]	48	92 100[L]		Fitzmayer *et al.*, 1982
Daphnia pulex	Water flea[C]	48	424 000[L]	197 612	Fitzmayer *et al.*, 1982
Gammarus fasciatus	Scud[C]	48	ffi100 00ð		Sanders, 1970
Gammarus lacustris	Scud[C]	96	13 000[L]		Montague, 2000
Gammarus lacustris	Scud[C]	48	21 000[L]	16 523	Sanders, 1970
Penaeus duorarum	Pink shrimp[C]	96	113 000[E]		Montague, 2000
Pteronarcys californica	Stonefly[I]	96	1900[E]		Johnson and Finley, 1980
Terbutryn					
Anacystis nidulans	Bluegreen[P]	120	9[E]		Hatfield *et al.*, 1989
Anacystis nidulans	Bluegreen[P]	120	47[E]		Hatfield *et al.*, 1989
Anacystis nidulans	Bluegreen[P]	120	32[E]		Hatfield *et al.*, 1989
Anacystis nidulans	Bluegreen[P]	120	95[E]	34	Hatfield *et al.*, 1989
Carassius carassius	Goldfish[F]	96	1400[L]		Bathe *et al.*, 1975
Carassius carassius	Goldfish[F]	96	4000[L]		Bathe *et al.*, 1975
Carassius carassius	Goldfish[F]	96	4000[L]	2819	Bathe *et al.*, 1975
Ctenopharyngodon idella	Grass carp[F]	96	5800[L]		Tooby *et al.*, 1980
Ctenopharyngodon idella	Grass carp[F]	48	8900[L]	7185	Tooby *et al.*, 1980
Ictalurus sp	Catfish[F]	96	3000[L]		Bathe *et al.*, 1973
Lepomis macrochirus	Bluegill[F]	96	2700[L]		Johnson and Finley, 1980
Lepomis macrochirus	Bluegill[F]	96	2720[L]		Mayer and Ellersieck, 1986
Lepomis macrochirus	Bluegill[F]	96	4000[L]		Bathe *et al.*, 1973
Lepomis macrochirus	Bluegill[F]	48	6000[L]	3644	Bathe *et al.*, 1973
Oncorhynchus mykiss	Rainbow trout[F]	48	820[L]		Mayer and Ellersieck, 1986
Oncorhynchus mykiss	Rainbow trout[F]	96	1800[L]		Bathe *et al.*, 1973
Oncorhynchus mykiss	Rainbow trout[F]	48	3000[L]	1642	Bathe *et al.*, 1973
Daphnia magna	Water flea[C]	48	7100[E]		Marchini *et al.*, 1988

[a] For similar information on atrazine see Giddings *et al.* (2005).

[b] Code for Group.
P ffl Plant
F ffl Fish
C ffl Crustacea
M ffl Mollusca
I ffl Insect.

[c] Code for Measure
E ffl EC_{50} Effective concentration causing a specified effect in 50% of the tested population
L ffl LC_{50} Lethal concentration causing death in 50% of the population tested
* ffl INH 65

Table 28.2 Regression coefficients and intercepts for the toxicity data distributions for acute exposures of aquatic organisms to some triazine herbicides

Triazine toxicity to aquatic organisms	y ffi ax ffl b[a]		Regression intercepts (μg/L)			
	a	b	r^2	10%	5%	n[b]
Hexazinone, aquatic plants	0.45	3.91	1.00	0.4	0.1	2
Prometryn, aquatic plants	0.82	3.77	0.95	0.9	0.3	8
Cyanazine, aquatic plants	1.72	2.92	0.98	2.9	1.8	5
Ametryn, aquatic plants	1.81	2.31	0.96	6.0	3.8	15
Metribuzin, aquatic plants	2.17	1.76	0.79	7.9	5.4	3
Atrazine, aquatic plants	1.33	2.09	0.90	16.6	8.9	20
Simazine, aquatic plant	1.90	0.50	0.88	48.9	31.5	10
Prometon, aquatic plants	1.32	1.27	0.85	70.0	37.3	5
Terbutryn, aquatic plants	–	–	–	–	–	1
Atrazine, aquatic arthropods	0.99	1.13	0.94	405	174	17
Terbutryn, aquatic animals	2.99	┘ 5.66	0.93	1379	1042	6
Ametryn, aquatic animals	1.88	┘ 2.21	0.94	1426	914	14
Simazine, aquatic animals	1.01	0.52	0.98	1449	634	25
Prometryn, aquatic animals	1.80	┘ 2.00	0.95	1519	954	14
Cyanazine, aquatic animals	1.88	┘ 2.70	0.98	2628	1684	14
Atrazine, fish	2.17	┘ 3.98	0.92	3592	2442	16
Metribuzin, aquatic animals	1.56	┘ 2.29	0.89	7074	4140	9
Prometon, aquatic animals	3.62	┘ 11.08	0.98	12312	9771	10
Hexazinone, aquatic animals	2.28	┘ 7.50	0.88	81109	56246	12

[a] The equation for the linear regression y ffl a(slope) x ⎪ b(intercept) is derived from $\log_{10}$ and probit data transformed for the purposes of regression. Back-transforms were used to calculate intercepts.
[b] n is the number of data points in the data set.

were taken from a previous publication (Solomon *et al.*, 1996) and reanalyzed using the procedures described below. These data are included in Table 28.2 for comparison. It should be noted that a more recent risk assessment of atrazine has been completed and has incorporated additional toxicity data (Giddings *et al.*, 2005). The results of this new assessment are similar to those presented in Table 28.2.

Several recent papers have suggested that atrazine may have endocrine-mediated effects on frogs at environmentally relevant concentrations (Hayes *et al.*, 2002; Hayes *et al.*, 2003; Hayes, 2004; Hayes *et al.*, 2006a, b). Others have failed to be able to repeat these observations at small concentrations in laboratory studies (Carr *et al.*, 2003; Coady *et al.*, 2004; Coady *et al.*, 2005), under semi-field conditions (Jooste *et al.*, 2006; Du Preez et al., 2007), or in the field where frogs have been exposed to triazines used in corn production for many years (Du Preez *et al.*, 2005a, b; Smith *et al.*, 2005). The putative mechanism – that of induction of the enzyme aromatase – has not been observed in atrazine-exposed frogs in the field (Hecker *et al.*, 2004) or in the laboratory (Hecker *et al.*, 2005). In addition, robust populations of frogs have been reported in areas close to agricultural production, and no apparent relationship to atrazine was observed (Knutsen *et al.*, 2004). The Australian Pesticides and Veterinary Medicines Authority (APVMA, 2004) recently reviewed the available studies on the potential effects of atrazine on amphibians. The APVMA (2004) concluded that: 'Inconsistencies between studies, the difficulty in replicating the low dose effects of atrazine in amphibians and the likely influence of other stressors, together with the occurrence of healthy amphibian populations at sites where atrazine is present, indicate that it is unlikely that atrazine is impacting adversely on populations of Australian amphibians at current levels of exposure.'

In light of all of the lines of evidence, atrazine does not cause endocrine-modulated or reproductive effects in amphibians at small concentrations such as are found in the environment (Solomon *et al.*, 2005). The USEPA recently reviewed the literature and a comprehensive laboratory study and concluded that atrazine does not affect gonadal development in amphibians (USEPA, 2007). Given that atrazine does not cause these responses, it is unlikely that other structurally related chlorotriazines cause responses in frogs; however, no studies on reproductive or endocrine effects of other triazines on amphibians have been reported in the literature.

Because of the greater toxicity of the triazines toward plants, these data were analyzed separately. Inspection of the data revealed insufficient tests for some triazines in certain groups of fish, arthropods, and other organisms. Therefore, the data for aquatic animals were not separated into groups. Normally, this is a useful technique for differentiating the assessment of risks in organisms, such as plants and groups of animals that may not be able to tolerate the same return frequencies of adverse effects to the same degree. However, because of the richness of the data set for atrazine, aquatic animals were divided into fish and arthropods for the distributional analysis.

Some of the toxicity data were obtained from tests with formulated products. Toxicity data for formulated products were generally similar to those for the technical material and, for this reason, were included in the data sets. In all cases, the effect concentration was converted to active ingredient to allow for combination and comparison. Where data from multiple studies on the same species were available, the geometric mean of the toxicity values was used to represent the species. In the few instances where sensitivity of different life stages of the same species were reported, these did not differ greatly and the geometric mean for these data were also used to represent the species in the distribution. The geometric mean results in a relatively conservative combination of data from different tests, allowing all the data to be used in the distributions without assigning greater weight to a species or particular test with more data. In some cases, particularly for insensitive organisms, toxicity values were reported as 'greater than' a certain concentration. These data were omitted from regressions of the cumulative frequency distributions, but they were included in the calculation of ranks. The likelihood that these concentrations would ever be exceeded is extremely small, and they are thus of minimal significance in the risk assessment process. Although some reported EC_{50} and LD_{50} values were close to the reported water solubility values for the herbicides, these did not greatly exceed the maximum water solubility and were not excluded from the analysis.

The plotting positions for the graphs were calculated from the formula $100 - i/(n \mid 1)$ (Parkhurst *et al.*, 1996), where i is the rank of the datum and n is the total number of data in the set. These plotting positions are expressed as percentages. Data were plotted using a log-normal transformation and linear regressions that were performed with the aid of the SigmaPlot 6 graphics package (SPSS, 2000). Although a number of other models might produce a better fit (Versteeg *et al.*, 1999), use of the log-normal model for characterizing toxicity distributions has been recommended (Burmaster and Hull, 1997) and is supported by observations in other studies (Klaine *et al.*, 1996; Solomon and Chappel, 1998; Giesy *et al.*, 1999; Giddings *et al.*, 2000; Hall *et al.*, 2000; Solomon *et al.*, 2001, 1996).

Lower centiles of the toxicity distribution of a substance may be used as a convenient working criterion (assessment endpoint) for characterizing toxicity. From a theoretical point of view, any measure such as the 5th, 10th, 20th, or the 25th centiles could be used for assessment purposes, provided that this measure could be validated against a knowledge and understanding of ecosystem structure and function or calibrated in tests conducted in microcosms, mesocosms, or in the field. The 10th centile of LC_{50} data has been observed to be conservative when compared to other responses in mesocosms (Solomon *et al.*, 1996; Giesy *et al.*, 1999; Giddings *et al.*, 2000, 2001). Based on these observations, the 10th centile may be useful as a primary assessment measure; however, other centiles were calculated for comparison.

Results

Toxicity Distributions

Toxicity distributions for the triazines are shown in Figures 28.1–28.3 and are summarized as the 10th and 5th centile values in Table 28.2. The distribution for atrazine is not presented in a graph, but is similar to what was previously published (Solomon *et al.*, 1996) and recently updated (Giddings *et al.*, 2005). For both plants and animals, the data in Table 28.2 are presented in descending order of toxicity. Several of the triazines had limited data for assessing their sensitivity to aquatic plants. Only a single data point was available for terbutryn, while hexazinone, metribuzin, cyanazine, and prometon had 2, 3, 5, and 5 data points, respectively. For smaller data sets, the point estimates of the lower centiles are conservative (Solomon, 1996) so that the low position of hexazinone in rank is partially accounted for. Except for hexazinone and prometryn, the slopes of the distributions were similar, indicating a similar range of sensitivity to the plants tested. The most sensitive plants were generally algae. This may be due to their inherent sensitivity, but more likely is due to the nature of the test and its endpoint, which is growth rather than toxicity. A more recent review of toxicology data for atrazine has shown that macrophytes are slightly more sensitive than algae (Giddings *et al.*, 2005). A 50% reduction in growth would be expected to occur at a lower concentration than 50% mortality (Faber *et al.*, 1997). The range of sensitivity in aquatic plants was about 80, with prometryn having the lowest 10th centile and prometon the greatest. There are several possible explanations for this observation. These include differences in binding to the active site, differences in rate of detoxification, and differences in biological availability from the surrounding matrix.

Aquatic animals were less sensitive to the triazines than were aquatic plants. Toxicity to fish and arthropods varied, with fish or crustaceans sharing the position of most sensitive aquatic animal, depending on the herbicide. In the case of atrazine, arthropods were generally more sensitive than fish. Ratios of the 10th centiles between animals and plants were varied. The animal/plant ratio for atrazine (arthropods) was 24, while hexazinone was 202 800. The hexazinone data were from a small data set, and the atrazine data were for arthropods and could not be compared to data for the other triazines. However, the ratios for the other triazines also varied over an order of magnitude, from 176 for prometon to 1688 for prometryn. Since the molecular weights of the triazines are similar, one would expect their

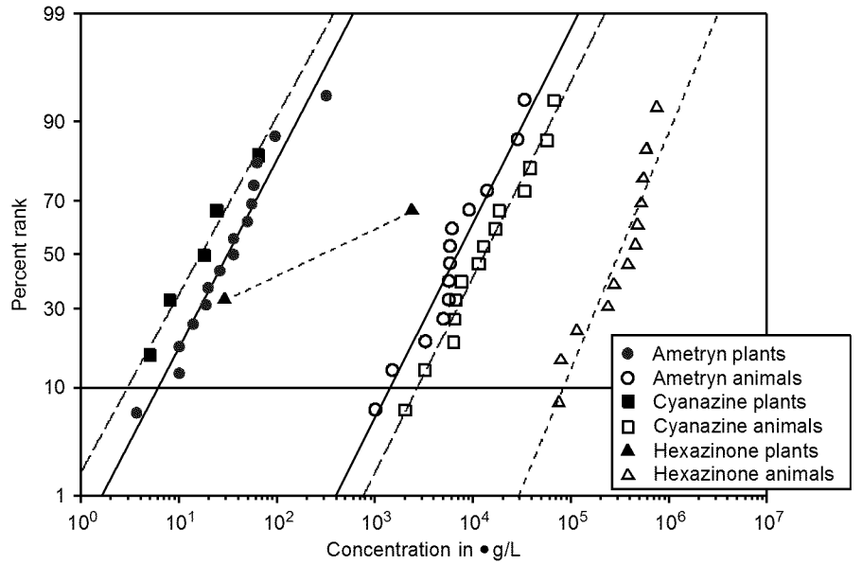

Figure 28.1 Distributions of acute toxicity values (LC-and EC-50s)[a] for ametryn, cyanazine, and hexazinone to aquatic plants and aquatic animals.
[a] EC_{50} ffl effective concentration causing a specifi ed effect in 50% of the tested population. LC_{50} ffl lethal concentration causing death in 50% of the population tested.

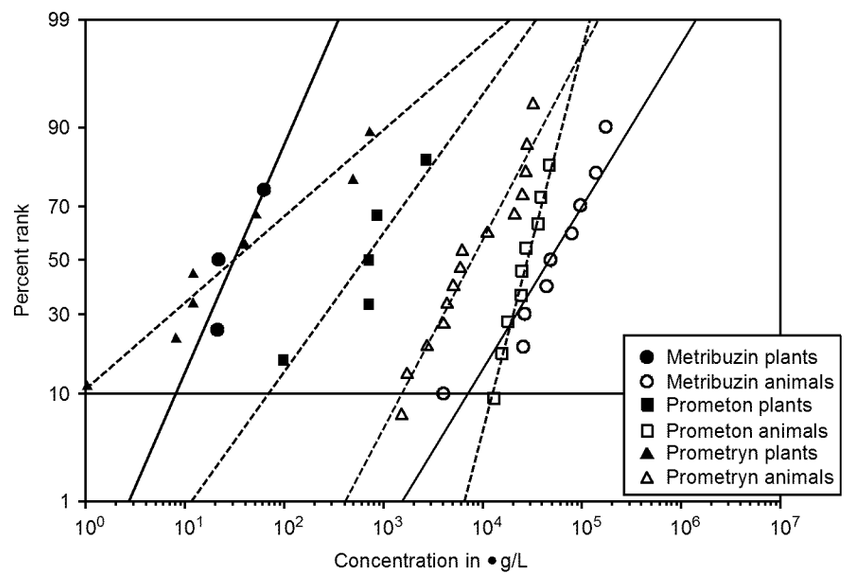

Figure 28.2 Distributions of acute toxicity values (LC-and EC-50s)[a] for metribuzin, prometon, and prometryn to aquatic plants and aquatic animals.
[a] EC_{50} ffl effective concentration causing a specifi ed effect in 50% of the tested population. LC_{50} ffl lethal concentration causing death in 50% of the population tested.

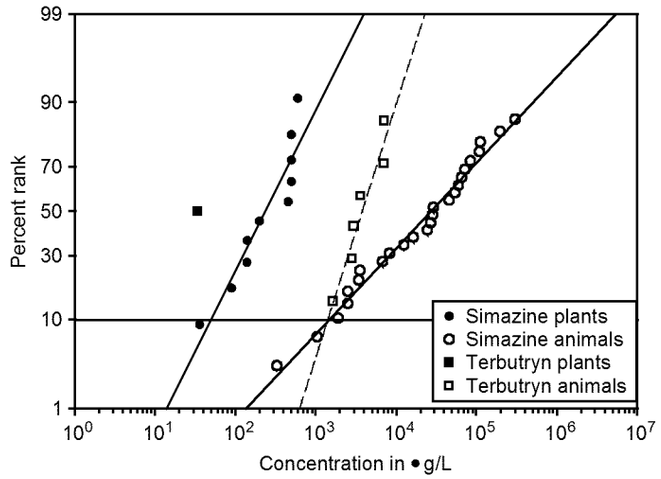

Figure 28.3 Distributions of acute toxicity values (LC- and EC-50s)[a] for simazine and terbutryn to aquatic plants and aquatic animals.
[a] EC_{50} ffl effective concentration causing a specifi ed effect in 50% of the tested population. LC_{50} ffl lethal concentration causing death in 50% of the population tested.

toxicity to nontarget organisms to be similar and likely mediated by nonspecific or narcotic mechanisms (Lipnick, 1993). That this is not the case indicates that different triazines may affect nontarget aquatic organisms via different mechanisms, or that the pharmacokinetics of these substances may be different.

The lower centiles derived from the toxicity distributions of the triazine herbicides could be used to assess the relevance of environmental concentrations, especially if these concentrations are also analyzed through the use of distributional approaches (Solomon, 1996; Solomon *et al.*, 1996). These toxicity distributions may also be used to refine monitoring and stewardship programs (Solomon, 1999; Giddings *et al.*, 2005).

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Chapter 29

Atrazine and Simazine Monitoring Data in Community Water Systems in the United States during 1993 to 2000

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Summary

A population-linked database was developed to assess exposure to the herbicides atrazine and simazine in the drinking water of community water systems (CWS) fed by groundwater and surface water sources in 32 major-use states. These states represent about 99% of the annual atrazine and simazine use in the United States. Herbicide concentration and population data from 1993 through 2000 were paired for each water system and then aggregated to construct state and multistate exposure profiles.

There are 41 362 CWS in these 32 states, serving a population of 213 million. Sixty-eight percent of the systems (28 280) had monitoring data for atrazine. Sixty-eight percent (27 959) had data for simazine. Eighty-five percent (182 million) of the population was assessed for atrazine and 86% (183 million) for simazine. The assessed populations in these 32 states represent 78% of the US population.

The majority of the population served by these systems had no detectable exposure to atrazine and simazine during the eight monitoring years. Overall, 92.3% of the atrazine samples from CWS on groundwater and surface water did not have detections of atrazine, and 98.8% of the samples did not have detections of simazine. All of the simazine data (100% of the population) and 99.9992% of the atrazine data reflected multiyear means during the 8-year period of 1993–2000 that were below the respective lifetime maximum contaminant levels (MCLs) for drinking water. Three of the 28 280 water systems with atrazine data had multiyear mean concentrations above the MCL of 3.0 ppb, ranging from 3.30 to 3.41 ppb. The margin of safety for the populations associated with these three CWS (1495 people) was nearly 1000, based on the United States Environmental Protection Agency (USEPA) lifetime drinking water reference dose. None of the CWS exceeded short-term health advisory levels. The margins of safety for exposures to atrazine for the populations served by the other 28 277 water systems were at least 10 000 for 88% of the population and between 1000 and 10 000 for 11.99% of the population, based on the USEPA's lifetime drinking water reference dose. Exposure to simazine for 100% of the assessed population had a margin of safety of at least 10 000 based on the USEPA's lifetime drinking water reference dose.

Introduction

Atrazine and simazine are triazine herbicides that exhibit herbicidal activity on certain annual broadleaf and grass weeds through inhibition of photosynthesis. In the United States, annual atrazine use is the greatest on corn (83%), followed by sorghum (11%), and sugarcane (4%). In 1998, the annual use of atrazine and simazine in the 32 major-use states (Figure 29.1) accounted for 99.3% and 99.9% of the estimated US use, respectively, based on product use survey data. Simazine is used less extensively than atrazine on corn. The major uses of simazine are on fruit (especially citrus, grape, and apple), nuts, and corn crops. In contrast to atrazine, the greatest use of simazine occurs in Florida and California rather than in Midwestern corn-growing states. After an extensive review, atrazine and simazine have been reregistered in the United States (USEPA, 2006).

Atrazine has been detected in surface water and groundwater in several of the major-use states (Keck, 1991; Thurman *et al.*, 1991; Goolsby *et al.*, 1991a, b; Hall *et al.*, 1999; USGS, 2006). Typically, groundwater detections of atrazine occur much less frequently than surface water detections. Also, groundwater detections are usually lower in concentration than those in surface water. In surface water, atrazine concentrations in streams and rivers are episodic,

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Figure 29.1 Thirty-two major-use states for atrazine and simazine are darkened.

with major peaks in the spring and early summer after field application in April and May. In impounded water bodies (reservoirs), the peak concentrations are usually lower than in rivers and also occur in April and May; however, the duration may be longer due to longer hydraulic residence time. Simazine is detected less frequently than atrazine in groundwater and surface water in the United States and at lower concentrations (Keck, 1991; Thurman *et al.*, 1991; Goolsby *et al.*, 1991a; Baker, 1998).

The United States Geological Survey (USGS) has provided extensive reviews on the distribution, trends, and governing factors on pesticides in groundwater and in surface water (Barbash and Resek, 1996; Larson *et al.*, 1997; USGS, 2006). These reviews included data on several triazines and chlorotriazine metabolites and are helpful in understanding the environmental fate of triazines within the hydrologic cycle in the Midwestern United States.

In the late 1980s, the USEPA conducted a drinking water national survey of rural individual and CWS wells for more than 100 pesticides, including atrazine and simazine (USEPA, 1990). This survey included only groundwater sources. Iowa (Iowa Department of Natural Resources, 1988; Kross, 1990), Minnesota (Klaseus, 1988), and Wisconsin (Wisconsin Department of Agriculture, Trade and Consumer Protection, 1989) also conducted private well and CWS pesticide surveys of drinking water in the late 1980s and early 1990s. Atrazine and simazine were included. From 1992 to 1996, the USGS monitored a certain number of domestic and community system wells in each of the 60 nationwide watershed study-unit basins in the National Water Quality Assessment Program (NAWQA) (Gilliam *et al.*, 1995; USGS, 2006). The primary focus of many of the drinking water studies was groundwater, and the studies were often limited to only 1 year of data. Typically a linkage of population to exposure was not made in these studies.

A more specific population-linked drinking water exposure assessment was conducted in Ohio for several herbicides, including atrazine, for CWS on groundwater and surface water sources and individual wells (Baker and Richards, 1990). This exposure assessment used drinking water data from community systems and ambient surface water monitoring data from other sites. Similarly, Richards *et al.* (1995) conducted a drinking water exposure assessment for atrazine in three key use states (Illinois, Iowa, and Ohio) for populations served by community systems and rural individual wells. However, actual finished drinking water data were not uniformly available for atrazine and simazine from CWS on groundwater and surface water sources at that time.

Table 29.1 Health Advisory Levels (HALs) and Maximum Contaminant Levels (MCLs) for atrazine and simazine from USEPA (1989a, 2000)

		Atrazine			Simazine		
		MCL	HAL	Safety factor	MCL	HAL	Safety factor
Exposure	Duration	(ppb)	(ppb)		(ppb)	(ppb)	
1-day, child	5 consecutive days	–	100	100	–	500	100
10-day, child	14 consecutive d	–	100	100	–	500	100
7-year, child	Approx. 7 years	–	50	100	–	70	100
7-year, adult	Approx. 7 years	–	200	100	–	70	100
70-year, adult	Lifetime	3	3	1000	4	4	1000

In this report, monitoring data collected for January 1993 to December 2000 from CWS in 32 states expands on these past assessments of atrazine and simazine in drinking water. Herbicide exposure and population data from 1993 through 2000 are reported. These results provide a more complete assessment of the herbicides' frequency of occurrence and concentrations for populations served by community groundwater and surface water sources through the development of a population-linked exposure (PLEX) database. The results add substantially to the body of knowledge on drinking water exposure to atrazine and simazine and allow the evaluation of exposure relative to the established federal drinking water standards. This review also shows the variation in drinking water exposure between groundwater and surface water sources serving CWS. Two other chlorotriazine herbicides, cyanazine and propazine, could not be included in the exposure assessment due to insufficient monitoring data.

USEPA Drinking Water Standards

USEPA developed drinking water health advisory levels for atrazine and simazine in 1988 (USEPA, 1989a). Health advisory levels are defined as the concentration of a chemical in drinking water that is not expected to cause any adverse effects for up to a certain number of consecutive days of exposure or a certain number of years of exposure, calculated with a margin of safety (Table 29.1). The USEPA Safe Drinking Water Act (SDWA) also established MCL and monitoring requirements to be initiated in 1993 for several pesticides, including atrazine and simazine (USEPA, 1991). The recommended health advisory levels (HAL) and enforceable MCLs are permissible concentrations in drinking water at which adverse health effects would not be expected to occur for the specified duration of exposure. Both HALs and MCLs are based on the 'no observable effect' level in animal toxicity studies.

Beginning in 1993, the USEPA initiated compliance monitoring of finished water for atrazine, simazine, and several other chemicals. Surface water supplies were monitored quarterly, and groundwater supplies were monitored once or twice annually. The purpose was to assess annual running mean concentrations of atrazine and simazine for each CWS for compliance with their respective MCLs (Table 29.1).

Methods

Triazine Herbicide Major-Use States

A hierarchical protocol was developed to determine the segments of the United States population served by CWS (USDA, 1992) with potential exposure to atrazine and/or simazine. Based on agricultural land-use data from the United States Department of Agriculture (USDA, 1992), product-use survey data by county from 1990 to 1998, and summary national herbicide survey information (USEPA, 1992a, b), the 32 major-use states were selected for quantitative exposure assessment (Figure 29.1). These 32 states represent 83% (234 million) of the total US population (United States Bureau of Population Census, 1994) and 99.3% and 99.9% of the estimated annual atrazine and simazine use in pounds, respectively, in the United States in 1998. The highest atrazine use states are generally in the Midwest and include Illinois, Iowa, Nebraska, Indiana, Kansas, Ohio, and Missouri. The highest simazine-use states are California and Florida.

PLEX Database

At the time the PLEX database was initiated, drinking water is provided to nearly 243 million people, or 94% of the total US population, by 58 000 community-based water systems (USEPA, 1993). The other 15 million people (6%) received drinking water from private wells or other nonregulated systems (USEPA, 1993). A CWS, as regulated under

the SDWA, is defined as a facility that provides piped water for human consumption to at least 15 service connections and provides water to the same population year round. A community system can use different raw water sources: groundwater, surface water (rivers, lakes, and reservoirs), or blends of both.

There are 41 362 community systems in the 32 major-use states. These facilities provide drinking water to 91% (213 million) of the 234 million people in these states (Table 29.2). SDWA quarterly compliance-monitoring data for atrazine and simazine from community systems in the 32 major-use states were obtained from the state regulatory agencies. These primary data represent an 8-year period (January 1993 to December 2000). There are 28 280 CWS (68%) with 146 683 samples analyzed for atrazine in the PLEX database (Table 29.3) and 27 959 community systems (68%) with 137 956 simazine data points (Table 29.4).

The majority (78%) of the CWS in the 32 states use groundwater as the raw water source (Table 29.2). Thus, the two herbicides' databases contain more groundwater (four to five times) than surface water samples (Tables 29.3 and 29.4). The most frequently used limits of quantification (LOQ) for the analysis of atrazine were equal to or less than 0.5 ppb (1/6 of MCL) in 28 states, 0.6 ppb in one state, and 1.0 ppb and 2.5 ppb in the other two states, respectively. Prior to 1997, two states had an LOQ at the MCL of 3 ppb. The LOQs were lowered in 1997 and subsequent years to less than 1 ppb. The LOQs for simazine were equal to or less than 0.8 ppb (~1/5 of MCL) in 27 of the 31 states. LOQs of 1.0 ppb (1/4 of MCL) and 2.0 ppb (1/2 of MCL) were used in the other four states. By 2005, almost all CWS in the United States used LOQs of 0.1 ppb or lower.

Since the PLEX databases for atrazine and simazine were dominated by samples with nondetections, the exposure profiles (Tables 29.5 and 29.6) are primarily driven by the LOQ concentration. To develop the PLEX databases, a numerical value had to be assigned to the samples with nondetectable residues. Following USEPA guidance, a concentration of one-half the detection limit was assigned to all samples reported as nondetections (USEPA, 1989b). The substitution value is arbitrary (Helsel, 1990; Helsel and Hirsch, 1992) and provides no actual knowledge of the concentration values below the reporting limit. However, it does provide a conservative estimate of drinking water exposure by assuming that all nondetection samples have atrazine or simazine present at one-half the LOQ.

The CWS with atrazine and simazine monitoring data served populations of 182 and 183 million, respectively (Tables 29.3 and 29.4). The populations associated with atrazine monitoring data represent 85% of community systems and 78% of total populations, respectively, in the 32 states (Table 29.3). The populations assessed for simazine exposure were 86% and 78% of the assessed water systems and total population, respectively (Table 29.4).

Herbicide concentration and population data were then paired for each CWS and aggregated to construct state and multistate exposure profiles for each herbicide. All data were entered into individual state PLEX databases along with population data and source water type (groundwater; surface water including river, reservoir, or lake; or 'other' for blended waters). Average concentrations for the two herbicides in finished drinking water were determined for each water system. When several annual means were available, the exposure concentration is the average of all available annual records since 1993. From these state-specific databases, an aggregate or multistate exposure profile was developed for each herbicide for the three source-water classifications. For each category, the number of water systems and the populations served by these facilities were totaled. In this manner the atrazine and simazine multistate exposure profiles were developed (Tables 29.5 and 29.6). USEPA guidance was used to develop protocols for data collection, database preparation, and data analysis (USEPA, 1989b).

Results

The exposure of populations served by CWS to atrazine and simazine in each of the 32 states was evaluated using SDWA compliance monitoring data collected between January 1993 and December 2000 (Tables 29.3 and 29.4). These data represented the best available information from state SDWA agencies in the 32 states. Drinking water data entered into the PLEX database provided a direct link between the population and the estimated concentration of atrazine and simazine in the drinking water.

Atrazine

Atrazine was not detected in 92.3% of the samples (Table 29.5). As expected, there were more nondetections in groundwater than in surface water sources.

Annual average atrazine concentrations from the 28 280 assessed water systems seldom exceeded the MCL of atrazine (3.0 ppb). Overall, 28 277 CWS serving 182 million (99.999%) had multiyear average atrazine concentrations that were either nondetectable or less than the 3.0 ppb MCLs over the 8-year period (Table 29.5).

Three of the 28 280 assessed systems had multiyear average concentrations above 3.0 ppb for the 8-year period (Table 29.5). Each obtained raw water from an impounded (reservoir) surface water source. The multiyear average for atrazine concentrations in these systems ranged from 3.30 to 3.41 ppb.

Table 29.2 Number of CWS, state, and CWS population[a] in the 32 atrazine/simazine major-use states

State	State population	Surface water CWS		Groundwater CWS		Other CWS		Total CWS		Population not served by CWS
		Number in group	Total population	Number in group	Total population	Number in group	Total population	Number in group	Total population	
Alabama	4447100	154	2290269	315	1392632	103	1278236	572	4961137	–
Arkansas	2673400	246	2322708	442	955887	38	148886	726	3427481	–
California	33871648	342	5313703	2446	7208058	567	21703115	3355	34224876	–
Colorado	4301261	263	3423806	565	424375	1	90	829	3848271	452990
Delaware	783600	1	140000	228	537565	1	36130	230	713695	69905
Florida	15982378	45	1349560	1969	14197328	2	123872	2016	15670760	311618
Georgia	8186453	194	5072469	1474	1488840	0	0	1668	6561309	1625144
Hawaii	1211537	6	50894	103	1168883	9	58389	118	1278166	–
Illinois	12419293	495	7574912	1245	2751539	54	520635	1794	10847086	1572207
Indiana	6080485	40	1036988	709	2059915	8	943658	757	4040561	2039924
Iowa	2926324	102	788688	1022	1663380	30	37449	1154	2489517	436807
Kansas	2688418	256	641734	570	702774	96	1088391	922	2432899	255519
Kentucky	4041769	310	3005056	91	287618	53	1034807	454	4327481	b
Louisiana	4468976	69	1897503	1130	2996919	12	96542	1211	4990964	b
Maryland	5296486	45	3495775	450	543956	16	574055	511	4613786	682700
Michigan	9938444	274	5286059	1122	1627620	15	299269	1411	7212948	2725496
Minnesota	4919479	24	715354	913	2410843	17	696817	954	3823014	1096465
Mississippi	2844658	12	324877	1309	2702410	0	0	1321	3027287	b
Missouri	5595211	170	1871411	1201	1666885	80	1262849	1451	4801145	794066
Nebraska	1711263	6	11888	603	863515	8	520237	617	1395640	315623
New Mexico	1819046	40	239240	601	1305024	0	0	641	1544264	274782
New York	18976457	758	12440184	1894	4113438	200	1140487	2852	17694109	1282348
North Carolina	8049313	345	4130755	1850	1295186	59	295981	2254	5721922	2327391
Ohio	11353140	285	6070556	1105	3723701	20	58696	1410	9852953	1500187
Oklahoma	3450654	580	2522024	549	657631	39	262596	1168	3442251	8403
Pennsylvania	12281054	242	5791846	1690	1359004	258	3235787	2190	10386637	1894417
South Carolina	4012012	168	2530277	508	658205	15	139769	691	3328251	683761
South Dakota	754844	112	152724	349	250442	12	237584	473	640750	114094
Tennessee	5689283	311	2808675	252	1485588	65	768759	628	5063022	626261
Texas	20851820	795	9035941	3519	6392418	221	5450482	4535	20878841	b
Virginia	7078515	302	5787063	1012	422971	7	68706	1321	6278740	799775
Wisconsin	5363675	39	1498186	1080	1966832	9	192822	1128	3657840	1705835
Total	234067996	7031	99621125	32316	71281382	2015	42275096	41362	213177603	20890393

[a] As of December 2000.
[b] Indicates that population not served by CWS could not be determined because population on CWS was greater than the state census population.

Table 29.3 Major-use states with Population-Linked Exposure (PLEX) data for atrazine over an 8-year period from January 1993 to December 2000.

Data	Groundwater	Surface water	Other/Blended	Totals
Number of samples	107 106	24 775	14 802	146 683
Number of CWS with data	21 380	5394	1506	28 280
Percent CWS with data	66.16	76.72	74.74	68.37
Major-use population				234 067 996
Population on CWS	71 281 382	99 621 125	42 275 096	213 177 603
Population served by CWS with data	58 423 916	84 164 592	39 298 183	181 886 691
Percent population assessed	24.96	35.96	16.79	77.71
Percent CWS population assessed	81.96	84.48	92.96	85.32

Table 29.4 The major-use states with Population-Linked Exposure (PLEX) data for simazine over an 8-year period from January 1993 to December 2000.

Data	Groundwater	Surface water	Other/Blended	Totals
Number of samples	101 728	21 995	14 233	137 956
Number of CWS with data	21 147	5308	1504	27 959
Percent CWS with data	65.44	75.34	75.20	67.60
Major-use population				234 067 996
Population on CWS	71 286 072	99 683 190	42 208 341	213 177 603
Population served by CWS with data	58 287 934	85 064 972	39 494 212	182 847 118
Percent population assessed	24.90	36.34	16.87	78.12
Percent CWS population assessed	81.77	85.34	93.57	85.77

Table 29.5 Multiyear mean exposure to atrazine compared to the atrazine lifetime MCL of 3.0 ppb over a multiyear average from January 1993 to December 2000[a] for 32 major-use states.

Group	Number	Population served	Percent of population
Surface water ffi3.0 ppb	3	1495	0.002
Surface water ffl3.0 ppb or not detected	5391	84 163 097	99.998
Groundwater ffi3.0 ppb	0	0	0.0
Groundwater ffl3.0 ppb or not detected	21 380	58 423 916	100.0
Other (blends) ffi3.0 ppb	0	0	0.0
Other (blends) ffl3.0 ppb or not detected	1506	39 298 183	100.0
Total ffi3.0 ppb	3	1495	0.0008
Total ffl3.0 ppb or not detected	28 277	181 885 196	99.9992

[a]Database dominated by samples with nondetections (92.3%).

Simazine

Simazine was detected less frequently than atrazine in the quarterly samples from CWS. It was not detected in 98.8% of the samples (Table 29.6). Again, groundwater samples had more nondetections than surface water.

Overall, 27 959 community systems serving 183 million (100%) had average simazine concentrations that were either nondetectable or less than the MCL (lifetime HAL) of 4.0 ppb over the 8-year period (Table 29.6). No community system had a multiyear mean for simazine concentration that was above the MCL of 4.0 ppb.

Data Limitations or Uncertainty

The PLEX database is considered to be a conservative representation (overestimation) of potential exposure for populations served by CWS. The population-linked estimates of atrazine and simazine concentrations represent exposure to persons consuming potable water from regulated water supplies. However, this exposure could be overestimated. The atrazine and simazine databases are dominated by nondetections (92.3% and 98.8%, respectively). Many water

Table 29.6 CWS population exposure to simazine compared to simazine lifetime MCL of 4.0 ppb over a multiyear average from January 1993 to December 2000[a] for 32 major-use states

Group	Number	Population served	Percent of population
Surface water ffi4.0 ppb	0	0	0.0
Surface water ffl4.0 ppb or not detected	5308	85 064 972	100.00
Groundwater ffi4.0 ppb	0	0	0.0
Groundwater ffl4.0 ppb or not detected	21 147	58 287 934	100.0
Other (blends) ffi4.0 ppb	0	0	0.0
Other (blends) ffl4.0 ppb or not detected	1504	39 494 212	100.0
Total ffi4.0 ppb	0	0	0.0
Total ffl4.0 ppb or not detected	27 959	182 847 118	100.0

[a]Database dominated by samples with nondetections (98.8%).

systems with an estimated atrazine exposure concentration of less than 3.0 ppb are based on samples reported as nondetections. Similarly, almost all of the systems with an exposure concentration for simazine of less than 4.0 ppb are based on nondetection samples. It is expected that the actual population exposure in these systems is lower than estimated because an exposure concentration equal to ½ of the LOQ was assigned to nondetection samples.

Other data limitations exist and introduce additional uncertainty. Not all CWS have finished-water monitoring data. The absence of monitoring data is usually due to two factors: (1) a monitoring waiver has been granted to the CWS by the state SDWA agency or (2) the system purchases finished water from another CWS source. In addition, populations on private wells within a state were not evaluated since they do not receive water from a community system. Some community systems, especially those fed by groundwater, are represented by exposure concentrations based on less than four quarterly samples.

Other Chlorotriazines and CWS Exposure Assessments

The four major chlorotriazines used in US agriculture over the past five decades are atrazine, cyanazine, propazine, and simazine. Atrazine typically had the largest annual use and widest use distribution throughout the United States. Propazine's annual use was usually the lowest of the four herbicides, and the use pattern was more geographically restricted due to its primary use on sorghum. Cyanazine was used primarily on corn and cotton, and its corn use pattern was similar to that of atrazine.

There are limited monitoring data for cyanazine and propazine from CWS with groundwater and surface water sources. USEPA (1989a) has established lifetime HALs of 1.0 and 10.0 ppb for cyanazine and propazine, respectively.

In the absence of drinking water monitoring data for CWS, a PLEX assessment for 1993–2000 was not conducted for either chemical. Cyanazine use in the United States was discontinued after 2002 under a negotiated phase-out settlement between the major registrant and USEPA. Since 1989, use of propazine has been limited to sorghum under localized, state-specific USEPA Section 18 authorizations, pursuant to Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) requirements. In 2007, propazine was registered for use in sorghum by the USEPA.

Triazine Metabolites in Drinking Water

The herbicides atrazine and simazine undergo physical, chemical, and biological degradation in the soil, as well as in the water column (Wackett *et al.*, 1998) and in sediment associated with bodies of water (Mersie *et al.*, 1998; Seybold *et al.*, 1999). Atrazine degrades to deethylatrazine (DEA) and deisopropylatrazine (DIA), while simazine degrades to DIA. Both DIA and DEA degrade further to a diaminotriazine metabolite (DDA). The three metabolites retain the chlorine molecule and are termed chlorotriazines. The chlorotriazine metabolites are considered toxicologically similar to the parent herbicides, atrazine and simazine, in mammalian systems (Breckenridge, 1997). For structures and further information on these and other metabolites of triazine herbicides, see Table 3A of Appendix. Some monitoring programs have included analyses of the three metabolites, and the USEPA has developed mathematical methodology using regression equations to estimate the levels of chlorotriazine metabolites in surface water (USEPA, 2003).

Federal drinking water standards in the United States have not been promulgated for the three chlorotriazine metabolites or for the hydroxylated metabolites. The government of Australia (National Registration Authority for Agriculture and Veterinary Chemicals, 2004) has recommended a lifetime drinking water standard (health value) of 40 ppb for atrazine. This recommendation includes consideration to include atrazine-specific metabolites with atrazine in the definition of a guideline value.

Exposure Reduction in Drinking Water

The adoption of 'best management practices' can reduce the storm-related runoff of atrazine, simazine, and other moderately soluble herbicides from fields into bodies of surface water. Several papers (Fawcett *et al.*, 1994; Hirsch *et al.*, 1997; USDA-NRCS, 2000; Krutz *et al.*, 2005) note how these in-field practices can be beneficial to water quality.

These practices, which include conservation tillage in concert with riparian and in-field vegetated buffer strips and grass waterways, affect the volume of both runoff water and eroded soil and the pesticide concentrations in both. Reductions in pesticide concentrations due to infiltration into the soil profile within the buffer strip can range from 11% to 100% (USDA-NRCS, 2000). Similarly, conservation tillage (e.g., no-till, reduced-till, and ridge-till) can reduce herbicide runoff by 42–70% as compared to the moldboard plow (Fawcett *et al.*, 1994). Together, these in-field best management practices can help reduce herbicide loss in surface runoff water and help protect the source water.

In addition to the 8-year drinking water (1993–2000) exposure assessment for CWS, monitoring studies from Iowa and Minnesota have shown a decrease in the concentration and/or frequency of occurrence of atrazine in groundwater and surface water. In 1998, Skopec and Hoyer analyzed pesticide monitoring data from the Iowa Pesticide Water Resources Database, which contains surface and groundwater monitoring data from a wide range of datasets (Skopec *et al.*, 1998). The rate of atrazine detection in groundwater (percent of samples with atrazine detected at any concentration) declined from about 30% in 1982 to about 5% in 1995. Linear regression analysis showed significant downward trends in atrazine concentrations in wells. A similar analysis of atrazine in surface water from the same database showed that atrazine detection rates declined from more than 90% in 1982 to about 60% in 1995. Linear regression analysis showed that atrazine concentrations declined significantly during the period. The authors attributed these water quality improvements to adoption of BMPs by Iowa farmers. More recently, a 2005 report of atrazine in Minnesota groundwater by the Minnesota Department of Agriculture stated, 'Results of trend analysis reveal that in all cases where a statistically significant trend is measurable the corresponding trend is always downward. This indicates clear evidence that, in a general sense, the concentration of atrazine and its degradates is declining in Minnesota's groundwater' (Minnesota, 2005).

Conclusions

This drinking water exposure assessment is the most comprehensive study to date in the United States to evaluate an agricultural product's presence in drinking water provided by CWS. It assesses a population of 183 million out of 213 million who receive drinking water from 41 362 water systems in the 32 major atrazine and simazine use states (Tables 29.2–29.4). The objective was to better assess the two products' frequency of occurrence and estimated exposure for populations over an 8-year (1993–2000) period.

The CWS monitoring data for the 32 major-use states represent a reasonably conservative estimate of the exposure of US populations to atrazine and simazine through drinking water. This is illustrated for atrazine (Figure 29.2) and simazine (Figure 29.3) by comparing the toxicological end-points (used by USEPA to establish a lifetime drinking water reference dose) with the individual herbicide's MCL, and the actual concentration profiles (1993–2000) for the three types of water source categories. The margin of safety is calculated from the 'no observable effect level' used to establish the drinking water reference dose for each chemical (USEPA, 1989a). Margin of safety equals the reference dose divided by the water exposure (margin of safety = reference dose/exposure concentration). The lifetime drinking water MCL for atrazine (3.0 ppb) has a 1000-fold safety factor, while simazine (4.0 ppb) has a 10 000-fold safety factor incorporated into the calculation. Exposure equal to or less than 0.3 ppb corresponds to a margin of safety of at least 10 000 from the 'no observable effect level' for the most sensitive species tested in animal toxicity studies (Figure 29.2). Additionally, the most exposed population for atrazine (mean concentration 3.30–3.41 ppb) had a margin of safety of approximately 1000 (Figure 29.2). If exposure to simazine was at or less than 0.25 ppb, the exposure corresponded to a margin of safety greater than 10 000 (Figure 29.3). In both Figures 29.2 and 29.3, it should be noted that both sets of data are dominated by samples with nondetections (92.3% for atrazine and 98.8% for simazine), thereby representing conservative exposure estimates.

These exposure concentrations in CWS represent conservative exposure scenarios for persons in the 32 atrazine/simazine major-use states and could be conservatively extrapolated to the other 18 states with populations using drinking water supplied by community systems. Those persons using individual wells and those served by CWS (groundwater and surface water) in the 18 minor-use states (approximately 1% of product use annually) were not assessed. However, it is expected that drinking water exposure to atrazine and simazine would not be greater than, and most likely would be much less than, the exposure profile observed in the community systems of the 32 major-use states (Figures 29.2 and 29.3). For the vast majority of the 47 million people in the 18 minor-use states, atrazine and simazine are not expected to be present in drinking water. Therefore, there is expected to be essentially no or very low exposure to populations relying on CWS in these 18 states (Figure 29.1).

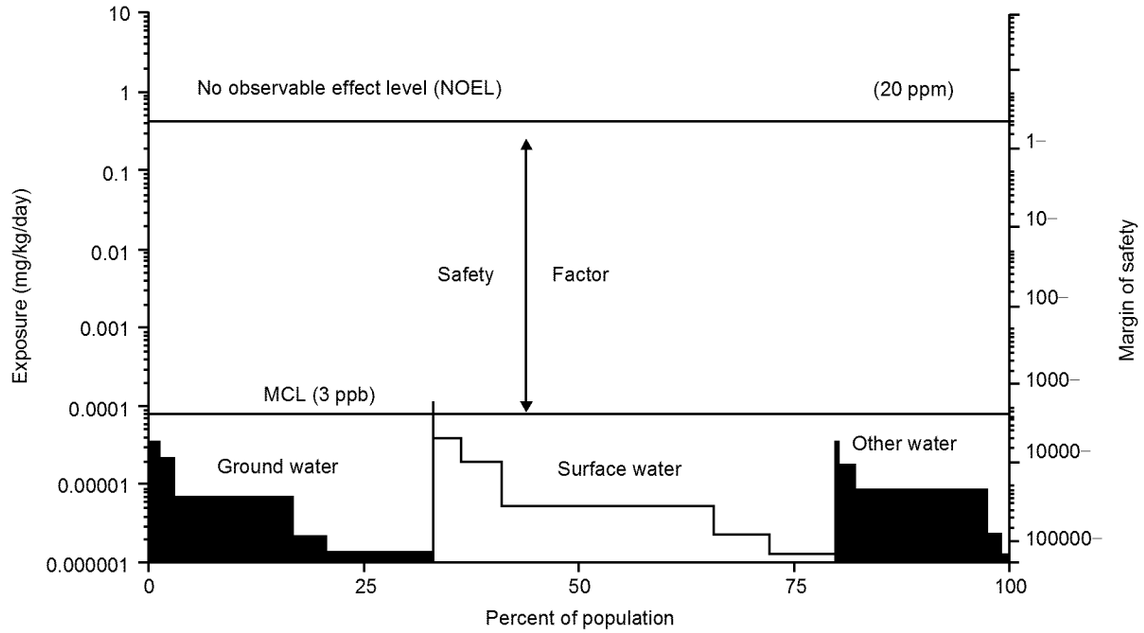

Figure 29.2 Atrazine CWS exposure profile for three water sources and associated percentage of assessed population. Bar width is proportional to the percent of the population in the 32 major-use states exposed in each water source category.

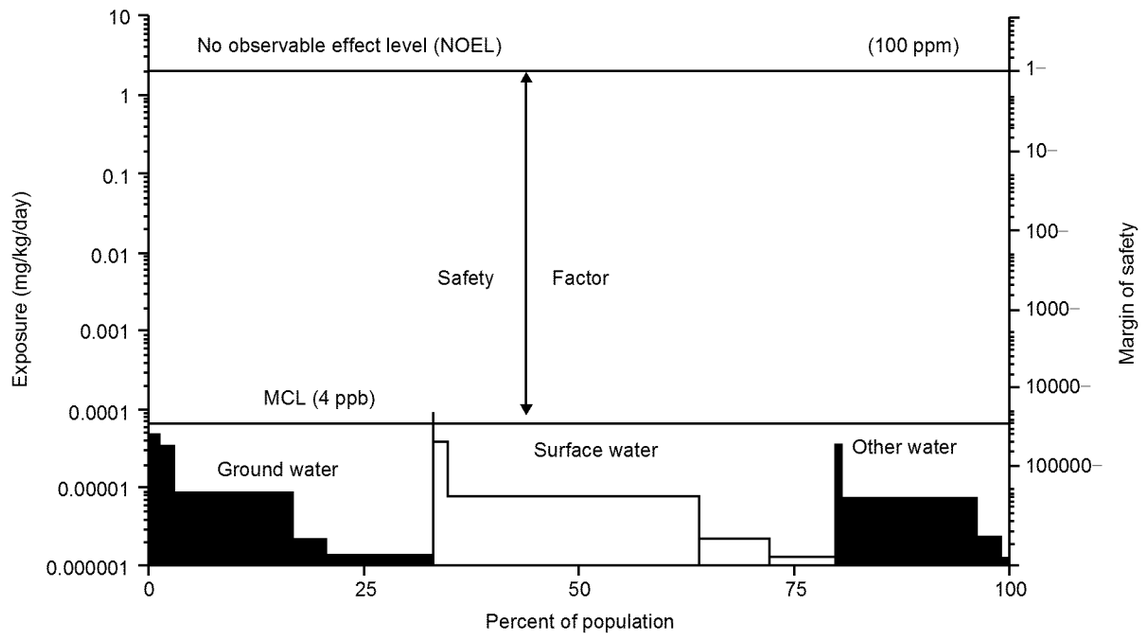

Figure 29.3 Simazine CWS exposure profile for three water sources and associated percentage of assessed populations. Bar width is proportional to the percent of the population in the 32 major-use states exposed in each water source category.

Private wells were not included in the PLEX database. Persons receiving potable water from private wells were not quantitatively assessed, but represent approximately 6% of the US population (USEPA, 1993). National groundwater studies of private wells have shown that more than 98% have nondetectable atrazine concentrations or concentrations | 0.02 ppb (Holden *et al.*, 1992; USEPA, 1992b), and more than 99.8% have simazine concentrations that are either

nondetectable or at levels below 0.38 ppb (USEPA, 1990). These reports indicate that human exposure from private wells is minimal.

The PLEX analysis indicates that exposure to atrazine and simazine in drinking water from community systems at concentrations above MCLs is rare and localized. Levels of atrazine and simazine in water are declining due to changes in agriculture use patterns and greater progress in stewardship practices, including best management practices used by farmers to improve water quality.

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Chapter 30

A Decade of Measuring, Monitoring, and Studying the Fate and Transport of Triazine Herbicides and their Degradation Products in Groundwater, Surface Water, Reservoirs, and Precipitation by the US Geological Survey

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Summary

A number of major studies to analyze triazine herbicides and their degradation products (i.e., metabolites) in water have been carried out by the United States Geological Survey (USGS), Water Resources Division, in the Toxic Substances Hydrology Program. These studies investigated four major water resources – groundwater, surface water, reservoirs, and precipitation.

Reconnaissance studies of groundwater wells in the midwestern United States identified the relationship between land use, groundwater age, and concentration and occurrence of herbicides and their degradation products in groundwater. The studies also described the frequency of herbicide detection in relation to analytical reporting limits, groundwater age in relation to the frequency of herbicide detections, and the persistence of degradation products. Studies revealed that pre-1953 groundwater (before most herbicides were used on a large scale) had a much lower herbicide detection frequency than post-1953 (ffi16% versus 70%). This is logical since the percentage of detections that occur in groundwater is a function of the detection limit of the method used and more sensitive methods continue to be developed. Parent herbicides are not detected as frequently in groundwater as are degradation products. Finally, herbicides that have a long half-life are detected more frequently in groundwater.

Surface water runoff studies in the midwestern United States began in 1989. A reconnaissance study of 147 streams was conducted to determine the geographic and seasonal distribution of herbicides. The data showed that herbicides were flushed from cropland and were transported through the surface water system as pulses in response to late spring and early summer precipitation. Median concentrations of atrazine and cyanazine increased by one order of magnitude and then decreased to near pre-planting levels by harvest sampling. Deethylatrazine (DEA) and deisopropylatrazine (DIA) also occurred in samples, indicating either that some of the parent herbicides remained from the previous year or there was early pre-plant atrazine application before the sampling period. The data also show that the ratio of DEA to atrazine (called the DAR), which has been used as an indicator of atrazine from non-point sources, may be used also as a tracer of movement into rivers. The concentrations of these degradation products vary with the hydrologic conditions of the basin and the timing of runoff. Also, changes in herbicide usage and best management practices have significantly decreased the amount of atrazine and cyanazine concentrations found in surface water. Trends for atrazine concentrations show a median of 10.9 micrograms per liter (μg/L) in 1989, 5.54 μg/L in 1995, and 4.27 μg/L in 1998. There was also a decrease of cyanazine concentration in surface water over years with a median of 2.65 μg/L in 1989, 1.35 μg/L in 1995, and 0.44 μg/L in 1998.

The third major water resource study involved 76 reservoirs located in 11 midwestern states. These studies determined the occurrence and temporal distribution of herbicides and their degradation products in the outflow from selected reservoirs in the upper Midwest; they also explored whether the occurrence of herbicides in the reservoir outflow could be related to drainage-basin characteristics, water and land use, herbicide use, and climate. It was found that reservoirs are repositories for herbicides from midwestern streams and that herbicides and their degradation products were detected more frequently throughout the year in reservoirs than in streams. Reservoirs hold runoff from cropland causing herbicide concentrations to decrease downstream, and they dampen the pulse of herbicides that occur during the spring flush.

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The fourth major water resource examined by these studies was precipitation. The area studied included 26 states from the upper Midwest (where herbicide use is prevalent) and the Northeast. The results identified the occurrence and temporal distribution of triazine herbicides and their degradation products in the Midwest. The highest concentrations occurred following herbicide application to cropland. From mid-April to mid-July in 1990 and 1991, volume-weighted concentrations of 0.2–0.4 μg/L for atrazine were typical throughout the Midwest, and volume-weighted concentrations as large as 0.6–0.9 μg/L occurred in precipitation at several sites. Atrazine was detected most often, followed by DEA, cyanazine, and DIA. Herbicide deposits were greatest in areas where herbicide use was high and decreased with distance from the Midwest.

Introduction

Triazine herbicides in groundwater, surface water, reservoirs, and precipitation have been found throughout the United States. As a result, numerous studies have been completed to document the formation, usage, degradation, fate, and transport of triazine herbicides and their degradation products in the environment. Agricultural practices in the United States often require extensive use of herbicides for crop production. Compiled data indicate that approximately 16% of the 209 million kg of all herbicides applied during 1997 in the United States were triazine herbicides used in crop production in the Midwest (Gianessi and Marcelli, 2000). Voluntary reductions in the recommended application rates have affected the frequency of detection of some of the triazine herbicides and their degradation products. Monitoring the effects of these changes is important in order to understand the occurrence, fate, and transport of these herbicides and their degradation products.

Throughout the decade of the 1990s, the occurrence, fate, and transport of agricultural chemicals have been studied by the USGS Toxic Substances Hydrology Program in the upper midwestern United States (Scribner *et al.*, 2005). The region was selected for study because it is the largest and most intensive area of row-crop agriculture in the country (sometimes referred to as the 'Corn Belt') (Figure 30.1). Consequently, much of the agricultural triazine herbicides used in the United States are applied to crops in this region. The major row crops include corn, sorghum, and soybean, to which approximately 24 million kg of herbicides – such as atrazine, cyanazine, and simazine – were applied in 1997 (Gianessi and Marcelli, 2000). Another important region selected for study by the USGS was the southern United States (Figure 30.1), which includes the Mississippi River Delta (often called the 'Cotton Belt'), the Playa Lakes of Texas, and the cotton- and rice-growing areas of Arizona, Arkansas, and California. Cotton and rice receive equal or greater application rates of herbicides per acre than do corn or soybean. More than 8 million kg of triazine herbicides were applied in these states during 1997 (Gianessi and Marcelli, 2000).

In conjunction with monitoring herbicide and degradation product occurrence, USGS research during the past decade has emphasized the development of methods for the analysis of triazine herbicides and their degradation products. To research and develop analytical methods for pesticides, a laboratory for organic geochemistry was established by

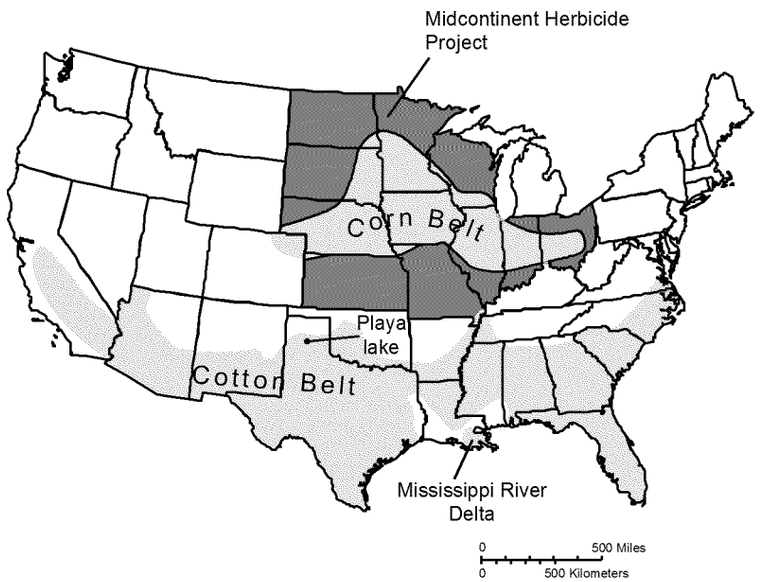


Figure 30.1 Location of USGS study areas for triazine herbicides in midwestern and southern United States.

the USGS in Lawrence, Kansas, in 1988. Analytical methods have been and continue to be developed at the laboratory to measure herbicides and their degradation product concentrations. For example, USGS methods development has included enzyme-linked immunosorbent assays (ELISA) (Thurman *et al*., 1990; Fallon and Thurman, 1996; Pomes *et al*., 1998), automated solid-phase extraction (Mills and Thurman, 1992; Meyer *et al*., 1993; Thurman and Mills, 1998; Thurman and Snavely, 2000), gas chromatography/mass spectrometry (GC/MS) (Thurman *et al*., 1990; Zimmerman and Thurman, 1999; Kish *et al*., 2000), high-performance liquid chromatography (HPLC) (Lerch *et al*., 1997; Zimmerman *et al*., 2000), and liquid chromatography/mass spectrometry (LC/MS) (Ferrer *et al*., 1997, 1999, 2000). Analyses of herbicides and degradation products are listed in Scribner *et al*. (2000b).

The study of the chemistry, fate, and transport of triazine herbicides and their degradation products in surface water and groundwater has also been a major USGS effort. A number of field-dissipation studies have been carried out for atrazine, cyanazine, propazine, and simazine. These dissipation studies have resulted in outlining the structure and transport of many degradation products of the triazine herbicides in aquatic environments (Mills and Thurman, 1992, 1994; Thurman *et al*., 1994). This chapter summarizes triazine herbicide usage, water-quality studies, occurrence and degradation of triazine degradation products, and the potential for transport of triazine degradation products in surface water and groundwater.

Triazine Herbicide Usage

This section discusses usage information for triazine herbicides – including atrazine, cyanazine, prometryn, and simazine. Data for propazine were not available because the herbicide was voluntarily removed from the market by the manufacturer in early 1990 for economic reasons. However, many farmers considered propazine a necessary herbicide and protested its removal (Griffin LLC, 2000). The manufacturer agreed to continue marketing the product in states that applied for temporary use under the Federal Section 18 Special Local Needs Permit. This continued until 1997 when the registrant stopped marketing propazine (Griffin LLC, 2000). Propazine was reregistered by USEPA for use in sorghum in 2007 (USEPA, 2007).

Approximately 31 million kg of triazines were applied to crops in the Corn Belt during 1997. In 1990, the manufacturers of atrazine voluntarily reduced the maximum recommended application rate from 4.5 to 3.4 kg of a.i. (active ingredient) per hectare per year (kg a.i./ha/yr) for corn and sorghum [US Environmental Protection Agency (USEPA), 1990]. The 1990 label change also restricted noncropland uses of atrazine to a maximum of 11.1 kg a.i./ha/yr. This label change was applied to all products released for shipment after September 1, 1990. In 1992, the manufacturers of atrazine again voluntarily reduced the maximum recommended application rate of atrazine on corn and sorghum from 3.4 kg a.i./ha/yr to a range of 1.8 to 2.8 kg a.i./ha/yr, depending on soil surface organic residue and erosion potential. As little as 0.60 kg a.i./ha/yr could be used in subsequent post-emergence applications, and the total of all applications (pre- and post-emergence) could not exceed 2.8 kg a.i./ha/yr (USEPA, 1993). A maximum of 1.8 kg a.i./ha/yr was recommended on soil with less than 30% plant residue remaining on the surface at planting. Most noncropland uses of atrazine are no longer recommended on the manufacturers' labels. This label change applied to all atrazine products shipped for use after August 1, 1992. As a result of these two voluntary label changes, the maximum recommended application rate for atrazine on corn and sorghum was reduced by approximately 50%. The average application rate for atrazine decreased approximately 10%, from an average of 1.38 kg/ha in 1990 to 1.20 kg/ha in 1994 and 1.23 kg/ha in 1995. Changes in herbicide use are shown in Figure 30.2. During the decade of the 1990s, atrazine use in the United States rose to 21 million kg in 1991 and decreased to 17 million kg in 1995, fluctuating to 24 million kg by the year 2001, then declined to 19 million kg during the next year. Besides voluntary reductions in application rates, herbicide use fluctuates in response to annual changes in planted corn and soybean acreage and the introduction of new herbicide products [US Department of Agriculture (USDA), 1991–2003].

Cyanazine was introduced in 1972. Use gradually increased from 9 million kg in 1990 (Gianessi, 1992) to 11 million kg in 1994 (USDA, 1991–1995), then decreased to 9 million kg in 1997 (Figure 30.2). During 1997, approximately 7 million kg of cyanazine were applied in the Corn Belt, and 1.5 million kg were applied in the Cotton Belt. In 1996, the manufacturer of cyanazine began voluntarily reducing the maximum recommended application rate. All products released for shipment after July 25, 1996, stated a reduction in maximum seasonal application rates from 7.3 to 5.6 kg/ha by January 1, 1997, to 3.4 kg/ha by January 1, 1998, and 1.12 kg/ha by January 1, 1999. The production of cyanazine was discontinued on December 31, 1999, and distribution of existing supplies continued through September 30, 2002. Since December 31, 2002, cyanazine is no longer used.

Approximately 2.3 million kg of simazine were applied to corn and various fruit crops throughout the United States during 1997. About 624 000 kg of simazine were used in the Corn Belt; more than 1 million kg of simazine were used in the Cotton Belt during the same timeframe. Prometryn is primarily used in Arizona, California, and the southern states of Arkansas, Louisiana, Mississippi, and Texas. Only 1 763 kg of prometryn were applied to cotton grown in the Corn Belt during 1997, while more than 756 000 kg were applied to cotton grown in the Cotton Belt during that same year.

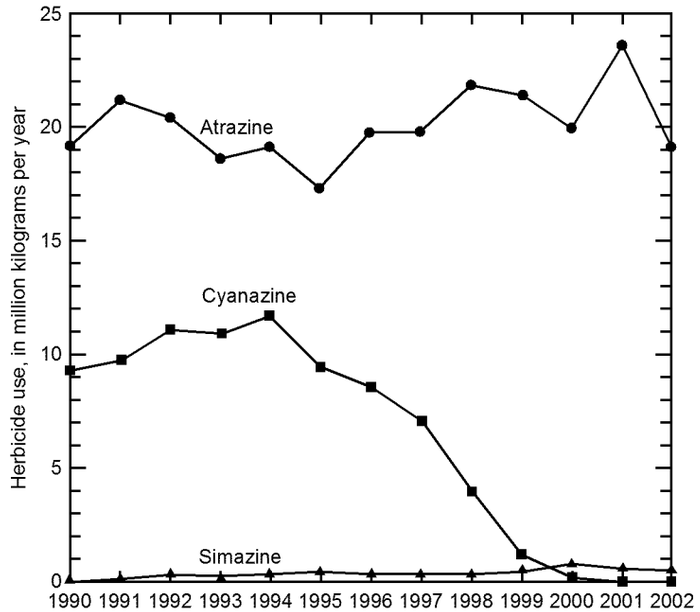


Figure 30.2 Herbicide use in midwestern United States, 1990–2002.
Data Source: USDA (1991–2003).

Water-Quality Studies

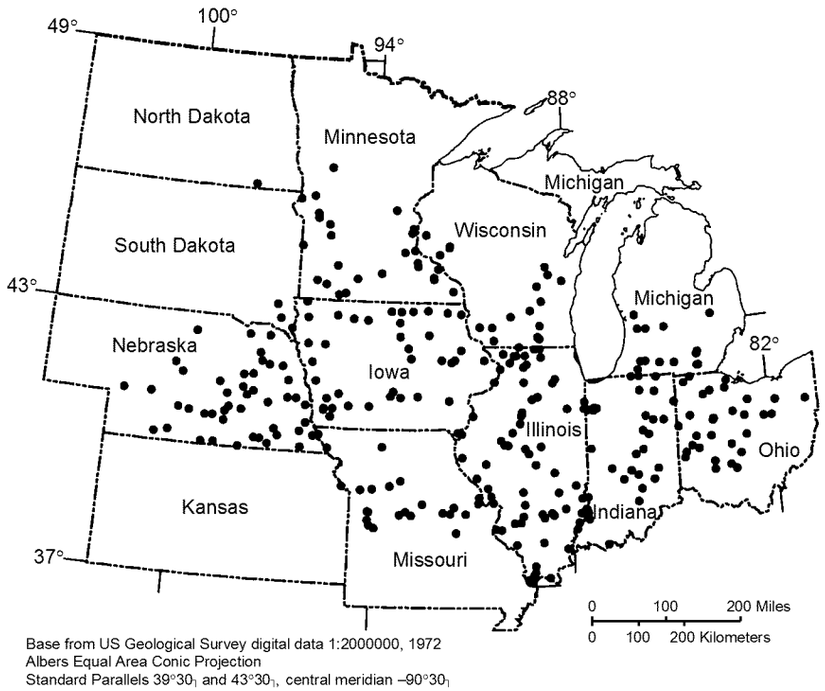
The first part of this discussion reviews studies that monitored herbicides and their degradation products in groundwater. The second part describes a regional reconnaissance of surface water that included streams whose drainage basins ranged in size from less than 100 miles^2 (260 km^2) in area to the entire Mississippi River drainage basin. The third part describes monitoring of reservoirs and the impact of the seasonal application of herbicides. The fourth part discusses herbicides and degradation products in precipitation, the regional deposit patterns of herbicides, and their impact on the Great Lakes.

Groundwater

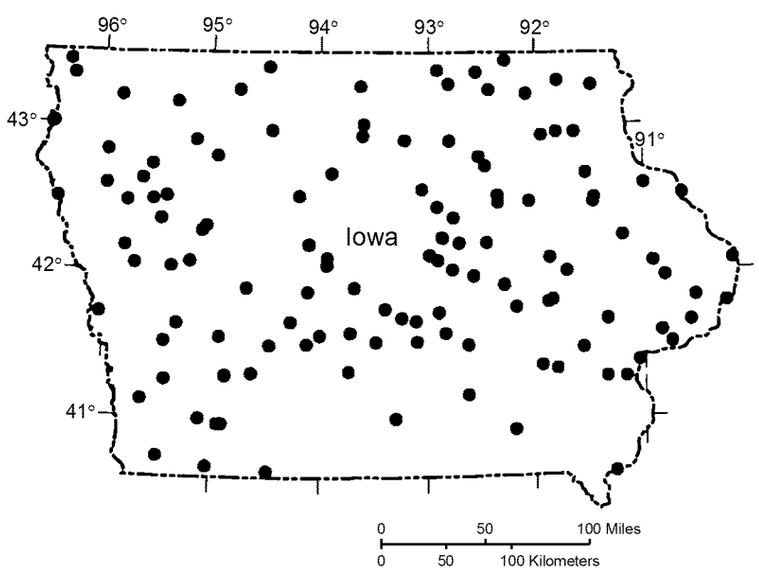
During the 1990s, research on agricultural chemical occurrence in groundwater focused on the midwestern United States because it is the area where the use of herbicides is most prevalent. The USGS designed a monitoring network that was geographically and hydrogeologically representative of near-surface aquifers in the corn and soybean producing areas of the Midwest (Figure 30.3). A series of publications (Kolpin and Burkart, 1991; Kolpin *et al.*, 1993, 1994, 1995, 1996, 1997, 1998, 2000; Kolpin and Thurman, 1995; Thurman *et al.*, 1998b) highlighted the major findings of these studies. Kolpin *et al.* determined the relationship between land use, groundwater age, and the concentration and occurrence of herbicides and their degradation products in groundwater. Groundwater age is typically calculated as the time between recharge at the land surface and sampling. For young groundwater, the time elapsed is measured by the amount of tritium incorporated from the atmosphere as a result of nuclear testing during the 1950s. Older water that precedes the 1950s can be aged-dated by other radioisotopes.

The paper that best summarizes these midwestern groundwater studies is Kolpin *et al.* (1993), which describes the exact locations, detailed land use, and local features surrounding the 303 wells sampled. All of the Kolpin groundwater studies, which address a 12-state area in the Corn Belt, use procedures described in this key paper.

Major findings from these groundwater studies include many interesting results. First, the number of detections of triazine herbicides in groundwater is a function of the age of the groundwater. Groundwater that is pre-1953 (before atrazine and most other herbicides were used) has a much lower herbicide detection frequency than post-1953 (ffi16% versus 70%). It is possible that mixing older groundwater with younger herbicide-contaminated groundwater could yield an older age by tritium analysis. Second, the percentage of detections that occur in groundwater are a function of the detection limit of the method used as more sensitive methods continued to be developed. For example, atrazine had less than 12% detections when the reporting limit was 0.1 μg/L, but this percentage increased to almost 40% when the reporting limit was 0.01 μg/L. Third, parent herbicides are not detected as frequently in groundwater as degradation products. Finally, herbicides that have a long half-life are detected more frequently in groundwater.



A. Well sampled during 1991–1994



B. Well sampled during 1995–1998

Figure 30.3 Location of wells in the USGS midwestern groundwater monitoring network. (A) Location of wells (mostly drinking water) sampled in the USGS midwestern groundwater monitoring network during 1991–1994. (B) Location of municipal drinking water wells sampled in Iowa during 1995–1998.

During 1991, water-quality samples were analyzed from a network of 303 wells across 12 states, while a subset of the 303 wells was sampled from 1992 to 1994 (Kolpin and Burkart, 1991; Kolpin *et al.*, 1993, 1994,1995, 1996, 1997, 1998, 2000) (Figure 30.3A). Atrazine was detected in samples from 22.4% of the 303 wells (Table 30.1). Two atrazine degradation products, DEA and DIA, also were some of the most frequently detected compounds in these studies. The trend, as shown by the frequencies of detection, reflects the relative stability of these compounds.

Table 30.1 Triazine herbicides and their degradation products in samples collected from USGS groundwater monitoring network in midwestern United States from 1991 to 1994[a]

Triazine herbicides or degradation products	Detection frequency (%)	Wells sampled	Reporting limit (μg/L)
Atrazine	22.4	303	0.05
Cyanazine	2.3	303	0.05
Simazine	2.6	303	0.05
Deethylatrazine (DEA)	22.8	303	0.05
Deisopropylatrazine (DIA)	10.2	303	0.05
Cyanazine amide (CAM)	11.0	100	0.05
Deethylcyanazine (DEC)	0	100	0.05
Deethylcyanazine amide (DCAM)	0	100	0.05

[a]Data reported by Kolpin *et al*. (1996).

Table 30.2 Triazine herbicides and their degradation products in samples collected from 131 wells in Iowa from 1995 to 1998[a]

Triazine herbicides or degradation products	Detection limit (μg/L)	Detection frequency (%)	Maximum concentration (μg/L)
Atrazine	0.05	37.4	2.1
Deethylatrazine (DEA)	0.05	32.1	0.59
Deisopropylatrazine (DIA)	0.05	21.4	1.1
Hydroxyatrazine (HA)	0.20	11.4	1.3
Cyanazine	0.05	6.1	0.51
Cyanazine amide (CAM)	0.05	19.8	0.64
Prometon	0.05	18.3	1.0
Prometryn	0.05	0	–
Propazine	0.05	0	–
Simazine	0.05	0	–

[a]Data reported by Kolpin *et al*. (2000).

Although used extensively during the early 1990s, cyanazine was detected in only 2.3% of the wells; however, cyanazine amide (CAM), a degradation product of cyanazine, was detected in samples from 11% of the wells (Table 30.1). This greater frequency of detection is due to an increase in degradation product mobility to groundwater after transformation from cyanazine. As with atrazine and cyanazine, simazine also can be transformed to DIA, but at a faster rate than atrazine (Mills and Thurman, 1994). Simazine was detected in samples from 2.6% of the wells (Table 30.1), and its dealkylation to DIA probably contributed little to the amount of DIA in groundwater.

Kolpin's studies to compare the occurrence of herbicide degradation products in groundwater with that of their parent compounds continued during 1995–1998. Samples were collected from 131 municipal wells covering all the major aquifer types in Iowa (Figure 30.3B). An important finding of this study was the high frequency with which degradation products were detected in groundwater. As shown in Table 30.2, the concentration of triazine degradation products accounted for 55.5% of the total atrazine concentration and for 85% of the total cyanazine concentration. Degradates were the major contributor to the measured concentration in groundwater for a given herbicide, even for a relatively persistent compound such as atrazine (Kolpin *et al*., 1998).

Table 30.2 shows that atrazine was detected most frequently with a maximum concentration of 2.1 μg/L, which is less than the health standard of 3 ppb set by USEPA. In fact, the majority of the detections for atrazine had a concentration range of 0.05–0.2 μg/L. DEA was detected in 32.1% of the samples and the highest concentration of DEA was 0.59 μg/L. Table 30.2 shows the maximum concentration of triazine herbicides detected.

Surface Water

During the 1990s, two major regional studies were the focus of research on agricultural chemical contamination in surface water. The first study was initiated by the USGS's Midcontinent Herbicide Project and involved the parent herbicides atrazine, cyanazine, propazine, and simazine, which are used extensively on corn and sorghum in the midwestern United States (Figure 30.1). A series of papers (Thurman *et al*., 1991, 1992; Goolsby *et al*., 1991, 1993; Battaglin *et al*., 1993; Scribner *et al*., 1993, 1994, 1998, 2000a, 2005) includes interesting data and major findings from this regional study. The highlights were as follows: (1) results indicate that the herbicides were flushed from

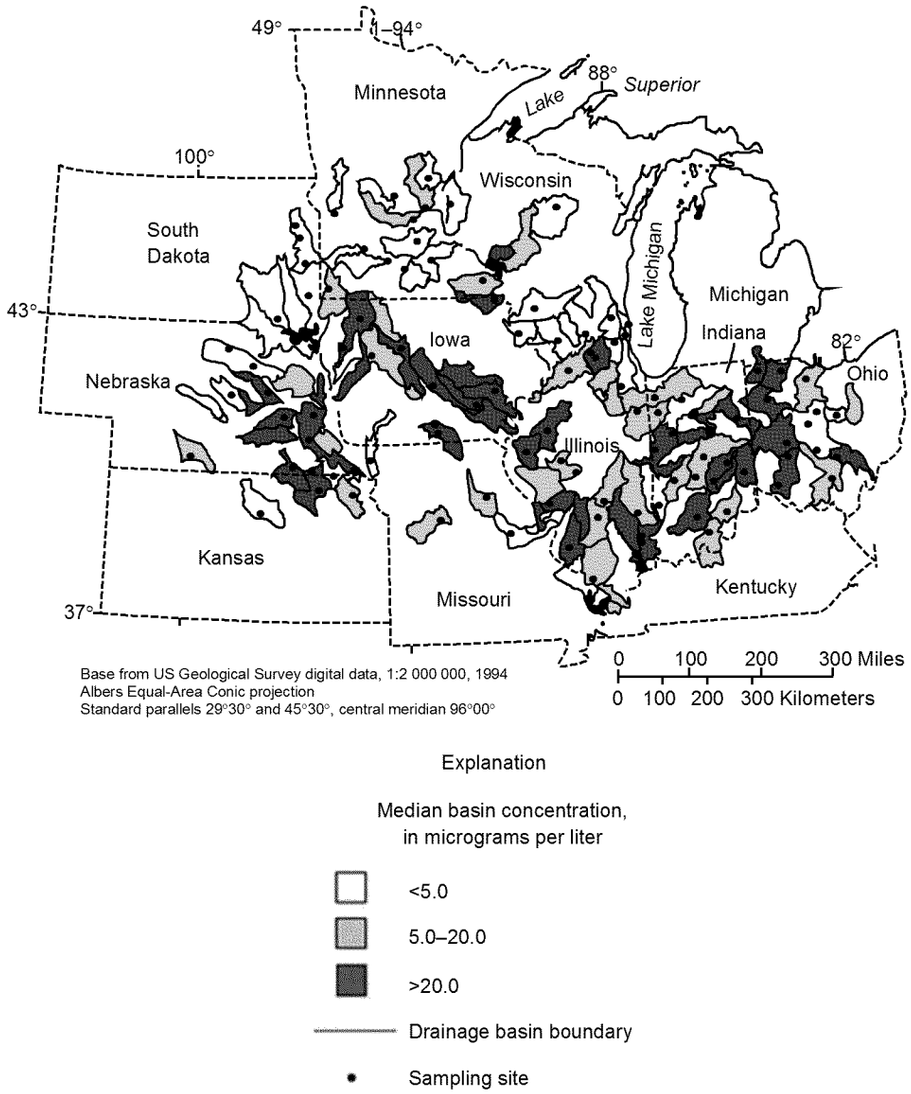


Figure 30.4 Geographic distribution of total herbicides by GC/MS for the post-planting sampling period, displayed by hydrologic cataloging unit based on samples collected at gaging stations located near the terminus of the basin.

cropland and transported through the surface water system in response to late spring and early summer precipitation, (2) concentrations of atrazine and cyanazine temporarily approached USEPA maximum contaminant levels (MCL) during the spring flush, (3) DEA and DIA occurred in many samples, which indicate that some of the parent herbicides may persist from year to year in soil and water, and (4) changes in herbicide usage and best management practices led to a decrease in the amount of atrazine and cyanazine concentrated in surface water.

The second regional study was conducted in the cotton-growing areas of the Mississippi River Delta and the Playa Lakes on the High Plains of West Texas (Figure 30.1). The major findings from the Mississippi River Delta were that the occurrence of cotton and rice herbicides in surface water of three streams is related to how those herbicides are used. High usage rates, more continuous application, long half-lives, and solubility all played important roles (Coupe *et al.*, 1998; Thurman *et al.*, 1998b). The major findings from the Texas Playa Lakes included: (1) atrazine and its degradation products were commonly found in the samples, (2) concentrations of degradation products were equal to or exceeded concentrations of the parent compound, and (3) the concentrations of hydroxyatrazine (HA) were notably high in comparison to concentrations in surface water in the midwestern United States (Thurman *et al.*, 2000).

Midcontinent Herbicide Project: During 1989, a reconnaissance study of 147 streams in 10 midwestern states was conducted to determine the geographic and seasonal distribution of herbicides and their degradation products. Sites were selected to ensure geographic distribution and regional-scale interpretation of the data (Figure 30.4). The streams were sampled before application of herbicides, during the first major runoff period after application of herbicides,

Table 30.3 Percent detections of triazine herbicides and degradation products in surface water of the midwestern United States during 1989–1990[a]

Triazine herbicides or degradation products	Pre-planting (%)	Post-planting (%)	Harvest (%)
Atrazine	91	98	76
Cyanazine	5	63	0
Prometon	0	23	6
Propazine	0	40	<1
Simazine	7	55	3
Degradation products			
Deethylatrazine (DEA)	54	86	47
Deisopropylatrazine (DIA)	9	54	0

[a]Data reported by Thurman *et al*. (1991) where there were 55 samples for pre-planting, 132 for post-planting, and 145 for harvest period.

and during a low-flow period in the fall when most of the streamflow was derived from groundwater. A follow-up sampling was conducted in 1990. The distribution of major herbicide concentrations detected in these streams was essentially the same in 1989 and 1990 for both pre- and post-application samples. Results further indicated that the flush of herbicides following application is an annual occurrence (Goolsby *et al*., 1991; Thurman *et al*., 1991, 1992; Scribner *et al*., 1993, 1998, 2000a; Goolsby and Battaglin, 1995).

The reconnaissance data showed that herbicides were flushed from cropland and transported through the surface water system as pulses in response to late spring and early summer precipitation (Figure 30.4). Median concentrations of atrazine and cyanazine increased by one order of magnitude and then decreased to near pre-planting levels by harvest sampling. Table 30.3 shows the percent detections of herbicides, which increased during post-planting and then decreased to near pre-planting levels by harvest sampling. Measurable amounts of atrazine, the most frequently detected herbicide, occurred in 91% of the pre-planting samples and 76% of the harvest samples, providing an indication of the fate of herbicides in surface water, at a detection limit of 0.05 μg/L. The distribution of atrazine concentrations was from <0.05 to ~1 μg/L. These findings are significant because they indicate either that some of the parent herbicides remained from the previous year, or there was early pre-plant atrazine application before the sampling period.

In 1994, 1995, and 1998, post-application runoff samples were collected at 53 of the sites sampled in 1989–1990 (Figure 30.4). These samples were collected to help determine if changes in the application rates recommended by the manufacturers of atrazine had resulted in a decrease in atrazine concentrations in post-application runoff (Scribner *et al*., 1998, 2000a). The percentage of samples at or above the analytical reporting limit was greater in 1995 than in 1989 for cyanazine, prometon, and propazine, and less in 1995 than in 1989 for simazine. Propazine was detected even though it was no longer used extensively. The detection frequency for atrazine was 100% for every year. The frequency of the detection of DEA was the same in 1989 and 1995 (96%), but the frequency of detection of DIA was greater in 1995 than in 1989.

The distribution of concentrations for atrazine, cyanazine, propazine, simazine, CAM, DEA, and DIA during post-application runoff in 1989–1990, 1994–1995, and 1998 is shown in Figure 30.5 using box plots. Nondetections are plotted at the GC/MS reporting limit of 0.05 μg/L for the individual compounds. Median concentrations of herbicides in midwestern streams during post-application runoff are listed below. The median concentrations of herbicides in these streams during post-application runoff were lower in 1995 than in 1989 for atrazine, cyanazine, propazine, and simazine. Prometon and prometryn were below the detection limit in both 1989 and 1995. The median concentration of both DEA and DIA decreased between 1989 and 1995. The median of the sum of parent herbicide concentrations was 22 μg/L in 1989, 11.4 μg/L in 1995, and 9.25 μg/L in 1998 (Scribner *et al*., 2000a).

Another part of the Midcontinent Herbicide Project involved nine stream sites equipped with automatic samplers that were monitored for triazine herbicides and their degradation products from April through July 1990. It was found that the use of microtiter plate ELISA provided a good semiquantitative measure of atrazine for concentrations less than 3.0 μg/L. At higher concentrations, the samples had to be diluted. Results from the ELISA screen compared with GC/MS results showed good agreement (Pomes and Thurman, 1991; Scribner *et al*., 1994).

Cotton Herbicide Project: Though herbicides applied to corn have an important impact on water quality, cotton and rice receive three to five times more herbicides per acre than either corn or soybean. Cotton-growing areas of the United States (Figure 30.1) extend from the East Coast (the Carolinas) to the Mississippi River Delta, the Texas High Plains, and the arid regions of the Southwest (Arizona and California). These areas of the country have different climate, precipitation, and soil types, which result in different weed and insect pressures and different runoff potentials; therefore, leaching patterns are also different. Because of these factors, the types and amounts of herbicides applied may vary considerably throughout cotton-growing areas (Thurman *et al*., 1998b).

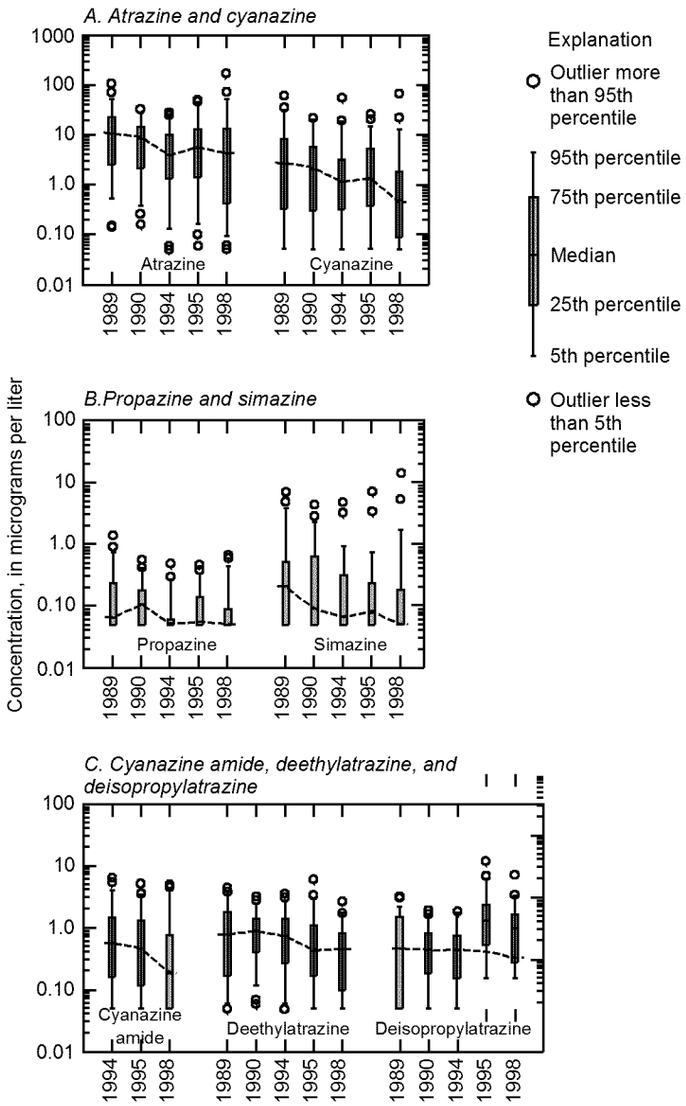


Figure 30.5 Concentrations in selected midwestern streams during post-application runoff in 1989, 1990, 1994, 1995, and 1999 for: (A) atrazine and cyanazine, (B) propazine and simazine, and (C) cyanazine amide, deethylatrazine, and deisopropyl atrazine.

A study in the Mississippi River Delta (Figure 30.1) during 1995–1997 included surface water samples collected primarily from Steele Bayou, Deer Creek, and Big Sunflower River, which are part of the Yazoo River Basin. This is Mississippi's largest river basin and is divided almost equally between the Mississippi alluvial plain, an intensive agricultural area of soybean, cotton, and rice production, and the uplands, which generally consist of forests, pastures, and small farms (Coupe *et al*., 1998).

In the Mississippi Delta, the long growing season allows farmers more flexibility in the types of crops planted and the length of the planting season compared to the Midwest. In addition, herbicides are applied to cotton as much as 12 weeks after planting, resulting in the occurrence of herbicides in surface water from early April until August (Figure 30.6). The concentrations of total herbicides peak in late June and early July, similar to the Corn Belt. There is a larger variety of herbicides in the Delta streams, and concentrations are more sustained with multiple peaks reflecting different application times and the post-emergent application to cotton and rice. For instance, atrazine, which is used on corn in the Delta, had its maximum concentrations in April rather than in May to June as in the Corn Belt (Coupe*et al*., 1998).

As shown in Figure 30.6, the triazine herbicides cyanazine and prometryn have been used extensively in the Mississippi River Delta. Approximately 68% of cotton was treated with cyanazine prior to its 2002 phaseout and 40% with prometryn (Coupe *et al*., 1998). CAM, a major degradation product of cyanazine, occurred with the parent compound at about one-half the concentration of the parent compound, and its concentration increased relative to

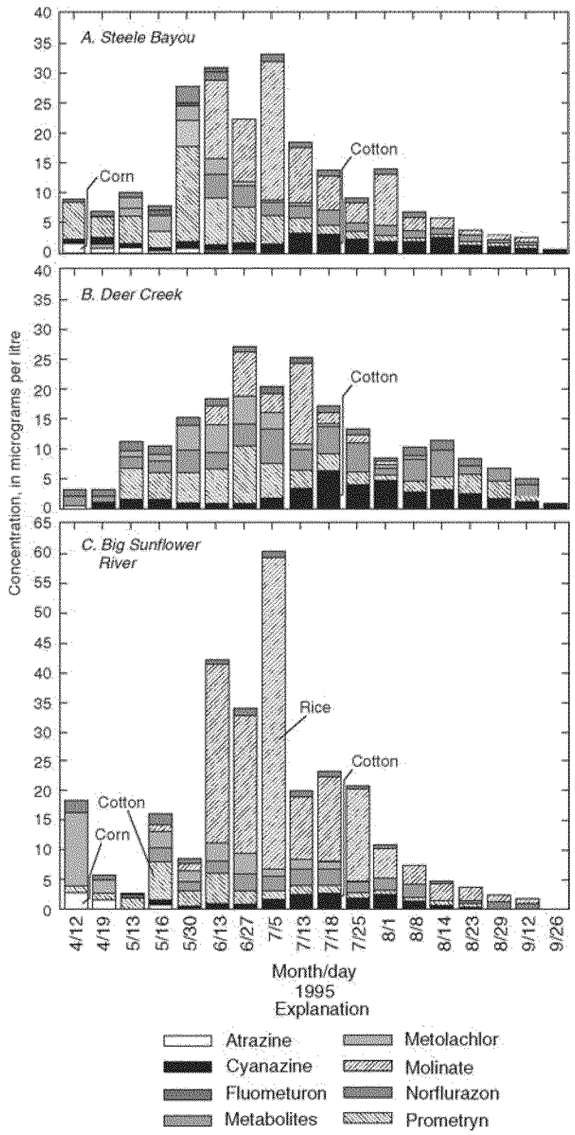


Figure 30.6 Total concentrations of herbicides and selected degradation products in (A) Steele Bayou, (B) Deer Creek, and (C) Big Sunflower River in the United States during 1995 (Coupe *et al.*, 1998).

the parent compound later in the growing season. Prometryn was frequently detected, but not above 1.0 μg/L, and its degradation product, deisopropylprometryn, was not detected above the reporting limit of 0.05μg/L.

During the summer of 1997, water samples were collected and analyzed for herbicides from 32 Playa Lakes of the High Plains that receive drainage from both cotton and corn agriculture in West Texas (Figure 30.1). The major cotton herbicides detected in the water samples were diuron, fluometuron, metolachlor, norflurazon, and prometryn. Atrazine and propazine also were routinely detected in samples from the Playa Lakes.

Degradation products were a significant proportion of the total herbicide concentration in the Playa Lakes samples. The median degradation product percentage was 27% of the total herbicide present. The highest degradation product percentage was 70.5%. Detections included three atrazine degradation products – DEA, DIA, and HA – and one prometryn degradation product, deisopropylprometryn. Of these degradation products, the most frequently detected was DEA, followed by deisopropylprometryn, HA, and DIA. The frequency of prometryn detections was 72%, with a mean concentration of 1.3 μg/L. The degradation product of prometryn was in nearly every sample that contained prometryn, with relative abundance of 0.1, or about 10% of the parent compound. Atrazine was detected in 72% of the samples, with a mean concentration of 0.47 μg/L. Propazine was detected in 59% of the samples, with a mean

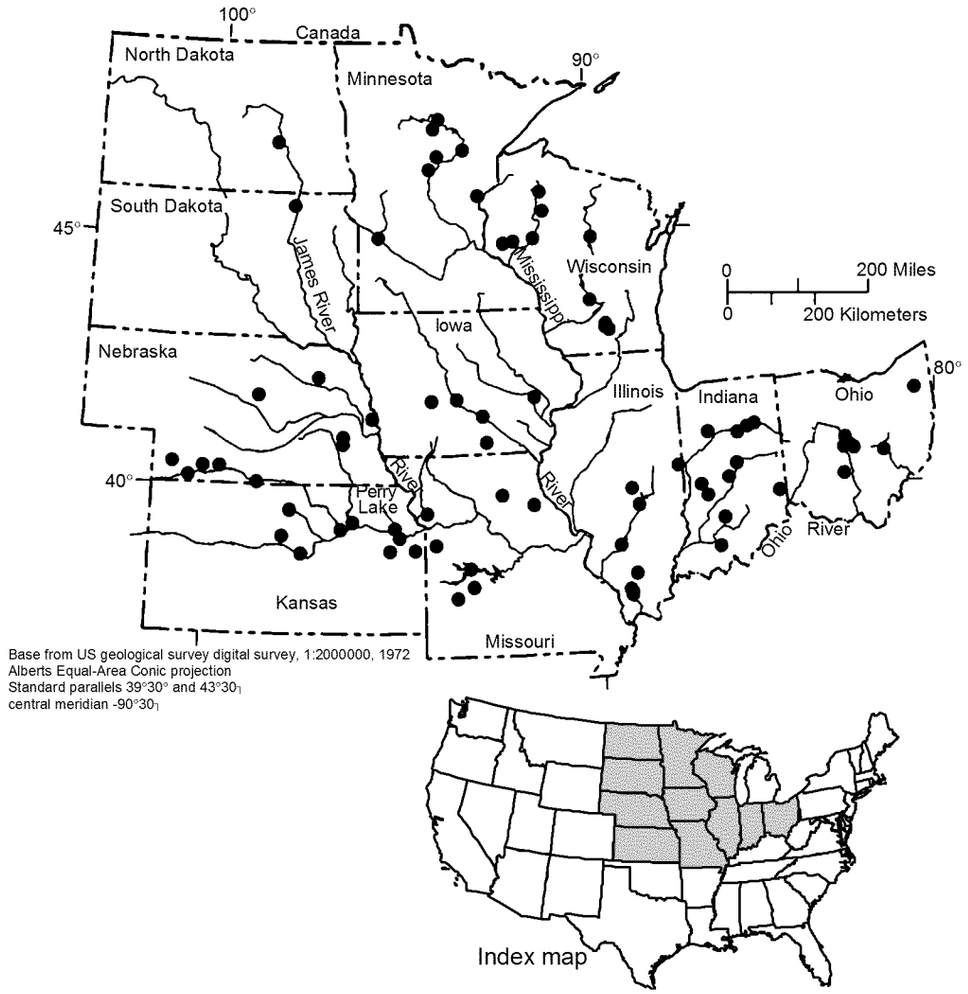


Figure 30.7 Location of 76 reservoirs sampled ( ) for herbicide analysis in the midwestern United States during 1992 and 1993.

concentration of 0.25 μg/L. DEA, a common degradation product of both atrazine and propazine, was found in 63% of the samples, with a mean concentration of 0.36μg/L (Thurman *et al.*, 2000).

Reservoirs and Lakes

Reservoirs in the midwestern United States were also part of the focus of the USGS study of agricultural chemical contamination from use of herbicides in this region. Reservoirs are an integral component of the supply, management, and quality of water resources in the United States. Reservoir water quality is critical to drinking water. Many reservoirs used for drinking water have drainage basins whose primary land use is crop production. Regional studies have shown that spring runoff may contain elevated concentrations of herbicides for several months of the year (Leonard, 1988; Baker and Richards, 1989; Pereira and Rostadt, 1990; Thurman *et al.*, 1991, 1992; Squillace and Thurman, 1992; Goolsby *et al.*, 1996).

The USGS designed a monitoring network that was hydrologically representative of reservoirs in the corn and soybean producing region. The paper that sets the stage for the reservoir study is Scribner *et al.* (1996), which describes the exact locations, selected characteristics, and features of the reservoirs sampled. During 1992 and 1993, 76 reservoirs were sampled in the same study area as the 1989–1990 surface water reconnaissance of herbicides in streams described earlier (Figure 30.7). Reservoirs were screened and selected from the reservoir database compiled by Ruddy and Hitt (1990).

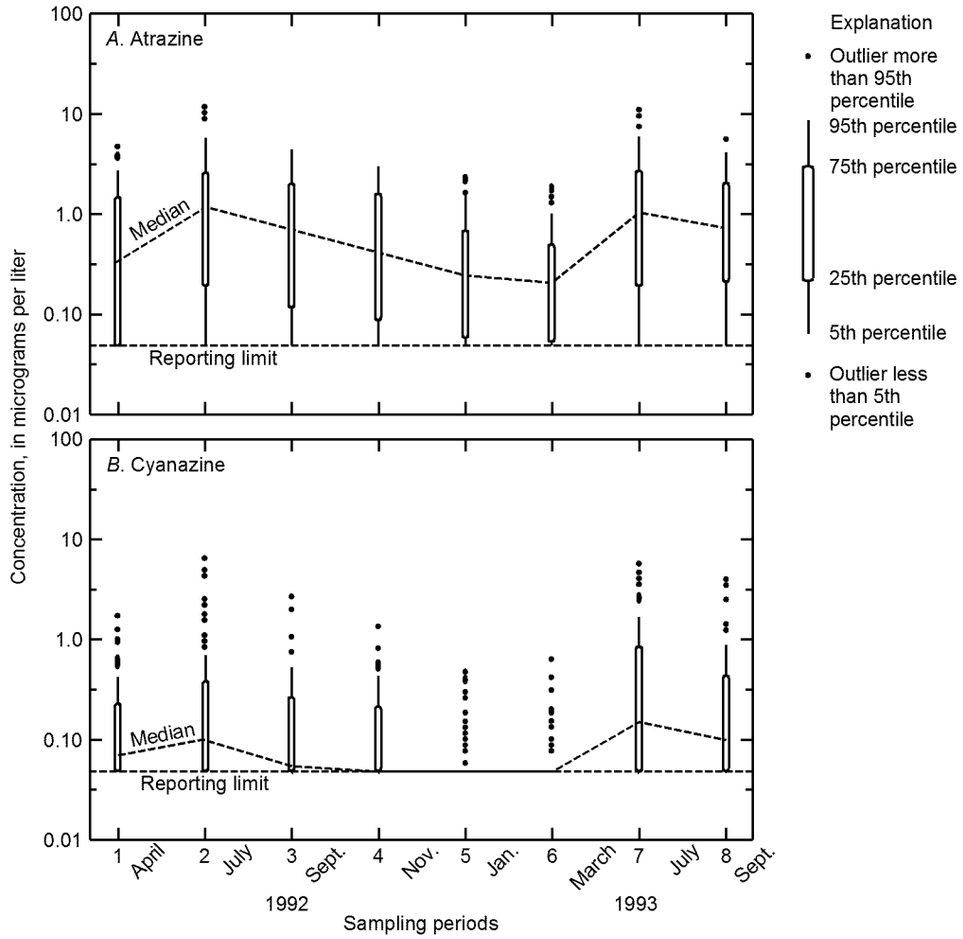


Figure 30.8 Concentrations of atrazine and cyanazine in water samples selected from reservoirs of midwestern United States during 1992 and 1993.

The major findings from this study include many interesting results. First, reservoirs are repositories for substances that are introduced into midwestern streams. Second, herbicides and degradation products are detected more frequently throughout the year in reservoirs than in streams. Third, long-term storage and mixing of water in reservoirs that originates as spring and summer storm runoff from cropland dampens the pulse of herbicides in surface water.

Analytical results from samples collected during 1992 indicate that the herbicides and degradation products present and detected were in 82% to 92% of the selected reservoirs during four sampling periods. One of the most notable differences between the occurrence of herbicides in reservoirs and streams was a much higher frequency of detection of cyanazine and DIA in reservoirs. A possible explanation for this observation is that these two compounds are much more stable in the water of lakes and streams than in soil, where organic matter and microorganisms promote their rapid biodegradation. Consequently, late spring and summer runoff can flush these two compounds into reservoirs, where they can persist for long periods of time. Neither cyanazine nor DIA was detected in streams during the fall because these compounds are no longer present in significant amounts on the agricultural fields where they were applied (Goolsby *et al.*, 1996). Thus, herbicide concentrations in reservoir outflows behave differently than those in unregulated streams (Stamer and Zelt, 1992; Fallon and Thurman, 1996; Stamer *et al.*, 1998a). The box plots in Figure 30.8 show the distribution of atrazine and cyanazine detections in midwestern reservoirs. The mean of the individual concentrations of atrazine and its degradation products was 1.9 μg/L. Similarly, the mean sum of the individual concentrations of cyanazine and its degradation products was 1.0 μg/L, which is also consistent with the fact that cyanazine usage in the study area was about half that of atrazine.

A detailed study of Perry Lake in northeast Kansas was carried out during 1992 and 1993. The sampling strategy consisted of two components. The first included five seasonal surveys of the reservoir, conducted before herbicide

application, after runoff during the growing season, and before herbicide application the following spring. Water samples were collected from one to four depths at up to 102 randomly and purposely selected sites. The number of samples collected during each survey ranged from 31 to 186. The second component of the sampling strategy was designed to monitor the inflow and outflow of the reservoir. Samples were collected from four locations upstream and downstream from the reservoir, as well as within the reservoir. Water samples were collected monthly throughout the year and from runoff during April to August (Fallon, 1994; Fallon and Thurman, 1996).

Atrazine concentrations in Perry Lake increased 48% after application to croplands (from 2.7 to 4.0 μg/L). Three-dimensional computer images of atrazine concentrations and DEA-to-atrazine ratio (DAR) values showed that recently applied atrazine mixed with atrazine applied the previous year as water moved sequentially through the reservoir (Figure 30.9). Changes in atrazine concentrations resulted from several factors, including herbicide application, precipitation, and reservoir residence time. Atrazine application fueled and reset the system. Precipitation drove the system by flushing atrazine into the reservoir. The timing of the precipitation and runoff affected how much atrazine flushed into the reservoir. The volume of precipitation and runoff affected how long atrazine remained in the reservoir. Precipitation shortened reservoir residence time by increasing inflow and outflow during wet periods. Below-normal precipitation in May and June 1992, combined with above-normal precipitation during the last 9 months of the study period, produced lower atrazine concentrations in the reservoir outflow than those found previously. Atrazine concentrations at the outflow were decreased and dampened by the pulse of water entering the reservoir, as water containing higher atrazine concentrations was temporarily stored and mixed with water having lower concentrations.

Precipitation

During the late spring and summer of 1990 and 1991, a USGS study focused on herbicide transport into the atmosphere by various processes. This study was conducted prior to significant label rate reductions for atrazine-containing products. Once in the atmosphere, these compounds can be dispersed by air currents and redeposited by precipitation, snow, and dry deposition on the land surface, lakes, and streams.

The overall objective of the precipitation study was to: (1) determine the occurrence and temporal distribution of herbicides and their degradation products in precipitation, (2) estimate the amounts of atrazine deposited by precipitation annually in individual states and over a large part of the United States, (3) relate annual deposition of atrazine to amounts applied annually, and (4) compare annual herbicide deposition by precipitation within the Mississippi River Basin to the estimated annual amount transported out of the basin in streamflow.

Herbicide concentrations exhibited distinct geographic and seasonal patterns. The highest concentrations occurred in midwestern Corn Belt states following herbicide application to cropland. Table 30.4 presents a summary of the occurrence and concentrations of triazine herbicides detected in 5 297 samples collected during the study period by immunoassay (ELISA). A confirmation by GC/MS was made of 2 085 of the precipitation samples (Pomes *et al.*, 1998).

The most frequently detected herbicide was atrazine, which was present in 30.2% of the samples analyzed. DEA was present in more than half of the samples that contained atrazine and was the third most frequently detected compound in the study. Trace concentrations of DEA were detected in 12 samples that contained no detectable atrazine. Cyanazine was detected in 7.2% of the samples. Although herbicides were detected in a significant number of samples, concentrations were relatively low. Atrazine also was detected in low concentrations at sites in Maine and on Isle Royale in northern Lake Superior, far from agricultural areas (Goolsby *et al.*, 1997; Stamer *et al.*, 1998b; Thurman and Cromwell, 2000).

Because of the large temporal and spatial variation in the amount of precipitation, it is difficult to make a meaningful comparison of herbicide concentrations among sites or over time on the basis of individual weekly samples. Therefore, comparisons were made with precipitation-weighted concentrations. Figure 30.10 shows the spatial distribution of precipitation-weighted concentrations of atrazine calculated for 13-week periods from mid-April through mid-July 1990 and 1991, when concentrations were the highest. Precipitation-weighted concentrations of 0.2–0.4 μg/L for atrazine were typical throughout the Midwest for this 13-week period, and weighted concentrations of 0.4–0.9 μg/L were recorded at sites in Iowa, Illinois, and Indiana. Overall, the spatial patterns of the weighted atrazine concentrations in 1990 and 1991 were similar and generally reflect atrazine use.

Figure 30.11 shows the regional patterns of atrazine deposited in precipitation during March through December 1990 and January through September 1991. Nearly all of the atrazine deposition occurred during April through July when concentrations were highest. Consequently, results shown should closely represent the total annual wet deposition of atrazine during the 2 years. Atrazine deposition rates ranged from more than 100 $\mu g/m^2/yr$ in the midwestern states to less than 10 $\mu g/m^2/yr$ in the northeastern states. Deposition rates throughout most of the Corn Belt ranged from 50 $\mu g/m^2/yr$ to more than 100 $\mu g/m^2/yr$ for atrazine.

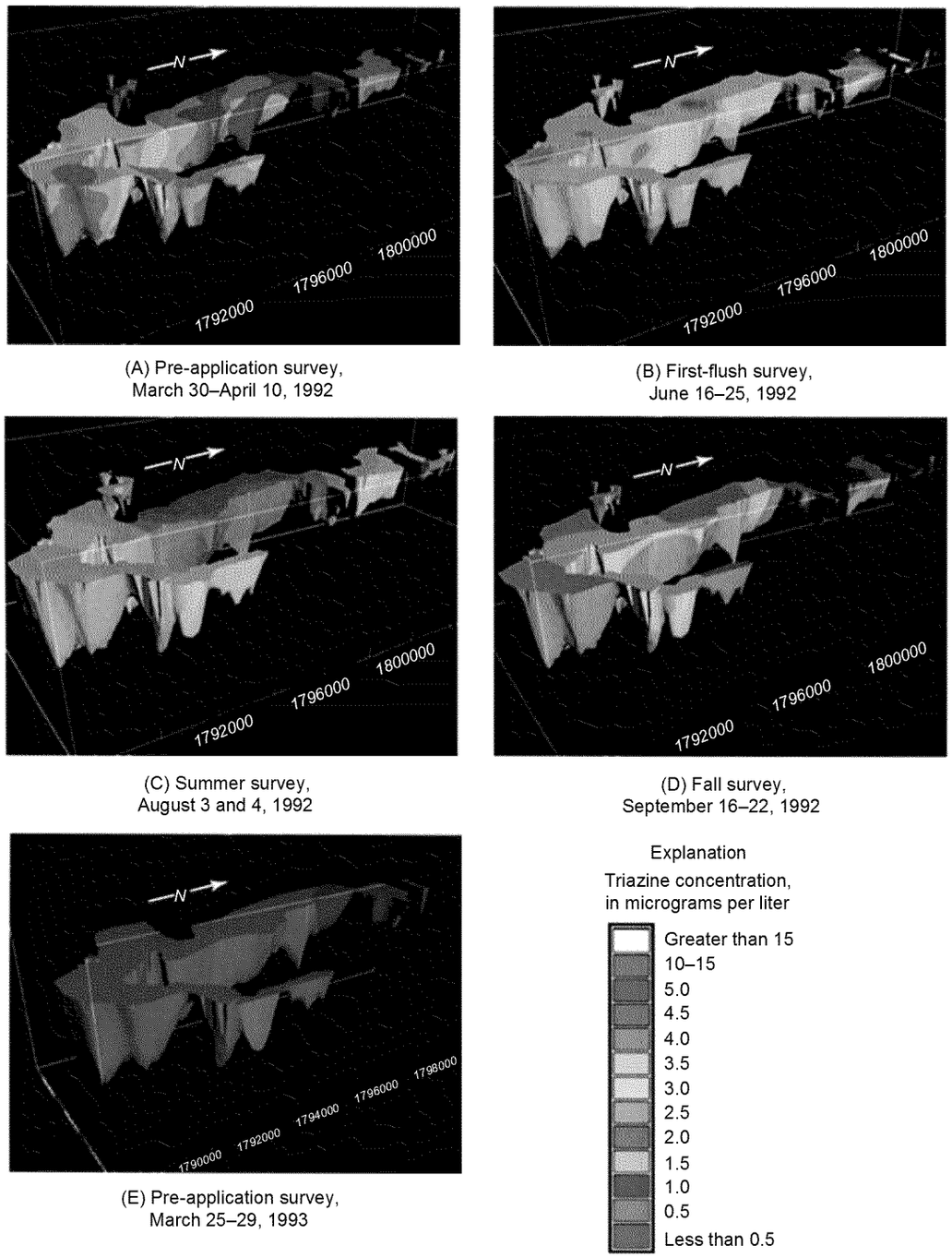


Figure 30.9 Three-dimensional computer images of atrazine and deethylatrazine-to-atrazine (DAR) concentrations in Perry Lake, Kansas during 1992 and 1993. (See Color Plate Section)

One of the sampling sites was located on Isle Royale in the northwest part of Lake Superior near the Canadian border and far from the US Corn Belt. Atrazine, presumably from the Corn Belt, was detected and verified by GC/MS analysis in samples from several rains at this site during June 1990. These data prompted the collection of water samples from Lake Superior and from four small lakes on Isle Royale in late September 1990. The atrazine concentration in these samples, determined by isotope dilution methods, was 6.5 nanograms per liter (ng/L) for Lake Superior,

Table 30.4 Triazine herbicide and degradation product concentrations measured in precipitation samples from 81 sites across the midwestern and northeastern United States from March 1990 to September 1991[a]

	Concentration in micrograms per liter for indicated percentiles					
Triazine herbicides	**Detections (%)[b]**	**75**	**90**	**95**	**99**	**100 (maximum)**
ELISA analysis: No. 5297 samples						
Triazine ELISA	25.5	0.10	0.24	0.42	1.3	16
GC/MS analysis prescreened by ELISA: No. 2085 samples						
Atrazine	30.2	0.07	0.23	0.40	1.0	10.9
Cyanazine	7.2		<0.05	0.07	0.27	2.0
DEA	17.4	<0.05	0.11	0.15	0.39	0.75
DIA	2.6			<0.05	0.17	1.2
Simazine	1.5			<0.05	0.07	1.5
Prometon	0.50				<0.05	0.21

[a]Goolsby *et al*. (1997).
[b]Reporting limits were 0.10 μg/L for triazines by ELISA and 0.05 μg/L for all compounds analyzed by GC/MS.

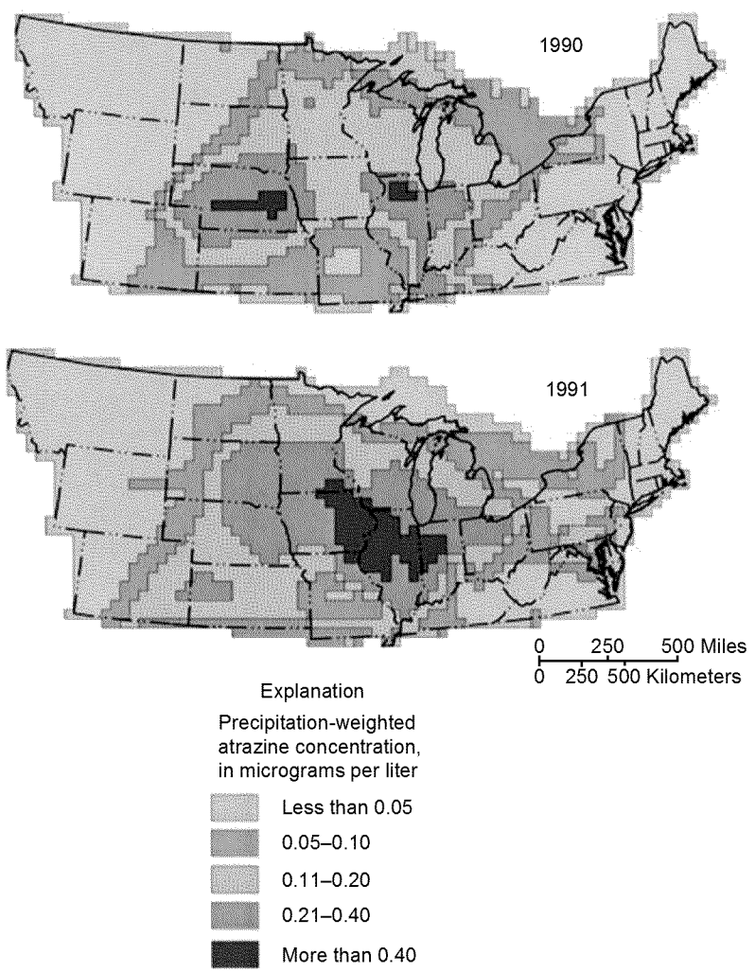


Figure 30.10 Precipitation-weighted concentrations of atrazine in water samples from northern and northeastern United States during mid-April and mid-July in 1990 and 1991 (Goolsby *et al*., 1997). (See Color Plate Section)

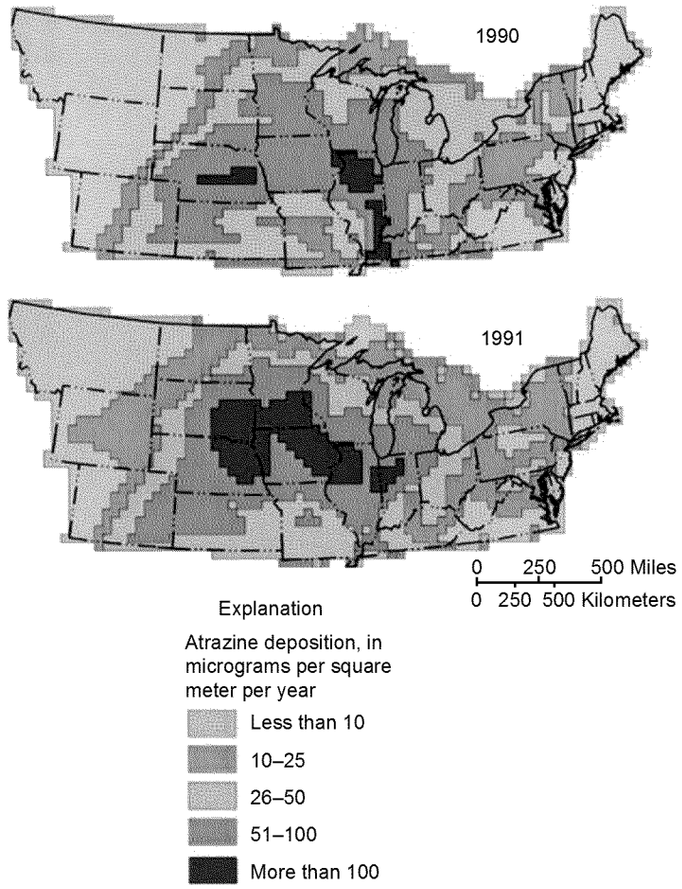


Figure 30.11 Estimated deposition of atrazine in precipitation from northern and northeastern United States during March through December 1990 and January through December 1991 (Goolsby *et al*., 1997). (See Color Plate Section)

and ranged from 2.5 to 20 ng/L for four lakes. The likely source of atrazine to Lake Superior and Isle Royale is atmospheric deposition.

Occurrence of Triazine Degradation Products

This section discusses the occurrence of the degradation products of atrazine, cyanazine, simazine, propazine, prometryn, and prometon, as well as their degradation pathways from soil into surface water. More detailed discussion of triazine degradation can be found in several other chapters of this book.

An important finding of USGS research was the occurrence of triazine herbicides in surface water. In a study by Thurman *et al*. (1991, 1994) measurable amounts of atrazine, the most frequently detected herbicide, occurred in 91% of the pre-planting samples, 98% of the post-planting samples, and 76% of the harvest samples. The atrazine degradation product DEA was found in many of the samples that contained atrazine. The frequency of detection or apparent order of stability of the herbicides and their degradation products is as follows: atrazine, DEA, DIA, and cyanazine. This stability order is based on results of field-dissipation studies on atrazine and cyanazine (Meyer, 1994; Mills and Thurman, 1994).

This stability relationship may be interpreted from the number of herbicide detections in both pre-planting and post-harvest samples and in the range of the distribution during sampling periods. For example, atrazine has a reported half-life of 60 days, while cyanazine has a half-life of 25 days. Work by Pereira and Rostadt (1990) indicates that cyanazine can be conservatively transported in an aquatic environment. Oxidation of the cyano group is the most rapid degradation pathway. CAM is an important degradation product of cyanazine and would result from the oxidation of the cyano group.

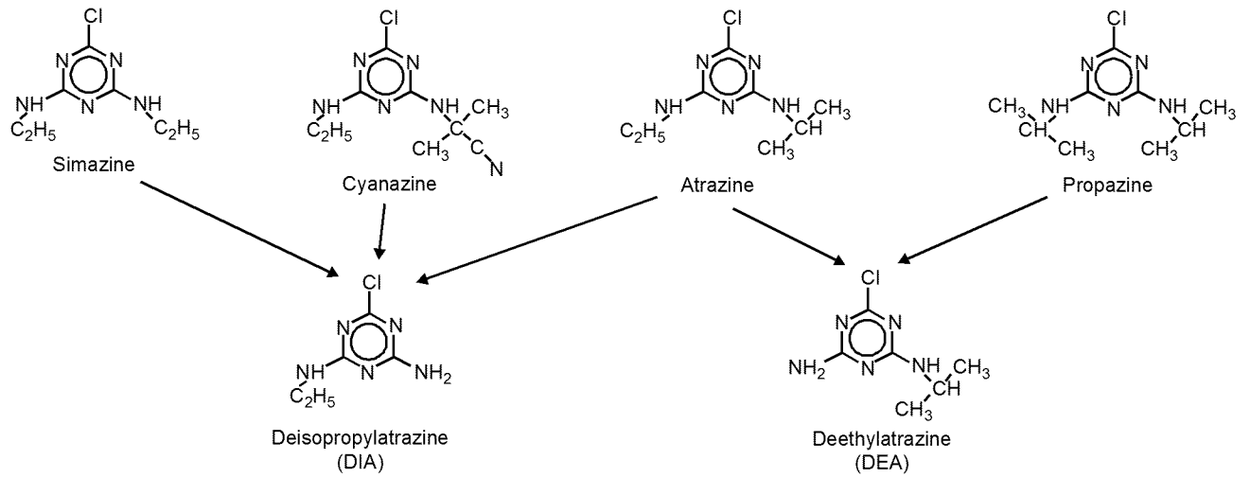


Figure 30.12 Degradation pathways for atrazine, cyanazine, propazine, and simazine to DEA and/or DIA.

Once it is applied, atrazine begins to degrade through the action of soil microbes, abiotic losses, and chemical reactions. Much of the past research on the fate of atrazine has addressed only the parent herbicide because most of the atrazine degradation products were known to be nonphytotoxic or much less phytotoxic than their parent (Shimabukuro and Swanson, 1969) and, therefore, were not of particular interest to the agronomist. In the 1990s, however, there was an increased focus on accounting for the ultimate fate of atrazine degradation products (Adams and Thurman, 1991; Thurman *et al.*, 1994).

Atrazine degradation pathways to DEA, DIA, deethylhydroxyatrazine (DEHA), deisopropylhydroxyatrazine (DIHA), and HA are shown in Figure 30.12, Chapter 22, and Chapter 7. These degradation products and didealkylatrazine (DDA) further degrade to ammeline, ammelide, cyanuric acid, and ring cleavage. Many studies focus on the chlorinated atrazine degradation products because of their greater water solubility and lower soil adsorption compared to the parent compound. However, hydroxylated atrazine degradation products, particularly HA, are the major degradation products of atrazine in most soils and these degradation products bind tightly to the soil. Hydroxylated atrazine degradation products (HADPs), which include HA, DEHA, and DIHA, are a major group of atrazine degradation products. In soil or water, their hydrolysis is enhanced by extremes in pH, dissolved organic matter, sorption to soil colloids, and the presence of photosensitizing compounds. HADPs have been shown to be more persistent in soil than atrazine and humic acids (Lerch *et al.*, 1998).

The DEA degradation product, mostly from atrazine, has been the most important degradation product found in groundwater studies throughout the midwestern United States. DIA also may occur from the degradation of cyanazine and simazine. DIA may be degraded further to DDA by removal of the remaining ethyl group. The production of DEA and the degradation of DIA proceed through removal of the isopropyl group and the ethyl group (Figure 30.12), respectively. Therefore, the higher levels of DEA and the lower levels of DIA during atrazine dealkylation may be a reflection of the greater ease of deethylation versus deisopropylation.

Preferential removal of an ethyl versus an isopropyl moiety is a concept that has been suggested by Leonard (1988). DIA occurs in surface water that has received parent atrazine, simazine, or cyanazine. DEA occurs in surface water that has received atrazine or propazine. The concentrations of DEA and DIA in surface water vary with the hydrologic conditions of the basin and the timing of runoff (Mills and Thurman, 1994; Thurman *et al.*, 1994, 1998a).

In colder climates and where crop rotation practices were used, some triazine herbicides persisted into the following year and interfered with some crop rotations; therefore, cyanazine was introduced in 1967 as an alternative to other triazines because of its shorter half-life. Studies showed that the half-life of cyanazine in the soil varied from 14 to 25 days (Meyer and Thurman, 1996). However, degradation products of cyanazine have been identified in the soil as long as 4 years after application (Meyer and Thurman, 1996) (Figure 30.13). The degradation process for herbicides generally increases the water solubility and the polarity of the compound. The increase in solubility is caused by the loss of carbon, the incorporation of oxygen, and the addition of carboxylic-acid functional groups. For every carbon atom that is removed, the water solubility may increase from two to three times (Meyer and Thurman, 1996).

A study by Meyer (1994) found that CAM dissipated rapidly and was not readily transported through the shallow soil (Figure 30.13). The concentration of CAM often was greater than cyanazine in both soil and pore water. By midseason,

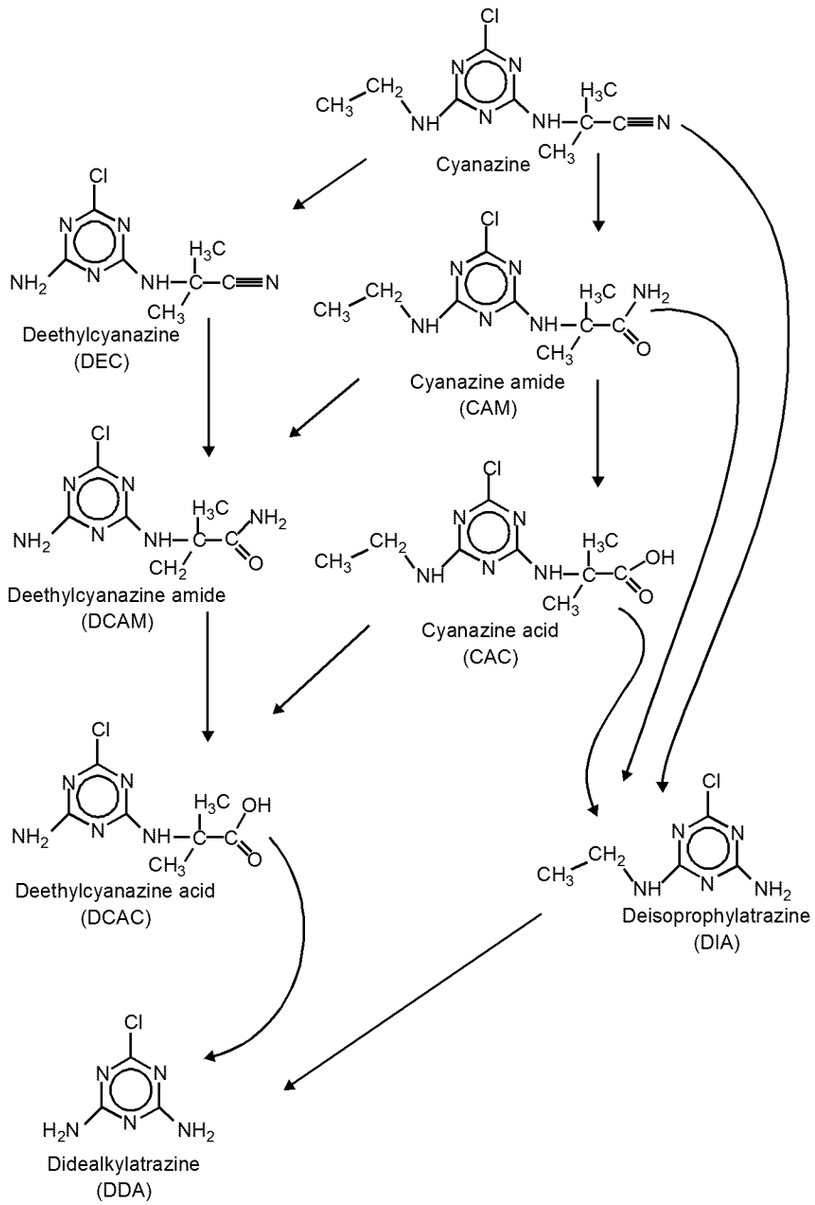


Figure 30.13 Degradation pathways for cyanazine.

studies indicated that CAM was more readily transported through the unsaturated zone than cyanazine. The deethylated cyanazine degradation products, deethylcyanazine (DEC) and deethylcyanazine amide (DCAM), were readily identified only in the early season when the concentrations of cyanazine and CAM were high. Furthermore, they were rapidly dissipated and were not transported at detectable levels below 30 cm. The primary degradation product of cyanazine that was measured with depth was DIA. It was detected as deep as 75 cm and at concentrations greater than DEA (Meyer, 1994). Also, DIA generally was detected later in the growing season and at deeper levels than either cyanazine or CAM. Thus, the detection of DIA at a greater depth is probably the result of the degradation of another cyanazine degradation product (e.g., cyanazine acid (CAC)) that is rapidly transported through the unsaturated zone. CAM and CAC both have modified isopropyl moieties that may be removed by microbial degradation. The rapid dissipation of CAM suggests that CAC is formed rapidly. Furthermore, because CAC is ionic at normal pH, it may be preferentially leached through the soil. As it is leached, it may undergo deethylation and/or dealkylation.

The structures and other properties for simazine, propazine, prometryn, prometon, and other triazine herbicides and many of their degradation products can be found in the Appendices, Tables A1, A2 and A3. As shown in Figure 30.12, atrazine and simazine degrade to DIA.

In a study of Playa Lakes in the High Plains of Texas (Thurman *et al.*, 2000), atrazine was detected in 72% of the samples (mean concentration of 1.3 μg/L), and propazine was detected in 59% of the samples. The common degradation product, DEA (Figure 30.12), was found in 63% of the samples with a mean concentration of 0.36 μg/L. Atrazine was responsible for the majority of DEA.

Prometryn also was detected frequently in the Playa Lakes sampling in Texas (Thurman *et al.*, 2000). The degradation product of prometryn, deisopropylprometryn, was detected in nearly every sample that contained prometryn.

The primary use of prometon, introduced in 1959, is for total vegetation control in noncrop areas around the farm, on industrial sites, and for use in and under asphalt. Application rates are from 10 and 60 lb/A/yr (11 and 67 kg/ha/yr), 10 to 30 times higher than for atrazine, but the treated areas are much smaller (Capel *et al.*, 1999). Prometon is highly persistent with an average field half-life of 500 days.

Chemistry and Transport of Triazine Degradation Products in Water

Herbicides derived from point and nonpoint sources can be transported to streams by runoff from agricultural and urban areas, discharge from reservoirs and aquifers, and precipitation. The physiochemical properties of the triazine herbicides, as well as other factors such as usage, precipitation patterns, and farming practices, are important in determining the amounts and concentrations of these chemicals in streams.

Transport Mechanisms for Degradation products

A study was designed to define the relative rates of dealkylation of selected triazine herbicides and two monodealkylated triazine degradation products in the unsaturated zone and in surface runoff. Atrazine and propazine degrade to DEA by deethylation and deisopropylation, respectively. Similarly, atrazine and simazine can both dealkylate to DIA by removal of an isopropyl and ethyl side chain, respectively (Figure 30.12). Differences in the concentration of the dealkylated degradation product from the two different sources should indicate any preferential removal of ethyl versus isopropyl side chain. Furthermore, because monodealkylated DEA and DIA have different side chains remaining, their relative rate of removal should provide additional information on the liability of the ethyl side chain versus an isopropyl side chain.

This study showed that under field conditions, the removal of an ethyl side chain from atrazine occurred more readily than the removal of an isopropyl side chain. Furthermore, deethylation rates of atrazine and simazine were comparable, and approximately two to three times more rapid than the rates of deisopropylation from atrazine and propazine, regardless of parent triazine. Continued dealkylation of the monodealkylated degradation products at 1 m in the unsaturated zone also shows a preferential removal of ethyl side chains over isopropyl side chains. Therefore, the small concentrations of DIA commonly reported in the environment do not result purely from a smaller production of the degradation product, but from a rapid removal once produced. This substantial turnover rate or flux of DIA in the environment is evidence for the presence of a didealkylated degradation product in the unsaturated zone (Mills and Thurman, 1994; Thurman *et al.*, 1994).

Further studies were conducted to look at the relation between the amount of cyanazine relative to atrazine and its effect on the production of DIA (Meyer and Thurman, 1996). A discrimination diagram, which differentiates between different sources, was used by Meyer *et al.* (2001) to determine the source of an herbicide degradation product from two different compounds. Field-dissipation studies demonstrated that about 6% of the atrazine degraded to DEA and about 3% degraded to DIA (Adams and Thurman, 1991; Mills and Thurman, 1994). Cyanazine degraded only to the common degradation product DIA. Thus, the ratio of DIA to DEA (D^2R) and the atrazine to cyanazine ratio were used to differentiate the nonpoint source of DIA to surface water. Next, Meyer *et al.* (2001) successfully tested the D^2R discrimination diagram in two basins with very different application rates of atrazine and cyanazine to check the validity of the D^2R. The D^2R was also used to show that cyanazine contributed a considerable amount (40%) of the DIA that was transported during flooding of the Mississippi River in 1993. The D^2R may become a useful water-quality monitoring tool to measure nonpoint-source contributions of DIA in the coming years as cyanazine has been removed from the market (Meyer *et al.*, 2001).

The DEA to Atrazine Ratio and Transport of Triazine Degradation Products

To understand the geochemistry of atrazine, it is valuable to compare the degradation product DEA to atrazine. The resulting ratio is called the DAR and is defined as:

$$\text{DAR} = \frac{(\text{deethylatrazine, mol/L})}{(\text{atrazine, mol/L})}$$

It is hypothesized that the DAR may be an indicator of point-source versus nonpoint-source contamination of groundwater by atrazine (Adams and Thurman, 1991). The DAR hypothesis is predicated on the assumption that atrazine degrades slowly in an aquifer because of low organic carbon concentrations, small microbial populations, and anaerobic conditions. This is substantiated by Wehtje *et al.* (1983) who determined that, under aquifer conditions, atrazine did not undergo deethylation or deisopropylation, and only slowly underwent abiotic degradation to HA.

The formation and transport of DEA and DIA in surface water were confirmed in a study by Thurman *et al.* (1994) in which field-dissipation studies and a regional study of nine streams in the Midwest Corn Belt showed that DEA and DIA occur frequently in surface water that has received storm runoff containing atrazine and cyanazine (Pomes and Thurman, 1991; Scribner *et al.*, 1994). The concentrations of DEA and DIA in surface water varied with the hydrologic conditions of the basin and the timing of runoff, with maximum concentrations reaching 5 μg/L (DEA | DIA). Early precipitation followed by a dry summer delayed the maximum concentrations, giving a 'second flush' of triazine degradation products to surface water. Replicated field-dissipation studies of atrazine and cyanazine indicate that DIA/DEA ratios varied from 0.4 − 0.1 when atrazine is the major triazine compound present, to 0.6 − 0.1 when significant amounts of cyanazine are present (Meyer *et al.*, 2001). A comparison of transport time of DEA and DIA

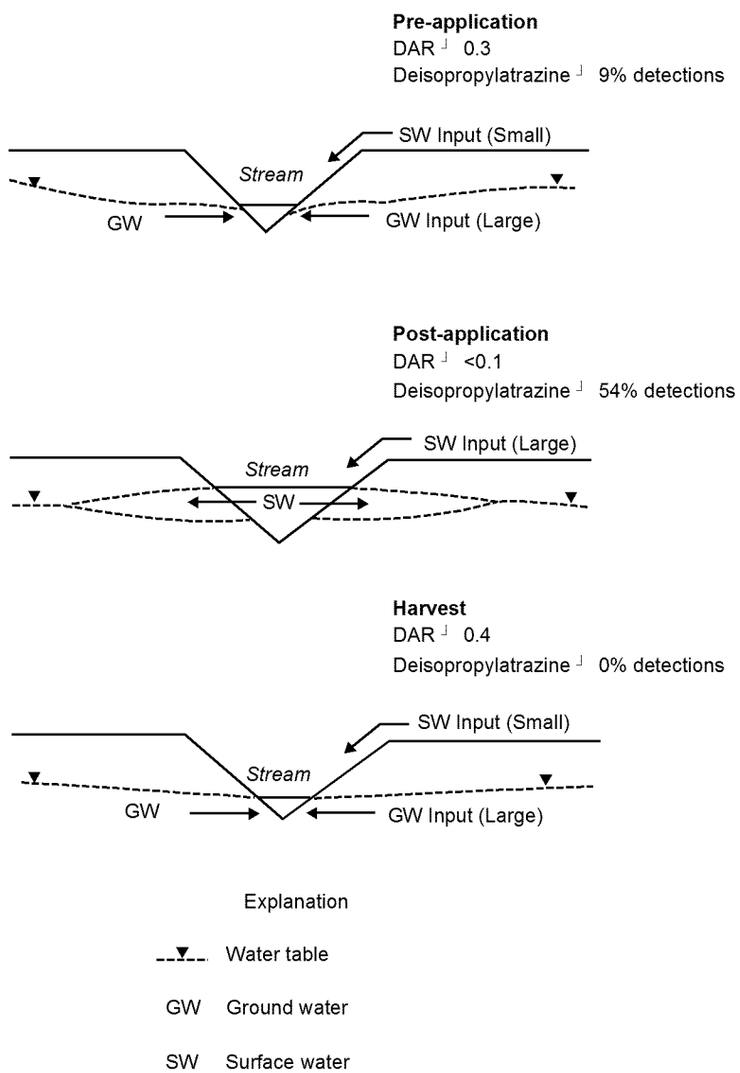


Figure 30.14 DAR for groundwater and surface water interactions during field-dissipation study at Topeka, Kansas.

from field plots to their appearance in surface water indicated that storage and dilution were occurring in the alluvial aquifers of the nine stream basins (Thurman *et al*., 1994).

Adams and Thurman (1991) found that atrazine transport through the unsaturated zone gave DAR values greater than 1.0, whereas atrazine transported off the field by surface runoff had DAR values much less than 1.0. DIA was rapidly degraded in the unsaturated zone, but was an important degradation product in surface runoff from the fields. Thus, the DAR may be indicative of groundwater recharge by water containing atrazine and may be used in studies of surface water transport. For instance, Figure 30.14 shows that pre-planting samples had a high DAR of 0.3, but also contained a few detections of DIA (9%). These results show both a surface water and groundwater origin of herbicides at this sampling period. The post-planting samples collected during this period had a low DAR (└ 0.1) and the greatest number of detections of DIA (54%), indicating that surface runoff is the major contributor of herbicides at this time. Finally, the post-harvest sampling during low streamflow period had the greatest DAR (0.4) and no detections of DIA, indicating the alluvial groundwater was likely the major source of herbicide at this time (Thurman *et al*., 1992).

In a study by Kolpin *et al*. (1994), data indicated that the ratio of the DAR provided useful information on the source of atrazine in groundwater. The fact that DEA was detected more frequently than DIA supports previous conclusions that deethylation is the preferred and more stable biotic degradation pathway. Also, this study confirmed that the more slowly infiltration through the soil occurs, the larger the DAR will become. As shown by Figure 30.15, the median DAR values from the groundwater reconnaissance for both pre- and post-planting sampling periods were about 0.7. The DAR ranged from about 0.1 to 8.4.

In a study by Fallon and Thurman (1996), a conceptual model was presented to explain how DAR could be used to follow runoff through Perry Lake in northeast Kansas. In the model, the reservoir already contained water with atrazine and DEA from the previous year's runoff. The arrows in Figure 30.16 show the direction of the flow for the dam. Near the dam, the water is older in age and contains water from the previous summer, whereas the upstream water contained herbicides that had entered the reservoir during the fall and winter. Therefore, the DAR values were higher on the upstream end of the reservoir, as shown in Figure 30.16 by the actual DAR values for each time period. The concept of the DAR has proved to be a useful tool in following water movement through a reservoir.

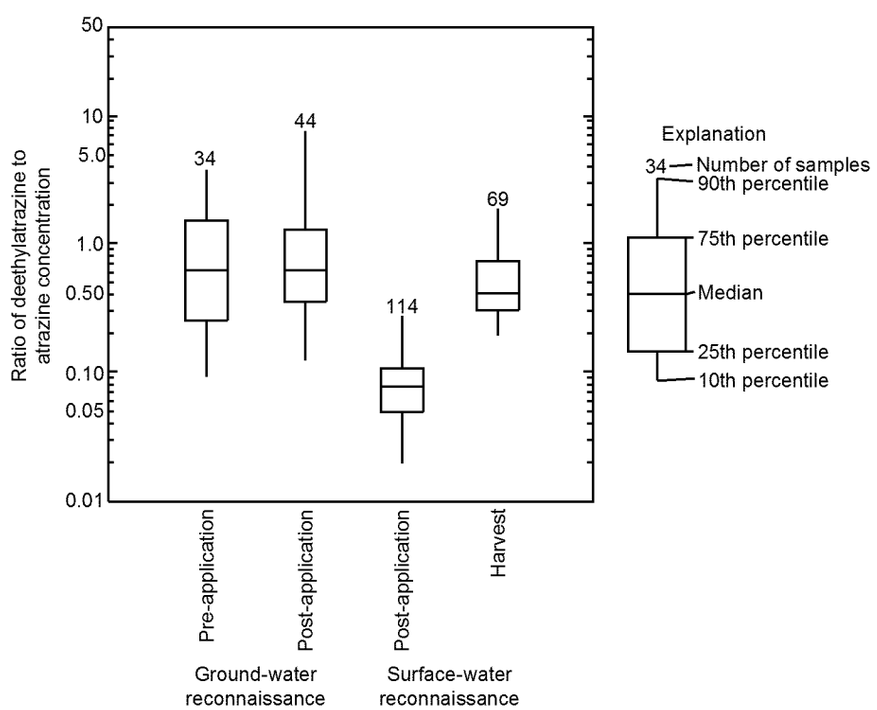


Figure 30.15 DAR for groundwater samples (Kolpin *et al*., 1994) and for surface water samples from midwestern United States (Thurman, 1992).

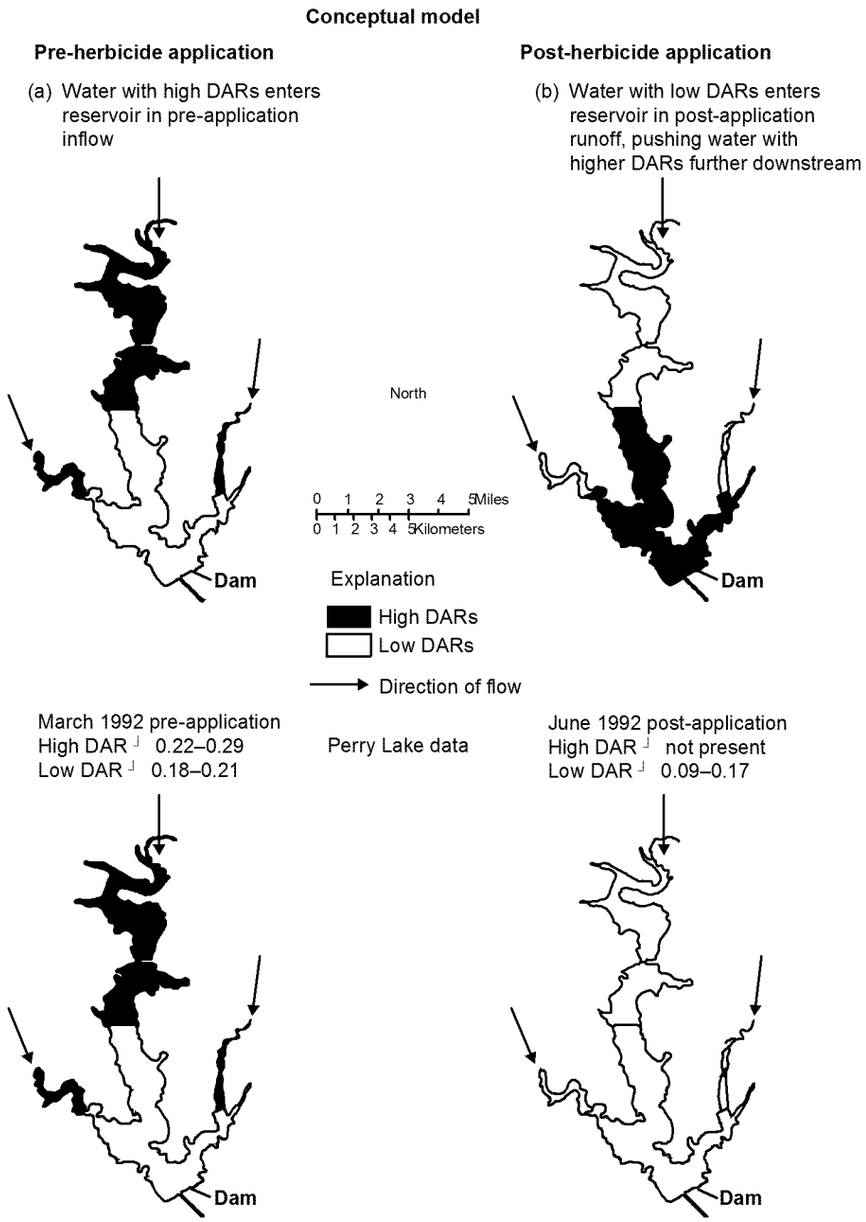


Figure 30.16 Conceptual model and data for DAR in Perry Lake, northeast Kansas during 1992. Modified from Thurman and Fallon (1996).

Conclusion

To understand nonpoint-source contamination of water resources, major water-quality research initiatives have been conducted in the United States since the 1990s. Investigations of herbicides in groundwater, surface water (including reservoirs), and precipitation have been carried out by the US Geological Survey. Studies of a network of groundwater wells in the midwestern United States identified the relationship between land use, groundwater age, and concentration and occurrence of herbicides and their degradation products. A reconnaissance study of 147 rivers was conducted to determine the geographic and seasonal distribution of herbicides. This study showed that large concentrations of herbicides were flushed from cropland and transported through the river system as pulses in response to spring and summer precipitation. The study also revealed the persistence of herbicides and their degradation products in rivers. A study of 76 reservoirs located in 11 midwestern states determined the occurrence and temporal distribution of triazine herbicides and their degradation products in the outflow could be related to reservoir and drainage-basin

characteristics, water and land use, herbicide use, and climate. The last study investigated precipitation in the upper Midwest, northeast to the Atlantic Ocean, and northward to the Canadian border. It was found the highest concentrations in precipitation occurred following herbicide application to cropland. The result of these studies is a clear understanding of the aquatic transport and fate of the triazine herbicides in the environment. Increased knowledge of the transport and fate of the triazines was an important goal of the monitoring effort of the past decade, in addition to providing the data for monitoring exposure and toxicity assessments of the triazines in the aquatic environment.

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Chapter 31

Probabilistic Risk Assessment Using Atrazine and Simazine as a Model

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Summary

Results of a probabilistic risk assessment indicate that neither occupational exposure nor environmental exposure to atrazine and simazine is likely to produce adverse health consequences in the US population. This conclusion is based on a quantitative risk assessment that potential human exposure to atrazine and simazine is much smaller than the intakes required to produce adverse health effects in animal experiments.

In human health risk assessments, the margin of exposure (MOE[1]) can be defined as the amount of a substance required to produce adverse health effects in animal experiments divided by the amount a human receives. The larger the MOE is, the safer the exposure.

Probabilistic techniques (including Monte Carlo methods) were used to incorporate the variation in individual human exposures and resulting intakes. The frequency distributions of individual intakes and MOEs in a population were estimated from the number of individuals in each of the population's component subpopulations and their corresponding intake distributions.

Even when an individual's atrazine and simazine intakes from drinking water ingestion (water) and food consumption (diet) were combined, 95% of the MOEs exceeded 30 000. The minimum acceptable MOE for human environmental exposure is usually in the range between 10 and 1 000; so the atrazine and simazine MOEs provide an ample safety margin. For atrazine and simazine combined, 95% of the MOEs are in excess of 38 000 for water alone and in excess of 280 000 for diet alone.

The MOEs were also calculated for herbicide handlers who used atrazine in conjunction with crop production (corn, sorghum, sugarcane, or sod), vegetation management, and residential lawn care, for herbicide handlers using simazine in crop production (corn or sod), and for both flowable and granular herbicide formulations. These MOEs included the combined atrazine and simazine intakes from water and diet. MOEs exceeded 3 000 in at least 95% of the calculations for each use, except for sugarcane, where the MOE exceeded 500. In comparison to a minimum acceptable MOE for occupational exposure (generally, 10–100), the calculated MOEs (including both occupational and environmental exposures) for atrazine and simazine provide an ample safety margin.

The MOEs for each herbicide use were calculated not only for the population of all such herbicide handlers, but also for several subpopulations (growers, commercial operators, mixer/loaders, applicators, etc.). The calculation of the frequency distribution of triazine herbicide intake from herbicide handling included data on the size of the different herbicide handler subpopulations, the frequency distribution of pounds of herbicide (active ingredient) applied, the frequency distribution of the amount of exposure inside normal clothing per pound of active ingredient applied (for each body part, herbicide formulation, method of mixing/loading, and method of application), and the frequency distribution of adult body weight.

The MOEs for water were calculated for each major-use state and for all major-use states combined. The calculation of the frequency distribution of triazine herbicide intake from water included the number of people using each community water supply system in each major-use state and the corresponding estimates of concentrations of the herbicide in the drinking water in these community water supply systems in each of the four seasons.

[1]For a description of this and other technical terms, see the Glossary at the end of this chapter.

ED_001334_00003007-00494 EPA-HQ-2017-008866

The MOEs for diet were calculated for different regions and for all regions combined. The calculation of the frequency distribution of herbicide intake from diet included all food potentially exposed to the herbicide, the average amount of each of these foods consumed per day in a lifetime, and the frequency distribution of the residue concentration (triazine herbicide and chloro-metabolites) in each of these foods.

Introduction

This chapter presents a quantitative probabilistic risk assessment for atrazine and simazine conducted for Syngenta Crop Protection, Inc. The risk of an effect is the likelihood that an individual will develop the effect as a result of that individual's exposure to atrazine and/or simazine. The risk assessment is quantitative because it characterizes the likelihood in numerical terms. The risk assessment does include some qualitative discussion of the uncertainties associated with the quantitative characterization of the likelihood. It also assumes relevance of an animal effect in humans even when a lack of human relevance has been established, as is the case with atrazine and simazine [US Environmental Protection Agency (USEPA), 2006].

The likelihood that an individual will develop a specified response depends on the dose the individual receives as a result of exposure and the relevance of the effect to humans. The dose of atrazine and/or simazine is measured as the intake in milligrams of herbicide per kilogram of body weight per day (mg/kg/day). The way in which the likelihood that an individual will develop a specified response is characterized depends upon the dose–response relationship (defined and discussed in the next three paragraphs).

The manner in which the proportion of animals developing a response changes as the dose level changes is the dose–response relationship. If the proportion decreases in parallel with decreasing dose (e.g., halving the dose halves the proportion), then the dose–response relationship is linear. However, if the proportion decreases faster than linearly (e.g., halving the dose results in either one-fourth the proportion or no occurrences of the adverse effect), then the dose–response relationship is sublinear (one type of nonlinearity).

In some types of experimental animals and at some high dose levels of atrazine or simazine, there were rodent health effects for which the observed proportion of animals developing the effect increased relative to the proportion in unexposed or control animals (Rinde, 1989; USEPA, 1989; Breckenridge, 1996a, b). For both atrazine and simazine, the incidence of mammary tumors in female Sprague-Dawley (SD) rats is the most sensitive effect in the most sensitive sex, strain, and species tested (Breckenridge, 1996a, b). The USEPA has concluded that the tumor response in the SD rats is not relevant to humans (USEPA, 2003).

The proportion of female SD rats developing mammary tumors decreases rapidly at lower experimental doses (Wingard, 1986; McCormick, 1988; Thakur, 1991, 1992). However, there is a high incidence of spontaneous mammary tumors in untreated female SD rats. The observed dose–response relationship is sublinear. Furthermore, the biological mechanism by which atrazine and simazine cause this response is a threshold mechanism; so the sublinear dose–response relationship contains a range of positive doses for which the frequency of the response is not increased above the background frequency at zero dose (Andersen *et al.*, 1998; Connor *et al.*, 1998; Eldridge *et al.*, 1998; Simpkins *et al.*, 1998). USEPA (2003) and a 2000 USEPA scientific advisory panel have concluded that exposure assessment should be based on a margin of safety or a MOE approach.

The MOE is defined as a benchmark dose divided by the dose from exposure. As the dose from exposure becomes smaller, the MOE becomes larger and the likelihood of any adverse health effect as a result of the exposure becomes smaller or zero. The larger the MOE is, the more confidence that no adverse health effect will be observed as a result of the exposure.

The MOE does not quantify risk (the increased probability of an adverse health effect). Instead, the MOE indicates how far the dose from exposure is below the benchmark dose. If the MOE is sufficiently large, then the increased probability of an adverse health effect is either zero (because the dose is below a threshold for the adverse health effect) or *de minimis* (without appreciable risk or practical certainty of no harm) and, hence, acceptable or safe.

In 1954, Lehman and Fitzhugh of the US Food and Drug Administration (USFDA) proposed the use of a 100-fold margin of safety. Factors of 10, 100, and 1 000 were used by the Drinking Water and Health Committee of the National Research Council (NRC, 1977). Typically, for environmental exposures (e.g., from drinking water and food), the minimum acceptable MOE is in the range from 10 to 1 000(e.g., Lu and Sielken, 1991; Dourson *et al.*, 1996). For occupational exposures, the minimum acceptable MOE is usually in the range from 10 to 100, and MOEs greater than 100 are generally sufficiently large to be acceptable.

Traditionally, the benchmark dose for noncancer endpoints has been the no observed effect level (NOEL), or the lowest observed effect level (LOEL) when the NOEL is not quantifiable. The NOEL is the highest dose of a substance for which there are neither statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control. Because the NOEL is limited to one of

the experimental dose levels and is sensitive to the sample size in the experiment, the effective dose (ED_{10}, ED_{05}, etc.) is increasingly being used as the benchmark dose (USEPA, 1996). For example, the ED_{10} is the dose corresponding to an increase of 0.10 in the probability of an adverse health effect above the background probability at dose zero. The ED_{10} corresponds to a well-defined increased risk, is usually in or near the range of the experimental doses, and its estimated value is usually relatively insensitive to the type of dose–response model fit to the observed experimental data. Use of the ED_{10} as the benchmark dose in an MOE characterization of risk for sublinear dose–response relationships is consistent with USEPA's guidelines for carcinogen risk assessment (USEPA, 2005).

The benchmark dose used in this MOE-based risk characterization for atrazine and simazine is the ED_{10} for the mammary tumor response observed in female SD rats in lifetime rat studies (Sielken *et al.*, 1996). The doses given to male SD rats and both sexes of Fischer 344 rats and CD-1 mice were large enough that there should have been an observed increment in their tumor response if they were as sensitive as the female SD rats; however, atrazine and simazine did not increase the incidence of mammary tumors in male SD rats, Fischer, rats or in mice, did not increase the incidence of other tumor types in either Fischer or SD rats or in mice, and did not increase the incidence of any adverse noncancer effects at doses as low as those increasing the incidence of mammary tumors in female SD rats (Breckenridge, 1996a). Thus, the ED_{10} for this tumor response is the ED_{10} for the most sensitive health effect observed in the most sensitive sex, strain, and species studied in animal chronic bioassays. Although this response has been determined to be not relevant to humans by USEPA (2003) and other regulatory bodies around the world [International Agency for Research on Cancer (IARC), 1998; United Kingdom (UK), 2000; Australian Pesticides and Veterinary Medicines Authority (APVMA), 2004], this response was used in order to conduct an extremely conservative risk assessment for the triazines.

For atrazine, the ED_{10} was calculated for each possible data combination (Sielken *et al.*, 1996). Two chronic bioassays involving female SD rats were used. There were three possible representations of each of the two bioassays. In the first bioassay, either all five doses, only the lowest four doses, or only the lowest three doses could be used. In the second bioassay, either the oncogenicity study, the hormone study, or both studies combined could be used. There were two different dose–response models – the multistage model that excludes time-to-response information and the multistage-Weibull model that includes the time-to-response information. There were two ways to fit the dose–response models to the tumor data: with the model forced to be linearly increasing at low doses and without the requirement to increase as soon as the dose increases above zero. Thus, there were 24 combinations (2 ┐ 3 ┐ 2 ┐ 2). The range of the 24 corresponding ED_{10}s was from 1.4 to 26.3 mg/kg/day.

Treating each of these 24 calculated ED_{10}s as equally likely and using the corresponding distribution to characterize the ED_{10} would have been a better reflection of the uncertainty in the dose–response characterization. However, the smallest of these 24 calculated ED_{10}s (approximately the 5th percentile in the distribution of estimates) is the value used in this chapter for the ED_{10} for atrazine. A similar lower bound (95% lower confidence limit) is used for the ED_{10} for simazine. The range of the eight calculated ED_{10}s for simazine was from 2.6 to 13.1 mg/kg/day. The lower bounds on the ED_{10}s are most conservative (by minimizing the MOE and maximizing the protection of human health). Our worst case estimates used the smallest dose that causes a 10% increase in tumor incidence in the SD rat studies on atrazine and simazine. These conservative values for the ED_{10}s for atrazine and simazine in animals are 1.4 and 2.6 mg/kg/day, respectively (Sielken *et al.*, 1996).

The doses of atrazine and simazine received by individual humans through either environmental (drinking water ingestion and food) or occupational (herbicide handling) exposures are much smaller than the doses required to observe health effects in the most sensitive sex, strain, and species of experimental animals. Thus, the MOEs for individuals exposed to atrazine and simazine are quite large, indicating a considerable margin of safety. Quantifying the magnitude of these MOEs is the main subject of the rest of this chapter.

The doses from exposure are characterized by distributions. For each possible dose level, these distributions quantify the probability that an individual in a specified population or subpopulation will receive that dose level as a result of exposure to atrazine and simazine through drinking water ingestion, dietary consumption, herbicide handling, or a combination of these potential exposure routes. For chronic toxic endpoints, the traditional (default) dose metric summarizing a lifetime of exposure is the lifetime average daily dose (LADD). Distributions of LADDs have been determined, and the corresponding distributions of the MOEs are presented herein.

Human health risk assessment has often been dominated by the use of default assumptions and worst case analyses, based on the use of upper bounds on the dose from exposure instead of distributional characterizations of that dose. There are severe limitations associated with the use of default assumptions and upper bounds instead of distributions when detailed exposure and/or dose–response data are available. The US National Academy of Sciences, the USEPA, and many others have recognized the need for new risk assessment methodology (NRC, 1983, 1993, 1994; USEPA, 1992; CRARM, 1997). This need has promoted the development of new quantitative risk assessment methods that use probabilistic techniques, especially Monte Carlo simulation and distributional characterizations of dose–response, exposure, and risk. For these reasons, this paper uses a probabilistic approach. An indication of some of these new methods and the type of results they produce are given below.

The methodology in the case study for chronic exposure, as well as several advances in probabilistic assessment methodology for acute exposure (e.g., a person's exposure on a single day), are being incorporated into the Cumulative and Aggregate Risk Evaluation System (CARES) begun in 2000 and being further developed with the International Life Sciences Institute (ILSI) in 2004.

Guidance on aggregate and cumulative risk assessment has recently been published (ILSI, 1998; USEPA, 2003), and the methodologies discussed (Sielken, 2000; Van Hemmen and Van der Jagt, 2001). New guidelines for carcinogen risk assessment have also been recently published (USEPA, 2005). Regulatory implementations of the recent guidance have been published for atrazine (USEPA, 2002b; PMRA, 2003), as well as for other pesticides (USEPA, 2002a).

Benefits of Using Probabilistic Techniques Instead of Default Constants

Risk assessments conducted by many federal and state agencies have generally relied on default constants. This technique uses a policy-driven choice of a single value for an unknown or uncertain component of the risk assessment. Each of these single values is generally selected to fulfill the goal of being health-protective; that is, it is selected to be reasonably certain that risk is not underestimated and to err on the side of overestimating risk. The use of default constants has several shortcomings that can be largely overcome by using probability distributions and probabilistic techniques.

In contrast to the use of a single (default) value for a risk parameter, a probability distribution can reflect the relative likelihood of the different possible values of the parameter. Thus, a probability distribution can reflect not only the largest and smallest possible values of a parameter, but also the probability of the occurrence of each of the values in its range.

If default constants are used for each of several different parameters in the risk assessment, then the conservative aspect of the individual components is compounded when they are combined in the risk characterization. Furthermore, the extent of the overestimation cannot be readily quantified, and so the magnitude of the overestimation of the risk is not identified. However, distributional techniques make it possible to combine exposures more realistically – whether from multiple years, subpopulations, exposure pathways, or chemicals – without having to assume the worst case for each component. By carrying all the information for each component of the risk assessment through to the end of the entire risk characterization, instead of requiring interim single-number characterizations, probabilistic techniques help avoid the compounding of the conservative aspect of multiple parameters.

The estimated upper bound on risk obtained by combining default constants provides no indication of the relative likelihood or frequency of that risk or any other risk between zero and the exaggerated upper bound. On the other hand, the risk characterization obtained by using probability distributions and probabilistic techniques provides a quantitative assessment of the relative likelihood of each of the different possible values for the risk.

Furthermore, default constants and assumptions do not explicitly address the uncertainty and variability that are an inherent part of human risk assessments; however, probability distributions can explicitly include both uncertainty and variability. The uncertainty here refers to lack of knowledge or the limitations in the current state of knowledge. Variability, on the other hand, refers to the parameter value differing from one individual to another individual in a population, or from one instance to another. Additional research may reduce uncertainty, but not variability.

Finally, probability distribution characterizations can describe the entire population (all of the people in the exposed population), rather than a hypothetical subpopulation.

Exposure Characterization

Exposure assessments characterize the water, diet, and herbicide handling exposure pathways for atrazine and simazine (Sielken *et al.*, 1996, 1998). For each exposure pathway, the chemical-specific doses (mg/kg/day) from each relevant route (ingestion, inhalation, and dermal) are summed. The total chemical-specific dose for each exposure pathway is characterized separately, and then these doses are aggregated by summing over the multiple exposure pathways. The pathway-specific and aggregate assessments are performed separately for atrazine and simazine. In addition, because atrazine and simazine are assumed to have a common mechanism of toxicity, a cumulative exposure assessment is performed combining the doses of atrazine and simazine.

The aggregate and cumulative assessments required by the 1996 Food Quality Protection Act (FQPA) when sufficient data are available combine the water, diet, and nondietary pathways (e.g., residential users), but exclude occupational pathways. The aggregate and cumulative assessments in this chapter include not only residential users, but also occupational herbicide handling by growers and commercial operators. Thus, the corresponding aggregate and cumulative assessments for atrazine and simazine in this chapter estimate more exaggerated doses than required by FQPA.

The exposure analyses are based on data provided by Novartis Crop Protection, Inc. to the USEPA on March 23, 1995, and updated on October 31, 1996. These data included several new studies that added to the state of knowledge about the potential human exposure to atrazine and simazine through drinking water (Clarkson, 1996), diet (Bray, 1996a, b, c), and herbicide handling (Selman, 1996a, b, c).

The distribution of the dose from exposure is characterized separately for the US population, four regional subpopulations, several states, and several different subpopulations of herbicide handlers that reflect different herbicide uses, formulations, and tasks. These distributions reflect the variability in the dose from individual to individual within the population (or subpopulation). Rather than focusing on an average exposure in a population, the distribution describes the relative frequency of each dose value. This means that these distributions indicate the dose that is most likely to occur, the range of doses expected in the population, and the relative likelihood of the different doses in that range. Each of the individual doses in the distribution is the best estimate of that individual dose and not an upper or lower bound.

This chapter provides an overview of the exposure, dose, and risk assessments for atrazine and simazine. The underlying databases and detailed algorithms used to produce the numerical results presented herein have been submitted to the USEPA (Sielken *et al.*, 1996).

The Role of Monte Carlo Simulation

The exposure from each of the routes of exposure (drinking water ingestion, dietary consumption, and herbicide handling) is described by an equation in the triazine assessment. Some of the components of these equations have values that are variable (e.g., varying from individual to individual, from one year to the next, from one serving of a specific food to another serving, and from one handling of a herbicide to another handling). These variable components of the exposure equations are described by probability distributions that reflect the relative frequency of the different values for the variable.

The outcome of the exposure equation is a dose. This dose varies because of the variability of the components in the equation. The probability distribution of the dose is generally quite difficult to calculate analytically, but can be fairly readily approximated using a Monte Carlo simulation. The simulation consists of numerous iterations. In an iteration, a single value for each component in the exposure equation is randomly sampled from its corresponding distribution. These component values are then substituted into the exposure equation, and the outcome (exposure) is explicitly calculated. The frequency distribution of the calculated values from numerous iterations is the simulated exposure distribution. The exposure equations and the probability distributions of the components are treated as known in the distributional results presented in this chapter. Thus, the simulated exposure distributions reflect exposure variability – but not uncertainty about these equations, the distributions of the components, and related assumptions. This uncertainty and its quantitative impact on the simulated exposure distribution are presented in Sielken *et al.* (1996).

In the Monte Carlo approach, there are no inherent limitations on the complexity of the exposure equation, the number of component variables, the probability distributions for the variable components, or the number of iterations. This freedom from limitations is especially useful in simulating the distributions of a LADD for the different exposure scenarios considered here. As its name suggests, a LADD is the average over all the days in an individual's lifetime of the dose of a chemical (e.g., atrazine, simazine, or both) received as a result of his or her exposure from one or more exposure pathways (e.g., water, diet, or herbicide handling). Because the exposure equation can explicitly consider each day individually, the values of the equation's variable components can vary from day to day and have different distributions for different ages and different lifespan projections.

Another powerful feature of the Monte Carlo approach is that when the values of the variable components in an exposure equation are being determined in an iteration, the value for one variable can depend on the value of another variable. For example, when determining the dose from drinking water ingestion in a region containing several states, the computer software used allowed the random selection of a state for each Monte Carlo iteration. Then, the concentration of atrazine or simazine in the individual's drinking water is selected from that state's distribution of drinking water concentrations, rather than from a national distribution. This capability of conditioning the distribution of one variable (e.g., the concentration in the drinking water) on the value of another variable (e.g., the state) helps advanced Monte Carlo implementations better reflect reality.

The Monte Carlo exposure calculations described in this chapter are carried out with a flexible computer software program named DistGEN (Sielken Inc., 1995). This program allows exposure equations to be specified in the general computer language called FORTRAN, so they can have practically any form. Furthermore, the user-specified distributions for the components of the exposure equations can be selected from a wide variety of classical statistical distributions (normal, log-normal, etc. with user-specified parameter values) or from sample data (either the sample

values themselves, frequency histograms, etc.). DistGEN incorporates a modification of the standard Monte Carlo procedure described above, called Latin Hypercube sampling. This modification allows for more accurate results with fewer iterations. Each Monte Carlo simulation described here is based on 10 000 iterations (10 000 evaluations of the exposure equations for individuals).

Margin of Exposure

The MOE is defined here as follows: MOE └ ED_{10}/(dose from exposure).

The dose from exposure is the LADD. In the next three sections the equations for calculating the daily doses going into the LADD are indicated for drinking water ingestion, dietary consumption, and herbicide handling, respectively, and the corresponding distributions of the MOE are displayed. Aggregate exposure is characterized separately for atrazine and simazine by the distribution of the MOEs aggregated across these pathways. Cumulative exposure is characterized by the distribution of the MOEs cumulated over atrazine, simazine, and all pathways.

Drinking Water Ingestion

The LADD (mg/kg/day) from drinking water ingestion for an individual is calculated using Equation (31.1):

LADD └ [concentration of herbicide in drinking water, mg/liter(L)]
┐ (amount of drinking water ingested per day, L/d)/(body weight in kg) (31.1)

Probability distributions for the LADD in the 18 states that use the most atrazine and simazine every year (approximately 90% of the total) were determined. In these major-use states, the concentration of herbicide in the drinking water varies too much between and within states to be accurately characterized by a single number. Instead, the database of observed individual concentrations collected by the states for local community water supplies, and the number of people served by each community water supply, were used in the Monte Carlo evaluations of Equation (31.1) and the corresponding LADD distributions. In determining these LADD distributions, the objective is to make the person whose drinking water herbicide concentration is used in Equation (31.1) equally likely to be each person served by community water supplies. For example, if the population of interest is a state, then the LADD distribution in that state is determined by randomly selecting a large number of individuals from that state and randomly selecting each individual's drinking water concentration from the database of drinking water concentrations for that individual's community water supply system. In order for the resulting distribution to correspond to the state's distribution, the selection process is done in a way that makes each person in the state equally likely to be selected and makes the likelihood of a community water supply being selected equal to its relative size within the state (i.e., the number of individuals served by the community water supply system divided by the number of individuals in the state). If the population of interest includes more than one state, then individuals are selected so that each individual in the population is equally likely to be selected, and the likelihood of each state is proportional to the relative size of the state within the total population.

Because the variability in the amount of drinking water ingested per day per kilogram of body weight is much smaller than the variability of the atrazine and simazine concentrations in the drinking water, Equation (31.1) is evaluated assuming a default upper bound value of 2 L/d and a default adult body weight of 70 kg/d.

The distributional analysis of the dose from exposure using Equation (31.1) indicates that for atrazine, at least 95% of the estimated LADDs from drinking water ingestion have an MOE of at least 50 000 in the 18 major-use states combined for atrazine (Figure 31.1). Figure 31.1 shows a histogram of the MOEs for atrazine in the 18 major-use states combined. The horizontal axis indicates intervals of possible MOE values, and the vertical axis indicates the proportion of individuals in the 18 major-use states that are estimated to have MOEs in that interval. For example, the smallest MOEs in the population are in the interval from 1 000 to 5 000; the proportion in this interval is only 0.0013 (0.13% of the population). The proportion of the population with MOEs below 50 000 is approximately 0.05 (0.0013┘ 0.0065┘ 0.0443 └ 0.0521). Hence, approximately 95% of the MOEs in the population are greater than 50 000. Figure 31.1 indicates not only the 95% lower bound on the MOE, but also the entire MOE distribution. This distribution covers a range from 1 000 to more than 10 billion, which indicates that the MOE in the population is quite variable and that most have MOEs considerably above the 95% lower bound. For simazine, at least 95% of the MOEs are greater than 200 000 in the 18 major-use states combined.

Figures 31.2 and 31.3 show the atrazine MOEs for the 18 individual major-use states separately. The entire histograms in these figures are not all easily seen, but what is important is that these major-use states have hardly any MOEs below 5 000 and that most of the people in every state have much larger MOEs.

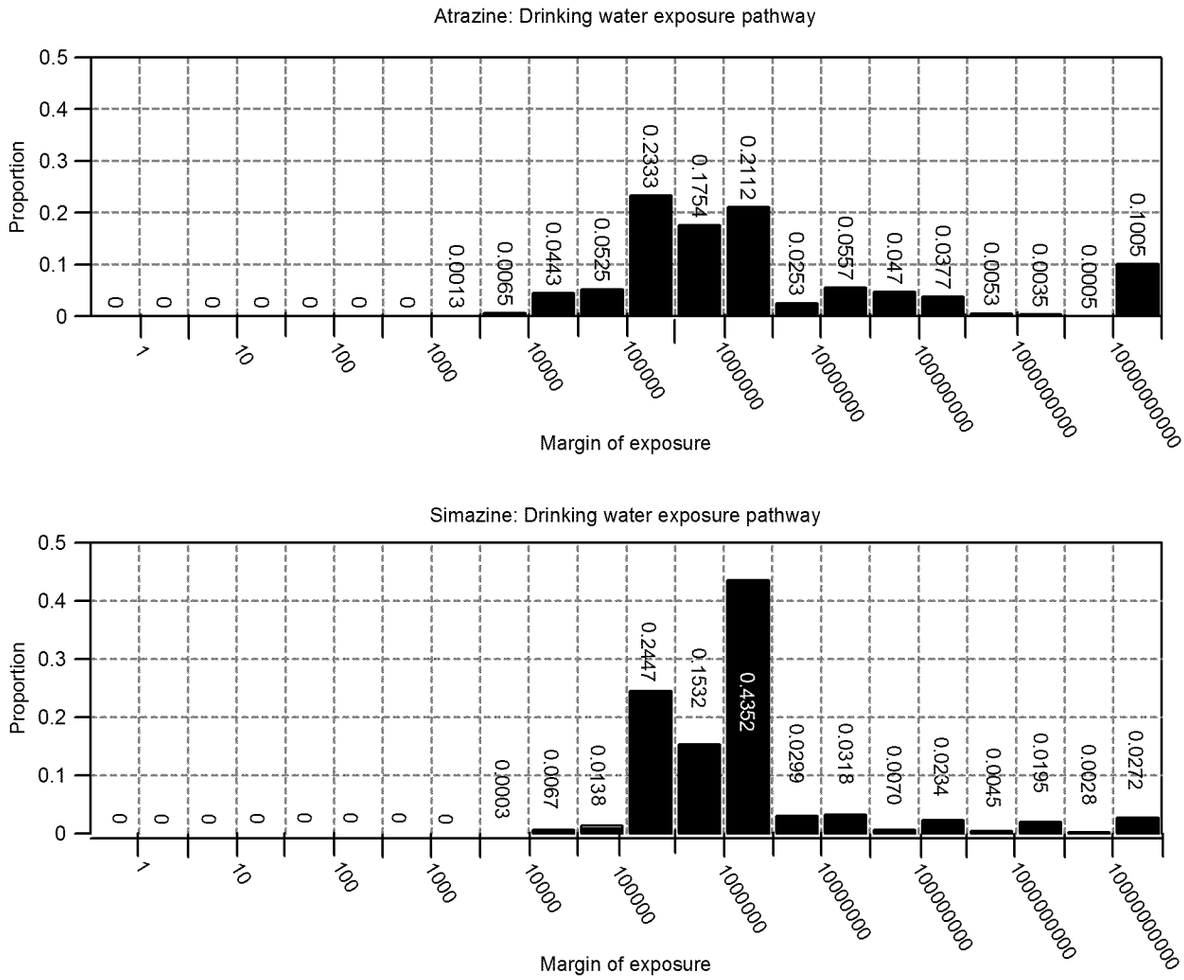

Figure 31.1 The distribution of the MOEs associated with atrazine and simazine from drinking water ingestion in the 18 major atrazine-use states combined. Based on drinking water data prior to June 1, 1994.

The margins of safety indicated by the MOEs in Figures 31.1–31.3 are even greater when the exposure evaluation is expanded to include the following alternatives:

- Drinking water consumption distribution and body weight distribution.
- Age-dependent drinking water consumption and body weight distributions.
- Year-to-year variability as opposed to the same concentration and consumption for 70 years.
- Exposure duration distributions corresponding to residential durations as opposed to 70 years.
- More recent water monitoring data. (Data used in this assessment were collected prior to June 1994, and atrazine levels in water are declining.)

Dietary Consumption

The LADD (mg/kg/day) from dietary exposure can be calculated for an individual in a specified population or subpopulation, using Equation (31.2):

LADD └ sum of the dose from each food
i └ number of foods
└ $\sum_{i└1}$ [(amount of food$_i$ consumed in a day per kilogram body weight, mg/kg/d)
┐ (residue concentration in raw agricultural commodity contributing to food$_i$, mg herbicide/mg food) ┐ (adjustment factor 1 for food$_i$) ┐ (adjustment factor 2 for food$_i$)] (31.2)

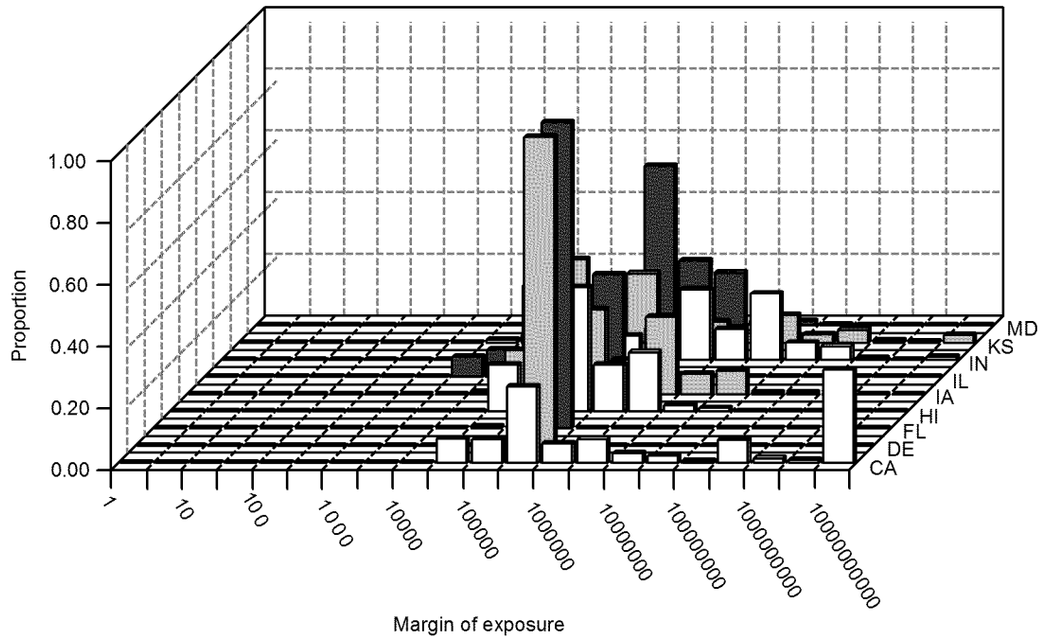

Figure 31.2 The distributions of the MOEs associated with atrazine from drinking water ingestion in nine of 18 major atrazine-use states using data prior to June 1, 1994.[a]
[a]CA └ California; DE └ Delaware; FL └ Florida; HI └ Hawaii; IA └ Iowa; IL └ Illinois; IN └ Indiana; KS └ Kansas; MD └ Maryland.

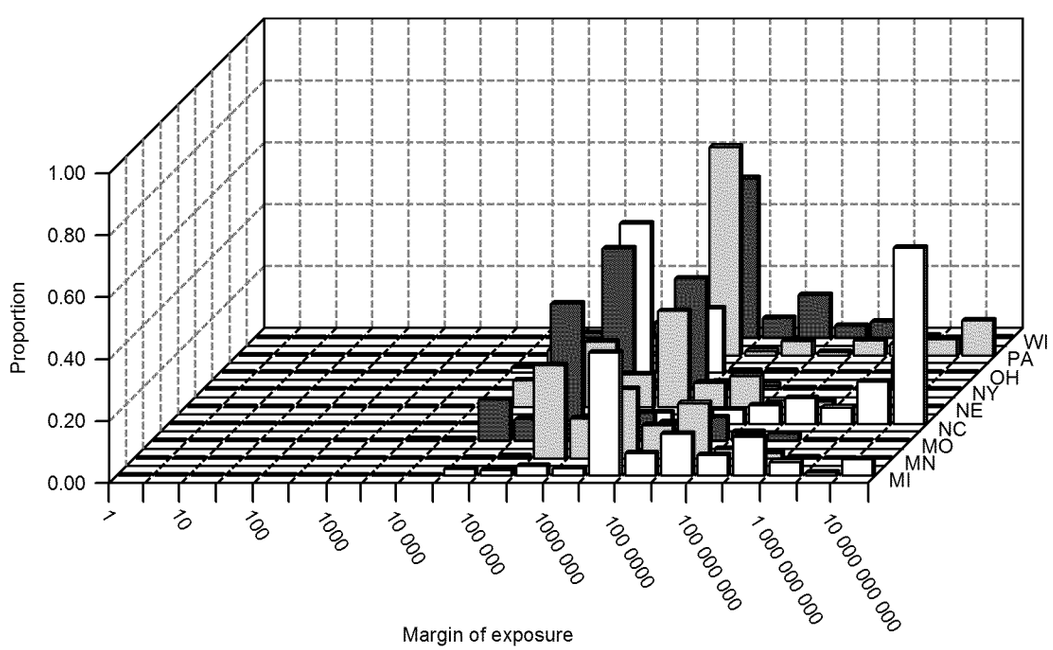

Figure 31.3 The distributions of the MOEs associated with atrazine from drinking water ingestion in nine of 18 major atrazine-use states using data prior to June 1, 1994.[a]
[a]MI └ Michigan; MN └ Minnesota; MO └ Missouri; NC └ North Carolina; NE └ Nebraska; NY └ New York; OH └ Ohio; PA └ Pennsylvania; WI └ Wisconsin.

In Equation (31.2), the amount of each type of food consumed in a day per unit body weight of the consumer is assumed to be a constant, equal to the corresponding food consumption value in the USEPA's database for Dietary Risk Exposure Assessments (DRES), which is an average chronic consumption value [US Department of Agriculture (USDA), 1983]. For most consumed foods, the food originates as a raw agricultural commodity. The fraction of the weight of the raw agricultural commodity that is the chemical of interest (e.g., atrazine or simazine) is the residue concentration. The residue concentration in the raw agricultural commodity is not necessarily the same as the chemical's concentration in the food as it is consumed. For example, the concentration of a chemical in an ear of corn when it is harvested in the field and the concentration after it has been cleaned and cooked may be different. This difference is accounted for by 'adjustment factor 1.' The values for adjustment factor 1 are the default constant values in DRES. During an individual's lifetime, some of the raw agricultural commodity in consumed food may come from crops treated with the chemical of interest, and some may come from untreated crops. An individual's lifetime average proportion from treated crops is assumed to equal the proportion of acres treated with the chemical. This proportion is reflected in Equation (31.2) as adjustment factor 2. The constant values for adjustment factor 2 were the data available on percent crop acreage treated. Ciba Crop Protection obtained these data in 1993 from Maritz Marketing Research Inc. and from Doane Marketing Research Inc., both of St. Louis, Missouri. In sensitivity analyses, adjustment factor 2 can be set at 1.0 to correspond to an individual's food being all locally produced and treated, instead of having residue concentrations corresponding to the national average.

Macadamia nuts, guava, refined sugar, and molasses are the only raw agricultural commodities treated with atrazine that are consumed as foods. There are no known residue concentrations of atrazine or its chloro-metabolites above their analytical limits of detection (LODs) in any of these four foods. In evaluating Equation (31.2), the residue concentration in each of these four foods is assumed to be equally likely to be any value between zero and its LOD (i.e., uniformly distributed between zero and the LOD).

For meat, milk, and eggs, the residue concentration in raw agricultural commodity contributing to food^{i} in Equation (31.2) is the concentration of the chemical of interest that results from some of the raw agricultural commodities in the diets of cattle and poultry being treated with that chemical. While the observed residue concentrations in meat, milk, and eggs are below their LODs, the concentrations of atrazine in the raw agricultural commodities used as feed for cows and poultry are sometimes quantifiable. Probability distributions on the anticipated residue concentrations of atrazine and its chloro-metabolites in meat, milk, and eggs are based on estimated diets for cows and poultry, the observed residue concentration distributions in the components of these diets, and proportionality constants relating high experimental concentrations in feed to resulting concentrations in meat, milk, and eggs (Sielken *et al.*, 1996). The estimated diets provided adequate nutrition to poultry and lactating dairy cattle and maximized the amount of feed items treated with atrazine.

Using Equation (31.2), distributional analyses of dietary exposure to atrazine and its chloro-metabolites in the United States and the four regions (Northwest, North Central, Southern, or Western) indicate that at least 95% of the estimated LADDs from dietary consumption have an MOE of at least 300 000 in each of the four regions and 330 000 in the United States as a whole (Figure 31.4).

The atrazine chloro-metabolites in the diet have been combined with atrazine in Figure 31.4. Atrazine's chloro-metabolites in the diet have been assumed to have the same toxicity as atrazine in calculating the MOEs in Figure 31.4.

There is not much variability in the distributions in Figure 31.4 because all of the components in Equation (31.2) have been assumed to be their lifetime average values, except for the residue concentrations. Thus, the only really important characteristic of the distributions in Figure 31.4 is that the MOEs are quite large.

For simazine, the residue concentrations in Equation (31.2) are constants (averages or upper bounds), determined directly from the most recent residue data on the commodities themselves or, for meat, milk, and eggs, determined indirectly from the diets of cattle and poultry. The corresponding MOE is at least 1 750 000 for each of the four regions and at least 2 000 000 for the United States as a whole.

While the following two observations are not critical in the distributional characterization of the intake of atrazine and simazine from dietary consumption, these observations can be important in other situations. First, making the assumption that the residue concentration in an individual's food is the same every time that food is consumed (as in Equation (31.2) exaggerates the variability in the intake distribution. Without this assumption, both the low and high percentiles of the intake distribution would be closer to the median intake, and the 95% lower bound on the MOE would increase. Second, when a sum is being characterized (such as the sum of intakes in Equation (31.2), it is important to determine explicitly the probability distribution of the entire sum and not to attempt to infer the characteristics of the distribution of the sum indirectly from the distributions of its components. For example, the 95th percentile of a sum may be much smaller than the sum of the 95th percentiles of its components.

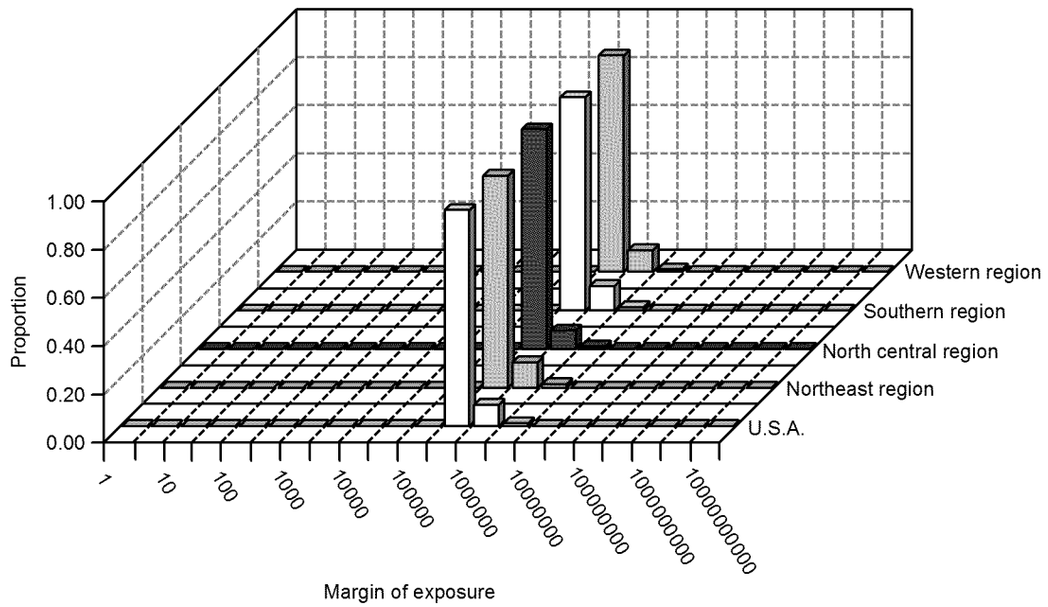

Figure 31.4 Distributions of the MOEs for atrazine plus its chloro-metabolites from dietary consumption.

Herbicide Handling by Workers

The LADD (mg/kg/d) from dermal absorption and inhalation due to herbicide handling exposure can be calculated for an individual in a specified population or subpopulation using Equation (31.3):

Dose └ {[(pounds of active ingredient applied per acre, lb a.i./A) ┐ (number of acres treated in a year, A/yr) ┐ (number of years in which treatments occur, yr)]/[(70 yr) ┐ (365.25 d/yr) ┐ (body weight, kg)]} (31.3)
┐ sum from k └ 1 to k └ 12 of [(fraction absorbed for the kth body part) ┐ (amount of exposure for the kth body part, mg/lb a.i.)]

In Equation (31.3), the application rate per acre is in terms of the pounds of active ingredient (i.e., atrazine or simazine), as opposed to the pounds of whatever mixture containing atrazine or simazine is actually applied to the area. The total amount of active ingredient (a.i.) applied per year is multiplied by the number of treatment years in a lifetime and divided by 70 years, 365.25 days per year, and body weight (kg) in order to convert to the lifetime average pounds of active ingredient applied per kg of body weight per day.

An individual's absorbed dose is assumed to be proportional to the amount of a.i. applied. In this paper that proportion (mg a.i. absorbed/lb a.i. applied) is derived from the exposure information in USEPA's Pesticide Handlers Database (PHED, 1992) and herbicide-specific absorption data. PHED provides exposure information on 12 parts of the body (as opposed to the body as a whole). For each body part, PHED provides data on the amount of active ingredient that comes into contact with that body part per pound of active ingredient applied (amount inhaled or amount of dermal contact per pound applied). The PHED data used here assume that the individual is wearing normal clothing and gloves but not additional protective devices such as aprons or respirators. Based on atrazine- and simazine-specific studies conducted by Syngenta, the fraction of atrazine and simazine absorbed as a result of dermal contact is 0.056 when the exposure is less than or equal to 8 $\mu g/cm^2$, 0.012 for exposures greater than or equal to 80 $\mu g/cm^2$, and a linear interpolated value for intermediate exposures. The fraction of the inhaled atrazine or simazine that is assumed to be absorbed is 1.0.

In applying Equation (31.3), the lb a.i./A was assumed to be a use-specific constant, and the number of acres treated in a year was assumed to be a use- and user-specific distribution. Use refers to crop (e.g., corn, sorghum, North American sugarcane, or Hawaiian sugarcane), vegetation management, sod, or lawn care, and user refers to a commercial operator, grower, or homeowner, as well as the method of herbicide mixing, loading, and application. The number of years in which treatments occur was assumed to be 10 years for commercial lawn care operators and 35 years for all other uses and users.

The distribution of body weights was assumed to be a normal distribution with mean 70 kg and a 20% coefficient of variation (i.e., a standard deviation equal to 14 kg). The amount of exposure for a body part was a PHED-based distribution, depending on the body part and the type of user, as well as the type of herbicide formulation used. The herbicide formulations were: granule (G) formulated with fertilizer and used by homeowners for residential lawn care; flowable formulation (FF), which is among the formulations classified as emulsifiable concentrate (EC) in PHED; and water-dispersible granule (WDG).

Using Equation (31.3), the distributions of exposure indicate that at least 95% of the estimated LADDs associated with herbicide handling exposure have an MOE of at least between 500 and 11 000 for atrazine, and between 10 000 and 20 000 for simazine, depending on the herbicide use (e.g., corn, sod, etc.) (Figures 31.5–31.7).

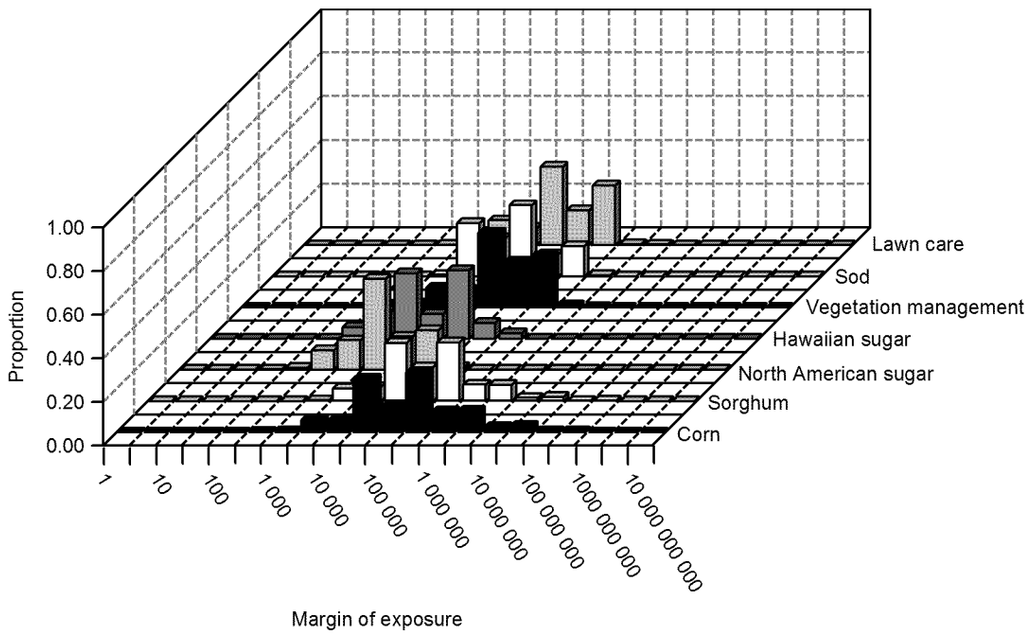

Figure 31.5 Distributions of the MOEs for atrazine from herbicide handling with flowable formulation for different use populations.

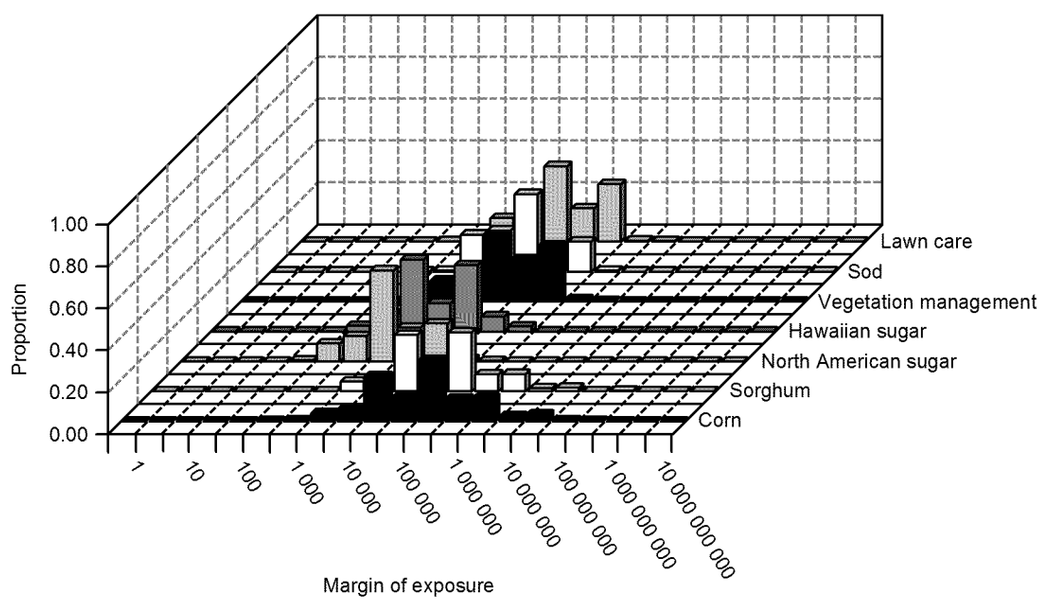

Figure 31.6 Distributions of the MOEs for atrazine from herbicide handling with WDG formulation for different use populations.

Even though the smallest MOEs in Figures 31.5–31.7 are relatively large, their true values are probably even larger. In statistical terms, this probable underestimation of the smaller MOEs occurs because the variance of an average of several variable events is less than the variance when every event is assumed to have the same value. Thus, the lower percentiles in the distribution for an average of several events are larger than the lower percentiles in the distribution when every event is assumed to be the same. For the same reason, the upper percentiles in the distribution for an average of several events are smaller than the upper percentiles in the distribution when every event is assumed to be same. In simpler terms, the LADD is almost always the average of a large number of different daily doses, corresponding to different exposure events – not the result of the same daily dose and exposure event repeated throughout the lifetime. However, Equation (31.3) implicitly assumes that all of the daily doses and exposure events are the same. For example, the lb a.i./A is implicitly assumed in Equation (31.3) to be the same for every year in which treatments occur. The number of acres treated in a year is assumed to be the same for every year. The fraction of the amount of herbicide applied that the body is exposed to and absorbs is assumed to be the same for every year in which treatments occur. If these factors were allowed to vary within the lifetime of an individual, then the 95% lower bounds on the MOEs shown in Figures 31.5–31.7 would be larger.

For each herbicide use, the whole population of herbicide handlers and each of several subpopulations of potential interest are explicitly evaluated. For example, for crops, the following subpopulations are explicitly evaluated:

1. All growers
2. Growers who do mixing/loading
3. Growers who do applications
4. Growers who do both mixing/loading and application
5. All commercial herbicide handlers
6. Commercial herbicide handlers who use the herbicides for ground application
7. Commercial ground mixer/loaders
8. Commercial ground applicators
9. Commercial herbicide handlers who use the herbicides in aerial applications
10. Commercial aerial mixer/loaders
11. Commercial aerial applicators (pilots)

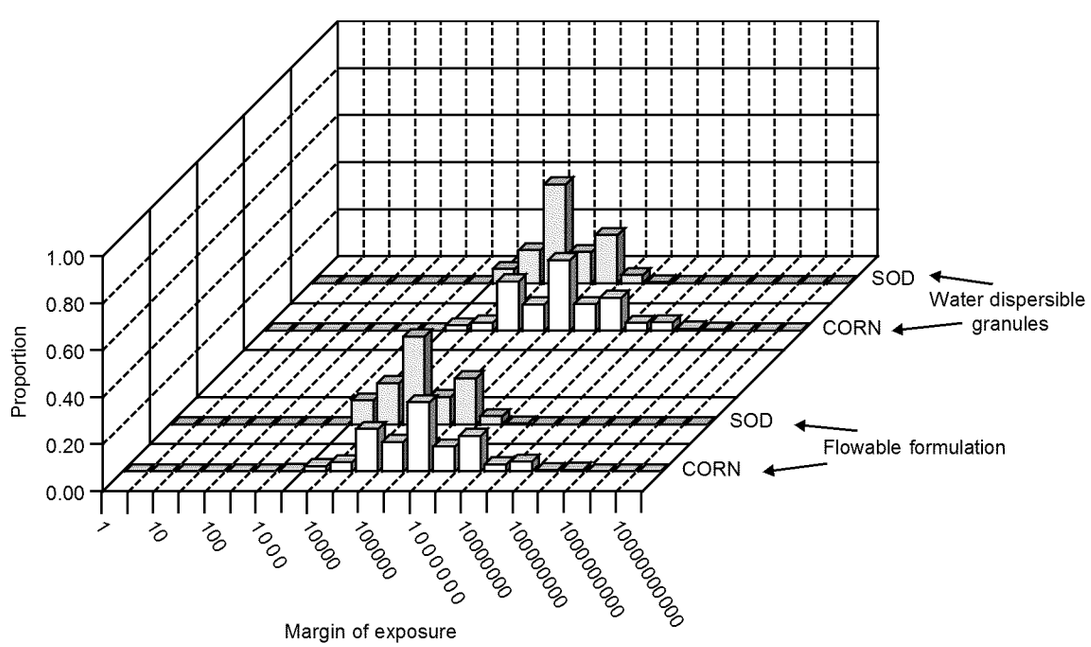

Figure 31.7 Distributions of the MOEs for simazine from herbicide handling with flowable and WDG formulations for corn and sod use populations.

These subpopulations are also further subdivided by:

(a) Herbicide formulation (flowable or WDG).
(b) Type of mixing/loading operation.
(c) Type of application.

Monte Carlo techniques allow the exposure characterizations for subpopulations to be properly aggregated into a population characterization that reflects the relative subpopulation sizes and the different exposure distributions, without having to assume the worst case for the population or a specific subpopulation. For example, Figure 31.8 shows how the population of herbicide handlers involved in the production of corn crops is related to its component subpopulations. The Monte Carlo simulation for the population of herbicide handlers involved in the production of corn crops can be done in such a way that 97% of the iterations in the simulation are expected to be for grower and 3% for commercial. Among the iterations for grower, one-third are expected to be for mixer/loader, etc. Thus, a Monte Carlo simulation correctly characterizes a population by sampling its subpopulations with the appropriate frequencies – rather than incorrectly characterizing a population solely in terms of its most-exposed subpopulation, or incorrectly characterizing an individual's exposure as the weighted average of exposures from different subpopulations.

Figures 31.9 and 31.10 indicate the distributions of the MOEs in the atrazine handling population involved in corn production, and each of its subpopulations for the flowable and WDG formulations, respectively.

Aggregate Exposure

Monte Carlo techniques allow the distribution of the LADDs for the combined exposure pathways for atrazine or simazine to be appropriately determined. It is assumed here that it is appropriate to add together the absorbed doses from each route (ingestion, inhalation, and dermal), and each pathway (drinking water, diet, and herbicide handling). Thus, the LADD distribution is the distribution among individuals of the lifetime average of the sum of the individual's daily doses from the different exposure pathways and routes. The individual's LADDs from the different pathways and routes are summed, and then the distribution of these sums in a population or subpopulation is determined. For example, this approach combines an individual's dose from drinking water ingestion with that same individual's dose from dietary consumption, rather than combining one person's dose from drinking water ingestion with a different person's dose from dietary consumption. Similarly, the 95th percentile for the combined pathway exposure is not determined by summing the 95th percentiles for the different pathways.

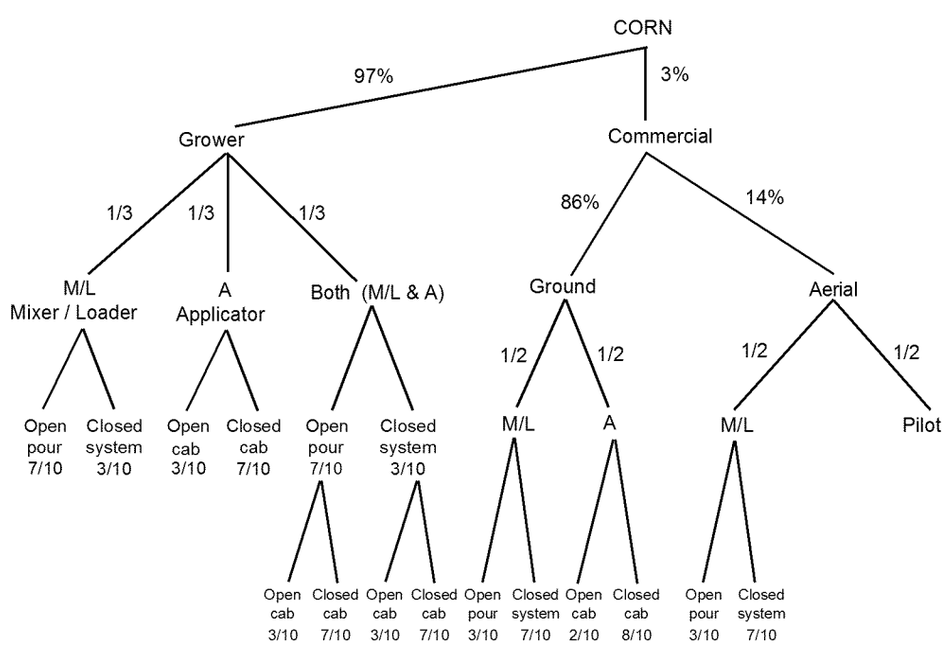

Figure 31.8 The population of all herbicide handlers using atrazine in corn production, shown by component subpopulations and the relative number of herbicide handlers in each subpopulation.

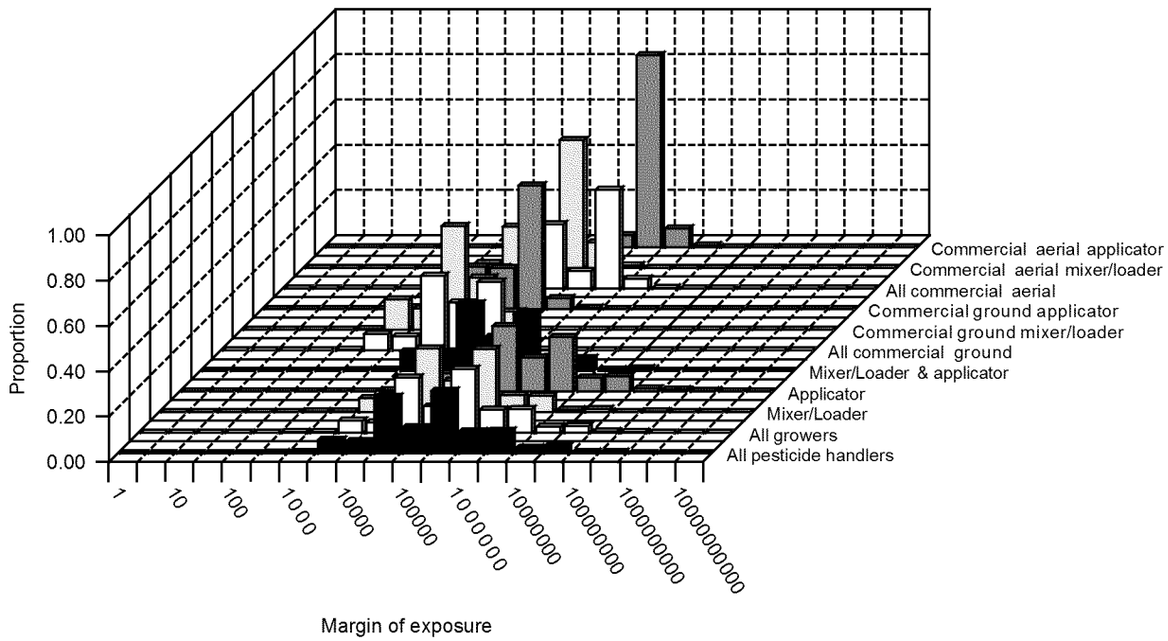

Figure 31.9 Distributions of the MOEs for atrazine from herbicide handling with fbwable formulation in corn production, shown for the entire population and its component subpopulations.

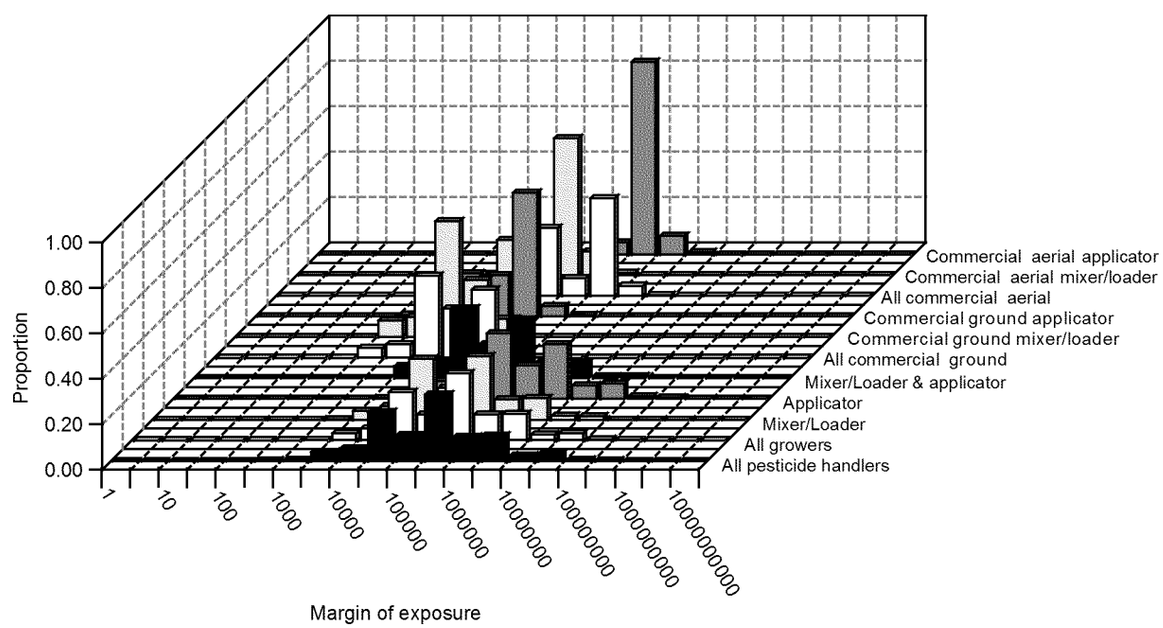

Figure 31.10 Distributions of the MOEs for atrazine from herbicide handling with WDG formulation in corn production, shown for the entire population and for its component subpopulations.

The distributions of the LADDs for atrazine or simazine contain only very small values for water, diet, and the combination of water and dietary exposures. Therefore, the corresponding MOEs are quite large, even when the water and dietary pathways are combined (Figures 31.11 and 31.12).

The LADD distributions for herbicide handlers contain slightly larger values for fl owable formulations than for WDGs, and both distributions contain considerably larger values than the distributions for water, diet, and the

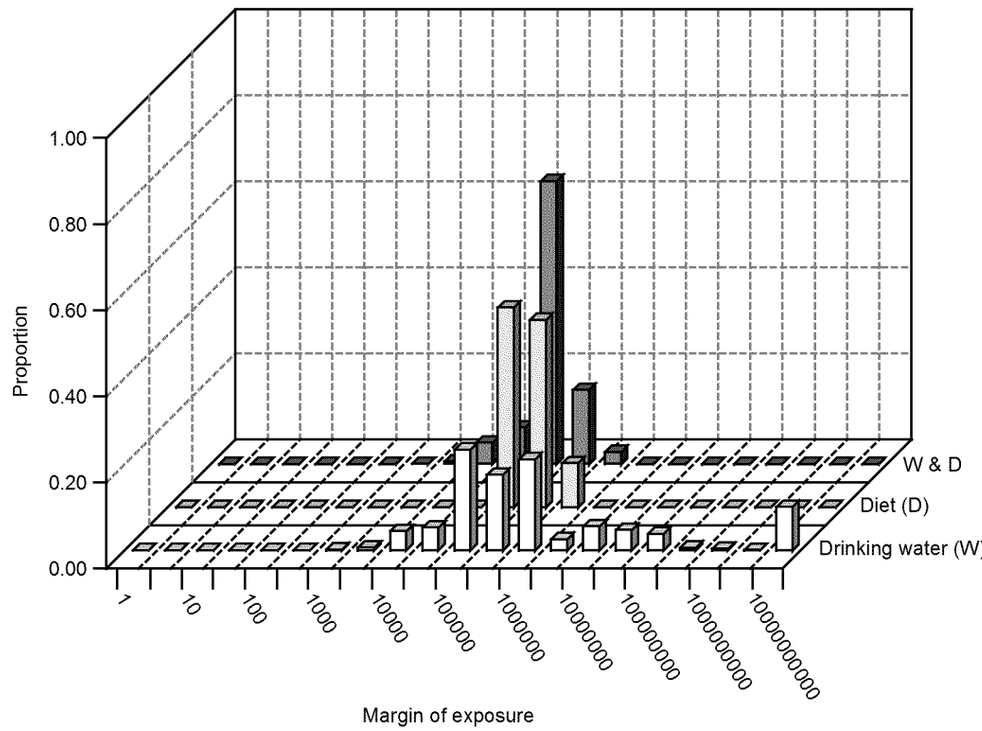

Figure 31.11 Distributions of the MOEs for atrazine from drinking water ingestion, dietary consumption, and both exposure pathways combined.

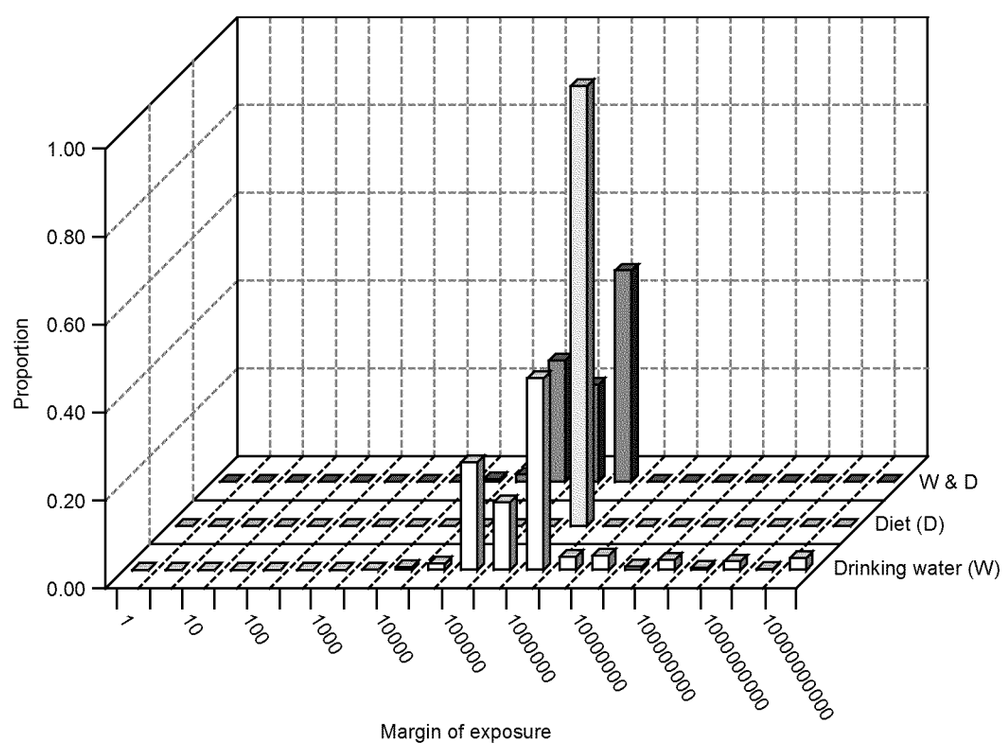

Figure 31.12 Distributions of the MOEs for simazine from drinking water ingestion, dietary consumption, and both exposure pathways combined.

combination of drinking water ingestion and dietary consumption. Finally, the values in the LADD distributions for herbicide handlers are not substantially increased by the addition of drinking water and dietary exposure pathways. Thus, the large MOEs for herbicide handlers remain large even when their doses from drinking water and dietary consumption are added to their doses from herbicide handling (Figures 31.13 and 31.14).

Cumulative Exposure: Atrazine and Simazine Combined

MOE distributions for atrazine and simazine combined contain only very large MOEs for water, diet, and the combination of water and dietary exposures (Figure 31.15). Distributions for herbicide handlers who apply either atrazine or simazine, and who are also exposed to atrazine and simazine through the water and dietary pathways, are almost the same as the distributions corresponding to the herbicide handling component alone (Figure 31.16).

The cumulative MOE for atrazine and simazine is calculated using Equation (31.4):

$$MOE_{atrazine\ and\ simazine} = 1/([1/MOE_{atrazine}] + [1/MOE_{simazine}]) \quad (31.4)$$

Because Equation (31.4) is mathematically equivalent to either Equation (31.5) or (31.6) (shown below), the formula for $MOE_{atrazine\ and\ simazine}$ in Equation (31.4) is of the proper form – namely, a benchmark dose corresponding to a known amount of toxicity, divided by a cumulative dose from exposure that reflects the relative toxicity of the cumulated chemicals.

$$MOE_{atrazine\ and\ simazine} = ED_{10,atrazine}/(1 + [dose_{atrazine}] + [ED_{10,atrazine}/ED_{10,simazine}] + [dose_{simazine}]) \quad (31.5)$$

$$MOE_{atrazine\ and\ simazine} = ED_{10,simazine}/([ED_{10,simazine}/ED_{10,atrazine}] + [dose_{atrazine}] + 1 + [dose_{simazine}]) \quad (31.6)$$

For example, in Equation (31.5) the multiplier of the dose due to simazine exposure ($dose_{simazine}$) is the toxic equivalency factor: $(ED_{10,atrazine}/ED_{10,simazine}) = [(1.4\ mg/kg/d)/(2.6\ mg/kg/d)] = 0.5385$.

The above equations are appropriate for atrazine and simazine because these herbicides appear to have a common mechanism of action, and the toxicological endpoints for the ED_{10}s are the same. The toxicological endpoint is the incidence of mammary tumors in female SD rats in similar experiments.

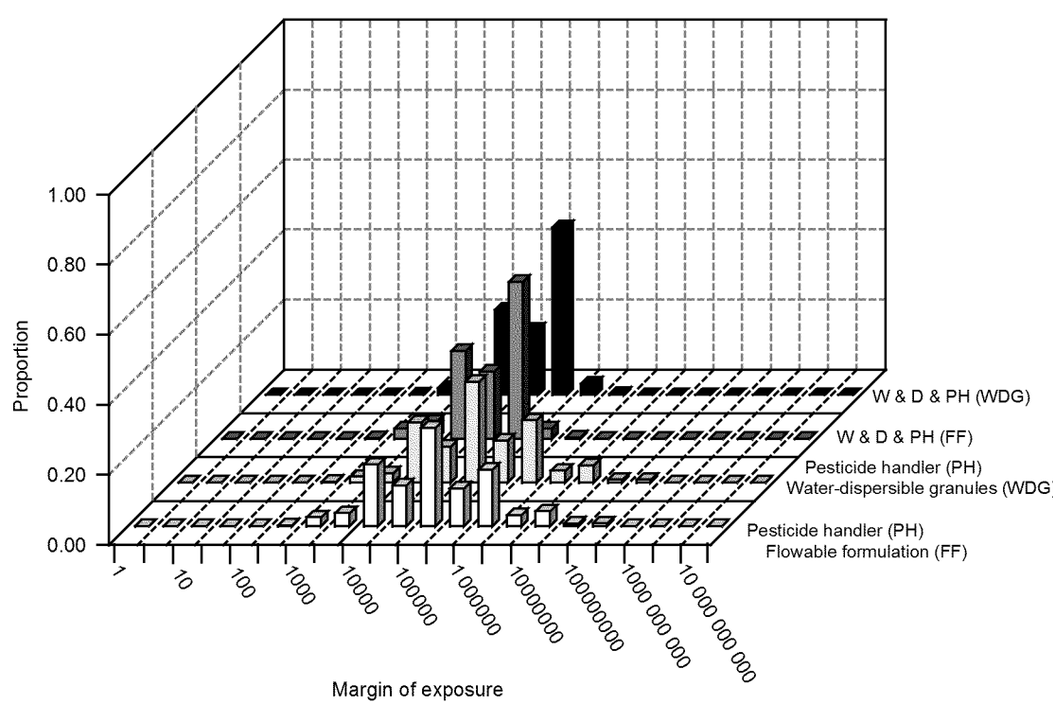

Figure 31.13 Distributions of the MOEs for atrazine herbicide handlers involved in corn production from their use of flowable formulation or WDGs and from their herbicide handling combined with both drinking water ingestion and dietary consumption.

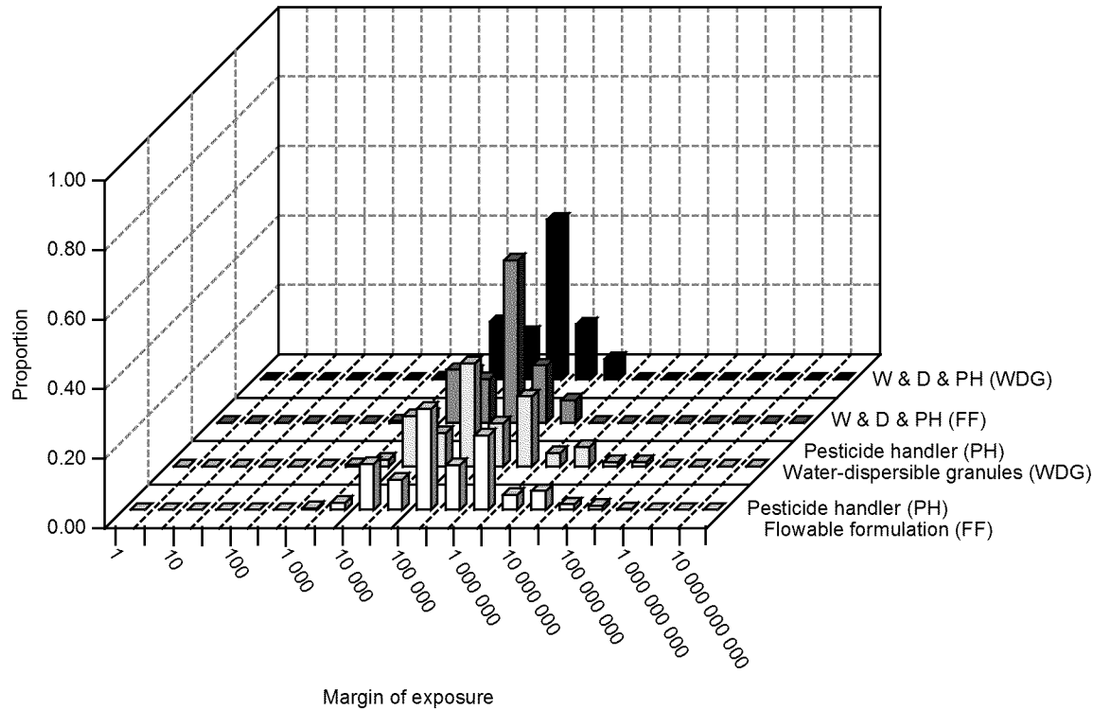

Figure 31.14 Distributions of the MOEs for simazine herbicide handlers involved in corn production from their use of flowable formulation or WDGs and from their herbicide handling combined with both drinking water ingestion and dietary exposure.

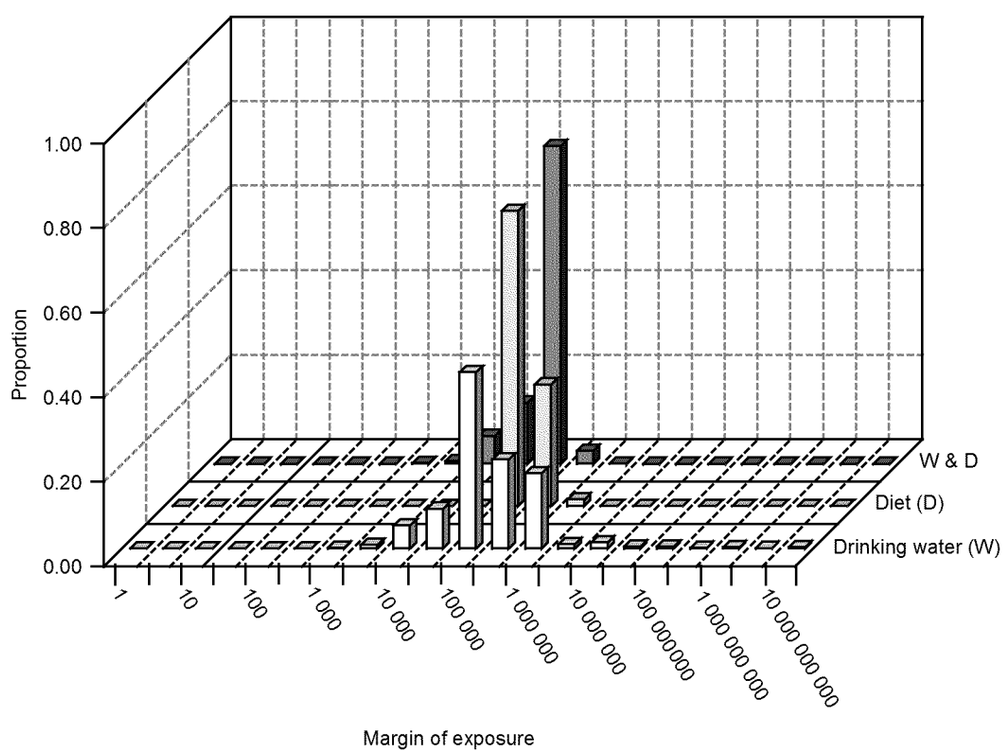

Figure 31.15 Distributions of the MOEs for atrazine and simazine, shown from drinking water ingestion, dietary consumption, and both exposure pathways combined.

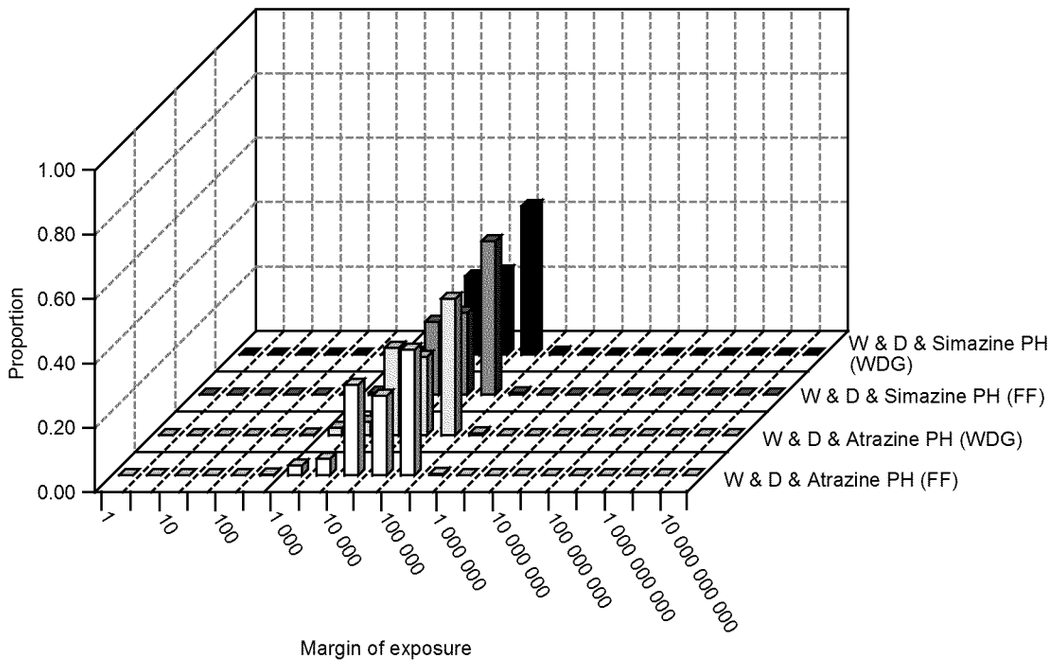

Figure 31.16 Distributions of the MOEs for herbicide handlers who apply either atrazine or simazine and also are possibly exposed to both via water and dietary pathways.

These equations do make the implicit assumption that the atrazine and simazine doses are additive and that the relative impacts of atrazine and simazine at low doses are the same as they are at the ED_{10}s. That is, whatever the shapes of the dose–response relationships are for atrazine and simazine in the low-dose region, a low dose from simazine has only approximately half the impact as the same dose from atrazine.

Conclusion

The Monte Carlo-based distributional characterizations of the MOE indicate that neither occupational exposure nor environmental exposure to atrazine and simazine is likely to produce adverse health consequences in the US population. The MOEs are very large and suggest an ample margin of safety (Tables 31.1 and 31.2). In the distributions, the MOEs are generally well above 1 000 for drinking water and dietary consumption and well above 100 for herbicide handling.

Distributions of the MOE have been presented for individual exposure pathways (drinking water ingestion, dietary consumption, and herbicide handling), for the combined exposure pathways, and for atrazine and simazine both separately and combined. The MOEs have been calculated using a lower bound on the ED_{10} for the most sensitive effect in the most sensitive sex, strain, and species studied in chronic animal bioassays (i.e., mammary tumors in female SD rats). This mammary tumor response in the SD rat is not relevant to humans (IARC, 1999; United Kingdom, 2000; USEPA, 2003; Australia, 2004).

Probabilistic techniques and distributions are used in the triazine quantitative risk assessment to incorporate more of the available data and to reflect more accurately the variability associated with the components of the risk assessment. The MOEs include drinking water concentrations, food residue concentrations, and herbicide handling exposures associated with different user subpopulations, crops, herbicide formulations, and techniques of mixing/loading and application. Specifically, these probabilistic techniques allow the variables in the exposure equations to be described in terms of data-based distributions – reflecting the relative likelihood of the different possible variable values, rather than restricting the characterization of these variables to a single summary value. Furthermore, Monte Carlo techniques make it possible to combine exposures more realistically from multiple pathways, multiple chemicals, and multiple subpopulations, without having to assume the worst case for each component. Thus, probabilistic techniques, including Monte Carlo simulation, help the risk assessor avoid the pitfalls of compounding multiple conservatisms and decrease the exaggeration of the magnitude of exposure and of risk in human health risk assessments. Probabilistic techniques also facilitate risk characterizations that reflect and explicitly quantify the relative likelihood

Table 31.1 MOE assessment for herbicide handlers using flowable or WDG formulations of atrazine or simazine, and including drinking water and dietary exposures to atrazine and simazine combined[a]

	Distributions of MOE			
Herbicide and use	**50th percentile**	**95th percentile[b]**	**50th percentile**	**95th percentile[b]**
Atrazine formulations	Flowable	Flowable	Granular	Granular
Residential lawn care	44 000	5 000	44 000	5 000
Sorghum	68 000	8 000	71 000	8 000
Corn	76 000	7 000	83 000	8 000
Sod	21 000	4 000	25 000	5 000
Vegetation management	48 000	3 000	51 000	4 000
Hawaiian sugarcane	10 000	970	11 000	1 200
North American sugarcane	5 000	530	5 000	650
Simazine formulations	Flowable	Flowable	Granular	Granular
Corn	96 000	11 000	92 000	12 000
Sod	33 000	6 000	39 000	8 000

[a]Lower bounds on atrazine ED_{10} └ 1.4 mg/kg/d and simazine ED_{10} └ 2.6 mg/kg/d.
[b]95% of the distribution exceeds the indicated 95th percentile.

Table 31.2 MOE assessment for atrazine and simazine from water and diet, separately and combined[a]

	Distributions of MOE		
	50th percentile	**75th percentile[b]**	**95th percentile[c]**
Atrazine			
Water[d]	980 000	240 000	48 000
Diet[e]	520 000	410 000	320 000
Water and diet[d,e]	320 000	170 000	44 000
Simazine			
Water[d]	1 800 000	450 000	180 000
Diet[e]	2 000 000	2 000 000	2 000 000
Water and diet[d,e]	980 000	370 000	170 000
Atrazine & simazine combined[f]			
Water[d]	380 000	150 000	38 000
Diet[e]	420 000	350 000	280 000
Water and diet[d,e]	190 000	110 000	34 000

[a]Lower bounds on atrazine ED_{10} └ 1.4 mg/kg/d and simazine ED_{10} └ 2.6 mg/kg/d.
[b]75% of the distribution exceeds the indicated 75th percentile.
[c]95% of the distribution exceeds the indicated 95th percentile.
[d]Using concentration data collected prior to June 1, 1994 for the combination of 18 major-use states.
[e]Dietary consumptions on a national basis.
[f]Margin of Exposure └ $1/([1/MOE_{atrazine}]$ ┘ $[1/MOE_{simazine}])$
└ 1/([total atrazine dose/atrazine ED_{10}] ┘ [total simazine dose/simazine ED_{10}]

of different risk values in the overall population, as well as component subpopulations. These Monte Carlo-based distribution characterizations of risk provide greater information to risk managers than single-number summaries or bounds and, hence, should lead to better risk-management decisions.

Glossary of Terms

Active ingredient is the amount of biologically active chemical (e.g., the triazine) in the pesticide formulation.

Adverse effect is functional impairment or pathological lesion that affects the performance of the organism or reduces its ability to respond to additional challenge. Adverse effects are intended to be effects that have an adverse health consequence, as opposed to just any effect.

Aggregate exposure is the exposure to a single substance from all exposure pathways and routes.

Application of a pesticide is the treatment (spraying, etc.) of a field, lawn, or house either by hand or using equipment. Application does not include the preparation (e.g., mixing or loading) of the pesticide.

Benchmark dose is one of a number of possible specified dose values used as a reference point. For example, a NOEL and an effective dose (e.g., ED_{10}) are both benchmark doses.

Bound is usually the upper or lower limit of a range within which an unknown quantity is likely to be found (e.g., a 95% upper confidence limit, a sample percentile, or a result of methodology that tends to overestimate or underestimate the true value). For example, an upper bound on risk attempts to be sufficiently large so that it is unlikely that the true risk is greater than the upper bound. A bound is a limit on where something could be, whereas a point estimate attempts to be an accurate prediction of where something actually is (i.e., its specific value). For example, 90% might be an upper bound on an absorption percentage, whereas a best estimate might be 10%. A bound is usually not a limit that is impossible to exceed.

Commercial operator is a person who handles pesticides for multiple clients, as opposed to a farmer who handles pesticides in conjunction with his own crop production.

Conservative is used in risk assessment to describe a policy or choice attempting to be health-protective; that is, selected to be reasonably certain that exposure or risk is not underestimated and to err on the side of overestimating the exposure or risk.

Cumulative exposure is the exposure to multiple substances from multiple exposure pathways and routes. *Cumulative dose* is the dose resulting from the cumulative exposure, and *cumulative risk* is the risk due to the cumulative dose.

Cumulative distribution function (cdf) for a random variable, say X, is a function, say F, such that for any value t, $F(t)$ is the probability that X is less than or equal to t.

Default is a choice used in the absence of any sufficiently defensible alternative. In risk assessment, a default is usually a policy-driven assumption, choice, or value intended to make it reasonably certain that exposure or risk is not underestimated and to err on the side of overestimating.

Distribution is the set of all possible values for a random variable and the relative likelihood of each of the possible values (e.g., the distribution of dose in a population is the set of all doses in the population and the relative likelihood or frequency of each dose).

Distributional technique is an analytical method incorporating uncertainty and/or variability into a distribution of values. Distributional techniques are probabilistic techniques and include Monte Carlo simulation.

Dose is the amount of a specified chemical (parent compound and/or specified metabolites) or other substance that an individual receives as a result of exposure.

Dose–response relationship is the relationship between the magnitude of the dose and the probability of a specified response (adverse health effect).

Exposure is a situation in which there is an opportunity for an individual to receive a dose of a chemical or substance, including drinking, eating, dermal contact, or breathing from activities like recreation or work.

Effective dose (ED) is a dose corresponding to a specified increase in the probability of a specified adverse health effect. For example, the ED_{10} is the dose corresponding to an increase of 0.10 in the probability of a specified adverse health effect above the probability at the zero dose.

Exposure pathway is a way for a substance to reach an individual.

Exposure route is usually ingestion, inhalation, or dermal absorption, but sometimes includes more exotic means for a specified substance to enter the body (like injection).

Formulation is the form in which the pesticide is delivered to the pest. Some types of triazine formulations are flowable formulation (FF), emulsifiable concentrate (EC), and water-dispersible granule (WDG).

Grower is a farmer who handles pesticides in conjunction with his own crop production, as opposed to a commercial operator who handles pesticides for multiple clients.

Histogram is a graphical display in which the range of possible values is subdivided into intervals, and the frequency, percentage, or proportion of values in each interval is indicated by the height of the bar drawn above that interval.

Lifetime average daily dose (LADD) is the total dose of a specified substance an individual receives in a lifetime, divided by the total number of days in that lifetime. The LADD is frequently expressed on a per unit body weight basis (e.g., mg/kg/d).

Linear dose–response relationship is a dose–response relationship in which the probability of a specified response changes as a linear function of the dose.

Lowest observed effect level (LOEL) is the lowest dose of a substance that has been observed to produce either a statistically or biologically significant increase in the frequency or severity of an effect, compared to the frequency or severity at zero dose.

Margin of exposure (MOE) is a specified dose (e.g., NOEL or benchmark dose) divided by the dose from exposure.

Mixing/loading is mixing the active pesticide ingredient with other substances, such as water, and/or loading the pesticide into application equipment.

Monte Carlo is a probabilistic technique for simulating the outcome of an equation or model involving random variables. The *frequency distribution of simulated outcomes* is an estimate of the distribution of random outcomes from the equation or model that is being simulated.

No observed effect level (NOEL) is the highest dose of a substance at which there are neither statistically nor biologically significant increases in the frequency or severity of effects between the exposed population and the unexposed population.

Percentile is the smallest value such that the variable is less than or equal to that value at least the specified percentage of the time (e.g., if every number from 0 to 2 000 is equally likely, then 100 is the 5th percentile).

Pesticide handler is one who prepares a pesticide for application and/or applies the pesticide.

Population is the set of all individuals being characterized. The exposure may vary among the individuals in the population. A population may be composed of multiple subpopulations (e.g., the US population can be partitioned/subdivided into 50 state subpopulations).

Positive number is a number greater than zero.

Probability is a number indicating either the relative frequency of an event (e.g., the chance that a person randomly selected from a population will be age 30 years) or the chance that something is true (e.g., the subjective probability that humans are more sensitive to a particular substance than mice and rats).

Random variable is a quantity that takes on numerical values depending upon an experimental outcome. For example, the unknown number of liters of drinking water consumed in a day is a random variable, and the *observed value of the random variable* might be 1.4. Different values may have different probabilities.

Relative likelihood indicates the chance that a value or an event will occur. If the random variable is a discrete random variable, then the relative likelihood of a value is the probability that the random variable equals that value. If the random variable is a continuous random variable, then the relative likelihood at a value is the same as the probability density function at that value.

Risk is the likelihood that an individual will develop a specified adverse health effect. Risk can be characterized in quantitative terms, such as the probability of the adverse health effect or the MOE.

Risk assessment is frequently described as involving four components – hazard identification, exposure assessment, dose–response assessment, and risk characterization. Risk assessment may be an input to risk management.

Risk characterization is either the quantitative or qualitative description of risk. For example, a quantitative risk characterization could be either a point estimate of risk (a single value for the risk as opposed to a range of values), an upper bound on risk (which implies a range of values for the risk), or a distribution of risk (which implies a range of values for the risk and the relative likelihood of each value in that range).

Sublinear dose–response relationship is a dose–response relationship in which the probability of a specified adverse health effect is either not increasing at all or increasing slower than linearly (i.e., slower than in direct proportion to the increase in dose).

Subpopulation is a subset or subgroup within a larger population (e.g., the US population can be partitioned into male and female subpopulations, and the male subpopulation can be subdivided into five subpopulations – infants, children 1–6 years old, children 7–12 years old, teenagers 13–17 years old, and adults).

Uncertainty is lack of knowledge or less than complete information, as opposed to certainty. Sometimes the term uncertainty is used to include both uncertainty and variability.

Variability is lack of constancy, such as when something changes from one person to another, one day to another, etc.

Worst case is used in risk assessments to describe an extreme assumption, choice, or value intended to fulfill the goal of being health-protective – that is, selected to be reasonably certain that exposure or risk is not underestimated and to err on the side of overestimating the exposure or risk. For example, a worst case assumption in evaluating dermal exposure to swimming pool water would be to assume that a person swims for 24 h/day and is always 100% immersed.

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Chapter 32

Progress in Best Management Practices

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Summary

Four broad approaches have been identified for preventing soil degradation and improving water quality, while sustaining a profitable agricultural sector: (1) conserve and enhance soil quality as the fundamental first step to environmental improvement; (2) increase the resistance of farming systems to erosion and runoff; (3) increase efficiencies of nutrient, pesticide, and irrigation use in farming systems; and (4) make greater use of field and landscape buffer zones [National Academy of Sciences (NAS), 1993]. Major Farm Bill programs have been implemented to conserve and enhance soil quality, improve wildlife habitat, and increase resistance of farming systems to erosion and runoff. These programs include conservation tillage, as part of the Conservation Compliance Provisions, and field and landscape buffer zones, as part of the Conservation Reserve Program (CRP) and Wetland Reserve Program (WRP).

Use of the combination of nutrient management and best management practices (BMPs) with conservation tillage and vegetative buffer zones will reduce nutrient and pesticide loads in the environment. Many US farmers have adopted these BMPs. Conservation tillage acres increased from 26% of US annually planted acres (A) in 1990 to 41% of acres in 2004. Herbicides such as the triazines have been vital components of the growth in acreage under conservation tillage. In response to the CRP, over 35 million A of farmland, a large percentage of which was environmentally sensitive, has been placed into grassland, trees, wildlife habitat, and conservation buffers in one of the most beneficial conservation programs in US history. BMPs have made a major contribution to the reduction in soil erosion and are expected to have an increasing impact on the removal of sediment, nutrients, pesticides, and bacteria in surface runoff.

Introduction

Runoff of soil, agricultural chemicals, fertilizers, bacteria, natural toxins and other pollutants can flow into streams and rivers and can subsequently affect surface water supplies or leak vertically into shallow alluvial aquifers (Burkart *et al*., 1997).

Farmers are environmental stewards and are applying many BMPs to reduce erosion and improve water quality. BMPs can be structural (e.g., a mixing–loading pad) or nonstructural (e.g., a tillage practice, vegetative buffer strip, or other agricultural practices). As the adoption of BMPs increases, levels of pesticides, nutrients, and sediment in water will continue to decline.

In the mid to late 1990s, scouting for weeds and pests and implementing crop rotation were widely adopted for Integrated Pest Management (IPM). Other BMP practices for IPM are growing, such as buffer strips, conservation tillage, utilization of economic threshold infestation levels with scouting, and alternating pesticide modes of action. Farmers' increasing awareness of the need to alternate pesticide modes of action is being precipitated by pressure from weeds and pests resistant to control measures. Rapid adoption of crops resistant to pests and herbicides through plant breeding and biotechnology is valuable to IPM, but is adding to pest and weed resistance. To further the adoption of IPM techniques, critical barriers need to be addressed. These barriers include the lack of viable nonchemical alternatives for pest control due to efficacy issues; limited availability of pesticides or herbicides with alternate modes of action to control certain resistant pests or resistant weeds; potential decreases in crop quality or yields; potential increases in production costs; increased time requirements for land management; and the need for further education on how BMPs can reduce erosion and improve water quality.

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The Impact of Erosion

The status of the US water resources (rivers, lakes, and estuaries) is assessed by state personnel and Native American Tribes in accordance with Section 305(b) of the Clean Water Act. Each state and each tribe rates whether their water quality is high enough to support fully a healthy community of aquatic organisms, as well as human activities such as swimming, fishing, and drinking. Table 32.1 gives the US Environmental Protection Agency's (USEPA's) list of the top 15 sources of impairment in US rivers and lakes as of July 2005.

Soil sediment is a major source of water quality impairment from agriculture. River and stream bank erosion, erosion from forest and farmland, and soil loss from urban and industrial development are the main sources of this sediment. In 2001, wind, sheet, and rill (water) erosion removed nearly 1.8 billion tonnes (1.6 billion metric tonnes) [down from 3.1 billion tonnes (2.8 billion metric tonnes) in 1982] of soil from US cropland [US Department of Agriculture (USDA)-Natural Resources Conservation Service (NRCS), 1992; USDA-NRCS, 2003], not including ephemeral gully erosion. Studies in 19 states estimated that ephemeral gully erosion contributed another 21–275% of the estimated sheet and rill erosion soil loss from the field (USDA-NRCS, 1996). Although not all soil from sheet, rill, ephemeral, and wind erosion reaches rivers and streams, enough does so to cause significant water quality problems.

Sediment and associated contaminant damages from erosion have been estimated to be between $3.2 and $13 billion per year with about $2.2 to $3.5 billion from eroding cropland (Clark *et al.*, 1985; Ribaudo, 1989). These damages include reduced recreation opportunities, increased water treatment costs to remove sediment, sedimentation of reservoirs, increased dredging of navigation channels, increased silting in drainage and irrigation channels, and flood damages.

Soil erosion and degradation are the major issues impacting agricultural sustainability worldwide. In 1984, World Watch conservatively estimated that the world was losing 23 billion metric tons of soil from croplands in excess of new soil formation (Brown and Wolf, 1984). This loss translated to a staggering average of 18 tons (16.3 metric tonnes) per worldwide cropland hectare (ha). Oldeman *et al.* (1990) estimated that almost 4.9 billion A (2 billion ha) of land worldwide were degraded over a 45-year period. The Food and Agricultural Organization (FAO) of the United Nations estimated losses of as much as 12–17 million A (5–7 million ha) of good, arable land per year because of soil degradation (FAO, 1992). Sediment-loaded rivers are clearly evident around the world as a result of this degradation and erosion.

Soil sediment is also an important source of nutrients in water, particularly of phosphorus, in the form of soil and vegetative matter. A portion of this phosphorus is available for aquatic biota [USDA-The Cooperative State Research, Education, and Extension Service (CSREES), 1998]. The large number of US soils now testing 'high' or 'excessive' for phosphorus has increased concerns about the transport of phosphorus in surface and subsurface flow from soils to streams, rivers, lakes, and eventually oceans. In a National Water Quality Assessment (NAWQA), the United States Geological Survey (USGS), determined that 57% of sampled streams in 20 of 50 study units had concentrations of phosphorus above national average background levels and the USEPA desired goal of 0.1 mg/L (USGS, 1999). The NAWQA Program focuses on water quality in more than 50 major river basins and aquifer systems that cover about 50% of the land area in the United States. Elevated phosphorus levels in water can lead to accelerated growth of algae and submerged plants, depleting the water column of dissolved oxygen necessary to maintain populations of fish and desirable plant species.

Table 32.1 Listing of the top 15 sources of impairments of US rivers and lakes as of July 2005 (USEPA, July 2005)

Top 15 sources of impairments	Water bodies (% of total impairment)
Metals	19.21
Pathogens	13.16
Nutrients	9.31
Sediment/siltation	8.41
Organic enrichment/low dissolved oxygen	7.34
Fish consumption advisory	5.30
pH	4.84
Other habitat alterations	3.98
Thermal modifications	3.67
Biological criteria	3.53
Flow alteration	2.63
Pesticides	2.45
Turbidity	1.90
Salinity/total dissolved solids/chlorides	1.66
Suspended solids	1.61

Another important nutrient from the water quality standpoint is nitrogen. Nitrogen, primarily found in the soil as nitrate-nitrogen, is soluble and is transported in surface runoff, in subsurface drains, or with leachate. In the NAWQA study, the USGS determined that 61% of sampled streams in 20 of 50 study units had concentrations of total nitrogen and nitrate-nitrogen above national average background levels of 1.0 and 0.6 mg/L, respectively. In addition, 23% of sampled streams showed ammonia concentrations in excess of the background concentration of 0.1 mg/L (USGS, 1999). Two years of monitoring data from the Mississippi River and its tributaries show that nitrate-nitrogen concentrations were between 1 and 5 mg/L (Coupe *et al.*, 1995) at three locations: Clinton, Iowa; Thebes, Illinois; and Baton Rouge, Louisiana. Subsurface drainage is a management tool that reduces erosion and phosphorus enrichment of surface waters from agricultural activities by reducing total runoff (Management Systems Evaluation Area (MSEA), 1998). However, nitrate-nitrogen exported from drainage conduits to surface waters continues to be a water quality concern. About 75.5 million A (30.6 million ha) of cropland have been drained in the United States (MSEA, 1998). Jaynes and Hatfield (1994) showed that nitrate-nitrogen movement was related to the levels of discharge through subsurface drain lines. Soenksen (1996) found that subsurface drainage dominated the movement of water and the discharge of nitrate-nitrogen into the stream channel as compared to other flow paths. Nitrate-nitrogen is one of the factors associated with hypoxia in the Gulf of Mexico and nutrient enrichment with Pfeisteria outbreaks in the Chesapeake Bay (MSEA, 1998; USEPA, 1998b).

In the NAWQA studies, USGS found that nitrate-nitrogen exceeded the 10 mg/L drinking water standard in about 15% of the groundwater at 0–100 ft (30 m). The incidence decreased with increasing soil depth, with no samples showing nitrate-nitrogen in excess of the drinking water standard at depths below 200 ft (61 m) (USGS, 1999). In a one-time survey of 686 private rural water supply wells in Iowa, nitrate-nitrogen exceeded 10 mg/L in 18% of the well water samples (Hallberg *et al.*, 1992).

Additional NAWQA monitoring data from 1992 through 2001 show that pesticides or their degradates are commonly detected in surface water streams and groundwater (USGS, 2006). Groundwater contamination from agriculturally applied pesticides in the United States does not often result in exceedances of drinking water standards (USEPA, 1990; Hallberg *et al.* 1992; USGS, 2006, 1999). River and stream data show that the maximum contaminant levels (MCLs) and the human health benchmarks are seldom exceeded in annual averages, but single samples can exceed the levels set for the MCLs for brief periods during the heavy discharge period in the spring of each year (Johnson and Baker, 1982, 1984; Coupe *et al.*, 1995; USGS, 2006). Levels of pesticides in water are trending downward (Kolpin *et al.*, 1997; Skopec and Hoyer, 1998). With the further implementation of BMPs, such as buffer strips and conservation tillage, these levels should continue to decline.

Policies and Government Programs

Agricultural environmental issues have expanded beyond the soil erosion and sediment issues precipitated by the Dust Bowl and the soil erosion focus of the 1985 and 1990 Farm Bills in the United States. In 1989, the Board on Agriculture of the National Research Council was asked to convene a committee to assess the science, technical tools, and policies needed to protect soil and water quality while providing for the production of food and fiber from US croplands (NAS, 1993). Their report defines four broad approaches that hold the most promise for preventing soil degradation and water pollution, while sustaining a profitable agricultural sector. They recommended that programs should seek to: (1) conserve and enhance soil quality as the fundamental first step to environmental improvement; (2) increase the resistance of farming systems to erosion and runoff; (3) increase nutrient, pesticide, and irrigation use efficiencies in farming systems; and (4) make greater use of field and landscape buffer zones. These four approaches are interrelated, and the combination of a quality soil with high infiltration rates and a vegetative buffer strip along a waterway will result in less runoff of nutrients, pesticides, and soil into surface water.

Many of the BMPs and United States Farm Program elements to support these practices lend themselves very well to local and watershed partnership initiatives. The USEPA has long encouraged the formation of watershed partnerships to identify water quality issues and, in the case of agriculture, to implement a systems approach to deal with these issues (USEPA, 1998a). As a result of the 'Know Your Watershed Program' initiated by the Conservation Technology Information Center (CTIC) in West Lafayette, Indiana, more than 1200 active watershed partnerships have been identified and captured in a National Watershed Network Database. This database provides a tremendous tool for promoting and tracking the adoption of BMPs.

Conservation Compliance

In 1982, cultivated highly erodible land (HEL) accounted for nearly 60% of total erosion on US cropland (USDA-NRCS, 1992). HEL is land that has an erodibility index of eight or more. The erodibility index of a soil is determined

by dividing the potential erodibility for each soil by the soil loss tolerance value established for the soil, with the tolerance value representing the maximum annual rate of soil erosion that could take place without causing a decline in long-term productivity. The 1985 Farm Bill required farmers who produced agricultural commodities on HEL land to implement approved erosion control plans by January 1, 1995 to maintain eligibility for USDA agricultural program benefits (USDA-Economic Research Service (ERS), 1997). The 1996 Farm Bill included several modifications to reduce compliance costs and monitoring costs: (1) expedited variances for timely responses to producer requests for relief from climatic or economic hardship, (2) grace periods for good-faith violations to provide producers with unintended violations to come into compliance without penalty, (3) on-farm conservation research authority to examine innovative conservation systems, and (4) provisions to allow farmers to report residue measurements.

Conservation Reserve Program

CRP was introduced as part of the 1985 Farm Bill. This voluntary long-term cropland retirement program provides farm owners and operators with an annual per-acre rent and half the cost of establishing a permanent land cover (usually grass or trees) in exchange for retiring highly erodible and/or environmentally sensitive cropland for 10–15 years (USDA-ERS, 1997). By 1990, 33.9 million A (13.7 million ha) had been enrolled. The 1990 Farm Bill extended the CRP enrollment period through 1995 and redirected part of the program toward improving water quality. The CRP was continued through 2002 in the 1996 Farm Bill. The USDA was given authority to reenroll existing CRP contracts, as well as enroll new acreage, subject to a maximum annual enrollment of 36.4 million A. In the 1995 program, emphasis on more environmentally sensitive acreage was strengthened, as well as cropland eligibility criteria. These new criteria have been applied to land resubmitted from expiring contracts and to new land submissions. Bids are ranked by an environmental benefits index, and those with the highest environmental benefits relative to government cost are enrolled. A continuous sign-up was included as a provision in the 1996 Farm Bill CRP extension for filter strips, riparian buffers, grassed waterways, field windbreaks, shelterbelts, living snow fences, salt-tolerant vegetation, shallow water areas for wildlife, and wellhead protection areas designated by the USEPA. Producers wishing to enroll acres for these practices may do so at any time, thus avoiding the need to wait for a discrete CRP sign-up period. In response to the continuous sign-up program, USDA established a goal to sign up 2 million miles (3.22 million km) of conservation buffers by the year 2002. The 2002 Farm Bill extended the CRP through calendar year 2007 and authorized USDA to expand the CRP enrollment up to 39.2 million A from the previous cap of 36.4 million A. Of the total amount available, about 3.0 million A are reserved for special initiatives within CRP, including: (1) A continuous sign-up program; (2) Planting flood plains to bottomland hardwood trees to help sequester greenhouse gases, improve water quality and restore wildlife habitat; (3) The Bobwhite Quail Initiative that allows for enrollment of field borders to provide valuable habitat for quail and other upland birds; (4) The Wetlands Initiative that allows enrollment of larger wetland complexes and playa lakes beyond the 100-year floodplain; (5) The Conservation Reserve Enhancement Program (CREP), which is a federal-state partnership to target additional resources in defined geographic areas for conservation practices such as buffer and filter strips; and (6) The Farmable Wetland Program to protect certain farmed and prior converted wetlands.

WRP and Swampbuster Program

The WRP was established in the 1990 Farm Bill and had a goal to restore 975 000 A (395 000 ha) to wetlands by 2002. The 1996 Farm Bill reaffirmed the enrollment goal and required one-third of enrollments each in 30-year easements, cost-share agreements, and permanent easements. The WRP program funds USDA to restore wetlands and purchase permanent or long-term easements to restrict agricultural use of the restored wetlands (USDA-ERS, 1997). The landowner is allowed certain economic uses of the restored wetland that may reduce the cost of the easement. These uses include hunting, fishing, or other recreational activity, grazing during prescribed times, and selective timber harvesting that is compatible with wetland restoration. The landowner is paid up to 75% of the cost of restoring the former wetland. The 2002 Farm Bill continues the WRP through 2007, increases the overall program acreage cap to 2 275 000 A and caps annual acreage enrollment at 250 000 A. The Emergency WRP was established in 1993, using funds from the Emergency Watershed Protection Program authorized under emergency supplemental appropriations after the Midwest flood. The voluntary program helped landowners convert flood-damaged cropland to wetlands if the cost of the levee restoration and cropland renovation exceeded the value of the land. Voluntary cost share programs such as the United States Fish and Wildlife Service's Partners for Wildlife and the North American Waterfowl Management Plan (NAWMP) have also made major contributions to wetland restoration.

The Swampbuster program, originally part of the 1985 Farm Bill, eliminates eligibility for USDA agricultural program benefits for those producers who convert wetlands to agricultural commodity production, unless the USDA determines that the conversion would have only minimal effect on wetland hydrology and biology. The 1996 Farm

Bill changed the Swampbuster program to give USDA participants greater flexibility to comply with wetland conservation requirements and to make wetlands more valuable and functional. Some examples of these changes include expanding areas where mitigation can be used; providing more options for mitigation; encouraging effective and timely use of 'minimal effect' determinations; and providing USDA program participants the opportunity to request review of previous wetland determinations to verify their accuracy. The only modification under the 2002 Farm Bill included a provision to clarify that only a qualified NRCS employee has the authority to make determinations regarding compliance with the program.

The Environmental Quality Incentives Program

The 1996 Farm Bill consolidated the functions of the Agricultural Conservation Program, the Water Quality Incentives Program, the Great Plains Conservation Program, and the Colorado River Salinity Control Program into the Environmental Quality Incentives Program (EQIP) (USDA-ERS, 1997). EQIP was reauthorized in the 2002 Farm Bill through 2007 with greater funding resources. EQIP gives priority to areas where state and local governments offer financial or technical assistance, or where agricultural improvements will help meet water quality objectives. The program initially established 5- to 10-year contracts to provide technical assistance and education. However, the 2002 Farm Bill modified the contracting length provision to stipulate a minimum term that ends 1 year after the implementation of the last scheduled practices and a maximum term of 10 years. EQIP pays up to 75% of the costs of structural practices, such as manure management systems, terraces, and filter strips. The 2002 Farm Bill provided additional authority to allow up to a 90% cost-share for beginning or limited resource farmers and ranchers. Additionally, the 2002 Farm Bill provides an overall payment limit of $450 000 over the authorized life of the 2002 Farm Bill per producer, regardless of the number of farms or contracts. Producers who implement land management practices such as nutrient, IPM, and tillage management can receive technical assistance, education, and incentive payment amounts to be determined by the USDA Secretary. Activities must be carried out according to a conservation plan.

Conservation Security Program

The 2002 Farm Bill created a new program, the Conservation Security Program (CSP), to reward stewardship and create incentives for efforts to address concerns on working agricultural lands (cropland, grassland, prairie land, rangeland, improved pasture and some forested land). CSP is authorized through 2007. CSP provides financial and technical assistance to help producers address soil, water, air and energy conservation. CSP sign-up was offered in select watersheds in 2004, 2005 and 2006. Producers complete a self-assessment that is then used to determine eligibility and level of benefit. There are three established tiers: For Tier I, the producer must have addressed soil quality and water quality to the described minimum level of treatment for eligible land uses on part of the agricultural operation prior to acceptance. For Tier II, the producer must have addressed soil quality and water quality to the described minimum level of treatment on all eligible land uses on the entire agricultural operation prior to acceptance and agree to address one additional resource by the end of the contract period. For Tier III, the producer must have addressed all applicable resource concerns to a resource management system level that meets the NRCS Field Office Technical Guide standards on all eligible land uses on the entire agricultural operation before acceptance into the program.

Section 319 of the Clean Water Act

This act authorizes the USEPA to issue annual grants to assist states in implementing their USEPA-approved nonpoint source management programs. The funds are used by states for public education and outreach, technical assistance, and specific project support to prevent or reduce nonpoint source pollution at the watershed level. The funding for these grant funds was approximately $237 million for fiscal year 2004.

In addition to the above federal programs, states have appropriated about $1 billion for conservation initiatives. These cost-share and incentive programs are resulting in increasing adoption of BMPs. The most important of these for soil, water, and wildlife quality are conservation tillage, field and vegetative buffers (conservation buffers), wetland restoration, nutrient management, and IPM.

Adoption and Effectiveness of BMPs

Conservation Tillage and the CRP

Efforts to decrease soil degradation and erosion have been demonstrated by the rapid adoption of conservation tillage and no-till by farmers within the United States as shown in Table 32.2.

Table 32.2 Conservation tillage and other tillage types in the United States in millions of acres (CTIC, 2004)

	1990	1992	1994	1996	1998	2000	2002	2004
Conservation tillage[a] types – over 30% cover after planting								
No-till[b]	16.9	28.1	38.9	42.9	47.8	52.2	55.3	62.4
Ridge-till[c]	3.0	3.4	3.6	3.4	3.5	3.3	2.8	2.2
Mulch-till[d]	53.3	57.3	56.8	57.5	57.9	53.5	45.0	48.0
Other tillage management types – less than 30% cover after planting								
Reduced-till[e] (15–30% cover)	71.0	73.4	73.2	74.8	78.1	61.3	64.1	59.6
Conventional-till[f] (0–15% cover)	136.7	120.8	111.4	111.6	106.1	127.1	114.3	104.4
US Total planted acres	280.9	282.9	283.9	290.2	293.4	297.5	281.4	276.6

[a]Conservation Tillage (no-till, strip-till, ridge-till, and mulch-till) is any tillage and planting system with 30% or more residue remaining on the soil surface after planting to reduce soil erosion by water. Where soil erosion by wind is the primary concern, conservation tillage is any system that maintains at least 1000 pounds per acre (lb/A) of flat, small grain residue equivalent on the surface throughout the critical wind erosion period.
[b]No-till and strip-till leave soil undisturbed from harvest to planting, except for strips up to 1/3 of the row width. Planting or drilling is accomplished using disk openers, coulters, row cleaners, in-row chisels, or rototillers.
[c]Ridge-till leaves the soil undisturbed from harvest to planting, except for strips up to 1/3 of the row width. Planting is completed with sweeps, disk openers, coulters, or row cleaners on the ridge, and usually involves removal of the top of the ridge. Plant residue is left on the surface between ridges. Weed control is accomplished with herbicides and/or cultivation. Ridges are rebuilt during the last cultivation.
[d]Mulch-till is full width tillage, which disturbs all of the soil surface prior to and/or during planting. Tillage tools such as chisels, field cultivators, disks, sweeps, or blades are used, leaving ample residue cover on the soil surface as per the definition of conservation tillage above.
[e]Reduced-tillage types leave 15–30% residue cover on the soil surface after planting, or 500–1000 lb/A (560–1120 kg/ha) of small grain residue equivalent throughout the critical erosion period.
[f]Conventional-till leaves less than 15% residue cover on the soil surface after planting, or less than 500 lb/A (560 kg/ha) of small grain residue equivalent throughout the critical erosion period. Generally involves plowing or intensive (numerous) tillage trips.
Source: Conservation Technology Information Center (2004) National Crop Residue Management Survey. www.ctic.purdue.edu/CTIC/CTIC.html.

The Conservation Compliance Provisions of the 1985 and 1990 Farm Bills, together with active promotion by the public and private sectors of crop residue management as a means to reduce erosion, resulted in rapid adoption of conservation tillage in the United States. This rapid adoption was the result of weed management (broader herbicide choices for preplant burndown and in-crop management) and equipment technology, combined with input cost reductions as a result of conservation tillage and the use of crop residue management as a tool to reach conservation compliance. Crop residue management with conservation tillage rapidly became the preferred method (83%) of soil erosion reduction in conservation compliance plans on highly erodible farmland. Terraces, which require significant capital investment, were used in 14% of the plans (USDA-ERS, 1997). From 1990 to 2004, conservation tillage grew from 26% of annually planted cropland acreage to 41% of these acres. In this time frame, no-till crop production grew from 17 to 62 million A. The rapid adoption of these practices between 1989 and 1994 reduced cropland erosion (sheet, rill, and wind erosion) from an average of about 8 tonnes (18 metric tons/ha) per acre per year in 1982 to about 5.2 tonnes/A/year (11.7 metric tonnes/ha/year) in 1995 (USDA-NRCS, 1992, 1998). As a result of reduced erosion, total soil loss from US cropland has been reduced from about 3.4 to 2 billion tonnes per year (7.6 to 4.5 billion metric tonnes). National off-site benefits from controlling erosion have been estimated at $0.56 per tonne and include commercial and recreational uses, water storage, and reduced fbod damage (Ribaudo and Young, 1989).

As of June 2005, over 35 million A of cropland were enrolled in CRP. In 1996, due to early-out provisions and contract terminations, the acreage in CRP was 33 million A (13.4 million ha). As of October 1, 1999, due to more selective reenrollment of expiring and new enrollment acreage, the total enrolled was at 31.3 million A (12.7 million ha). CRP is a major contributor to soil erosion reduction. As of December 1996, this reduction totaled 626 million tonnes of soil or about 19 tonnes per acre (43 metric tonnes/ha) (USDA-ERS, 1997). Most CRP acres were planted to grass, but also included 2.4 million A (1 million ha) of trees, 1.6 million A (0.65 million ha) of special wildlife practices, and 8100 miles (13 040 km) of filter strips along waterways. In 1990, when the CRP had enrolled 33.9 million A (13.7 million ha), the Economic Research Service estimated net social benefits (extent to which social benefits of CRP exceeded its social costs) of $4.2–9 billion in present value over the life of the program (Osborn and Konyar, 1990). Social benefits included increases in net farm income ($2.1–6.3 billion), the value of future timber ($3.3 billion), preservation of soil productivity ($0.6–1.7 billion), improved surface water quality ($1.3–4.2 billion), and lower damages due to windblown soil ($0.3–0.9 billion). In 1994, the United States Fish and Wildlife Service estimated additional wildlife benefits of $4.1 billion for nonconsumptive wildlife benefits such as bird and wildlife watching (Johnson *et al.*, 1994). The overall net benefit estimate of CRP ranges from $9.7 to $14.5 billion. Net government costs of the program (rental rates and other costs minus savings in commodity program payments) were estimated at $6.6–9.3 billion.

Table 32.3 The adoption of conservation tillage practices in important agricultural countries other than the United States

Country	Tillage type	1989	1991	1993	1995	1997	1998	% of area[c]
		Millions of acres						
Argentina[a]	No-till	0.2	1.2	4.2	6.9	11.9	18.0	32
Australia	Conservation tillage	NA	NA	24.2	28.3	32.9	33.6	58
Brazil[a]	No-till	2.2	3.3	7.4	13.6	27.9	30.0	23
Canada	Conservation tillage	NA[b]	NA	15.8	20.4	24.9	27.1	29

[a]CAAPAS (1999).
[b]NA: Not available.
[c]To convert acres to hectares, multiply by 0.4047.

The environmental and wildlife benefits of conservation tillage resulting from carbon sequestration include improved water infiltration, reduced runoff due to surface crop residue, and improvements in soil structure. Lake Erie and the Raccoon Creek in Indiana are important examples of the effectiveness of this practice that bear examination. The impending eutrophication of Lake Erie due to excessive soil sediment and phosphorus loading in the early 1970s led to the establishment of the Lake Erie Agricultural Systems for Environmental Quality (LEASEQ) project. Most of this loading comes from row crop agriculture on almost 5.2 million A (2.1 million ha) of land in Ohio's Maumee and Sandusky River watersheds. The first 20 years of this project resulted in significant reductions in phosphorus and sediment loadings in the Maumee River basin, resulting in measurable improvements in the western basin of Lake Erie (Richards and Baker, 1997). These trends show that the more efficient nutrient use and the adoption of conservation tillage practices have contributed to these reductions. At the beginning of the study period, only 5% of the acreage in the Maumee and Sandusky watersheds was under conservation tillage, as compared to 50% in 1995.

Soil sediment from erosion was found in the Big Raccoon Creek in Indiana in the early 1980s. Some dry spells in the 1980s improved the situation, due to lower sediment loads. Rainfall was much heavier in the 1990s. However, sediment erosion has decreased due to the adoption of conservation tillage. In 1989, about 10% of corn and soybean acreage was no-till. By 1997, 84% of soybean was no-till, and 41% of corn was no-till or strip-till (Brunoehler, 1998). With the increased adoption of conservation tillage, we are likely to see continual improvements in water quality and increased fish and wildlife populations.

Rapid adoption of conservation tillage also occurred in a number of other key agricultural countries in the 1990s (Table 32.3) [CTIC, 1998; Confederation de Asociaciones Americanas para la Agricultura Sustentable (CAAPAS), 1999].

The global conservation tillage adoption rate has continued to escalate in more recent years. For example, adoption of no-till in Argentina has increased from a few hundred thousand hectares in 1990 to more than 16 million ha in 2003, accounting for approximately 65% of Argentina's grain hectares (Peiretti, 2003; McKell and Peiretti, 2004).

Conservation Buffers

Conservation tillage is playing a major role in the improvement of both soil and water quality. However, in extreme rainfall events, even soils with excellent water penetration rates and good residue cover can reach saturation, resulting in runoff carrying soil, nutrients, and pesticides into rivers and streams. Additionally, stream bank erosion can be an important source of soil sediment. It has been estimated that 30–80% of the soil in Iowa streams is from bank and streambed erosion (Odgaard, 1984). Therefore, the integration of vegetative buffer systems in the landscape and in riparian zones is an important component of a total management system. Much research is beginning to emerge on the efficiency of vegetative buffers for removal of soil sediment, nutrients, pesticides, and bacteria.

Vegetative Buffers

Vegetative buffers and filter strips are areas of permanent vegetation located within and between agricultural fields and the water courses to which they drain (Helmers *et al.*, 2006). These buffers are intended to intercept and slow runoff, thereby providing water quality benefits. Vegetative buffers encompass a range of terms, including grassed waterways, contour buffer strips, vegetative barriers, field borders, filter strips, and riparian zones. Vegetative buffers may be constructed or naturally vegetated, within or along fields, or adjacent to drainage ditches, streams, lakes, ponds,

and wetlands. Grassed waterways carry surface water to a stable outlet. Contour buffer strips are alternated with the principal crop to slow runoff. The term riparian zone refers to a multispecies buffer – often including trees – located along a stream, lake, pond, or wetland. Although the definitions of these terms vary, especially among countries, most tend to utilize perennial, closely seeded crops, though annuals are sometimes used in contour buffer strips.

The purpose of the vegetative buffer is to reduce the water flow rate and to remove sediment and chemicals from runoff or wastewater by filtration, deposition, infiltration, absorption, volatilization, vegetative consumption, and decomposition [Kansas Department of Health and Environment (KDHE), 1995; USDA-Soil Conservation Service (SCS), 1986]. Bharati (1997) measured five times as much water infiltration in a multispecies riparian buffer as in a grazed pasture and cultivated fields. Species used in the vegetative buffer range from traditional crops planted in narrow rows, to pasture and rangeland grasses and legumes, to shrubs and trees. The benefits of vegetative buffers have been apparent for some time. Even in 1991, a survey of Illinois farmers indicated that 29% of respondents had grass waterways or filter strips, and 90% of those with ponds had grass or legume buffer strips around the ponds, with an average buffer width of 55 meters (m) above the ponds (Pike *et al.*, 1994).

Several recent reviews are very helpful in understanding the current state of buffer knowledge. Polyakov *et al.* (2005) gave an overview of research on riparian buffers, discussed the importance of local conditions on performance, and presented approaches for precision buffer design. Hickey and Doran (2004) concluded that buffer strip effectiveness in removing nutrients is highly variable, that significant removal comes from studies undertaken in riparian buffers greater than 30-m wide, and that more research is needed on the 1–10-m width typical on farms. Karthikeyan *et al.* (2004) reviewed plant remediation literature and provided numerous reminders of the variety of direct and indirect pesticide detoxification mechanisms operating in plants in filter strips, riparian buffers, and vegetated remediation environments. Lyons *et al.* (2000) focused on the effects of grass versus woody riparian vegetation for small streams in the grassland/savannah region of central North America, additional research needs, and management implications.

Dabney *et al.* (2006) reviewed a variety of in-field, edge-of-field, and after-field buffers and noted four key principles: (1) even narrow buffers improve water quality, (2) buffers work best on slow, shallow diffuse flows, (3) buffers slow, trap, and enhance metabolism of pesticides, and (4) buffers are most valuable on shallow soils, which are most susceptible to runoff.

Krutz *et al.* (2005) did a comprehensive review of research on herbicide retention in vegetative filter strips and found that strips reduced herbicide transport by 27% or more in all papers except two. However, they noted that an understanding of strip efficacy as a function of flow rate was limited to a few experiments conducted under extreme conditions of inundation (saturation) or complete infiltration. Lacas *et al.* (2005) also reviewed the literature on grassed buffer strips and noted very variable results due to the number of interacting processes and dynamic contributing factors, some of which have not yet been quantitatively described or remain largely unknown (e.g., subsurface flow processes).

Impact of Buffers on Sediment

The positive effects of buffers on sediment trapping are well known. Fasching and Bauder (2001) showed that vegetative filter strips reduced the amount of sediment exiting various perennial grass strips by about 75–85%. Filter strips were effective for sediment removal when flow was shallow and uniform (Dillaha *et al.*, 1989). Van Dijk *et al.* (1996) conducted experiments on sloping loess soils in South Limburg (The Netherlands) and showed that 1, 4.5, and 10 m strips reduced sediment 55%, 75%, and 95%, respectively, if concentrated flow was absent.

Efficiency gains with wider filters are seldom linear. Mickelson and Baker (1993) obtained high trapping of sediment with 4.6 m (72%) and 9.1 m (76%) grass filters. The average total suspended solids trapping efficiency for 2, 5, 10, and 15 m strips was 50%, 72%, 87%, and 86%, respectively (Lalonde *et al.*, 1998).

Grass and riparian filter effectiveness in the North Carolina Piedmont varied based on storm intensity and watershed erosiveness, but decreased total sediment from 60% to 90% across a wide variation of natural rainfall (Daniels and Gilliam, 1996).

A laboratory simulation indicated that the denser roots and more erect growth of sheeps fescue resulted in significantly lower soil loss compared to Kentucky bluegrass (Tadesse and Morgan, 1996), but Melville and Morgan (2001) found no differences. The age of the grass had a great impact on its stiffness (Vuurmans and Gelok, 1993) and density (Barfield *et al.*, 1979). Sod-forming grasses prevented sediment transport, while bunch-type grasses did not (Choi, 1992).

There are numerous models involving sediment removal by vegetative filter strips, including Tollner *et al.* (1977); Barfield *et al.* (1979); Flanagan *et al.* (1989); and Vache *et al.* (2002). Munoz-Carpena (1993) developed a model to study hydrology and sediment movement in vegetative filter strips and identified soil moisture and grass spacing as

two very sensitive parameters. Barfield *et al.* (1979) used modeling to predict that sediment load would have little impact on outflow concentration leaving the filter strip until the buffer was inundated. Verstraeten *et al.* (2006) used the soil erosion and sediment delivery model WATEM/SEDEM and, while 70% or greater sediment reduction by a riparian vegetative filter strip was comparable to other studies, it was only about 20% for the entire catchment due to overland flow convergence and sediment bypasses through ditches, sewers, and road surfaces.

Pearce *et al.* (1998) showed that sediment movement was influenced by many factors, including percent surface vegetation cover, aboveground biomass, surface roughness coefficient, soil texture of introduced sediment, percent bare ground, distance downslope, vegetation density and height, and percent shrubs, grasses, and sedges. Helmers *et al.* (2006) estimated that properly located, designed, and maintained buffers may be expected to trap on the order of 50% of incoming sediment.

The Impact of Buffers on Phosphorus

Total phosphorus in surface runoff can be estimated from total suspended sediments (Bolton *et al.*, 1991). In an agricultural watershed in Indiana, about 90% of the total phosphorus transported was bound to sediment (Monke *et al.*, 1981).

Schwer and Clausen (1989) reported that a fescue/ryegrass/bluegrass filter strip retained 89% of the phosphorus from dairy milk-house wastewater. Vought *et al.* (1994), summarizing his own research on phosphorus removal from surface runoff, noted exponential removal with 66% and 95% of soluble phosphorus retained in the first 8 and 16 m of buffer strip, respectively. Daniels and Gilliam (1996) determined that fescue and riparian filter strips reduced total phosphorus load by 50%, but that 80% of the soluble phosphorus frequently moved through the strips.

Field experiments with artificial runoff on 20 filters showed an average phosphorus trapping efficiency of 61%, which was highly dependent on filter length (31% and 89% in 2- and 15-m filters, respectively) (Abu-Zreig *et al.*, 2003). Daily phosphorus yields from six Wisconsin watersheds over a 2-year period indicated that the shape, continuity, and uniformity of the riparian buffer strip were more correlated than the strip width with phosphorus yield (Reed and Carpenter, 2002). Sediment equilibrium phosphorus concentration measurements suggested that sediments may be releasing dissolved inorganic phosphorus during winter and spring and serve as a temporary sink of dissolved inorganic phosphorus during summer and fall (Chaubey *et al.*, 2007), adding to the complexity of the situation.

Kronvang *et al.* (2005) summarized the effects of buffer zones on phosphorus losses and some of the other primary mitigation practices implemented to date, and discussed factors that may delay or even counteract these practices when monitored at the catchment scale.

The Impact of Buffers on Nitrogen

In general, vegetative buffer strips are not as effective in reducing nonsediment-bound nutrients. Nitrate is water soluble and thus often leaches to shallow groundwater, subsequently moving to streams unless degraded or intercepted by denitrification and other processes. Conversely, ammonium-nitrogen in runoff water can be adsorbed to soil colloids and organic matter, which are more likely trapped by a filter strip.

Only 50% of total nitrogen was bound to sediment (Schreiber *et al.*, 1980). Nitrate uptake appeared to be linear with distance from the source, with an average of 20% and 50% removal in the first 8 and 16 m of buffer strip, respectively (Vought *et al.*, 1994). Jordan *et al.* (1993) found that most of the drop in nitrate levels within the riparian zone occurred abruptly at the edge of a floodplain within the forest, where the water table was nearest the surface and strong reducing conditions existed.

High rates of nitrogen removal can occur. On a private farm along Bear Creek in Iowa, Lee *et al.* (2003) reported 80% of total nitrogen and 62% of nitrate nitrogen were removed by a 7-m switchgrass buffer, and even greater amounts (94% and 85%, respectively) by a 16.3 m switchgrass/woody buffer containing a variety of shrub and tree species. A 10-m wide giant cane (*Arundinaria gigantea*) buffer with high-infiltration capability trapped all dissolved nitrate and dissolved and total ammonium nitrate (Schoonover *et al.*, 2005). Nitrogen immobilization was greater in an Iowan poplar/switchgrass buffer than in the cropped fields (corn–soybean) or cool-season grass buffer (Tufekcioglu *et al.*, 2003).

Nitrogen removal by buffers can be enhanced in various ways. Buffers cut with nylon line trimmers used 2.3 times as much nitrogen as uncut buffers, corresponding to increased growth following cutting (Bedard-Haughn *et al.*, 2005). A design having stiff-stemmed switchgrass barriers *above* fescue filter strips trapped 4.9 times more organic nitrogen and 2.3 times more ammonium nitrate during concentrated flow from simulated rainfall than fescue filter strips alone (Blanco-Canqui *et al.*, 2004).

The Impact of Buffers on Herbicides

Herbicide trapping by predominantly smooth bromegrass/Kentucky bluegrass filter strips ranged from 11% to 100% for atrazine, 16% to 100% for metolachlor, and 8% to 100% for cyanazine for six runoff events during a 2-year period, the variability due to the saturation level of the soil (Arora *et al.*, 1996). Herbicide reductions were similar (34–41%) for atrazine, metolachlor, and cyanazine for two ratios of drainage to vegetated area (15:1 and 30:1) (Misra *et al.*, 1996). With simulated runoff (Arora *et al.*, 2003), a 30:1 ratio buffer strip performed as well as a 15:1 strip, with average reductions of 46.8% and 52.5% (atrazine) and 48.1% and 54.4% (metolachlor), respectively.

A vegetative filter strip reduced losses of metribuzin and metolachlor by more than 85% (Webster and Shaw, 1996). Grassed waterways reduced loads of 2,4-D by 69% and 71% under wet and dry conditions, respectively (Asmussen *et al.*, 1977), while trifluralin retention dropped from 96% under dry conditions to 86% under wet conditions (Rhode *et al.*, 1980). A 6-m vegetative buffer strip composed of trees, shrubs, and grass almost completely removed terbuthylazine from runoff (Vianello *et al.*, 2005). Oats as a strip crop below corn reduced atrazine runoff losses by 91% and 65% after applications of 2.2 and 4.5 kg/ha, respectively (Hall *et al.*, 1983). Atrazine and metolachlor concentrations in runoff were reduced 83–94% and 82–96%, respectively, with 4.3- and 8.5-m vegetative filter strips (Barone *et al.*, 1998).

Webster and Shaw (1996) showed the importance of vegetative density on effectiveness of the filter strip. In the first years of strip establishment, total herbicide losses from no-till doublecrop soybeans were similar with and without filter strips, while metolachlor and metribuzin losses were reduced as much as 90% with the more established, denser filter strips in the third year. Both fresh and thatch switchgrass residue in vegetative filter strips can intercept and sorb herbicides (Mersie *et al.*, 2006).

Runoff of pesticides can be increased if sediment in the water reduces infiltration, indicating another important reason to prevent soil erosion. With 1 mg/L of herbicide applied in simulated runoff to smooth bromegrass filter strips, atrazine, cyanazine, and metolachlor losses were reduced 83–85% with no sediment present, but only 53–58% with 10 000 mg/L sediment (Misra, 1994).

Belden and Coats (2004) studied the impact of grass presence and species on atrazine, metolachlor, and pendimethalin fate in a soil column and showed that average infiltration time of simulated runoff decreased from 7.5 h to 3.4 h with vegetation. Although the type of grass had no impact on infiltration, degradation of atrazine and metolachor was significantly greater in soil under mixed prairie than under fescue. Since there were no differences in the levels of microbial activity, the authors hypothesized that 'some grass types may be creating an environment that selects for microbes that are capable of pesticide degradation.' Conversely, Lin *et al.* (2004) concluded from two studies that switchgrass, tall fescue, and smooth bromegrass were the best grass candidates for atrazine and isoxaflutole trapping and metabolism in tree–shrub–grass riparian buffer systems.

Characteristics of the pesticide can have a large impact on the effectiveness of vegetative filter strips (Boyd *et al.*, 2003). In a large-scale field experiment using a corn source area and an established bromegrass (81%)/bluegrass (12%)/other (7%) vegetative filter strip, pesticides that move predominantly in the water phase (atrazine and alachlor) depended mainly on infiltration capability of, rather than sediment reduction by, the strip. Vellidis (2002) indicated that a restored (2–3 year old) riparian forest buffer and a mature buffer (from a previous study) retained atrazine and alachlor similarly.

In recent years, the effect of buffers on the major metabolites of herbicides has begun to receive attention. Gay *et al.* (2006) monitored atrazine and three major degradation products in groundwater, soil, and runoff water for 11 months after application to a 0.1 ha strip immediately upslope from a restored forested riparian buffer in southern Georgia. Removal efficiency from groundwater (84.2–99.5%), surface runoff water (92–100%), and surface runoff sediment (67.4–92.0%) was significant for all four compounds (ranges in parentheses).

Krutz *et al.* (2005) reviewed studies investigating the effectiveness of vegetative filter strips on reducing herbicide runoff and methods of evaluating herbicide retention. They concluded that parameters affecting herbicide retention include width of vegetative filter strips, area ratio, species established, time after establishment of the vegetative filter strips, antecedent moisture content, nominal herbicide inflow concentration, and herbicide properties. The Natural Resources Conservation Service (USDA-NRCS, 2000) published a review of the effectiveness and proper installation and maintenance of conservation buffers to reduce runoff of pesticides. A summary of studies comparing herbicide runoff with BMPs to runoff without BMPs shows a significant reduction in runoff through the use of BMPs (Figure 32.1) (Ciba-Geigy Technical Report: 10-92).

Government organizations, universities, grower groups, dealers, pesticide producers, and others in the agricultural community have been very involved in product stewardship by encouraging the use of BMPs to protect the environment from runoff into streams, rivers, reservoirs, and lakes. The label directions on several pesticide products now include BMPs. The adoption of BMPs in many crops can be attributed in part to specific use directions on the labels

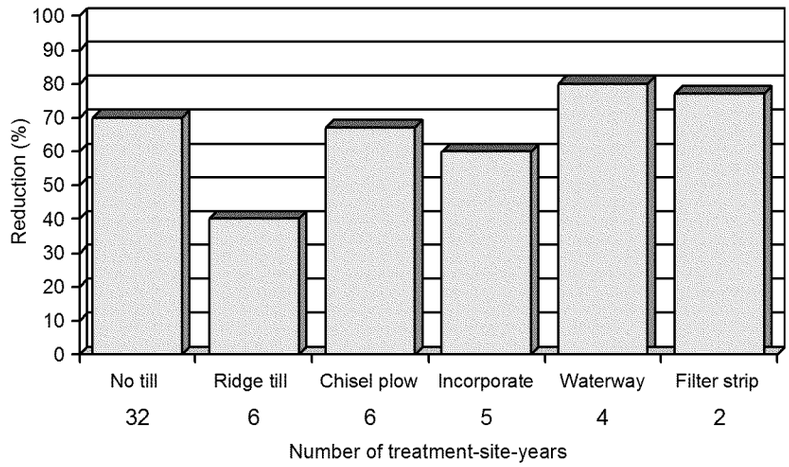

Figure 32.1 Reduction in herbicide runoff with BMPs as compared to runoff in the absence of BMPs.

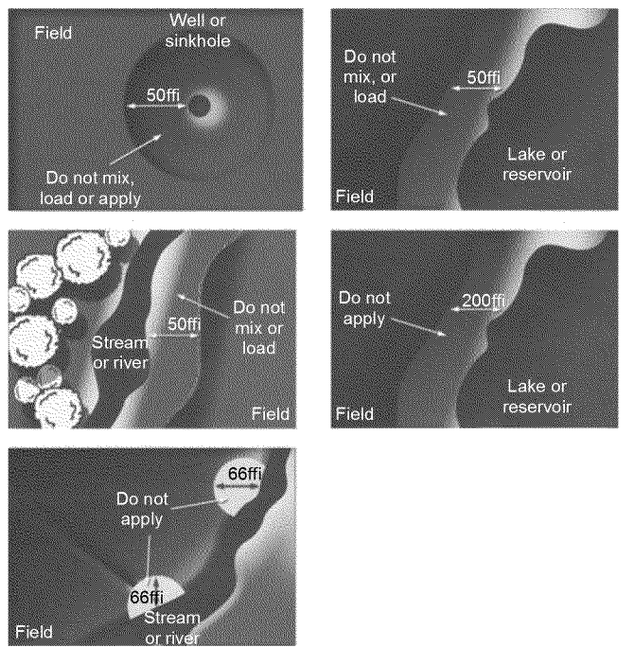

Figure 32.2 Examples of BMPs included on atrazine product labels. From Syngenta Crop Protection (2005).

of key pesticides, including the triazines. Examples of BMPs on atrazine product labels appear in Figure 32.2. The setbacks on the atrazine label were instrumental in helping farmers throughout the United States to understand the importance of vegetative buffers to improve water quality.

Crop Nutrient Management

The impact of soil management on water quality and the greater use of vegetative buffers is only a part of the picture. Efficient management of inputs is both profitable and environmentally beneficial. Several states require livestock farms to have comprehensive nutrient management plans that account for all sources of nutrients and match nutrient application and availability to crop needs (USDA-ERS, 1997).

Government agencies and the agricultural industry are helping farmers with nutrient management planning. The CTIC mailed 12 085 questionnaires on nutrient management planning to certified crop advisors in the United States. Sixty-one percent of the 1924 respondents reported that they were responsible for the development of Crop Nutrient Management Plans on 23.1 million A (9.35 million ha) of cropland (CTIC, 1999). The percentage of acres containing the key nutrient management planning components is presented in Table 32.4.

Table 32.4 Percent of acres or hectares containing various nutrient management planning components in the United States during 1998

Component	Percent of area
Field map	57
Soil test	83
Crop sequence	75
Estimated yield	75
Sources and forms	85
Sensitive areas	56
Recommended timing	69
Recommended rates	91
Recommended methods	72
Annual review and update	60

Certified advisors are including a broad base of components in the nutrient management plans they recommend to farmers. Some 83% of the respondents were employed by local agribusiness (62%) or were self-employed crop consultants (21%), indicating strong private sector support for nutrient management planning. A survey showed that retailers are well-positioned to help farmers with nutrient management planning (Agricultural Retailers Magazine Survey, 1997), with 96% making fertilizer recommendations. In addition, 90%, 54%, 49%, and 35% of retailers offer services for soil testing, tissue testing, field mapping, and site-specific farming services, respectively.

Site-specific farming (precision farming) is a growing trend that is likely to have a major impact on nutrient and pesticide use efficiency. A combination of yield monitoring, soil mapping, site-specific soil sampling, and remote sensing can identify places in the field where additional nutrient use will increase yield and thus farm income, by more than the added cost. It can also identify places to target reduced input in order to reduce costs while maintaining yield. Therefore, site-specific field management has the potential to reduce off-site transport of agricultural chemicals with surface runoff, subsurface drainage, and leaching (Baker *et al*., 1997; Johannsen *et al*., 1999).

In a survey sponsored by the Agricultural Publishers Association (1998), 1202 larger producers were interviewed (Agricultural Publishers Association, 1998). Some 11% of the respondents were already using Global Positioning Satellites on their farms, and 37% were using variable-rate chemical applications. This same survey indicated that rapid growth in the use of these technologies is expected.

Integrated Pest Management

On September 22, 1993, the USEPA, USDA, and the US Food and Drug Administration (USFDA) presented joint testimony to Congress on a comprehensive interagency effort to reduce the pesticide risks associated with agriculture (USDA-ERS, 1997). This testimony also expressed support for IPM and set a goal for using these programs on 75% of total US crop acreage. In 1996, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) defined IPM as: 'A sustainable approach to managing pests by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental risks.' BMPs are integral to any farming system using IPM.

For the 2000 growing season, the National Agricultural Statistics Service (NASS) of the USDA surveyed adoption of individual IPM practices (USDA-NASS, 2001). A summary of results for the major field crops, fruits and nuts, and vegetables is presented in Table 32.5. The adoption of systematic scouting for pests (weeds, insects, or plant diseases) looks very promising.

Crop rotation is widely used to avoid pest problems and is practiced on approximately 80% of corn and soybean acres, 87% of vegetable acres, 41% of cotton acres, and 65% of wheat acres.

There are serious challenges in certain cropping systems due to the development of insect, disease, and weed resistance to pesticides. As a result, farmers are alternating modes of action of pesticides in order to reduce the development of pest resistance. The use of alternate mode of action pesticides to manage resistant pests varies with different crops and ranges from 30 to 72% of crop acres (Table 32.5).

Conclusions

Significant progress in the adoption of BMPs has been made by the farming community. Setbacks from waterways added to herbicide product labels such as atrazine-containing products have contributed significantly to buffers being established on corn and sorghum farmland. Continued emphasis on the reduction of erosion and surface water runoff,

Table 32.5 Adoption of IPM practices in 2000 in field crops, vegetables, and fruit and nuts in the United States as adopted from USDA-NASS 2001 (a partial listing)

	Crops					
Crop production practices	**Corn**	**Soybean**	**Wheat**	**Cotton**	**Fruit/ nuts**	**Vegetables**
Surveyedarea (1000 A)	80 187	72 375	65 871	13 392	NA[g]	NA
			Percent of acres			
Prevention practices						
Tillage etc. to manage pests[a]	53	54	54	73	60	45
Remove or plow down crop residue	28	23	36	49	38	43
Water management practices	21	19	16	57	29	43
Avoidance practices						
Adjust planting and harvesting dates	17	0	32	63	21	21
Crop rotation	81	80	65	41	5	87
Monitoring practices						
Scouting for pests	58	56	50	86	72	75
Records to track pests	30	25	24	69	44	37
Field-mapping weeds[b]	30	29	27	44	20	31
Soil analysis for pests	22	27	13	42	38	37
Weather monitoring	30	32	33	56	54	41
Suppression practices						
Herbicide-resistant seed varieties	6	54	0	26	0	0
Threshold-based scouting[c]	34	34	26	62	41	35
Biological pesticides	18	7	5	47	30	27
Physical barriers[d]	24	23	31	26	33	33
Adjust planting methods[e]	12	19	11	10	5	25
Alternate pesticides[f]	30	45	41	67	66	72

[a]Tillage, mowing, burning, or chopping of field lanes or roadways to manage pests.
[b]To assist weed management decisions.
[c]Scouting data that was compared to university or extension guidelines for infestation thresholds to determine when to take measures to control pests.
[d]Ground covers, mulches, or physical barriers to reduce pest problems.
[e]Adjustments of row spacing, plant density, or row direction to control pests.
[f]Alternating pesticides to keep pests from becoming resistant.
[g]NA: Not available or not applicable.

combined with the necessary research, local support, and risk management incentives to help farmers adopt profitable cropland management practices and rotational systems that improve soil quality, will increase adoption of BMPs. Implementation of vegetative buffers and soil conservation practices has had a great impact on the removal of sediment, nutrients, pesticides, and bacteria from surface runoff. A recent study of 13 large watersheds in Iowa indicates that seven major conservation practices significantly reduced total nitrogen, nitrate, and phosphorus levels (Kling *et al.*, 2007). Model simulations for permanent vegetative filter strips along primary streams and/or grass contour buffer strips indicated that each alone reduced sediment load at the watershed outlet by more than 40%, but that both together gave a 71% reduction in load (Tim and Jolly, 1994).

Many of the gains in soil conservation accomplished over the last decade can be traced to adoption of conservation tillage. Herbicides such as the triazines are integral to the ability for corn and sorghum farmers to use no-till farming and other conservation tillage practices. Herbicides are responsible for declines in soil erosion because of their importance in conservation tillage. Several different conservation tillage systems have also been effective in reducing pesticide runoff. Many common herbicides run off treated fields primarily in the solution phase rather than being adsorbed to sediment. However, because conservation tillage slows water runoff, and often increases water infiltration as well as reducing erosion, herbicide runoff is usually reduced. Conservation tillage systems reduced herbicide runoff, with average reductions of approximately 70% for no-till and chisel plowing and approximately 40% for ridge-till (Figure 32.1). In addition, setbacks from waterways and reservoirs on atrazine-containing product labels have resulted in the establishment of buffer strips on corn and sorghum acreage throughout the United States.

Adoption of appropriate BMPs, including conservation tillage, yields positive results, as measured by declining levels of nutrients, pesticide, and sediment contamination in surface water (Fawcett *et al.*, 1994). It is critical that participants in conservation programs be given tools for evaluating the economic benefits of BMPs. These data could then be used at local levels to promote additional adoption of conservation and IPM practices. Government agencies in collaboration with the private sector have an opportunity to look at system approaches, programs, and resources that will influence the adoption of these best management systems in the next Farm Bill. Excellent progress in

improving the balance between economic agricultural production and environmental quality will continue as research on the benefits of BMPs continues and decision-making tools are enhanced (Hatfield, 2005).

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Chapter 33

Environmental Benefits of Triazine Use in Conservation Tillage

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Summary

Conservation tillage systems, which leave crop residues on the soil surface, have been widely adopted by farmers in the United States and elsewhere. In these systems, herbicides are substituted for part or all of the tillage normally performed to prepare seedbeds and control weeds. The triazine herbicides are uniquely suited to conservation tillage, and their use has facilitated adoption of these systems. Triazines control weeds emerged at the time of application and residues of the herbicides in soil control later-germinating weeds. Among the properties of triazines are low vapor pressure and weak adsorption to crop residue. Consequently, a triazine intercepted by surface crop residue during application shows minor volatilization loss. Most of the herbicide washes from the crop residue with rainfall and enters the surface soil, where it is active in controlling weeds.

Crop residues left on the soil surface following conservation tillage protect the soil from the erosive impacts of water and wind, preserving agricultural sustainability. Water infiltration is increased, thereby reducing runoff, which may carry sediment, nutrients, and pesticides into surface water resources. Conservation tillage fields, especially those in no-till, more closely resemble natural ecosystems than conventionally tilled agricultural fields. Reductions in tillage result in more biological diversity. Microbial populations increase, and invertebrates such as earthworms increase. Wildlife populations increase because of improved cover, more abundant food sources, and fewer disruptions caused by tillage and other trips across fields.

Soil structure improves with conservation tillage. The improvement includes increased soil organic matter content. Intensive tillage releases large amounts of carbon dioxide into the atmosphere because of increased microbial degradation of organic matter. No-till systems cause atmospheric carbon dioxide to be sequestered in the soil, reducing the release of this greenhouse gas. Conservation tillage keeps more herbicides, nutrients, and soil on fields and out of water. It also results in many other environmental benefits.

Introduction

Conservation tillage, defined as tillage systems leaving at least 30% of the soil surface covered by crop residue at crop planting, has been widely adopted by farmers in the United States and other regions of the world. In 2004, 112.6 million A (45.6 million ha) or 40.7% of total cropland acres in the United States were under some form of conservation tillage, according to the Conservation Technology Information Center (CTIC, 2004). These cropping systems have been adopted to conserve soil and water, save time and fuel, and provide other environmental benefits.

Development of Conservation Tillage

Edward Faulkner was one of the earliest proponents of eliminating the use of the moldboard plow and striving to leave more plant residues on the soil surface. In his book, *Plowman's Folly* (Faulkner, 1943), he called the plow the 'villain in the world's agricultural drama.' He concluded that plowing crop residues deep into the soil, rather than leaving them near the soil surface, was detrimental to crops and reduced the productivity of the soil. Faulkner wrote: 'Had we not originally gone contrary to the laws of nature by plowing the land, we would have avoided the problems … the erosion, the sour soils, the mounting floods, the lowering water table, the vanishing wildlife, the compact and

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Table 33.1 Conservation tillage in the United States as a percent of total crop acres[a]

Tillage system	1990	1992	1994	1996	1998	2000	2002	2004
No-till	6	10	14	15	16	18	20	23
Ridge-till	1	1	1	1	1	1	1	1
Mulch-till	19	20	20	20	20	18	16	17
All conservation tillage	26	31	35	36	37	37	37	41

[a]Conservation Technology Information Center (2004). Numbers rounded to the closest whole percentage.

impervious soil surfaces.' Although many of Faulkner's predictions of benefits from what was later to be called conservation tillage systems turned out to be true, poor weed control experienced when tillage was reduced prevented most farmers from adopting conservation tillage until the introduction of herbicides.

One of the earliest attempts to grow row crops without any preplant tillage was made by Barrons and Fitzgerald (1952), who also reported successful production of corn and soybean in ladino clover sod that had been killed with trichlorophenoxyacetic acid (2,4,5-T). DNBP (2-sec-butyl-4,6-dinitrophenol) and dinoseb were applied prior to planting and as directed sprays on both crops, and 2,4-dichlorophenoxyacetic acid (2,4-D) was applied postemergence to corn. While this study proved the viability of the concept of no-till planting, the practice wasn't adopted because of the lack of herbicides to provide residual control of weeds that germinate later in the season. The introduction of atrazine resulted in a renewal of interest in no-till planting and was, to a large degree, responsible for the birth of no-till and other conservation tillage systems in corn production. Atrazine controlled sod plants, as well as most annual weeds, while causing no corn injury.

Intensive research on no-till corn production was conducted in Virginia after a test in 1960 on orchardgrass sod was highly successful (Moody *et al.*, 1961). Orchardgrass killed with 4.4 kg/ha atrazine was compared with tilled plots. Stover yields were 33% higher on the untilled plots with atrazine.

No one herbicide will do the job and control all of the many weeds in most fields. Farmers require combinations of effective and dependable herbicides that will work under a wide variety of weed species and densities, soil types, and climatic conditions. Many years of research, trial, error, and experience have been required for farmers to arrive at the greater than 22% of all corn acreage in no-till, and 112.6 million A (45.6 million ha) or more than 40% of our total United States cropland acres in some form of conservation tillage systems in 2004 (CTIC, 2004). Table 33.1 shows the trend toward various types of conservation tillage in recent years.

Triazine Herbicides in Conservation Tillage

Triazine herbicides are particularly well suited for conservation tillage because they provide foliar and residual control of a broad spectrum of weeds. Atrazine, simazine, and metribuzin are used in corn, atrazine and propazine in sorghum, metribuzin in soybean, and simazine reduces tillage required for weed control in many perennial and tree crops. Atrazine is also used extensively in chemical fallow cropping systems in rotations involving corn, sorghum, and wheat. Cyanazine was also used extensively in corn and cotton until 2002.

Triazine herbicides such as atrazine and cyanazine are not tightly adsorbed to surface crop residue, allowing rainfall to wash intercepted herbicide into the soil. Low vapor pressures also avoid excessive vapor losses of residue-intercepted triazine herbicides. When atrazine was applied to corn-stalk residue, 52% of the herbicide washed off the stalk residue by the first 0.5 cm of simulated rainfall (Martin *et al.*, 1978). After 3.5 cm of rain, 89% of the intercepted atrazine had washed off the residue. Similarly, in another study (Baker and Shiers, 1989), 75% of applied cyanazine washed off corn-stalk residue with 0.7 cm of simulated rain, and an additional 11% was recovered from the residue.

Even when rainfall washes herbicides intercepted by the crop residue into the soil, some herbicides may be less effective because of altered distribution within the soil. Weeds may germinate under crop residue and escape contact with herbicides as they emerge. If an herbicide must be shoot adsorbed, weeds may not be controlled. Because triazines are root absorbed and relatively stable in the soil, they can kill small weed seedlings after emergence as roots grow to and encounter the herbicides in the soil. This property has made triazines highly popular in conservation tillage, used either alone or in combination with shoot-adsorbed, grass-controlling herbicides. Weeds escaping a shoot-adsorbed herbicide due to interception by crop residue can be controlled by residual activity of the triazines.

With conventional tillage, conditions for weed germination and growth are relatively similar each year; weeds emerging prior to crop planting are killed by tillage and the soil surface is devoid of crop residue. With conservation

tillage, differing weather conditions each year have a much greater impact in changing weed germination and growth patterns; weeds germinating prior to planting often must be controlled by herbicides. Stage of growth and species mix of these early germinating weeds vary from year-to-year, depending on weather. Surface crop residue reduces soil temperatures and delays weed seed germination. In the absence of a tillage operation, which stimulates more uniform weed seed germination, weed seed germination in conservation tillage is often delayed and more sporadic (Fawcett, 1987). The triazines are popular in conservation tillage due to their consistent performance under a wide variety of environmental, soil, and surface crop residue conditions and their residual soil activity, which controls late-germinating weeds.

Surface-applied herbicides require timely rainfall to incorporate them into the soil prior to weed germination. Timely rains after application often are more important in no-till systems than with tillage; weeds may have germinated (but not emerged) several days prior to planting and herbicide application, and thereby escape foliar nonselective herbicides. By the time rainfall activates the chemical, these weeds may be too large to control. Mechanical controls such as rotary hoeing and cultivation may be difficult or impossible in no-till due to heavy crop residue. The early preplant herbicide application program was developed to eliminate the weed control limitations of no-till systems and to allow growers more time flexibility to apply herbicides. Using the preplant program, residual herbicides are applied up to several weeks prior to planting, well before most weeds emerge. Early application allows more time for rains to occur before weed germination, reducing chances for dry-weather herbicide failure. Often, the need for a foliar nonselective herbicide, such as paraquat or glyphosate, is eliminated, as weeds are killed before or during emergence.

In an Iowa study at nine locations, traditional no-till corn herbicide programs using foliar nonselective herbicides combined with residual herbicides were compared with early preplant herbicide programs (Fawcett *et al.*, 1983). Traditional programs averaged 86% weed control, while all early preplant programs averaged 92% weed control. Because of its residual activity and broad spectrum of control, atrazine is one of the most effective herbicide alternatives applied early preplant. The postemergence activity of atrazine provides control of small emerged weeds from no-till planting-time treatments, often eliminating the need for nonselective herbicides.

In the western United States and other arid regions of the world, fallowing land for 1 year or a portion of a year stores some soil moisture, so water availability is sufficient to facilitate germination and better growth of grain crops the following year. However, weeds must be controlled during the fallow period to prevent evapotranspiration water losses. Repeated tillage had been traditionally used to control weeds. However, tillage increases water and wind erosion, increases evaporation losses, disturbs wildlife habitat, and expends extra fuel and labor.

Triazine herbicides have been integral components in the development of chemical fallow systems. Atrazine is used during the fallow period for weed control in wheat–sorghum–fallow, wheat–corn–fallow, and wheat–fallow–wheat rotations. Atrazine's low cost and broad spectrum weed control have made these fallow rotations profitable in areas where grain production otherwise would not be economically feasible. Greater water storage with chemical fallow, compared with conventional tillage fallow, has increased profitability and reduced risk associated with grain production in the Great Plains of the United States (Norwood, 1994).

Conversion from conventional tillage to conservation tillage involves considerable operator learning and crop production risk. Farmers reluctant to change to conservation tillage consistently rank concern about weed control as their primary reason for not converting to conservation tillage. Farmers who have successfully converted to conservation tillage have relied on many years of research and have invested many years of experience on their own farms. Confidence in the consistent weed control provided by triazine herbicides has encouraged these farmers to make a major management change and has allowed them to reap the economic and environmental benefits of the crop production system. If triazine herbicides were not available, major changes in weed control programs for conservation tillage would be necessary, increasing yield risk and uncertainty and hindering grower acceptance of conservation tillage. Thus, adoption of conservation tillage would be slowed or perhaps reversed.

Environmental Benefits

Tillage is a highly effective weed control technique, but it can have profound environmental impacts. Tillage increases wind and water erosion, threatening agricultural sustainability and causing off-site impacts such as sedimentation of aquatic ecosystems. Tillage expedites mineralization of organic matter in soil, depleting this important contributor to desirable soil structure and releasing carbon dioxide into the atmosphere. Water infiltration is often reduced, causing greater surface runoff, which carries soil and other contaminants to surface water. Because surface plant residues are buried, invertebrate and microbial populations are altered, and terrestrial wildlife habitats are disrupted.

Conservation tillage, which relies heavily on triazine herbicides, produces many environmental benefits. Herbicides allow tillage to be reduced or, in some cases, eliminated except for disturbance caused by the planter.

Soil Erosion

The problem of soil erosion into waterways has been correctly termed our most present form of water pollution (Pimental *et al.*, 1995). Soil erosion reduces water quality and disrupts agricultural sustainability.

Crosson (1994) observed that though the public tends to be concerned about minute and innocuous quantities of herbicides in the environment, there is little awareness or concern about the threat of sediment damage to surface water quality. Muddy water does not arouse moral indignation, even though its damage to the social welfare may be considerably greater than some other environmental threats. Crosson (1994) concluded that 'The public evidently is prepared to accept this damage even though its costs, present, and prospective, arguably are substantially higher than the costs of habitat loss and pesticide damage. If so, the costs of the damage are consistent with the sustainability of the country's agricultural system. Whether acceptance reflects full public awareness of the relative size of the sediment costs remains, for me, an open question.' Crosson further predicted that although the amounts of sediment delivered to surface water will not increase much, if any, under present agricultural production systems with conservation tillage, the cost of sediment damage will grow because of continuing increases in the economic and environmental value of water and the absence of effective erosion control policies.

Soil erosion, therefore, is the greatest threat to the economic and environmental sustainability of United States agriculture (Mueller, 1995). Pimentel *et al.* (1995) concluded that soil erosion is a major environmental and agricultural problem worldwide, and efforts to reduce these losses must be supported. Erosion rates have exceeded replacement values on most sloping fields since the Europeans introduced plowing and grain farming as North America was settled.

Ten to 20 cm of topsoil enriched with organic matter, plus a suitable subsoil, are needed for efficient crop growth. Under the best conditions, nature needs 30 years or more to develop 2 cm (about 3.36×10^8 kg/ha) of good topsoil (Hall *et al.*, 1981). The average annual loss of soil by erosion in the United States is about 1.0×10^4 kg/ha. However, erosion from sloping fields is often much higher, with losses exceeding 4.5×10^4 kg/ha common in some areas. Estimated annual soil regeneration rates are calculated for specific soils and called '*T*' values. Erosion rates exceeding *T* values are considered excessive. Typical *T* values for many agricultural soils in the United States are between 7×10^3 and 11×10^3 kg/ha. In 1977, 39 million ha of cropland (23% of the total) exceeded *T* values [United States Department of Agriculture (USDA), 1978].

In addition to the long-term effects on topsoil losses, soil erosion also causes off-site impacts. In 1989, the USDA calculated annual off-site damage impacts from soil erosion at $5 to $17 billion (Riboudo, 1989). Estimates for annual erosion damage categories are given in Table 33.2. The 1992 National Water Quality Inventory [United States Environmental Protection Agency (USEPA), 1994a] reports that sedimentation is the greatest polluter of rivers and streams, impairing 45% of assessed miles. Nutrients were the second most prevalent pollutant, impairing 37% of assessed miles. Eroded sediment carries most of the nutrients leaving farm fields. Sediment also pollutes our rivers and water supplies with other natural toxins and biological contaminants.

Sediment in rivers, streams, and lakes destroys aquatic habitats, decreases storage capacity of reservoirs, and interferes with navigational and recreational uses of water. The damage from sediments includes the loss of fish spawning sites, the cost of dredging ports and navigable rivers, and the cost of cleaning water for industrial and household users.

Table 33.2 Estimates of annual off-site damage from soil erosion by damage category (Riboudo, 1989)

Damage category	Off-site damage (in million $)
Freshwater recreation	2 080
Municipal and industrial use	1 196
Water storage	1 090
Flooding	978
Municipal water treatment	964
Navigation	749
Marine recreation	599
Roadside ditches	535
Marine commercial fishing	390
Irrigation ditches	118
Freshwater commercial fishing	60
Steam power cooling	24
Total	8783

Conservation tillage is one of the most practical and economical ways to reduce soil erosion. Surface crop residue protects the soil from the erosive impacts of wind and rain. Reductions in erosion are proportional to the soil coverage of crop residue. From 78% to 89% of the variance in erosion between tillage systems is explained by the percentage of soil coverage by plant residue (Laflen *et al.*, 1978). No-till systems, which leave nearly all surface plant residue in place, usually reduce erosion by 90% or more.

Use of conservation tillage was one of the primary ways United States farmers met 'Conservation Compliance' requirements of the 1985 Farm Bill. More than 75% of conservation plans for fields with 'Highly Erodible Land' mandated some level of crop residue cover achievable through the use of conservation tillage.

The 2001 Natural Resources Inventory (Natural Resources Conservation Service, 2003) shows dramatic decreases in erosion in the United States since 1982. Much of this reduction can be credited to adoption of conservation tillage, partly as a result of 'Conservation Compliance.' Sheet and rill erosion fell from an average 9.2×10^3 kg/ha/yr in 1982 to 6.2×10^3 kg/ha/yr in 2001, a 33% drop. The average wind erosion rate also dropped 36% during the same period.

One or more of the triazine herbicides has served as an essential component of virtually all conservation tillage programs in corn, sorghum, and many other crops. Due largely to these conservation tillage systems, it has been estimated that erosion of topsoil has been reduced by at least 50% in North America and Europe. By depending largely on herbicides rather than mechanical tillage for weed control, soil loss has been reduced at least 50% (Ray and Guzzo, 1993) and in some cases more than 90% (Laflen *et al.*, 1978), especially on relatively steep and windswept soils. Not only do conservation tillage systems greatly reduce the loss of valuable topsoil, but they also conserve much of the plant nutrients and organic matter that would otherwise be washed off the land along with soil to pollute our water systems further.

Soil Properties

Tillage speeds the mineralization of organic matter in soil by increasing oxygen availability. Mineralization releases large amounts of carbon dioxide, which contributes to atmospheric carbon dioxide levels. The organic matter content of agricultural soils in the United States has declined by as much as 50% because of this phenomenon. For example, the Morrow Plots at the University of Illinois were established in 1876 and have been maintained in constant cropping systems. Soil organic matter was first measured in 1903, when levels were about 90 000 kg/ha. By 1973, under corn production and conventional tillage, organic matter content had dropped to 45 000 kg/ha (Odel *et al.*, 1984).

Converting from conventional to conservation tillage can increase the organic matter in soil, rather than continuing to deplete it. Organic matter has increased by as much as 2 000 kg/ha/yr in long-term no-till studies (Reicosky *et al.*, 1995). Besides improving soil properties, this storing of carbon reduces atmospheric carbon dioxide concentrations.

Soil organic matter is the largest terrestrial carbon pool and a source of carbon dioxide, methane, and other greenhouse gases. Projected changes in atmospheric carbon over a 30-year period ending in 2020 were calculated under several United States tillage scenarios (Kern and Johnson, 1993). Minimum tillage (not including no-till) conserved existing levels of organic carbon in soil. Including the benefit of lower fuel use, minimum tillage produced a net reduction in carbon emissions equivalent to 0.7% to 1.1% of total projected United States fossil fuel emissions during the 30 years. If 57% of all cropland were in no-till, soil organic carbon would increase 80 to 129×10^{12} g C. With 76% of cropland in no-till, a rise of 286 to 468×10^{12} g C in soil organic carbon was projected.

Tillage and the multiple machinery passes used in conventional tillage systems can lead to soil compaction, which increases water runoff, alters the desirable mix of air and water in soil pores, and reduces crop yields. Tractor wheel traffic reduced corn yields by as much as 50% in a Canadian study (Raghavan *et al.*, 1978).

Tillage buries plant residues, exposing the soil surface to direct sunlight. Soil temperature fluctuations, water evaporation, and oxygen concentrations are increased, profoundly affecting microorganisms and invertebrates. Tillage favors microorganisms with higher turnover rates, such as bacteria and bactivorous fauna, including protozoa and nematodes (Hendrix *et al.*, 1986; Beare *et al.*, 1992). Decomposition processes in no–tillage agroecosystems, which leave crop residue on the soil surface, are controlled primarily by fungi, with fungivorous microarthropods, nematodes, and earthworms dominant in subsequent steps in the food web (Hu *et al.*, 1995). Fungal-dominated microbial communities within no-till systems store organic material for longer periods, resulting in higher steady-state levels of organic matter. Fungal-mediated aggregation is an important factor in promoting retention of soil carbon and develops desirable soil structure. Fungal hyphae contribute to the formation of macroaggregates by physically enmeshing microaggregates. In addition, the extracellular polysaccharides of fungi are important in forming soil aggregates.

Total microbial populations are often higher in no-till soils. In a study comparing surface soils from long-term no-till and conventional tillage plots at seven United States locations, counts of aerobic microorganisms, facultative anaerobes, and denitrifiers in no-till soils were 1.14–1.58, 1.57, and 7.31 times higher, respectively, than in the surface of plowed soils (Doran, 1980).

Many beneficial predatory arthropods, including ground beetles and spiders, are increased by no-till. For example, no-till soybean had 17.6 carabid beetles/m^2, compared with 0.38/m^2 in plowed soybean fields (House and Parmalee, 1985). Higher beneficial arthropod populations have been correlated with reductions in crop losses due to certain pests.

Water Quality

Conservation tillage slows and usually reduces water runoff. No-till has often produced dramatic decreases in water runoff and increases in water infiltration, especially in long-term studies. Several paired watershed studies produced no seasonal runoff from no-till fields, while conventional tillage watersheds had significant water runoff, soil erosion, and pesticide runoff (Glenn and Angle, 1987; Hall *et al.*, 1991). An Ohio study (Edwards *et al.*, 1988) compared total water runoff from a 0.5 ha watershed with 9% slope that had been farmed for 20 years in continuous no-till corn with runoff from similar but conventionally tilled watershed. Over 4 years, runoff was 99% less under the long-term no-till system. The lower runoff was attributed to increases in water infiltration with no-till because soil macropores developed in the absence of tillage. Cracks, root channels, and earthworm holes allow water to bypass upper soil layers when rainfall exceeds the capillary flow infiltration capacity of the soil (Edwards *et al.*, 1989).

Because conservation tillage systems reduce soil erosion and water runoff, the runoff of both sediment-adsorbed pesticides and dissolved pesticides is usually reduced. No-till has sometimes resulted in complete elimination of pesticide runoff (Glenn and Angle, 1987; Hall *et al.*, 1991). A summary of published natural rainfall studies comparing no-till with moldboard plowing and involving 32 treatment-site years of data (Fawcett *et al.*, 1994) showed that on average, no-till resulted in 70% less herbicide runoff, 69% less water runoff, and 93% less erosion than plowing.

Much of the ability of conservation tillage to reduce runoff of pesticides such as the triazines is related to increases in water infiltration due to improved soil structure and slowed water runoff. However, if water infiltration is prevented by a high water table or restrictions to permeability such as claypans, conservation tillage may not reduce pesticide runoff.

Some studies comparing no-till with tilled soil (Hall *et al.*, 1989) have shown increased leaching of certain pesticides to shallow depths, while other studies have documented less pesticide leaching with no-till (Fermanich and Daniel, 1991; Levanon *et al.*, 1993). Reductions in pesticide leaching with no-till may be due to greater microbial activity degrading the pesticide, greater organic matter adsorbing the pesticide, and water bypassing upper layers of soil containing the pesticide by flowing down macropores.

Aquatic Ecosystems

Because soil sediment causes the greatest harm to aquatic systems (USEPA, 1994b), erosion reductions credited to conservation tillage provide major benefits to aquatic ecosystems. Sediment covers gravel stream beds needed for habitat by fish and crustaceans. Sediment also clouds water, reducing sunlight penetration, and diminishing photosynthesis of submerged plants and algae, which causes a cascading effect through food chains. Effects of sediment in reducing photosynthesis are usually much greater than those of herbicides present in runoff (Solomon *et al.*, 1996; Wood and Armitage, 1997). Sediment can also carry ammonium–nitrogen, which is toxic to fish, and phosphate, which contributes to eutrophication of lakes.

Terrestrial Wildlife

Conservation tillage benefits wildlife by providing more crop residues for cover, more food sources (waste grain and weed seed left on the soil surface, as well as a greater number and variety of invertebrates), and less disturbance by field operations.

Studies in Iowa (Basore *et al.*, 1986), Illinois (Warburton and Klimstra, 1984), and Indiana (Castrale, 1985) have shown that no-till row-crop fields have higher densities of birds and bird nests, and they are used by a greater variety of bird species during their breeding season than tilled fields. Foliage- and litter-dwelling arthropods are important food sources for many birds. Bobwhite quail behavior in no-till and conventional soybean fields was studied in North Carolina (Palmer, 1995), determining the hours needed for quail chicks to obtain their minimum daily requirement of insects. It took chicks 22 h to obtain their minimum daily requirement in conventionally tilled soybean fields, illustrating the unsatisfactory habitat tilled fields provide. In no-till soybean fields, only 4.2 h were required to obtain the minimum daily insect requirement, less than the 4.3 h required in undisturbed areas believed to be ideal quail habitat.

Small mammals are also favored by conservation tillage. In Illinois, no-till corn fields had more abundant and more diverse populations of invertebrates, birds, and small mammals than conventionally tilled corn (Warburton and Klimstra, 1984). A greater diversity and proportionately more predators were noted within the invertebrate community in no-till fields. Small mammal populations also were more stable in no-till.

Conclusions

Triazine herbicides have been important tools in the development and implementation of conservation tillage systems. Postemergence and residual activity, low vapor pressure, weak adsorption to surface crop residue, root uptake, broad weed control spectrum, and crop safety make these herbicides compatible with the high crop residue conditions of conservation tillage.

Conservation tillage fields more closely resemble undisturbed ecosystems than conventionally tilled fields. This resemblance is most apparent in no-till fields. Soil erosion is reduced. Aquatic life and aquatic systems are protected from sedimentation. The water cycle is more similar to the cycle in undisturbed systems (more water infiltration and less runoff). Consequently, there is less runoff of pesticides and nutrients. As significant areas of crop production are converted to conservation systems by use of herbicides for weed control in place of tillage, streams are fed more by subsurface flow rather than surface runoff. This allows better use of water and nutrients by crops and allows soil colloids and biological activity to filter the water before it becomes surface water. Surface water should more closely resemble shallow groundwater than it did when tilled fields dominated the landscape. Also, flooding should be less pronounced and cause less damage because of greater water infiltration.

Conservation tillage fields are more biologically diverse than intensively tilled fields. Untilled soils are dominated by fungal rather than bacterial microorganisms, resulting in greater storage of carbon and improved soil structure. Releases of carbon dioxide caused by tillage are reduced. Invertebrate populations become more numerous and diverse as tillage is reduced, providing more food sources for birds and wildlife. Beneficial predator insect population increase as tillage is reduced. Surface crop residue provides cover for wildlife, and waste grain and weed seeds provide a food source. With fewer trips over the fields, there is less disruption and injury to wildlife. Conservation tillage systems have enabled a dual use of agricultural land: efficient production of crops and creation of better wildlife habitat.

Soil erosion has received limited attention outside of agricultural circles in the past, sometimes being considered a natural, unavoidable consequence of agriculture. Typically those concerns that have been raised have involved a feared loss of crop yield or productivity due to loss of fertile soil. However, off-site impacts of soil erosion are of greater ecological and economic magnitude than are on-site impacts. Sediment damages water quality for recreation and fish habitat. It also increases the cost of dredging ports and navigable rivers, and the cost of treating water to remove sediment for industrial and household users. A 1989 USDA study (Crosson, 1994) showed that the annual costs of damage to water quality by sediment from farm fields was $4 to $5 billion in the mid-1980s. The water sedimentation costs amounted to 20–25% of net farm income exclusive of direct government subsidies paid to farmers.

The use of conservation tillage, made possible by triazine and other herbicides, has dramatically reduced soil erosion and its on- and off-site impacts. Significant benefits from the use of herbicides such as the triazines can be realized because conservation tillage reduces erosion, sedimentation, and flooding. By creating a crop field with many features comparable to those in natural areas, conservation tillage also provides other benefits to the environment and to wildlife.

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Chapter 34

Role of Triazine Herbicides in Sustainable Agriculture: Potential of Nonchemical Weed Control Methods as Substitutes for Herbicides in United States Corn Production

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Summary

In recent years, much attention has been given to sustainable agriculture by Land Grant University administrators and agricultural policy makers. The philosophy has, in turn, greatly influenced the research agenda within the United States Department of Agriculture (USDA) and State Agricultural Experiment Stations. Because triazine herbicides are often used by university scientists as standards for weed control in efficacy tests, there is excellent information available on their benefits. The triazines are key components of long-term sustainable agriculture based on efficient food and feed production and on a profitable yield and return to the farmer, while preserving soil for future generations.

As a result of modern agricultural technology and farmer trial and error, great progress has been made toward the development of systems that provide long-term sustainability with reasonable use of agricultural chemicals. Farmers are concerned about weed control, weather, soil conditions, crop yield, and environmental stewardship. Alternatives to herbicides often come with costs or tradeoffs, such as increased soil erosion, lowered operational efficiency, more land needed, or reduced profits.

At the present time and for the foreseeable future, the most sustainable and most profitable approach to weed management for most US corn and sorghum growers is to continue to use and improve the no-till or conservation tillage systems that are based on the judicious application of atrazine, simazine, and other herbicides. This is especially true where soil erosion and other environmental damage from excessive tillage may otherwise occur. Atrazine and simazine still provide the most efficient, effective, environmentally safe, beneficial, and dependable weed management available for many growers of corn, sorghum, and certain other crops. The use of the triazines is sustainable in that herbicides such as atrazine can provide higher yields, facilitate conservation tillage practices, help control weeds resistant to other herbicides, and provide higher returns on a grower's investment than most other crop production expenditures.

Introduction

The use of herbicides to control weeds in corn represents the largest single crop use of pesticides in the United States in terms of quantity of active ingredient (a.i.), accounting for approximately 25% of the nation's total pesticide use (Gianessi and Marcelli, 2000). Surveys indicate that the percentage of corn treated with herbicides reached 79% in 1971 and has remained at more than 95% since 1982 (Andrilenas, 1974; USDA ERS, 1983), with 98% of corn treated in 1999 (USDA NASS, 2000). The major herbicide used on corn is atrazine, which represents 34% of the total volume of herbicides used by US corn farmers (Gianessi and Marcelli, 2000). Surveys conducted by the National Agricultural Statistics Service (NASS) show that atrazine was used on 55% of the nation's corn in 1971, 59% in 1982, 70% in 1999, and 66% in 2005.

Studies have analyzed the potential of nonchemical weed control technologies to serve as replacements for herbicides in US corn production. A Weed Science Society of America (WSSA) committee surveyed research and extension weed scientists in all states and asked them to estimate the percentage yield reductions that would occur if farmers used best management practices without herbicides (Bridges and Anderson, 1992). In the US Corn Belt,

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Table 34.1a Average control efficacy of atrazine for selected weed species in the United States[a]

Weed species	Type	% control
Giant foxtail	G[b]	70
Common lambsquarters	B[c]	90
Redroot pigweed	B	95
Velvetleaf	B	80
Pennsylvania smartweed	B	97
Common cocklebur	B	90
Common ragweed	B	90
Fall panicum	G	40
Jimsonweed	B	95
Morningglories	B	90
Giant ragweed	B	80
Black nightshade	B	90
Large crabgrass	G	50

[a] Anderson *et al.* (1994).
[b] G ffi grass weeds.
[c] B ffi broadleaf weeds.

Table 34.1b Weed infestations and potential yield loss in Illinois field corn[a]

Weed species	% area infested	Potential % yield loss
Giant foxtail	95	30
Common lambsquarters	60	40
Redroot pigweed	60	50
Velvetleaf	70	30
Pennsylvania smartweed	60	30
Common cocklebur	30	20
Common ragweed	30	20
Fall panicum	25	20
Jimsonweed	20	10
Morningglories	20	10
Giant ragweed	20	40
Black nightshade	20	5
Large crabgrass	20	10

[a] Pike and Knake (1994).

losses of corn yields to weed competition were estimated to rise from 7% with current methods to 32% if farmers relied on increased tillage and other practices currently available for controlling weeds without herbicides. A similar survey of weed scientists in 1990 concluded that US corn yields would decline 30% without the use of herbicides for weed control (Smith *et al.*, 1990). In the study, additional cultivations were assumed to substitute for herbicides, and no changes in crop rotation practices were considered.

The focus of this chapter is on various nonchemical weed control techniques and the challenges involved when they are used as replacements for herbicides in corn production.

Current Atrazine Use

Atrazine is widely used in corn production due to its efficacy, application flexibility, low cost, and crop safety. Its efficacy ratings for some common weed species are listed in Table 34.1a. Table 34.1b presents estimates of the infestation of these important weeds in Illinois and of the potential yield loss that would occur if these weed species were uncontrolled. These data indicate that atrazine provides significant levels of control for most of the important weed species that are widely distributed and those that would cause significant corn yield loss if left uncontrolled.

Although atrazine is used on approximately 65–70% of the nation's corn acreage, today it is often used in premixes with other herbicides. Survey data for 1992 indicated that atrazine by itself represented only 6% of the herbicide acre treatments in corn (USDA ERS, 1993b). Atrazine is more frequently used in formulations or in tank mixtures with other herbicides to broaden the weed control spectrum, particularly with regard to broadleaf species. The average

Table 34.2 Atrazine use in corn by state during 1999[a]

State	Total area Planted ffi 1000		Percent of area treated	Number of applications	Average rate per application		Average rate per crop year	
	Acres	Hectares			(lb/A)	(kg/ha)	(lb/A)	(kg/ha)
Colorado	1230	500	42	1.1	0.58	0.65	0.64	0.72
Illinois	10800	4370	84	1.1	1.08	1.21	1.25	1.40
Indiana	5800	2350	91	1.0	1.25	1.40	1.26	1.41
Iowa	12100	4900	65	1.3	0.78	0.87	1.04	1.17
Kansas	3150	1280	89	1.1	0.98	1.10	1.09	1.22
Kentucky	1320	540	87	1.0	1.54	1.73	1.57	1.76
Michigan	2220	900	69	1.0	1.25	1.40	1.25	1.40
Minnesota	7100	2870	24	1.0	0.61	0.68	0.61	0.68
Missouri	2650	1070	95	1.1	1.35	1.51	1.54	1.73
Nebraska	8600	3500	87	1.0	1.01	1.13	1.10	1.23
North Carolina	750	300	69	1.0	1.04	1.17	1.06	1.19
Ohio	3450	1400	83	1.0	1.30	1.46	1.33	1.49
South Dakota	3600	1460	42	1.0	0.69	0.77	0.71	0.80
Texas	1950	800	79	1.0	1.07	1.20	1.16	1.30
Wisconsin	3600	1460	37	1.0	0.80	0.90	0.80	0.90

[a] USDA NASS (2000).

number of herbicide active ingredients used on corn acreage is 2.4. Only 14% of corn acreage is treated with a single herbicide. Another 43% is treated with two active ingredients, 30% with three active ingredients and 10% with four or more (USDA ERS, 1997a).

Typically, the inclusion of atrazine in an herbicide program results in good-to-excellent control of broadleaf and grass weed species. In a summary of 750 Midwest research trials conducted from 1972 through 1991, control efficacy was 80–100% in 74% of the broadleaf weed control trials in which atrazine was used (Pike *et al.*, 1994). When atrazine was not used and other herbicides were used, broadleaf control in the 80–100% range was obtained only 54% of the time.

Atrazine is usually applied to corn at the early pre-plant, pre-plant incorporated, or pre-emergence stages. If applied pre-emergence, atrazine can provide season-long control of many germinating weeds (Jordan *et al.*, 1985). When applied post-emergence, atrazine will successfully control many grass and most broadleaf weed species if applied when the weeds are relatively small. Atrazine is widely used regardless of the tillage system used by the corn growers.

Atrazine is used in all corn-growing regions of the United States. Estimates of the frequency and extent of atrazine use in corn by leading states are shown in Table 34.2. Atrazine is usually applied once during the growing season (USDA NASS, 2000). The average number of herbicide applications on the typical US corn crop is 1.5 (USDA ERS, 1997a). One herbicide application includes mixtures of herbicides being applied at the same time. Fifty-five percent of the corn acreage receives one herbicide application, 35% receives two applications, and 6% receives three or more applications.

Another major advantage of atrazine is its low cost – approximately $4/A ($10/ha) (Witt *et al.*, 1993). The cost of alternative nontriazine herbicides that would control many of the same weed species is $8 to $18/A ($20 to $45/ha). Although 2,4-D post-emergence would be cheaper than atrazine ($1.25/A or $3.10/ha), such applications would control only broadleaf weed species, often only temporarily, and could result in some crop injury.

An additional advantage of atrazine is that it can be applied safely to corn without the potential for injury. Nontriazine alternatives to atrazine for broadleaf and grass weed control have some potential to harm corn plants (Anderson *et al.*, 1994).

Nonchemical Weed Control Techniques

Before the introduction of herbicides, weed management relied on cultural and mechanical control methods that increased soil erosion. Herbicides markedly improved weed management and began to replace tillage and cultural practices for weed control in corn in the early 1950s. But as late as 1959, 50% of surveyed Illinois corn growers still relied upon cultural practices, cultivation, rotary hoeing, and hand weeding as the sole methods for controlling weeds (Gianessi and Marcelli, 2000).

Crop Rotation

The open growth pattern of corn and relatively wide rows allow invasive weed growth between the rows. Problem weeds in corn generally have life cycles that are similar to the crop. By rotating to a winter annual crop with an entirely different growth pattern, the problem weed often is less able to compete with the new crop. Also, rotation with a high-density crop such as drilled soybean or alfalfa provides competition to the weeds by reducing the space and light available to them (Brust and Stinner, 1991).

Crop rotations are a desirable agronomic practice. For example, rotating corn with soybean reduces the need for a soil-applied insecticide in first-year corn. Eighty percent of the corn in the Corn Belt is estimated to be rotated with soybeans. Smaller percentages of corn acreage are grown in rotation with small grain, alfalfa, and other legumes. Including alfalfa in a rotation subjects weeds to several mowings and reduces the nitrogen fertilizer needs in the subsequent crop. Crop rotations are beneficial in controlling weeds for two reasons. The first is the disruption of certain weed species due to the planting of a crop with different growth characteristics. The second benefit is that the practice allows growers to use herbicides to control weeds in one crop that are difficult to control selectively in another crop.

Before the advent of selective herbicides in the mid-1940s, it was a common practice to grow alfalfa and corn in long rotations. Alfalfa fixed nitrogen in the soil. With the development and use of synthetic fertilizers, it was no longer necessary to use alfalfa as a nitrogen source. Some weed control benefits for corn were attributed to alfalfa in the agronomic literature of the early 1900s. Rotating to alfalfa has been shown to reduce the prevalence of some weeds through competition. For instance, rotating from corn to alfalfa almost completely eliminates wild proso millet as a weed, which is particularly hard to control in corn (Doll, 1988). Alfalfa controls the weed because the plants are very competitive before the soil is warm enough for wild proso millet to germinate.

The management of alfalfa also facilitates the control of some other weed species. Alfalfa is a perennial plant, and stands may last from 3 to 10 years. It is harvested three to six times during a year, depending on the length of the growing season. Repeated mowing of alfalfa helps to suppress populations of perennial weed species that propagate from rootstocks (e.g., hemp dogbane and common milkweed). As a result of mowing alfalfa stands, many weed species are less of a problem during the subsequent year when corn is planted. Another way that rotating corn with alfalfa helps to manage weeds is through suppression of those species with relatively short seed longevity in the soil. For example, the seed longevity of many annual grasses can be relatively short. If these grasses do not produce new seeds while fields are in alfalfa, the seed reservoir is reduced significantly. However, this approach has less effect on weeds such as velvetleaf, wild mustard, redroot pigweed, and common lambsquarters, which have very long-lived seeds (Doll, 1988). Alfalfa stands can be plagued with certain weed species due to the compatibility of growth patterns. In Minnesota, the average alfalfa stand is reestablished every third year, partly because weeds take over, stands become unproductive, or they produce low-quality feed (Durgan, 1988).

Weeds are often a problem in spring-planted alfalfa because many weed seeds germinate and emerge soon after the alfalfa is planted. By the time the alfalfa seedlings emerge from the soil, weed seedlings such as quackgrass, yellow nutsedge, yellow rocket, and dandelion frequently have a competitive advantage (Peters and Peters, 1972). Winter annual weeds, such as common chickweed and shepherd's purse, can become serious problems in summer alfalfa plantings by germinating from late summer through winter and continuing to grow after the alfalfa becomes dormant. These weeds often become so dense that they seriously reduce alfalfa stand and yield, especially if the mature alfalfa stands are not dense and growing vigorously. Well-established alfalfa prevents weed seedlings from becoming established, but weeds will thrive when alfalfa stands become so thin that sunlight reaches the ground.

Many alfalfa producers feed the crop to farm animals, so weed control is not a high priority. Thus, many alfalfa stands become heavily infested with weeds in 2–3 years and are plowed down, returning many weed seeds to the soil for later germination. When the field is planted to corn, the bare ground and wide rows again make it possible for certain weed species to proliferate, such as quackgrass and those with long-lived seeds (Bowman, 1992).

Many of the weed species controlled by atrazine are precisely the weed species that are not adequately controlled by alfalfa stands. While herbicide use on hay, pasture, or other crops is slightly lower than that in other rotations, the proportion of acreage treated remains at 89% (USDA ERS, 1996). Thus, many farmers who rotate into alfalfa for 3–5 years use atrazine to control weeds in a subsequent corn planting.

Growers who practice long-term alfalfa–corn rotations generally subdivide their farms into fields that are on different rotational cycles. Thus, a farmer may have 1/4 of the farm's acreage in corn one year, while 3/4 of the acreage is in alfalfa. The use of atrazine in corn actually facilitates this type of farming, eliminating the need for farmers to cultivate corn for weed control purposes at a time when they need to be harvesting their first cut of hay (Mt. Pleasant *et al.*, 1994). Many farmers who practice sustainable agriculture use atrazine to replace cultivation. A notable example is a farm in Pennsylvania that has been extensively studied as an example of a low-input, sustainable farming operation (Culik *et al.*, 1983; NRC, 1989). The farm produces alfalfa and red clover hays, barley, oat, rye, wheat, corn for grain

and silage, and soybean. Finished livestock include beef cattle, hogs, and laying hens. One hundred acres (40 ha) are owned by the operator's family or rented from a neighbor, while another 220 A (90 ha) are rented from an organic farming research center. Herbicides such as atrazine are used in the corn, but only on the nonorganic land. This alleviates the need to cultivate some of the row crop acreage at a time when the farmer should be harvesting the first hay crop.

It is important to consider the consequences of a large-scale shift to an alfalfa rotation on the nation's field-corn acreage. Currently, less than 2% of US corn is rotated to hay (USDA ERS, 1997a). Additional land would need to be brought into corn production to make up for acreage being put into rotation. Alfalfa usually is a low-value crop, much of it used by growers to feed their own livestock. It is questionable if there would be a market to support a large increase in alfalfa production.

Cover Crops

Cover crops, which include legumes and cereals, are grown specifically to protect the soil from erosion, enhance soil fertility, and suppress pests, including weeds (Lal *et al.*, 1991). Cover crops are often grown not for harvest, but for soil enrichment. In some cases, a rye cover crop is harvested as forage, which provides additional feed for livestock producers (Curran *et al.*, 1994b). Many different cover crops are used, but the most extensively used is winter or annual rye (Johnson *et al.*, 1993).

Cover crop residues can reduce weed seed germination and seedling growth by shading, lowering soil temperature, and acting as a physical barrier (Curran *et al.*, 1994a). For example, fall-planted winter rye, killed with an herbicide in the spring, has the ability to suppress annual weeds (Wyse, 1994). Successfully established cover crops may develop dense enough canopies in the fall to interfere with the growth of perennial and winter annual weeds (Swanton and Weise, 1991). Certain plants used as mulches such as rye contain allelochemicals that may further suppress weeds.

In other mulch research, row crops such as corn are typically seeded into a low-growing, pre-established winter grain, perennial legume, grass sod, or winter annual legume cover crop. With few exceptions, these mulches have shown little selectivity because mulches effective in controlling weeds also tend to suppress the row crop. Cover crops are most effective when killed in the spring by use of herbicides, partial tillage, or mowing to reduce their competition to row-crop establishment, growth, and yield (Johnson *et al.*, 1993). The most common herbicides for killing or suppressing a living cover crop in the spring are paraquat and glyphosate. In a series of experiments in Pennsylvania, researchers examined no-till corn production in pre-established crownvetch sod (Hartwig, 1988). Corn yields were comparable to the long-term average yields under conventional systems when crownvetch sods were suppressed from 50% to 67% (Hartwig and Hoffman ,1975; Linscott and Hagan, 1975). When crownvetch was not controlled or suppressed, corn yields and weed growth were significantly reduced over a 3-year trial (Echtenkemp and Moomaw, 1989; Lal *et al.*, 1991).

Small grain cover crops have been successfully killed by mowing. For this to be effective, however, the cover crop has to be near maturity so that it does not grow back. Mowing a cover crop can eliminate the need for a 'burndown' herbicide and still maintain mulch on the soil surface to prevent soil erosion, but the success of mowing is variable (Curran *et al.*, 1994a). An experiment in Missouri resulted in decreased corn stands from rye and hairy vetch in mowed plots (Johnson *et al.*, 1993). Mowing hairy vetch prior to the mid-bloom stage of growth failed to control the vetch adequately (Hoffman *et al.*, 1993). Mowing a winter rye cover crop at corn planting was also ineffective in killing the rye, which subsequently competed with the corn crop (Curran *et al.*, 1994a). In a 1992 experiment in Pennsylvania, hairy vetch was in the late vegetative stage of development at corn planting because of cool and wet conditions. This caused the winter annual legume to resume growth following some of the mowing treatments.

Planting a corn crop when the cover crop is near maturity reduces the possibility of competition between the two crops. Vetch did not compete with corn when corn was planted at the vetch mid-bloom stage, which allowed maximum vetch shoot development and weed suppression; no treatment was needed for its control at corn planting (Hoffman *et al.,* 1993). From the standpoint of weed control, vetch may be of greater benefit for corn planting dates timed to coincide with vetch mid-bloom (May) versus early bud (April) growth stages. However, delayed corn planting can potentially result in reduced corn yields.

Selection of mulch with a winter annual life cycle gave promising results in weed control and corn yield in New Jersey (Enache and Ilnicki 1990; Lal *et al.,* 1991). This 3-year study showed that corn planted into a living stand of unsuppressed subterranean clover yielded as well as or better than corn with conventional herbicidal weed control and no mulch. Subterranean clover planted in the fall goes dormant during the winter and resumes and completes growth during the spring, giving minimal competition to the corn. Subterranean clover leaves a weed-suppressive residue after senescence, and it reseeds itself. The choice of a cover crop with a winter annual life cycle seems logical for living mulch systems in the northeastern and north central United States. However, lack of sufficient winter-hardiness is a

major obstacle in living mulch technology for weed control. For example, in experiments in Nebraska, white clover did not survive the winter in two out of three years, and ladino clover did not survive one winter (Echtenkemp and Moomaw, 1989).

Research in Minnesota has focused on the use of short-term, spring-seeded cover crops that might avoid the problems associated with winter hardiness (DeHaan *et al.*, 1994). The yellow mustard has a short life cycle of 4 to 6 weeks. At high seeding rates within 24 hours of corn planting, the yellow mustard reduced weed dry weight by 82%, but it also reduced average corn yields 19%. At a lower seeding rate, the yellow mustard reduced weed dry weight an average of 51% and resulted in an average corn yield reduction of 4%. Researchers concluded that if progress is to be made, new smother plants will have to be bred (Wyse, 1994).

One drawback in using cover crops to control weeds is the potential depletion of soil moisture levels during the growth of the cover crop in the spring, leading to reduced crop yields in the following corn crop (Wyse, 1994). In a Nebraska experiment, a winter rye cover crop killed with glyphosate prior to corn planting provided 60% to 95% control of weeds, but reduced corn yields 34% and 37% in two out of four experiments due to competition for soil moisture (Echtenkemp and Moomaw, 1989; Lal *et al.*, 1991). In a 3-year Illinois study, it was found that fall-planted rye killed at or prior to corn planting depleted soil moisture and reduced crop yield in years when precipitation was below normal (Stoller *et al.*, 1989; Lal *et al.*, 1991). Rye was ineffective in controlling common lambsquarters, an early-emerging weed (Stoller *et al.*, 1989; Lal *et al.*, 1991). In Nebraska, chemically suppressed chewings fescue remained green during the growing season and gave good weed control, but it reduced field corn yield when precipitation was below normal (Echtenkemp and Moomaw, 1989). A Missouri experiment showed that a rye cover crop may also decrease corn yields in normal and wet years (Johnson *et al.*, 1993). This yield decrease may be from reduced nitrogen availability, as nitrogen can be immobilized by decomposed rye or other small grains used as cover crops prior to planting corn.

Residues from a killed cover crop can provide good weed control for a short period of time, but research has shown that for season-long weed control additional weed control measures are necessary (Lal *et al.*, 1991). For example, in a Kentucky experiment, killed cover crops were effective in suppressing weed growth for 45 days, but significant weed growth existed in all killed cover crops after 60 days (Weston, 1990). Corn yields were not determined due to substantial weed interference. In North Carolina, redroot pigweed control 4 weeks after planting no-till corn was 81% in rye, 79% in subterranean clover, 72% in crimson clover, and 41% in hairy vetch, but post-emergence herbicides were needed later in the season for complete weed control (Worsham, 1991).

In a study of hairy vetch flail-chopped after flowering in late May, the dead mulch suppressed weeds effectively for 6 weeks after corn was planted, but weed dry weight at the end of the season was equal to that of the weedy check (Janke and Peters, 1989; Lal *et al.*, 1991; Hoffman *et al.*, 1993). These researchers also found that delayed kill of hairy vetch resulted in greater weed suppression.

Residues of hairy vetch following desiccation with a contact herbicide in Maryland reduced weed emergence during the first 4 weeks after desiccation (Teasdale, 1993; Teasdale and Daughtry, 1993). However, as hairy vetch residues decomposed, weed emergence became similar to that without a cover crop and a comparable weed biomass resulted. A living mulch of hairy vetch in the no-treatment control or a dead mulch in the mowed treatment improved weed control during the first 6 weeks of the season, but weed control deteriorated thereafter.

Comparing the effectiveness of cover crops to herbicides for weed control in corn generally shows a corn yield penalty with cover crops. Competition from weeds or uncontrolled vetch in the treatments without herbicides reduced corn yield in 3 of 4 years by an average of 46% as compared with a standard herbicide treatment of paraquat plus atrazine plus metolachlor (Teasdale, 1993). In a Missouri experiment, giant foxtail and common cocklebur were controlled 57–69% and 63–69%, respectively, with rye and hairy vetch as killed cover crops (Johnson *et al.*, 1993). By comparison, alachlor and atrazine applied pre-emergence provided 90–93% control of giant foxtail and 86–99% control of common cocklebur. Associated corn yields were 81% and 93% higher in the herbicide-treated plots than in the rye and hairy vetch cover plots in the 2 years of the experiment. The researchers concluded that weed suppression with cover crops may be at the expense of corn emergence, growth, and grain yield.

There are disadvantages and challenges with the use of cover crops. These include the cost of establishment, difficulty in killing cover crops (especially legumes), leaching of nitrates from legumes, lowering of soil temperatures in spring, depleting soil moisture in the spring, releasing natural phytotoxins into the soil environment, and possibly increasing certain insects and diseases (Worsham, 1991). In addition, use of currently available cover crops and smother plants generally requires increased management as compared to chemical weed control (DeHaan *et al.*, 1994).

Cultivation

Mechanical tillage (cultivation) continues to be one of the principal nonchemical means of weed control (Anderson, 1983). Primary tillage is the initial ground-breaking in preparation for crop production. Secondary tillage is performed

to smooth and level the ground prior to planting (Ashton and Monaco. 1991). Primary and secondary tillage are used to prepare a suitable crop seedbed free of established weeds. Row crop cultivation occurs during the growing season, and its primary objective is to control weeds.

Primary tillage, especially soil inversion with a moldboard plow, buries weed seeds and places many weed seeds in an unfavorable environment for germination. The equipment is used to break and loosen the soil to depths of 6–10 in. (15–25 cm), burying weed species deeply in the soil. This seed burial can reduce the weed populations the year after heavy seed production by uncontrolled weeds (Ashton and Monaco, 1991). Moldboard plowing kills many annual weeds and perennials with a simple taproot system (e.g., dandelions, yellow rocket, alfalfa, white cockle, etc.). But perennials with rhizomes (e.g., quackgrass) or creeping vegetative roots (e.g., Canada thistle, field or hedge bindweed, hemp dogbane, and common milkweed) will survive plowing (Doll, 1988).

Secondary tillage equipment is used to work the soil to depths of less than 6 in. (15 cm). These tools include harrows, field cultivators, and tandem disks. A large portion of the potential weed population can germinate and emerge before corn planting if planting is delayed. Reworking the ground with harrows just before planting will kill most of these weeds. Tillers of the rigid, forked type – such as sweeps, shovels, spikes, knives, and spike- or spring-toothed harrows – can control weeds by disturbing the soil about their roots and dragging them from the soil to its surface (Anderson, 1983).

Cultivation during the growing season can be accomplished with rotary hoes or row cultivators with knives, teeth, shovels, or sweeps. Typical corn acreage is cultivated once after planting (USDA ERS, 1994) because herbicides are used on most corn acreage to control weeds. In Illinois in 1990, 25% of the corn acres received post-plant cultivation with rotary hoes, while 63% were cultivated with row cultivators (Pike, 1991). Surveys in Iowa indicate that 75% and 21% of the corn acres are cultivated and rotary hoed, respectively (Hartzler and Wintersteen, 1991). Cultivation can be an effective means of controlling weeds in row crops, but several passes across the field are generally required (Mt. Pleasant *et al.*, 1994).

Row crop cultivation is used once the corn plants are more than 3-in. (8 cm) tall. Seedling weeds can be killed by cultivating 1- to 2-in. (2.5–5 cm) deep. Larger weeds require deeper cultivation. Care needs to be taken to protect the crop root system, especially when the plants are 10 in. (25 cm) or more in height (Doll, 1988). Row crop cultivation achieves weed control primarily by the burial of small annual weeds with soil thrown over them through the action of tillage tools and the disruption of the intimate relationship between the weed roots and the soil. Loosening the soil about the roots disrupts water absorption and often results in death by desiccation. In other instances the plant is cut off below ground (Anderson, 1983). Best results from cultivation are obtained with small (ffl2.5 in. or 6.4 cm) weeds. Larger weeds are difficult to bury and often have sufficient roots to escape total separation from the soil. Cultivation equipment can also be clogged by the larger weeds (Ashton and Monaco, 1991).

The rotary hoe consists of a series of pronged wheels (about 18–20 in. or 45–50 cm in diameter) mounted about 6 in. (15 cm) apart on an axle. The rotary hoe often can be operated right over the row of corn plants, as well as between the rows. The rapidly moving, shallow operations of the rotary hoe uproot small weed seedlings, while the deeper-rooted and stronger corn plants survive (Ashton and Monaco, 1991). Weed seedlings more than 0.5- to 1-in. (1- to 2.5-cm) tall are not easily controlled by the rotary hoe (Doll, 1988). Large-seeded weeds like velvetleaf, shattercane, and perennial species are not adequately controlled by rotary hoeing. Use of the rotary hoe should be avoided if the corn plants are very turgid, as excessive breakage might occur.

The principal disadvantage of row cultivation is its inability or difficulty in controlling weeds close to or between the crop plants in the seed row. In order to control weeds within the rows, growers in the early 1900s would 'check plant' corn fields. That is, they used a wire with 'knots' evenly spaced to trip the planter so the hills of corn would be lined up in two directions, allowing cultivation in two directions (Knake, 1990). Many farmers cultivated three or four times. Of course, by growing the individual corn plants far enough apart to allow for cultivation on all four sides of the plant, fewer corn plants could be grown per acre. In the 1950s, about 12 000 corn plants were grown per acre (30 000 ha); in the 1980s the number of corn plants per acre had risen to 20 000/A (50 000/ha). Today, corn populations of 25 000–30 000/A (60 000–75 000/ha) are common. Average corn yields doubled from 1950 to 1967 as the number of plants per acre increased and other corn production practices improved, especially weed control.

Spyders are another cultivation tool that may be used to control weeds in the corn row. Spyders use toothed disks to move soil *away from* the crop row on the first two cultivations and to move soil *into* the crop row on the third cultivation to cover up small weed seedlings (Schweizer *et al.*, 1994). Tools that uproot weeds in the row are called torsion weeders, spinners, and spring-hoe weeders. These designs cause the cultivator to create a vibration as the implement passes through the soil near the crop row, effectively dislodging the weed seedlings (Doll and Francis, 1992).

Effective cultivation needs relatively dry soil, both at the surface and below the depth of cultivation. Cultivation should throw dry soil into the crop row to cover small weeds. Dry soil also permits desiccation of the uprooted weeds. Cultivation when the soil is too wet will often transplant weeds, especially the vegetative reproductive organs

of perennial weeds (Ashton and Monaco, 1991). The same problem can occur if rainfall occurs soon after cultivation. Ample moisture in the soil will permit greater weed survival after cultivation.

Untimely rains that delay the use of cultivation can also result in large, uncontrollable weeds (Ashton and Monaco, 1991). In an experiment in New York, nine mechanical cultivation regimes (including Lely weeders, Lilliston rolling cultivators, Bezzeride's spyders, spring hoes, torsion weeders, and spinners) were compared to a broadcast application of pendimethalin and atrazine (Mohler *et al.*, 1994). In 1991, dry weather and late planting led to low weed densities, resulting in no differences in yields due to weed control treatments. In 1992, wet weather caused cultivation to be late and prevented the final cultivation entirely. Consequently, yields were substantially higher with the herbicide treatments than with most of the cultivator treatments. In a Colorado experiment, corn yields were reduced when weeds emerged simultaneously with corn and when rain delayed the first cultivation by 10 days (Schweizer *et al.*, 1994).

Growers who successfully use mechanical weed control strategies often plant corn approximately 2 weeks later than the average planting date for their location (Fernholz, 1990; Hartzler *et al.*, 1993). Delaying planting allows early-germinating weeds to be controlled with pre-plant tillage and favors rapid crop establishment. However, delaying corn planting in Iowa from May 1 to May 20 resulted in an average yield loss of 8%.

Farmers use cultivation and herbicides in their weed control programs because of their complementary nature in controlling weed species that are missed if exclusive reliance were to be placed on either technique alone. One way to decrease herbicide use with additional cultivation while controlling weeds in the corn row is to band the herbicide over the row of corn plants. Essentially, the herbicide controls the weeds within the row, while the weeds between the rows are effectively controlled with mechanical cultivation.

Banding of herbicides is not a new practice. In fact, when herbicides were first introduced for corn production, it was common to band herbicides in order to keep herbicide costs low. However, farmers have largely replaced banding of herbicides in corn with broadcast sprays over the entire field. In 1993 in the US Corn Belt, 16% of the corn acreage was banded (USDA ERS, 1994). The rate of banding was highest in Nebraska (50%) and lowest in Illinois (3%).

Rural sociologists at the University of Missouri studied the reasons why farmers stopped banding herbicides in corn (Rikoon *et al.*, 1993). The primary reason given for not relying on banded herbicide treatments was the amount of time, labor, and equipment needed to cultivate in the untreated areas between the rows. By operating a six-row cultivator on 30-in. rows at 3 mph (75 cm at 4.8 km/h), 5.45 A (2.2 ha) can be covered per hour (Bowman, 1991). Thus, it would take about 18 h to cultivate 100 A at 3 mph (40 ha at 4.8 km/h). Estimates of the amount of diesel fuel required for weed control operations are as follows: cultivator row crop (4.2 L/ha), rotary hoe (2.3 L/ha), and herbicide sprayer (0.9 L/ha) (Alder *et al.*, 1976). Also, when wet spring weather prevented cultivation, farmers relied more on herbicides for weed control. Essentially, these are the reasons that cultivation is no longer relied upon as the primary method of weed control. With the decline in availability of hired labor in most rural communities, it was difficult to find labor to complete cultivation on a timely basis. Eighty-five percent of the growers sampled in the University of Missouri study used little or no hired help (Rikoon *et al.*, 1993).

Mandated and voluntary soil conservation policies have led to reduced tillage in US corn. The Farm Bill of 1985 introduced the Conservation Compliance Program, which required growers with highly erodible land to implement soil conservation plans in order to participate in other federal assistance programs. Nearly a third of total US cropland is designated as highly erodible and subject to Conservation Compliance. Reduced tillage practices are commonly part of the management systems intended to reduce soil erosion (USDA ERS, 1997b).

Conservation tillage systems must leave 30% or more of the soil surface covered with prior crop residue (USDA ERS, 1993a). If less than 30% residue is left, the system is called "conventional tillage." Use of the moldboard plow leaves about 2% of the plant residue remaining on the soil surface after planting. The use of the moldboard plow on corn acres in the United States declined from 20% of the acreage in 1988 to 9% in 1995. The use of a no-tillage system, in which no residue-disrupting tillage operations are performed prior to planting (65% residue remaining), rose from 7% to 18% of US corn acreage in the 1988 to 1995 time period (USDA ERS, 1993a, 1997a). Fifty-one percent of the corn acreage in 1995 was classified as conventionally tilled – without the moldboard plow and with 17% residue at planting. Eighteen percent of the acreage was in a mulch-till system with 38% residue, and 3% of the acres were in a ridge-till system with 45% residue. Ridge tillage use in 1995 was highest in Nebraska, with 16% of the corn acreage in ridge tillage (CTIC, 1999).

Conservation tillage systems tend to rely more on herbicides to control weeds that might otherwise be controlled through cultivation. The amount of cultivation that is possible on reduced tillage acreage is very limited. However, ridge tillage systems are composed of a series of permanent ridges that are re-formed each year at last cultivation. At planting time, 5–10 cm of soil and the residue from the previous crop are scraped from the top of the ridge ahead of the planter to provide a clean and relatively weed-free strip for a seedbed. A rotary hoe is used once before emergence and often a second time shortly after crop emergence to control weeds in the row on top of the ridge. Weeds between the rows are managed by cultivating a few weeks later when the crop is small, using shields on the no-till

cultivator. This is followed by a more aggressive cultivation that rebuilds the ridge for next year's crop (Doll and Francis, 1992).

The ridge-plant system is generally limited to row crops with a row width of 30–40 in. (75–100 cm). Building or maintaining the ridges on hillside slopes of 6–8% is a problem because of the downslope movement of equipment and soil (Randall, 1987). Also, running the row up and down slopes of greater than 6–8% can result in some interrow gullying in a corn–soybean rotation. Continuous corn with its higher amounts of residue will allow erosion control on slopes greater than 8%, depending on slope length, soil permeability, and residue conservation. Timing of the ridging operation is critical. Corn is best ridged when 18- to 36-in. (46–90 cm) tall. Farmers on large acreage may have difficulty getting all their land ridged during this period of rapid corn growth if it rains frequently. An option following soybean crops is to ridge after fall harvest, but this may leave the soil more prone to erosion. Because the larger amount of residue interferes with the ridging, fall ridging is not an option following corn. Ridge-till planting is not a practical alternative on some fine-textured soils. For example, on a Charity clay in Michigan, ridges could not be constructed in one or even two cultivations without damage to the corn crop (Robertson and Erickson, 1983).

Ridge tillage is most widely practiced in Nebraska. This is the result of large areas of continuous, furrow-irrigated corn. Since the irrigation water requires furrows between the rows of corn plants, building ridges between the furrows is practical. Ridge-till systems may incorporate reduced herbicide application rates, banding herbicides on the tops of ridges, eliminating the need for rotary hoeing in most years, and slightly increasing the variable costs (Doll and Francis, 1992). Farmers have made ridge-till more effective by applying herbicides 1–3 weeks before planting. Most ridge-till farmers apply herbicides pre-emergence in a band over the row to improve intrarow weed control and use two cultivations to control interrow weeds. Under heavy weed densities, a broadcast herbicide application is often used (Klein *et al.*, 1996). Most of the ridge-tilled corn in Nebraska is treated with atrazine. Winter annual weeds in Nebraska (such as downy brome, tansy mustard, and field pennycress) increase with ridge planting. A late March or early April application of atrazine at 1.1 kg/ha controls these weeds and early germinating broadleaf weeds – such as kochia and common lambsquarters (Wicks, 1986).

Bioeconomic Models

Several US weed scientists have conducted research to develop weed–corn bioeconomic simulation models to help guide decisions regarding herbicide use (Lybecker *et al.*, 1991). Weed seed numbers in soil are used to make decisions regarding the application of soil-applied herbicides. Weed densities after corn emergence are used to make post-emergence herbicide application decisions. Bioeconomic models are seen as a potential tool for integrated weed management, allowing growers to tailor their weed management programs to suit the specific weed species and densities in their fields.

Two bioeconomic weed control models have been developed through cooperative work between USDA's Agricultural Research Service (ARS) and university experts: WEEDCAM (Colorado) and WEEDSIM (Minnesota). WEEDSIM was field tested for 1991–1994. After 4 years of applying WEEDSIM recommendations to the same plots, there were no increases in annual weed densities or decreases in weed control or crop yields as compared to standard herbicide management systems for the region (Forcella *et al.*, 1996). In most cases, the model-generated treatments controlled weeds as well as the standard herbicide treatment. The quantity of herbicide active ingredient applied decreased 27% with the seedbank model and 68% with the seedling model, relative to the standard herbicide treatment (Buhler *et al.*, 1996).

These initial results show the potential usefulness of these models, however, the major limitation to widespread adoption is the amount of information needed on biology and ecology of common weeds within any particular region. Such information is not readily available for most weed species (Forcella *et al.*, 1996). Furthermore, growers would be required to sample their fields to measure weed seedbanks and to scout for emerged weeds. The increased time and management requirements for growers to perform the detailed weed assessments are substantial (Vangessel *et al.*, 1996).

Researchers have found tremendous variability in weed seedbank numbers. Recent research in Iowa counted from 113 million to 613 million weed seeds per acre (280 million to 1515 million/ha) (Hartzler, 1993), but there was as much variation in size of the weed seedbank within individual fields as there was between different fields. Because of the extreme variability of seedbanks within individual fields, Hartzler concluded that measurements of the weed seedbank do not appear to be an accurate tool for predicting weed populations. He suggested that the precision of such measurements could probably be improved by dividing fields into smaller units for samples and by increasing the number of samples collected. However, he concluded that the cost and time demands of this type of sampling would be prohibitive.

A practical limitation to the weed seed counting method is the need to wash the soil from the seeds and then count and identify them (Doll and Francis, 1992). This is a laborious and time-consuming task. Greenhouse grow-out tests

may hold potential as an alternative to weed extraction and counting. However, in an Iowa experiment, greenhouse grow-outs underestimated the size of the seedbank (Hartzler, 1993). One possible factor accounting for the underestimation may have been the effect of seed dormancy.

Researchers have investigated methods for reducing scouting time requirements. In a 1994 study, visual assessments of weed seedling emergence resulted in the same recommendation from WEEDCAM as a detailed assessment (Vangessel *et al.*, 1996). As farm size continues to increase, weed management methods that conserve grower time requirements tend to be used more extensively.

Future research is needed to determine the factors that govern seedling emergence from seedbanks. Measurements taken in eight Midwest sites showed tremendous variation in the emergence percentages for several weed species. For example, in an Iowa location, 0.6% of the redroot pigweed emerged from the seedbank, while 13% emerged at an Ohio location (Forcella *et al.*, 1992). Also, 8% of the giant foxtail seeds emerged in an Illinois location, 10% in Minnesota, and 35% in Ohio. The currently available models must make many assumptions regarding weed seedling emergence due to the lack of statistically reliable relationships between weed emergence and environmental conditions.

There is a recognized need to develop multiyear data to relate the impact of uncontrolled weeds in a given year on weed densities and potential crop losses in future years (Lybecker *et al.*, 1991). Individual weed seedlings can produce a tremendous number of seeds per plant, which if left uncontrolled in any year, can quickly replenish the seedbank (Chapter 6).

The use of atrazine in corn has helped to reduce significantly the number of weed seeds in the soil. In a Colorado experiment, a field that began with 1.3 billion buried weed seeds per acre (2.7 billion/ha) was treated with atrazine for 6 years. Afterwards, the weed seed population had been reduced to 20 million/A (50 million/ha). In this experiment, atrazine was discontinued on half the plot after the third year, when 407 million buried weed seeds were counted per acre (1006 million/ha). After three additional years of no atrazine use, the weed seed population had increased to 648 million/A (1600 million/ha) (Schweizer and Zimdahl, 1984).

Long-term research in Sweden (1973–1989) demonstrated that with no control, weed populations increased an average of 25% per year (Bellinder *et al.*, 1994). Weed populations decreased an average of 10% per year with a standard herbicide treatment. The major conclusion expressed by Swedish researchers and accepted by Swedish policymakers is that skipping herbicide applications will lead to a gradual weed population increase, and that this will eventually require applications of greater quantities of herbicides to bring the populations back to acceptable levels.

Farmers Currently Practicing Nonchemical Weed Control

USDA-ARS has compiled agronomic, economic, and environmental data from a small Iowa farm that stopped chemical weed control in 1968, as well as from a neighboring conventional farm for comparative study (USDA NSTL, 1993). The information that follows describes the experiences of the small farm using nonchemical weed control methods.

Many of the Iowa farm's weed control experiments and whole farm experiences have confirmed the limitations of several nonchemical weed control practices. About 200 A (80 ha) of the farm followed a 5-year rotation of corn–soybean–corn–oat–hay. Corn following hay consistently produced the lowest returns due to consistently lower yields, which result from limited moisture and reduced potassium. In a 6-year rotation on 80 A (32 ha), with 3 years in a hay crop, Canada thistle became a problem at the end of the hay rotation and prior to corn planting. Herbicides were occasionally applied to the Canada thistles in the hay fields.

In the 1980s, a variety of cover crops were planted in the farm's corn fields. The rye cover crop was difficult to remove. In 1984, a very dry year, the rye depleted soil moisture and corn yields were reduced by 40 bu/A (2700 kg/ha). In 1988, corn yields on the farm using nonchemical methods of weed control were reduced by 16 bu/A (1075 kg/ha) when rye was used as a cover crop prior to corn. This was a severe drought year, and it was concluded that grain rye production for grain ahead of corn is not compatible. In 1989 and 1990, pre-plant tillage was used as a way of removing the cover crop. However, weed populations increased due to additional weed seeds being brought to the surface by the tillage.

Research by an Iowa State University botanist concluded that on the nonchemical farm, weed populations in the plots hoed four times were similar to those in herbicide-treated fields on other farms (Jurik, 1993). He also estimated that seed production from redroot pigweed that emerged on the farm was between 40 million and 400 million total seeds per acre (100 million to 1000 million seeds per ha). In an effort to improve the efficacy of the rotary hoe operations, experiments have been conducted using double rotary hoe passes on fields on the same day (Thompson and Thompson, 1994).

Unlike many farm operations, the Iowa nonchemical farm is small and grows approximately 100 A (40 ha) of field corn, 50 A (20 ha) of soybean, 50 A (20 ha) of oat, 50 A (20 ha) of hay, with 32 A (13 ha) in pasture (NRC, 1989). A 1993 survey of north central Illinois grain farms revealed that the average acreage of tillable land managed by a single operator is 800 (324 ha), with about 400 A (162 ha) in corn and soybean, respectively (Lattz *et al.*, 1994). With

a 30-ft (9 m) rotary hoe, 150 A (61 ha) of corn and soybean can be covered in a single day (NRC, 1989). Operators with larger corn and soybean acreages to manage could not do the same. Thus, weather becomes an important risk management factor in controlling weeds on larger farms.

A publication from the Land Stewardship Project describes four sustainable farms in Minnesota (Chan-Muehlbauer *et al.*, 1994). None of the four farmers use herbicides in their corn fields. For weed control they rely on extended crop rotations with alfalfa, mechanical cultivation with a rotary hoe and cultivator, and late planting of corn to allow mechanical control of the first flush of weeds. One farmer reported that the corn crop is cultivated three to five times. The report details the 1992 corn yields at these four farms in comparison to the average yields in the same regions. In all cases, the yields of the sustainable farms were lower than the average yields in their regions. The reduced yields for the four farms were ↓ 38%, ↓ 11%, ↓ 7%, and ↓ 5%.

A 1991 *New Farm* article profiled two Ohio corn farmers who used a rotary hoe for weed control (Culp, 1991). One farmer intended to continue with the rotary hoe. He estimated that it works ideally 2 years out of 10, gets shut out by weather about as often, and is usable to some degree in the other 6 years. If wet weather prevents timely use of the rotary hoe, this farmer uses a rescue treatment of herbicides. The other farmer was selling his rotary hoe because late-season weeds in the corn row depressed corn yields by 30% in the rotary-hoed fields, compared to fields where herbicides were used. In addition, this farmer estimated that cultivations cost significantly more per acre than herbicides: $25/A ($62/ha) for three rotary hoeings and two cultivations, versus $13 to $16/A ($32 to $40/ha) for herbicides.

Evaluation of Aggregate Studies

Several studies have been conducted regarding the potential of nonchemical weed control techniques to serve as adequate replacements for herbicides in US corn production. The WSSA and Texas A&M University released separate reports that estimate US corn yields would decline approximately 30% without the use of herbicides (Smith *et al.*, 1990; Bridges and Anderson, 1992). These studies were based on a survey of weed researchers and extension weed scientists from individual states who were asked to estimate the percentage yield reductions that would occur if farmers used best management practices without herbicides. Gianessi and Reigner (2007) estimated a 20% reduction in corn yields without herbicides compared to tillage or hand weeding.

A major constraint on conducting a credible aggregate study to estimate corn yields without herbicide use is the lack of a unified database concerning the efficacy of most nonchemical control methods. While it is relatively straightforward to compare the efficacy of individual chemical active ingredients in controlling individual weed species (Table 34.1a covers atrazine efficacies), no such database is available on nonchemical control methods.

Another limitation with the assessment of nonchemical weed control methods concerns reliability under a wide variety of environmental and agronomic conditions. The weed control efficacies of atrazine (Table 34.1a) apply to normal applications with a high degree of reliability – even after accounting for yearly variations in rainfall, soil type, size of farm, etc. Most of the nonchemical weed control methods have an unknown degree of failure, complicating an aggregate assessment. For example, the efficacy of mechanical cultivation varies considerably by rainfall amounts and timeliness of use. It is difficult to estimate and predict how many farmers are unable to perform timely cultivations because of wet field, large size of operations, or other demands on their time.

Conclusions

Triazine herbicides provide cost-effective, broad-spectrum weed control and are key tools in conservation tillage. Although the chemical alternatives to the triazines are more costly and generally less efficacious, they are considerably more reliable as weed control methods and more compatible with current farming operations than available nonchemical weed control methods. Even as more corn acreage shifts to herbicide-tolerant corn, the need remains for residual herbicides such as the triazines to manage resistant weeds and to avoid the need for multiple tillage passes for weed control.

The use of cover crops and bioeconomic models to reduce herbicide use are still in research stages. Crop rotations have many benefits, but provide only partial weed control in subsequent corn plantings. Growers who currently practice nonchemical weed control in corn fields typically cultivate their fields three or more times during the growing season. Growers using herbicides typically cultivate only one time, if at all. The use of cultivation alone for weed control has the following challenges:

- Due to the size of most farms, labor for cultivation is not available or affordable.
- Cultivation is time-consuming.
- Cultivation often costs more than herbicide applications.
- Cultivation is unreliable as a weed control method within the rows and when fields are wet.

- Cultivation increases soil erosion.
- Close and frequent cultivations can cause crop damage by root pruning.
- Corn yield losses can reach 50% in years when cultivation fails to control weeds.

On farms practicing no-till corn production, herbicides are the main tool for weed control. Without herbicides, no-till farmers would have to switch to some other form of tillage operation. They could no longer use no-tillage methods, resulting in much more soil erosion. Triazine and other herbicides in corn will continue to represent the main weed control technology for the vast majority of corn acreage.

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Chapter 35

Environmental Stewardship: The Roots of a Family Farm

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Summary

Corn and atrazine are so intimately and essentially connected that they have become almost synonymous with each other over 50 years. Atrazine has made corn production what it is today, and corn has made atrazine the essential agricultural tool that it has become.

This chapter covers agriculture and farming in general and corn production in particular. It presents the story of a specific farm family, the McCauleys, over a period of more than 120 years. This chapter describes many of the challenges and changes faced by the David McCauley family from the time they purchased land in northeast Kansas in the 1880s to the present. Ken McCauley (David's great-great grandson) is the 2006–2007 president of the National Corn Growers Association. This story is illustrative and reminiscent of the many challenges faced and overcome by generations of farm families, and it is also typical of many homestead farmers in the Great Plains.

Farmers are avid and committed environmentalists, especially as it pertains to care and preservation of their land. They became environmentalists long before it was popular or faddish. To be successful, farmers must be innovators and experimenters – flexible, willing, and ready to change. They must be willing to try new methods and to adopt new and better farming systems once they see them work. Progress and major breakthroughs often must wait until new tools, equipment, or chemicals become available. Atrazine became available at precisely the right time. Without this herbicide, the McCauley family could not have fully utilized the soil conservation programs and farming methods so much needed following the Dust Bowl years.

As farm labor became less available or too costly following World War II and the Korean War, atrazine allowed the McCauleys to manage their weed problems more efficiently and with less labor. Also, atrazine allowed the McCauleys to expand their corn acreage greatly, to begin reduced tillage practices, and to become better overall farm managers.

This common, yet remarkable story of the McCauley family is one that we should understand and appreciate. It directly relates to why scientific progress and new agricultural technologies have enabled us to feed the world's increasing population. The future of farm productivity will likely depend on whether present and future technology (e.g., herbicides, biotechnology, etc.) will be available to our farmers without burdensome or unjustified restrictions.

Early Beginnings of US Agriculture and Conservation

Native Americans began sustainable environmental practices related to gathering and hunting food generations ago. Many of their practices were a mix of stewardship and religious beliefs. In 1681, William Penn established what likely was the first environmental stewardship program for new inhabitants in the 'New World' with his declaration that 'for every 5 A of forest cleared, one acre shall remain.' Interestingly enough, Penn was still in England when he created this policy. Penn arrived in 1682 in what would later become the United States, years after King Charles II bequeathed him the Western Jersey Territory to repay an $800 000 debt owed his father (Beatty, 1939).

In the mid-1800s, Henry David Thoreau lived at and wrote of Walden Pond, and his writings still serve as a mantra for those looking for a simpler form of life (Thoreau, 1854, 1885). During this same period, John Muir emerged as a leader in the conservation movement. In 1867, Muir was blinded while working in his Wisconsin framing shop. He decreed '… that if he gets his vision back he will live for the inventions of God, not for the inventions of man.'

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His vision returned, and in 1868 he walked from Wisconsin to the Gulf of Mexico and continued on to Yosemite Valley by ship and on foot, becoming a strong advocate for the conservation of our natural resources.

With the efforts of conservation-minded citizens like President Theodore Roosevelt and Gifford Pinchot, America's first native-born professional forester, the United States Government soon became involved in preserving land and forming national parks. In fact, Roosevelt was so impressed with Muir and the need to protect our environment that he created five national parks overall. He added 140 million A (57 million ha) to the national forest system and set aside 18 areas of historic or natural interest (Badé, 1924). Yellowstone became the first national park in 1872, and with Muir's help was followed by the establishment of the Yosemite, Grand Canyon, Sequoia, and Mount Rainier national parks. In 1892, Muir established an organization that is now called the Sierra Club.

Early Beginning of the McCauley Family Farm

A prominent pioneer of the Great Plains agricultural movement in the 1880s, David McCauley heeded the advice of Horace Greeley to 'go west young man' and pulled up roots in Ohio to head west. The challenge and opportunity must have seemed as vast as the state now known as Kansas, which became McCauley's new home. Even with the vision that inspired him to journey from his home in Flatrock, Ohio, McCauley could not have known what changes lay in store for his family and descendants while farming in this new environment.

David McCauley began his new life with the purchase of 480 A in the Wolf River Township of Doniphan County, Kansas. The McCauley family still owns and operates a family farm on some of that land, plus several thousand more acres added over the years. The McCauley farm began producing corn as its major commodity in the late 1800s and has continued to pioneer corn production into the 21st century.

Progress in Corn Production

Corn (*Zea mays* L.) is a leading cereal crop in the United States and is also referred to as 'maize.' Corn is classified in the tribe *Maydeae* of the *Gramineae* or grass family. The corn plant may have developed from teosinte, a wild grass found in Mexico and Guatemala. The oldest evidence of corn found in South America dates back to about 1000 BC and in North America to at least 2000 BC. Corn was a major food and daily bread of the Mayans, Aztecs, and Incas of Central and South America. Spaniards who came with Christopher Columbus and were sent to explore the interior of Cuba in 1492 returned with a report of 'a sort of grain they call maize which was well tasted, baked, dried, and made into flour' (Wallace and Brown, 1956).

Although corn has always been a valuable crop, especially in North and South America, the reason for its continually increasing importance throughout the world, and for the McCauley family continuing with corn as their major commodity, has been the many advances in: corn genetics; weed, disease, and insect control; production; harvesting; and other agricultural technologies (Wallace and Bressman, 1949; Sprague, 1955). Among the first who learned how to grow corn in the 'New World' were the early settlers at Jamestown, Virginia, who learned from the Indians in 1609 (Carrier, 1957). The Pilgrims at Plymouth, Massachusetts, were taught to grown corn by the Indian Squanto in 1621 (Bradford, 1856).

During the 1800s and 1900s, much research and progress in agriculture helped the McCauley family succeed with producing corn. A few of these breakthroughs include:

1813: John Lorain began discussing the value of cross-breeding corn to obtain higher yields (Wallace and Brown, 1956).
1839: The double row, horse-drawn corn planter was patented by D.S. Rockwell (Church, 1935).
1846: Robert Reid developed a new variety of corn known as 'Reid's Yellow Dent,' which eventually dominated the Corn Belt (Rasmussen, 1975).
1846, June 26: Great Britain repealed the Corn Laws, greatly increasing the import of agricultural products from the United States (Merk, 1934).
1846: Commercial corn and wheat belts began to develop. Wheat occupied the newer and cheaper areas and was constantly being forced westward by rising land values and the encroachment of corn; however, New York, Pennsylvania, and Ohio were still the chief wheat-producing states (Clark, 1966).
1853, August 2: A patent for a widely used corn planter was granted to G.W. Brown (Schlebecker, 1975).
1869, November: W.O. Atwater published an analysis of corn (Moore, 1947).
1881: W.J. Beal of Michigan Agricultural College crossed two varieties of corn by detasseling one of them, hybridizing the corn for the sole purpose of utilizing the vigor of the first-generation hybrid to increase corn yields (Jenkins, 1936).
1905: George H. Shull and Edward M. East produced the first hybrid corn (Jenkins, 1936).

1916, December 1: Federal grading standards for shelled corn became effective (Jenkins, 1936).
1917: A system for growing modern hybrid seed corn was developed by Donald F. Jones (Allard, 1960).
1926: Henry A. Wallace developed commercial hybrid seed corn (Crabb, 1947), and in 1928 he and Lester Pfister made hybrid pollination completely workable commercially. Eventually, hybrids accounted for nearly all corn production in the United States.

These landmarks in American agriculture, and many others dated from before 1600 to 1990, are documented by Smith and Roth (1990). In 1958 and 1959 another major milestone in corn production was attained when the triazine herbicides (simazine and atrazine) were registered for weed control in corn.

Land Stewardship

By 1880 plow agriculture was beginning to extend into the Great Plains. This movement, encouraged by population pressure and facilitated by the development of barbed wire fencing and the windmill, advanced in spite of the resistance of many cattlemen (Webb, 1931). There were also some innovative, conservation-minded corn farmers who were trying different approaches to long-term or sustainable agriculture. John McCauley, David's son, continued the family's personal style of stewardship and sustainability in the early 1900s. While terms like 'conservation' were not spoken as frequently as today, farming at the beginning of the 20th century required all the skill and talent the McCauleys could muster.

Leon McCauley, John's son, born in 1898, left the farm to serve his country in World War I. He was fortunate to return and continue the legacy created by his grandfather David. While the normal farmer stockmen of the time would feed their own cattle, Leon McCauley was an entrepreneur and wanted to get extra value for the grain he produced in excess. He acquired additional livestock and would feed more than a thousand, a very large number in those days.

One of the most significant events in United States agriculture during the 20th century was the Dust Bowl of the 1930s. In fact, on April 14, 1935, the powder-dry soil of the Great Plains created the awesome 'black blizzard' (Hurt, 1977, 1981). It followed a time when tremendous numbers of grassland acres were plowed under and planted to wheat and other crops. This practice might have worked in a time of adequate rainfall, but not after several years of drought. Instead, plowing contributed significantly to the Dust Bowl by promoting wind erosion (Worster, 1979). Other factors contributed to the problem, such as exposed erodible soils, deforestation, and overgrazing. Dust on the roads in east central Kansas would be several inches deep, almost like a thick layer of talcum powder. On the McCauley farm, the pressures of a national economic depression and years of drought took their toll, to be sure. But a more balanced approach to land usage provided a better safety net, both for conserving the soil and sustaining the farm enterprise.

The serious problem of soil erosion and loss was not totally unexpected. In 1928, a United States Department of Agriculture (USDA) bulletin by Hugh Hammond Bennett and W.R. Chapline warned the nation of the danger of soil erosion (Brink, 1951). Also in 1928, the United States Congress appropriated the first funds for soil erosion research through the Buchanan Amendment to the USDA Appropriation Act (Looper, 1970).

In September 1933, the Soil Erosion Service, which later became the Soil Conservation Service, was created in the United States Department of the Interior (Simms, 1970). The next month on October 10, 1933, the first soil erosion control project of the Soil Erosion Service was established in Coon Valley, Wisconsin (Geiger and Keller, 1970).

The disastrous events of the Dust Bowl led to the Soil Erosion Service Act of 1935. On April 27 of that year, the United States Congress declared soil erosion a national menace in an act directing the USDA to establish a Soil Conservation Service (Wehrwein, 1938). Also in 1935, the Soil Erosion Service was transferred from the United States Department of the Interior to the USDA with Hugh Hammond Bennett as its head (Morgan, 1965). This was soon followed by the Soil Conservation and Domestic Allotment Act of 1936. The State Soil Conservation Districts model law of 1937 was designed to customize soil conservation measures to reflect more local needs, placing more public focus on agricultural production methods.

In 1940, ecologist Aldo Leopold helped create the conservation organization Friends of the Land. Leopold was a great spokesperson for the value of the conservation movement to society (Leopold, 1999). In 1949, he wrote: 'The problem is how to bring about a striving for harmony with the land among a people many of whom have forgotten there is any such thing as land, among whom education and culture have become synonymous with landlessness. This is the problem of conservation education.' (Leopold, 1949).

In 1837 when John Deere began manufacturing plows with steel share and smooth wrought iron moldboards in Illinois, the development was hailed by many as a great step forward in agriculture (Kendall, 1959). Not all observers, though, were enthusiastic. Edward H. Faulkner (1943) in his book *Plowman's Folly* contended that moldboard plowing contributed to soil erosion, the depletion of soil fertility, and other detrimental results. Faulkner felt that plowing evolved as a custom and had no scientific basis. He supported leaving crop stubble in the field and using minimum tillage.

While not generally supported by researchers and producers at the time, attempts to find alternatives to moldboard plowing did produce some new implements, including the one-way disk plow. However, the one-way disk plow along with high temperatures and low rainfall were the primary causes of the Dust Bowl in the Great Plains.

Researchers working for the Nebraska Agricultural Experiment Station developed the stubble-mulch or crop residue mulch practice, a highly effective method of soil erosion control (Douley and Russel, 1939). Stubble mulching used subsurface tillage implements that left crop residues on the soil surface and provided some protection against wind and water erosion (Zingg and Whitfield, 1957).

Other methods of erosion control became more popular as farmers began recovering from the Dust Bowl and the depression that had ravaged the Great Plains. Ridge planting or tilling, an old practice, was adapted for use in fields in which row crops were planted. Although ridge planting eliminated the level field created by conventional plowing, the soil was still bare. A variety of conservation and limited tillage systems were researched, developed, and found successful by farmers in various areas of the United States and elsewhere around the world (Phillips, 1964; Hoefer *et al.*, 1981; Shear, 1985).

Effects of World War II

World War II was in full force when Leon McCauley's son Stanley graduated from high school and joined the military service. Agricultural deferments were available because of the critical role of food production to the war effort. But Stan had friends, relatives, and many acquaintances willing to serve, so for Stan the decision to enlist was simple.

World War II brought many changes and problems to much of the world, including a severe shortage of food. The United States did not get fully involved in the war until Japan attacked Pearl Harbor on December 7, 1941 (Sanders, 1967). On March 1, 1943, fruits and vegetables began to be rationed under a point system – the first major food rationing program in the history of the United States (Russell and Fantin, 1947). On March 26, 1943, the Food Production and Distribution Administration, later known as the War Food Administration, was established within the USDA by Executive Order (Baker, 1951).

In connection with the war effort, some useful discoveries were made that were later important for agriculture. In 1942, P.W. Zimmerman and A.E. Hitchcock discovered the growth regulation property of 2,4-D, later widely used as a weed killer (Peterson, 1967). With the termination of the war in 1945, food rationing ended on all products except sugar (Redford, 1947). Sugar rationing was discontinued in 1947 (Rasmussen and Baker, 1952). Stanley McCauley returned to Kansas and the family farm.

Northern Corn Belt states and New England states were slower to adopt conservation tillage practices that left crop residues on the soil. The soil in those Northern states warmed more slowly, and these areas received much more rainfall. The addition of crop residues slowed the warming of soil even more, delaying seed germination. Researchers and farmers in Northern states found that ridge tilling raised seedbeds and enabled soil to warm more quickly.

Progress in University, Government, and Industry Research

Many advances in soil conservation and agricultural practices were made, or at least started, then demonstrated and taught to farmers by university and USDA scientists and extension personnel. The communication and close on-farm relationship between the research, extension, and farm personnel – especially over the past 60 years – have been models in the advancement and application of practical farming technology within North America. In the United States in 1953, the Agricultural Research Service (ARS) was established in the USDA, replacing the Agricultural Research Administration (ARA) (Moore, 1967). However, this change from ARA to ARS had little significance to or impact on those employed by USDA at that time. Also in 1953, the Cooperative Research and Service Division, formerly a part of Farm Credit Administration, became the Farmer Cooperative Service. Under provisions of the Farm Credit Act of 1953, the Farm Cooperative Service remained in the USDA, while the remainder of the Administration became an independent agency (McKay and Abrahamsen, 1962; Stucker and Collins, 1986).

In the Great Plains, researchers experimented with various implements that performed types of minimum tillage. By the end of the 1960s, many soil preparation methods in corn were available to help reduce soil erosion, such as mulch tillage, double-cut plowing, manure mulching, ridge-row tillage, listing, and corn–sod intercropping. With the advantages of chemical fertilizers and herbicides (including atrazine), hybrid seeds, and improved implements, corn growers could use any of a number of conservation tillage techniques to help prevent soil erosion (Anonymous, 1971).

Over the years, much progress has been made on the quality, utilization, and distribution of corn. In 1966, the development of high lysine corn was announced, bred to enhance protein values of the grain. Globally, corn is ranked next to wheat and rice in total area planted. About half of the world corn production is in the United States. While some corn is grown in all states of the United States, the Corn Belt states represent more than half of this production. Corn has become a

very important crop in other countries, including China, Brazil, Mexico, Argentina, India, Indonesia, South Africa, and the Philippines. It is grown on more than 320 million A (130 million ha) each year. Before hybrid corn and double-cross corn seed became available, yields were about 30 bu/A(2020 kg/ha).In many states, yields of more than 130 bu/A(8750 kg/ha) are now routine. These remarkable corn yield increases over the years were due to a number of genetic and machinery changes and to improved production practices, with superior weed control being an important component. Corn is utilized mainly for livestock feed, human food, and as a raw material for many industrial purposes (Sprague, 1955). Mechanical harvesting of corn also has contributed much to improving production efficiency (Anonymous, 1971). In 1970, approximately 70% of corn produced in the five principal Corn Belt states was harvested by combines equipped with corn heads.

During the early 1960s, Stanley McCauley was putting new conservation practices in place on the family farm in Kansas. The McCauleys were the first farmers in the region to incorporate a system of parallel terraces, designed to provide the benefits of traditional land terraces while providing easier use to the farmer with modern farm equipment. The McCauley farm's adoption of minimum tillage techniques coincided with the introduction of new tools for agricultural production; among the most significant was the new herbicide atrazine. According to Stanley, it was only with the use of atrazine that tillage could be reduced at all. Not controlling weeds was simply not an economical option, and over the next 40 years it would be atrazine that provided the basis for much of the McCauley farm's improvements in corn production.

In 1969, man first walked on the moon, giving us a new view of our world. The following year the first Earth Day was celebrated. During this time, a new breed of McCauley children began to finish their education. Ken McCauley, Stan's son, had finished school and married his high school classmate, Mary Pauly, while the whole country was in turmoil. It was at the height of the Vietnam conflict, a time when many young American men and women were confused about the future. Ken and Mary did not lack a sense of direction. They knew that their future was back in northeast Kansas. Their road trip from Kansas State University to Doniphan County very well might have been filled with a different kind of wonder and apprehension than that felt by David McCauley (Ken's great-great grandfather) so many years before, but the commitment was much the same.

In the 1970s and 1980s, changes in agricultural technology took place at the pace of a Kansas tornado. In the mid-1970s, cropland in the United States totaled approximately 450 million A (182 million ha) (Mayes, 1978; Walsh and Johnson, 1980). Some form of conservation tillage was being used on about 40 million of those A (16 million ha). In the 1980s 'no-till' or 'low-till' methods of preparing land for planting were used by more farmers on a variety of crops. The objective was to enhance crop yields while lessening erosion. More herbicides were used than under high-till conditions, and a greater degree of management control was required (Anonymous, 1985). By 1987, almost 100 million A (40 million ha) were under some form of conservation tillage. In addition to the incentives to conserve the productivity of their land, many growers embraced reduced tillage practices to achieve soil loss standards required by the Food Security Act of 1985. For Ken and Mary McCauley, reduced tillage and then the complete elimination of subsurface tillage went from an experiment on a few of their farming acres to the main tillage system on their entire farm.

During the 1990s, the McCauley farm embraced even newer methods of farming, and the operation thrived. The Ken McCauley farm was now without livestock for the first time. But cow chips were soon replaced with microchips. New global positioning systems were teamed with grid sampling for nutrient needs. The McCauleys provided their fields for research on variable-rate planter technology. This system allowed the optimum number of corn seed kernels to be planted for the yield potential of that particular area in the field, which of course was determined by the yield data collected the previous year and linked via satellite. This modern and sophisticated technology is relatively new to farming, but is advancing rapidly (Steven, 1997).

Another modern technology that is rapidly changing things on the farm is biotechnology, including genetic engineering of crops. While many farmers and some members of the general public, especially in Europe, are cautious about using such powerful and revolutionary tools, properly tested and applied they have great potential to improve crop production and quality. They also can be used to improve our environment, while providing a better diet and life to hungry people in developing countries of the world (Avery, 2002). In 1985, 20 million dollars in competitive grants were authorized by USDA for research in biotechnology (Kerr, 1987). Change is the one constant on a modern farm.

New Millennium and Beyond

There is a new McCauley on the scene in this new millennium. Brad McCauley finished his education at Kansas State University in 2001. He has acquired land and equipment in Doniphan County. While the previous generations of his family must be part of his desire to return to his roots, Brad knows that the wonder of agricultural technology for him is only beginning. A new twist for the McCauley's is their investment in value-added agriculture, including both biofuels and food.

Biotechnology, precision farming, ultra high technology equipment, and the challenge of competing in a true global economy are in the future for Brad McCauley. Yet he also knows the value of keeping things that produce positive results working for the McCauley farm. And so it is with techniques like terraces and conservation tillage. They are long-term fixtures on the farm. Crop protection tools are evaluated the same way. A change must pay its way at the farm gate. In spite of all the new products that are out in the marketplace today, in spite of all the new technologies that have been tried and implemented over the past several decades on the McCauley farm, atrazine is still the foundation of the McCauley farm herbicide program. Atrazine helps conservation tillage work for the McCauleys. And without the availability of atrazine and other crop protection tools, conservation tillage could not be used on their farm. It is a partnership – the land, the tools, the farmer, all working in harmony. The McCauleys would not have it any other way.

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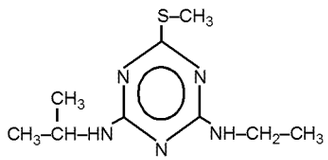
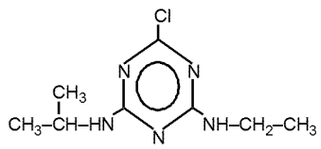
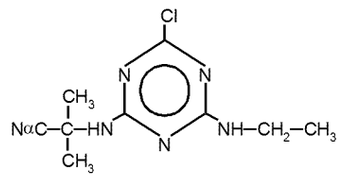
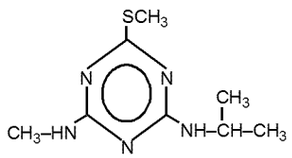
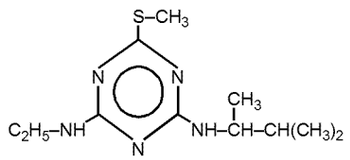
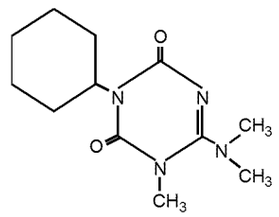
Appendix

Table A1 Chemical structures, names, and molecular weights of triazine herbicides[a,b]

Common name	Ametryn	Atrazine	Cyanazine
Tradename(s)	Evik, Gesapax	AAtrex, Gesaprim	Bladex, Envoy
Code	G-34162	G-30027	SD-15418, WL-19805, DW-3418
Chemical structure			
Formula	$C_9H_{17}N_5S$	$C_8H_{14}ClN_5$	$C_9H_{13}ClN_6$
Molecular weight	227.3	215.7	240.7
Chemical names original	2-methylthio-4-ethylamino-6-isopropylamino-*s*-triazine	2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine	2-chloro-4-(1-cyano-1-methylethylamino)-6-ethylamino-1,3,5-triazine
IUPAC	N^2-ethyl-N^4-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine	6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine	2-(4-chloro-6-ethylamino-[1,3,5]triazine-2-ylamino)-2-methylpropionitrile
CAS	*N*-ethyl-*N*ffi-(1-methylethyl)-6-methylthio-1,3,5-triazine-2,4-diamine	6-chloro-*N*-ethyl-*N*ffi-(1-methylethyl)-1,3,5-triazine-2,4-diamine	2-[[4-chloro-6-(ethylamino)-1,3,5-triazine-2-yl]amino]-2-methylpropanenitrile
CAS number	834-12-8	1912-24-9	21725-46-2
Abbreviation	SEIT	CEIT	CCyET
Common name	Desmetryn	Dimethametryn	Hexazinone
Tradename(s)	Semeron	Dimepax	Velpar, Pronone
Code	G-34360	C-18898	DPX-3674
Chemical structure			
Formula	$C_8H_{15}N_5S$	$C_{11}H_{21}N_5S$	$C_{12}H_{20}N_4O_2$
Molecular weight	213.3	255.4	252.3
Chemical names original	2-methylthio-4-methylamino-6-isopropylamino-*s*-triazine	2-methylthio-4-ethylamino-6-(1,2-dimethylpropyl)-amino-*s*-triazine	NA
IUPAC	N^2-isopropyl-N^4-methyl-6-methylthio-1,3,5-triazine-2,4-diamine	N^2-(1,2-dimethylpropyl)-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine	3-cyclohexyl-6-diethylamino-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione
CAS	*N*-methyl-*N*ffi-(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine	*N*-(1,2-dimethylpropyl)-*N*ffi-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine	3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione
CAS number	1014-69-3	22936-75-0	51235-04-2
Abbreviation	SMIT	SEDT	NA

(*Continued*)

Table A1 (Continued)

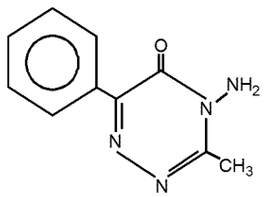
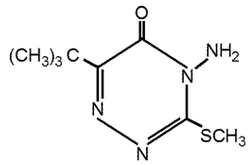
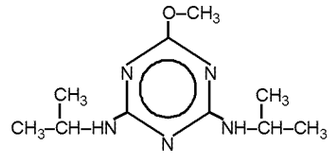
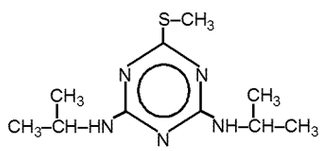
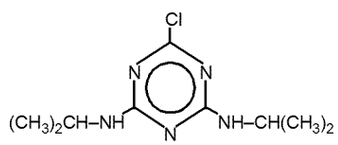
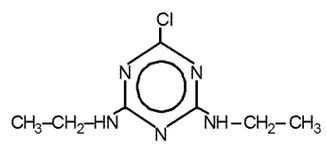
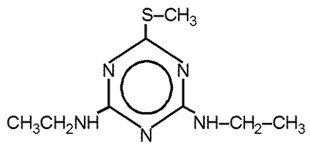
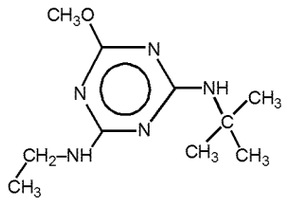
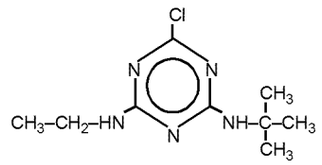
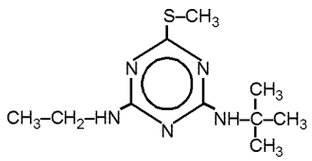
Common name	Metamitron	Metribuzin	Prometon
Tradename(s)	Goltix	Sencor, Lexone	Pramitol, Gesafram
Code	BAY DRW1139, BAY-134028	BAY-94337, BAY DIC 1468, DPX 2504	G-31435
Chemical structure			
Formula	$C_{10}H_{10}N_4O$	$C_8H_{14}N_4OS$	$C_{10}H_{19}N_5O$
Molecular weight	202.2	214.3	225.3
Chemical names original	NA	4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazine-5(4*H*)-one	2-methoxy-4,6-bis(isopropyl amino)-*s*-triazine
IUPAC	4-amino-4,5-dihydro-3-methyl-6-phenyl-1,2,4-triazin-5-one	4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazine-5-one	*N*,*N*ffi-diisopropyl-6-methoxy-1,3,5-triazine-2,4-diamine
CAS	4-amino-3-methyl-6-phenyl-1,2,4-triazine-5(4*H*)-one	4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazine-5(4*H*)-one	6-methoxy-*N*,*N*ffi-bis (1-methylethyl)-1,3,5-triazine-2,4-diamine
CAS number	41394-05-2	21087-64-9	1610-18-0
Abbreviation	NA	NA	MIIT
Common name	Prometryn	Propazine	Simazine
Tradename(s)	Caparol, Gesagard	Milogard, Gesamil	Princep, Gesatop
Code	G-34161	G-30028	G-27692
Chemical structure			
Formula	$C_{10}H_{19}N_5S$	$C_9H_{16}ClN_5$	$C_7H_{12}ClN_5$
Molecular weight	241.4	229.7	201.7
Chemical names original	2-methylthio-4,6-bis(isopropyl amino)-*s*-triazine	2-chloro-4,6-bis-isopropylamino-*s*-triazine	2-chloro-4,6-bis(ethylamino)-*s*-triazine
IUPAC	*N*,*N*ffi-diisopropyl-6-methylthio-1,3,5-triazine-2,4-diamine	6-chloro-N^2,N^4-diisopropyl-1,3,5-triazine-2,4-diamine	6-chloro- N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine
CAS	*N*,*N*ffi-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine	6-chloro-*N*,*N*ffi-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine	6-chloro-*N*,*N*ffi-diethyl-1,3,5-triazine-2,4-diamine
CAS number	7287-19-6	139-40-2	122-34-9
Abbreviation	SIIT	CIIT	CEET

Table A1 (Continued)

Common name	Simetryn	Terbumeton	Terbuthylazine
Tradename(s)	Gy-bon	Caragard	Gardoprim
Code	G-32911	GS-14259	GS-13529
Chemical structure			
Formula	$C_8H_{15}N_5S$	$C_{10}H_{19}N_5O$	$C_9H_{16}ClN_5$
Molecular weight	213.3	225.3	229.7
Chemical names original	2-methylthio-4,6-bis-ethyl amino-*s*-triazine	2-*tert*-butylamino-4-ethylamino-6-methoxy-*s*-triazine	2-chloro-4-ethylamino-6-*tert*-butylamino-*s*-triazine
IUPAC	N^2,N^4-diethyl-6-methylthio-1,3,5-triazine-2,4-diamine	N^2-*tert*-butyl- N^4-ethyl-6-methoxy-1,3,5-triazine-2,4-diamine	N^2-*tert*-butyl-6-chloro-N^4-ethyl-1,3,5-triazine-2,4-diamine
CAS	*N*,*N*ffi-diethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine	*N*-(1,1-dimethylethyl)-*N*ffi-ethyl-6-methoxy-1,3,5-triazine-2,4-diamine	6-chloro-*N*-(1,1-dimethylethyl)-*N*ffi-ethyl-1,3,5-triazine-2,4-diamine
CAS number	1014-70-6	33693-04-8	5915-41-3
Abbreviation	SEET	NA	CBET

Common name	Terbutryn
Tradename(s)	Igran
Code	GS-14260
Chemical structure	
Formula	$C_{10}H_{19}N_5S$
Molecular weight	241.4
Chemical names original	2-methylthio-4-ethylamino-6-*tert*-butylamino-*s*-triazine
IUPAC	N^2-*tert*-butyl-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine
CAS	*N*-(1,1-dimethylethyl)-*N*ffi-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine
CAS number	886-50-0
Abbreviation	SETT

[a]Includes triazine herbicides in commercial use in 2000.
[b]For additional details, see *Herbicide Handbook 2002*, 8th edn. W.K. Vencill, ed., WSSA, Lawrence, Kansas, or *The Pesticide Manual: A World Compendium*. 1994. 10th edn. Clive D.S. Tomlin, ed., British Crop Protection Council and The Royal Society of Chemistry, The Bath Press, Bath, United Kingdom.
[c]NA: Not available.

Table A2 Physical/chemical properties of triazine herbicides[a,b]

Name	Description of pure compound	Density (at 20°C) g/mL	Melting point (°C)	Boiling point	Vapor pressure (mm Hg 293°K)	pKa (at 20°C)	K_{ow}[c] at ┐ 20–25°C and pH ┐ 7	Dipole moment[d]	Water solublity (ppm at ┐ 20°C and pH ┐ 7	Molar water solubility (ffi10^{ffl4})	
										pH 3	pH 7
Ametryn	White crystalline solid	1.19	84–85	Unknown	8.4 ┘ 10^{ffl7}	4.1	427	2.10	200	17.8	8.57
Atrazine	White crystalline solid	0.19	175–177	Unknown	2.9 ┘ 10^{ffl7} at 298°K	1.7	316	2.46	33	1.44	1.61
Cyanazine	Colorless crystals	1.29	167.5–169	Unknown	1.6 ┘ 10^{ffl9}	5.1	127	4.91	171	NA[e]	3.65
Desmetryn	White crystalline solid	1.172	84–86	Unknown	1.6 ┘ 10^{ffl6}	4.0	2.40	2.34	580	NA	24
Dimethametryn	Colorless crystals	1.098	65	151–153°C/ 0.05 mm Hg	0.186 mPa	4.1	7000	2.26	50	NA	NA
Hexazinone	Colorless crystals, negligible odor	1.25	115–117	Unknown	2 ┘ 10^{ffl7} at 298°K (extrapolated)	NA	11.3	NA	33 000	NA	NA
Metamitron	Colorless crystal	1.35	166.6	Unknown	0.86 µPa	Non-dissociated	6.8	2.46	1700	NA	NA
Metribuzin	White crystalline solid, technical has a slight sulfurous odor	1.31	125.5–126.5	Unknown	1.2 ┘ 10^{ffl7}	1.1	44.7	2.76	1050	NA	NA
Prometon	Colorless powder	1.088	91–92	Unknown	2.3 ┘ 10^{ffl6}	4.28	492	2.94	750	44.4	30.1
Prometryn	White crystalline	1.157	118–120	Unknown	1.0 ┘ 10^{ffl6}	4.09	1212	3.54	33	8.53	1.67
Propazine	Colorless crystalline solid	1.162	212–214	Unknown	2.9 ┘ 10^{ffl8}	1.70	NA	4.52	5.0	0.21	0.20
Simazine	White crystalline solid	1.30	225–227	Unknown	6.1 ┘ 10^{ffl9}	1.7	122	2.99	6.2	0.29	0.25
Simetryn	Whitish crystals	1.02	81–82.5	Unknown	71 ┘ 10^{ffl7}	4.0	NA	NA	400	31.7	20.8
Terbumeton	Colorless crystals	1.08	123–124	Unknown	0.27 ┘ 10^{ffl6}	4.6	1097	3.19	130	NA	NA
Terbuthylazine	Colorless powder	1.188	177–179	Unknown	1.12 ┘ 10^{ffl6}	2.0	1096	NA	8.5	NA	NA
Terbutryn	White crystalline	1.12	104–105	154–160°C	9.6 ┘ 10^{ffl6}	4.3	4470	NA	22	NA	2.41

[a]This table covers herbicides still in use in 2000.
[b]For further information, see *Herbicide Handbook 2002*, 8th edn. W.K. Vencill, ed., WSSA, Lawrence, Kansas.
[c]Much of these data are taken from: Reddy, K.N. and M.A. Locke (1996). Molecular properties as descriptors of octanol-water partition coefficients of herbicides.
The Herbicide Handbook, 2002 or the Pesticide Manual, 1994. Water Air Soil Pollut., 86: 389–405.
[d]In Debye units, as measured in dioxane at 20°C.
[e]NA: Not available.

Table A3 Selected metabolites of various triazine herbicides listed by metabolic processes or by individual compound

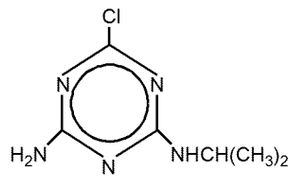
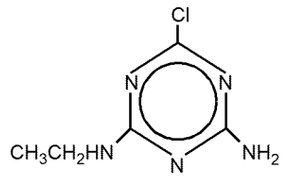
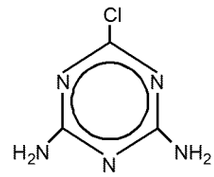
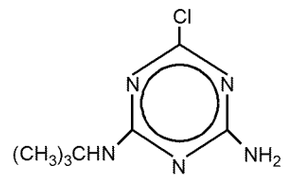
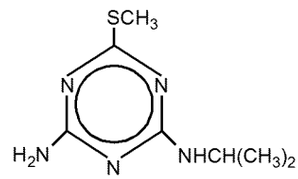
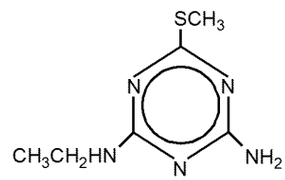
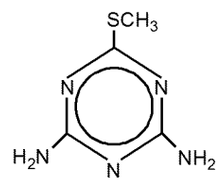
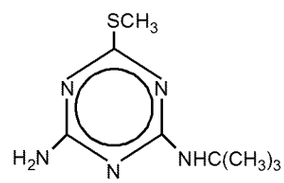
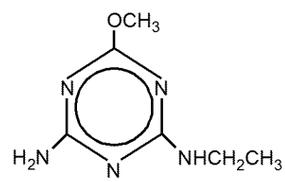
Common name(s)	Original, CAS or IUPAC names	Structure	Abbreviations or code
Metabolic processes			
N-Dealkylation			
Deethylatrazine	6-chloro-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine or 2-amino-4-chloro-6-isopropylamino-*s*-triazine		DEA or CAIT G-30033
Deisopropylatrazine *De-2-methyl propionitrile cyanazine *Deethylsimazine	6-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine or 2-amino-4-chloro-6-isopropylamino-*s*-triazine		DIA or CAET or CEAT G-28279

Table A3 (Continued)

Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
*Diaminochloro-*s*-triazine	6-chloro-1,3,5-triazine-2,4-diamine or 2-chloro-4,6-diamino-*s*-triazine		DDA or CAAT G-28273
Deethyl-terbuthylazine	2-amino-4-*tert*-butylamino-6-chloro-*s*-triazine		CBAT or CABT G-26379
Deethylametryn	6-(methylthio)-*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine		SAIT GS-11354
Deisopropylametryn	6-(methylthio)-*N*-ethyl-1,3,5-triazine-2,4-diamine		SAET GS-11355
Diaminoametryn	6-(methylthio)-1,3,5-triazine-2,4-diamine		SAAT GS-26831
Deethylterbutryn	2-amino-4-*tert*-butylamino-6-methylthio-*s*triazine		GS-26575
Debutylsecbumeton	2-amino-4-ethylamino-6-methoxy-*s*-triazine		MAET GS-31709

(*Continued*)

Table A3 (Continued)

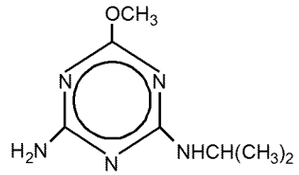
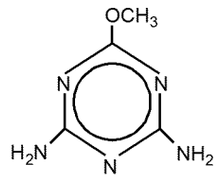
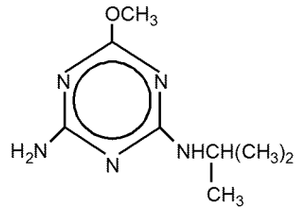
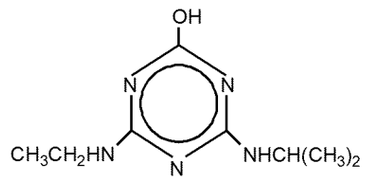
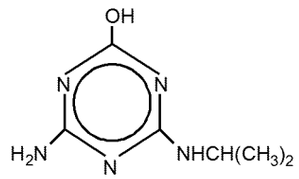
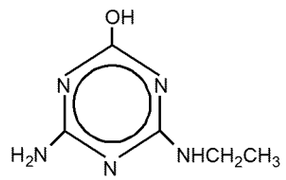
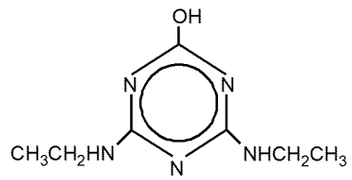
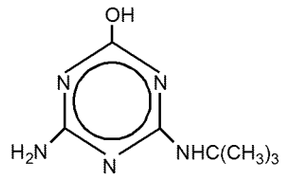
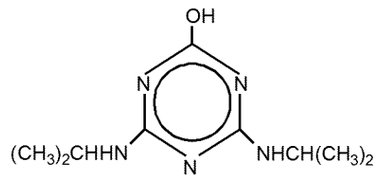
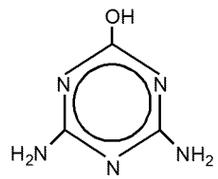
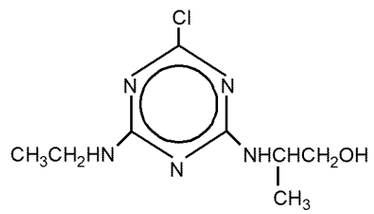
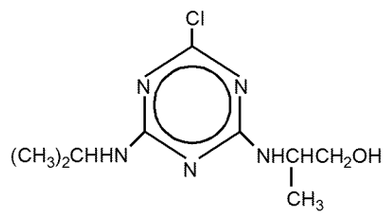
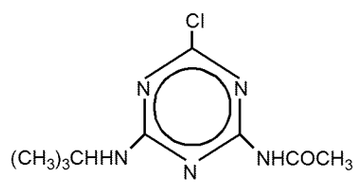
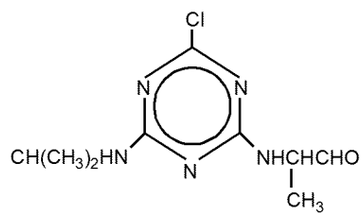
Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
Deisopropylprometon	2-amino-4-isopropylamino-6-methoxy-*s*-triazine		MAIT
Diaminoprometon	2,4-diamino-6-methoxy-*s*-triazine		MAAT GS-12853
Deethylsecbumeton	2-amino-4-*sec*-butylamine-6-methoxy-*s*-triazine		GS-25433
Hydroxylation			
Hydroxyatrazine *Hydroxyametryn	4-(ethylamino)-6-[(1-methylethyl)amino]-1,3,5-triazine-2(1*H*)-one		ATOH or OEIT or HA G-34048
Desethylhydroxy-atrazine *Desethylhydroxy ametryn	4-amino-6-[(1-methylethyl)amino]-1,3,5-triazine-2(1*H*)-one		OAIT or DEHA GS-17794
Desisopropylhydroxy atrazine *Desethylhydroxy simazine *Des-2-methylpropionitrile hydroxy cyanazine	4-amino-6-(ethylamino)-1,3,5-triazine-2(1*H*)-one		OAET or OEAT or DIHA GS-17792
Hydroxysimazine	4,6-bis(ethylamino)-1,3,5-triazine-2(1*H*)-one		OEET G-30414

Table A3 (Continued)

Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
Desethyl hydroxyterbutryn	2-amino-4-*tert*-butylamino-6-hydroxy-*s*-triazine		OIAT or AOIT GS-28620
Hydroxypropazine	2-hydroxy-4,6-bis(isopropylamino)-*s*-triazine		OIIT GS-11526
Ammeline	4,6-diamino-1,3,5-triazine-2(1*H*)-one		OAAT or AAOT GS-17791
Oxidation			
NA[a]	2-[[4-chloro-6-(ethylamino)-1,3,5-triazine-2*yl*]amino]-1-propanol		CEPT
NA	2-[[4-chloro-6-(1-methylethylamino)-1,3,5-triazine-2*yl*]amino]-1-propanol		CPIT
NA	Acetamide, *N*-[6-chloro-4-(1-methylethyl)]-1,3,5-triazine-2-*yl*-(9Cl)		CDIT
NA	2-(4-chloro-6-ethylamino-[1,3,5-triazine-2*yl*]amino)-propionaldehyde		CYIP

(*Continued*)

Table A3 (Continued)

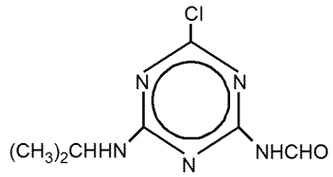
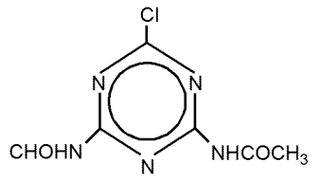
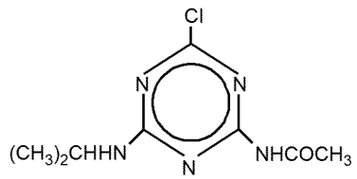
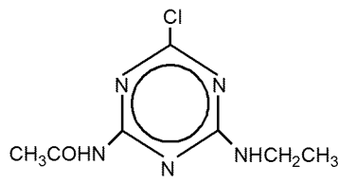
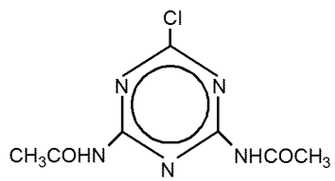
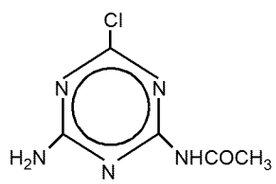
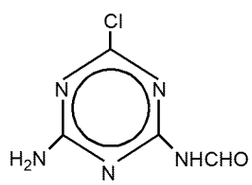
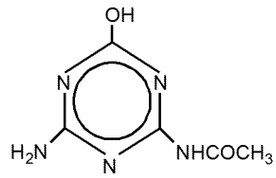
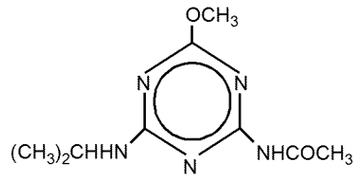
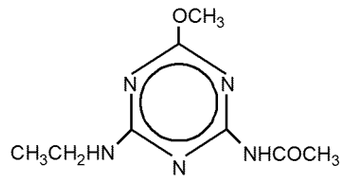
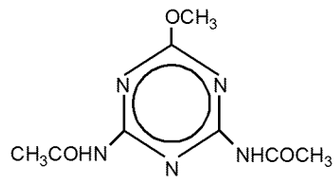
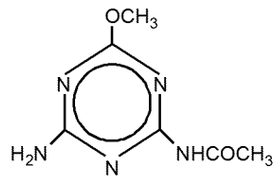
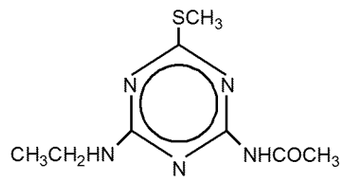
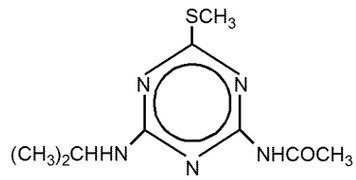
Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
NA	Formamide, *N*-[4-(1-methylethyl)-6-chloro]-1,3,5-triazine-2,4-diamine		CFIT
NA	Acetamide, *N*-[4-(*N*-formyl)-6-chloro]-1,3,5-triazine-2,4-diamine		CDFT
NA	Acetamide, *N*-[4-(1-methylethyl)-6-chloro]-1,3,5-triazine-2,4-diamine		CDIT
NA	Acetamide, *N*-(4-ethyl-6-chloro)-1,3,5-triazine-2,4-diamine		CDET or CEDT
NA	Acetamide, *N,N*ffi-(6-chloro)-1,3,5-triazine-2,4-diamine		CDDT
NA	Acetamide, *N*-(6-chloro)-1,3,5-triazine-2,4-diamine		CDAT or CADT
NA	Formamide, *N*-(6-chloro)-1,3,5-triazine-2,4-diamine		CAFT

Table A3 (Continued)

Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
NA	Acetamide, *N*-(6-hydroxy)-1,3,5-triazine-2,4-diamine		ODAT or OADT
NA	Acetamide, *N*-[4-(1-methylethyl)-6-methoxy]-1,3,5-triazine-2,4-diamine		MDIT
NA	Acetamide, *N*-[4-(ethyl)-6-methoxy]-1,3,5-triazine-2,4-diamine		MDET
NA	Acetamide, *N*-*N*ffi-(6-methoxy)-1,3,5-triazine-2,4-diamine		MDDT
NA	Acetamide, *N*-(6-methoxy)-1,3,5-triazine-2,4-diamine		MADT
NA	Acetamide, *N*-(4-ethyl-6-thiomethyl)-1,3,5-triazine-2,4-diamine		SDET
NA	Acetamide, *N*-[4-(1-methylethyl)-6-thiomethyl]-1,3,5-triazine-2,4-diamine		SDIT

(*Continued*)

Table A3 (Continued)

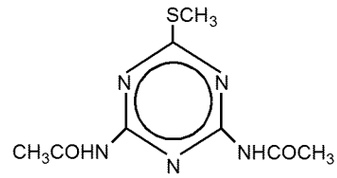
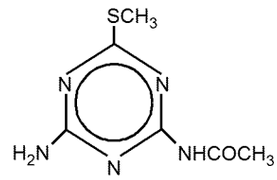
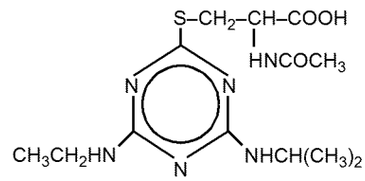
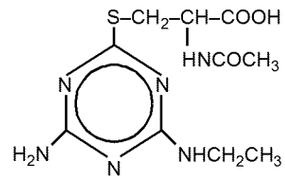
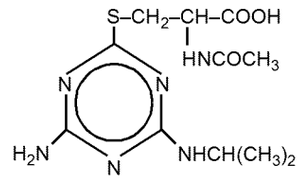
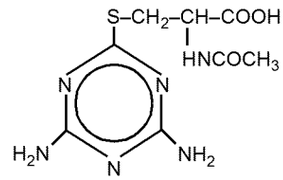
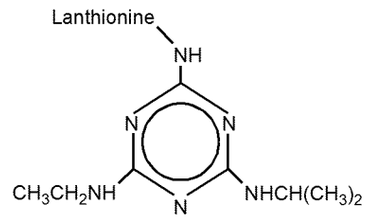
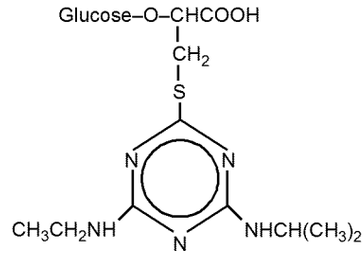
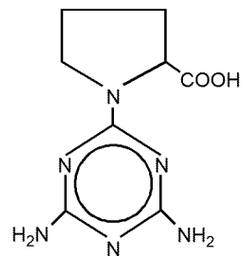
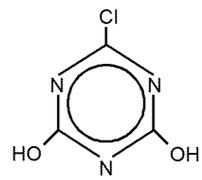
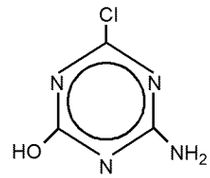
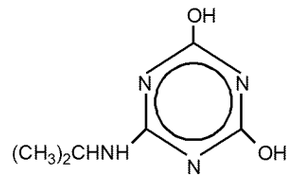
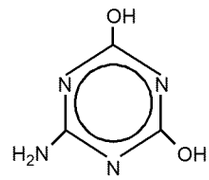
Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
NA	Acetamide, *N*-*N*ffi-(6-methylthio)-1,3,5-triazine-2,4-diamine		SDDT
NA	Acetamide, *N*-(6-methylthio)-1,3,5-triazine-2,4-diamine		SADT
Conjugation			
Atrazine mercapturate	*N*-acetyl-*S*-[4-(ethylamino)-6-[(1-methylethyl)amino]-1,3,5-triazine-2-*yl*]-L-cysteine		
Desisopropylatrazine mercapturate *Desethylsimazine mercapturate *Des-2-methylpropionitrile cyanazine mercapturate	*N*-acetyl-*S*-[4-amino-6-ethylamino-1,3,5-triazine-2-*yl*]-L-cysteine		
Desethylatrazine mercapturate	*N*-acetyl-*S*-[4-amino-6-[(1-methylethyl) amino]-1,3,5-triazine-2-*yl*]-L-cysteine		
Diaminochloro triazine mercapturate	*N*-acetyl-*S*-(4,6-diamino-1,3,5-triazine-2-*yl*)-L-cysteine		
Lanthionine-conjugate of atrazine	*N*-(4-ethylamino-6-isopropylamino-*s*-triazinyl-2)lanthionine		

Table A3 (Continued)

Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
Glucose-Thiolactic acid conjugate of atrazine			
Proline conjugate of diamino chloro-*s*-triazine	L-proline 1-(4,6-diamino-1,3,5-triazine-2-*yl*)		(PC-DAC)
Deamination			
NA	6-chloro-1,3,5-triazine-2,4(1*H*,3*H*)-dione		COOT
NA	2-amino-4-chloro-6-hydroxy-*s*-triazine		COAT or CAOT
	2,4-dihydroxy-6-isopropylamino-*s*-triazine		OOIT GS-11957
Ammelide	6-amino-1,3,5-triazine-2,4(1*H*,3*H*)-dione		OOAT G-35713

(*Continued*)

Table A3 (Continued)

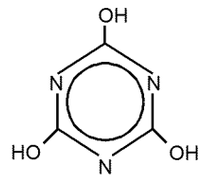
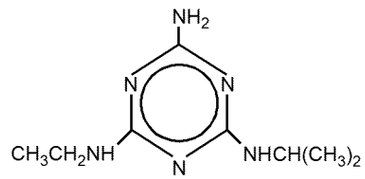
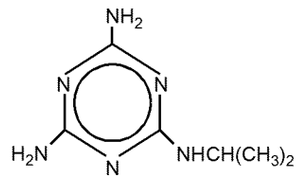
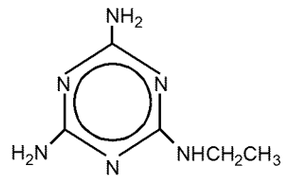
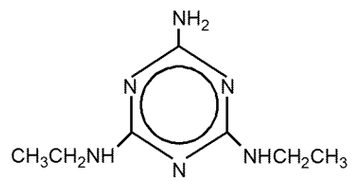
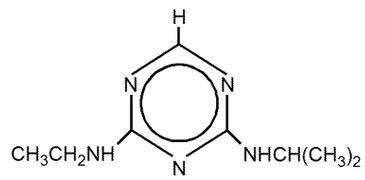
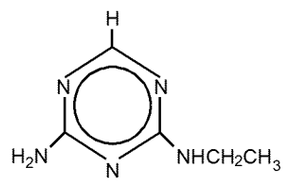
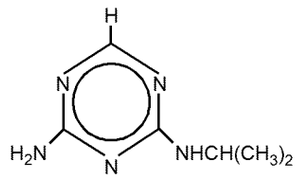
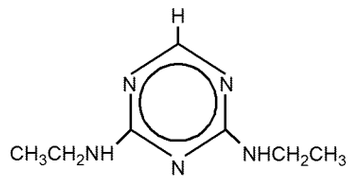
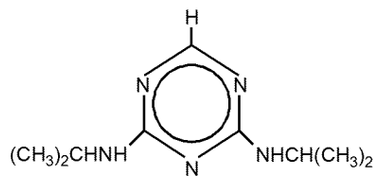
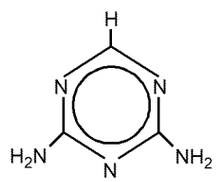
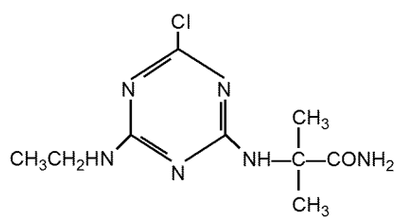
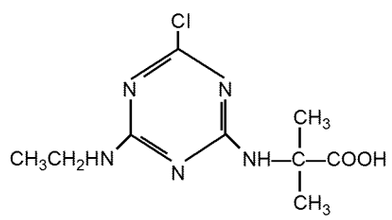
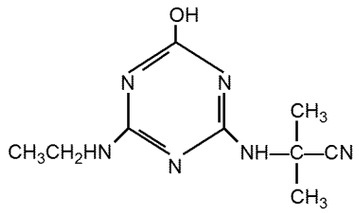
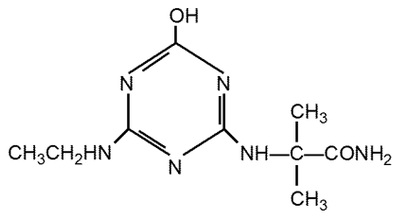
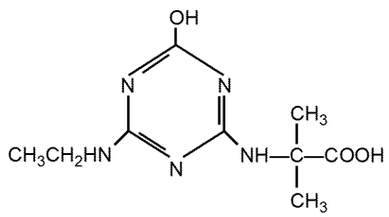
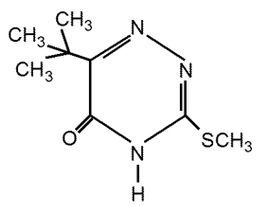
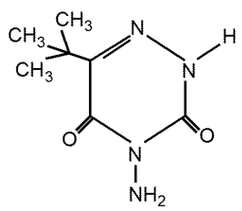
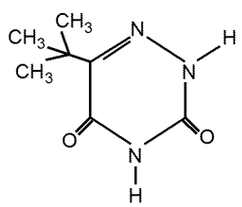
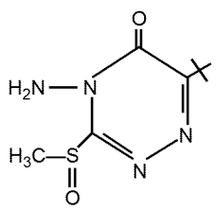
Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
Cyanuric acid	1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione		OOOT G-28251
Amination			
Aminoatrazine	*N*-ethyl-*N*ffi-(1-methylethyl)-1,3,5-triazine-2,4,6-triamine		AEIT GS-12517
Aminodesethyl atrazine	*N*-(1-methylethyl)-1,3,5-triazine-2,4,6-triamine		AAIT CGA-101248
Aminodeisopropyl atrazine	*N*-ethyl-1,3,5-triazine-2,4,6-triamine		AAET CGA-74650
Aminosimazine	*N*,*N*ffi-ethyl-1,3,5-triazine-2,4,6-triamine		G-30705
Reductive dehalogenation			
NA	*N*-ethyl-*N*ffi-(1-methylethyl)-1,3,5-triazine-2,4-diamine		HEIT
NA	*N*-ethyl-1,3,5-triazine-2,4-diamine		HAET

Table A3 (*Continued*)

Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
NA	*N*-(1-methylethyl)-1,3,5-triazine-2,4-diamine		HAIT
NA	*N*-*N*ffi-ethyl-1,3,5-triazine-2,4-diamine		HEET
NA	*N*-*N*ffi-(1-methylethyl)-1,3,5-triazine-2,4-diamine		HIIT
NA	1,3,5-triazine-2,4-diamine		HAAT
Compound			
Cyanazine metabolites			
Cyanazine amide	4-chloro-6-ethylamino-[1,3,5-triazine-2*yl*]-2-methylpropionamide		Compound II CAM
Cyanazine acid	4-chloro-6-ethylamino-[1,3,5-triazine-2*yl*]-2-methylpropanoic acid		Compound III CAC

(*Continued*)

Table A3 (Continued)

Common name	Original, CAS or IUPAC names	Structure	Abbreviations or code
Hydroxycyanazine	2-[(4-hydroxy-6-ethylamino-1,3,5-triazine-2-*yl*]amino)-2-methylpropanenitrile		
Amiodohydroxy-cyanazine	2-[(4-hydroxy-6-ethylamino)-1,3,5-triazine-2-*yl*]amino-2-methylpropionamide		
Carboxylic acid hydroxycyanazine	2-[(4-hydroxy-6-ethylamino)-1,3,5-triazine-2-*yl*]amino-2-methylpropanoic acid		Compound IV
Metribuzin metabolites			
Deaminated metribuzin	6-t-butyl-4H-3-methylthio-1,2,4-triazoyl-*s*-one		DA
Diketometribuzin	4-amino-6(1,1-dimethylethyl)-1,2,4-triazine-3,5-(2*H*)-dione		DK
Deaminodiketo-metribuzin	6-(1,1-dimethylethyl)-1,2,4-triazine-3,5-(2*H*,4*H*)-dione		DADK
Sulfoxide of metribuzin	4-amino-6-*t*-butyl-3-methylsulfoxo-1,2,4-triazinyl-5-one		

[a]NA: not available.
*This structure is a common metabolite of multiple herbicides.

Table A4a Scientific and common names of weeds mentioned in this book in alphabetic order by scientific name

Genus and species	Common name
Ageratum conyzoides L.	ageratum, tropic
Abutilon theophrasti Medicus	velvetleaf
Acanthospermum spp.	starbur spp.
Agrostis spp.	bentgrass spp.
Alchemilla arvensis (L.) Scop.	parsley-piert
Alisma plantago-aquatica L.	waterplantain, common
Alopecurus japonicus Steud.	foxtail, Japanese or setagaya (J.)[1]
Alopecurus myosuroides Huds.	blackgrass or slender foxtail
Amaranthus albus L.	pigweed, tumble
Amaranthus arenicola I.M. Johnst.	amaranth, sandhills
Amaranthus blitoides S. Wats.	pigweed, prostrate
Amaranthus bouchonii Thell.	amaranth, bouchons (D.)[2]
Amaranthus cruentus L. or *A. hybridus* L. var. *patulus* (Bertol.) Thell.	redshank, red amaranth or Italian amaranth
Amaranthus hybridus L. or *A. chlorostachys* Willd.	pigweed, smooth
Amaranthus lividus L.	amaranth, livid
Amaranthus palmeri S. Wats.	amaranth, Palmer
Amaranthus powellii S. Wats.	amaranth, Powell or green pigweed
Amaranthus quitensis L.	pigweed or quitensis (S.)[3]
Amaranthus retrofl exus L.	pigweed, redroot
Amaranthus rudis Sauer	waterhemp, common
Amaranthus spinosus L.	amaranth, spiny
Amaranthus spp.	pigweed spp.
Amaranthus tuberculatus (Moq.) J.D. Sauer	waterhemp, tall
Ambrosia artemisiifolia L.	ragweed, common
Ambrosia trifi da L.	ragweed, giant
Ammannia auriculata Willd.	redstem
Ammannia coccinea Rottb.	ammannia, purple or long-leaved loosestrife
Ampelamus albidus (Nutt.) Britt.	milkweed or honeyvine
Andropogon gerardii Vitman	bluestem, big
Anthemis cotula L.	chamomile, mayweed
Apera spica-venti (L.) Beauv. or *Agrostis spica-venti* L.	windgrass or silky bentgrass
Arenaria serpyllifolia L.	sandwort, thymeleaf
Argemone glauca L.	prickly poppy, smooth
Aristida oligantha Michx.	threeawn, prairie
Artemisia vulgaris L.	mugwort
Asclepias syriaca L.	milkweed, common
Atriplex patula L.	orach, spreading
Avena fatua L.	oat, wild
Bacopa rotundifolia (Michx.) Wettst.	waterhyssop, disk or ukiazene (J.)[1]
Barbarea vulgaris R. Br.	rocket, yellow
Beckmannia syzigachne (Steud.) Fernald	sloughgrass, American
Bidens bipinnata L.	spanishneedles
Bidens pilosa L.	beggarticks, hairy
Bidens subalternans DC.	beggarticks or amor seco (S.)[3]
Bidens tripartita L.	beggarticks, bur
Bouteloua curtipendula (Michx.) Torr.	grama, sideoats
Brachiaria mutica (Forsk.) Stapf	paragrass
Brachiaria plantaginea (Link) A.S. Hitchc.	alexandergrass
Brachiaria platyphylla (Griseb.) Nash	signalgrass, broadleaf
Brachypodium distachyon (L.) P. Beauv.	brome, false
Brassica kaber (DC.) L.C. Wheeler or *Sinapis arvensis* L.	mustard, wild
Brassica rapa L. or *B. campestris* L.	mustard, birdsrape
Brassica L. spp. or *Sinapis* L. spp.	mustard spp.
Brassica tournefortii Gouan	mustard, African or turnip, wild
Bromus secalinus L.	cheat
Bromus L. spp.	brome spp.
Bromus tectorum L.	brome, downy
Buddleia L. spp.	butterfly bush spp.
Calystegia sepium (L.) R. Br.	bindweed, hedge
Camelina microcarpa Andrz. ex DC.	falseflax, smallseed
Capsella bursa-pastoris (L.) Medicus	shepherd's purse
Carex L. spp. or *Cyperus* L. spp.	sedge spp.
Cassia obtusifolia L.	sicklepod

(*Continued*)

Table A4a (Continued)

Genus and species	Common name
Cenchrus echinatus L.	sandbur, southern
Chenopodium album L.	lambsquarters, common
Chenopodium fi cifolium J.E. Sm.	goosefoot, figleaved
Chenopodium gigantospermum Aellen	goosefoot, mapleleaf
Chenopodium missouriense Aellen	goosefoot, Missouri
Chenopodium murale L.	goosefoot, nettleleaf
Chenopodium polyspermum L.	goosefoot, manyseeded
Chenopodium rubrum L.	goosefoot, red
Chenopodium strictum Roth var. *glaucophyllum* (Aellen) H.A.Wahl	goosefoot, lateflowering
Chloris barbata Sw.	fingergrass, swollen
Chloris radiate (L.) Sw.	fingergrass, radiate
Chrysanthemum coronarium L.	chrysanthemum, Garland or crown daisy
Chrysanthemum segetum L.	marigold, corn
Cirsium arvense (L.) Scop.	thistle, Canada
Coix lacryma-jobi (*lachryma-jobi*) L.	Job's tears
Commelina benghalensis L.	spiderwort, tropical
Convolvulus arvensis L.	bindweed, field
Conyza bonariensis (L.) Cronq. or *Erigeron bonariensis* L.	fleabane, hairy
Conyza canadensis (L.) Cronq. or *Erigeron canadensis* L.	horseweed
Conyza fl oribunda H.B.K.	fleabane, tall
Crataegus L. spp.	hawthorn spp.
Crepis L. spp.	hawksbeard spp.
Crepis tectorum L.	hawksbeard, narrowleaf
Crotalaria spectabilis Roth	crotalaria, showy
Croton capitatus Michx.	croton, woolly
Crypsis schoenoides (L.) Lam. or *Heleochloa schoenoides* (L.) Host ex Roem	timothy, swamp
Cuscuta campestris Yuncker	dodder, field
Cynodon dactylon (L.) Pers.	bermudagrass
Cyperus difformis L.	sedge, smallflower umbrella
Cyperus esculentus L.	nutsedge, yellow
Cyperus rotundus L.	nutsedge, purple
Cyrtococcum Stapf or *Ottochloa* Dandy L. spp.	panicgrass spp.
Dactylis glomerata L.	orchardgrass
Dactyloctenium aegyptium (L.) Willd.	crowfootgrass
Damasonium minus Buchen.	starfruit
Datura stramonium L.	jimsonweed
Descurainia spp.	tansymustard spp.
Descurainia sophia (L.) Webb. ex Prantl	flixweed
Digitaria decumbens Stent.	pangolagrass
Digitaria horizontalis Willd.	capim-colchao (P.)[4] or tiende capote (S.)[3]
Digitaria ischaemum (Schreb. ex Schweig.) Schreb. ex Muhl.	crabgrass, smooth
Digitaria sanguinalis (L.) Scop.	crabgrass, large
Dioscorea bulbifera L.	air-potato
Diplotaxis tenuifolia (L.) DC.	rocket, wall
Echinochloa colona (L.) Link	Junglerice
Echinochloa crus-galli (L.) Beauv.	barnyardgrass
Echium plantagineum L.	Paterson's curse or salvation jane
Eichhornia crassipes (Mart.) Solms	waterhyacinth
Elatine triandra Schkuhr	waterwort or mizohakobe (J.)[1]
Eleusine indica (L.) Gaertn.	goosegrass
Elodea canadensis L.C. Rich.	elodea, common
Elytrigia repens (L.) Nevski	quackgrass
Epilobium adenocaulon Hausskn. or *E. ciliatum* Raf.	willowherb, American
Epilobium adenocaulon Hausskn. or *E. ciliatum* Raf.	willowweed, American
Epilobium adnatum Griseb. or *E. tetragonum* L.	willowherb, square-stalked
Epilobium angustifolium L.	fireweed
Eragrostis P. Beauv spp.	lovegrass spp.
Eremochloa ophiuroides (Munro) Hack.	centipedegrass
Eupatorium capillifolium (Lam.) Small	dogfennel
Euphorbia esula L.	spurge, leafy
Euphorbia heterophylla L.	poinsettia, wild
Euphorbia maculate L.	spurge, spotted

Table A4a (Continued)

Genus and species	Common name
Festucoideae spp. or *Festuca* L. spp.	fescue spp.
Fimbristylis miliacea (L.) Vahl	fringerush, globe
Foeniculum vulgare Mill.	fennel
Galeopsis tetrahit L.	hempnettle, common
Galinsoga ciliata (Raf.) Blake	galinsoga, hairy
Galinsoga parvifl ora Cav.	galinsoga, smallflower
Galium aparine L.	bedstraw, catchweed
Galium spurium L.	cleavers, false
Geranium carolinianum L.	geranium, Carolina
Gramineae spp.	grasses
Helianthus annuus L.	sunflower, common
Hieracium vulgatum Fries	hawkweed, common
Hordeum glaucum Steud.	wall barley
Imperata cylindrica (L.) Beauv.	cogongrass
Ipomoea alba L.	moonflower
Ipomoea L. spp.	morningglory spp.
Iva xanthifolia Nutt.	marshelder
Kochia scoparia (L.) Schrad.	kochia
Lactuca serriola L.	lettuce, prickly
Lamium amplexicaule L.	henbit
Lamium purpureum L.	deadnettle, purple
Lantana camara L.	lantana, largeleaf
Lepidium virginicum L.	pepperweed, Virginia
Lespedeza spp.	lespedeza spp.
Lespedeza striata (Thunb.) H. and A.	lespedeza, common
Limnocharis fl ava (L.) Buchen.	velvetleaf, yellow or burhead
Limnophila erecta Bentham	marshweed
Limnophila sessilifl ora (Vahl) Blume	marshweed, Asian or limnophila
Lindernia attenuata L.	falsepimpernel, short-stalked
Lindernia dubia (L.) Pennell	falsepimpernel, low
Lindernia dubia var. *major* L.	falsepimpernel, major low
Lindernia micrantha or *L. angustifolia* Wettst.	azetogarashi (J.)[1] or falsepimpernel, Japanese
Lindernia procumbens (Krock.) Philcox or *L. pyxidaria* L.	falsepimpernel, common or azena (J.)[1]
Lolium multifl orum Lam.	ryegrass, Italian
Lolium perenne L.	ryegrass, perennial
Lolium rigidum Gaudin	ryegrass, rigid or annual ryegrass
Lophochloa cristata (L.) Hyl. or *L. phleoides* (Vill.) Rchb.	catstail, annual
Malva neglecta Wallr.	mallow, common
Matricaria matricarioides (Less.) C.L. Porter or *M. discoidea* DC. or *Chamomilla suaveolens* (Pursh) Rydb.	pineapple-weed or rayless mayweed or disk mayweed
Medicago arabica (L.) Huds.	burclover, spotted
Mesembryanthemum crystallinum L.	Iceplant
Momordica charantia L.	balsamapple, bitter
Monochoria hastata (L.) Solms	monochoria, arrowleaved
Monochoria korsakowii Regel and Maack	mizuaoi (J.)[1] or moolokzam (K.)[5]
Monochoria vaginalis (Burm. F.) C. Presl. ex. Kunth	monochoria
Monochoria vaginalis Presl. var. *plantaginea* (Roxb.) Solms-Laub.	konagi (J.)[1]
Morrenia odorata (H. and A.) Lindl	stranglervine or milkweedvine
Myosoton aquaticum (L.) Moench or *Stellaria aquatica* (L.) Scop. or *Malachium aquaticum* (L.) Fries	starwort, water or water chickweed or giantchickweed
Nelumbo nucifera Gaertn.	lotus, Indian
Neslia paniculata (L.) Desv.	mustard, ball
Oenothera laciniata Hill	eveningprimrose, cutleaf
Oxalis L. spp.	woodsorrel spp.
Panicoideae spp.	panicum spp.
Panicum adspersum Trin.	broadleaf panicum
Panicum antidotale (L.) Retz	blue (or giant) panicgrass
Panicum capillare L.	witchgrass
Panicum dichotomifl orum Michx.	panicum, fall
Panicum fasciculatum Sw.	panicum, browntop
Panicum maximum Jacq.	guineagrass
Panicum miliaceum L.	millet, wild-proso

(*Continued*)

Table A4a (Continued)

Genus and species	Common name
Panicum repens L.	torpedograss
Panicum texanum Buckl.	panicum, Texas
Panicum virgatum L.	switchgrass
Papaver rhoeas L.	poppy, corn
Parietaria fl oridana Nutt.	Florida pellitory
Parthenium hysterophorus L.	parthenium, ragweed
Parthenocissus quinquefolio (L.) Planch.	Virginia creeper
Paspalum dilatatum Poir.	dallisgrass
Paspalum notatum Fluegge	bahiagrass
Paspalum paniculatum L.	rivergrass, Russell
Paspalum urvillei Steud.	vaseygrass
Pennisetum americanum (L.) Leeke	foxtail, yellow (pearl millet)
Pennisetum purpureum Schumach.	napiergrass
Pentzia suffruticosa Hutch. ex Merxm.	sheepbush or karoobush
Phalaris minor Retz.	canarygrass, littleseed
Phalaris paradoxa L.	canarygrass, hood
Phleum pratense L.	timothy
Physalis longifolia Nutt.	groundcherry, longleaf
Picris hieracioides L.	oxtongue, hawkweed
Plantago lagopus L.	plantain or pie de liebre (S.)[3]
Plantago lanceolata L.	plantain, buckhorn
Plantago L. spp.	plantain spp.
Poa annua L.	bluegrass, annual
Polygonum aviculare L.	knotweed, prostrate
Polygonum caespitosum Blume var. *longisetum* (DeBruyn) A.N. Stewart	knotweed, tufted
Polygonum convolvulus L.	buckwheat, wild
Polygonum hydropiper L.	smartweed, marshpepper
Polygonum lapathifolium L.	smartweed, pale
Polygonum pensylvanicum L.	smartweed, Pennsylvania
Polygonum persicaria L.	ladysthumb
Polypogon monspeliensis (L.) Desf.	polypogon, rabbitfoot
Portulaca oleracea L.	purslane, common
Pyracantha Roem. spp.	firethorn
Ranunculus L. spp.	buttercup spp.
Raphanus L. spp.	charlock or radish
Raphanus raphanistrum L.	radish, wild
Raphanus sativus L. var. *niger* (Mill.) Pars.	radish, garden
Rapistrum rugosum (L.) All.	turnipweed
Reseda luteola L.	rocket, dyers
Richardia scabra L.	pusley, Florida
Rotala indica (Willd.) Koehne	toothcup, Indian or kikashigusa (J.)[1]
Rottboellia cochinchinensis (Lour.) W. Clayton	itchgrass
Rumex crispus L.	dock, curly
Sagina procumbens L.	pearlwort, birdseye
Sagittaria guayanensis H.B.K.	arrowhead-lily or swamp-potato
Sagittaria latifolia Willd.	arrowhead, common
Sagittaria montevidensis Cham. and Schlecht.	arrowhead, California
Sagittaria pygmaea Miq.	arrowhead, dwarf or urikawa (J.)[1]
Salsola iberica Sennen and Pau.	thistle, Russian
Scirpus fl uviatilis (Torr.) Gray or *S. maritimus*	bulrush, river
Scirpus juncoides Roxb. var. *ohwianus* T. Koyama	inuhotarui (J.)[1] or Japanese bulrush
Scirpus mucronatus L.	bulrush, ricefield
Scoparia dulcis L./Benth.	broomweed, sweet or goatweed
Senecio vulgaris L.	groundsel, common
Setaria faberi Herrm.	foxtail, giant
Setaria glauca (L.) Beauv. or *S. lutescens* Weigel (F.T. Hubb)	foxtail, yellow
Setaria italica (L.) Beauv.	foxtail, millet
Setaria uniseta Fourn. ex Hemsl. or *Ixophorus unisetus* (Presl) Schult./Schlecht.	hatico (S.)[3] or Spanish foxtail
Setaria verticillata (L.) Beauv.	foxtail, bristly or rough bristlegrass
Setaria viridis (L.) Beauv.	foxtail, green
Setaria viridis var. *major* (Gaudin) Pospichel	foxtail, giant green

Table A4a (Continued)

Genus and species	Common name
Setaria viridis var. *robusta-alba* Schreiber	foxtail, robust white
Sida spinosa L.	sida, prickly
Silene alba (Mill.) E.H. Krause or *Melandrium album* (Mill.) Garcke	campion, white or white cockle
Sisymbrium orientale Torn.	mustard, Oriental or Indian hedge
Sisymbrium thellungii Schulz	turnipweed, African
Solanum americanum Mill	nightshade, American black
Solanum carolinense L.	horsenettle
Solanum nigrum L.	nightshade, black
Solanum ptycanthum Dun.	nightshade, eastern black
Soliva pterosperma (Juss.) Less.	burweed, lawn
Sonchus asper (L.) Hill	sowthistle, spiny
Sonchus oleraceus L.	sowthistle, annual
Sorghastrum nutans (L.) Nash ex Small	indiangrass, yellow
Sorghum bicolor (L.) Moench	shattercane
Sorghum halepense (L.) Pers.	johnsongrass
Sorghum spp.	sorghum, wild
Sporobolus indicus (L.) R. Br.	smutgrass
Stachys recta L.	hempnettle, upright or betony
Stellaria media (L.) Vill	chickweed, common
Striga asiatica (L.) Ktze.	witchweed
Tagetes L. spp.	marigold spp.
Taraxacum offi cinale Weber in Wiggers	dandelion
Thlaspi arvense L.	pennycress, field
Toxicodendron radicans (L.) Ktze.	poison-ivy
Trifolium arvense L.	clover, rabbitfoot
Trifolium aureum Pollich	clover, hop
Triodanis perfoliata (L.) Nieuwl.	venuslookingglass, common
Tsuga Carr. spp.	hemlock
Urochloa panicoides Beauv.	liverseedgrass
Urtica urens L.	nettle, burning or stinging nettle
Verbascum thapsus L.	mullein, common
Veronica arvensis L.	speedwell, corn
Xanthium strumarium L.	cocklebur, common

[1] J: Japanese
[2] D: German
[3] S: Spanish
[4] P: Portuguese
[5] K: Korean

Table A4b Common and scientific names of weeds mentioned in this book in alphabetic order by common name

Common name	Genus and species
ageratum, tropic	*Ageratum conyzoides* L.
air-potato	*Dioscorea bulbifera* L.
alexandergrass	*Brachiaria plantaginea* (Link) A.S. Hitchc.
amaranth, bouchons (D.)[2]	*Amaranthus bouchonii* Thell.
amaranth, Italian or redshank or red amaranth	*Amaranthus cruentus* L. or *A. hybridus* L. var. *patulus* (Bertol.) Thell.
amaranth, livid	*Amaranthus lividus* L.
amaranth, Palmer	*Amaranthus palmeri* S. Wats.
amaranth, Powell or green pigweed	*Amaranthus powellii* S. Wats.
amaranth, red or redshank or Italian amaranth	*Amaranthus cruentus* L. or *A. hybridus* L. var. *patulus* (Bertol.) Thell.
amaranth, sandhills	*Amaranthus arenicola* I. M. Johnst.
amaranth, spiny	*Amaranthus spinosus* L.
ammannia, purple or long-leaved loosestrife	*Ammannia coccinea* Rottb.
amor seco (S.)[3] or beggarticks	*Bidens subalternans* DC.
arrowhead, California	*Sagittaria montevidensis* Cham. and Schlecht.
arrowhead, common	*Sagittaria latifolia* Willd.
arrowhead, dwarf or urikawa (J.)[1]	*Sagittaria pygmaea* Miq.

(*Continued*)

Table A4b (Continued)

Common name	Genus and species
arrowhead-lily or swamp-potato	*Sagittaria gauyanesis* H.B.K.
azena (J.)[1] or falsepimpernel, common	*Lindernia procumbens* (Krock.) Philcox or *L. pyxidaria* L.
azetogarashi (J.)[1] or falsepimpernel, Japanese	*Lindernia micrantha* or *L. angustifolia* Wettst.
bahiagrass	*Paspalum notatum* Fluegge
balsamapple, bitter	*Momordica charantia* L.
barnyardgrass	*Echinochloa crus-galli* (L.) Beauv.
bedstraw, catchweed	*Galium aparine* L.
beggarticks or amor seco (S.)[3]	*Bidens subalternans* DC.
beggarticks, bur	*Bidens tripartita* L.
beggarticks, hairy	*Bidens pilosa* L.
bentgrass spp.	*Agrostis* spp.
bermudagrass	*Cynodon dactylon* (L.) Pers.
betony or hempnettle, upright	*Stachys recta* L.
bindweed, field	*Convolvulus arvensis* L.
bindweed, hedge	*Calystegia sepium* (L.) R. Br.
blackgrass or slender foxtail	*Alopecurus myosuroides* Huds.
blue (or giant) panicgrass	Panicum antidotale (L.) Retz
bluegrass, annual	*Poa annua* L.
bluestem, big	*Andropogon gerardii* Vitman
bristlegrass, rough or foxtail, bristly	*Setaria verticillata* (L.) Beauv.
broadleaf panicum	Panicum adspersum Trin.
brome spp.	*Bromus* L. spp.
brome, downy	*Bromus tectorum* L.
brome, false	*Brachypodium distachyon* (L.) P. Beauv.
broomweed, sweet or goatweed	*Scoparia dulcis* L./Benth.
buckwheat, wild	*Polygonum convolvolus* L. or *Fallopia convolvulus* (L.) A. Loeve
bulrush, Japanese or inuhotarui (J.)[1]	*Scirpus juncoides* Roxb. var. *ohwianus* T. Koyama
bulrush, ricefield	*Scirpus mucronatus* L.
bulrush, river	*Scirpus fl uviatilis* (Torr.) Gray or *S. meritimus*
burclover, spotted	*Medicago arabica* (L.) Huds.
burhead or yellow velvetleaf	*Limnocharis fl ava* (L.) Buchen
burweed, lawn	*Soliva pterosperma* (Juss.) Less.
buttercup spp.	*Ranunculus* L. spp.
butterflybush spp.	*Buddleia* L. spp.
campion, white or white cockle	*Silene alba* (Mill.) E.H. Krause
canarygrass, hood	*Phalaris paradoxa* L.
canarygrass, littleseed	*Phalaris minor* Retz.
capim-colchao (P.)[4] or tiende capote (S.)[3]	*Digitaria horizontalis* Willd.
catstail, annual	*Lophochloa cristata* (L.) Hyl. or *L. phleoides* (Vill.) Rchb.
centipedegrass	*Eremochloa ophiuroides* (Munro) Hack.
chamomile, mayweed	*Anthemis cotula* L.
charlock or radish	*Raphanus* L. spp.
cheat	*Bromus secalinus* L.
chickweed, common	*Stellaria media* (L.) Vill
chickweed, water or water starwort or giantchickweed	*Myosoton aquaticum* (L.) Moench or *Stellaria aquatica* (L.) Scop. or *Malachium aquaticum* (L.) Fries
chrysanthemum, Garland or crown daisy	*Chrysanthemum coronarium* L.
cleavers, false	*Galium spurium* L.
clover, hop	*Trifolium aureum* Pollich
clover, rabbitfoot	*Trifolium arvense* L.
cockle, white or white campion	*Silene alba* (Mill.) E.H. Krause or *Melandrium album* (Mill.) Garcke
cocklebur, common	*Xanthium strumarium* L.
cogongrass	*Imperata cylindrica* (L.) Beauv.
crabgrass, large	*Digitaria sanguinalis* (L.) Scop.
crabgrass, smooth	*Digitaria ischaemum* (Schreb. ex Schweig.) Schreb. ex Muhl.
crotalaria, showy	*Crotalaria spectabilis* Roth
croton, woolly	*Croton capitatus* Michx.
crowfootgrass	Dactyloctenium aegyptium (L.) Willd.
daisy, crown or Garland chrysanthemum	*Chrysanthemum coronarium* L.
dallisgrass	*Paspalum dilatatum* Poir.
dandelion	*Taraxacum offi cinale* Weber in Wiggers
deadnettle, purple	*Lamium purpureum* L.
dock, curly	*Rumex crispus* L.

Table A4b (Continued)

Common name	Genus and species
dodder, field	*Cuscuta campestris* Yuncker
dogfennel	*Eupatorium capillifolium* (Lam.) Small
elodea, common	*Elodea canadensis* L.C. Rich.
eveningprimrose, cutleaf	*Oenothera laciniata* Hill
falseflax, smallseed	*Camelina microcarpa* Andrz. ex DC.
falsepimpernel, common or azena (J.)[1]	*Lindernia procumbens* (Krock.) Philcox or *L. pyxidaria* L.
falsepimpernel, low	*Lindernia dubia* (L.) Pennell
falsepimpernel, major low	*Lindernia dubia* var. *major* L.
falsepimpernel, Japanese or azetogarashi (J.)[1]	*Lindernia micrantha* or *L. angustifolia* Wettst.
falsepimpernel, short-stalked	*Lindernia attenuata* L.
fennel	*Foeniculum vulgare* Mill.
fescue spp.	*Festucoideae* spp. or *Festuca* L. spp.
fingergrass, radiate	*Chloris radiate* (L.) Sw.
fingergrass, swollen	*Chloris barbata* Sw.
firethorn	*Pyracantha* Roem. spp.
fireweed	*Epilobium angustifolium* L.
fleabane, hairy	*Conyza bonariensis* (L.) Cronq. or *Erigeron bonariensis* L.
fleabane, tall	*Conyza fl oribunda* H.B.K.
flixweed	*Descurainia sophia* (L.) Webb. ex Prantl
Florida pellitory	*Parietaria fl oridana* Nutt.
foxtail, bristly or rough bristlegrass	*Setaria verticillata* (L.) Beauv.
foxtail, giant	*Setaria faberi* Herrm.
foxtail, giant green	*Setaria viridis* var. *major* (Gaudin) Pospichel
foxtail, green	*Setaria viridis* (L.) Beauv
foxtail, Japanese or setogaya (J.)[1]	*Alopecurus japonicus* Steud.
foxtail, robust white	*Setaria viridis* var. *robusta-alba* Schreiber
foxtail, slender or blackgrass	*Alopecurus myosuroides* Huds.
foxtail, Spanish or hatico (S.)[3]	*Setaria uniseta* Fourn. ex Hemsl. or *Ixophorus unisetus* (Presl) Schult./Schlecht.
foxtail, yellow	*Setaria glauca* (L.) Beauv. or *S. lutescens* Weigel (F.T. Hubb)
fringerush, globe	*Fimbristylis miliacea* (L.) Vahl
galinsoga, hairy	*Galinsoga ciliata* (Raf.) Blake
galinsoga, smallflower	*Galinsoga parvifl ora* Cav.
geranium, Carolina	*Geranium carolinianum* L.
giantchickweed or water chickweed or water starwort	*Malachium aquaticum* (L.) Fries or *Stellaria aquatica* (L.) Scop. or *Myosoton aquaticum* (L.) Moench
goatweed or sweet broomweed	*Scoparia dulcis* L./Benth.
goosefoot, figleaved	*Chenopodium fi cifolium* J. E. Sm.
goosefoot, lateflowering	*Chenopodium strictum* Roth var. *glaucophyllum* (Aellen) H.A.Wahl
goosefoot, manyseeded	*Chenopodium polyspermum* L.
goosefoot, mapleleaf	*Chenopodium gigantospermum* Aellen
goosefoot, Missouri	*Chenopodium missouriense* Aellen
goosefoot, nettleleaf	*Chenopodium murale* L.
goosefoot, red	*Chenopodium rubrum* L.
goosegrass	*Eleusine indica* (L.) Gaertn.
grama, sideoats	*Bouteloua curtipendula* (Michx.) Torr.
grasses	*Gramineae* spp.
groundcherry, longleaf	*Physalis longifolia* Nutt.
groundsel, common	*Senecio vulgaris* L.
guineagrass	*Panicum maximum* Jacq.
hatico (S.)[3] or Spanish foxtail	*Setaria uniseta* Fourn. ex Hensl. or *Ixophorus unisatus* (Presl.) Schult./Schlecht.
hawksbeard spp.	*Crepis* L. spp.
hawksbeard, narrowleaf	*Crepis tectorum* L.
hawkweed, common	*Hieracium vulgatum* Fries
hawthorn spp.	*Crataegus* L. spp.
hedge, Indian or Oriental mustard	*Sisymbrium orientale* Torn.
hemlock	*Tsuga* Carr. spp.
hempnettle, common	*Galeopsis tetrahit* L.
hempnettle, upright or betony	*Stachys recta* L.
henbit	*Lamium amplexicaule* L.
honeyvine or milkweed	*Ampelamus albidus* (Nutt.) Britt.
horsenettle	*Solanum carolinense* L.

(*Continued*)

Table A4b (Continued)

Common name	Genus and species
horseweed	*Conyza canadensis* (L.) Cronq. or *Erigeron canadensis* L.
iceplant	*Mesembryanthemum crystallinum L.*
indiangrass, yellow	*Sorghastrum nutans* (L.) Nash ex Small
inuhotarui (J.)[1] or Japanese bulrush	*Scirpus juncoides* Roxb. var. *ohwianus* T. Koyama
itchgrass	*Rottboellia cochinchinensis* (Lour.) W. Clayton
jimsonweed	*Datura stramonium* L.
Job's tears	*Coix lacryma-jobi (lachryma-jobi)* L.
johnsongrass	*Sorghum halepense* (L.) Pers.
junglerice	*Echinochloa colona* (L.) Link
kikashigusa (J.)[1] or Indian toothcup	*Rotala indica* (Willd.) Koehne
knotweed, prostrate	*Polygonum aviculare* L.
knotweed, tufted	*Polygonum caespitosum* Blume var. *longisetum* (DeBruyn) A.N. Stewart
kochia	*Kochia scoparia* (L.) Schrad.
konagi (J.)[1]	*Monochoria vaginalis* Presl. var. *plantaginea* (Roxb.) Solms-Laub.
ladysthumb	*Polygonum persicaria* L.
lambsquarters, common	*Chenopodium album* L.
lantana, largeleaf	*Lantana camara* L.
lespedeza spp.	*Lespedeza* spp.
lespedeza, common	*Lespedeza striata* (Thunb.) H. and A.
lettuce, prickly	*Lactuca serriola* L.
limnophiloa or Asian marshweed	*Limnophila sessilifl ora* (Vahl) Blume
liverseedgrass	*Urochloa panicoides* Beauv.
loosestrife, long-leaved or purple ammannia	*Ammannia coccinea* Rottb.
lotus, Indian	*Nelumbo nucifera* Gaertn.
lovegrass spp.	*Eragrostis* P. Beauv. spp. or *Eragrostoideae*
mallow, common	*Malva neglecta* Wallr.
marigold spp.	*Tagetes* L. spp.
marigold, corn	*Chrysanthemum segetum* L.
marshelder	*Iva xanthifolia* Nutt.
marshweed	*Limnophila erecta* Bentham
marshweed, Asian or limnophila	*Limnophila sessilifl ora* (Vahl) Blume
mayweed, rayless or pineapple-weed or disk mayweed	*Matricaria matricarioides* (Less.) C. L. Porter or *Chamomilla suaveolens* (Pursh) Rydb.
milkweed, common	*Asclepias syriaca* L.
milkweed or honeyvine	*Ampelamus albidus* (Nutt.) Britt.
milkweedvine or stranglervine	*Morrenia odorata* (H. and A.) Lindl.
millet, foxtail	*Setaria italica* (L.) Beauv.
foxtail, yellow (pearl millet)	*Pennisetum americanum* (L.) Leeke
millet, wild-proso	*Panicum miliaceum* L.
mizohakobe (J.)[1] or waterwort	*Elatine triandra* Schkuhr
mizuaoi (J.)[1] or moolokzam (K.)[5]	*Monochoria korsakowii* Regel and Maack
monochoria	*Monochoria vaginalis* (Burm. F.) C. Presl. ex. Kunth
monochoria, arrowleaved	*Monochoria hastata* (L.) Solms
moolokzam (K.)[5] or mizuaoi (J.)[1]	*Monochoria korsakowii* Regel and Maack
moonflower	*Ipomoea alba* L.
morningglory spp.	*Ipomoea* L. spp.
mugwort	*Artemisia vulgaris* L.
mullein, common	*Verbascum thapsus* L.
mustard spp.	*Brassica* L. spp. or *Sinapis* L. spp.
mustard, African or wild turnip	*Brassica tournefortii* Gouan
mustard, ball	*Neslia paniculata* (L.) Desv.
mustard, birdsrape	*Brassica rapa* L. or *B. campestris* L.
mustard, Oriental or Indian hedge	*Sisymbrium orientale* Torn.
mustard, wild	*Brassica kaber* (DC.) L. C. Wheeler or *Sinapis arvensis* L.
napiergrass	*Pennisetum purpureum* Schumach.
nettle, burning or stinging nettle	*Urtica urens* L.
nettle, stinging or burning nettle	*Urtica urens* L.
nightshade, American black	*Solanum americanum* Mill
nightshade, black	*Solanum nigrum* L.
nightshade, eastern black	*Solanum ptycanthum* Dun.
nutsedge, purple	*Cyperus rotundus* L.
nutsedge, yellow	*Cyperus esculentus* L.
oat, wild	*Avena fatua* L.

Table A4b (Continued)

Common name	Genus and species
orach, spreading	*Atriplex patula* L.
orchardgrass	*Dactylis glomerata* L.
oxtongue, hawkweed	*Picris hieracioides* L.
pangolagrass	*Digitaria decumbens* Stent.
panicgrass spp.	*Cyrtococcum* Stapf spp. or *Ottochloa* Dandy L. spp.
panicgrass, blue	*Panicum antidotale* (L.) Retz
panicgrass, giant	*Panicum antidotale* (L.) Retz
panicum spp.	*Panicoideae* spp.
panicum, broadleaf	*Panicum adspersum* Trin.
panicum, browntop	*Panicum fasciculatum* Sw.
panicum, fall	*Panicum dichotomifl orum* Michx.
panicum, Texas	*Panicum texanum* Buckl.
paragrass	*Brachiaria mutica* (Forsk.) Stapf
parsley-piert	*Alchemilla arvensis* (L.) Scop.
parthenium, ragweed	*Parthenium hysterophorus* L.
Paterson's curse or salvation jane	*Echium plantagineum* L.
pearlwort, birdseye	*Sagina procumbens* L.
pennycress, field	*Thlaspi arvense* L.
pepperweed, Virginia	*Lepidium virginicum* L.
pie de liebre (S.)[3] or plantain	*Plantago lagopus* L.
pigweed, green or Powell amaranth	*Amaranthus powellii* S. Wats.
pigweed spp.	*Amaranthus* spp.
pigweed or quitensis (S.)[3]	*Amaranthus quitensis* L.
pigweed, prostrate	*Amaranthus blitoides* S. Wats.
pigweed, redroot	*Amaranthus retrofl exus* L.
pigweed, smooth	*Amaranthus hybridus* L. or *A. chlorostachys* Willd.
pigweed, tumble	*Amaranthus albus* L.
pineapple-weed or rayless mayweed or disk mayweed	*Matricaria matricarioides* (Less.) C. L. Porter or *Chamomilla suaveolens* (Pursh) Rydb.
plantain spp.	*Plantago* L. spp.
plantain, buckhorn	*Plantago lanceolata* L.
plantain or pie de liebre (S.)[3]	*Plantago lagopus* L.
poinsettia, wild	*Euphorbia heterophylla* L.
poison-ivy	*Toxicodendron radicans* (L.) Ktze.
polypogon, rabbitfoot	*Polypogon monspeliensis* (L.) Desf.
poppy, corn	*Papaver rhoeas* L.
pricklypoppy, smooth	*Argemone glauca* L.
purslane, common	*Portulaca oleracea* L.
pusley, Florida	*Richardia scabra* L.
quackgrass	*Elytrigia repens* (L.) Nevski
quitensis (S.)[3] or pigweed	*Amaranthus quitensis* L.
radish, garden	*Raphanus sativus* L. var. *niger* (Mill.) Pars.
radish or charlock	*Raphanus* L. spp.
radish, wild	*Raphanus raphanistrum* L.
ragweed, common	*Ambrosia artemisiifolia* L.
ragweed, giant	*Ambrosia trifi da* L.
redshank or red amaranth or Italian amaranth	*Amaranthus cruentus* L. or *A. hybridus* L. var. *patulus* (Bertol.) Thell.
redstem	*Ammannia auriculata* Willd.
rivergrass, Russell	*Paspalum paniculatum* L.
rocket, dyers	*Reseda luteola* L.
rocket, wall	*Diplotaxis tenuifolia* (L.) DC
rocket, yellow	*Barbarea vulgaris* R. Br.
ryegrass, annual or rigid ryegrass	*Lolium rigidum* Gaudin
ryegrass, Italian	*Lolium multifl orum* Lam.
ryegrass, perennial	*Lolium perenne* L.
ryegrass, rigid or annual ryegrass	*Lolium rigidum* Gaudin
salvation jane or Paterson's curse	*Echium plantagineum* L.
sandbur, southern	*Cenchrus echinatus* L.
sandwort, thymeleaf	*Arenaria serpyllifolia* L.
sedge spp.	*Carex* L. spp. or *Cyperus* L. spp.
sedge, smallflower umbrella	*Cyperus difformis* L.
setogaya (J.)[1] or foxtail, Japanese	*Alopecurus japonicus* Steud.
shattercane	*Sorghum bicolor* (L.) Moench

(*Continued*)

Table A4b (Continued)

Common name	Genus and species
sheepbush or karoobush	*Pentzia suffruticosa* Hutch. ex Merxm.
shepherd's purse	*Capsella bursa-pastoris* (L.) Medicus
sicklepod	*Cassia obtusifolia* L.
sida, prickly	*Sida spinosa* L.
signalgrass, broadleaf	*Brachiaria platyphylla* (Griseb.) Nash
sloughgrass, American	*Beckmannia syzigachne* (Steud.) Fernald
smartweed, marshpepper	*Polygonum hydropiper* L.
smartweed, pale	*Polygonum lapathifolium* L.
smartweed, Pennsylvania	*Polygonum pensylvanicum* L.
smutgrass	*Sporobolus indicus* (L.) R. Br.
sorghum, wild	*Sorghum* L. spp.
sowthistle, annual	*Sonchus oleraceus* L.
sowthistle, spiny	*Sonchus asper* (L.) Hill
spanishneedles	*Bidens bipinnata* L.
speedwell, corn	*Veronica arvensis* L.
spiderwort, tropical	*Commelina benghalensis* L.
spurge, leafy	*Euphorbia esula* L.
spurge, spotted	*Euphorbia maculate* L.
starbur spp.	*Acanthospermum* spp.
starfruit	*Damasonium minus* Buchen.
starwort, water or water chickweed or giantchickweed	*Myosoton aquaticum* (L.) Moench or *Stellaria aquatica* (L.) Scop. or *Malachium aquaticum* (L.) Fries
stranglervine or mildweedvine	*Morrenia odorata* (H. and A.) Lindl.
sunflower, common	*Helianthus annuus* L.
swamp-potato or arrowhead-lily	*Sagittaria gauyanesis* H.B.K.
switchgrass	*Panicum virgatum* L.
tansymustard spp.	*Descurainia* spp.
thistle, Canada	*Cirsium arvense* (L.) Scop.
thistle, Russian	*Salsola iberica* Sennen and Pau.
threeawn, prairie	*Aristida oligantha* Michx.
tiende capote (S.)[3] or capim-colchao (P.)[3]	*Digitaria horizontalis* Willd.
timothy	*Phleum pratense* L.
timothy, swamp	*Crypsis schoenoides* (L.) Lam. or *Heleochloa schoenoides* (L.) Host ex Roem
toothcup, Indian or kikashigusa (J.)[1]	*Rotala indica* (Willd.) Koehne
torpedograss	*Panicum repens* L.
turnip, wild or mustard, African	*Brassica tournefortii* Gouan
turnipweed	*Rapistrum rugosum* (L.) All.
turnipweed, African	*Sisymbrium thellungii* Schulz
ukiazene (J.)[1] or waterhyssop, disk	*Bacopa rotundifolia* (Michx.) Wettst.
vaseygrass	*Paspalum urvillei* Steud.
velvetleaf	*Abutilon theophrasti* Medicus
velvetleaf, yellow or burhead	*Limnocharis fl ava* (L.) Buchen.
venuslookingglass, common	*Triodanis perfoliata* (L.) Nieuwl.
Virginia creeper	*Parthenocissus quinquefolio* (L.) Planch.
wall barley	*Hordeum glaucum* Steud.
waterhemp, common	*Amaranthus rudis* Sauer
waterhemp, tall	*Amaranthus tuberculatus* (Moq.) J.D. Sauer
waterhyacinth	*Eichhornia crassipes* (Mart.) Solms
waterhysop, disk or ukiazene (J.)[1]	*Bacopa rotundifolia* (Michx.) Wettst.
waterplantain, common	*Alisma plantago-aquatica* L.
waterwort or mizohakobe (J.)[1]	*Elatine triandra* Schkuhr
willowherb, square-stalked	*Epilobium adnatum* Griseb. or *E. tetragonum* L.
willowweed, American	*Epilobium adenocaulon* Hausskn. or *E. ciliatum* Raf.
willowherb, American	*Epilobium adenocaulon* Hausskn. or *E. ciliatum* Raf.
windgrass or silky bentgrass	*Apera spica-venti* (L.) Beauv. or *Agrostis spica-venti* L.
witchgrass	*Panicum capillare* L.
witchweed	*Striga asiatica* (L.) Ktze.
woodsorrel spp.	*Oxalis* L. spp.

[1] J: Japanese
[2] D: German
[3] S: Spanish
[4] P: Portuguese
[5] K: Korean

Label Changes for Atrazine in the United States

During the almost 50 years that atrazine has been registered, there have been a number of label changes to reduce total use of atrazine and 'maximum use' allowable rates, to remove certain uses, and to reduce environmental loading. In addition, Best Management Practices (BMPs) have reduced the potential exposure of nontarget organisms. Table A5 captures important label changes for atrazine in the United States over time. Refer to the current product label and follow all directions for use when applying atrazine or any other triazine-containing product.

Table A5 Examples of label changes implemented for Syngenta atrazine products

Date	Uses	Mitigation/Stewardship Baseline mitigating statements
Prior to 1970	All	Do not contaminate domestic or irrigation water supplies or lakes, streams, or ponds.
	All	Avoid (aerial) application where excessive spray drift may occur.
	All	Care should be taken to avoid using where adjacent desirable trees, shrubs, or lawns might be injured.
	Macadamia nuts	Do not make aerial applications.
	Conifers	To avoid crop injury, do not apply to seedbeds.
Industry-wide adjustment for anti-siphoning provisions		
1978	Corn	Apply only through irrigation systems containing anti-siphon and check valves to prevent contamination of well during shutdown and overflow of solution tank.
Industry-wide drift management: Adjustments made on product-by-product basis		
1982	All	Do not apply directly to any body of water.
	Chemical fallow, sugarcane, conifers-aerial application	In order to assure that spray will be controllable within the target area when used according to label directions, make applications at a maximum height of 10 ft, using low-drift nozzles at a maximum pressure of 40 psi, and restrict application to periods when wind speed does not exceed 10 mph.
	Chemical fallow, sugarcane, conifers-aerial application	To assure that spray will not adversely affect adjacent, sensitive, nontarget plants, apply alone by aircraft at a minimum upwind distance of 400 ft from sensitive plants.
USEPA label statements required for all agriculture products affected by off-target movement		
1984	All	To avoid spray drift, do not apply under windy conditions.
	All	Do not apply where runoff is likely to occur.
	All	Do not apply when weather conditions favor drift from treated areas.
Baseline mitigating statements *New use*		
1984	Roadsides	Applications must be made in the fall before the ground freezes, or after thawing in the spring.
USEPA label statements required for terrestrial-use agricultural products affected by off-target movement		
1986	All	Do not apply directly to water or wetlands.
	All	Runoff and drift from treated areas may be hazardous to aquatic organisms in neighboring areas.
1990 label changes for atrazine products		
1990	All	Do not apply through any type of irrigation system.
	All	Restricted use pesticide (groundwater) – For retail sale to and use only by certified applicators or persons under their direct supervision.
	All – specific geographies	Certain states may have established rate limitations within specific geographical areas. Consult your state lead pesticide control agency for additional information.
	Corn, sorghum	1 lb a.i./A rate reduction to new maximum rate of 3 lb a.i./A.
1990	Proso millet	Deleted all proso millet uses.
	All	Required 50 ft well setbacks for mixing, loading or use; required for all wells.
	Rangeland	Deleted all rangeland uses.
	Pineapple	Deleted all pineapple uses.
	Nonselective control on noncrop	30 lb a.i./A rate reduction to new maximum rate of 10 lb a.i./A.

(*Continued*)

Table A5 (Continued)

Date	Uses	Mitigation/Stewardship Baseline mitigating statements
1992 label changes for atrazine products		
1992	All	Restricted use classification (surface water mitigation)
	All	This product may not be mixed or loaded within 50 ft of intermittent streams and rivers, natural or impounded lakes and reservoirs.
	All	This product may not be applied aerially or by ground within 66 ft of the points where field surface water runoff enters perennial or intermittent streams and rivers or within 200 ft around natural or impounded lakes and reservoirs.
	All	If this product is applied to highly erodible land, the 66 ft buffer or setback from runoff entry points must be planted to crop, seeded with grass or other suitable crop.
	All	Do not apply directly to water, to areas where surface water is present, or to intertidal areas below the mean high-water mark.
	All	Do not contaminate water when disposing of equipment wash waters.
	All – specific geographies	Where there are state/local requirements regarding atrazine use (including lower maximum rates and/or greater setbacks) that are different from the label, the more restrictive/protective requirements must be followed.
	Corn, sorghum	Preemergence rate reduction to maximum rate of 1.6–2 lb a.i./A.
	Corn, sorghum	Postemergence rate reduction to maximum rate of 2 lb a.i./A.
	Corn, sorghum	Split rate reduction to maximum of 2.5 lb a.i./A – pre plus post.
	Corn, sorghum	Restriction on those soils defined by Soil Conservation Service as highly erodible: If conservation tillage is practiced, leave at least 30% of the soil covered with plant residues at planting, apply a maximum of 2 lb a.i./A as a broadcast spray. If the soil coverage with plant residue is less than 30% at planting, a maximum of 1.6 lb a.i./A may be applied.
	Nonselective control on noncrop	Deleted all nonselective weed control uses on noncrop land.
New use site for atrazine products		
1993	Conservation Reserve Program (CRP)	To assure that drift will not adversely affect adjacent sensitive nontarget plants, apply AAtrex 4L by aircraft at a minimum upwind distance of 400 ft from sensitive plants.
	CRP	Fall applications for renovation must be made before the ground freezes.
	CRP	Make applications at a maximum height of 10 ft above vegetation. Use low-drift nozzles at a maximum pressure of 40 psi. Restrict application to periods when wind speed does not exceed 10 mph to control drift.
Mitigation for tile-outlets in terraced fields for atrazine products		
1996	All	To ensure protection of surface water from runoff through standpipes with tile-outlets in terraced fields, one of the following options may be used: 1. Do not apply this product within 66 ft of standpipes in tile-outletted terraced fields. 2. Apply to the entire tile-outletted terraced field and immediately incorporate to a depth of 2–3 in. in the entire tile-outletted terraced field. 3. Apply to the entire tile-outletted terraced field under a no-till practice only when a high crop residue management practice is used. With high crop residue management practices, little or no crop residue is removed from the field during and after crop harvest.
USEPA reregistration use directions for atrazine products		
2005		Rates for corn and sorghum remain the same as 1992 rates. Roadside rates reduced to 1 lb a.i./A. Ecofallow rates reduced to 2.25 lbs a.i./A. Single turf rate reduced to 1 lb a.i./A, and maximum per year reduced to 2 lbs a.i./A in all states except Florida. Macadamia nut annual limit reduced to 8 lbs a.i./A. A toll-free watershed information phone number and website were added to the label. In Florida sod production, the maximum is a 4 lbs a.i./A single application, followed by a 2 lbs a.i./A application.

Index

AAtrex 4L, 36
AAtrex Nine-O®, 40
Acetolactate synthase (ALS) inhibitors, 136
  resistance to, 134, 135, 136–42
    cross-resistance, 140–2
    distribution of, 138–9
    fitness of, 137
    mechanisms, 137–40
    multiple resistance, 142
Acetyl coenzyme carboxylase (ACC) inhibitors, 136
  resistance to, 135
Aerosols, photolysis and, 333, 342–3
Aging of residues in soil:
  microbial degradation and, 317–18
  soil interactions and, 290–1
Agriculture:
  advances, 10
  trends:
    Europe, 57–61
    North America, 46–8
  yields, 1, 2
    triazine benefits, 8, 9, 155–7
  See also Specifi c crops
AGSIM aggregate economic model, 157–8, 160, 168, 170
Alachlor, 54
Alfalfa:
  crop rotation, 530–1
  secbumeton metabolism, 89–90
Almond, 218
Amenity weed control, 61
Ametryn, 27, 549
  dietary exposure, 420
  fruit crops, 204–5, 220
  metabolism of, 85–6
  photolysis, 333
  registration, 38
  sugarcane crops, 191, 194
  toxicity to aquatic organisms, 427, 434
Amphibians, atrazine effects on, 432
Analysis methods, 243–61
  detection technology, 248–60
    capillary electrophoresis, 251–2
    gas chromatography, 248–9
    high-performance liquid chromatography (HPLC), 250–1
    hyphenated chromatographic techniques, 252
    immunoassay, 252–60
    thin-layer chromatography, 251
  food extraction, 248
  soil extraction, 247
  water extraction, 244–7
Animal studies, *See* Safety; Sprague-Dawley (SD) rat model
Apple, 218
  simazine metabolism, 82
  See also Fruit crops
Apricot, 219
  See also Fruit crops
Aquatic organisms:
  atrazine toxicity assessment, 425–35
  conservation tillage benefits, 524
Aquatic Risk Assessment and Dialogue Group, 425
Arthropods, triazine toxicity assessment, 425–35
Asulam, 192
Asymmetrical triazines, 73, 74
  metabolism of, 90–6
  registrations, 39
  See also Specifi c triazines
Atomic emission detector, 248–9
Atraton, 27
  photolysis, 333, 337
  registration, 37
Atrazine, 549
  benefits of, 8, 9, 168–71
    application flexibility, 169
    broad-spectrum weed control, 169
    crop tolerance, 169
    economic benefits, 154–61, 169–70
    tillage compatibility, 169, 170
    weather insensitivity, 169
  combined use, 4, 169, 171, 528–9
    premixes, 40–1, 167, 528–9
  conifers, 228–9, 231
  corn crops, 163–73, 527–8
  DEA to atrazine ratio (DAR), 469–72
  dietary exposure, 417
    monitoring data, 420, 421
  ecofallow role, 177, 178, 180–1
  fruit crops, 213
    citrus, 205
  future prospects, 171–2
  history of, 25, 26, 69–70
    discovery, 22
    introduction in the United States, 39–40, 54, 163–4
    production, 32
  hydrolysis, 347, 349
  immunoassay validation studies, 260
  label changes, 573–4
  management of resistance to other herbicides, 145–6
  metabolism of, 75–82, 83
  microbial degradation, 302–4, 306–9, 313–19
  oil and, 40
  ornamentals, 227
  persistence, 231–2, 369–73
  photolysis, 333, 336–40, 342
  probabilistic risk assessment, 477–95
    aggregate exposure, 489–92
    cumulative exposure, 492–4
    dietary consumption, 483–6
    drinking water ingestion, 482–3
    handling by workers, 486–9
  registrations, 35–6
  reregistration, Europe, 9
  safety, 169, 394–5
    mammary tumor formation in rats, 399–409
    toxicity to aquatic organisms, 432
  soil interactions, 278
  soil movement, 357–8, 360, 361–7, 368

Atrazine (*continued*)
sorghum crops, 163–73
sugarcane crops, 190–1, 194, 196
tolerance, 169, 228
turf, 235–7, 239
usage, 4
Europe, 60
market share, 171
United States, 3, 171, 172, 453, 528–9
water:
degradation products, 466–7
exposure reduction in drinking water, 446
groundwater, 454–6
monitoring data, 439–40, 442–4, 446–8
precipitation, 463–6
reservoirs, 461–3, 469
surface runoff water, 456–61, 466
weeds controlled, 237
Yield Check Program, 39–40
See also Triazines
Atrazine chlorohydrolase (*atzA*) enzyme, 310–11
Australia:
scientific reviews, 9, 42
sugarcane crops, 193–4
Aziprotryn, 38

Bagasse, 187
Banana, ametryn metabolism, 85, 86
Best management practices (BMPs), 501–14
adoption and effectiveness of, 505–11
buffer impact on herbicides, 510–11
buffer impact on nitrogen, 509
buffer impact on phosphorus, 509
buffer impact on sediment, 508–9
conservation buffers, 507
conservation tillage, 505–7
vegetative buffers, 507–8
crop nutrient management, 511–12
integrated pest management, 512, 513
policies and government programs, 503–5
Clean Water Act, Section 319, 505
conservation compliance, 503–4
Conservation Reserve Program (CRP), 504
Conservation Security Program, 505
Environmental Quality Incentives Program, 505
Swampbuster Program, 504–5
Wetland Reserve Program (WRP), 504
Biodegradation, *See* Microbial degradation
Biofuel production, *See* Fuel production
Bioherbicide, 203
Biological control, citrus, 203
Bioremediation, 320–1
Black carbon, 277
Bobwhite Quail Initiative, 504
Brazil:
citrus crops, 199
fuel production, 1, 3
sugarcane crops, 194
Bromacil, 205, 206
Buffers:
conservation, 507
impact on herbicides, 510–11
impact on nitrogen, 509
impact on phosphorus, 509
impact on sediment, 508–9
vegetative, 507–8
Burning, 203
Butylate, 54–5

Cancer classification, 394–5
See also Carcinogenicity
Caneberry, 220
Capillary electrophoresis, 251–2
Carcinogenicity:
bioassays, 390–3
chlorotriazine cancer classification, 394–5
epidemiology, 394
Carfentrazone, 192
CDAA, 53–4
Celery, prometryn metabolism, 87, 88
Charcoal, 277
Check planting, 50
Chemical weed control, 68–9
ecofallow, 177
history, 51–6
See also Herbicides; Weed control
Cherry, 219
See also Fruit crops
China, fuel production, 1, 2
Chloramben, 54
Chlorazine, 22, 25
Chlorotriazines, 70, 73
cancer classification, 394–5
history of, 25–6
metabolism of, 73, 74, 77–85
registrations, 32–7
See also Specifi c triazines
Christmas trees, 225–6, 228–9, 231
Chromatography:
capillary electrophoresis, 251–2
gas, 248–9
high-performance liquid, 246, 250–1
hyphenated chromatographic techniques, 252
thin-layer, 251
Ciba Crop Protection benefits model, 153, 158–60
Citrus:
biological weed control, 203
chemical weed control, 203–6
postemergence herbicides, 206
preemergence herbicides, 204–6
success factors, 206
crops, 199–200
weeds in, 200–2
cultural methods of weed control, 203
economic benefits analysis, 160
physical methods of weed control, 202–3
weed prevention, 202
Clay, 286–8
Clean Water Act, Section 319, 505
Clomazone, 180, 192
Community water systems (CWS), *See* Water
Comprehensive Environmental Economic Policy Evaluations System (CEEPES), 153, 155, 156–7
Conifers, 225–32
tolerance of triazines, 229
weed management importance, 225–7, 230–1
Conservation Reserve Enhancement Program (CREP), 504
Conservation Reserve Program (CRP), 504

Conservation Security Program, 505
Conservation Technology Information Center (CTIC), 177, 503, 511
Conservation tillage, 5–6, 41, 55, 178, 519–25
adoption and effectiveness of, 505–7
cost and yield changes, 159–60
definition, 519
development of, 519–20
ecological benefits, 521–4
aquatic ecosystems, 524
soil erosion, 522–3
soil properties, 523–4
terrestrial wildlife, 524
water quality, 524
runoff and, 368
triazine herbicides in, 520–1
atrazine importance, 170
See also Best management practices (BMPs)
Continuing Survey of Food Intake by Individuals (CSFII), US, 414
Controlled-release formulations, 368
Corn:
crop rotation, 50–1, 530–1
fuel production, 1, 2
herbicide resistance, 55
herbicides used, 5
nonchemical weed control techniques, 529–37
aggregate studies, 537
bioeconomic models, 535–6
cover crops, 531–2
crop rotation, 530–1
cultivation, 532–5
farmers currently practicing, 536–7
popcorn, 160
production, 4, 527–8
Europe, 59–61
McCauley family farm, 541–6
sweet corn, 160
triazine benefits, 8, 9, 163–73
analysis approach, 167–8
conservation tillage and, 159–60
economic benefits analysis, 156–7, 158–61, 535–6
triazine metabolism, 82, 84, 85
Corn Belt, United States, 452, 453, 527–8, 544
Corn drill, 57–8
Cotton Herbicide Project, 458–61
Cotton, prometryn metabolism, 87
Cover crops, 203, 531–2
Crop rotation, 58, 512
comparisons, 179–80
corn, 50–1, 530–1
microbial degradation and, 319
Norfolk four-course rotation, 58
stacked rotations, 180
weed management and, 66, 530–1
Crop selectivity, 112–14
Cross-resistance, 106–7
negative cross-resistance, 127
risk from, 126–8
to ALS inhibitors, 136, 140–2
Crustaceans, triazine toxicity assessment, 425–35
Cultivation, *See* Tillage
Cunninghamella echinulata, 316
Cyanazine, 166, 549
dietary exposure, 420–1
ecofallow and, 178, 180
history of, 28
hydrolysis, 348
metabolism of, 84–5
microbial degradation, 316
persistence, 371
registrations, 36–7
soil movement, 358, 363–4, 366
toxicity to aquatic organisms, 427–8, 434
usage, 453
water, 445
degradation products, 467–8
groundwater, 456
precipitation, 463
reservoirs, 462
surface runoff water, 457, 458, 459
Cyanuric acid, 304, 310

D1 protein:
identification of, 102–3
rapid turnover significance, 107–8
resistance mechanisms, 106, 114
genetics of, 124–5
triazine 3D orientation in, 104–6
2,4-D, 52
ecofallow and, 178, 181
sugarcane crops, 191
N-Dealkylation, 74–5, 112–13
Deethylatrazine (DEA):
DEA to atrazine ratio (DAR), 469–72
water monitoring, 359, 467–9
groundwater, 360, 365–7, 455–6
surface runoff water, 457–8, 460–1, 470
Degradation, *See* Hydrolysis; Microbial degradation; Photolysis
Dehalogenation, 74
Deisopropylatrazine (DIA), water monitoring, 359, 467–9
groundwater, 360, 365–7, 455–6
surface runoff water, 457–8, 460, 470
Desmetryn, 27, 549
registration, 38
Detection technology, *See* Analysis methods
Developmental toxicity, 390, 391
Devine™, 203
Dicamba:
ecofallow and, 178, 181
sugarcane crops, 192
Dichlormid, 54
Dietary exposure, 413–21
food consumption surveys, 414
residues in animal commodities, 417–19
risk assessment, 483–6
process, 414–17
triazine monitoring data, 419–21
See also Food analysis; Water
Dietary Exposure Evaluation Model (DEEM), 414
Dietary Risk Evaluation System (DRES), 414, 485
Dimethametryn, 38, 549
Dipropetryn, 38
Dissipation, *See* Hydrolysis; Microbial degradation; Photolysis
Dissolved organic carbon (DOC):
hydrolysis and, 349
photolysis and, 341–2

Diuron:
citrus crops, 205
sugarcane crops, 192, 194, 196
Drinking water, *See* Water
Dust Bowl, United States, 5–6, 176, 543
See also Great Plains
Dust–mulch fallow, 176

Earthworm burrows, soil movement and, 360
Ecofallow, 165–6, 175–82
crop rotation and tillage systems, 179–80
herbicides for, 178
insect and plant disease control, 180
native prairie conversion to cropland, 176–8
plant residue effects, 178–9
triazine role, 180–1
water-use, 178, 179, 180
See also Conservation tillage
Ecological risk assessment, 425–6
Economic benefits assessment, 153–61
conservation tillage and, 159–60
weed control in corn, 535–6
Effective dose (ED_{10}), 479, 494
Electrospray ionization-liquid chromatography (ESI-LC), 245, 251
Environmental dissipation, *See* Hydrolysis; Microbial degradation; Photolysis
Environmental issues, 55
benefits of triazines, 5–7
atrazine, 169
See also Conservation tillage; Ecofallow; Toxicity; Water
Environmental Quality Incentives Program, 505
Enzyme immunoassay (EIA), 253, 255, 258
Enzyme-linked immunosorbent assay (ELISA), 253, 255, 256, 258
Estrogen-related activity, Sprague-Dawley rat, 401–2
Estrous cycling effects, Sprague-Dawley rat, 402–5, 407
Ethanol production, 1, 2–3
S-ethyl dipropylthiocarbamate (EPTC), 54–5
Europe:
agricultural trends, 57–61
corn production, 59–61
reregistration issue, 9
scientific review, 42
Exposure, *See* Dietary exposure; Probabilistic risk assessment; Water
Extraction:
food, 248
liquid-liquid (LLE), 244, 247
soil, 247
solid-phase (SPE), 244–7
solid-phase microextraction (SPME), 246–7
supercritical fluid (SFE), 247
water, 244–7

Farm size trends, 46
Farm-Pak® Mini-Bulks, 40
Farmable Wetland Program, 504
Farming, *See* Agriculture
Faulkner, Edward, 519–20, 543
Ferric ion, photolysis and, 338–40
triazine removal from water, 347
Fertilizers, microbial degradation and, 314–15
Fish, toxicity assessment, 425–35
Food analysis:
extraction, 248
immunoassay, 257
See also Dietary exposure
Food Quality Protection Act (FQPA), US, 413, 480
Forestry, 225–7, 230–2
microbial degradation, 319
France:
corn production, 59–60
scientific review, 10, 42
Frogs, atrazine effects on, 432
Fruit crops, 211–12
weed control, 213–20
herbicides used, 216–20
principles, 215
triazine foliar symptoms, 216
triazine movement and irrigation, 215–16
triazine residues in soils, 216
weed problems, 212–13
See also Citrus
Fuel production, 1, 2–3
Fulvic acid, 341
hydrolysis and, 349
photolysis and, 341–2

Gas chromatography, 248–9
atomic emission detector, 248–9
column technology, 248
high-resolution (HRGC), 248, 249
injection systems, 248
mass spectrometry, 249
Gast, Albert, 13, 21, 23
Glutathione (GHS), 112
conjugation, 75–7, 111, 112
resistance mechanism, 112, 115
Glyphosate:
citrus crops, 205
crop resistance, 55
ecofallow and, 178, 181
sugarcane crops, 192
weed resistance, 142–3
Grape, 219–20
simazine metabolism, 82
See also Fruit crops; Vineyards
Grass, *See* Turf
Grazing, 203
Great Plains, United States, 175–6, 544
crop rotation and tillage systems, 179–80
Dust Bowl, 5–6, 176
ecofallow, 175–81
herbicides for, 178
native prairie conversion to cropland, 176–7
triazine herbicide role, 180–1
water use efficiency, 178–9
limited irrigation, 180
Groundwater, 232, 451, 454–6
triazine degradation products, 360, 365–7
triazine movement to groundwater, 359–67

Hairy vetch cover crop, 532
Halosulfuron, 192
Hansch equation, 104
Hazard assessment, *See* Safety
Hazard quotient (HQ), 425

Health risk assessment, *See* Risk assessment
Herbicide Resistance Action Committee (HRAC), 123, 137
Herbicides:
banding of, 534
bioherbicide, 203
history of use, 51–6, 68–9
weed biology and, 66–7
See also Chemical weed control; *Specifi c herbicides*
Hexazinone, 70, 74, 549
conifers, 228–9, 231
hydrolysis, 348
metabolism of, 90, 91
ornamentals, 227
persistence, 232, 371
photolysis, 345
registration, 39
soil movement, 364
sugarcane crops, 191, 194, 196
tolerance, 228
toxicity to aquatic organisms, 428–9, 434
High Plains, *See* Great Plains, United States
High-performance liquid chromatography (HPLC), 246, 250–1
column technology, 250
mass spectrometry, 250–1
UV detectors, 250
High-resolution gas chromatography (HRGC), 248, 249
Highly erodible land (HEL), 503–4
Hoeing, 202–3, 533
Hot water for weed control, 203
Humic substances, 277–8
hydrolysis and, 349
photolysis and, 341–2
Hydrogen peroxide, photolysis and, 340–1
triazine removal from water, 346–7
Hydrolysis, 283–4, 347–9
DOC effect, 349
in aqueous solution, 347–8
in presence of soil, 349
Hydrolytic dehalogenation, 74
Hydroxyatrazine, 308, 312, 313
Hyphenated chromatographic techniques, 252

Illinois, 46–9
Immunoassay, 252–60
advantages and disadvantages, 260
antibody specificities, 254
background, 252–3
food analyses, 257
innovations, 258–60
pesticide applicators, 257
soil analyses, 256–7
types of, 253
water analyses, 253–6
validation studies, 260
Insects, biological weed control, 203
Integrated pest management (IPM), 7–8, 41, 134, 501, 512, 513
Integrated weed management (IWM), 134, 143–4
International Agency for Research on Cancer (IARC), 10, 42
Ipaton, 27
Ipazine, 26
Irrigation:
Great Plains, 180
triazine movement and, 215–16
Isoxaben, 239–40

Klebsiella pneumonia, 306
Knüsli, Enrico, 13–21

Lake monitoring, 461–3
Lake Erie Agricultural Systems for Environmental Quality (LEASEQ) project, 507
Land Stewardship Project, 537
Large volume on-column injection (LVI), 248
Leaching Estimation and Chemistry Model-Pesticide (LEACHP), 373
Leaching, *See* Persistence; Soil movement
Lethal Concentration 50 (LC_{50}), 388
Lethal Dose 50 (LD_{50}), 388, 400
Lifetime average daily dose (LADD), 479, 481
aggregate exposure, 489–92
cumulative exposure, 492–4
dietary consumption, 483, 485
drinking water ingestion, 482
herbicide handling by workers, 486–8
Lipids, in soil, 277
Liquid-liquid extraction (LLE), 244, 247
Lo-tillage systems, 177–8
See also Conservation tillage
Lowest observed effect levels (LOELs), 387, 388–90, 478
Luteinizing hormone surge, Sprague-Dawley rat, 405–7

McCauley family farm, 541–6
Mammary tumor formation in rat, *See* Sprague-Dawley (SD) rat model
Manure, microbial degradation and, 315
Margin of exposure (MOE), 414, 477–9, 482, 494
cumulative exposure, 492–4
dietary consumption, 483–6
drinking water ingestion, 482–3
herbicide handling by workers, 488–9
Mass spectrometry (MS), 249, 250–1
multiple stages of (MS^n), 249, 251
Mass spectrometry/mass spectrometry (MS/MS), 249, 251
Mauritius, sugarcane production, 195
Maximum Tolerated Dose (MTD), 390, 400
Metabolism, 74–96
atypical triazines, 90–6
chlorotriazines, 77–85
early research, 74–5
glutathione conjugation, 75–7
metabolites, 552–62
methoxytriazines, 89–90
methylthiotriazines, 85–9
resistance mechanisms, 115–16, 125
Metamitron, 550
photolysis, 344
registration, 39
Methoprotryn, 27
registration, 38
Methoxytriazines, 70, 74
history of, 26–7
metabolism of, 73, 89–90
registrations, 37–8
See also Specifi c triazines
Methylthiotriazines, 70, 73–4
history of, 27–8
metabolism of, 73, 85–8
registrations, 38–9
See also Specifi c triazines

Metolachlor, 167
Metribuzin, 54, 70, 74, 550
dietary exposure, 420, 421
intermediate resistance to, 127
metabolism of, 90–6
long-term, 94–6
short-term, 90–4
microbial degradation, 318
persistence, 371–2
photolysis, 343–4
registration, 39
soil movement, 358–9, 364, 366
tolerance mechanisms, 113
toxicity to aquatic organisms, 429, 434
turf, 236, 238, 240
weeds controlled, 237
Micellar electrokinetic chromatography (MEKC), 251–2
Microbial degradation, 301–21, 355–6
bioremediation, 320–1
early research, 304–5
environmental biodegradation, 312–19
aging effect, 317–18
agricultural practices and, 319
biotic versus abiotic degradation, 312
electron acceptors, 318–19
exogenous organic matter effect, 314–15
mixtures of herbicides, 315–16
moisture effect, 316
pH effect, 314
presence of degrading microorganisms, 313–14
rhizosphere soil, 318
soil depth and, 316–17
soil organic matter effect, 314
temperature effect, 316
enzymology, 310–11
evolution of microbial abilities, 305–6
genetics of, 306–9
microorganisms capable of degrading triazines, 302
Molasses, 187
Molecularly imprinted polymers (MIP), 247
Monte Carlo simulation, 479–80, 481–2, 489, 494
Movement, *See* Soil movement
Mowing, 203
MSMA, 236
Mulching, 202–3
dust–mulch fallow, 176
stubble–mulch fallow, 176–7, 179
Multiple stages of mass spectrometry (MS^n), 249
Multiple-resistance, 127
in ALS-resistant biotypes, 142
Mutagenicity, 390, 392

Naphthalic anhydride, 167
National Agricultural Statistics Service (NASS), 512
National Food Processors Association (NFPA), US, 421
National Water Quality Assessment (NAWQA), 502–3
Nationwide Food Consumption Survey (NFCS), US, 414
Natural Resources Conservation Service, 510
Natural Resources Inventory, 523
Negative cross-resistance, 127
Nitrate, photolysis and, 337
No observed effect levels (NOELs), 387–90, 400, 414, 478–9
No tillage systems, 177–8, 179, 181, 520
See also Conservation tillage
Nonaqueous capillary zone electrophoresis (NA-CZE), 252
Noraton, 27
Norazine, 22, 25–6
Norflurazon, 205
Norfolk four-course rotation, 58
North American Herbicide Resistance Action Committee (NAHRAC), 123, 137
Nurseries, *See* Ornamentals
Nut crops, 211–13, 218
triazine foliar symptoms, 216

Orchards:
weed control, 213–20
herbicides used, 216–20
weed problems, 212–13
See also Fruit crops
Ornamentals, 225–32
triazine role, 227–32
triazine tolerance, 228, 230
weed management importance, 225–7
Oryzalin, 205
Oxadiazon, 238

Paraquat, 178
Peach, 218–19
See also Fruit crops
Pear, 218
See also Fruit crops
Pecan, 218
Pendimethalin, 191
Persistence, 369–74
forest soils, 231–2
See also Hydrolysis; Microbial degradation; Photolysis; Soil movement
pH:
hydrolysis and, 347–8
microbial degradation and, 314
triazine–soil interactions and, 288
Phanerochaete chrysosporium, 314, 315
Photolysis, 331–47
direct, 331, 333–6
hexazinone, 345
in aerosols, 333, 342–3
in aqueous solutions, 332, 333–42
involving energy transfer, 336–7
involving hydroxyl radical formation, 337–42
removal of triazines from water, 345–7
indirect, 331, 336–42
metamitron, 344
metribuzin, 343–4
products, 334–5
thin film studies, 332–3
Photosynthesis inhibition:
binding to herbicide-binding protein, 104–6
quantitative structure activity relationships, 104
rapid D1 protein turnover significance, 107–8
target protein identification, 102–3
Phytoremediation, 320
Pineapple, 220
See also Fruit crops
Pituitary LH surge, Sprague-Dawley rat, 405–7
Plant residues, ecofallow, 178–9
Plant roots, soil movement and, 360, 361

Plum, 219
See also Fruit crops
Point of zero net charge (PZNC), 280
Polyoxometalates, photolysis and, 340
triazine removal from water, 347
Popcorn, 160
Population-linked exposure (PLEX) database, 441–5
Potato, cyanazine metabolism, 84
Probabilistic risk assessment, 477–95
aggregate exposure, 489–92
benefits of, 480
cumulative exposure, 492–4
dietary consumption, 483–5
drinking water ingestion, 482–3
exposure characterization, 480–1
herbicide handling by workers, 486–9
margin of exposure, 482
Prometon, 26, 469, 550
metabolism of, 89
registration, 37–8
toxicity to aquatic organisms, 429, 434
Prometryn, 27, 550
dietary exposure, 420
metabolism of, 87, 88
registration, 38
soil movement, 359, 364
toxicity to aquatic organisms, 430, 434
water monitoring, 459–461, 469
Propachlor, 54
Propazine, 26, 166, 550
ecofallow and, 178
metabolism of, 82
photolysis, 333, 337
registration, 37
soil movement, 364
water monitoring, 445, 458, 460–1
Prune, 219
PS II inhibition:
binding to herbicide-binding protein, 104–6
quantitative structure activity relationships, 104
rapid D1 protein turnover significance, 107–8
target protein identification, 102–3
psbA gene:
identification, 103
mutation in resistance plants, 124–5
Pseudomonas, 305–11, 313, 316, 318, 320

Quantitative structure activity relationships (QSAR), 104

Ralstonia pickettii, 313, 315–16
Rat studies, *See* Safety; Sprague-Dawley (SD) rat model
Reduced-tillage systems, 177–8, 181
Registrations:
asymmetrical triazines, 39
chlorotriazines, 32–7
methoxytriazines, 37–8
methylthiotriazines, 38–9
Regulatory reviews, 41–2
Reproductive toxicity, 390
Reregistrations, 8–10
Research, 10
Reservoir monitoring, 451, 461–3
Resistance, 114, 227, 238
crop resistance, genetic modification for, 55
cross-resistance, 106–7
risk from, 126–8
distribution of, 120–4, 134
documentation of, 123
fitness and, 124
genetics of, 124–5
history of, 119–20, 121–2
management of, 7–8, 126
modeling role, 144
triazine role in management of resistance to other herbicides, 145–6
mechanisms, 114–16, 238
enhanced herbicide metabolism, 115–16
target site-based resistance, 106, 114–15
multiple-resistance, 127
significance of, 134–6
to nontriazine herbicides, 133–46
ALS inhibitors, 136–42
glyphosate, 142–3
Rhizobium, 306
Rhizosphere soil, 318
Rhodococcus, 305, 306–7, 316
Ridge tillage system, 534–5
Risk assessment, 478–80
dietary exposure, 414–17
ecological, 425–6
See also Probabilistic risk assessment; Toxicity
Row crop management, 49–51, 533
Runoff, 7, 501
management effects, 367–9
See also Surface runoff water
Rye cover crop, 532

Safe Drinking Water Act (SDWA), US, 441
Safeners, 54
Safety, 395
acute toxicity studies, 388, 389
advances in testing, 10
atrazine, 169
carcinogenicity, 390–4
bioassays, 390–3
epidemiology, 394
developmental and reproductive toxicity, 390, 391
mutagenicity, 390, 392
toxicity after repeat exposure, 388–90
Scientific reviews, 8–10
Secbumeton, 27
metabolism of, 89–90
registration, 38
Selectivity:
triazine-tolerant crops, 112–14
triazine-tolerant weeds, 114
See also Resistance
Sethoxydim, 55
Simazine, 550
combined use, 227
conifers, 228–9, 231
dietary exposure, 417
monitoring data, 420, 421
fruit crops, 213–14, 215, 218–20
citrus, 204, 205
history of, 23–5, 69, 166
discovery, 22
production, 32

Simazine (*continued*)
metabolism of, 74–5, 82
microbial degradation, 302–4, 305, 316, 317
nut crops, 218
ornamentals, 227–8, 230
persistence, 231–2, 372
photolysis, 333, 337
probabilistic risk assessment, 477–95
aggregate exposure, 489–92
cumulative exposure, 492–4
dietary consumption, 483–6
drinking water ingestion, 482–3
handling by workers, 486–9
registrations, 32–5
reregistration issue, Europe, 9
soil movement, 359, 364–5, 368–9
sugarcane crops, 190
toxicity to aquatic organisms, 430–1, 435
turf, 235–8, 239
usage, United States, 4, 453
water:
exposure reduction in drinking water, 446
groundwater, 454–6
monitoring data, 439–40, 444, 446–8
surface runoff water, 458
weeds controlled, 237
See also Triazines
Simeton, 26
Simetryn, 27, 551
registration, 38
Sod, 203
production of, 238–9
Soil analysis:
extraction, 247
immunoassay, 256–7
Soil erosion, 5–6
conservation tillage benefits, 522–3
impact of, 502
United States Dust Bowl, 5–6, 176, 543
Soil interactions, 275–92
hydrolysis of triazines, 283–4
quantification of, 284–91
aging effect, 290–1
clay content effect, 286–8
concentration effect, 289
dissolved organic carbon effect, 288–9
organic matter effect, 286
pH effect, 288
temperature effect, 291
water content effect, 291
with inorganic soil materials, 279–83
permanent charge surfaces, 281–3
uncharged surfaces, 279
variable charge surfaces, 280
with organic matter in soil, 276–8, 286
See also Soil movement; Soils
Soil movement, 355–74
downward movement, 360
management effects, 367–9
to groundwater, 359–67
to surface runoff water, 356–9, 470–1
See also Persistence; Soil interactions; Soils
Soils:
conservation tillage effects, 523–4
herbicide activity and, 216
herbicide residues in, 216
hydrolysis in, 349
microbial degradation in, 312–19
organic matter, 276–8
microbial degradation and, 314
triazine–soil interactions and, 286
organic waste addition, 368–9
persistence in, 369–74
forests, 231–2
rhizosphere soil, 318
vadose zone, 316–17
See also Soil analysis; Soil erosion; Soil interactions; Soil movement
Solid-phase extraction (SPE), 244–7
Solid-phase microextraction (SPME), 246–7
Sorghum:
crop rotation systems, 179
fallow, 177
triazine benefits, 163–73
analysis approach, 167–8
conservation tillage and, 159–60
economic benefits analysis, 156–7, 158–61
triazine metabolism, 75–7, 82–4, 87
Sorption, *See* Soil interactions
South Africa, sugarcane production, 195–6
Soybean, 50
corn/soybean crop rotation, 50–1
herbicide resistance, 55
metribuzin metabolism, 93–6
row crop management, 49–51
Sprague-Dawley (SD) rat model:
carcinogenicity bioassays, 390–3
mammary tumor formation, 399–409, 478
bioassay data, 400–1
estrogen-related activity tests, 401–2
estrous cycling effects, 402–5, 407
pituitary LH surge effect, 405–7
Sprigging, turf, 236–7
Spyders, 533
Stewardship practices, 10, 543–4
Strong cation exchange (SCX) cartridge, 245–6
Stubble–mulch fallow, 176–7, 179
Sucrose, 186
Sugarcane, 185–7
economic benefits analysis, 160
fuel production, 1, 3
losses imposed by weeds, 189
production of, 186–7
atrazine metabolism, 77–81, 83, 85, 90
weed biology and, 188–9
weed control, 187–8, 189–96
animal power, 188
Australia, 193–4
Brazil, 194
by hand, 187–8
chemical control, 189–96
Mauritius, 195
mechanization, 188
South Africa, 195–6
weed targets, 192–193
Sulfentrazone, 192
Supercritical fluid extraction (SFE), 247
Surface runoff water, 356–9, 451, 456–61
Surfactants, photolysis and, 342
Sustainable agriculture, 527–38

Swampbuster Program, 504–5
Sweet corn, 160

Target site-based resistance, 106, 114–15
Tebuthiuron, 194
Temperature:
  hydrolysis and, 347
  microbial degradation and, 316
  soil–triazine interactions and, 291
Terbacil, 205
Terbumeton, 38, 551
Terbuthylazine, 9, 26, 61, 551
  metabolism of, 82–4
  microbial degradation, 315
  photolysis, 341, 342–3
  registrations, 37
Terbutryn, 27, 551
  ecofallow and, 178, 180
  metabolism of, 87, 89
  registrations, 38–9
  toxicity to aquatic organisms, 431, 435
Thin-layer chromatography, 251
Tillage systems, 521
  atrazine compatibility, 169
  comparisons, 179–80
  ecofallow, 177–8
  fruit crops, 213–14
    citrus, 202
  persistence and, 373
  primary, 532–3
  ridge tillage system, 534–5
  runoff and, 368
  secondary, 532–3
  weed biology and, 65–6
  weed control, 532–5
  See also Conservation tillage
Tolerance:
  conifers, 228–9
  crops, 112–14, 169
  dietary, 414, 416
  ornamentals, 228, 230
  weeds, 114
  See also Resistance
Tomato, metribuzin metabolism, 92–3
Toxicity:
  acute toxicity studies, 388, 389
  after repeat exposure, 388–90
  aquatic organisms, 425–35
    toxicity distributions, 433–5
  carcinogenicity, 390–4
    bioassays, 390–3
    epidemiology, 394
  developmental, 390, 391
  reproductive, 390
Tractors, introduction of, 46
Transport in soils, *See* Soil movement
Trappea darkeri, 314
Triazines, 330–1
  chemistry of, 276, 330, 549–52
  conservation tillage and, 520–1
  economic benefits assessment, 153–61
  future prospects, 171–2
  history of, 25–7, 69–70
    discovery, 1, 13–25
  metabolism of, 74–96
    metabolites, 552–62
    resistance mechanisms, 115–16, 125
  mode of action, 101–6
    binding to herbicide-binding protein, 104–6
    quantitative structure activity relationships, 104
    significance of rapid D1 protein turnover, 107–8
    target protein identification, 102–3
  production, 32
  resistance to, *See* Resistance
  selectivity, 112–14
  usage:
    primary uses, 3
    United States, 1, 3–4, 440, 453
  weeds controlled, 237
  See also Specifi c triazines
Trietazine, 22, 25
  registrations, 37
Trifloxysulfuron, 192
Trifluralin, 54, 191
Tungstates, photolysis and, 340
Turf, 235–40
  alternatives to triazines, 239–40
  sod production, 238–9
  warm-season turfrass establishment, 236–8
  weed control, 235–6

Ultra pressure liquid chromatography (UPLC), 250
Ultraviolet (UV):
  detectors, 250
  triazine removal from water by photolysis, 345–7
United Kingdom:
  amenity weed control, 61
  scientific reviews, 9, 42
United States:
  agricultural yields, 2
  best management practices (BMPs), 501–15
    adoption and effectiveness of, 505–11
    policies and government programs, 503–5
  conservation tillage, 5–6, 159–60, 505–7
  corn production, 527–8
  ecofallow, 175–81
  ethanol production, 1, 2
  fruit crops, 211
    citrus crops, 200
  future directions, 171–2
  ornamentals, 227
  scientific reviews, 8–9, 41–2
  triazine benefits, 8, 9
    corn crops, 163–73
    economic benefits analysis, 153–61
    sorghum crops, 163–73
  triazine introduction, 39–42
  triazine usage, 1, 3–4, 451, 453, 528–9
  water:
    drinking water monitoring data, 439–48
    groundwater, 451, 454–6
    precipitation, 452, 463–6
    reservoirs, 451, 461–3
    surface runoff water, 451, 456–61
  weed control practices, 45–56
    history, 46–8
  See also Great Plains
United States Department of Agriculture (USDA), 413–14, 419
  triazine monitoring, 420–1

United States Environmental Protection Agency (USEPA):
Pesticide Handlers Database (PHED), 486
Pesticide Toxicity Database, 426
public document (PD-1), 167
Safe Drinking Water Act (SDWA), 441
solid-phase extraction method, 244
Special Review, 153, 154, 167–71
toxicity studies, 388
United States Food and Drug Administration (USFDA), 419
triazine monitoring, 419–20
United States Geological Survey (USGS):
Midcontinent Herbicide Project, 457–8
Toxic Substances Hydrology Program, 452
University Corn Model, 168
UV, *See* Ultraviolet (UV)

Vadose zone:
microbial degradation in, 316–17
Vegetative filter strips (VFS), 369
Vineyards:
triazine foliar symptoms, 216
weed control, 213, 219–20
weed problems, 212–13
See also Fruit crops; Grape

Walnut, 217, 218
Water analysis:
extraction, 244–7
immunoassay, 253–6
Water conservation, 7, 178
plant residue effects, 178–9
precipitation storage as plant-available soil water, 179
Water, 55
conservation tillage benefits, 524
drinking water monitoring data, 439–48
exposure reduction in drinking water, 446
groundwater, 232, 359–67, 451, 454–6
precipitation, 452, 463–6
reservoirs, 451, 461–3
risk assessment, 482–3
surface runoff water, 356–9, 451, 456–61
triazine degradation products, 359, 365–7, 466–9
Weed control:
aggregate studies, 537
amenity weed control, 61
conifers, 225–32
costs and benefits, 212
See also Economic benefits assessment
development of, 67–8
fruit crops, 213–20
citrus, 202–6
history, 46–8, 163
chemical weed control, 51–6
row crop management, 49–51, 533
'no seed' threshold, 66
nonchemical, 527–8, 529–37
bioeconomic models, 535–6
cover crops, 531–2
crop rotation, 530–1
cultivation, 532–5
farmers currently practicing, 536–7
North America, 45–56
objectives, 68
ornamentals, 225–32
sugarcane, 187–96
turf, 235–6
See also Chemical weed control; Herbicides; *Specific methods*
Weed Science Society of America (WSSA), 123, 527
Weed thresholds, 143–4
WEEDCAM model, 535–6
Weeds, 563–72
definition, 64
ecology of, 63–4
herbicides and, 66–7
resistance to triazines, *See* Resistance
seed biology, 64–5
seed predation, 65–6
tillage systems and, 65–6
tolerance of triazines, 114
See also Weed control
WEEDSIM model, 535–6
Wetland Reserve Program (WRP), 504
Wetlands Initiative, 504
Wheat:
crop rotation systems, 179
fallow, 177
stubble effects, 178–9
triazine metabolism, 84–5, 87, 89
yields, 58–9
World Health Organization, International Agency for Research on Cancer (IARC), 10, 42

Yields, 1, 2
triazine benefits, 8, 9, 155–7