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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

July 12, 1994

108801

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on **METOLACHLOR (4th)**

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (7509c)

TO: Addressees

Attached for your review is a package on **METOLACHLOR** prepared by Dr. Stephen Dapson.

A meeting to consider the carcinogenicity classification of this chemical is scheduled for **Wednesday July 27, 1994**, at **10:00 am in Room 817, CM2.**

Addressees

P. Fenner-Crisp
R. Schmitt
S. Irene
R. Engler
W. Burnam
K. Baetcke
M. Van Gemert
K. Dearfield
H. Pettigrew
B. Fisher
L. Brunsman
E. Doyle
S. Dapson
M. Ioannou
R. Hill
Y. Woo
R. DiLavore/L.Brennecke

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PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Metolachlor, 4th.

TO: Esther Rinde, Ph.D.
Manager, Peer Review for Carcinogenicity
Science Analysis Branch
Health Effects Division (7509C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/7/94
Senior Pharmacologist, Review Section I
Toxicology Branch II/Health Effects Division (7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. Y M Ioannou 7/7/94
Section Head, Review Section I
and
Marcia van Gemert, Ph.D. Y M Ioannou for MvG - 7/7/94
Branch Chief
Toxicology Branch II/Health Effects Division (7509C)

Attached is the additional information provided by the registrant for consideration of the weight-of-evidence on the carcinogenicity potential of Metolachlor by the Health Effects Division Peer Review Committee for Carcinogenicity.

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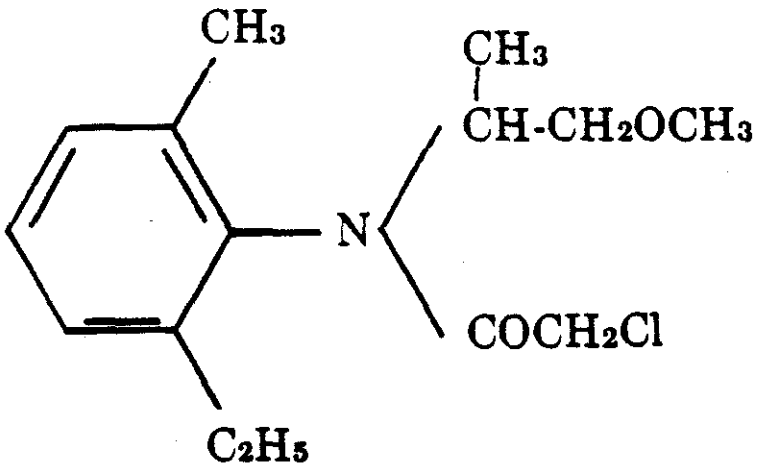
Proposed metabolic pathways for Metolachlor
Previous Peer Review Document #3
Toxicology One-Liners for §82 series and higher
Documents from Ciba-Geigy
Metabolism data from 1988 document and SAP submission
Documents from Monsanto relating to metabolism of alachlor and metolachlor
Statistical Analysis Document

I. Background

Metolachlor is the common name for 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide, one of the family of chloracetanilides. The CAS number is 51218-45-2, EPA Pesticide Chemical Code is 108801, and the Toxicology Chemical Number is 188DD.

Metolachlor is a selective herbicide registered for use (CFR 180.368) for the control of most annual grasses and certain broadleaf weeds in corn, soybeans, peanuts, grain sorghum, potatoes, pod crops, cotton, safflower, and woody ornamentals.

STRUCTURE OF METOLACHLOR



Metolachlor

II. Previous considerations by the Carcinogenicity Peer Review Committee:

i. First Peer Review

The Toxicology Branch Peer Review Committee met to discuss and evaluate the data base on Metolachlor, with particular reference to the carcinogenic potential of the chemical on May 30, 1985.

A. Individuals in Attendance:

1. Peer Review Committee: Theodore M. Farber, Ph.D., Richard Hill, M.D., Harry Milman, Ph.D., Louis Kasza, D.V.M., Herbert Lacayo, Ph.D., John A. Quest, Ph.D.

2. Reviewers: Gary Burin, M.P.H., D. Steven Saunders, Ph.D., Laurence D. Chitlik, D.A.B.T.

B. Material Reviewed:

The material reviewed by the Peer Review Committee (PRC) consisted of Data Evaluation Records (DER's) of rat and mouse chronic carcinogenicity studies, laboratory audit reports and related memoranda on the studies, risk assessment information, data on other toxicology studies (mutagenicity, subchronic), developmental toxicity (reproduction and teratology studies), and the one-liners for Metolachlor.

C. Classification of Carcinogenic Potential from the 5/30/85 meeting:

The PRC concluded that the data available for Metolachlor provides weak evidence of carcinogenicity. Before making a final conclusion on the carcinogenic potential of Metolachlor, the Committee recommended that the registrant provide:

(1) the full mutagenicity battery required by EPA; and

(2) metabolism studies as required by the 1982 guidelines.

Subsequent to receipt of this information, the Committee was to reconvene to consider classification of the carcinogenic potential of the chemical and possible recalculation of the Q_1* (potency factor).

D. Toxicology Issues reviewed in the 5/30/85 meeting:

Metolachlor was observed to produce primary liver tumors (neoplastic nodules plus hepatocellular carcinomas combined) in female rats at the highest dose level tested in two separate chronic feeding studies sponsored by the registrant, Ciba-Geigy Corp.

The initial rat chronic feeding study was performed at Industrial Biotest Laboratories (IBT) and was classified as **Core-Supplementary Data**. A repeat rat chronic feeding study was performed at Hazleton-Raltech, Inc., and was classified as **Core Minimum Data**. Although both studies were given careful consideration, the PRC focused attention primarily on data in the Hazleton-Raltech rat study because of the **Core Minimum Data** classification.

The registrant also evaluated the carcinogenic potential of Metolachlor in two mouse carcinogenicity feeding studies. One of the studies was performed at IBT, and a repeat study was performed at Hazleton-Raltech, Inc. Both mouse studies were classified as **Core Minimum Data**, and no evidence of carcinogenicity was found in either study.

ii. Second Peer Review

The Health Effects Division Peer Review Committee for Carcinogenicity met to discuss and evaluate the weight-of-the-evidence on Metolachlor, with particular reference to the carcinogenic potential of the chemical on April 17, 1991.

A. Individuals in Attendance:

1. Peer Review Committee: Penelope A. Fenner-Crisp, William L. Burnam, Reto Engler, Karl Baetke, Marcia Van Gemert, Esther Rinde, Kerry Dearfield, Marion Copley, Robert Beliles, Juli Du, Yin-Tak Woo, Hugh Pettigrew.

2. Reviewers: Stephen Dapson, M. Ioannou

B. Material Reviewed:

The material reviewed by the Peer Review Committee (PRC) consisted of the previous peer review committee discussion, Data Evaluation Records (DER's), one-liners, and other data summaries prepared by HED scientists and an executive summary of data provided by the registrant.

C. Classification of Carcinogenic Potential from the 4/17/91 meeting:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered. The Peer Review Committee agreed that metolachlor should be classified as Group C, a Possible Human Carcinogen, and recommended that a linear low dose extrapolation model be applied to the incidence of hepatic neoplasia observed in the rat for the purpose of risk characterization. This recommendation was based on the replication of the finding of hepatic neoplasia in female rats and the apparent induction of a small number of nasal turbinate tumors, an uncommon tumor induced by certain analogs of metolachlor, at the high dose level in the second rat study. Application of a linear low dose extrapolation model will facilitate comparison of the cancer potency of metolachlor with other members of this class of herbicide.

The PRC recommended that an *in vivo/in vitro* unscheduled DNA synthesis assay be conducted to fulfill the data gap for genotoxicity testing.

D. Toxicology Issues reviewed in the 4/17/91 meeting:

Metolachlor was found to induce hepatic neoplasia in female rats in two separate studies at a dose level of 3000 ppm. Most of the tumors which were induced by metolachlor were benign. The small number of nasal turbinate tumors at the high dose level in the second rat study (2 adenocarcinomas and 1 fibrosarcoma) are suggestive of a response at that site although not standing alone, convincing evidence of carcinogenicity. Nasal turbinate tumors are considered to be rare in oral carcinogenicity bioassays but have been associated with the dietary administration of two analogs (acetochlor and alachlor). Although no statistically significant increase in neoplasia was observed in male rats, there was a positive trend for hepatic neoplastic nodules. No increase in the incidence of neoplasia was associated with metolachlor administration in mice. The limited genotoxicity data for metolachlor are negative. Metolachlor is structurally related to several compounds which induce neoplasia in rats and/or mice.

iii. Third Peer Review

The Health Effects Division Peer Review Committee for Carcinogenicity met to discuss and evaluate the weight-of-the-evidence on Metolachlor, with particular reference to the carcinogenic potential of the chemical on April 14, 1993.

A. Individuals in Attendance:

1. Peer Review Committee: Penelope A. Fenner-Crisp, Reto Engler, William L. Burnam, Karl Baetke, Marion Copley, Kerry Dearfield, Julie Du, Hugh Pettigrew, Esther Rinde.

2. Reviewers: Stephen Dapson, M. Ioannou, Lori Brunsman, Lucas Brennecke.

B. Material Reviewed:

The material reviewed by the Peer Review Committee (PRC) consisted of the previous peer review committee discussion, Data Evaluation Records (DER's), one-liners, SAP meeting noted, a pathology report and new data provided by the registrant.

C. Classification of Carcinogenic Potential from the 4/14/93 meeting:

The PRC considered the criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986} for classifying the weight of evidence for carcinogenicity. The PRC agreed that the classification for Metolachlor should be Group C - possible human carcinogen and recommended that, for the purpose of risk characterization, a low dose extrapolation model be applied to the experimental animal tumor data (q_1*) for quantification of human risk. The q_1* will be re-calculated based on hepatic neoplasia (combined adenoma and carcinoma) in female rats using the data in the pathology report by Dr. Jerry Hardisty (dated July 24, 1984, Table 4).

The PRC recommended that the registrant perform additional tests in stages. First it was recommended that the *in vivo/in vitro* unscheduled DNA test be repeated to fulfill the data gap for genotoxicity testing. If this proved negative, the PRC recommended that the registrant provide data on the identification of metabolites from the submitted study "Disposition of Metolachlor in the rat (general metabolism)" to resolve issues and fulfill the data requirements. Finally, a comparative metabolism study was recommended in order to provide information on the metabolic generation of quinone imine and formaldehyde and to support the assertion that the metabolism of Metolachlor is substantially different from its major structurally analogous compounds. After the completion and evaluation of the recommended studies, the PRC agreed to reconsider the weight-of-evidence for the classification of Metolachlor.

D. Toxicology Issues reviewed in the 4/14/93 meeting:

This meeting was held to consider the additional data submitted by Ciba-Geigy pertaining to Metolachlor's carcinogenicity potential (even though the information had previously been submitted to the PRC as part of the April 17, 1991 peer review package), since the registrant felt that the data were not adequately considered by the committee; specifically, the submitter requested a formal review of the document submitted in 1988, and a review of the company's document entitled "Metolachlor. Briefing Paper for the Scientific Advisory Panel" submitted to the SAP in September 1991. The registrant originally stated that Metolachlor is not carcinogenic; however, in the SAP briefing they call it a "weak C" carcinogen.

iv. Scientific Advisory Panel (SAP)

Metolachlor was taken before the SAP on September 18, 1991. The Panel considered the PRC's recommendations for the carcinogenicity evaluation of metolachlor with specific comment to the weight given to Structure-Activity Relationships (SAR), particularly when the same effects are evident in the same species. The Panel considered two issues to be central to the question posed by the Agency:

1. Is the effect of Metolachlor well established in animals with regard to liver or nasal turbinate tumors?

The Panel considered the tumor incidence in female rats that have been treated with high dose of Metolachlor to be adequate in assessing its carcinogenic effects in that species. A positive dose-dependent trend was also evident in the treated male rats. In both, the tumors were benign and hyperplasia was evident.

The incidence of nasal turbinate tumors in rats was low and not statistically significant. Although no evidence for nasal turbinate tumorigenicity of Metolachlor existed, other structurally related herbicides do cause nasal turbinate tumors. This raised some concern of the Panel as to the biological significance of the small but statistically insignificant number of nasal tumors found in the metolachlor-treated animals.

2. Are SAR and metabolism findings important for interpretation of adverse effects from Metolachlor?

The similarity of chemical structure in these herbicides may nor result in a similarity in metabolism, either in a qualitative of quantitative sense, or in biologic effect. Evidence exists that metabolites of metolachlor are in fact different from those of the

other dialkylbenylamino herbicides, for example, the lack of formation of the quinone imine from metolachlor. To further distinguish Metolachlor from other compounds, the pharmacokinetics were stated as dose dependent. However, this was based upon the amount of total ^{14}C in urine (the individual metabolites were not identified in this study). The assertion that Metolachlor went from a 2 to a 1 compartment open model at high doses is highly presumptuous and may have little to do with the SAR issue.

The Panel considered the evidence to be minimal but sufficient for the classification of Metolachlor as a Group C carcinogen.

III. Additional data provided by the registrant since the previous meetings.

i. Metabolism

The registrant provided a metabolism study in rats (§85-1) to replace previous unacceptable studies. The study consisted of a combination of 2 separate reports, *Metabolism of [Phenyl-(u)-14C] Metolachlor in Rats (Preliminary and Definitive Phases* [HWI 6117-208] and *Characterization and Identification of Metabolites in Rats Treated with ^{14}C-Metolachlor* [ABR-94001] (Ciba-Geigy Corporation and Hazleton Wisconsin, Inc. for Ciba-Geigy Corporation, December 8, 1992 and March 11, 1994). The following are the conclusions from the review:

In this study, the disposition of radiolabeled Metolachlor was examined in male and female Sprague-Dawley rats in order to fulfill the §85-1 guideline requirement for metabolism, as well as to provide information to the Peer Review Committee in reevaluating the weight of evidence for the carcinogenicity classification of metolachlor.

This study contained a single low oral dose group, a single intravenous dose group, a repeated low oral dose group, and a single high oral dose group. The low dose used was 1.5 mg/kg/day, while a high dose of 300 mg/kg/day was employed. Groups of 5 rats/sex/dose group received these doses as appropriate, and excreta samples collected for up to 7 days post-dose.

Intravenous data which summarize the extent of radioactivity found in 0-168 hour urine, tissue residues, and residual carcass show that a total of 46.4% of the dose was recovered in male rats of this group, while a total of 60.1% of the dose was recovered in female rats of this same group. In all of the other dose groups, the recovery of radioactivity from these same routes was similar to that of the intravenous dose group. Thus, as stated in the report, 69.8% to 93.2% of the oral dose was absorbed in both male

and female rats of all dose groups. The high oral dose had no apparent effect on the extent of absorption. However, repeated oral dosing appeared to increase the rate of absorption as judged from urinary excretion patterns between dose groups B and C, while at the high oral dose, absorption appeared delayed, again based on comparison of urinary excretion between groups B and D.

Distribution data at 168 hours post-dose indicated that very few tissues harbored significant amounts of radioactivity, either in terms of total administered dose or in terms of µg equivalents/g tissue. Residual carcass and red cells appeared to be the only tissues where any significant residual radioactivity was found, but these levels were by themselves low (0.9 - 2.2% of the administered dose for residual carcass for males and females inclusive of all dose groups; between 0.95- 1.53 µg equivalents/gram in blood cells for all low dose male and female rats). In the high dose male and female rats, levels of 143.79 and 227.40 µg equivalents/gram were reported in male and female rats, respectively, but these levels were within the proportionality of the doses used (~ 200-fold difference). It is noted that red blood cells were also found to be a site for significant amounts of residual radioactivity after administration of acetochlor (HED document # 008478)

Excretion data showed that excretion of metolachlor derived radioactivity was almost equally divided between urinary and fecal excretion. The similar amounts of fecal excretion observed after i.v. vs oral administration indicate the bile as the source for fecal radioactivity and that absorption was essentially complete. Recovery of radioactivity was good among all dose groups. In the low dose groups, the urine appeared more of a predominant route for excretion in female rats than in males, where fecal excretion was slightly higher. However, at the high oral dose, there were no apparent sex-related differences in the pattern of urinary excretion. Examination of urinary excretion data as presented in graphical format indicated that at the 300 mg/kg dose, excretion was delayed vs the low oral dose, suggesting saturation of elimination.

Characterization of urinary and fecal metabolites in the present study was very extensive, as several metabolites were found in both urine and feces of all dose groups. In urine, 26 new metabolites were identified as well as 6 previously identified structures. The "major" urinary metabolite found in all dose groups was the metabolite designated CGA-46129. This metabolite was present as 5.6-13.1% of the TRR in rat urine across all dose groups, and was highest in the intravenously dosed group. In the orally dosed rats, the percentage of this metabolite decreased from approximately 13% of TRR to between 5.6-9.2% of TRR. Other metabolites identified in urine which constituted near or at 5% of TRR were U10 (CGA-37735), U13, U17, U1, "polar 1", and "polar 2."

The radioactivity constituting the 'polar 1' and 'polar 2' regions was further broken down to at least 12 components by TLC, but the identity of the metabolites in these regions was not identified.

In feces, a similarly complex metabolite profile was obtained. The "major" metabolite observed in feces, F9, was identical to U7, or CGA-46129. Except for intravenously dosed rats, where the percentage of this metabolite in feces was equivalent in male and female rats (11.6 and 13.2% of TRR, respectively), the percentage of F9 in feces of orally dosed rats was always higher in males than in females. Other fecal metabolites identified at or near 5% of TRR in feces included F2 (CGA-41638), F3 (CGA-133275), F7, F8 and F8', F14, F16, and F17.

Based on the metabolite identification in this and previous work with metolachlor, two schemes for transformation of metolachlor were proposed (attached). Of note is the observation that CGA-46129 was identified as the major urinary metabolite in a previous study (MRID # 401144-01) as well as in the present study. These two schemes show that metolachlor has the following major degradative pathways: 1) cleavage of the methyl ester; 2) oxidation of the resultant alcohol; 3) conjugation of the chloroacetyl group with glutathione followed by hydrolysis; and 4) oxidation of the aryl methyl and/or ethyl groups to benzylic alcohols followed in some cases by cyclization.

Of interest is the depiction of the formation of methylethylaniline, which was not previously mentioned in the prior metabolism study or in the SAP document on metolachlor. At the Science Advisory Panel meeting on metolachlor, data were presented contrasting the metabolism of metolachlor with alachlor and acetochlor. It was shown diagrammatically that formation of a di-substituted aniline would be more likely from metabolism of acetochlor and alachlor than metolachlor, based on the presence of a 2-carbon bridge as part of the ether linkage in metolachlor vs alachlor and acetochlor. According to the report, "the extremely low levels of methylethylaniline found (<0.00055% of the dose) could not be quantitated nor determined to be radiolabeled and could have been present as a minor impurity in the technical material used to dilute the 14-C test material."

With regard to the toxicity of di-substituted anilines, the available evidence indicates that monocyclic aromatic amines require high dosages in animal studies to produce a carcinogenic effect, and that even in situations of occupational exposure, monocyclic aromatic amines have not been associated with cancer. The central question, then in this study relates to whether the potential formation of methylethylaniline, claimed to be extremely minor, poses a significant issue related to the carcinogenicity of metolachlor. This question cannot be addressed here, but it is noted that in the present study, several new metabolites of

metolachlor were isolated, some of which still remain unidentified.

Metolachlor has been considered to be related to alachlor and acetochlor during the previous PRC meetings. The following are the conclusions from the review of the Alachlor metabolism study (Monsanto Agricultural Products Company, MSL-3198, October 21, 1983):

Alachlor was rapidly metabolized in rats and equally eliminated in urine and feces as conjugates of mercapturic acid, glucuronic acid, and sulfate. Approximately 89% of the initial dosages (7 mg/kg and 700 mg/kg) for the low and high dosage groups respectively) were eliminated within 10 days of the initial exposure; most of this elimination occurred within the first 48 hours of exposure. Elimination through CO_2 was minimal (0.02% of the dosage within 48 hours). The rate of elimination through urine and feces was biphasic: an initial rapid phase with a half life of 0.2 to 10.6 hours followed by a slower phase with a half life of 5 to 16 days.

After 10 days of an initial exposure to the above mentioned low and high dosage levels, 1.3% to 2.2% residual alachlor/metabolites were detected in blood; 0.66% to 1.27% in carcass; and a total of 0.24 to 0.32% in organ/tissues.

Levels of residual alachlor in spleen, liver, kidney and heart were relatively high. However, the radioactivity measured in these organs may be largely due to the presence of blood in these tissues.

Although the detected radioactivity in tissues/organs was dose-related, it appears that the eyes and brain in both sexes, the stomach in males (and to a much lesser extent in females), and the ovaries were target organs for a higher accumulation of alachlor/metabolites. These organs were the sites of specific lesions in the 2-year rat feeding study (BD-77-421, 11/13/81 by Bio/dynamics Inc.).

For the metabolism of acetochlor, a five part study was conducted (Huntingdon Research Centre Ltd. and ICI Central Toxicology Laboratory for ICI-Zeneca, Laboratory Projects Numbers HRC/STR 18/88502, HRC/STR 18/89184, HRC/STR 18/89487, HRC/STR 18/89603, and CTL/P/2809, February, April, June 1987, June 1989, and March 1990), the following are the conclusions from the review:

In this study, the disposition and metabolism of acetochlor was investigated in male and female rats. Data were presented

demonstrating the absorption, distribution, and excretion of ^{14}C-acetochlor in male and female rats at a single low oral dose (10 mg/kg, repeated low oral doses (10 mg/kg x 14 days) and a single high oral dose (200 mg/kg) along with the biotransformation and identification of acetochlor metabolites in urine, bile, and feces.

Absorption of a dose of ^{14}C-acetochlor appeared complete at both 10 and 200 mg/kg by comparison of excretion data in bile duct cannulated and non-cannulated rats. While a significant percentage of ^{14}C-acetochlor derived radioactivity was found in feces of rats (between 13-26% at the 10 mg/kg dose level, and between 26-37% at the 200 mg/kg dose level), it was demonstrated in bile duct cannulated rats that fecal excretion of ^{14}C-acetochlor derived radioactivity was of biliary origin and did not represent unabsorbed acetochlor.

Excretion of a dose of ^{14}C-acetochlor in urine was relatively rapid at the 10 mg/kg dose level, with the majority of radioactivity (59-67%) eliminated in the urine by 24 hours. At the 200 mg/kg dose level, a lower percentage of ^{14}C-acetochlor was eliminated in urine by 24 hours (43-51%), but a greater percentage was found in feces at this time point. Although this altered pattern of elimination may be the result of enterohepatic recirculation of radioactivity eliminated in bile at the high dose, there was no apparent difference in the percentage of ^{14}C-acetochlor derived biliary radioactivity eliminated at both doses. In addition examination of the time course of urinary elimination shows that a much smaller percentage of ^{14}C-acetochlor derived radioactivity was eliminated in urine between 0-12 hours. Thus, some delay in urinary elimination of ^{14}C-acetochlor derived radioactivity is apparent. At all doses examined, the percentage of ^{14}C-acetochlor derived radioactivity eliminated in urine of female rats was somewhat higher than that of male rats, with a corresponding decrease in fecal elimination.

The concentration of ^{14}C-acetochlor derived radioactivity in tissues at 120 hours showed that radioactivity was concentrated in those tissues receiving the greatest amount of cardiac output (heart, lungs, liver, kidneys, and spleen). Few sex- or dose-dependent differences were noted, but at each dose level, the greatest concentration of ^{14}C-acetochlor derived radioactivity was found in whole blood. This is reflected in the ratio of whole blood to plasma radioactivity, which exceeded 100 at all doses in both sexes. Thus, binding of acetochlor or a metabolite to red blood cells is extremely likely from review of these data. Identification of the chemical species responsible for binding was not performed in the present studies. However, it should be noted that conjugation with glutathione is a major biotransformation pathway for acetochlor as shown by the profile of urinary metabolites, indicating the formation of a potentially reactive

electrophilic species capable of such binding.

Metabolites of acetochlor in urine, feces, and bile were characterized and identified. From the results of these studies, the ethoxymethyl side chain and the chlorine atom of acetochlor were identified as major reaction sites for biotransformation. It is proposed, from analysis of acetochlor metabolites in bile and urine, that initial conjugation with glucuronic acid and glutathione occurs in liver, with subsequent excretion of the glucuronide conjugate (B9), the mercapturic acid conjugate (B15), and the glutathione conjugate of N-dealkylated acetochlor (B5) in bile. The two glutathione conjugates are reabsorbed, with further metabolism to the mercapturic acid conjugate which is excreted in urine. Since the amount of mercapturic acid metabolite in urine exceeded the combined amounts of glutathione conjugated in bile, the additional mercapturic acid found in urine is proposed to come from hydrolysis of the glucuronide conjugate in the gut, with subsequent reabsorption and metabolism to the glutathione conjugate which is then excreted in urine as the mercapturic acid conjugate.

The limited data presented on *in vivo* plasma kinetic of acetochlor demonstrated that the elimination of ^{14}C-acetochlor derived radioactivity in plasma was at least biexponential. While it is possible to approximate the half life of elimination of ^{14}C-acetochlor by inspection of graphical data, the observation that acetochlor and/or a metabolite binds to red blood cells does not give much meaning to an evaluation, as $t_{1/2}$ would likely be different for data on whole blood levels of ^{14}C-acetochlor derived radioactivity over time. The lack of intravenous data (acetochlor is of low solubility) does not make it possible to distinguish which chemical species might be responsible for binding to red cells, as binding likely occurs prior to the peak plasma levels reported. The avid binding of ^{14}C-acetochlor derived radioactivity to red blood cells makes it likely that volume of distribution for acetochlor is small (limited to total body water).

ii. Mutagenicity

The registrant also conducted two genotoxicity studies, as requested by the Agency, an *in vivo/in vitro* UDS assay and a cell proliferation assay. The following presents the **DRAFT** Executive Summaries from these reviews:

Metolachlor technical was tested in an *in vivo/in vitro* UDS assay in rat primary hepatocytes, following oral doses of 1250, 2500, and 4000 mg/kg in males and 500, 1000, and 1500 mg/kg in females. Rats were sacrificed 2 or 15 hours after dosage, with a minimum of 3 animals/sex/dose/timepoint. There were sufficient indications of toxicity and/or cytotoxicity at the highest dose levels tested.

Metolachlor technical is negative for induction of UDS under the conditions of this assay. However, significant increases in percentages of cells in S-phase were observed in primary hepatocytes from female rats dosed with 500 (but not at 1000 or 1500) mg/kg and sacrificed at 15 hours.

Metolachlor technical was administered by oral gavage at doses of 150, 500, and 1000 mg/kg to groups of 5 male and 5 female rats. The animals were sacrificed 72 hours later. Metolachlor was positive for induction of cell proliferation of hepatocytes from males receiving 500 mg/kg (preparations from 1000 mg/kg males were not examined) and from females receiving 1000 mg/kg.

IV. Questions to the Peer Review Committee for Carcinogenicity

The data presented in this document were requested in the decisions by the previous Peer Review Committee meetings where the PRC recommended that the registrant perform additional tests in stages.

1. It was recommended that the *in vivo/in vitro* unscheduled DNA test be repeated to fulfill the data gap for genotoxicity testing.

2. If this proved negative, the PRC recommended that the registrant provide data on the identification of metabolites from the submitted study "Disposition of Metolachlor in the rat (general metabolism)" to resolve issues and fulfill the data requirements.

3. Finally, a comparative metabolism study was recommended in order to provide information on the metabolic generation of quinone imine and formaldehyde and to support the assertion that the metabolism of Metolachlor is substantially different from its major structurally analogous compounds.

After the completion and evaluation of the recommended studies, the PRC agreed to reconsider the weight-of-evidence for the classification of Metolachlor.

The question for the PRC is, do the additional data provided by the registrant change the previous PRC recommendations?[1]

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[1]Ciba-Geigy has requested that the PRC reconsider the q_1* in light of the provided additional data.

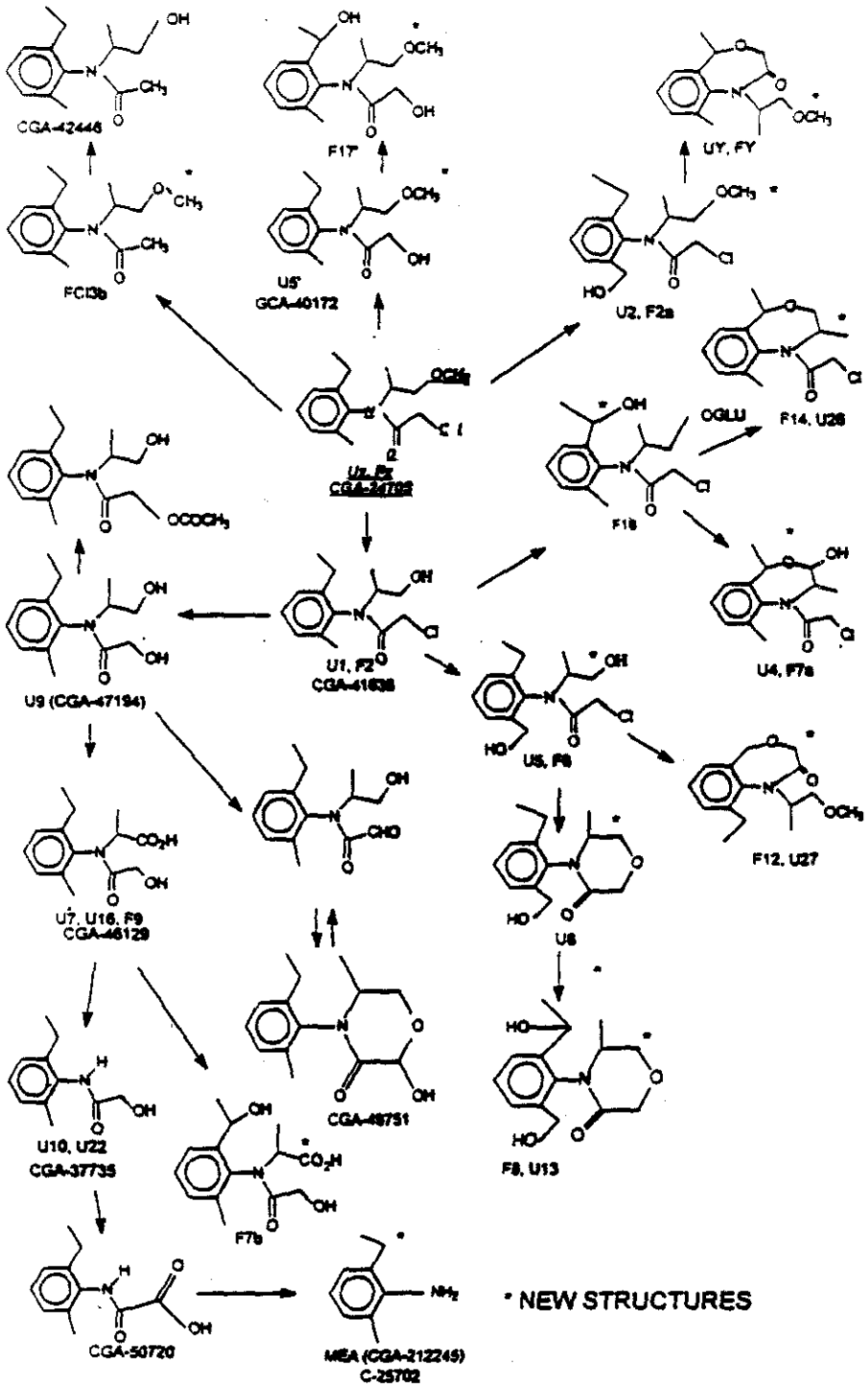

Figure 108. Proposed Pathway I

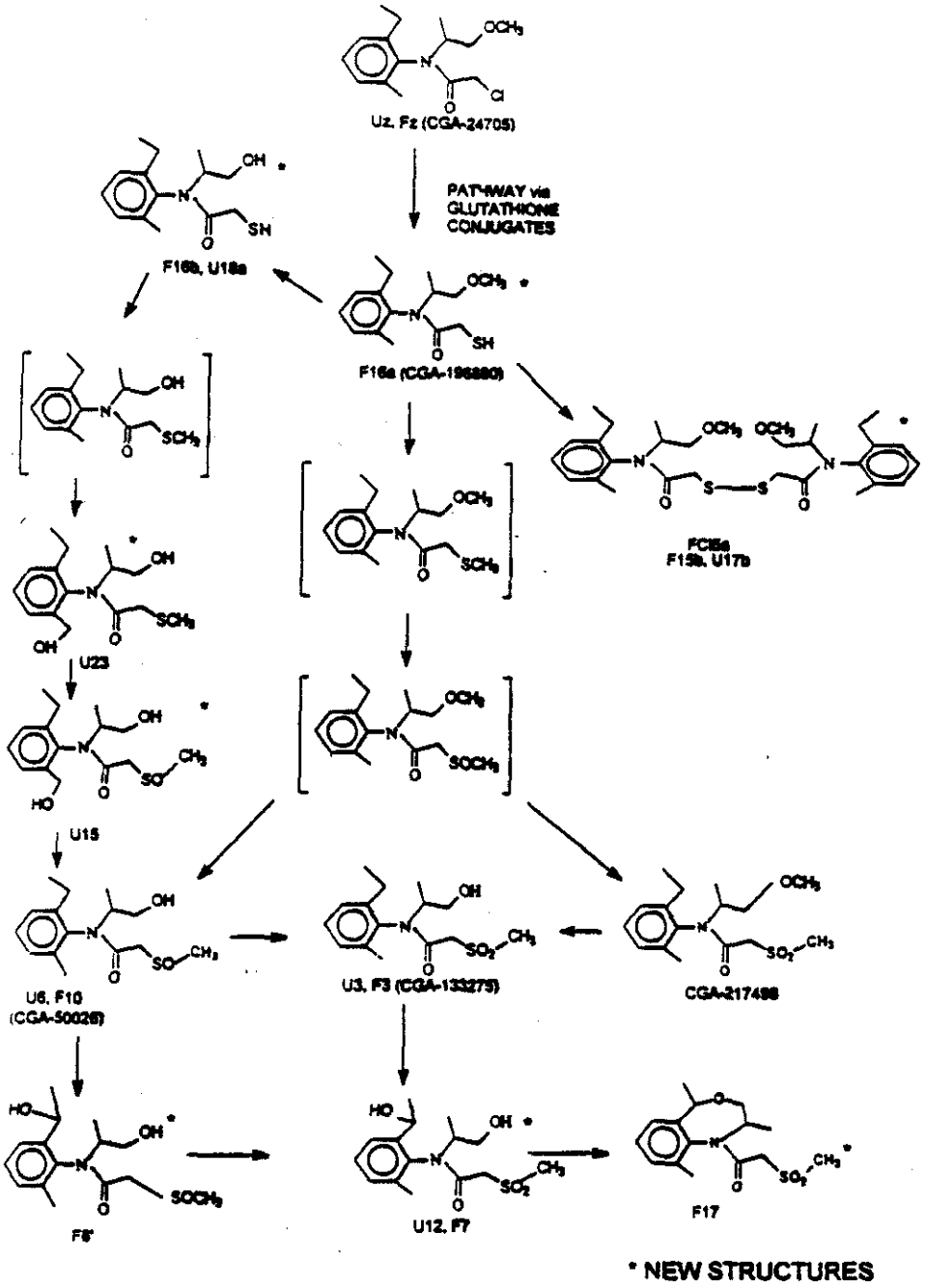

Figure 109. Proposed Pathway II

M. Ioannou

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 21 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Metolachlor (3rd)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 6/29/93
Toxicologist, Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

and

Esther Rinde, Ph.D. Esther Rinde
Manager, Carcinogenicity Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Joanne Miller
Product Manager #23
Herbicide-Fungicide Branch
Registration Division (H7505C)

and

Walter Waldrop
Section Head
Accelerated Reregistration Branch
Special Review and Reregistration Division (H7505W)

Penelope A. Fenner-Crisp
THROUGH: Penelope Fenner-Crisp, Ph.D.
Director, Health Effects Division (H7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (PRC) met on May 30, 1985 (first meeting), April 17, 1991 (second meeting) and on April 14, 1993 (present meeting) to discuss and evaluate the weight-of-the-evidence on Metolachlor with particular reference to its carcinogenic potential.

The Peer Review Committee agreed that Metolachlor should be classified as Group C - possible human carcinogen and recommended that for the purpose of risk characterization a low dose extrapolation model applied to the experimental animal tumor data should be used for quantification of human risk (q_1*).

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A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penny Fenner-Crisp	Penelope A Fenner-Crisp
Reto Engler	
William L. Burnam	
Karl Baetcke	
Marion Copley	Marion P. Copley
Kerry Dearfield	
Julie Du	
Hugh Pettigrew	Hugh M. Pettigrew
Esther Rinde	Esther Rinde

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Stephen C. Dapson[1]	Stephen C. Dapson
Mike Ioannou	J. M. Ioannou
Lori Brunsman	
Lucas Brennecke[2] (PAI/Clement)	Lucas H. Brennecke

3. Other Attendees:

Linda Kutney, Jim Rowe (HED)

Diane Mandell (Clement)

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

B. **Material Reviewed:**

The material available for review consisted of DER's, one-liners, and other data summaries prepared by Dr. Stephen Dapson, pathology report analysis by L. Brennecke, the FIFRA Scientific Advisory Panel (SAP) meeting notes on Metolachlor, new data provided by the registrant, and tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report. The data reviewed are based on studies submitted to the Agency by Ciba-Geigy Corporation.

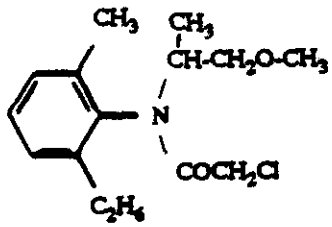
C. **Background Information:**

Metolachlor is the common name for 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide, one of the family of chloroacetanilides.

Metolachlor is a selective herbicide registered for use (CFR 180.368) for the control of most annual grasses and certain broadleaf weeds in corn, soybeans, peanuts, grain sorghum, potatoes, pod crops, cotton, safflower, and woody ornamentals.

The Caswell (or Tox Chem) Number of Metolachlor is 188DD. The Chemical Abstracts Registry Number (CAS No.) is 51218-45-2.

The structure of Metolachlor is shown below:



The PRC first met on May 30, 1985 to review the evidence for the carcinogenic classification of Metolachlor. The Committee concluded that "the data available for Metolachlor provide weak evidence of carcinogenicity," but did not assign a carcinogenicity classification. Their evaluation was based on a chronic Industrial Biotest Laboratories (IBT) rat study (IBT rat study No. 622-07925), and a second chronic (2-year) rat study performed by Hazleton-Raltech Inc. (No. 80030); a 2-year chronic mouse study performed by IBT (No. 622-07925), and a second 2-year chronic mouse study by Hazleton-Raltech Inc.; two genotoxicity assays (mouse dominant lethal study, Salmonella mutagenicity assay); and four developmental studies using Metolachlor. Before making a final evaluation regarding the classification and calculation of a potency factor (q_1^*) for Metolachlor, the PRC requested additional information from the registrant to fulfill

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the mutagenicity battery and metabolism study requirements, based on 1982 EPA testing guidelines.

The second peer review meeting on Metolachlor took place on April 17, 1991. The PRC recommended that Metolachlor be classified as Group C, and that quantification of risk be performed using a linearized multistage model (q_1^*). This recommendation was based on the replication of hepatic neoplasia in female rats in the first (IBT) and second (Hazleton-Raltech) rat carcinogenicity studies, and the apparent induction of a small number of nasal turbinate tumors (an uncommon tumor induced by certain analogs of Metolachlor) at the highest dose tested (HDT) in the second rat study. The material reviewed included the database from the first PRC meeting and supplemental pathology reports and historical control data submitted by the registrant in 1988. Additional data on metabolism and genotoxicity were also reviewed. The PRC recommended the completion of an in vivo/in vitro unscheduled DNA synthesis assay.

A q_1^* was calculated for Metolachlor of 2.1×10^{-3} $(mg/kg/day)^{-1}$ (q_1^* from Memorandum dated January 29, 1982, G.J. Burin to R. Mountfort). Exposure assessment was conducted by the Occupational and Residential Exposure Branch (Memorandum dated May 10, 1990, C. Lunchick to R. Ikeda) and an average daily exposure of 1.1×10^{-4} mg/kg/day was calculated. The estimated risk is 2.3×10^{-7}.

The SAP met on September 18, 1991 to review the classification of Metolachlor. The SAP considered the evidence to be "minimal but sufficient for the classification of Metolachlor as a Group C carcinogen." According to the registrant, the information provided to the SAP in a briefing paper was supplemental to the data submitted in 1988, and served to clarify and correct several data points in the 1988 submission (Dr. William Field's reading of control male adenocarcinoma and male 300 ppm adenomous polyp is stated to be overestimated). The registrant further claimed that "application of quantitative risk assessment to a marginal Class C oncogen like Metolachlor in order to facilitate comparison of cancer potency of Metolachlor with other members of this class of herbicides as stated in EPA's second Peer Review, is scientifically unfounded."

The third peer review meeting (present meeting, April 14, 1993) was held to consider the additional data submitted by Ciba-Geigy pertaining to Metolachlor's carcinogenicity potential (even though the information had previously been submitted to the PRC as part of the April 17, 1991 peer review package), since the registrant felt that the data were not adequately considered by the committee; specifically, the submitter requested a formal review of the document submitted in 1988, and a review of the company's document entitled "Metolachlor, Briefing Paper for the Scientific Advisory Panel" submitted to the SAP in September

1991. The registrant originally stated that Metolachlor is not carcinogenic; however, in the SAP briefing paper they call it a "weak C" carcinogen.

D. Evaluation of Carcinogenicity Evidence:

1. Charles River CD Rat Chronic Feeding Carcinogenicity Study

Reference: Chronic toxicity/carcinogenicity study in Charles River Crl:CD(SD)BR albino rats. MRID No. 129377-00. Study No. 80030. Hazleton Raltech Incorporated, Madison, WI; for Ciba-Geigy Corporation, Greensboro, NC. Submitted May 2, 1983.

a. Experimental Design

Metolachlor was administered in the diet to 70 animals per sex at doses of 0 or 3000 ppm, and to 60 animals per sex at doses of 30 or 300 ppm. Ten animals per sex in the control and 3000 ppm dose groups were designated for interim sacrifice, five at week 53 and five at week 57 following a 4-week recovery period.

b. Discussion of Tumor Data

Initially, all slides were read by Hazleton Raltech, Incorporated. Ciba-Geigy Corporation subsequently requested Dr. Jerry F. Hardisty of Experimental Pathology Laboratories, Incorporated, (EPL) to re-read only the liver slides (issued on July 20, 1984). EPL then conducted their own internal peer review of Dr. Hardisty's read of the slides by presenting only those slides of animals on which Dr. Hardisty found liver lesions and an additional six female control blanks to a panel of six pathologists, including Dr. Hardisty. For this reason, statistical analysis was not conducted. In addition, Ciba-Geigy requested Dr. Kevin T. Morgan, under contract to EPL, to re-read only the nasal passages slides [issued May 4, 1988 (Attachments 2 and 4 of MRID No. 409344-01 from Ciba-Geigy dated November 18, 1988)]. A memorandum from Dr. Lucas H. Brennecke to Dr. Stephen Dapson dated July 20, 1992 was also considered by the PRC.

Statistical analysis of tumor rates was based on the Exact Trend Test and Fisher's Exact Test for the pair-wise comparison of controls and each treated group, since there were small occurrences of tumors and no significant statistical evidence of mortality with increasing doses of Metolachlor.

Significant increasing trends in liver adenomas ($p < 0.01$, Hazleton; $p < 0.05$, Hardisty) and combined liver adenomas and/or carcinomas ($p < 0.05$ for both reports) were observed in male rats (Tables 1 and 2). There were no significant differences in the pair-wise comparisons of the controls with the dosed groups.

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Table 1. Metolachlor - Charles River Crl:CD(SD)BR Rat Study

Male Liver Tumor Rates[+] and Exact Trend Test
and Fisher's Exact Test Results (p values)

Pathology Report By:
Hazleton Raltech, Incorporated
May 2, 1983

	Dose (ppm)			
Tumors:	0	30	300	3000
Neoplastic Nodules (Adenomas)	0/58	0/57	0/59	4[a]/60
(%)	(0)	(0)	(0)	(7)
p =	0.004**	1.000	1.000	0.064
Hepatocellular Carcinomas	2[b]/58	1/57	3/59	2/60
(%)	(3)	(2)	(5)	(3)
p =	0.432	0.507[n]	0.508	0.678
Combined	2/58	1/57	3/59	6/60
(%)	(3)	(2)	(5)	(10)
p =	0.027*	0.507[n]	0.508	0.147

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[n]Negative change from control.

[a]First neoplastic nodule observed at week 82, dose 3000 ppm.

[b]First hepatocellular carcinoma observed at week 74, dose 0 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 2. Metolachlor - Charles River Crl:CD(SD)BR Rat Study

Male Liver Tumor Rates+ and Exact Trend Test
and Fisher's Exact Test Results (p values)

Pathology Report By:
Dr. Jerry F. Hardisty
July 20, 1984

	Dose (ppm)			
Tumors:	0	30	300	3000
Hepatocellular Adenomas (%)	1/58 (2)	1/57 (2)	0/59 (0)	4/60 (7)
p =	0.035*	0.748	0.496n	0.193
Hepatocellular Carcinomas (%)	2/58 (3)	1/57 (2)	3/59 (5)	3/60 (5)
p =	0.247	0.507n	0.508	0.516
Combined (%)	3/58 (5)	2/57 (4)	3/59 (5)	7/60 (12)
p =	0.037*	0.508n	0.652	0.175

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

nNegative change from control.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

In female rats, an increased trend is indicated in liver adenomas and combined liver adenomas and/or carcinomas ($p < 0.01$ for both parameters in both reports) (Tables 3 and 4). Significant pair-wise comparisons were found in the 3000 ppm dose group for liver adenomas ($p < 0.05$) based on the Hardisty report, and for combined liver adenomas and/or carcinomas ($p < 0.05$, Hazleton; $p < 0.01$, Hardisty).

Dr. Hardisty's concluded that, "the increased incidence of hepatocellular neoplasms observed in female rats given 3000 ppm is considered to be equivocal due to their low incidence and similarity to published incidences for aged rats of this strain." The PRC disagreed with this conclusion. Furthermore, according to

a memorandum from Dr. Lucas H. Brennecke to Dr. Stephen Dapson, July 20, 1992,

> "The 1988 re-evaluation of liver slides for all female rats by a panel of five pathologists revealed that the incidences of adenomas and adenomas/carcinomas (combined) were significantly ($p \leq 0.05$) greater than controls. In only one of the 12 studies showing historical control incidence of liver tumors in Sprague-Dawley rats at Hazleton did the incidence of adenomas or combined adenomas and carcinomas exceed that noted in the Metolachlor study. In that study (#12), the historical incidence of liver tumors was clearly higher (more than twice) than in any other study. As such, it should not be given much weight in considering the real historical incidence at Hazleton."

Table 3. Metolachlor - Charles River Crl:CD(SD)BR Rat Study

Female Liver Tumor Rates* and Exact Trend Test
and Fisher's Exact Test Results (p values)

Pathology Report By:
Hazleton Raltech, Incorporated
May 2, 1983

	Dose (ppm)			
Tumors:	0	30	300	3000
Neoplastic Nodules (Adenomas)	0/58	0/60	1[a]/58	4/57
(%)	(0)	(0)	(2)	(7)
p =	0.005**	1.000	0.500	0.057
Hepatocellular Carcinomas	0/58	0/60	0/58	2[b]/57
(%)	(0)	(0)	(0)	(4)
p =	0.059	1.000	1.000	0.244
Combined	0/58	0/60	1/58	6/57
(%)	(0)	(0)	(2)	(11)
p =	0.000**	1.000	0.500	0.013*

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First neoplastic nodule observed at week 104, dose 300 ppm.

[b]First hepatocellular carcinoma observed at week 90, dose 3000 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 4. Metolachlor - Charles River Crl:CD(SD)BR Rat Study

Female Liver Tumor Rates[+] and Exact Trend Test
and Fisher's Exact Test Results (p values)

Pathology Report By:
Dr. Jerry F. Hardisty
July 20, 1984

	Dose (ppm)			
Tumors:	0	30	300	3000
Hepatocellular Adenomas	0/58	1/60	2/58	6/57
(%)	(0)	(2)	(3)	(11)
p =	0.002**	0.509	0.248	0.013*
Hepatocellular Carcinomas	0/58	0/60	0/58	1/57
(%)	(0)	(0)	(0)	(2)
p =	0.245	1.000	1.000	0.496
Combined	0/58	1/60	2/58	7/57
(%)	(0)	(2)	(3)	(12)
p =	0.001**	0.509	0.248	0.006**

[+]Number of tumor bearing animals/Number of animals examined; no tumors were found in animals that died or were sacrificed before week 53.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for the incidence of hepatocellular tumors in female rats for contemporary studies conducted at Hazleton Laboratories in Madison, Wisconsin, five years prior to and after the Metolachlor study are shown below:

Hepatocellular lesion type	Historical control range[a] Mean (%)	Range (%)
Adenoma	2.1	0-11.5[b]
Carcinoma	0.2	0- 1.9
Combined	2.3	0-13.5[c]

[a]Based on 12 studies conducted at Hazleton Laboratories in Madison, Wisconsin between the years 1980 and 1984.
[b]The distribution of adenoma (percent incidence) from the 12 individual studies was as follows: 0, 0, 3.3, 0, 0, 4.0, 5.1, 0, 0, 1.4, 1.7, 11.5.
[c]The distribution of combined adenoma/carcinoma (percent incidence) from the 12 individual studies was as follows: 0, 0, 3.3, 0, 0, 4.0, 5.1, 0, 0, 1.4, 1.7, 13.5.

No statistical analysis was performed on the nasal pathology report by Dr. Morgan of EPL because the tumor occurrences were too small to indicate statistical trends or pair-wise comparisons at any level of significance. The PRC agreed that it would be inappropriate to combine these lesions because the three neoplasms were not of the same type, nor did they originate in similar tissue (Memorandum from Dr. Lucas H. Brennecke to Dr. Stephen Dapson, July 20, 1992). Therefore, the PRC agreed to accept the conclusion of Dr. Morgan that "there is no evidence of treatment-related nonneoplastic or neoplastic responses in the nasal passages of male or female Charles River CD Albino rats given 30, 300, or 3000 ppm Metolachlor in this study." In addition, the PRC discussed the possibility that there may be in situ formation of formaldehyde from Metolachlor; no data were available to support or contradict this possibility. Nasal tumor data from the Morgan report are presented in Tables 5 and 6 for males and females, respectively.

Table 5. Metolachlor - Charles River Crl:CD(SD)BR Rat Study
Male Nasal Tumor Rates*

Pathology Report By:
Dr. Kevin T. Morgan
May 4, 1988

Tumors:	Dose (ppm) 0	30	300	3000
Polypoid Adenomas (Respiratory Epithelium)	1/64	0/56	0/53	1/67
Adenocarcinomas (Nasal Glands)	0/64	0/56	0/53	1/67
Neurofibrosarcomas (Peripheral Nerve)	0/64	0/56	0/53	1/67
Malignant Lymphomas (Metastatic)	0/64	2/56	0/53	0/67

*Number of tumor bearing animals/Number of animals examined.

Table 6. Metolachlor - Charles River Crl:CD(SD)BR Rat Study
Female Nasal Tumor Rates*

Pathology Report By:
Dr. Kevin T. Morgan
May 4, 1988

Tumors:	Dose (ppm) 0	30	300	3000
Polypoid Adenomas (Respiratory Epithelium)	0/67	0/51	1/56	0/67
Odontomas	1/67	0/51	0/56	1/67
Squamous Papillomas	0/67	0/51	0/56	1/67

*Number of tumor bearing animals/Number of animals examined.

c. Non-neoplastic Lesions and Other Observations

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Metolachlor in male or female rats (Tables 7 and 8).

Table 7. Metolachlor - Charles River Crl:CD(SD)BR Rat Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose(ppm)	1-26	27-52	53[i]	53-78	79-105[f]	Total
0	1/70	1/69	10/68	9/58	16/49	27/60 (45)
30	0/60	3/60	0/57	5/57	18/52	26/60 (43)
300	1/60	0/59	0/59	3/59	31/56	35/60 (58)
3000	0/70	0/70	10/70	5/60	21/55	26/60 (43)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 104.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 8. Metolachlor - Charles River Crl:CD(SD)BR Rat Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose(ppm)	1-26	27-52	53[i]	53-78	79-105[f]	Total
0	0/70	2/70	10/68	7/58	18/51	27/60 (45)
30	0/60	0/60	0/60	12/60	18/48	30/60 (50)
300	0/60	2/60	0/58	7/58	22/51	31/60 (52)
3000	2/70	1/68	10/67	3/57	14/54	20/60 (33)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 104.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Female rats had reversible weight loss from study weeks 2 to 104; food consumption was reduced in female rats during intermittent periods during the study. Male rats exhibited testicular atrophy with degeneration of tubular epithelium. The severity of the effect was reported to be similar in all treated groups but the time of onset was sooner in all treated males. Finally, there was an increased incidence of eosinophilic foci in the livers of both male (10/59 control, 15/59 low, 14/60 mid, 21/60 high) and female (4/60 control, 7/60 low, 5/60 mid, 23/60 high) rats upon histological examination.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be marginally adequate for assessing the carcinogenic potential of Metolachlor, based on decreased body weight gain (13%) and decreased food consumption in 3000 ppm females, testicular atrophy and degeneration in treated

males, and liver histopathology in male and female rats. The PRC had difficulty establishing if the doses used in this study were high enough to establish without a doubt that the nasal tumors that were seen in rats dosed with 3000 ppm Metolachlor were indeed spurious.

2. Other carcinogenicity studies

Not considered by the PRC was a chronic feeding study in rats submitted by Industrial Biotest Laboratory (MRID No. 244166, 099628, 099626, 070048; study No. 622-07925, Submitted February 9, 1979). This study was classified as Core-Supplementary because of inadequate clinical chemistry determinations and dietary preparation records. Although not discussed in detail, the PRC noted that liver hyperplastic nodules and carcinomas occurred in female rats at the HDT (3000 ppm) in this study; these agreed with the results of the repeat Hazleton rat study. Further detail is provided in Section D.1 of the second peer review document.

See Sections D.3. and D.4. of the second peer review document for description of two negative mouse carcinogenicity studies (IBT's 2-year mouse study No. 622-07925; Hazleton-Raltech 2-year repeat mouse study No. 79020). These mouse studies were not discussed further by the PRC.

E. Additional Toxicology Data on Metolachlor:

1. <u>Metabolism</u>

Reference: Disposition of Metolachlor in the Rat (General Metabolism). MRID No. 401144-01. Study No ABR-8611. Ciba-Geigy Corporation. Submitted February 17, 1987.

A single low (1.5 mg/kg), a single high (300 mg/kg), and repeated low (1.5 mg/kg/day for 15 days) oral doses of Metolachlor were used. These doses were found to be readily absorbed and excreted by male and female rats. The urinary and fecal elimination of radioactivity was essentially complete within 48 to 72 hours postdosing. Approximately 48 to 64 % of the radiolabel was recovered within 7 days with similar amount in the feces. Low levels of radioactivity were found in the tissues of all animals at 7 days postdosing.

This study is classified as Core-Supplementary Data since the study did not provide the purity of the test compound used and the metabolites were not identified. This study alone does not satisfy the guideline requirement (§85-1) for a metabolism study in the rat, and only provides supplementary information on the metabolism of single low, single high, and repeated oral doses of Metolachlor.

Reference: Kinetics of Metolachlor, a report by I.W.F. Davidson, October 14, 1988. In this report, a kinetics modelling system was applied to data from the following DER:
Disposition of Metolachlor in the Rat (General Metabolism). MRID No. 401144-01. Study No ABR-8611. Ciba-Geigy Corporation. Submitted February 17, 1987.

The results of this analysis indicated that, after oral dosing of rats with Metolachlor, renal excretion of the radiolabel followed first-order kinetics for both the low dose (1.5 mg/kg) and the high dose (300 mg/kg). Low dose kinetics exhibited two components of excretion of ^{14}C-activity, while high dose kinetics exhibited a single component of excretion. No sex differences were noted. Additionally, virtually all of the administered labeled compound was accounted for by the ^{14}C-activity excreted in the urine and in feces.

Reference: Metabolism of CGA 24 705 in the Rat and Addendum to Project 7/74: Metabolism of CGA 24 705 in the Rat, MRID No's. 00015655, 00039193 and 00015425. Study No.'s 7/74 and 12/74. Ciba-Geigy Ltd. Submitted September 26, 1974, November 25, 1975, March 26, 1975.

A separate metabolism study was submitted by the registrant which deals with the identification of the metabolites. The results are summarized below:

Urinary metabolites were identified following oral administration of 28, 33, and 52 mg/kg to male rats. Two metabolites of the organic extractable urinary radioactivity were identified from oral administration of the test compound.

The major metabolic pathway proposed from analysis of urinary as well as fecal metabolites is one of cleavage of the ether bond and subsequent oxidation to the carboxylic acid, as well as hydrolytic removal of the chlorine atom. Conjugation of the parent or metabolites with glucuronic acid or sulfate does not appear to occur.

The aqueous extractable urinary radioactivity contained 58% of the total urinary radioactivity and was composed of 5 different radioactive fractions which were not identified.

This study did not follow current guideline recommendations as to dose levels or the use of both sexes. Therefore, if the metabolic pattern is altered by dose or repeated exposure, this cannot be determined by these data. Further, the doses tested in this study were not equivalent to those tested in the previously discussed study (MRID No. 401144-01). The study is classified as Core-Supplementary Data and does not fulfill the data requirements (§85-1) for a general metabolism study in rats.

The Agency recommends that the registrant provide data on the identification of urinary and fecal metabolites from the submitted study, "Disposition of Metolachlor in the Rat (General Metabolism)" (Ciba-Geigy Corporation, MRID No. 401144-01, Study No. ABR-8611, 2/17/87) to resolve this data gap.

2. Genotoxicity

Reference: Evaluation of Metolachlor Technical in the *in vivo*/*in vitro* Rat Hepatocyte Unscheduled DNA Synthesis Assay, Hazleton Biotechnologies Company, HBC Project No. 20991, August 10, 1988, MRID No. 420433-01.

Under the conditions tested, Metolachlor was inactive in this *in vivo*/*in vitro* rat hepatocyte assay at the dose levels tested (females: 3.07, 31.49, 291.9, and 519.5 mg/kg; males: 2.88, 31.91, 301.03, and 474.5 mg/kg). The study has been classified as Unacceptable and does not satisfy the guideline requirements for a mutagenicity study (§ 84-4) because i) it did not report the batch number or the purity of the test compound and ii) there were no overt signs of toxicity (i.e. dosing could be higher). It was noted that S phase induction was significantly increased in females and only slightly so in males.

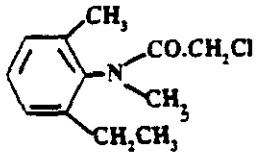
Further discussion about the genotoxicity of Metolachlor can be found in the second peer review document. In general, the PRC did not feel that Metolachlor posed a high degree of risk for genotoxicity; however, they recommended upgrading the *in vivo*/*in vitro* unscheduled DNA test to fulfill the data gap for genotoxicity testing.

3. Structure-Activity Correlations

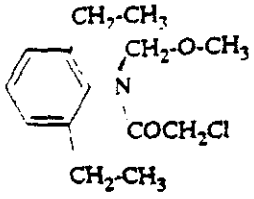
Metolachlor is structurally related to Acetochlor, Alachlor, Allidochlor, Butachlor, and Propachlor.

Figure 1

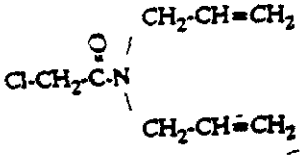
Structural Analogs of Metolachlor



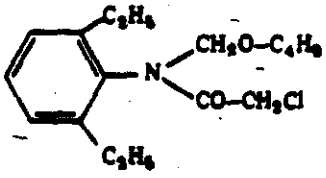
Acetochlor



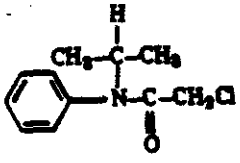
Alachlor



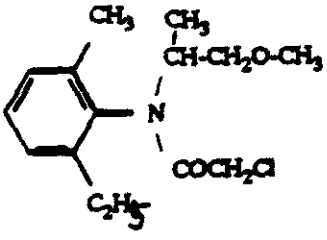
Allidochlor



Butachlor



Propachlor



Metolachlor

Acetochlor has been classified as a Group B2 (Probable Human) Carcinogen by the PRC and the SAP. This was based on the evidence that Acetochlor caused an increased incidence of benign and malignant tumors at multiple sites in Sprague-Dawley rats at 1000 ppm (papillary adenomas of the nasal turbinates in both sexes; hepatocellular carcinomas in both sexes and thyroid follicular cell adenomas in males) and an increased incidence of benign and malignant tumors at multiple sites in Swiss albino CD-1 mice (hepatocellular carcinoma in both sexes at 5000 ppm; lung carcinomas in females at 500, 1500, and 5000 ppm; uterine histiocytic sarcoma and benign ovarian tumors in females at 500, 1500, and 5000 ppm; and a positive trend in kidney adenomas in females). Acetochlor caused point mutations in a Chinese hamster ovary test both with and without metabolic activation, gene mutations in the mouse lymphoma assay (with activation), aberrations in human lymphocytes and unscheduled DNA synthesis (UDS) in an *in vivo*/*in vitro* UDS assay. There were no mutagenic

effects in gene mutation tests in bacteria, a micronucleus assay in mice, an in vivo cytogenetics assay in rats and an UDS assay in rat primary hepatocytes in vitro.

The PRC has classified Alachlor as a Group B2 carcinogen. In a dietary study in Long-Evans rats, nasal turbinate tumors were found at 42 mg/kg and squamous cell tumors of the stomach were found in both sexes at 126 mg/kg as well as thyroid follicular adenomas in males at 146 mg/kg. In a dietary study in mice, there was an increased incidence of liver tumors in females at 260 mg/kg. Alachlor was positive in a DNA damage/repair (in vivo/in vitro UDS) assay, while it was negative in bacterial assays, in vivo/in vitro cytogenetics, and a CHO/HGPRT assay. Some metabolites of Alachlor are positive in the Salmonella assay. The registrant used the different rat strain used in the carcinogenicity study as an explanation for the difference in nasal tumor response.

Allidochlor has no acceptable chronic or mutagenicity studies.

Butachlor is carcinogenic in rats after dietary administration, including stomach tumors in females (3000 ppm), adenomas and carcinomas of the nasal mucosa in both sexes (1000 ppm and above), follicular adenomas/carcinomas of the thyroid in females (1000 ppm) and in both sexes at higher levels (3000 ppm), and renal cortical cell tumors in both sexes (3000 ppm). Mice developed alveolar/bronchiolar adenomas/carcinomas at the 2000 ppm level. It was weakly mutagenic in one Salmonella assay, and negative in a bacterial rec (DNA damage) assay and for reversion.

Propachlor showed possible evidence of increased "C" cell tumors of the thyroid and ovarian neoplasia in rats at 500 ppm; however, this study did not test at high enough levels to adequately assess the carcinogenic potential of Propachlor. A carcinogenicity study in mice did not test at high enough levels to adequately assess carcinogenicity potential (500 ppm). Propachlor was positive in a chromosome aberration assay, and suggestive of a positive response in a CHO/HGPRT assay. It was negative in a rat bone marrow cytogenetics assay and in an in vitro UDS assay.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Metolachlor in a weight-of-the-evidence determination of carcinogenic potential.

1. Metolachlor administration resulted in significant dose-related increasing trends in liver neoplastic nodules (adenomas) and combined liver adenomas and/or carcinomas in male rats for both the Hazleton and Hardisty pathology reports. In addition, female rats had significant dose-related increasing trends in liver neoplastic nodules (adenomas) and combined liver adenomas and/or carcinomas for both the Hazleton and Hardisty pathology

reports. There were significant differences in the pair-wise comparisons of the controls with the 3000 ppm dose group for liver adenomas for the Hardisty report and for combined liver adenomas and/or carcinomas for both the Hazleton and Hardisty reports. These tumor incidences were greater than the contemporary historical control range.

2. The PRC agreed that the incidence of nasal tumors in female rats at the HDT was not related to Metolachlor administration at doses of 3000 ppm. However, this dose was determined to be only marginally adequate for evaluation of carcinogenicity. Therefore, the issue of whether Metolachlor would or would not produce nasal tumors cannot be unequivocally resolved.

3. No increase in the incidence of neoplasia was associated with Metolachlor administration in mice.

4. Although the PRC did not feel that Metolachlor posed a high degree of risk for genotoxicity, they recommended repeating the in vivo/in vitro unscheduled DNA test to fulfill the data gap for genotoxicity testing.

5. Metolachlor is structurally similar to Acetochlor and Alachlor, two Group B2 carcinogens with genotoxic activity. The metabolic and biological similarity or dissimilarity of Metolachlor to these compounds has not yet been adequately established. The PRC agreed that the registrant should provide data on the identification of metabolites from the submitted study "Disposition of Metolachlor in the rat (general metabolism)" to resolve issues and fulfill the data requirements.

G. Classification of Carcinogenic Potential:

The PRC considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity. The PRC agreed that the classification for Metolachlor should be Group C - possible human carcinogen and recommended that, for the purpose of risk characterization, a low dose extrapolation model be applied to the experimental animal tumor data (q_1^*) for quantification of human risk. The q_1^* will be re-calculated based on hepatic neoplasia (combined adenoma and carcinoma) in female rats using the data in the pathology report by Dr. Jerry Hardisty (dated July 20, 1984, Table 4).

The PRC recommended that the registrant perform additional tests in stages. First it was recommended that the in vivo/in vitro unscheduled DNA test be repeated to fulfill the data gap for genotoxicity testing. If this test proves negative, the PRC recommended that the registrant should provide data on the identification of metabolites from the submitted study "Disposition of Metolachlor in the rat (general metabolism)" to resolve issues and fulfill the data requirements. Finally, a comparative metabolism study was recommended in order to provide information on the metabolic generation of quinone imine and

formaldehyde and to support the assertion that the metabolism of Metolachlor is substantially different from its major structurally analogous compounds. After the completion and evaluation of the recommended studies, the PRC agreed to reconsider the weight-of-evidence for the classification of Metolachlor.

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

CASWELL#: 188DD
CAS-REG#: 51218-45-2

P.C. CODE 108801- 2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide FILE LAST PRINTED: 03/14/94

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-2(b) Carcinogenic Species: mice IBT 622-07925; 12/15/77	CGA 24705 Tech. (Metolachlor)		Oncogenic LOEL => 3000 ppm (HDT) IBT valid (audited by Ciba-Geigy) Doses: 0, 30, 1000 & 3000 ppm		Minimum 000434
83-1(a) and 83-2(a) Feeding/carcinogenic-2 year Species: rat Hazleton Raltech 80030; 4/29/85	Metolachlor Technical FL-800362	250369 250370 250375 245958 258390	Oncogenic LOEL = 3000 ppm (incr. incid. of neoplastic nodules/hepatocellular carcinomas). Systemic NOEL = 300 ppm. LEL = 3000 ppm. (Decr. body wt. gain). Doses: 0, 30, 300 & 3000 ppm in CD Crl:CD(SD)BR rats. Addendum- Examination of nasal turbinates revealed additional evid. of weak oncogenicity at this site. Addendum - 5/2/93: A very slight effect was noted on body weight gain in the high dose males with a significant effect noted in high dose females from about week 4 to the end of the study. There was an apparent increase in liver absolute (7%) and relative (13%) weights for the high dose males Although these differences were not statistically significant- DER 010251 ' The RfD Committee accepted the conclusions of the DERs, 8/25/93.		Supplementary 001374 Minimum 004200 Minimum 006444 Minimum 010251 010661
83-1(a) and 83-2(a) Feeding/carcinogenic-2 year Species: rat IBT 622-07926; 2/9/79	Metolachlor Tech 99.9% Batch # FL-750227 & FL-752105	244166 099628 099626 070048	Sys. NOEL < 30 ppm (LDT) (decr spleen wt.). Onco LEL = 1000 ppm (hepatocellular carcinoma and cystic cholangioma) Doses in Charles River str: 0, 30, 300 & 1000 ppm IBT - validated		Supplementary 001051
83-1(a) and 83-2(b) Feeding/carcinogenic-2 year Species: mice Hazleton Raltech 79020; 8/13/82	Metolachlor Tech.	248722	Oncogenic LOEL > 3000 ppm (HDT). Systemic NOEL = 1000 ppm Systemic LEL = 3000 ppm (decr. wt. gain, decr. survival of females.) Levels tested: 0, 300, 1000 and 3000 ppm.		Minimum 003885 Minimum 004725
83-1(b) Feeding-1 year Species: dog Ciba-Geigy Corp. Inc. 862253; 1/23/89	Metolachlor tech., batch FL-861768, purity 97%	409807-01 411645-01	Systemic toxicity NOEL - males: 300 ppm. Sys. LOEL - males: 1000 ppm based on an increased alkaline phosphatase activity. Sys. NOEL - females: 100 ppm. Sys. LOEL - females: 300 ppm based on decreased body weight gains. Dietary levels of: 0, 100, 300 & 1000 ppm for 13 or 52 weeks. Mean daily intake: Males: 3.5, 9.7 & 32.7 mg/kg/day. Females: 3.6, 9.7 & 33.0 mg/kg/day. Strain tested: beagles from Marshall farms. Study can be upgraded by identification of compound purity. Review of additional submitted data and identification of compound purity has allowed upgrading of this study. (DER 009699, 08/27/92). ' The RfD Committee recommended that a RfD of 0.097 mg/kg/d be established. This was derived from a NOEL of 9.7 mg/kg in the 1 yr. dog feeding study. (8/25/93).		Supplementary 008442 Guideline 009699 010088 010251 010661

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U.S. ENVIRONMENTAL PROTECTION AGENCY
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CASWELL#: 188DD
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P.C. CODE 108801- 2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide FILE LAST PRINTED: 03/14/94

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(a) Developmental Toxicity Study Species: rat Ciba-Geigy Corp. Inc. 227625; 6/21/76	CGA 24705 Technical (Metolachlor)		Teratogenic NOEL > 360 mg/kg. Fetotoxic NOEL > 360 mg/kg Maternal NOEL > 360 mg/kg. Doses by gavage in Sprague Dawley: 0, 60, 180 and 360 mg/kg/day		Minimum 000434
83-3(a) Developmental Toxicity Study Species: rat Argus Research Labs 203-004; 08/06/85	CGA-24705 (Metolachlor FL-841697, 96.4% a.i.) Lot# B-203-004-A	00151941	Maternal NOEL = 300 mg/kg/day. Maternal LOEL = 1000 mg/kg/day, based on deaths, incr. incid. of clinical signs, lower body weight gains & decr. food consumption. Developmental NOEL = 300 mg/kg/day. Developmental LOEL = 1000 mg/kg/day based on reduced number of implantation per dam, with a resulting reduced litter size, a slight increase in resorptions per dam & the resultant slight increase in postimplanation loss. Also, a decrease in mean fetal body weight. CGA-24705 was administered by oral gavage to pregnant CrL:COBS(CD)(SD)BR rats from Charles River KIngston Breeding Lasb, Inc. at dose levels of: 0, 30, 100, 300 or 1000 mg/kg/day by gavage on gestation days 6-15 inclusive. This study satisfies the guideline requirement (83-3(a)) study in rats. ' RfD Committee recommends that the NOEL and LOEL be revised to 100 & 300 mg/kg/d based on the increased salivation in a significant number of animals. 8/25/93.		Minimum 009509 010661
83-3(b) Developmental Toxicity Study Species: rabbit Argus Research Labs 203-001; 07/16/80	CGA-24705 tech. (Metolachlor FL-7911174, 95.4%)	00041283	Fetotoxic NOEL > 360 mg/kg/day. Teratogenic NOEL > 360 mg/kg Maternal NOEL = 120 mg/kg. Levels tested gavage: 0, 36, 120, 360 mg/kg Maternal NOEL = 120 mg/kg/day. Maternal LOEL = 360 mg/kg/day. Developmental NOEL => 360 mg/kg (HDT). CGA-24705 was administered by oral gavage to pregnant NZW rabbits from Dutchland Lab at dose levels of: 0, 36, 120, and 360 mg/kg/day from gestation days 6-18 inclusive. Maternal toxicity was noted in the high dose group in the form of an increase in clinical observations and lower body weight gain. No developmental toxicity was noted in the dose levels tested. Reevaluation (DER 010088). RfD Committee accepted the conclusions of the DERs. (8/'25/93)		Minimum 001051 010088 010661
83-4 Reproduction-3 generation Species: rat IBT 622-07928; 1/4/78	CGA 24705 Technical (Metolachlor)		NOEL > 1000 ppm (HDT) IBT Supplementary (1/31/80). Doses: 0, 30, 300 & 1000 ppm		Minimum 000434 Supplementary 000438 Supplementary 002265 Supplementary 003197

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U.S. ENVIRONMENTAL PROTECTION AGENCY
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CASWELL#: 188DD
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P.C. CODE 108801- 2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide FILE LAST PRINTED: 03/14/94

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-4 Reproduction-2 generation Species: rat Toxigenics Inc. 450-0272; 8/31/81	*Metolachlor (Tech)*	245959 245960 245961	*Reproductive NOEL = 300 ppm. Reproductive LEL = 1000 ppm (reduced pup* weights & reduced parental food consumption. Doses: 0, 30, 300 or 1000 ppm ' RfD Committee accepted the conclusions of DER 001374. (8/25/93).		Guideline 001374 010088 010661
82-1(b) Feeding- 6 months Species: dog Interntl. Res. and Develop. Co 382-054; 11/2/79	CGA 24705 Tech (metolachlor)	00032174	NOEL = 100 ppm LEL = 300 ppm (based on a lower rate of decrease for SAP levels) Doses: 0, 100, 300, and 1000 ppm in beagles Addendum: Systemic NOEL = 300 ppm. Systemic LOEL = 1000 ppm based on reduced body weight gains and food consumption in the high dose males & females. An effect was noted on the APTT in the mid & high dose males & females (not statistically significant in high dose females) at the end *of the study; however, the biological relevance of these observations is* unknown. Also, a stat. sig. increase in alkaline phosphatase activity was noted in the mid dose males & high dose males & females, again the biological relevance of this observation is unknown as it is not accompanied by gross or histopathological observations. This study is acceptable as a range finding study for a chronic in the dog. Dose levels: 0, 100, 300 & 1000 ppm in the diet to beagle dogs from Ridglan res farms, Inc, for 6 months. Major deficiency: the purity of the test article was not provided. ' The RfD Committee agreed that this 6 month study was useful in support of the 1 yr. study that revealed a 9.7 mg/kg/d NOEL. (DER 010661). *(8/25/93).*		Minimum 000432 010251 010661
82-1(a) Feeding-3 month Species: rat Oncins Research and Breeding 3/1/74	Metolachlor Technical	112841	NOEL = 1000 ppm		Supplementary 000434 000436 000437 000438
82-1(b) Feeding-3 month Species: dog Oncins Research and Breeding 1974	CGA 24705 (metolachlor)		NOEL = 500 ppm		Minimum 000438 000436 000434

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
82-2 Dermal-3 week Species: rabbit Ciba-Geigy Ltd. 862012; 11/16/87	Metolachlor Tech. (FL - 841697) 96.4% a.i.	418331-01	Metolachlor tech. was applied dermally, daily for 6 hrs/day for at least 21 consec. days. There were 5 male & 5 female rabbits at each dose of: 0, 10, 100, & 1000 mg/kg/day. Very slight or moderate erythema was observed in all treatment groups; other signs of dermal irritation included dry skin, wrinkling, & fissuring. There was a dose-related incr. in the incidence of minor histopathological alterations of the skin. Statistically significant dose-related increases in total bilirubin were observed in females from the 100 & 1000 mg/kg/day dose group. Statistically significant dose-related increases in the mean absolute & relative liver weight values were observed in males from the 1000 mg/kg/d dose group. A statistically significant increase was observed in relative kidney weight in females from the 1000 mg/kg/d dose group. Systemic NOEL (F) = 10 mg/kg/d. Systemic NOEL (M) = 100 mg/kg/d. Systemic LOEL (F) = 100 mg/kg/d. Systemic LOEL (M) = 1000 mg/kg/d. Study used male & female NZW rabbits from H.A.R.E., Inc. of Hewitt, N.J.; test compound was a liquid. The study does not satisfy the guideline requirement (82-2) for a 21 day dermal study in rabbits, information on the purity and stability of the test article is required. Additionally provided data allows this study to be upgraded to Guideline (DER 010315).		Supplementary 009553 Guideline 010315
84-2(b) Mutagenic-dominant lethal test Species: mice Ciba-Geigy Corp. Inc. 9/8/76	CGA 24705 (metolachlor)		Negative mutagen		Minimum 000434
84-2(b) Mutagenic-micronucleus assay Species: Chinese hamster Ciba-Geigy Corp. Inc. 831498; 10/84	Metolachlor Tech 95.9%	258390	No evidence of mutagenicity. No evid. that test material was absorbed or reached the target tissue bone marrow. Doses: 0, 1250, 2500 & 5000 mg/kg by gavage in Chinese hamsters (str. unspecified) A Range Finding dose study by the Registrant resolved the rationale for dosing and this study is now Acceptable (DER 006444).		Inconclusive 004725 Acceptable 006444
84-2(b) Mutagenic-DNA damage/repair Species: Fibroblasts Ciba-Geigy Corp. Inc. 831499; 11/84	Metolachlor Tech 95.9%	258390	No evidence of mutagenicity. Unacceptable because no rationale for the selection of doses. Doses: 0.125, 0.625, 3.125, & 15.625 nl/ml in in human fibroblasts (CRL 1121, ATCC.) A Range Finding dose study by the Registrant resolved the rationale for dosing and this study is now Acceptable (DER 006444).		Unacceptable 004725 Acceptable 006444

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-2(b) Mutagenic-DNA damage/repair Species: rat hepatocytes Ciba-Geigy Corp. Inc. 831497; 11/84	Metolachlor Tech 95.9%	258390	No mutagenicity. Unacceptable; no rationale for doses. Doses: 0.25, 1.25, 6.25 & 31.25 in hepatocytes from adult male (Tif:RAIF[SPF]]) A Range Finding dose study by the Registrant resolved the rationale for dosing and this study is now Acceptable (DER 006444).		Unacceptable 004725 Acceptable 006444
84-2(a) Mutagenic- lymphoma mutation Species: mice Ciba-Geigy Ltd. 831500; 12/5/84	Metolachlor Tech 95.9%	262713	Not a mutagen in both presence and absence of metabolic activator.		Acceptable 006444
84-4 Mutagenic-unscheduled DNA synt Species: rat hepatocytes Hazleton Biotechnologies Corp. 8147; 08/10/88	Metolachlor Tech. (FL 841 697 96.4% a.i.)	420433-01	Metolachlor technical was inactive in this in vivo/vitro rat hepatocyte assay at the dose levels tested; however dose levels were not adequate. Dose levels tested: 3.07, 31.49, 291.9, & 519.5 mg/kg for females; 2.88, 31.91, 301.03, & 474.5 mg/kg in males). Deficiencies: lack of information on the purity and batch no. of Metolachlor. Ths missing information was provided and is acceptable to the Agency; however the study is not upgraded since the HED Peer Review Committee has determined that the study used an inaequate range of doses and the test must be repeated at higher dosages.		Unacceptable 008880 010315
Registration standard	Metolachlor		Toxicology Chapter - 6/2/86		005504
Risk assessment Species:	Metolachlor		Document date - 1/8/85. Title 'Recommendation for Revision of Metolachlor Risk Assessment'.		004200
Carcinogenicity- Peer Review Species: 8/13/91	Metolachlor		Reviewer's Peer Review Package. Date: 04/02/91		008523
Carcinogenicity- Peer Review Species: 07/21/93	Metolachlor		Peer review Document - Date: 07/21/93 Classification: Cq1*		010490
RfD Committee Report 08/25/93	Metolachlor		The Committee recommended that an RfD of 0.097 mg/kg/day be established. This was derived from an NOEL of 9.7 mg/kg/day in a 1 year dog study. (8/25/93).		010661

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U.S. ENVIRONMENTAL PROTECTION AGENCY
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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
RfD Committee Report 08/25/93	Metolachlor		A Reference Dose (RfD) for Metolachlor was assessed by the Health Effects Division RfD Committee on March 21, 1986 and verified by the Agency RfD Work Group on April 22, 1986, The RfD was then reassessed on April 7, 1988 and verified by the Agency Work Group on June 22, 1988. The current RfD value on IRIS is 0.15 mg/kg/day based on a NOEL of 15 mg/kg/day for decr. body wt. gain observed at 150 mg/kg/day in a long term feeding study in rats using an uncertainty factor of 100.		010661
85-1 Metabolism Species: rat Ciba-Geigy Ltd. 26/81; 8/20/81	Metolachlor C14	262713	Approximately 90% of the amount administered was excreted over 3 days. Elimination of label was about equal between urine & feces		Minimum 006444
85-1 Metabolism Species: rat Ciba-Geigy Corp. Inc. 7/74; 12/74	CGA 24 705; purity 99& un labeled; > 99% (labeled)	not given	Identification of urinary metabolites of CGA 24 705 was assessed after oral administration of 52 mg/kg, 28 mg/kg, and 3 mg/kg to male rats. Two metabolites, each comprising approx. 5% of chloroform extractable radioactivity, were identified (CGA 37 735 and CGA 46 129). In study 12/74, the % found as CGA 46 129 was between 20-25% of urinary radioactivity, & CGA 37 735 represented between 3-5% of urinary radioactivity. Conjugation of CGA 24 705 was not observed. Aqueous extractable radioactivity contained 58% of the total urinary radioactivity and was comprised of 5 different fractions, but was not identified. A metabolic pathway for CGA 24 705 was proposed from these data.		Supplementary 008442
85-1 Metabolism Species: rat Ciba-Geigy Corp. Inc. ABR-8611; 2/17/87	Unlabeled Metolachlor, purity not spec.; C-14 metolachlor, radio purity > 99%	401144-01	A single low (1.5 mg/kg), a single high (300 mg/kg) , and a repeated low (1.5 mg/kg/day for 15 days) oral doses of metolachlor were used. These doses were found to to be readily absorbed and excreted by male & female rats. The urinary and fecal elimination of radioactivity was essentially complete within 48 to 72 hrs post-dosing. Approx. 48-64% of the radiolabel was recovered within 7 days with similar amount in the feces. Low levels of radioactivity were found in the tissues of all animals at 7 days postdosing. The study did not provide the purity of the test compound used and the metabolites were not identified. Upgrading is possible by identification of test compound purity and metabolites.		Supplementary 008442
85-2 Metabolism - dermal absorption Species: rat Ciba-Geigy Corp. Inc. ABR-87051; 08/25/87	Metolachlor - phenyl ring 14C-CGA-24705	418331-02	Metolachlor was tested at doses of 0.01, 0.1, and 1.0 mg/cm2. The compound was rapidly absorbed with significant bioaccumulation noted. The absorption at 24 hrs was 62.84, 26.85, and 16.15% of the respective administered doses with 30.66, 13.66 and 10.07% of the respective administered dose remaining in the carcass and 11.09, 19.4, and 15.49% remaining on the washed skin. Washing at 10 & 24 hrs showed significant absorption of skin residues. Study use male CrL:CD(BR) rats from Charles River with the administered dose suspended in deionized water. This study satisfies the guideline requirement (85-2) for a dermal absorption study in rats.		Acceptable 009553

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
88 Carcinogenicity- Peer Review Species: 2nd Meeting 09/10/91	Metolachlor		Second Peer Review Document - Date: Sep 10, 1991 Classification: Cq		009074
Laboratory audit - oncogenic Species: rat			This audit found no study deficiencies which could preclude this study from being classified as core minimum - 7/20/84		004199
Metabolites			2,6-Dinitro-4-trifluoromethyl phenol		004200
Peer Reviews Species: ? 8/23/85	Metolachlor		Current Agency Decision: Pending; Additional studies requested: mutagenicity & metabolism. Peer Review documented: 08/23/85		007809
81-1 Acute oral LD50 Species: rat	CGA 24705 Technical		LD50 = 2780 mg/kg	3	000436 000428
81-1 Acute oral LD50 Species: dog	CGA 24705		At 19 mg/kg emesis		000436
81-1 Acute oral LD50 Species: rat	CGA 24705 6EC		LD50 = 4286 mg/kg (male) LD50 = 2828 mg/kg (female)	3	000436
81-1 Acute oral LD50 Species: dog	CGA 24705 6EC		24.5 mg/kg - emesis	3	000436
81-1 Acute oral LD50 Species: rat IBT 8530-10822; 10/28/77	Dual 8E (Metolachlor)		LD50 = 2533.5 mg/kg (1888.5 - 3398.9)	3	Minimum 000430
81-1 Acute oral LD50 Species: rat Interntl. Res. and Develop. Co 382-043; 10/17/78	Milocep (Metolachlor)		LD50 = 3868 mg/kg (M & F) (3142- 4761) LD50 = 4811 mg/kg (M). (3771-6139) LD50 = 2944 mg/kg (F). (2185-3965)	3	Minimum 000431

VOLUME 2 OF 2 OF SUBMISSION

TECHNICAL METOLACHLOR

EXAMINATION OF THE ONCOGENICITY POTENTIAL FOR METOLACHLOR

EPA GUIDELINES REQUIREMENT: NOT APPLICABLE

AUTHOR - DR. A. W. PHELPS

REPORT COMPLETED: NOVEMBER 18, 1988

PERFORMING LABORATORY: NOT APPLICABLE

STUDY NO.: NOT APPLICABLE

VOLUME 1 OF 1 OF STUDY

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AGRICULTURAL DIVISION
CIBA-GEIGY CORPORATION
POST OFFICE BOX 18300
GREENSBORO, NC 27419

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METOLACHLOR

BRIEFING PAPER FOR THE SCIENTIFIC ADVISORY PANEL

PREPARED BY

C. BRECKENRIDGE, J. CHANG, B. SIMONEAUX,
J. STEVENS and D. SUMNER

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CIBA-GEIGY CORPORATION
AGRICULTURAL DIVISION
POST OFFICE BOX 18300
GREENSBORO, NC 27419

METOLACHLOR: BRIEFING PAPER FOR THE SCIENTIFIC ADVISORY PANEL

Executive Summary

Metolachlor is a chloroacetanilide herbicide that is used extensively throughout the U.S. for control of grasses, primarily in corn and soybeans. In the past, dietary exposure to metolachlor has been regulated by using a safety factor approach to set food crop tolerances. If the EPA proposal before the Scientific Advisory Panel is implemented, future tolerances would be determined on the basis of quantitative risk assessment. CIBA-GEIGY believes that such an approach is not scientifically defensible. **In 1988, CIBA-GEIGY submitted critical data pertinent to the issues discussed in the EPA second Peer Review; this information was not considered.**

Metolachlor has a low order of acute toxicity and was not mutagenic in a series of standard assays. Metolachlor is not a reproductive toxin and it is not a teratogen. The liver has been identified as a target organ at high doses in the rat, mouse and dog in subchronic and chronic toxicity studies.

Metolachlor is completely metabolized and rapidly excreted in the feces and urine; the pharmacokinetics for metolachlor are non-linear because at low doses, elimination follows a 2 compartment model and at high doses, a 1 compartment model. Metolachlor is found primarily in red blood cells, liver, kidney, lung and heart. Approximately 36-66% of the radiolabelled dose has been accounted for with an additional 25-30% found as non-extractable compounds in the feces. Less than 2% are dealkylated metabolites. This is consistent with data that shows that metolachlor cannot be dealkylated even by strong acid hydrolysis.

No evidence of an oncogenic response to metolachlor has been observed in the mouse or male rat at feeding levels up to the maximum tolerated dose of 3000 ppm. In female Sprague-Dawley rats, a slightly increased incidence of benign liver tumors (0/60 vs. 6/60) was seen at the maximum tolerated dose of 3000 ppm. This response was considered marginal because the control group had a low spontaneous incidence of tumors in the liver and the high dose females survived longer and therefore were at risk for a longer period of time than the control group. Furthermore, evidence of non-linear kinetics of elimination at 3000 ppm and increased hepatocyte turnover, presumably due to cytotoxicity, suggest that these findings may have no relevance to man.

Based on nasal tumors observed in other structurally similar acetanilides, notably alachlor and acetochlor, the EPA proposed that metolachlor should be regulated using mathematical risk assessment. However, metolachlor does not produce nasal tumors nor is it metabolized to 2,6-methyl-ethylaniline. Alachlor and acetochlor do induce nasal turbinate tumors and are biotransformed to the diethyl- or methylethyl- aniline, respectively. Thus a similar tumor profile and similarities in metabolism of alachlor, acetochlor and the positive control 2,6-dimethylaniline, provide a clear contrast to metolachlor.

Based on these considerations, CIBA-GEIGY believes that the application of quantitative risk assessment to a marginal Class C oncogen in order to "facilitate comparison of cancer potency of metolachlor with other members of this class of herbicides," as stated in EPA's second Peer Review, is scientifically unfounded. From a regulatory perspective it is inappropriate to regulate dietary exposure to Class B oncogens like alachlor and acetochlor in exactly the same way as metolachlor.

1.0 OBJECTIVES

A concise evaluation of the toxicological data on metolachlor will be presented, including additional critical information that was submitted to the EPA in December, 1988, but which was not incorporated into EPA's second Peer Review (See Appendices 4, 5, and 6). This will include an evaluation of the following:

1) The significance of benign liver tumors noted only in female Sprague-Dawley rats at the highest feeding level of 3000 ppm. The relevance of this tumor to man will be discussed in the context of a negative mutagenicity profile, variability of diagnosis, historical control incidence, the effects of increased survival in the high dose group, and the impact of metabolic overload and possible increased hepatocellular turnover at high doses of metolachlor.

2) The incidence of nasal turbinate tumors in other bioassays on several chloroacetanilide herbicides.

   These data will be compared to the incidence reported by NTP for 2,6-dimethylaniline and will be contrasted with the results obtained in the oncogenicity studies on metolachlor.

3) Possible differences in metabolism between metolachlor and the other chloroacetanilide herbicides that might account for the absence of an effect on nasal turbinate tumors in the metolachlor study compared to the clear dose response noted for acetochlor, alachlor, and 2,6-dimethylaniline.

Based on these considerations, CIBA-GEIGY hopes to convince the Scientific Advisory Panel and the EPA that the application of quantitative risk assessment to a marginal Class C oncogen like metolachlor in order to "facilitate comparison of cancer potency of metolachlor with other members of this class of herbicides," as stated in EPA's second Peer Review, is scientifically unfounded.

2.0 INTRODUCTION

2.1 Summary

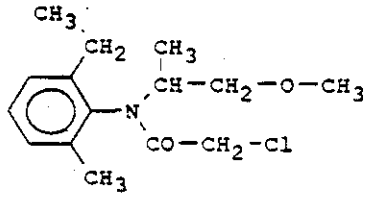
Metolachlor is a chloroacetanilide herbicide that is used extensively throughout the U.S. for control of grasses primarily in corn and soybeans. In the past, dietary exposure to metolachlor has been regulated by using a safety factor approach (100X) to set food crop and water tolerances[1,3,4] (also see Appendix 1, page 6). If the EPA proposal[2] before the Scientific Advisory Panel is implemented, future tolerances would be determined on the basis of quantitative risk assessment. CIBA-GEIGY believes that such an approach is not scientifically defensible.

2.2 Chemical Structure

Metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl) N-(2-methoxy-1-methylethyl) acetamide is a chloroacetanilide herbicide with the following chemical structure:

Figure 1

Chemical Structure of Metolachlor



2.3 Formulations

Metolachlor is formulated in various concentrations as a liquid (Dual®, Dual 8E, Pennant® Liquid) or granule (Dual 25G , Pennant 5G). It is also formulated in combination with triazine herbicides such as atrazine (Bicep® 6L), simazine (Derby™) or cyanazine (Cycle®).

2.4 Uses/Tolerances

Metolachlor is applied pre-emergence, for the control of many annual grasses primarily in corn and soybeans but also in safflower, stone fruits, vegetables, peanuts, and tree nuts. Food crop tolerances have been established for metolachlor in these agricultural products as well as in milk, meat, poultry and eggs.

3.0 HAZARD ASSESSMENT

3.1 Acute Toxicity

Metolachlor has a low order of acute toxicity by the oral[5], dermal[6], and inhalation[7] routes of exposure. Metolachlor is slightly irritating to the eyes[8] and minimally irritating to skin[9]. A positive dermal sensitization reaction has been observed in the Guinea pig[10].

3.2 Mutagenicity

In studies conducted by CIBA-GEIGY, neither metolachlor nor its metabolites formed as a result of metabolic activation in vitro or in vivo show any evidence of mutagenic potential. Metolachlor does not induce point mutation in either bacterial[11] or mammalian cells[12]; it does not cause chromosome aberrations[13,14] nor DNA damage in human fibroblasts[15] or in rat hepatocytes[16,17] and it does not have dominant lethal effects in germ cells[18].

EPA's comment that there is not an acceptable mutagenicity study in the "other mechanisms of mutagenicity category" is inconsistent with recent communications from EPA, which states that the study requirements were met (Appendix 1). An in vivo/in vitro study submitted in December, 1988, on unscheduled DNA synthesis in rat hepatocytes[17], which the EPA apparently did not review, should resolve this question.

3.3 Reproductive and Developmental Toxicity

There were no reproductive effects of metolachlor in Sprague-Dawley rats in multigeneration reproduction studies conducted at feeding levels up to 3000 ppm[19,20]. Metolachlor was not teratogenic in the rat[21] or rabbit[22] at a maximum tolerated dose of 360 mg/kg.

3.4 Subchronic and Chronic Toxicity

No specific target organ toxicity has been identified in subchronic[23] or chronic[24] dog studies at feeding levels up to 2000 ppm. Evidence of increased liver weight was observed in subchronic[25] and chronic[26,27] rat studies.

3.5 Metabolism

3.5.1 Distribution and Excretion

As stated in the second EPA Peer Review, metolachlor is rapidly and extensively excreted by rats[28,29]. About 90% (89.9%) of the administered was excreted in 3 days following dosing. Elimination of the radiolabelled dose was about equal between urine and feces with no demonstrable differences between low (1.5 mg/kg) and high dose (300 mg/kg) groups.

The kinetics of elimination between the low and high dose groups however were remarkably different[30]. The elimination of metolachlor in the urine for the 1.5 mg/kg group is best described by a 2 compartment model, whereas at 300 mg/kg, urinary excretion followed a 1 compartment model. These differences in elimination kinetics ,when going from low to high doses, may indicate a shift in metabolism, i.e., the existence of a metabolic threshold.

Tissue distribution studies indicate the highest concentration of labeled metolachlor appeared in the erythrocyte[29]. Highly perfused and/or metabolically active tissues such as the liver, kidney, lung, and heart also showed significant residues while the lowest levels were found in brain, bone and muscle.

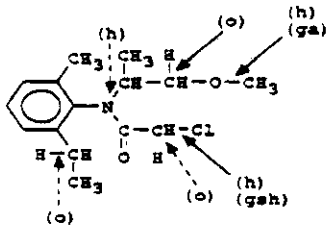
3.5.2 Degradation Pathways

The major pathway of degradation involves hydrolysis of the methylether and the chloroacetyl groups[30,31]. Minor pathways involve conjugation with glutathione (chloroacetyl group), conjugation with glucuronic acid and oxidation (alcohol resulting from hydrolysis of the ether), side chain hydroxylation, and dealkylation of the amide. An additional minor reaction involves the cyclization of the acid alcohol to a lactone.

The active sites of metabolism are identified in Figure 2 and the structure of the major metabolites are given in Figure 1, Appendix 2. The percent of the total radiolabelled dose accounted for by each degradation pathway appears in Table 1.

Figure 2

Active Sites of Metabolism



H: Hydrolysis
O: Oxidation
GA: Glucuronic acid conjugation
GSH: Glutathione conjugation

TABLE 1

Summary of the Metabolic Degradation of Metolachlor

Pathway/Reaction	Structures[1]	Percent Found[1]
Methyl-Ether Hydrolysis	II	15-32%
Chloroacetyl Hydrolysis	III	~1%
Alcohol Oxidation	IV	5-15%
Glutathione Conjugation	V - VII	7-8%
Glucuronic acid Conjugation	VIII - XI	~6%
Side-chain Hydroxylation	XI - XIII	1-2%
Amide Dealkylation	XIV - XVI	1-2%
Nonextractable (Feces)		25-30%
TOTAL		61-96%

[1]See Appendix 2, Figure 1.

3.5.3 Difference in Metabolism Between Alachlor, Acetochlor & Metolachlor

The sulfate conjugate of the p-hydroxylated disubstituted aniline appears to be a significant metabolite of both alachlor[38] and acetochlor[41] whereas, there is no evidence that it is formed for metolachlor.

Dealkylation and deacylation are precursor reactions for the formation of the disubstituted aniline. The amount of amide dealkylated metabolite is very minor (1-2%). This has also been reported by Feng and Wratten[47] for the in vivo metabolism of metolachlor by rat liver microsomal enzymes.

Administration of labelled ethyl-methyl aniline[32] has shown that the major excretory product is the sulfate conjugate of the p-hydroxylated aniline. The chromatographic behavior of this compound is unique and none was observed in the excreta of the metolachlor treated rats (See Appendix 2).

A mechanism to explain why alachlor and acetochlor are more susceptible to dealkylation than metolachlor is proposed in Appendix 2, Figure 3. The 2 carbon bridge between the amide nitrogen and the ether oxygen in metolachlor provides less reactivity than for acetochlor and alachlor, therefore, making metolachlor less susceptible to hydrolysis.

Chemically, when metolachlor is subjected to strong acid hydrolysis, the rate of production of the methyl-ethyl aniline is not significant and, therefore, this technique is not used as a method for residue analysis, whereas, the residue method for

alachlor is based on quantitative conversion to aniline. The difference in susceptibility to acid hydrolysis between metolachlor and alachlor has been documented by EPA's Residue Chemistry Branch (Appendix 3).

3.5.4 EPA Classification of CIBA-GEIGY Metabolism Studies

EPA indicated in its second Peer Review document that the rat metabolism studies for metolachlor did not satisfy guidelines. However, it appears that EPA has reached this conclusion because the metabolism reports were submitted in two sections, one with the excretion and distribution data and the second with the metabolite identification data. CIBA-GEIGY believes that together, these studies meet the FIFRA requirement.

4.0 ONCOGENIC POTENTIAL

4.1 Mouse Oncogenicity Study

No evidence of an oncogenic response was noted in two separate studies conducted in CD-1 mice fed metolachlor technical for 104 weeks at levels of 0, 30, 300, 1000, or 3000 ppm[33,34]. Based on reduced body weight gain, it was concluded that 3000 ppm was the maximum tolerated dose. The no-observable effect level was 1000 ppm.

4.2 Chronic Toxicity/Oncogenicity Study in Rats

4.2.1 Liver Tumors

Sprague-Dawley rats were fed metolachlor technical in the diet for 104 weeks at levels of 0, 30, 300, 1000 or 3000 ppm in two separate studies[25,27]. No evidence of an oncogenic response was noted in males at the maximum tolerated dose of 3000 ppm.

An increased incidence of benign liver tumors noted in females at 3000 ppm prompted CIBA-GEIGY to have a panel of pathologists re-examine the liver slides from both studies using the most recent NTP diagnostic criteria. This information, which was tabulated and submitted to the EPA in December, 1988, was unfortunately not considered in EPA's second Peer Review of metolachlor.

Table 2, which summarizes this information for the IBT study, shows that considerable differences exist between the original pathologist's results and the subsequent evaluation. These differences are attributed to a sharpening of the distinction between hyperplasia and adenomas in the liver.

TABLE 2

Summary of the Liver Tumor Incidence in the IBT
Chronic Rat Study on Metolachlor - Females

	Original Pathologist			1988 Re-evaluation[1]		
Feeding Level	Hyperplastic Nodules	Carcinomas	Combined	Adenomas	Carcinomas	Combined
0	1/54	0/54	1/54	0/70	0/70	0/70
30	1/58	0/58	1/58	0/59	0/59	0/59
300	3/60	0/60	3/53	0/60	0/60	0/60
1000	3/60	0/60	3/60	1/60	0/60	1/60
3000	9/60	2/60	11/60	3/70	2/70	5/70*

[1]Six pathologists re-examined the liver slides for all females; the results represent the majority opinion; this evaluation included interim sacrifice animals.

*Significantly different from the control group; $P \leq 0.05$.

Table 3, which represents similar information for the Hazleton study, shows that the original pathology read was more in line with current NTP criteria than for the IBT study. One additional adenoma was diagnosed in each of the 30 and 300 ppm groups and one of the carcinomas in the 3000 ppm group was reclassified as an adenoma and an additional adenoma was found in this group.

The historical control incidence of liver tumors in 12 chronic rat studies at Hazleton (Table 4), which was provided in the 1988 submission, indicates that the control group was at the lowest end of the control range.

TABLE 3

Summary of the Liver Tumor Incidence in the Hazleton
Chronic Rat Study on Metolachlor - Females

	Original Pathologist			1988 Re-evaluation[1]		
Feeding Level	Hyperplastic Nodules	Carcinomas	Combined	Adenomas	Carcinomas	Combined
0	0/60	0/60	0/60	0/60	0/60	0/60
30	0/.60	0/60	0/60	1/60	0/60	1/60
300	1/60	0/60	1/60	2/60	0/60	2/60
3000	4/60	2/60	6/60	6/60*	1/60	7/60*

[1]Five pathologists examined the liver slides for all females; the results reflect the majority opinion.
*Significantly different from the control group by survival adjusted prevalence analysis $P \leq 0.05$.

TABLE 4
Hepatocellular Lesions in Female Sprague-Dawley Rats
Hazleton Historical Control Data

Study Number

	1	2	3	4	5	6	7	8	9	10	11	12
Adenoma	0/47	0/60	2/60	0/55	0/70	2/50	2/39	0/51	0/46	1/70	1/59	6/52
Carcinoma	0/47	0/60	0/60	0/55	0/70	0/50	0/39	0/51	0/46	0/70	0/59	1/52
Combined	0/47	0/60	2/60	0/55	0/70	2/50	2/39	0/51	0/46	1/70	1/59	7/52

In EPA's second Peer Review document, it is stated that "the survival of the animals at 24 months was 54%, 57%, 42% and 57% for the control, low-, mid-, and high dose, respectively." The correct survival incidence at study termination was in fact 33/60 (55%), 30/60 (50%), 29/60 (48%) and 40/60 (67%) for the 0, 30, 300 and 3000 ppm females, respectively. A Kaplan-Meier survival analysis, shown in Figure 3, was statistically significant overall even though the individual group paired comparisons were not significant (Table 5).

FIGURE 3

KAPLAN-MEIER SURVIVAL CURVE
METOLACHLOR FEMALE RAT (HAZELTON)

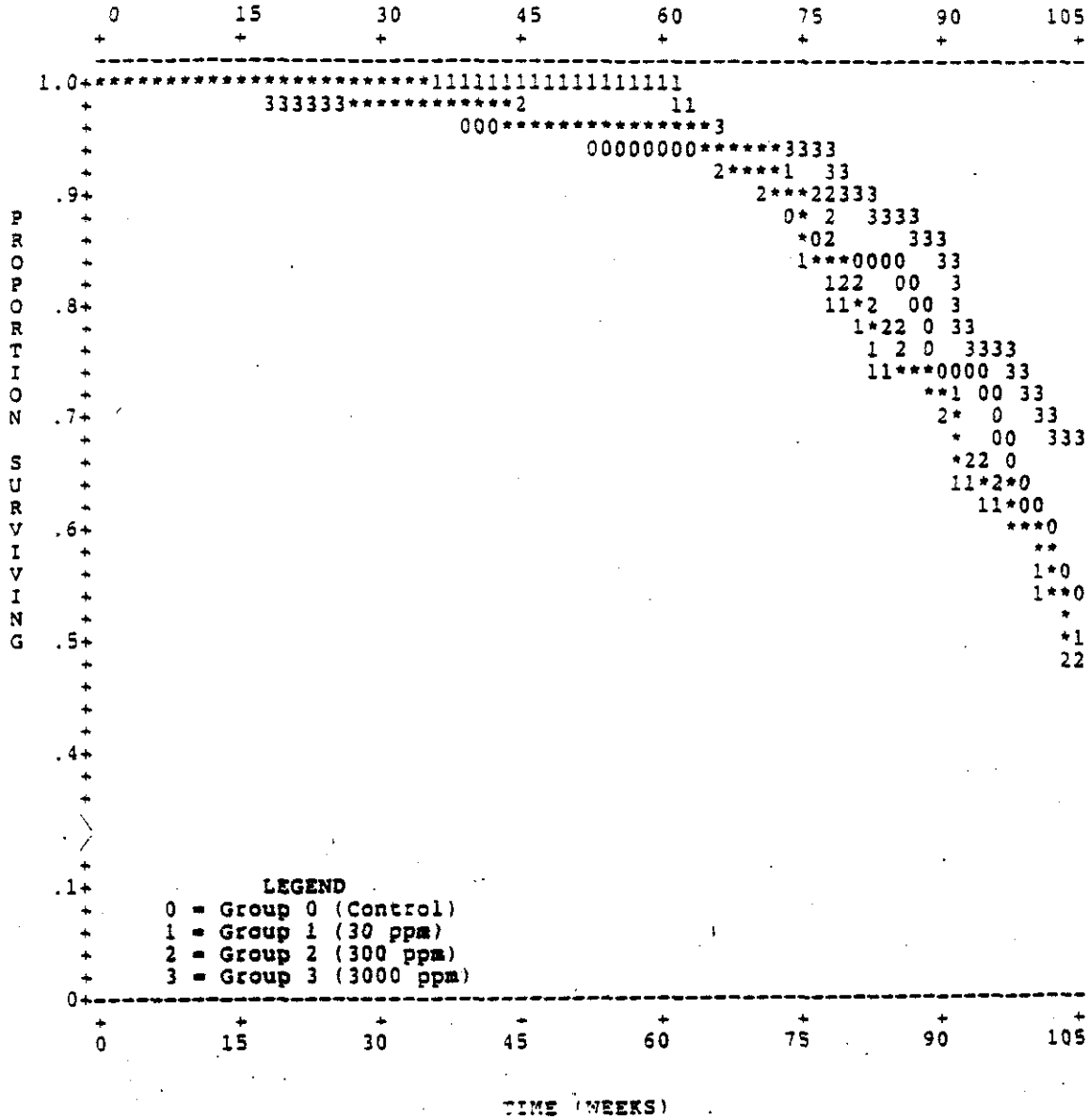


TABLE 5

Statistical Analysis for Hazleton's
Chronic Rat Study on Metolachlor - Females

	Group	0	1	2	3
	Level:	0 ppm	30 ppm	300 ppm	3000 ppm
Mortality Unadjusted (%)		0.550	0.500	0.483	0.678
Kaplan-Meier Adjusted Point Estimate (%)		0.550	0.500	0.483	0.678
S.E.		0.064	0.065	0.065	0.061

	Probability Values	
	Cox's Test	Gehan-Breslow
Trend p (1-tailed)	0.0356*	0.0392*
Group 0 vs 1	0.6555	0.5467
Groups 0 vs 2	0.5910	0.5409
Groups 0 vs 3	0.2059	0.1676

* = Statistical significance at $p \leq 0.05$.

A tumor prevalence analysis adjusted for differences in survival was performed using the 1988 incidence data shown in Table 3. The p-value for the paired comparison between the high dose and the control group for adenomas was 0.0248 (0/60 vs. 6/60) and the corresponding p-value for adenomas plus carcinomas (0/60 vs. 7/60) was 0.0112. An alternate scenario based on one control animal having an adenoma at study termination generated p-values of 0.0544 and 0.0120, respectively. This result illustrates the borderline nature of the response noted in the liver of female rats.

That the female rat may be metabolically compromised at the 3000 ppm level is supported by a shift to non-linear kinetics of elimination at high doses (See Appendix 4). There is also evidence of hepatocelluar toxicity, as indicated by an increased proportion of cells in S-phase in female rats, but not males that received an oral dose of metolachlor equivalent to that received by the 3000 ppm animals (See Appendix 5).

The results suggest that the marginal liver tumor response noted in females likely has little relevance to man under expected exposure conditions because:

1) Metolachlor is not genotoxic.

2) The liver response is restricted to the high dose female rat.

3) There is evidence of a possible saturation of elimination.

4) There is evidence of hepatocellular toxicity and increased cell turnover suggesting that a non-genotoxic mechanism may be operating at high doses[48].

4.2.2 Nasal Turbinate Tumors

A retrospective, microscopic evaluation of the nasal turbinate region of rats from the chronic study conducted at Hazleton gave the results shown in Tables 6 and 7 for males and females, respectively. When these data were reviewed by EPA[35,36], concerns were raised about the possible relationship of these findings to treatment. This prompted CIBA-GEIGY to have a recognized expert in the field of nasal carcinogenesis, Dr. Kevin Morgan, re-examine the slides from the study. Unfortunately, the results of this re-evaluation (See Tables 6 & 7), which were submitted to the EPA in December, 1988 (MRID No. 40934401), apparently were not considered in EPA's second Peer Review.

TABLE 6

Summary of Nasal Turbinate Tumor Incidence in the Metolachlor Chronic Rat Study - Males

	Dr. William Fields (1981)			Dr. Kevin Morgan (1988)[1]		
Feeding Level	Adenomatous Polyp	Adeno-carcinoma	Fibro-adenoma	Polypoid Adenoma[2]	Adeno-carcinoma[3]	Neurofibro-sarcoma[4]
0	1/67	1/67	0/67	1/57	0/57	0/57
30	0/59	0/59	0/59	0/59	0/59	0/59[5]
300	1/53	0/53	0/53	0/53	0/53	0/53
3000	0/69	2/69	1/69	1/59	1/59	1/59

[1]None of the 1-year interim sacrifice animals were re-examined; none of these animals had nasal turbinate tumors in the original evaluation.
[2]Tumors of this type associated with the respiratory epithelium.
[3]Tumors of this type associated with nasal glands.
[4]Tumor associated with the peripheral nerve.
[5]Two metastatic, malignant lymphomas were also found in the 30 ppm group.

TABLE 7

Summary of Nasal Turbinate Tumor Incidence in the Metolachlor Chronic Rat Study - Females

	Dr. William Fields (1981)			Dr. Kevin Morgan (1988)[1]		
Feeding Level	Adenoma Papilloma	Squamous cell Papilloma	Odontoma Adenoma[1]	Polypoid Papilloma[2]	Squamous Papilloma[3]	Odontoma[4]
0	0/67	0/67	1/67	0/57	0/57	1/57
30	0/59	1/59	0/59	0/57	0/57	0/57
300	1/53	0/53	0/53	1/59	0/59	0/59
3000	0/69	1/69	0/69	0/59	1/59	0/59

[1]None of the 1-year interim sacrifice animals were re-examined; none of these had nasal turbinate tumors in the original evaluation.
[2]Tumors of this type associated with the respiratory epithelium.
[3]Tumors of this type associated with buccal mucosa.
[4]Tumor associated with the teeth.

Based on this re-evaluation, Dr. Morgan concluded that there was no evidence of a treatment-related neoplastic response in the nasal passages of male or female Sprague-Dawley rats at feeding levels up to 3000 ppm (See Appendix 6).

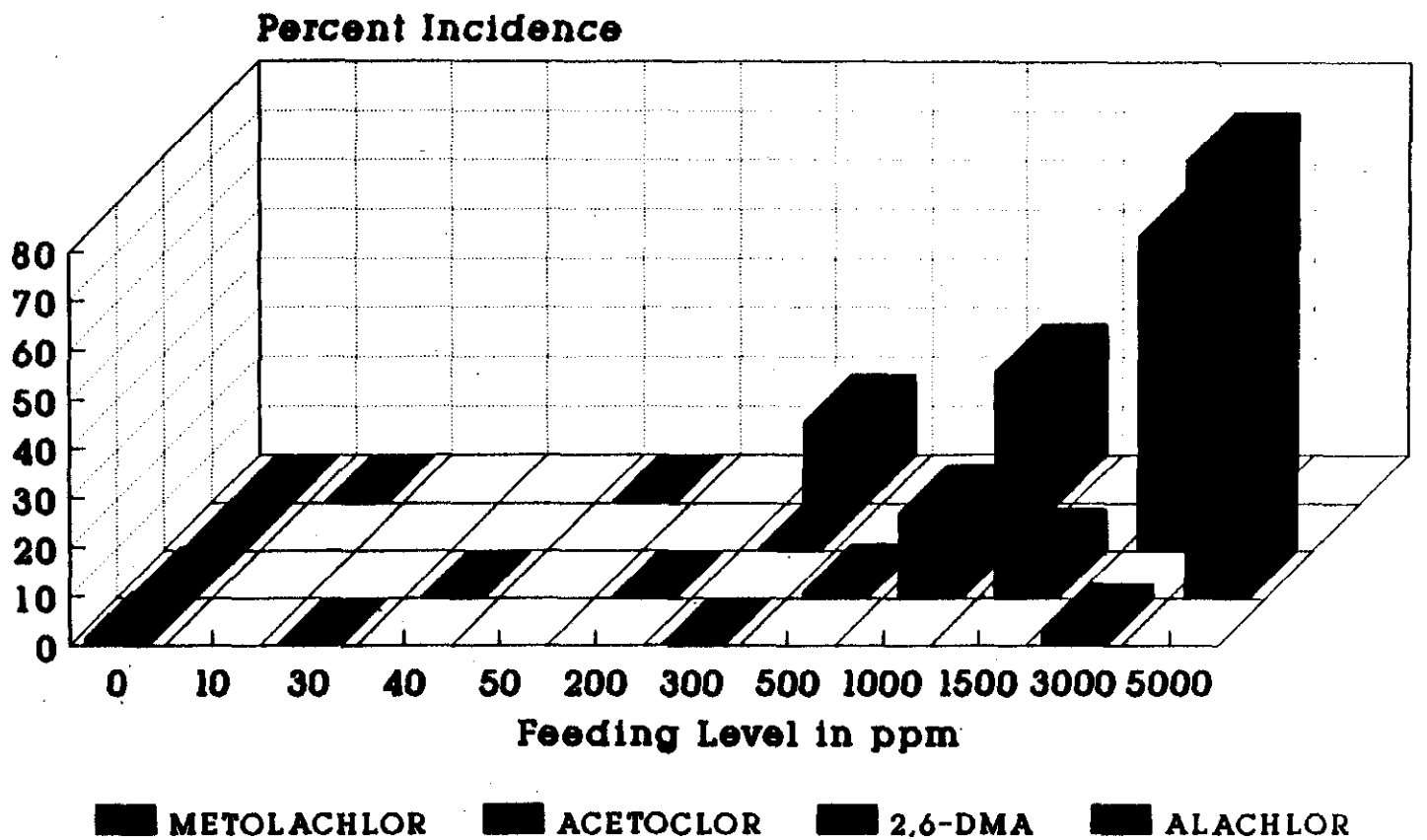
In contrast, the incidence of nasal turbinate tumors in studies on 2,6-dimethylaniline[37], alachlor[38] (metabolite is 2,6-diethylaniline) and acetochlor[38] (metabolite is 2-methyl, 6-ethylaniline) which are summarized in Tables 8 & 9 and Figures 4 & 5, show a clear relationship to treatment at feeding levels of $\geq$ 1000 ppm for acetochlor and alachlor. The reference compound, 2,6-dimethylaniline produced clear effects at 3000 ppm and equivocal results at 1000 ppm.

4.2.3 Tumors Profiles Comparisons

Table 10 provides a weight of evidence comparison for alachlor, acetochlor, 2,6-dimethylaniline and metolachlor. From this summary, it can be seen that alachlor, acetochlor and 2,6-dimethylaniline are mutagenic. They significantly increase both benign and malignant tumors in two species with two common sites of response; whereas, metolachlor is non-mutagenic, producing only a weak benign response in one sex and species at one site. Therefore, as shown in this summary of biological data, one can state that activitywise, alachlor, acetochlor and dimethylaniline are similar. Metolachlor is clearly biologically different.

Further the potency estimates, based on the nasal tumor again shows activity, clearly separating metolachlor from the others.

Figure 4. Combined Incidence of Nasal Tumors in male rats: Metolachlor, Alachlor, Acetochlor and 2,6-DMA*



* 2,6-dimethylaniline NTP study

TABLE 8

Summary of the Nasal Tumor Incidence for Males in the Chronic Rat Studies on 2,6-Dimethylaniline, Alachlor, and Acetochlor

Feeding Level	2,6-Dimethylaniline		Alachlor[1,2]		Acetochlor		Metolachlor	
	Adenoma	Carcinoma[3]	Adenoma	Carcinoma[4]	Adenoma	Carcinoma[5]	Adenoma	Carcinoma
0	0/56	0/56	0/90	0/90	1/139	0/139	1/57	0/57
10	----	----	0/47	0/47	----	----	----	----
30	----	----	----	----	----	----	0/59	0/59
40	----	----	----	----	1/70	0/70	----	----
50	----	----	0/44	0/44	----	----	----	----
200	----	----	----	----	0/70	0/70	----	----
300	0/56	0/56	15/91	0/91	----	----	0/53	0/53
500	----	----	----	----	1/70	0/70	----	----
1000	2/56	0/56	10/41	1/41	12/70	0/70	----	----
1500	----	----	----	----	6/69	0/69	----	----
3000	10/56	26/56	65/103[b]	7/103[b]	----	----	1/59	1/59
5000	----	----	----	----	18/69	2/69	----	----

[1]Doses of 0.5, 2.5, 14 or 15, 42, and 126 mg/kg/day are represented as equivalent feeding levels of 10, 50, 300, 1000, and 3000 ppm respectively.

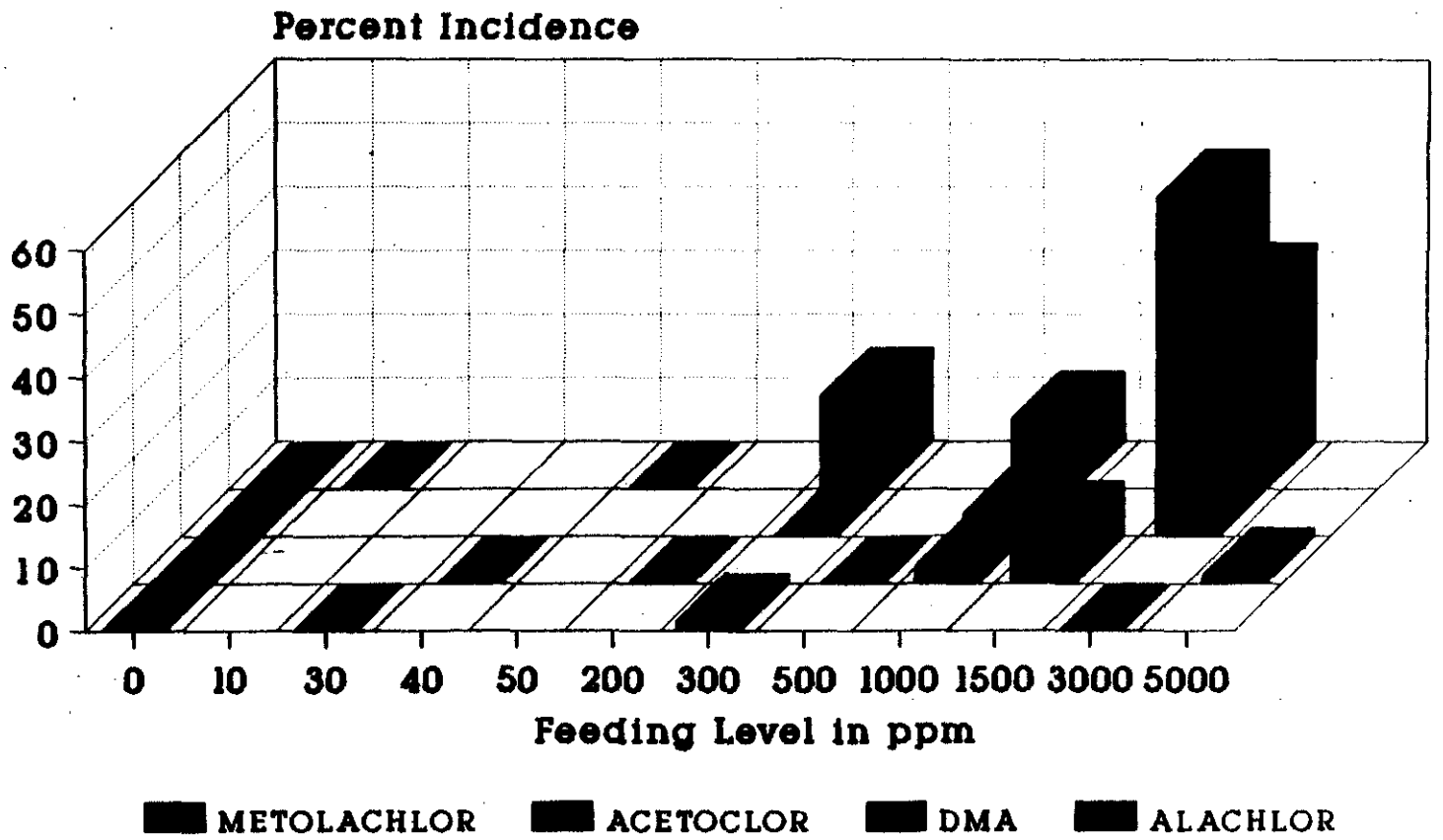
[2]Combined from Daly 1981b and Stout, 1983a and Stout, 1983b as listed on page 12, Table 3 of the alachlor special review document[38].

[3]Identified as carcinoma or adenocarcinoma in Table 12 of NTP Report # 278.

[4]Identified as papillary adenoma in the acetochlor peer review document.[40]

[5]Identified as papillary adenocarcinoma in the acetochlor document.[40]

Figure 5. Combined Incidence of Nasal Tumors in female rats: Metolachlor, Alachlor, Acetochlor, and 2,6-DMA*



* 2,6-demethylalanine NTP study

TABLE 9

Summary of the Nasal Tumor Incidence in the Chronic Rat Studies on 2,6-Dimethylaniline, Alachlor, and Acetochlor - Females

Feeding Level	2,6-Dimethylaniline		Alachlor[1,2]		Acetochlor		Metolachlor	
	Adenoma	Carcinoma[3]	Adenoma	Carcinoma[4]	Adenoma	Carcinoma[5]	Adenoma	Carcinoma
0	0/56	0/56	0/91	0/91	0/139	0/139	0/57	0/57
10	----	----	0/42	0/42	----	----	----	----
30	----	----	----	----	----	----	0/59	0/57
40	----	----	----	----	0/70	0/70	----	----
50	----	----	0/47	0/47	----	----	----	----
200	----	----	----	----	0/70	0/70	----	----
300	0/56	0/56	14/95	0/95	----	----	1/59	0/59
500	----	----	----	----	0/68	0/68	----	----
1000	1/56	1/56	4/45	1/45	2/70	0/70	----	----
1500	----	----	----	----	6/69	0/70	----	----
3000	6/56	24/56	21/73[b]	2/73[b]	----	----	0/59	0/59
5000	----	----	----	----	1/69	0/69	----	----

[1]Doses of 0.5, 2.5, 14 or 15, 42, and 126 mg/kg/day are represented as equivalent feeding levels of 10, 50, 300, 1000, and 3000 ppm respectively.

[2]Combined from Daly 1981b and Stout,1983a and Stout,1983b as listed on page 12, Table 3 of the alachlor special review document[38].

[3]Identified as carcinoma or adenocarcinoma in Table 12 of NTP Report # 278.

[4]Identified as papillary adenoma in the acetochlor peer review document[40].

[5]Identified as papillary adenocarcinoma; squamous cell carcinoma or carcinoma in situ are not included here[40].

TABLE 10

Weight of Evidence Summary for
2,6-Dimethylaniline (DMA), Alachlor, Acetochlor, Metolachlor

Weight of Evident Class : Subclass		DMA	Alachlor	Acetochlor	Metolachlor
Genotoxicity		+	+	+	-
Tumors:	Benign	+	+	+	+
	Malignant	+	+	+	
Species:	One				+
	Two	+	+	+	
Sex	One				+
	Two	+	+	+	
Organ/Site:	One				+
	Multiple[1]	+	+	+	
Potential to form Quinone imine		+	+	+	-

[1]Alachlor, acetochlor and 2,6-dimethylaniline all produced nasal tumors in rats of both sexes and lung tumors in female mice.

Based on this summary, it is clear that the biological response to metolachlor is qualitatively and quantitatively different than that observed for other chloroacetanilides in this class and for 2,6-dimethylaniline.

4.2.4 Discussion

The rat metabolism studies reported by Monsanto and summarized by EPA for alachlor[38] and acetochlor[39] demonstrate that these chemicals both form a sulfate derivative of the disubstituted aniline. Subsequent publications by Monsanto scientists[42,44,45] and others[43,46], suggest that the metabolism of alachlor to 2,6-dimethylaniline by the rat is the first step in forming the ultimate reactive species, a quinone imine[42,46]. This biotransformation may occur in the liver but also possibly in the nasal mucosa[42,47].

Since metolachlor does not readily undergo dealkylation, the production of a disubstituted aniline or the quinone imine appear unlikely. It is proposed that this difference in metabolism accounts for the marked differences in the nasal turbinate tumor profile of metolachlor compared to alachlor, acetochlor and 2,6-dimethylaniline.

5.0 WEIGHT OF EVIDENCE SUMMARY

Metolachlor is:

- NOT acutely toxic.
- NOT teratogenic in the rat or rabbit.
- NOT a reproductive hazard (2 multiple generation studies).
- NOT mutagenic.
- NOT carcinogenic in the mouse or the male rat (2 studies each).

Metolachlor may:

- Cause a marginally increased incidence of benign liver tumors in female Sprague-Dawley rats, but ONLY at the highest level of 3000 ppm.

Metolachlor does not:

- Cause an increased incidence of nasal turbinate tumors.

SAR analysis indicates that:

- the tumor profile observed with metolachlor is qualitatively and quantitatively different than that reported for alachlor or acetochlor.

- the response profile noted for alachlor and acetochlor is similar to that reported by NTP for 2, 6-dimethylaniline.

- metolachlor is not readily dealkylated to form the disubstituted aniline, whereas dealkylation of alachlor and acetochlor has been reported to be an important route of metabolic degradation.

Therefore:

- Since the metabolic and bioassay response profile for metolachlor is significantly different from alachlor and acetochlor, less weight should be given to the fact that the compounds are structurally similar and more weight given to qualitative and quantitative differences in the response profiles.

- Some doubt can be raised as to whether metolachlor is appropriately classified as a Class C oncogen based on a slightly increased incidence of benign liver tumor in high dose female rats because:

  1) Metolachlor is not genotoxic.

  2) The high dose group survived longer than the control group.

  3) The control incidence was low compared to historical controls.

  4) There is evidence of saturation kinetics at $\geq$ 3000 ppm.

  5) There is evidence of hepatocellular toxicity and cell turnover suggestive of a non-genotoxic mechanism activated only at high doses.

- The use of a safety factor approach is the most appropriate approach for regulating metolachlor exposure.

6.0 SUMMARY AND CONCLUSIONS

No evidence of an oncogenic response to metolachlor has been observed in the mouse or male rat at feeding levels that were at the maximum tolerated dose.

The slightly increased incidence of benign liver tumors observed in female Sprague-Dawley rats at the highest feeding level of 3000 ppm has an equivocal relationship to treatment because of variability in diagnosis, an unusually low control incidence and lengthened survival in the high dose females compared to the control group.

The occurrence of 1 polypoid adenoma in the nasal turbinates, in a 3000-ppm male and 1 adenoma in a 300-ppm female were not considered treatment-related because a similar tumor was observed in the control males and the finding in females did not display a dose-response relationship since no tumors were found in the 3000 ppm group.

The single occurrence of 1 adenocarcinoma, a tumor associated with the nasal gland, in a 3000 ppm male was not definitive in indicating a relationship to treatment.

Based on a comparison of the tumor profile for acetochlor, alachlor and 2,6-dimethylaniline, with metolachlor, it is concluded that there is no justification for using quantitative risk analysis to obtain an estimate of relative potency. Furthermore, based on the equivocal incidence of benign liver tumors in female rats, there is some doubt that metolachlor should be classified as a Class C oncogen.

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APPENDIX 2

METABOLISM OF METOLACHLOR AND RELATED CHLOROACETANILIDES

PREPARED BY

D. SUMNER AND B. SIMONEAUX

SEPTEMBER 9, 1991

CIBA-GEIGY CORPORATION
AGRICULTURAL DIVISION
POST OFFICE BOX 18300
GREENSBORO, NC 27419

Metabolism of Metolachlor and Related Chloroacetanilides

1.0 Chemical Class

Metolachlor belongs to the chloroacetanilides class of herbicides that also include acetochlor, alachlor, propachlor and butachlor. Others compounds belonging to the more general class of acetanilides include the drugs, acetaminophen and phenactin.

2.0 Rationale for Comparative Analysis

Bioassay results for alachlor and acetochlor, especially those which show an increased incidence of nasal turbinate tumors at feeding levels $\geq$ 1000 ppm, have prompted EPA to question whether the isolated occurrence and low incidence of nasal turbinate tumors in the metolachlor chronic rat study may be due to treatment[1]. Since the significance of the bioassay results have been discussed elsewhere in detail, the purpose of this document is to more fully elaborate the chemical hypothesis concerning the production of what may be the ultimate carcinogen, the quinone imine. By considering how the quinone imine is produced by the metabolism of 2,6-dimethylaniline, alachlor and acetochlor, but not metolachlor, a logical basis for understanding the differences in the bioassay results may be reached.

3.0 Proposed Metabolic Pathway for Metolachlor (Figure 1)[2]

3.1 Major Pathways

No unmetabolized metolachlor (I) was recovered after oral administration to rats[3-7]. The initial reaction in the major pathway involves hydrolysis of the methyl-ether to produce the alcohol (II, 15-32%). Hydrolysis of the chloroacetyl group in II produced the diol (III, ~ 1%). Oxidation of the alcohol formed by hydrolysis of the methyl ether produced a second major metabolite the acid alcohol (IV, 5-15%). Thus up to 48% of the administered dose is recoverable directly from this pathway.

3.2 Minor Pathways

Minor pathways and branches from the major pathway involve conjugation of the chloroacetyl group with glutathione. The products observed from this conjugation were the sulfoxide and sulfone of the thiomethyl ether arising from this pathway. (Compounds V, 1-2%; VI, ~4%; and VII, ~2%).

A minor amount of glucuronic acid conjugation of the alcohol metabolites was also observed[7] (Compounds VIII, ~2%; IX, ~1%; X, ~2%; and XI, ~1%).

Side chain hydroxylation was also observed in small

quantities (Compounds XI, XII, and XIII 1-2% or less).

Dealkylation of the amide was observed to only a minor extent (Compounds XIV, XV, and XVI 1-2% or less).

The metabolites above account for up to 65% of the administered radioactive material. An additional 25 - 30% of the dose was present in the feces as non-extractable material. Therefore approximately 61-95% of the administered dose has been accounted for as summarized in Table 1.

Table 1

Summary of the Metabolic Degradation Metolachlor

Pathway/Reaction	Structures	Percent Found*
Methyl-Ether Hydrolysis	II	15-32%
Chloroacetyl Hydrolysis	III	~ 1%
Alcohol Oxidation	IV	5-15%
Glutathione Conjugation	V - VII	7-8%
Glucuronic acid Conjugation	VIII - XI	~ 6%
Side-chain Hydroxylation	XI - XIII	1-2%
Amide Dealkylation	XIV - XVI	1-2%
Nonextactable (Feces)		25-30%
Total		61-96%

* The metabolism of metolachlor has been evaluated in several studies. The reported ranges are approximations based upon the amount of radiolabel recovered in the urine and feces.

3.3 Metabolic Pathways not Observed

The absence of certain pathways may be as important as those which have been observed in considering the toxicology of metolachlor. The percent degradation by amide dealkylation is very minor and there is no evidence of deacylation of the amide. Notably there is no evidence of production of methyl ethyl-aniline (MEA) or its daughter product (the sulfate conjugate of the para-hydroxylated methyl-ethyl aniline). Sixty to 77% of the sulfate was found in urine of rat that had received an oral dose of radio-labelled MEA[6].

The absence of MEA following metolachlor dosing can be determined by comparison of the chromatography of the urine extracts of rats treated with metolachlor with those treated with MEA. The chromatographic behavior of metolachlor metabolites from rats is shown in Figure 2[5]. It is worth noting that in solvent system 1 the MEA sulfate has an Rf of approximately 0.6; in system 6 the Rf was 0-0.05[6]. Therefore, if MEA were to have been produced, a radioactive zone would have appeared to the far left midpoint up the

plate. Since no radioactivity was observed in this area, we conclude that no significant production of MEA occurs in the rat.

The stability of the amide in metolachlor and lack of MEA formation is further reinforced by the residue method which even under strong hydrolytic conditions does not hydrolyze the amide (Appendix 3).

4.0 Differences in the Degradation of Metolachlor & Alachlor

4.1 Metabolism

The major difference between metolachlor and alachlor lies in the configuration about the amide nitrogen. Considering that the first major reaction with metolachlor is hydrolysis of the methyl-ether, it is logical to assume the same reaction would occur with alachlor. Figure 3 illustrates a possible facilitated hydrolysis of alachlor which is not possible with metolachlor.

Alternatively, the differences may be viewed as the oxidation state of the alkyl carbon atom bound to the amide nitrogen. Metolachlor is extremely stable to metabolic and chemical hydrolysis of the amide because of the steric hindrance provided by the substituents on the nitrogen atom. In alachlor the alkyl carbon atom attached to the amide nitrogen exists in an oxidation state equivalent to an aldehyde. Therefore, it is much more susceptible to hydrolysis. With removal of the alkyl group, steric hindrance about the amide would be reduced and hydrolysis to the aniline could be expected. A similar reaction is expected for acetochlor, as illustrated in Figure 3.

4.2 Chemical Hydrolysis

The difference between metolachlor and alachlor are further illustrated in the residue methods used for each. Metolachlor does not hydrolyze to the aniline at a sufficient rate for practical use as a residue method. Whereas residues of alachlor are quantitatively converted to the aniline in plant and animal tissues (See Appendix 3).

4.3 Formation of the Quinone Imine

Alachlor has been reported to exert its toxicologic effects through metabolism to the aniline and formation of a reactive quinone imine[9-13]. This is also the likely ultimate carcinogen for 2,6-dimethylaniline as also illustrated in Figure 3.

5.0 Discussion

The special review position document on alachlor[38] and subsequent publications by Monsanto scientists[9,11,12] and others[10,13] provide evidence that a significant fraction of alachlor is metabolized to 2,6-diethylaniline by the rat. This biotransformation may occur in the liver but also possibly in the nasal mucosa[9,14]. Since it has been shown that alachlor can be metabolically transformed to the reactive quinone imine derived from diethylaniline, it is probable that this reactive species may be the ultimate carcinogen in the nasal mucosa.

Since metolachlor does not metabolize to a reactive quinone imine a significant incidence of nasal turbinate tumors are not observed.

6.0 Conclusions

Metabolic differences between metolachlor on the one hand and alachlor and acetochlor on the other hand, may account for the dramatic differences in the tumor response profiles observed in the chronic bioassays on these chemicals. Specifically, the the two carbon bridge between the amide nitrogen and the ether oxygen prevents the hydrolysis of the amide thereby blocking the production of methyl-ethyl aniline. Acetochlor and alachlor readily form this compound which is subsequently biotransformed to the reactive species, the quinone imine. This chemical may be the ultimate carcinogen responsible for the nasal turbinate tumors noted in the chronic rat studies on alachlor, acetochlor and 2,6-dimethylaniline.

7.0 References

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Fig 1. Proposed metabolic pathways of metolachlor in the rat.

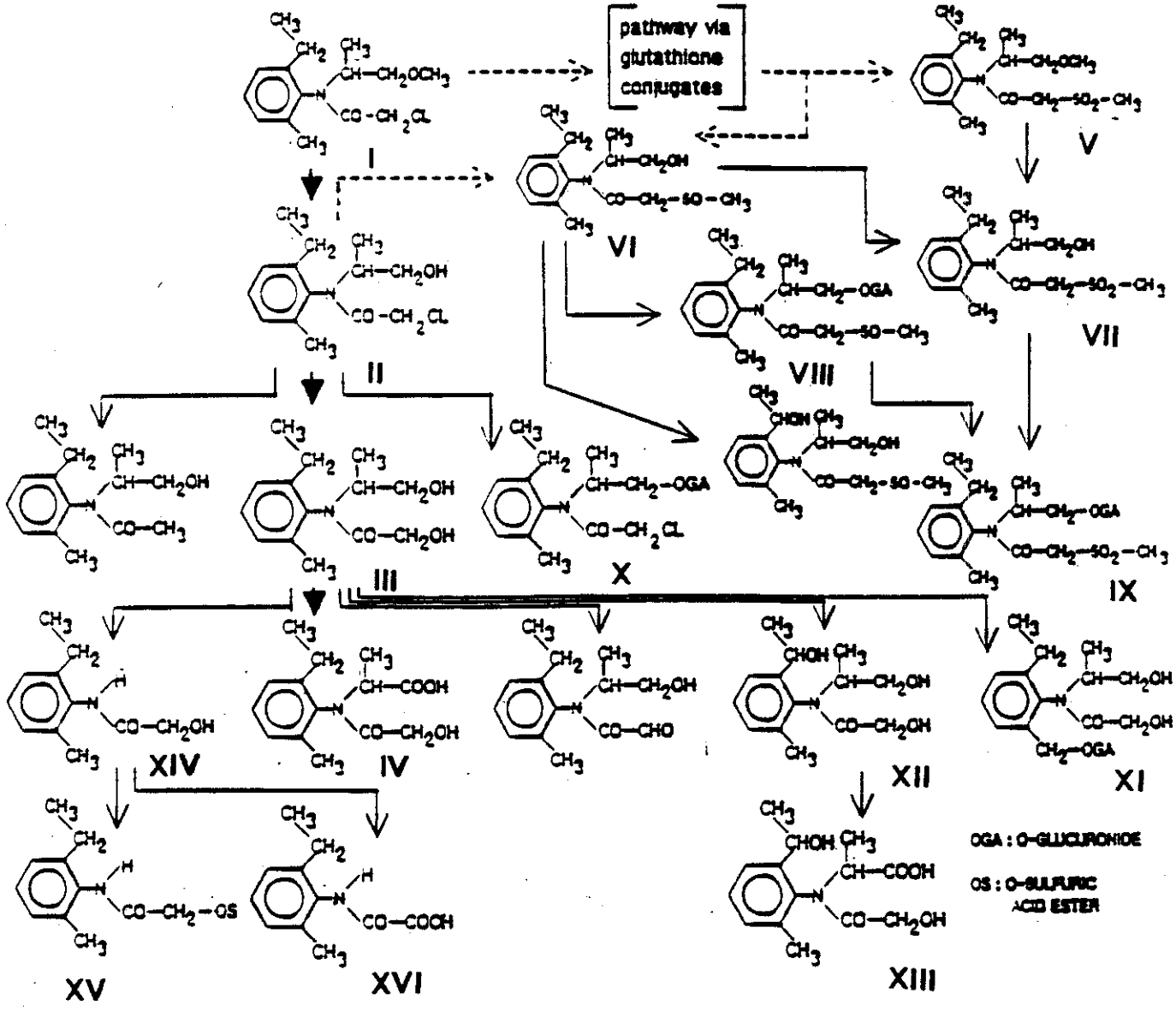
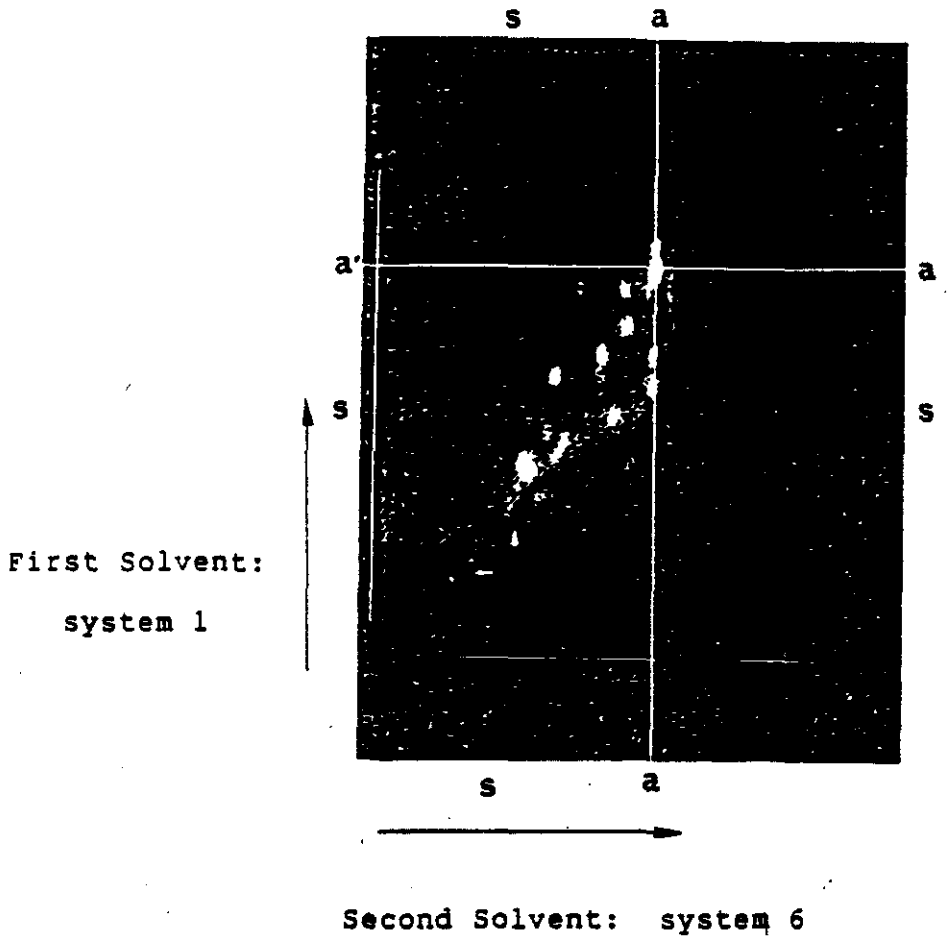


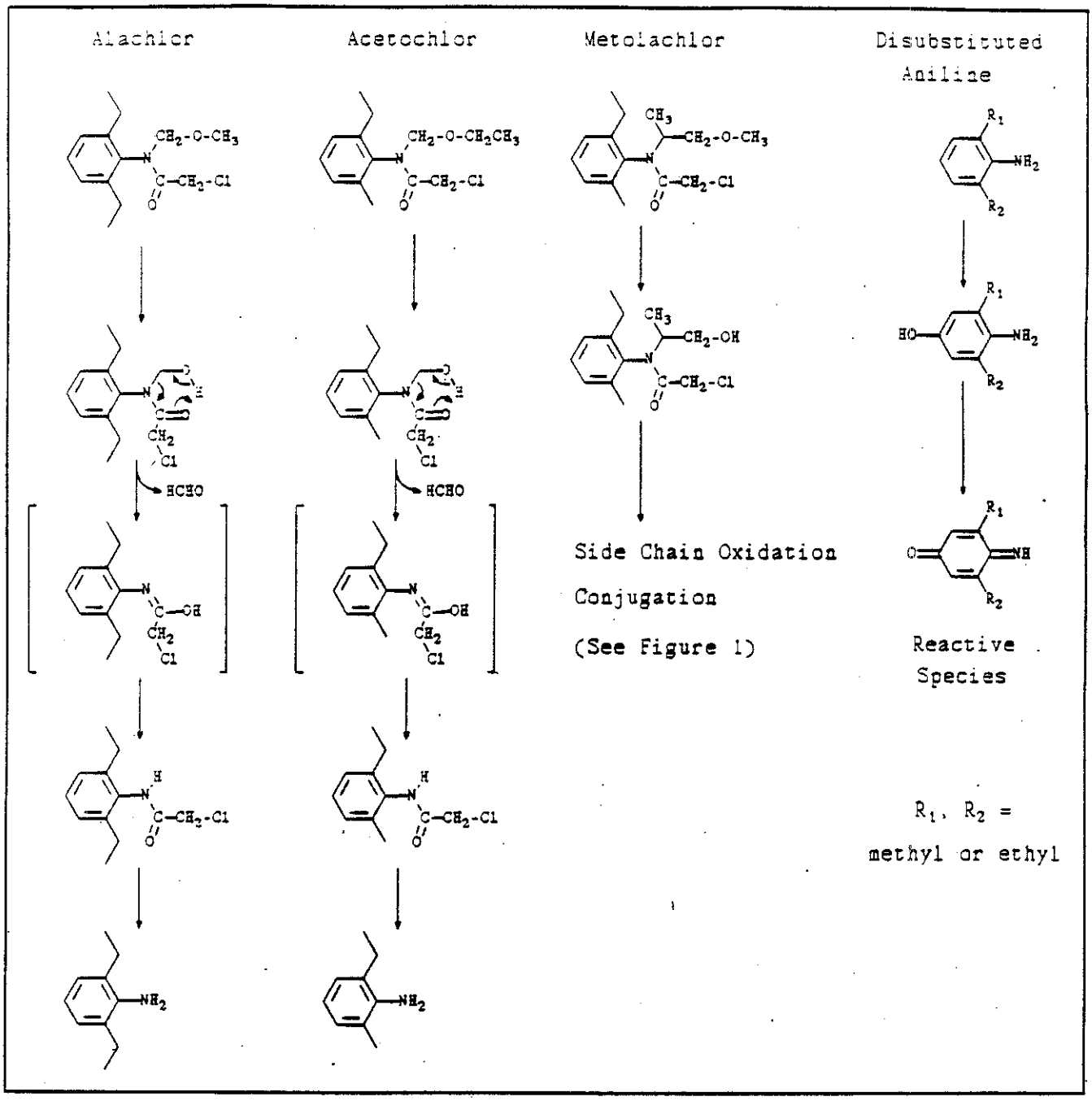
FIGURE 2 TWO DIMENSIONAL THIN LAYER CHROMATOGRAPHY OF THE AQUEOUS PHASE OF THE URINE FROM RATS DOSED WITH ^{14}C-CGA-24705



If aniline were formed from metolachlor in the rat, its sulfate conjugate would have been observed as a radioactive spot at the Rf values (Rf = .6 in system 1 and .05 in system 6) marked "s" (ref. CIBA-GEIGY report 29/82)

The Rf values marked "a", designate the location for the carboxylic acid metabolite IV from Figure 1 (ref CIBA-GEIGY report 12/74).

Figure 3 Proposed Pathway for Formation of the Disubstituted Aniline and the Quinoneimine



Monsanto

MONSANTO AGRICULTURAL COMPANY
800 N. Lindbergh Boulevard
St. Louis, Missouri 63167
Phone: (314) 694-1000

September 16, 1991

Mr. Robert B. Jaeger
Designated Federal Official
FIFRA Scientific Advisory Panel (H7509C)
Office of Pesticide Programs
U.S. Environmental Protection Agency
Room 821C, CM#2
1921 Jefferson Davis Highway
Arlington, VA 22202

Subject: FIFRA Scientific Advisory Panel review of Metolachlor; comments regarding structure activity comparisons with Alachlor and Acetochlor.

Dear Mr. Jaeger,

Monsanto has an interest in the proposed classification of metolachlor as a Group C, possible oncogen, because Agency documentation provided to the Scientific Advisory Panel includes a structure activity comparison two other chloroacetanilide herbicides, alachlor and acetochlor. Alachlor and acetochlor are both Monsanto products and have been classified as Group B-2 oncogens, in part because they produce nasal tumors in rats following chronic oral exposure. A number of other members of this class of chemistry have also been shown to produce nasal tumors in rats following chronic oral exposure. Investigations conducted by Monsanto indicate that metabolism is critical to tumor formation in rats. The attached document entitled, "Oncogenic Potential of Alachlor in Rats: Relevance For Human Risk Assessment", describes Monsanto's metabolic hypothesis for the formation of nasal tumors in rats. This hypothesis involves the oxidation of alachlor to a primary amine metabolite, 2,6-diethylaniline, which is further oxidized to an electrophilic quinone imine intermediate. We expect the same metabolic pathway to be operative for other chloroacetanilides, although quantitative differences in relative rates of quinone imine formation are expected. As described in the attached publication by Feng and Wratten, there are many similarities between alachlor and metolachlor metabolism. The other enclosed publications provide additional information on alachlor metabolism. This information should be of use to the Panel in its deliberations.

Monsanto is not requesting time for an oral presentation before the Panel on September 18, but will have its toxicology and metabolism experts available to answer any questions from the Panel.

Requests for additional information can be made through Dr. Kevin F. Cannon in Monsanto's Washington Office, 202-783-2460.

Sincerely,

Dennis P. Ward, Ph.D.
Toxicology Manager

Enclosures

cc: Kevin F. Cannon (Monsanto Washington Office)

Also note: Attached for your additional review is the executive summary of the "Alachlor Review Board In Canada".

ONCOGENIC POTENTIAL OF ALACHLOR IN RATS:

RELEVANCE FOR HUMAN RISK ASSESSMENT

Monsanto Agricultural Company
September 16, 1991

ONCOGENIC POTENTIAL OF ALACHLOR IN RATS
RELEVANCE FOR HUMAN RISK ASSESSMENT

Alachlor was developed by Monsanto Company and introduced in 1967 for pre-emergence or pre-plant control of a broad spectrum of grass, sedge and broadleaf weeds in corn, soybeans, dry beans, cotton, grain sorghum, sunflowers, peanuts and other crops. Alachlor is the herbicidal active ingredient in LASSO® herbicide. Alachlor (Figure 1) is a member of the chloroacetanilide class of chemistry.

The oncogenic potential of alachlor (1, see Figure 1) has been assessed in a series of rodent chronic bioassays. Two bioassays were conducted with the Long-Evans strain of rat. The first was conducted at Bio/dynamics, Inc. between 1978 and 1980 (study no. BDN-77-421) and the second was conducted at the Monsanto Environmental Health Laboratory (EHL) between 1980 and 1982 (study nos. ML-80-186/ML-80-224). A third bioassay, with CD-1 mice, was conducted between 1978 and 1980 and provided no evidence of oncogenic potential for alachlor in this specie.

On the Bio/dynamics study male and female Long-Evans rats (50/sex/group) received alachlor through their diet at dose levels equivalent to 0, 14, 42 and 126 mg/kg/day. Surviving male rats were sacrificed after 27 months and surviving females were sacrificed after 24 months on study. On the EHL study male and female Long-Evans rats received alachlor through their diets at dose levels equivalent to 0, 0.5, 2.5, 15 and 126 mg/kg/day for approximately 25 months. The high dose group on the EHL study included extra animals specifically for the purpose of investigating the progression and reversibility of an ocular lesion that was first diagnosed on the Bio/dynamics study. A portion of the animals in the 126 mg/kg/day group were administered alachlor treated diet for approximately 6 months and then switched to control diet for the remaining 19 months of the study. In essence, this study included a 'recovery group' at the high dose level. Due to the special investigations conducted on the high dose animals, the data for this group were reported separately under study no. ML-80-224. Data for the 0, 0.5, 2.5 and 15 mg/kg/day groups were reported under study no. ML-80-186. Although the results of these EHL studies were reported separately, all animals (and groups) were in fact part of a single bioassay (*i.e.* all rats were from the same supplier and shipment, and all were housed in the same animal room over the same period of time).

The high dose level of 126 mg/kg/day is considered to have exceeded a maximum tolerated dose (MTD), as evidenced by decreased survival, excessive body weight loss (>30%), and hepatocellular necrosis. Neoplastic responses attributable to alachlor administration were observed in the nasal turbinate mucosa and glandular stomach mucosa of both sexes and in the thyroid follicular epithelium of males. Significant increases in stomach and thyroid tumor incidences were restricted to the highest dose tested (*i.e.* 126 mg/kg/day). As indicated above, the EHL study contained a recovery group which received 126 mg/kg/day of alachlor for the 6 months followed by control diet for 19 months. In this recovery group only the incidence of nasal mucosal tumors was significantly increased above control incidences. Based on the results of these chronic bioassays, alachlor is considered to be oncogenic in the rat. In an extensive battery of genetic toxicity tests alachlor has not demonstrated any consistent or appreciable mutagenic activity.

Since 1985, Monsanto has had an active research program directed at elucidating alachlor's oncogenic mechanism of action in the rat. Metabolic activation of alachlor appears to be a

prerequisite for tumor formation in rats. Several lines of evidence indicate that the parent molecule, alachlor, is not the molecular entity responsible for producing tumors in the rat. Results from a number of mutagenicity systems indicate that parent alachlor is not directly genotoxic. The structural similarity of the alachlor metabolite, 2,6-diethylaniline, to the known rat nasal carcinogen, 2,6-dimethylaniline, implicates metabolism in process of rat carcinogenicity. Finally, the observed specie and tissue specificity of alachlor induced neoplasia argues against a direct mode of action. Extensive investigations into the metabolism and biodisposition of alachlor have demonstrated significant species differences in the way that this molecule is degraded and eliminated from the body. These differences in metabolism and biodisposition most likely account for alachlor's oncogenic potential in rats and its lack of oncogenic potential in mice. An understanding of metabolic differences is of prime importance in the extrapolation of oncogenic responses from one specie to another.

The metabolism of alachlor has been studied in rats, mice, monkeys and humans. Alachlor metabolism in rodents is very complex due to extensive biliary excretion, intestinal microbial metabolism, and enterohepatic circulation of metabolites. In excess of 30 discrete metabolites of alachlor have been identified in rat excreta, with approximately equal quantities appearing in urine and feces. Qualitatively, alachlor metabolism in the mouse is similar to the rat; however, there are significant quantitative differences between the two species. In contrast, alachlor is metabolized in monkeys to a limited number of glutathione and glucuronide conjugates which are excreted primarily *via* the kidney. Excretion in monkeys is more rapid than in rodents, with 90% of an administered dose being excreted in the urine within 48 hours. Limited investigations indicate that humans metabolize alachlor in a manner similar to monkeys. The extent to which alachlor metabolites are excreted in bile accounts for many of these species differences in metabolism and pharmacokinetics. A physiological phenomenon, commonly referred to as the molecular weight threshold for biliary excretion, explains the differences in rodent and primate elimination profiles. Being of intermediate molecular weight (*i.e.* 300 to 500 g/mole), alachlor liver metabolites are good candidates for biliary excretion in rodents, but not primates (Millburn, 1975; Williams, 1971). Bile cannulation studies in rats have confirmed that alachlor metabolites undergo extensive biliary excretion.

Once secreted into the intestine of the rat, glutathione-derived conjugates (2) of alachlor are cleaved by C-S lyase enzymes present in gut microflora (refer to Figure 1). Many of the resulting sulfur-containing metabolites (3) are then absorbed back into the systemic circulation and subject to further metabolic modification. This process of enterohepatic circulation contributes to the complexity of alachlor metabolites and their longer residence time in the rat. In the mouse, this process of enterohepatic circulation takes place to a lesser extent because a much smaller percentage of alachlor metabolites are absorbed back into the systemic circulation. Thus, a greater percentage of an administered dose is excreted in the feces. Alachlor metabolites do not undergo enterohepatic circulation to any significant extent in primates. Alachlor metabolites in rodents consist primarily of sulfoxides and sulfones and other products resulting from the oxidation of mercapturic acid pathway metabolites. For the most part, the major site of oxidation by mixed function oxidases (*i.e.* cytochrome P-450) is the C-1 position on the ethyl side chain. A major difference between rat and mouse metabolism of alachlor is the more extensive formation of glucuronic acid conjugates of these hydroxylated metabolites in mice.

The likelihood that species differences in alachlor metabolism accounts for induction of tumors in rats, but not mice, is best illustrated by a series of autoradiographic studies. Following oral

gavage administration of radiolabeled alachlor, radioactivity was found to specifically localize in the nasal mucosa of rats, but not mice or monkeys. In another autoradiographic study, the tertiary amide sulfide metabolite of alachlor (3), a metabolite arising only through the enterohepatic circulation process, was orally administered to rats. Specific localization of radioactivity in nasal and stomach mucosa was observed. The intensity of the labeling in these tissues was greater than that observed when parent alachlor was administered to the animals. This finding demonstrates the importance of enterohepatic circulation in the localization of alachlor metabolites in rat target tissues.

Over the last several years an increasing number of chemicals of diverse structure have been shown to produce neoplastic responses in the nasal mucosa of rats (Hecht, *et al.*, 1983). These same chemicals do not produce nasal tumors when tested in mice. Many investigators have found that rat nasal tissue possesses unusually high levels of metabolic activation enzymes; much higher than in nasal tissue from mice, monkeys and humans (Dahl *et al.*, 1982; Dahl, 1986; Dahl *et al.*, 1988; Hadley and Dahl, 1983; Longo *et al.*, 1986; Longo *et al.*, 1989)). In the rat this activity is highly concentrated in the ethmoidturbinates, the region of the nose in which alachlor induces tumors. Furthermore, detoxification enzymes (*e.g.* epoxide hydrolase) have been found at reduced levels in rat nasal tissue when compared to other species, including humans (Gervasi, *et al.*, 1991; Longo, *et al.*, 1988).

Monsanto scientists have developed a mechanistic hypothesis for nasal tumor formation in rats. This hypothesis proposes that metabolic products of alachlor enterohepatic circulation are converted by rat nasal mucosa to an electrophilic, diethylaniline quinone imine, metabolite (9). Figure 1 provides a schematic of the metabolic pathway leading to quinone imine formation in the rat. This hypothesis, supported by *in vitro* metabolic studies, describes the dealkylation of the tertiary and secondary amide sulfide metabolites of alachlor (3,4) to 2,6-diethylaniline (5), which is then hydroxylated to 4-amino-3,5-diethylphenol (7). This phenolic metabolite (7) can then undergo oxidation to a reactive quinone imine intermediate (9). Sulfate conjugation of (7) to 4-amino-3,5-diethylphenol sulfate (8) and conjugation of the quinone imine (9) with glutathione or cysteine represent detoxification reactions. Evidence for the formation of a quinone imine intermediate has been provided by the identification of its glutathione adduct (10) in homogenates of rat nasal mucosa. Quinone imines are highly electrophilic and have an affinity for amino acid sulfhydryl groups (11) (Pascoe, *et al.*, 1988). Evidence from *in vitro* mutagenicity assays suggests that metabolites of this pathway are not genotoxic. Alachlor and its secondary methylsulfide metabolite (4) were tested in Ames assays employing rat nasal turbinate metabolic activation. These assay conditions were expected to generate metabolites leading to formation of the quinone imine (9). No evidence of mutagenicity was observed for either molecule.

Formation of the quinone imine (9) from the secondary amide sulfide (4) and 2,6-diethylaniline (5) metabolites of alachlor involves the enzymes aryl amidase and aniline hydroxylase, respectively. In rat nasal tissue homogenates, the overall conversion rate of the alachlor secondary amide sulfide metabolite (4) to the quinone imine precursor (7) has been shown to be 30-40 times higher than in mouse or monkey nasal tissue (Feng, *et al.*, 1990; Monsanto unpublished data). Species differences in the rate of quinone imine formation are even greater if the initial conversion of alachlor *via* glutathione transferase is considered (Feng and Patanella, 1988; Feng and Patanella, 1989). Table 1 describes an interspecies comparison of *in vitro* nasal tissue activities for those enzymes involved in the conversion of sulfur-containing alachlor metabolites to 4-amino-3,5-diethylphenol (7). Enzymes involved in the conjugation (detoxification) of these metabolites (*e.g.* sulfotransferase) are present at lower levels in the nasal

mucosa of rats than mice.

Table 1. Comparative arylamidase and aniline hydroxylase activities for rat, mouse and monkey *in vitro* nasal tissue preparations. Activity is expressed as initial reaction velocities (*Vi*) in nmoles of reaction product per mg protein per minute. Reaction rates measured for the conversion of metabolite (4) to (5) and the conversion of metabolite (5) to (7).

	Rate [a]			Ratio [b]	
Reaction	Rat	Mouse	Monkey	Rat:Mouse	Rat:Monkey
Arylamidase	0.008	0.0004	0.002	20	4
Aniline hydroxylase	1.54	0.811	0.203	1.9	7.6
Combined reactions [c]				38	30

a - Initial reaction velocities (*Vi*) in nmoles/mg/min.
b - Interspecies ratio of initial reaction velocities.
c - Combined reaction ratios calculated as product of individual reaction ratios.

Higher rates of enterohepatic circulation, intestinal microbial metabolism, and target tissue metabolism result in a greater potential for quinone imine formation in rat nasal mucosa, as compared to the mouse or monkey. These differences in metabolic capability would explain the formation of nasal tumors in rats and their absence in mice, and would make their formation in primates highly unlikely. This conclusion is supported by autoradiographic studies showing the accumulation of alachlor derived carbon-14 in the nasal turbinates of rats, but not mice or monkeys.

This hypothesis is consistent with the observation of nasal epithelial tumors on rat chronic bioassays with the structurally similar analogues acetochlor, butachlor, metolachlor, 2,6-dimethylaniline and phenacetin (refer to Figure 2). These compounds do not produce nasal tumors in mice. Autoradiographic studies with both 2,6-diethylaniline (5) and 2,6-dimethylaniline have revealed specific localization of radioactivity in the nasal turbinates of rats, but not mice. This provides strong evidence to indicate that nasal tumor formation in rats is a specie-specific response. There is also evidence of differential strain susceptibility to nasal tumor formation. Autoradiographic studies with the Long-Evans, Sprague-Dawley and F-344 rats have revealed marked differences in nasal tissue localization of alachlor-derived radioactivity. Significant labeling of the nasal turbinates was apparent in the Long-Evans rat, but hardly detectible in the Sprague-Dawley or F-344 rat. Butachlor and phenacetin produce nasal tumors in Sprague-Dawley rats (unpublished Monsanto data; Isaka *et al.*, 1979), but not in F-344 rats (NCI, 1978). Acetaminophen, a primary metabolite of phenacetin, does not produce nasal tumors in F-344 rats (NTP, 1990). When the phylogenetically similar rat and mouse do not respond alike, or when different strains of the same specie do not respond alike, it is difficult to justify the extrapolation of these rat nasal tumors to humans.

Alachlor has demonstrated no significant genotoxic activity in an extensive battery of standard mammalian short-term assays. An increasing number of chemicals which are generally negative for genotoxic activity are being found to be oncogenic in chronic rodent bioassays (Cohen and Ellwein, 1990). The mechanism of carcinogenicity for many of these non-genotoxic chemicals is reported to involve the process of cell proliferation (Ames and Gold, 1990a; Busser and Lutz, 1989; Preston *et al.*, 1990; Schulte-Hermann, 1985). Cell proliferation would appear to be a

prerequisite for a number of stages in the carcinogenic process. For non-genotoxic agents, cell proliferation may lead to enhanced tumor formation by increasing the frequency of spontaneous mutations. Restorative cell proliferation in response to cytotoxicity, has been suggested as a critical step in the mechanism by which phenacetin causes nasal tumors in rats (Bogdanffy, *et al.*, 1989). Phenacetin, like alachlor, is a non-genotoxic agent that induces nasal tumors in rats following chronic oral exposure. Phenacetin also shows pronounced localization in nasal tissue as demonstrated by whole body autoradiographic techniques. This same type of process appears to be operative for alachlor.

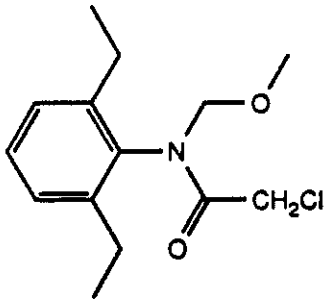
In a series of recently completed studies, alachlor produced a sustained and dose-dependent increase in cell proliferation in rat nasal tissue. In addition, the removal from alachlor exposure resulted in a reversal of this proliferative response to control levels. No evidence of increased cell proliferation was found in mouse nasal tissue. At high dose levels, alachlor also increased cell proliferation in the glandular stomach mucosa of rats. These data strongly invoke the role of cell proliferation in rat tumors produced by alachlor. Furthermore, these data support a non-genotoxic mechanism for rat carcinogenicity and the existence of a threshold.

Increased incidences of stomach and thyroid neoplasms were associated only with levels of exposure to alachlor that resulted in reduced animal survival, excessive body weight loss (>30%), and tissue necrosis. Neoplastic responses generated under such conditions are of doubtful relevance for predicting human cancer risk (Ames and Gold, 1990b). At this excessively toxic level normal cellular physiology and homeostasis would be disturbed. It is also likely that detoxification and repair processes would be compromised. Disturbance of cellular homeostasis following chronic high level exposure has been invoked as the mechanism by which a number of chemical agents induce thyroid follicular neoplasia (Paynter, *et al.*, 1988). Such disturbances can lead to sustained elevation of thyroid stimulating hormone (TSH) or impairment of the conversion of T_4 to T_3 in the liver. A chronic proliferative state and/or neoplasia can follow. Hormonal carcinogenesis is considered to be a receptor-mediated threshold phenomenon. Thus, small or short-term elevations in TSH would not be expected to result in neoplasia.

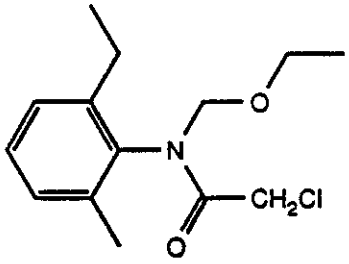
Male rats appear to be particularly susceptible to the development of thyroid tumors. Numerous structurally diverse chemicals and drugs induce these tumors in rats, but not in other species. A number of aniline derivatives (*e.g.* sulfonamides, *p*-aminobenzoic acid and amphenone) that share structural similarities with alachlor, have been characterized as inhibitors of thyroid function. Alachlor is most likely producing thyroid follicular tumors in male rats through a similar mechanism. The available data supports the conclusion that induction of thyroid tumors by alachlor is a high dose phenomenon that would not be expected to occur in humans. Support for this hypothesis is provided by the observation that thyroid follicular tumors were not elevated in male rats of the alachlor high dose recovery group. A study to investigate the potential of alachlor to interfere with thyroid hormone regulation is in progress.

In summary, alachlor appears to be producing oncogenic effects in rats through non-genotoxic mechanisms for which thresholds will exist and adequate margins of safety can be established. Furthermore, observed species differences in physiology and metabolism indicate that the rat is particularly susceptible to the induction of nasal, thyroid and possibly stomach tumors, following chronic exposure to this agent. Consequently, humans would be at significantly lower risk for development of these tumors and possibly facing no risk at all. Additional studies to support these hypotheses are ongoing.

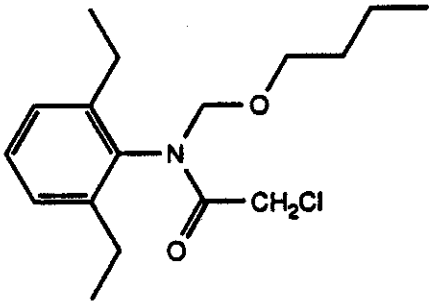
Figure 2. Chemical structures for a series of substituted aniline molecules.



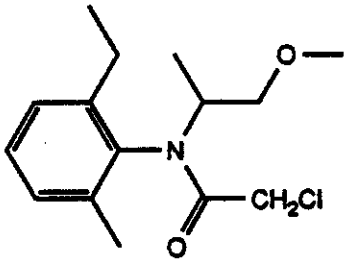
Alachlor



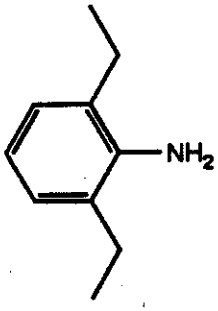
Acetochlor



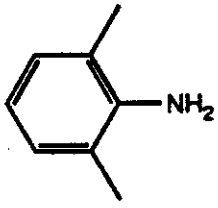
Butachlor



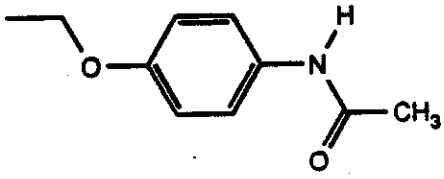
Metolachlor



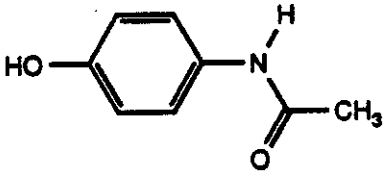
2,6-Diethylaniline



2,6-Dimethylaniline



Phenacetin



Acetaminophen

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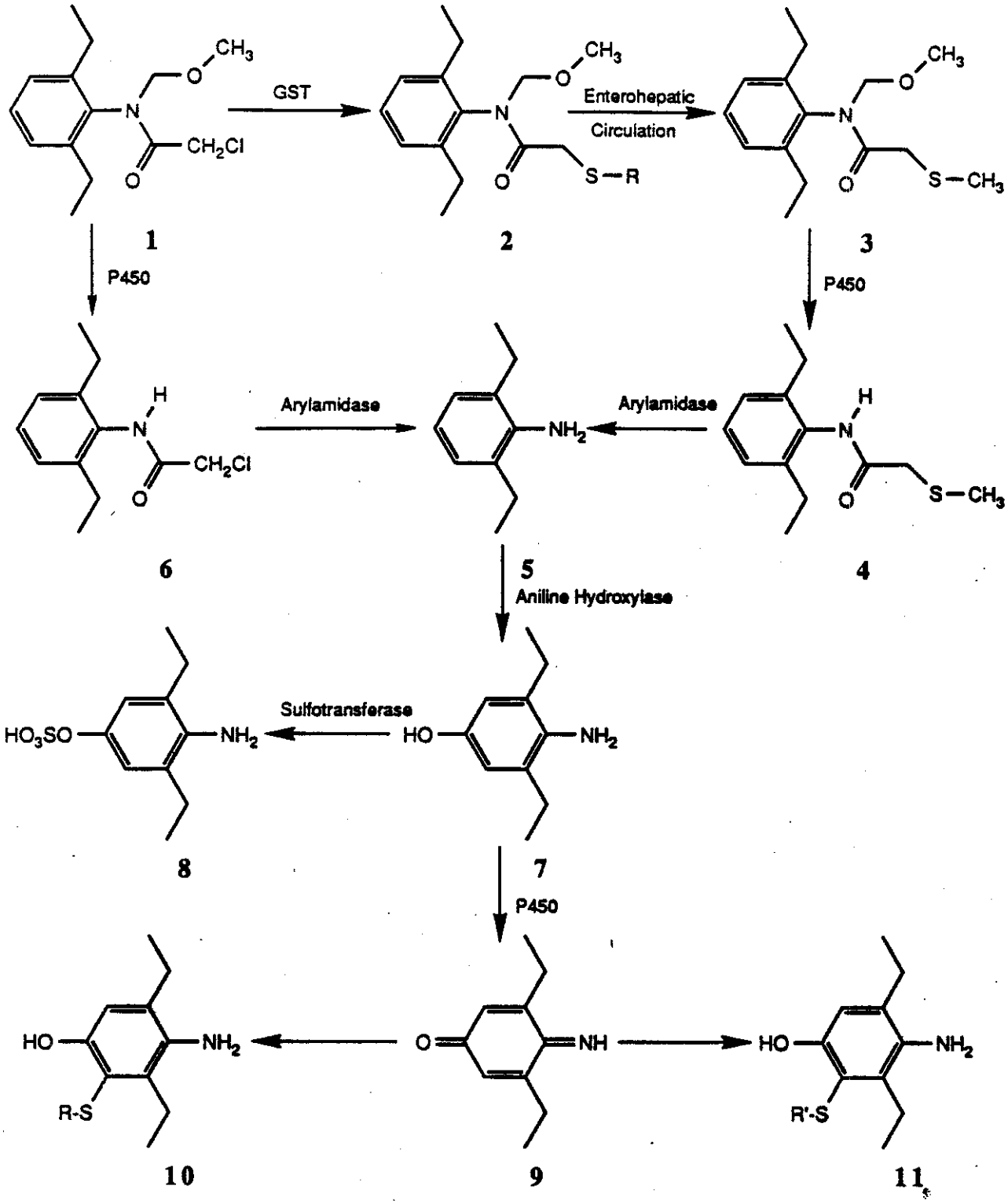
Figure 1. Pathway for metabolic activation of alachlor in the rat.



GST: glutathione-s-transferase; R: glutathione, cysteine or N-acetylcysteine; R': protein

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