Case No. 22-70118

UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

CENTER FOR FOOD SAFETY,
Petitioners,

v.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, *et al.*,
Respondents,

and

SYNGENTA CROP PROTECTION, LLC,
Respondent-Intervenor.

On Petition for Review of an Order of the
United States Environmental Protection Agency

PETITIONERS' MOTION FOR SUMMARY VACATUR

CENTER FOR FOOD SAFETY
Amy van Saun
2009 NE Alberta St., Suite 207
Portland, OR 97211
T: (971) 271-7372
avansaun@centerforfoodsafety.org

Sylvia Shih-Yau Wu
Meredith Stevenson
303 Sacramento Street, 2F
San Francisco, CA 94111
(415) 826-2770
swu@centerforfoodsafety.org
mstevenson@centerforfoodsafety.org

Counsel for Petitioners

TABLE OF CONTENTS

INTRODUCTION AND SUMMARY OF ARGUMENT 1

FACTUAL BACKGROUND 3

PROCEDURAL HISTORY 5

JURISDICTION 8

STANDARD OF REVIEW 11

ARGUMENT 13

I. EPA's Endangered Species Act Violation Is Clear Error. 13

II. EPA's Lack of Substantial Evidence Under FIFRA Is Clear Error 18

III. Vacatur Is the Proper Remedy. 23

CONCLUSION 27

CERTIFICATE OF COMPLIANCE 28

CERTIFICATE OF SERVICE 29

TABLE OF AUTHORITIES

Page(s)

Federal Cases

All. for the Wild Rockies v. U.S. Forest Serv.,
907 F.3d 1105 (9th Cir. 2018) 23, 24

Coal. to Protect Puget Sound Habitat v. U.S. Army Corps. of Eng'rs,
466 F. Supp. 3d 1217 (W.D. Wash. 2020) 25

Conner v. Burford,
848 F.2d 1441 (9th Cir. 1988) 12

Ctr. for Food Safety v. Vilsack,
734 F. Supp. 2d 948 (N.D. Cal. 2010) 25

Defs. of Wildlife v. Adm'r, EPA,
882 F.2d 1294 (8th Cir. 1989) 16

Ellis v. Housenger,
252 F. Supp. 3d 800 (N.D. Cal. 2017) 16

Farmworker Ass'n of Fla. v. EPA,
No. 21-1079, 2021 U.S. App. LEXIS 16882 (D.C. Cir. June 7, 2021) 2, 13, 15

Friends of the Earth, Inc. v. Laidlaw Envtl. Servs. (TOC), Inc.,
528 U.S. 167 (2000) 8, 9

Humane Soc'y of the United States v. Locke,
626 F.3d 1040 (9th Cir. 2010) 23

Idaho Farm Bureau Fed'n v. Babbitt,
58 F.3d 1392 (9th Cir. 1995) 23

Karuk Tribe of Cal. v. U.S. Forest Serv.,
681 F.3d 1006 (9th Cir. 2012) 13, 14, 16

Federal Cases (Cont'd) **Page(s)**

Lane Cty. Audubon Soc'y v. Jamison,
958 F.2d 290 (9th Cir. 1992) 16

Mass. v. EPA,
549 U.S. 497 (2007) 11

Motor Vehicle Mfrs. Ass'n v. State Farm Mut. Auto. Ins. Co.,
463 U.S. 29 (1983) 12

Nat. Res. Def. Council, Inc. v. EPA,
38 F.4th 34 (9th Cir. 2022) ..... *passim*

Nat. Res. Def. Council, Inc. v. FDA,
710 F.3d 71 (2d Cir. 2013) 10

Nat. Res. Def. Council v. *EPA*,
No. 20-70787 (9th Cir. filed Aug. 1, 2022) 26, 27

Nat'l Family Farm Coal. v. EPA,
960 F.3d 1120 (9th Cir. 2020) 3, 8, 18, 24

Nat'l Parks Conservation Ass'n v. Jewell,
62 F. Supp. 3d 7 (D.D.C. 2014) 24

Pollinator Stewardship Council v. EPA,
806 F.3d 520 (9th Cir. 2015) ..... *passim*

Salmon Spawning & Recovery All. v. Gutierrez,
545 F.3d 1220 (9th Cir. 2008) 11

Tucson Herpetological Soc'y v. Salazar,
566 F.3d 870 (9th Cir. 2009) 22

United States v. Hooton,
693 F.2d 857 (9th Cir. 1982) 11, 13, 18

W. States Petroleum Ass'n v. EPA,
87 F.3d 280 (9th Cir. 1996) 22

Federal Cases (Cont'd) **Page(s)**

W. Watersheds Project v. Kraayenbrink,
632 F.3d 472 (9th Cir. 2011) 13, 24

Wash. Toxics Coal. v. EPA,
413 F.3d 1024 (9th Cir. 2005) 16

Williams v. Hampton,
No. 19-56197, 2020 U.S. App. LEXIS 10473 (9th Cir. Apr. 2, 2020) 11, 13, 18

Federal Statutes

5 U.S.C. § 706(2)(A) 12, 13

7 U.S.C. § 136a(c)(2)(B) 19

7 U.S.C. § 136h(d)(1) 2, 20

7 U.S.C. § 136n(b) 8, 11, 13

16 U.S.C. § 1536(a) 14

16 U.S.C. § 1536(a)(2) 13, 14

FIFRA ..... *passim*

Rules

9th Cir. R. 3-6(a) 1

9th Cir. R. 3-6(a)(1) 11

9th Cir. R. 27-1 1

9th Cir. R. 27-1(2) 1

Fed. R. App. P. 27 1

Fed. R. App. P. 32.1 2

Regulations

40 C.F.R. § 23.6 8

40 C.F.R. § 152.112(b)-(c) 22

40 C.F.R. § 155.40(a)(1) 19

40 C.F.R. § 158.130(d)(6) 20

40 C.F.R. § 158.630(d), (e) 21

40 C.F.R. § 159.179(a) 20

50 C.F.R. § 402.01(b) 14

50 C.F.R. §§ 402.13-.14 14

50 C.F.R. § 402.14(a) 13, 14

Other Authorities

EPA, *Overview of the Ecological Risk Assessment Process* 46-47 (Jan. 2004), https://www.epa.gov/sites/default/files/2014-11/documents/ecorisk-overview.pdf 17

Pesticide Registration Review; Proposed Interim Decisions for Several Pesticides; Notice of Availability, 86 Fed. Reg. 41,838 (Aug. 3, 2021) 5

INTRODUCTION AND SUMMARY OF ARGUMENT

Petitioner Center for Food Safety (CFS) hereby moves this Court to summarily reverse and vacate the Respondent Environmental Protection Agency's (EPA or Respondent) interim registration of difenoconazole, an approval which violates both the Endangered Species Act (ESA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).[1] 9th Cir. R. 3-6(a); Fed. R. App. P. 27; 9th Cir. R. 27-1.[2] Pursuant to the ESA, Respondent's flouting of its consultation duties—for a fungicide that EPA knows causes harm to federally protected endangered species—is clear error warranting summary vacatur. And under FIFRA, EPA's decision to issue difenoconazole's interim registration without critical studies on the fungicide's potential harm to public health also warrants summary vacatur.

---

[1] *Difenoconazole: Interim Registration Review Decision*, Case Number 7014, EPA-HQ-OPP-2015-0401 (Interim Registration Review Decision), Attachment 1.

[2] Respondents reserve their position and Respondent-Intervenor opposes this motion. *See* 9th Cir. R. 27-1(2).

The D.C. Circuit recently granted summary vacatur in an analogous situation, for EPA's failure to comply with the ESA before registering the pesticide aldicarb, based on the "seriousness of the admitted error and the error's direct impacts on the merits of the EPA's registration decision." *Farmworker Ass'n of Fla. v. EPA*, No. 21-1079, 2021 U.S. App. LEXIS 16882 (D.C. Cir. June 7, 2021); *see* Fed. R. App. P. 32.1. The same is warranted here, for at least two reasons. First, EPA committed clear error in issuing the difenoconazole decision without ESA compliance. *Nat. Res. Def. Council, Inc. v. EPA*, 38 F.4th 34, 59 (9th Cir. 2022) (holding that interim registration review decisions like this one trigger ESA duties). And second, EPA issued its decision without critical studies that the agency itself requested over twenty years ago, studies on a topic (metabolism) which FIFRA itself requires, 7 U.S.C. § 136h(d)(1) (requiring EPA to support registrations with, *inter alia*, studies on "persistence, translocation and fate in the environment, and metabolism."). *See Pollinator Stewardship Council v. EPA*, 806 F.3d 520, 537 (9th Cir. 2015) (holding EPA pesticide approval lacking substantial evidence and vacating under FIFRA for lack of key studies). For either or both reasons, this Court should summarily vacate the

decision and remaexnd. *Nat'l Family Farm Coal. v. EPA*, 960 F.3d 1120, 1144–45 (9th Cir. 2020).

FACTUAL BACKGROUND

Difenoconazole is a broad-spectrum systematic fungicide.[3] Fungicides are a subclass of pesticides that target fungal pests like mold and mildew. Attach 1 at 24. Systemic fungicides like difenoconazole are absorbed and distributed throughout the plant's tissue, flowers, and fruits after application, increasing persistence of the fungicide in the plant and its new growth. *Id.*

Like all pesticides, fungicides like difenoconazole are toxic substances intentionally released or sprayed to kill pests, but that also kill and harm "non-target" plants and animals. As EPA recognized, difenoconazole potentially threatens a wide variety of species, from "mammals, birds, terrestrial invertebrates, freshwater and estuarine/marine fish, and aquatic invertebrates." *Id.* at 25.

---

[3] EPA, *Difenoconazole: Draft Ecological Risk Assessment for Registration Review* 5 (Sept. 16, 2020), Attachment 2.

Despite its potential harm to species and human health, over the years, EPA has approved difenoconazole use across a wide variety of landscapes. Initially approved as a seed treatment[4] on commodity crops, today the systemic fungicide is approved for many nationwide uses including: seed treatment on wheat and other cereal grains, cotton, and potatoes; direct spraying on major crops and commodities, including soybeans, sugar beets, various fruits and vegetables, tree nuts; and spraying on golf course turf grass and ornamental plants. Difenoconazole's use has increased dramatically since 2008, when EPA first approved direct spraying on soybeans, roughly 20-fold from 2008 to 2017 (from 25,000 to 500,000 lbs. per year), and continues to increase.[5] *See id.* at 12.

---

[4] Seed treatments involve coating crop seeds with systemic pesticides, which are absorbed into the plants' circulatory system as the plant grows. Attach. 1 at 25.

[5] Center for Food Safety, Comments on Proposed Interim Registration Decision for Difenoconazole 2, EPA-HQ-OPP-2015-0401, Attachment 4.

PROCEDURAL HISTORY

EPA published the proposed interim registration review decision for public comment on August 3, 2021.[6] Petitioner submitted comments. *See* Attach. 4. Among other critiques, Petitioner underscored EPA's failure to comply with the ESA and consult on difenoconazole with the expert wildlife agencies and obtain the critical studies on difenoconazole's risks to public health. *Id.*

In the ecological risk assessment accompanying the proposed interim registration, EPA acknowledged that difenoconazole application exceeds EPA's own acute and/or chronic "levels of concern" (LOCs) for numerous plants and animals, including fish, estuarine/marine invertebrates, aquatic invertebrates, honeybees, aquatic plants, and birds. Attach. 2 at 7, 9-13. Exposure occurs through runoff and spray drift to water and sediment, *id.* at 43-44, with difenoconazole's noted

---

[6] *Difenoconazole: Proposed Interim Registration Review Decision*, Case Number 7014, EPA-HQ-OPP-2015-0401-0049 ("Proposed Interim Registration Review Decision"); *Pesticide Registration Review; Proposed Interim Decisions for Several Pesticides; Notice of Availability*, 86 Fed. Reg. 41,838 (Aug. 3, 2021).

persistence increasing its accumulation in soil and aquatic environments. *Id.* at 8. Indeed, EPA identified chronic risk LOC exceedances for birds for up to 150 days after application in some scenarios, and after 56 days for mammals. *Id.* at 11.

In spite of these acknowledgments of harm to numerous categories of species throughout its risk assessment, EPA at the same time readily admits it has not complied with the ESA. Attach 1 at 4 ("The Agency has not yet fully evaluated difenoconazole's risks to federally listed species."); Attach. 2 at 13 ("Federally listed threatened and endangered species are not evaluated in the document."). Instead, EPA promises only to consult before its final registration decision—an entirely different federal action—and offered no timeline or work plan for when that may happen. Attach. 1 at 34-35.

EPA also issued its proposed interim registration without the critical information it requested *twenty-two* years ago on difenoconazole's potential adverse public health effects. Specifically, in 2000, EPA demanded pesticide registrants provide numerous studies to ensure that use of the fungicides in this class do not impair developing infants' brains and nervous systems, cause cancer, or disrupt hormonal

systems.[7] Then, EPA halted any further registrations of fungicides in the triazole class—to which difenoconazole belongs—over twenty years ago.[8] Attach. 1 at 9. Yet the proposed registration still contains the same glaring data gaps on difenoconazole's impacts on reproductive and developmental health.

EPA subsequently issued its final interim registration decision and finalized its draft risk assessments on March 31, 2022. Attach. 1 at 4. Regarding its ESA duties, EPA stated only that it would not undergo consultation until its separate final registration review decision, and still offered no timeline for that decision, in spite approving difenoconazole's continued use. *Id.* at 34-35. And under FIFRA, the human health studies EPA mandated more than twenty years ago still remained lacking, but EPA nonetheless issued the interim registration

---

[7] *See* EPA, *1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid: Human Health Aggregate Risk Assessment in Support of Reregistration and Registration Actions for Triazole-derivative Fungicide Compounds* 6 (Feb. 7, 2006), Attachment 3.

[8] Attach. 3, at 7.

by substituting a "conservative" uncertainty factor for actual studies. *Id.* at 9.

Petitioner timely filed this petition for review. ECF No. 1-6 (June 13, 2022). Registrant Syngenta intervened. ECF No. 9 (July 13, 2022); ECF No. 15 (July 27, 2022). The parties participated in this Court's required mediation process but their discussions failed to progress. Petitioner now files this motion seeking summary vacatur of the challenged decision in light of EPA's admitted ESA violation and continued lack of substantial evidence to support the interim registration approval of difenoconazole under FIFRA.

JURISDICTION

This Court has jurisdiction under FIFRA, which provides for direct review in the courts of appeals of "any order issued by [EPA] following a public hearing." 7 U.S.C. § 136n(b); *Nat'l Family Farm Coal. v. EPA*, 960 F.3d at 1131. EPA provided a "public hearing" by holding notice and comment. *Id.* Petitioner submitted comments and timely filed this petition for review. 7 U.S.C. § 136n(b); 40 C.F.R. § 23.6.

Petitioner has standing. *Friends of the Earth, Inc. v. Laidlaw Envtl. Servs. (TOC), Inc.*, 528 U.S. 167, 180-81 (2000). The

environmental interests at stake are germane to Petitioner's organizational mission, and this Court can redress the injuries to Petitioner's members. [9] *Id.* The procedural nature of Petitioner's injuries (Section 7 consultation sets a strict procedure to ensure compliance with ESA's substantive commands), calls for a relaxed causation and redressability standard. *Nat. Res. Def. Council*, 38 F.4th at 54. Namely, Petitioner must only show a "reasonable probability of the challenged action's threat to [Petitioner's] concrete interest," *id.* (citations omitted), and that "the agency decision 'could be influenced' by the procedures at issue." *Id.* at 56 (quoting *Hall v. Norton*, 266 F.3d 969, 977 (9th Cir. 2001).

This low bar is easily met here: EPA's failure to fulfill its ESA obligations injures Petitioner's members who have environmental, professional, recreational, and aesthetic interests in seeing, studying, and protecting dozens of ESA-protected species—including the smalltooth sawfish, whooping crane, and Everglade snail kite—all of

---

[9] *See* Attach. 5, Decl. Loda; Attach. 6, Decl. Naegele; Attach. 7, Decl. Schudda; Attach. 8, Decl. Wu.

which difenoconazole harms. Crops permitted for difenoconazole spraying and seed treatment overlap with the habitats and range of many ESA-protected species for which difenoconazole exposure exceeds EPA's levels of concern. Attach. 2 at 7, 9-13. *See* Attach. 9, Sinclair Decl. (mapping overlap of difenoconazole-approved crop uses with critical habitat for six ESA-protected species). Proper ESA compliance before issuance may have resulted in protective measures included in this decision for species to protect members' interests.

And Petitioner's members' exposure to potentially harmful difenoconazole residues also meets this standard. For injuries from exposure, Petitioner only needs to show "a credible threat of harm" due to exposure to "potentially harmful" difenoconazole residues, *Nat. Res. Def. Council, Inc. v. FDA*, 710 F.3d 71, 81 (2d Cir. 2013), as amended (Mar. 21, 2013) (quoting *Baur v. Veneman*, 352 F.3d 625, 641 (2d Cir. 2003) ("[T]he relevant 'injury' for standing purposes may be exposure to a sufficiently serious risk of medical harm—not the anticipated medical harm itself."). Petitioner's members regularly consume foods with potentially harmful difenoconazole residues, which EPA might have addressed had it properly collected and considered the public health

studies. *Salmon Spawning & Recovery All. v. Gutierrez*, 545 F.3d 1220, 1226-27 (9th Cir. 2008) (Petitioner "need[s] to show only that the relief requested—that the agency follow the correct procedures—may influence the agency's ultimate decision of whether to take or refrain from taking a certain action."); *Mass. v. EPA*, 549 U.S. 497, 518 (2007) ("[A] litigant has standing if there is some possibility that the requested relief will prompt the injury-causing party to reconsider the decision that allegedly harmed the litigant.").

STANDARD OF REVIEW

Summary reversal and vacatur is warranted when this Court concludes the respondent committed "clear error." 9th Cir. R. 3-6(a)(1); *Williams v. Hampton*, No. 19-56197, 2020 U.S. App. LEXIS 10473, at *1 (9th Cir. Apr. 2, 2020). Where, as here, the agency decision is "obviously controlled by precedent," then "summary disposition is of obvious benefit to all concerned," and should be issued. *United States v. Hooton*, 693 F.2d 857, 858 (9th Cir. 1982).

Under FIFRA, this Court may affirm EPA's difenoconazole's interim registration approval only if it is "supported by substantial evidence when considered on the record as a whole." 7 U.S.C. § 136n(b).

The FIFRA substantial evidence standard "affords an agency less deference than the arbitrary and capricious standard." *Pollinator Stewardship*, 806 F.3d at 533 (N.R. Smith, J., concurring) (citing *Universal Camera Corp. v. NLRB*, 340 U.S. 474, 477 (1951); *Union Oil Co. v. Fed. Power Com.*, 542 F.2d 1036, 1040-41 (9th Cir. 1976)). Therefore, if EPA's decision is arbitrary and capricious, it cannot be supported by substantial evidence. To avoid being arbitrary and capricious, EPA "must examine the relevant data and articulate a satisfactory explanation for its action including a rational connection between the facts found and the choice made." *Motor Vehicle Mfrs. Ass'n v. State Farm Mut. Auto. Ins. Co.*, 463 U.S. 29, 43 (1983) (internal citations and quotations omitted).

Similarly, EPA violated the ESA if its failure to consult the expert wildlife agencies was arbitrary, capricious, an abuse of discretion, or otherwise not in compliance with law. 5 U.S.C. § 706(2)(A); *see Conner v. Burford*, 848 F.2d 1441, 1453 (9th Cir. 1988). Agency actions subject to mandatory consultation include interim registration review decisions such as this one. *Nat. Res. Def. Council*, 38 F.4th at 59. The ESA requires that federal agencies consult the expert wildlife agencies before

taking any action that "may affect" any protected species or critical habitat, 50 C.F.R. § 402.14(a); *see* 16 U.S.C. § 1536(a)(2), and to do so "at the earliest possible time." 50 C.F.R. § 402.14(a); *Karuk Tribe of Cal. v. U.S. Forest Serv.*, 681 F.3d 1006, 1020 (9th Cir. 2012).

If the Court holds EPA unlawfully issued the interim registration of difenoconazole, it should "set aside," or vacate it. 7 U.S.C. § 136n(b); 5 U.S.C. § 706(2)(A); *Pollinator Stewardship*, 806 F.3d at 532-33; *see, e.g.*, *Farmworker Ass'n of Fla.*, 2021 U.S. App. LEXIS 16882.

ARGUMENT

I. EPA's Endangered Species Act Violation Is Clear Error.

EPA's violation of its ESA Section 7 consultation duties for difenoconazole is "clear error" that is "obviously controlled by precedent," and thus warrants summary reversal. *Williams*, 2020 U.S. App. LEXIS at *1; *Hooton*, 693 F.2d at 858.

Section 7(a)(2) is the "heart" of the ESA. *W. Watersheds Project v. Kraayenbrink*, 632 F.3d 472, 495 (9th Cir. 2011). It mandates that "[e]ach federal agency" "insure" that its action—here, EPA completing its interim registration review—is not likely to either jeopardize any endangered species or adversely modify any designated "critical"

habitat, by consulting with the U.S. Fish and Wildlife Service and National Marine Fisheries Service (the Expert Agencies). 16 U.S.C. § 1536(a)(2); 50 C.F.R. § 402.01(b). If an action agency makes a "may affect" determination, it must then insure, through consultation with the Expert Agencies, that the action is not likely to cause jeopardy or adversely modify designated critical habitat. 16 U.S.C. § 1536(a); 50 C.F.R. §§ 402.13-.14. The ESA commands that agencies complete this review and make this determination "at the earliest possible time." 50 C.F.R. § 402.14(a); *Karuk Tribe*, 681 F.3d at 1020.

Just two months ago, this Court made plain that EPA cannot flout its ESA duties as it did here when making an interim registration review decision. Rather as this Court held, interim registration review falls squarely within the purview of "agency action" and triggers mandatory consultation. *Nat. Res. Def. Council*, 38 F.4th at 58. In *Natural Resources Defense Council*, regarding the interim registration of the pesticide glyphosate, this Court explained that interim registrations are cognizable "agency actions" triggering consultation because 1) they are "affirmative" actions, and 2) they delineate the way in which producers may continue pesticide use consistent with FIFRA.

Id. at 58-59. Through the interim registration, EPA has discretionary control to benefit protected species through mitigation measures or otherwise, like deciding which factors to consider in the ecological risk assessment. *Id.*

The present case is no different than the glyphosate interim registration. In both instances, EPA issued a so-called "interim" registration that actually *finalized* the agency's ecological and human health risk assessments, yet deferred ESA consultation until a final registration review decision at some later, undetermined time. *See id.* at 43. As this Court found in *Natural Resources Defense Council*, EPA violated the ESA by failing to comply with the ESA consultation requirements before issuing the interim registration. *Id.* at 59.

In fact, EPA's violation of the ESA here is even more egregious than in *Natural Resources Defense Council*, because unlike in that case here EPA has not even *started* a draft ESA Biological Evaluation to make the required effects determination. Accordingly, pursuant to *Natural Resources Defense Council*, the ESA and its implementing regulations' plain language, as well as numerous other ESA precedent, EPA violated the ESA. *E.g., Farmworker Ass'n of Fla.*, 2021 U.S. App.

LEXIS 16882; *Wash. Toxics Coal. v. EPA*, 413 F.3d 1024, 1029 (9th Cir. 2005) (failure to consult for fifty-four pesticides); *Ellis v. Housenger*, 252 F. Supp. 3d 800, 820 (N.D. Cal. 2017) (failure to consult for dozens of pollinator-harming pesticides); *Lane Cty. Audubon Soc'y v. Jamison*, 958 F.2d 290, 294 (9th Cir. 1992) (interim management strategy designed to be implemented immediately constitutes agency action triggering consultation); *Defs. of Wildlife v. Adm'r, EPA*, 882 F.2d 1294, 1301 (8th Cir. 1989) (strychnine registration violated ESA).

And *had* EPA complied with the ESA and completed a Biological Evaluation, it almost certainly would have triggered formal ESA consultation. The "may affect" threshold triggering consultation is extremely low: "actions that have *any chance of affecting* listed species or critical habitat … *require* at least some consultation under the ESA." *Karuk Tribe*, 681 F.3d at 1027 (emphasis added); *id.* ("Any possible effect, whether beneficial, benign, adverse or of an undetermined character, triggers the requirement.") (citation and quotation omitted). Here, EPA admits in its ecological risk assessment that difenoconazole, approved for dozens of crops across the country, exceeds its own toxicity level of concern for exposures when used as approved for wide

categories of species, including fish, estuarine/marine and aquatic invertebrates, honeybees, aquatic plants, and birds. Attach 2 at 7, 9-13. EPA found the level of concern exceeded for non-federally protected species, meaning that under EPA's own risk assessment framework, the same difenoconazole uses would also potentially harm federally protected species belonging to the same categories.[10]

And there are *hundreds* of endangered species that fall into these broad species categories for which EPA acknowledges risk. To give just a few examples, extensive difenoconazole use: in California's Central Valley overlaps with California condor and conservancy fairy shrimp habitat; in the Midwest with whooping crane habitat; and in Florida with smalltooth sawfish and the Everglade snail kite habitat. *See* Attach. 9, Decl. Sinclair (and exhibits). But EPA admits it failed to do *any* analysis of these risks to ESA-protected birds, fish, and other species. Attach 1 at 3.

---

[10] *See* EPA, *Overview of the Ecological Risk Assessment Process* 46-47 (Jan. 2004), https://www.epa.gov/sites/default/files/2014-11/documents/ecorisk-overview.pdf (explaining that the level of concerns are set at lower thresholds for endangered species compared to their non-federally listed counterparts).

Because EPA's well-established ESA violation is "clear error" that is "obviously controlled by precedent," *Williams*, 2020 U.S. App. LEXIS at *1; *Hooton*, 693 F.2d at 858, this Court should grant summary reversal.

II. EPA's Lack of Substantial Evidence Under FIFRA Is Clear Error.

The ESA violation alone is more than sufficient ground on which to vacate the interim registration. However and additionally, EPA's failure to obtain the requisite studies on public health it mandated more than two decades ago also constitutes "clear error ... obviously controlled by precedent." *Williams*, 2020 U.S. App. LEXIS at *1; *Hooton*, 693 F.2d at 858. Without these studies, EPA cannot support its interim registration decision with substantial evidence as FIFRA requires, since the need for the health data prompted EPA to issue a moratorium on further registrations in 2000. Attach. 3; *see Nat'l Family Farm Coal.*, 960 F.3d at 1124.

This is impermissible. The very purpose of registration review requires EPA to revisit studies in light of improved ability to detect risks, policy changes, changes in pesticide usage practices, and

importantly here, evolving science that has occurred since the pesticide's last review. *See Nat. Res. Def. Council*, 38 F.4th at 40 (explaining that registration review "require[s] EPA to assess any new information regarding risks to human health and the environment that has emerged ... to verify that the pesticide continues to satisfy the FIFRA safety standard."). Specifically, EPA must base each re-registration on "*current* scientific and other knowledge regarding the pesticide, including its effects on human health and the environment," 40 C.F.R. § 155.40(a)(1) (emphasis added). Accordingly, EPA may identify and solicit data or information to aid in its review. *Id.* § 155.50(b)-(c). Under EPA's own FIFRA regulations, EPA must issue a data call-in "if" the agency determines that additional data "are required to maintain in effect an existing registration of a pesticide." 7 U.S.C. § 136a(c)(2)(B).

EPA did just that here. In 2000, EPA reviewed evolving science and issued a data call-in regarding the impact of 1,2,3-triazoles, a metabolite of difenoconazole and other triazole fungicides on developmental and reproductive health. Attach. 3 at 5. EPA admitted it lacked sufficient evidence for future registrations and uses to ensure

adherence to FIFRA's safety standard because available studies failed to assess risks to human health from triazole metabolites, and that additional animal studies would be required. *Id.* at 5-6 (recommending numerous toxicity animal studies on trazole metabolites as "a condition of registration"). EPA admittedly has "*no data*" to "directly evaluate the potential for carcinogenicity of 1,2.4-triazole," *id.* at 14 (emphasis added), evaluate dermal absorption of 1.2.4-triazole, *id.* at 22, or evaluate triazole alanine's potential to disrupt the nervous system. *Id.* at 79. EPA also admitted "free triazole *has not been adequately tested* in toxicity studies with the parent triazole fungicides." *Id.* at 79 (emphasis added).

EPA cannot support its decision with substantial evidence without metabolism studies like these. FIFRA makes plain that data required to support registration includes studies on pesticides' "persistence, translocation and fate in the environment, and *metabolism*." 7 U.S.C. § 136h(d)(1) (emphasis added). In turn, EPA's regulations require studies regarding metabolite toxicity to "increase[ ] the Agency's understanding of the behavior of the chemical when considering the human exposure anticipated from intended uses of the pesticide." 40 C.F.R. §§

158.130(d)(6),159.179(a); *Nat. Res. Def. Council*, 38 F.4th 34, 51 (in a pesticide registration challenge, holding that by failing to follow its own guidelines (there, cancer guidelines) EPA's interim registration of the pesticide could not survive substantial evidence review and was thus unlawful).

EPA went ahead here without the necessary studies. Attach 1 at 9. This is clear error. This Court has specifically held that EPA lacks substantial evidence when acts without necessary studies after a data call-in. In *Pollinator Stewardship*, the Court found sulfoxaflor's conditional registration unsupported because registrants had not yet submitted the Tier 2 semi-field tunnel studies on risks to pollinators the EPA had similarly identified as necessary. *Pollinator Stewardship*, 806 F.3d at 537; 40 C.F.R. § 158.630(d), (e) (requiring field testing for pollinators). There, as here, EPA's own regulations required these types of studies. And there, as here, EPA issued a data call-in to affirmatively prove that sulfoxaflor does *not* have unreasonable adverse effects on the environment after finding existing studies inconclusive.

EPA may not overcome this data requirement with its 10x database uncertainty factor and promise of future studies. In *Pollinator*

Stewardship, the Court found that lowering sulfoxaflor's maximum application rate could not overcome the data gaps because EPA still could not affirmatively prove that sulfoxaflor does not cause unreasonable adverse effects. *See Pollinator Stewardship*; *see also Tucson Herpetological Soc'y v. Salazar,* 566 F.3d 870, 879 (9th Cir. 2009) (finding that the Secretary of the Interior erred when he affirmatively relied on ambiguous and inconclusive studies to support a conclusion). The same must be true here: EPA enacted this safety factor twenty years ago solely as a temporary measure until it received the requisite studies. Attach. 3 at 67-68; *id.* at 73 ("A 10X database uncertainty factor is retained for the lack of the [mandatory] studies.").

A court should not defer to an agency's decision where, as here, the agency fails to adhere to its own regulations. *See, e.g., Pollinator Stewardship*, 806 F.3d at 537; *W. States Petroleum Ass'n v. EPA*, 87 F.3d 280, 283 (9th Cir. 1996). EPA's regulations require EPA to "[r]eview[ ] all relevant data in [its] possession " and to "determine[ ] that no additional data are necessary " to make determinations of no unreasonable adverse effects. 40 C.F.R. § 152.112(b)-(c). Accordingly, in the absence of the requisite scientific data, EPA's conclusion that

difenoconazole result in unreasonable adverse effects on the environment as unsupported by substantial evidence.

III. Vacatur Is the Proper Remedy.

Because EPA violated FIFRA and the ESA, the Court should set aside the decision. Vacatur is the default remedy for unlawful agency actions, including pesticide registrations. *All. for the Wild Rockies v. U.S. Forest Serv.*, 907 F.3d 1105, 1121-22 (9th Cir. 2018) ("presumption of vacatur," unless defendants meet their burden to show otherwise); *Nat. Res. Def. Council*, 38 F.4th at 51 ("[v]acatur is the traditional remedy for erroneous administrative decisions."); *Pollinator Stewardship*, 806 F.3d at 532 (remand without vacatur permitted only in "limited circumstances"); *Humane Soc'y of the United States v. Locke*, 626 F.3d 1040, 1053 n. 7 (9th Cir. 2010) ("rare circumstances"); *Idaho Farm Bureau Fed'n v. Babbitt*, 58 F.3d 1392, 1405 (9th Cir. 1995) (vacatur applies unless "equity demands" otherwise).

In determining whether the Respondent can meet its heavy burden to show that a case meets the "rare" or "limited" circumstances not to vacate, courts "weigh the seriousness of the agency's errors against the disruptive consequences of an interim change that may

itself be changed." *Nat. Res. Def. Council*, 38 F.4th at 51 (quoting *NFFC*, 960 F.3d at 1144). And in environmental cases like this one, the cognizable 'disruptive consequences' to be considered are *environmental* harms that flow *from* vacatur. *Id.* at 51-52; *NFFC*, 960 F.3d at 1144-45; *Pollinator Stewardship*, 806 F.3d at 532; *All. for the Wild Rockies*, 907 F.3d at 1122 (vacatur "appropriate when leaving in place an agency action risks more environmental harm than vacating it").

Just like in recent pesticide cases of *NRDC* and *Pollinator Stewardship*, EPA here substantially understated or entirely failed to acknowledge the risks, including numerous ESA-protected species and risks to public health. EPA's errors are very serious: A violation of ESA's Section 7 cuts to the "heart" of the statute, *W. Watersheds Project v. Kraayenbrink*, 632 F.3d at 495, and here it was done knowingly to boot. *Nat'l Parks Conservation Ass'n v. Jewell*, 62 F. Supp. 3d 7, 20-22 (D.D.C. 2014) (agency's failure to consult under ESA a serious error justifying vacatur of underlying agency action). Human health and environmental risks are also core considerations of FIFRA. *Nat. Res. Def. Council,* 38 F.4th at 52 (finding EPA's errors in assessing human-health risk serious); *Pollinator Stewardship*, 806 F.3d at 532 (EPA's

errors in assessing risks to pollinators under FIFRA serious and vacating).

And as in *Natural Resources Defense Council*, vacating the human-health portion of EPA's IRRD would not result in disruptive consequences. 38 F.4th at 52. In both cases, this portion "clearly weighs in favor of vacatur" because it simply maintains the status quo with no new mitigation measures for human health. *Id*; *see also* Attach. 1 at 40-45.

Nor would disruptive, environmental harms flow from vacating the ecological risk assessment portion. Disruptive consequences from vacatur are "weighty only insofar as the agency may be able to rehabilitate its rationale for the regulation." *Coal. to Protect Puget Sound Habitat v. U.S. Army Corps. of Eng'rs,* 466 F. Supp. 3d 1217, 1223–24 (W.D. Wash. 2020); *Ctr. for Food Safety v. Vilsack*, 734 F. Supp. 2d 948, 952 (N.D. Cal. 2010). The correct inquiry asks whether the "same rule would be adopted on remand," meaning the exact same action. *Pollinator Stewardship*, 806 F.3d at 532. But here any future decision will differ both procedurally and substantively because EPA will need to incorporate the substantive results of the ESA consultation

process. When "a different result *may* be reached," such as in this case, it undermines any "disruptive consequences of an interim change that may itself be changed" and supports vacatur. *Id.* (emphasis added).

In *Natural Resources Defense Council*'s similar situation, this Court chose only to partially vacate and not to vacate the ecological risk assessment as well only for "practical reasons" not present here. 38 F.4th at 61. There, vacating may have further delayed ESA consultation beyond the Congressionally mandated October 1, 2022 deadline because the Court had not yet received responsive briefing on ecological risk assessment's sufficiency, nor held oral argument. *Id.* at 61 (refusing to vacate because "while we hesitate to reward what some might consider sloth or indolence … fully litigating the issues could result in … more, and probably unnecessary, delay."). But here, EPA has no intention of completing its final registration review by October 1, nor could it.[11] In

---

[11] Even for glyphosate, for which EPA completed a draft Biological Evaluation, the agency subsequently asked this Court to vacate the ecological risk assessment because EPA cannot consult by October 1, 2022. Pet. Rehearing, *Nat. Res. Def. Council* v. *EPA*, No. 20-70787, at 5-6 (9th Cir. filed Aug. 1, 2022), ECF No. 141-1 (ESA consultations for pesticides typically take *years*); *id.* at 15 (final registration review

sum, the remedy here is plain under both the ESA and FIFRA: vacate the registration.

CONCLUSION

EPA's intentional disregard for its ESA and FIFRA duties in its interim registration decision for difenoconazole is manifest clear error, contrary to its Congressional mandates, and risks irreparable harm to wildlife on the verge of extinction as well as public health. The proper outcome and remedy need not be delayed any further. For these reasons, Petitioners respectfully request the Court should grant summary reversal and vacatur.

Respectfully submitted on September 1, 2022.

/s/ Meredith Stevenson
Meredith Stevenson
303 Sacramento Street, 2F
San Francisco, CA 94111
T: (415) 826-2770
mstevenson@centerforfoodsafety.org

Counsel for Petitioners

---

decision for glyphosate, including ESA consultation, will take until 2026); *see also* Order, *Nat. Res. Def. Council* v. *EPA*, No. 20-70787 (9th Cir. filed Aug. 5, 2022), ECF No. 142 (denying the petition for rehearing).

CERTIFICATE OF COMPLIANCE

I hereby certify that this brief complies with the requirements of Fed. R. App. P. 32(a)(5) and (6) because it has been printed in 14-point Century Schoolbook font, a proportionally spaced font. I further certify that this brief complies with Circuit Rule 27-1 because it contains 4,873 words, excluding the parts of the brief exempted under Rule 32(f), according to the count of Microsoft Word. *See* 9th Cir. R. 32-3(2).

/s/ Meredith Stevenson
Meredith Stevenson

CERTIFICATE OF SERVICE

I hereby certify that, on September 1, 2022, I electronically filed the foregoing PETITIONERS' MOTION FOR SUMMARY VACATUR with the Clerk of the Court for the United States Court of Appeals for the Ninth Circuit by using the appellate CM/ECF system.

I certify that all participants in the case are registered CM/ECF users, and that service will be accomplished by the appellate CM/ECF system.

/s/ Meredith Stevenson
Meredith Stevenson

Case No. 22-70118

UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

CENTER FOR FOOD SAFETY,
Petitioner,

v.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, *et al.*,
Respondents,

and

SYNGENTA CROP PROTECTION, LLC,
Respondent-Intervenor.

On Petition for Review of an Order of the
United States Environmental Protection Agency

VOLUME 1 OF 2 – ATTACHMENTS 1-4

CENTER FOR FOOD SAFETY
Amy van Saun
2009 NE Alberta St., Suite 207
Portland, OR 97211
T: (971) 271-7372
avansaun@centerforfoodsafety.org

Sylvia Shih-Yau Wu
Meredith Stevenson
303 Sacramento Street, 2F
San Francisco, CA 94111
(415) 826-2770
swu@centerforfoodsafety.org
mstevenson@centerforfoodsafety.org

Counsel for Petitioners

TABLE OF CONTENTS

VOLUME 1 – ATTACHMENTS 1-4	
Attach. No.	Title
1.	Interim Registration Review Decision; Case Number 7014 (March 2022), EPA-HQ-OPP-2015-0401 (IRRD)
2.	Difenoconazole: Draft Ecological Risk Assessment for Registration Review (Sept. 16, 2020) (Ecological Risk Assessment)
3.	Human Health Aggregate Risk Assessment in Support of Reregistration and Registration Actions for Triazole-derivative Fungicide Compounds (Feb. 7, 2006) (2006 Memo)
4.	Center for Food Safety; Comments on proposed interim registration decision for difenoconazole (Oct. 4, 2021) (CFS Comment)
VOLUME 2 – ATTACHMENTS 5-9	
Attach. No.	Title
5.	Declaration of Jennifer Loda
6.	Declaration of Alice Cimino Naegele
7.	Declaration of Carrie Santulli Schudda
8.	Declaration of Sylvia Shih-Yau Wu
9.	Declaration of Kristina Sinclair (with Exhibits A-I)

Attachment 1

Docket Number EPA-HQ-OPP-2015-0401
www.regulations.gov

Difenoconazole

Interim Registration Review Decision
Case Number 7014

March 2022

Approved by: _____
Mary Elissa Reaves, Ph.D.
Director
Pesticide Re-evaluation Division

Date: 03/31/2022

Table of Contents

I. INTRODUCTION 3
A. Updates to the Proposed Interim Decision 4
B. Summary of Public Comments on the Draft Risk Assessments and Agency Responses 4
II. USE AND USAGE 12
III. SCIENTIFIC ASSESSMENTS 13
A. Human Health Risks 13
1. Risk Summary and Characterization 13
2. Human Incidents and Epidemiology 17
3. Tolerances 17
4. Human Health Data Needs 18
B. Ecological Risks 18
1. Risk Summary and Characterization 18
2. Ecological Incidents 23
3. Ecological and Environmental Fate Data Needs 24
C. Benefits Assessment 24
IV. INTERIM REGISTRATION REVIEW DECISION 25
A. Risk Mitigation and Regulatory Rationale 25
4. Updated Glove Label Language 25
5. Soil Incorporation of Treated Seed 26
6. Seed Container Label Instructions 26
7. Use Restrictions for Foliar Rice Application 28
8. Standardize Environmental Hazard Language 29
9. Fungicide Resistance Management 29
10. Advisory Spray Drift Management 30
B. Environmental Justice 31
C. Tolerance Actions 31
D. Interim Registration Review Decision 33
E. Data Requirements 35
V. NEXT STEPS AND TIMELINE 35
A. Interim Registration Review Decision 35
Implementation of Label Changes 35
VI. REFERENCES 36
Appendix A: Summary of Labeling Actions for Difenoconazole 39
Appendix B: Labeling Changes for Difenoconazole Products 40
Appendix C: Listed-Species Assessment 46
Appendix D: Endocrine Disruptor Screening Program 47

I. INTRODUCTION

This document is the Environmental Protection Agency's (EPA or the Agency) Interim Registration Review Decision (ID) for difenoconazole (PC Code 128847, case 7014). In a registration review decision under the Federal Insecticide, Fungicide, Rodenticide Act (FIFRA), the Agency determines whether a pesticide continues to meet FIFRA's registration standard.[1] Where appropriate, the Agency may issue an interim registration review decision before completing a registration review.[2] Among other things, the interim registration review decision may determine that new risk mitigation measures are necessary, lay out interim risk mitigation measures, identify data or information required to complete the review, and include schedules for submitting the required data, conducting the new risk assessment and completing the registration review.[3] For more information on difenoconazole, see EPA's public docket (EPA-HQ-OPP-2015-0401) at www.regulations.gov.

FIFRA[4] mandates the continuous review of existing pesticides. All pesticides distributed or sold in the United States must be registered by EPA based on scientific data showing that they will not cause unreasonable risks to human health or to the environment when used as directed on product labeling. In 2006, the Agency began implementing the registration review program. EPA will review each registered pesticide every 15 years. Through the registration review program, the Agency intends to verify that all registered pesticides continue to meet the registration standard as the ability to assess and reduce risk evolves and as policies and practices change. By periodically re-evaluating pesticides as science, public policy, and pesticide-use practices change, the Agency ensures that the public can continue to use products in the marketplace that do not present unreasonable adverse effects. For more information on the registration review program, see http://www.epa.gov/pesticide-reevaluation.

The EPA is issuing an ID for difenoconazole so that it can (1) move forward with aspects of the registration review that are complete and (2) implement interim risk mitigation (see Appendices A and B). EPA is currently working with the U.S. Fish and Wildlife Service and the National Marine Fisheries Service (the Services) to improve the consultation process for national threatened and endangered (listed) species for pesticides under the Endangered Species Act (ESA).[5] The Agency has not yet fully evaluated difenoconazole's risks to federally listed species. However, EPA will complete its federally listed-species assessment and any necessary consultation with the Services before completing the difenoconazole registration review. Before completing registration review, EPA will also complete endocrine screening for difenoconazole under the Federal Food, Drug, and Cosmetic Act (FFDCA).[6]

Difenoconazole is a systemic triazole fungicide registered for foliar applications to numerous field crops, landscape ornamentals, golf course turf, and as a seed treatment for cereal crops, canola, cotton, and potato seed pieces. It is also registered for post-harvest use on various

---

[1] Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) § 3(g), 7 U.S.C. § 136a(g); 40 C.F.R. § 155.57.
[2] 40 C.F.R. §§ 155.56, 155.58.
[3] 40 C.F.R. § 155.56.
[4] As amended by the Food Quality Protection Act (FQPA) of 1996, Pub. L. No. 104-170, 110 Stat. 1489.
[5] Endangered Species Act (ESA) § 7, 16 U.S.C. § 1536.
[6] Federal Food, Drug, and Cosmetic Act (FFDCA) § 408(p), 21 U.S.C. § 346a(p).

tuberous and corm vegetables and pome fruits. Because it was registered after November 1984, difenoconazole was not subject to reregistration under FIFRA § 4(a).

This document is organized into five sections: *Introduction*, which includes this summary and a summary of public comments and EPA's responses; *Use and Usage*, which describes how and where difenoconazole is used and summarizes data on its use; *Scientific Assessments*, which summarizes EPA's risk and benefits assessments, and provides broader context with a discussion of risk characterization; *Interim Registration Review Decision*, which describes the mitigation measures to address risks of concern and the regulatory rationale for EPA's ID; and, lastly, *Next Steps and Timeline* for completion of this registration review.

A. Updates to the Proposed Interim Decision

In July 2021, EPA published the PID for difenoconazole. The Agency has made several changes to the PID in this ID. These changes include updating the water advisory to be more consistent with environmental hazard language in the Label Review Manual[7] as well as updating the labeling for seed treatments for clarity to be consistent with current fungicide seed treatment labeling. The PID proposed a dye statement for difenoconazole products that allow commercial seed treatment. The Agency has determined that a dye statement is not necessary because tolerances exist for residues of difenoconazole on treated seeds. According to 40 CFR 153.155(a) and explained the Label Review Manual,[8] the dye requirement does not apply if appropriate tolerances have been established under FFDCA for residues. The Agency has also streamlined the Personal Protective Equipment (PPE) labeling for seed treatment products to add clarity.

The revised labeling does not materially impact the instructions for users and should have no impacts on users. The Agency is also streamlining the personal protective equipment labeling proposed in the PID. See Section IV and Appendix B of this document for the revised labeling.

EPA has not updated the draft Human Health Risk Assessment and the draft Ecological Risk Assessment. This ID finalizes the Agency's draft supporting documents *Difenoconazole: Draft Human Health Risk Assessment for Registration Review* and the *Difenoconazole: Draft Ecological Risk Assessment for Registration Review*, which are available in EPA's public docket (EPA-HQ-OPP-2015-0401).

B. Summary of Difenoconazole Registration Review

On January 11, 2016, the Agency formally initiated registration review for difenoconazole with the opening of the registration review docket for the case.[9] The following summary highlights the docket opening and other significant milestones that have occurred thus far during the registration review of difenoconazole:

---

[7] See Chapter 8: Environmental Hazards section of the Label Review Manual at: https://www.epa.gov/sites/default/files/2015-03/documents/chap-08-sep-2012.pdf
[8] See Chapter 18: Unique Product Labeling section of the Label Review Manual at: https://www.epa.gov/sites/production/files/2014-06/documents/chap-18_0.pdf
[9] 40 CFR § 155.50

- January 2016 – EPA posted the *Difenoconazole Preliminary Work Plan Registration Review: Initial Docket Case Number 7014* (PWP) (December 21, 2015),the *Difenoconazole: Human Health Risk Scoping Document in Support of Registration Review* (October 1, 2015), the *Difenoconazole: Preliminary Problem Formulation for Environmental Fate, Ecological Risk, Endangered Species, and Drinking Water Exposure Assessments in Support of Registration Review* (November 5, 2015), and other supporting documents to the public docket for a 60-day public comment period.

- June 2016 – EPA posted the *Difenoconazole Final Work Plan* (FWP) (June 13, 2016) to the public docket. The Agency received five comments on the PWP. The comments did not result in changes to the registration review timeline or data needs as described in the FWP.

- January 2017 – EPA issued a generic data call-in (GDCI) for difenoconazole to obtain data needed to conduct the registration review risk assessments (GDCI-128847-1602). All data were submitted and reviewed. All data requirements have been fulfilled and the DCI is satisfied.

- November 2020 – EPA posted *Difenoconazole. Draft Human Health Risk Assessment for Registration Review* (September 18, 2020) and *Difenoconazole: Draft Ecological Risk Assessment for Registration Review* (September 16, 2020) for a 60-day public comment period. The Agency received comments from 6 commenters. The comments did not change the risk assessments or registration review timeline for difenoconazole.

- June 2021 – EPA completed the PID for difenoconazole and posted it to the public docket for a 60-day public comment period. The Agency received comments from 6 commenters. The comments did not change the risk assessments or registration review timeline for difenoconazole. However, comments did result in minor clarification changes to the labeling. Comments and responses to comments are summarized in section I.C below.

- March 2022 – EPA has completed the ID for difenoconazole and will post it to the public docket. Along with the ID, EPA will post the following documents to the public docket:
    - *Difenoconazole: EFED Response to Comments on the Proposed Interim Registration Review Decision (PID)* (November 30, 2021)
    - *Difenoconazole. Response to Comments on the Proposed Interim Decision* (March 10, 2022)

C. Summary of Public Comments on the Proposed Interim Registration Review Decision and Agency Responses

During the 60-day public-comment period for the difenoconazole Proposed Interim Registration Review Decision (which opened on August 3, 2021 and closed on October 2, 2021), the Agency

received six public comments. Comments were submitted by the United States Department of Agriculture (USDA), the Center for Food Safety (CFS), Syngenta Crop Protection, LLC (Syngenta), the Northwest Horticultural Council (NHC), the National Potato Council, and the American Soybean Association (ASA). The Agency has summarized and responded to all substantive comments and comments of a broader regulatory nature below. More detailed responses to comments can be found in the *Difenoconazole: EFED Response to Comments on the Proposed Interim Registration Review Decision (PID)* and the *Difenoconazole. Response to Comments on the Proposed Interim Decision*, which responds to comments relating to the human health risk assessment. Both are available on the public docket. The Agency thanks all commenters for participating and has considered all comments in developing this ID.

Comments Submitted by the Center for Food Safety (Docket ID: EPA-HQ-OPP-2015-0401-0053 to 0057)

Comments pertaining to the ecological risk assessment:

Center for Food Safety (CFS) submitted several comments related to environmental fate and ecological effects outlined in the Agency's ecological risk assessment. CFS mentioned that difenoconazole is extremely persistent in laboratory and field tests and is found in both water and soil. There is concern that EPA's exposure and risk assessments do not account for the accumulation of difenoconazole over a single season, or years.

CFS noted concern that the presented risks to terrestrial organisms do not capture the potential effect of difenoconazole on different taxa, specifically related to risk quotient (RQ) exceedances and difenoconazole's propensity to persist in the environment. It was stated that there are likely effects on ground-dwelling bees which was not adequately described in the Agency's risk assessment.

CFS stated that given the persistence of difenoconazole in terrestrial and aquatic environments repeated use can increase risks to aquatic organisms and increase the risks over time. CFS commented that difenoconazole can lead to bioaccumulation in fish and other aquatic organisms.

EPA Response: EPA considered the comment related to the persistence of difenoconazole in soil and water. The exposure modeling of aquatic environments is based on a collection of environmental fate properties as well as site-related input parameters. Additionally, variations in meteorological measurements were used to probabilistically estimate concentrations of difenoconazole in aquatic environments. These three things accounted for the accumulation of difenoconazole over a year and over multiple years.

The Agency's assessments rely on a surrogate species approach where a few tested species are used to represent sensitivity for all species. It is assumed that if honey bee data suggest level of concern (LOC) exceedances, then there is a risk concern for other bees, including ground-dwelling bees. When additional data are available for other terrestrial invertebrates, such as non-*Apis* bees, earthworms, and other soil dwelling invertebrates, that information is included in risk assessment as an additional line of evidence. For difenoconazole, no soil dwelling terrestrial invertebrate toxicity data are available, so refinement is not possible.

EPA acknowledges the risk for aquatic organisms and identified LOC exceedances for fish and aquatic invertebrates and disagrees with the comment that the Agency is not considering accumulation of difenoconazole over a year, or over multiple years. See the *Difenoconazole: EFED Response to Comments on the Proposed Interim Registration Review Decision (PID)* for more information.

Comments pertaining to the human health risk assessment:

CFS also submitted several comments related to the human health risk conclusions outlined in the Agency's human health risk assessment.

In 1994, the chronic reference dose EPA used was 0.01 mg/kg/day based on hepatocellular hypertrophy in male rats (MRID 42090019). In 2015, the chronic reference dose was retained, but the endpoint was changed to cumulative decreases in body weight gains. In the most recent 2020 human health risk assessment, the chronic reference dose was increased five-fold to 0.05 mg/kg/day and was based on a mouse instead of a rat study (MRID 42090015). CFS stated that EPA dismissed hepatocellular hypertrophy in male rats and that the current chronic reference dose value used for human health risk assessment is incorrect and should be reversed.

CFS noted that EPA originally classified difenoconazole as a Group C "Possible Human Carcinogen" in 1994, based on inducement of hepatocellular adenomas and carcinomas in a mouse study. In the 2020 human health risk assessment, EPA re-classified difenoconazole under the descriptor "Suggestive Evidence of Carcinogenicity." CFS suggested that the cancer classification should be reversed.

CFS asserted that a major metabolite of difenoconazole has been identified (CGA-205375) as present in humans, fish, and livestock. No toxicity data exists for the metabolite and CFS requested that EPA require that toxicity data be submitted.

Additionally, CFS asked EPA to require full dermal absorption data for various difenoconazole formulations and requested that the Agency conduct exposure assessments that incorporate a dermal absorption factor of 48% based on an older risk assessments, rather than the 6% dermal absorption factor derived from the dermal triple-pack approach.

CFS stated that triazole fungicides meet the criteria for designation as a common mechanism group and should have a cumulative assessment completed. CFS asserted that there are two endpoints, shared by most triazoles, that should be the focus of a cumulative risk assessment: fatty changes and carcinogenicity.

EPA Response: CFS correctly stated that the 2020 difenoconazole draft human health risk assessment used the no-observed adverse-effect level (NOAEL) from the mouse carcinogenicity study (4.7 mg/kg/day, from MRID 42090015) to derive a chronic population-adjusted dose (cPAD). That NOAEL is based on increased incidence of liver lesions (individual cell necrosis and bile stasis in males, hepatocyte hypertrophy in both sexes), and increased serum levels of serum sorbitol dehydrogenase (SDH) in males at a lowest-observed adverse-effect level

(LOAEL) of 46 mg/kg/day. Older risk assessments used a NOAEL from a combined rat chronic toxicity/carcinogenicity study (0.96 mg/kg/day, from MRID 42090019) to derive the cPAD, based on cumulative decreases in body weight gains. However, the difenoconazole toxicology database underwent extensive review for registration review in late 2020, and most studies were updated to reflect current toxicology evaluation practices. Changes in study LOAELs at that time prompted changes to the points of departure (PODs) selected for risk assessment. During the 2020 review, it was established that the rat combined chronic toxicity/carcinogenicity study determined the lowest-observed effect level (LOEL), not the lowest-observed adverse-effect level (LOAEL). Current Agency policy is to use only adverse effects, which are indicated by a LOAEL, not a LOEL, as a basis for risk assessment. The mouse study had a lower LOAEL than the rat study and was, therefore, selected to derive the cPAD.

EPA originally classified difenoconazole as a Group C Possible Human Carcinogen in 1994, based on clear inducement of hepatocellular adenomas and carcinomas in a mouse study. In 2007, in accordance with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*,[10] the Agency subsequently re-classified it under the descriptor Suggestive Evidence of Carcinogenicity based on liver tumors in male and female mice. This classification is consistent with current Agency guidelines. The reference dose would address the concern for chronic toxicity, including carcinogenicity, likely to result in exposure to difenoconazole.

EPA has reviewed the data that indicated a major metabolite of difenoconazole that has been identified (CGA-205375) as a residue of concern in humans, fish, and livestock. EPA uses structure-activity-relationship (SAR) analyses, as appropriate, to support decisions on residues of concern in situations where empirical data are lacking, and/or, to trigger the need for additional toxicology studies. In the case of difenoconazole, the SAR analysis of the metabolites using DEREK v.12 did not indicate any concerns for toxic effects that were not observed in the available difenoconazole toxicity database.

A dermal penetration study is conditionally required in the Code of Federal Regulations (CFR) Title 40 – Part §158.500, however, EPA has the flexibility to establish or modify data needs for individual pesticides and may require submission of additional data beyond what is specified. The dermal penetration study (guideline 870.7600) must be followed for *in vivo* studies in rodents, but it does not preclude use of other kinds of studies to derive a dermal absorption factor (DAF), such as *in vitro* skin absorption studies, or studies from the public scientific literature, or studies conducted under special protocols. Use of the triple pack approach is supported by EPA to refine dermal penetration results by accounting for differences between *in vitro* and *in vivo* absorption within a test species as well as species differences between animal and human skin. For registration review, the Agency used a DAF of 6% for human health risk assessment. The highest *in vivo* rat absorption from the available *in vivo* rat study was multiplied by the highest ratio of human vs. rat absorption from the available *in vitro* studies using technical difenoconazole.

The resulting DAF was considered a conservative estimate of absorption by human skin for difenoconazole at the time it was derived; however, the triple pack should be applied when

[10] https://www.epa.gov/risk/guidelines-carcinogen-risk-assessment

similar protocols are utilized across the *in vitro* and *in vivo* studies, including the same test substance and similar dosing. Therefore, the DAF for difenoconazole has been reevaluated and based on all the available data and current practices, EPA has concluded that a DAF of 8% should be applied for difenoconazole moving forward. The updated DAF of 8% is similar to the previous DAF of 6% that was applied in the 2020 human health risk assessment and, therefore, this change does not materially impact the human health risk assessment.

The triazole fungicides share the following common metabolites: 1,2,4-Triazole (1,2,4-T), triazole alanine (TA), and triazole acetic acid (TAA). In 2006, the Agency issued aggregate human health risk assessments for 1,2,4-T and the conjugated metabolites of 1,2,4-T, TA and TAA. The assessment was based on sufficient data to support a risk assessment for these metabolites. The Agency conducted two assessments: one for 1,2,4-T and one for combined exposure to TA and TAA. Both assessments are highly conservative, using the maximum combination of uncertainty factors and high-end estimates of both dietary and non-dietary exposures. In addition, the 2006 aggregate assessments retained a 10X database uncertainty factor to account for the data gaps associated with the toxicological database and were designed to be extremely conservative so that the assumptions will remain valid for anticipated registrations. The Agency has not received any new data following the 2006 assessments, however; several requests for new uses of triazole fungicides have been submitted to the Agency and have been evaluated with the same conservatisms that were in place for the 2006 aggregate risk assessments. EPA does not believe that exposure or risk has been underestimated through these risk assessment approaches. The Agency will continue to employ a protective screening approach for all actions involving the triazoles and will continue to evaluate the need for additional data.

The Agency has not assumed that difenoconazole has a common mechanism of toxicity with other substances at this time. The Agency will use the *Pesticide Cumulative Risk Assessment: Framework for Screening Analysis*[11] to determine if the available toxicological data for difenoconazole suggest that a candidate common mechanism group may be established with other pesticides. If a common mechanism group is established, a screening-level analysis may be conducted to provide an initial screen for multiple pesticide exposure.

See the *Difenoconazole. Response to Comments on the Proposed Interim Decision* for more information.

Comments pertaining to fungicide resistance:

CFS noted that the triazole fungicides are the dominant compounds used to treat crops, animals, and humans and are the only class used in both medicine and agriculture. Triazoles used in human medicine include compounds such as itraconazole, voriconazole, and posoconazole. CFS noted concern regarding the development of resistance because the drivers of resistance in plant and human pathogens share some similarities. CFS expresses concern that the widespread use of triazole fungicides in agriculture may contribute to the development of fungal resistance to

[11] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/pesticide-cumulative-risk-assessment-framework

medical-use triazole drugs. CFS requested that the Agency assess the public health threats posed by continued and expanding use of difenoconazole and other agricultural triazoles in terms of increasing resistance of human fungal pathogens such as *A. fumigatus* and *C. auris* to medical antifungal compounds.

EPA Response: EPA is aware of increases in the total agricultural usage of both difenoconazole between 2015-2019 and triazoles more generally. EPA is also aware of increased global incidence of triazole-resistant human fungal pathogens. The extent to which the continued use of triazole fungicides to control plant pathogens in agricultural production may contribute to the emergence of antifungal resistant human pathogens is unclear and a direct association between the quantity of U.S. agricultural triazole fungicide use and human fungal infections has not been established.[12] EPA is working with federal partners to assess the potential impact of increased plant agriculture fungicide (e.g., difenoconazole) use in the U.S. on the development of triazole-resistance in medical settings. The Agency considers it critical that a variety of mode of action (MOA) groups remain available for use in the interest of suppressing resistance development to both agricultural and public health pathogens. For more information, see Section IV of this document and PRN 2017-1 and PRN 2017-2, available at https://www.epa.gov/pesticide-registration/pesticide-registration-notices-year.

Comments Submitted by the United States Department of Agriculture (Docket ID: EPA-HQ-OPP-2015-0401

Comment: The United States Department of Agriculture (USDA) emphasized the importance of difenoconazole as a tool in specialty crop production, particularly to control certain fungal pests in almond, ginseng, guava, papaya, and parsley production. USDA noted that another key benefit of difenoconazole is its compatibility with other fungicides, which can broaden the spectrum of disease control and manage the development of resistance. USDA supports the re-registration of difenoconazole but is concerned about the withdrawal of EPA's previous recommendations to harmonize U.S. tolerances with Codex maximum residue limits (MRLs) for green onion, rapeseed, and wild rice. USDA stressed the importance of international harmonization and has requested that EPA reconsider this proposal.

EPA Response: The EPA thanks the USDA for its comments and will take them into consideration. Regarding the potential for additional harmonization with Codex MRLs, EPA attempts to harmonize existing U.S. tolerances with Codex MRLs where feasible. However, harmonization is not possible in some cases due to a difference in tolerance expression (*e.g.*, a difference in metabolites covered), a difference in commodity definition (*e.g.*, livestock meat versus livestock fat), or a difference in use pattern (*e.g.*, in season versus post-harvest). In addition, EPA does not routinely harmonize U.S. tolerances for pesticide residues in/on livestock feed commodities with Codex MRLs.

For green onion, rapeseed, and wild rice, the Agency originally proposed to harmonize with Codex, however Syngenta subsequently submitted confidential business information (CBI) with rationales for retaining the green onion/rapeseed tolerances and a preference for the wild rice

[12] https://www.cdc.gov/fungal/diseases/aspergillosis/antifungal-resistant.htm

tolerance. The human health risk assessment tolerance table contained an error: there is no Codex MRL for wild rice but there a Canadian MRL for wild rice. Anticipated tolerance revisions for difenoconazole are detailed in Section IV.C of this document. Tolerance amendments follow a notice and comment process separate from registration review and the Agency will consider any relevant comments submitted at that time, including USDA's comment. Any proposed tolerance changes will be announced in the *Federal Register* and will be subject to public comment prior to being finalized.

Comments Submitted by Syngenta (Docket ID: EPA-HQ-OPP-2015-0401)

Comment: Syngenta noted that the proposed drift/runoff, surface water advisory, and terrestrial use hazard statement should not be under the Directions for Use section of the label, but rather be placed under the Environmental Hazards section. Syngenta proposes that the drift and runoff statement ("Drift and runoff may be hazardous to aquatic organisms in water adjacent to treated areas") and the surface water advisory should not be added to the seed treatment labels because the advisory is intended for applications made to crops and not seed. Seed treatment product labels already have this text, which Syngenta argues is adequate: "This product is toxic to fish, shrimp, and other aquatic invertebrates. Do not apply directly to water, or to areas where surface water is present, or to intertidal areas below the mean high-water mark. Do not contaminate water when disposing of equipment washwater or rinsate."

Furthermore, Syngenta suggested using a streamlined personal protective equipment (PPE) statement on the seed tag and in the Directions of Use section of the seed treatment product labels: "Wear long-sleeved shirt, long pants, shoes, socks, and chemical-resistant gloves when loading or otherwise handling treated seed and when covering or collecting treated seeds spilled during loading and planting."

EPA Response: EPA thanks Syngenta for their comments on the difenoconazole PID and agrees that the noted advisory and hazard statements belong under the Environmental Hazards section of the label. Commercial seed treatments are likely to occur indoors, and environmental hazard statements intended for outdoor terrestrial uses would not apply. However, on-farm seed treatments can occur outdoors with portable treaters that can be used in the field, and on-farm seed treatment labels need to reflect any drift/runoff, surface water advisory, and terrestrial use environmental hazard statements specified for outdoor terrestrial uses as noted in Chapter 8 of the Label Review Manual. The Agency has clarified the label table (Appendix B) to note the types of uses affected for the drift/runoff, surface water advisory, and terrestrial use environmental hazard statements.

The Agency does not object to the proposed PPE statement, however, the gloves text needs to be updated to reflect the appropriate chemical-resistant glove types to use (*e.g.*, barrier laminate, butyl rubber, nitrile rubber, neoprene rubber, polyvinyl chloride, or viton) in accordance with Chapter 10 of the Label Review Manual. The revised PPE statement is as follows:

"When loading/pouring the treated seed/seed-pieces or covering/collecting spilled seed during loading and planting, wear long-sleeved shirt, long pants, shoes, socks, and chemical-resistant gloves [list specific glove types].

See Appendix B for the revised labeling, clarification on affected uses, and revised label placement.

Comments Submitted by Northwest Horticultural Council, National Potato Council, and American Soybean Association (Docket IDs: EPA-HQ-OPP-2015-0401-0051, -0052, and -003)

Comment: The Northwest Horticultural Council (NHC), the National Potato Council (NPC), and the American Soybean Council (ASC) submitted comments in support of the re-registration of difenoconazole. They stressed the importance of difenoconazole as an integral component of several fungicide combination products to control fungal diseases on specialty crops and promote effective resistance management. In addition, NHC and NPC indicated that adopting the label changes for difenoconazole are reasonable.

EPA Response: EPA thanks the Northwest Horticultural Council, the National Potato Council, and the American Soybean Association for their comments. The benefits of difenoconazole have been considered in developing this Interim Decision, see Section III.C of this document for a summary of the Agency's benefits analysis.

II. USE AND USAGE

Difenoconazole is a broad-spectrum systemic triazole fungicide first registered for use in the United States in 1994. Difenoconazole is registered for use as a foliar application to various fruit, nut, vegetable, and field crops as well as a seed treatment for a number of crops. Commodity postharvest applications including dip treatments and spray drenches registered for pome fruits (apple and pears) and tuberous and corm vegetables (such as arracacha, arrowroot, cassava, sweet potato, and yam). Seed treatment uses are also registered for barley, canola, sweet corn, cotton, oats, potatoes, rye, triticale, and wheat.[13]

Agricultural Usage

Seed treatment uses of difenoconazole have been registered for almost 30 years, but foliar uses were first registered in 2008. Usage for foliar uses is still increasing in some cases. For years 2015-2019, the annual average of foliar difenoconazole usage was 400,000 pounds of active ingredient (lbs. a.i.) on 3.7 million total acres treated.[14] Recent usage data for seed treatments and postharvest applications are not available; these usage estimates are not provided because reliable data sources are not available at present.

In terms of total acres treated and pounds of active ingredient applied, between 2015-2019 difenoconazole foliar usage was largely driven by soybeans with an annual average of 1,800,000

[13] United States Environmental Protection Agency (EPA). Biological and Economic Analysis Branch (BEAD). Science Information and Analysis Branch (SIAB). 2019. Difenoconazole (PC Code: 128847) Pesticide Label Use Summary (PLUS) Line by Line Report dated: 30/09/2019.
[14] Kynetec USA, Inc. 2020. "The AgroTrak® Study from Kynetec USA, Inc." Microsoft Access Database. Database Subset: 2015-2019. [Accessed April 2021].

total acres treated with an average of 200,000 lbs difenoconazole applied to soybeans, although this represents only 2 percent of the crop treated (PCT) for soybean. The leading crops in terms of PCT were tomatoes (45%), almonds (30%), sugar beets (30%), apples (30%), wine grapes (25%), and potatoes (15%). Although a significant percent crop treated of table grapes (30%) and watermelon (20%) were treated with difenoconazole, the total acres treated, and total pounds applied were relatively small in comparison to other sites. The average application rate across all crops was 0.1 lbs. ai/acre.[15]

Non-agricultural Usage

Difenoconazole is also registered for non-agricultural use on established ornamentals in landscaped areas, including recreational parks, institutional sites, roses, golf courses and residential properties treated by commercial and non-professional (*i.e.,* homeowner) applicators. These uses are surveyed but no recent usage has been reported, implying low or infrequent usage of difenoconazole.[16]

III. SCIENTIFIC ASSESSMENTS

A. Human Health Risks

A summary of the Agency's human health risk assessment is presented below. The Agency used the most current science policies and risk assessment methodologies available at the time to prepare a risk assessment in support of the registration review of difenoconazole. For additional details on the human health and drinking water assessments for difenoconazole, see the *Difenoconazole: Draft Human Health Risk Assessment for Registration Review,* and other supporting documents, which are available in the public docket.

1. Risk Summary and Characterization

Dietary Assessment

The Agency assessed exposure to difenoconazole in food and drinking water from the registered uses, since it may be applied directly to growing crops and application may result in difenoconazole reaching surface and ground water sources of drinking water. The acute dietary analysis assumed tolerance-level residues and 100% crop treated values for all registered crops. The chronic dietary analysis assumed tolerance-level residues and average percent crop treated information for some commodities.

Acute dietary risk estimates are not of concern at the 95th percentile of the exposure distribution for all population subgroups. Acute risk estimates for the general U.S. population equaled 17% of the acute population adjusted dose (aPAD), where risk estimates below 100% are not of

[15] Kynetec USA, Inc. 2020. "The AgroTrak® Study from Kynetec USA, Inc." iMap Software. Database Subset: 2015-2019. [Accessed April 2021].
[16] United States Environmental Protection Agency (EPA). Biological and Economic Analysis Branch (BEAD). Science Information and Analysis Branch (SIAB). 2019. Difenoconazole (PC Code: 128847) Summary Use and Usage Matrix dated: 28/08/2019.

concern. The most highly exposed population subgroup was all infants <1 year old which equaled 53% of the aPAD and not of concern.

Similarly, chronic risk estimates are not of concern. Chronic dietary risk estimates equaled 11% of the chronic population adjusted dose (cPAD) for the general U.S. population, where risk estimates below 100% are not of concern. The most highly exposed population subgroup was all infants less than 1 years old at 38% of the cPAD and not of concern.

Dietary Assessment—Triazole Metabolites

Difenoconazole belongs in the triazole group of fungicides, and triazole metabolites common to the group, 1,2,4-triazole (1,2,4-T), triazolylalanine (TA), and triazolylacetic acid (TAA) are residues of concern which are considered toxicologically different from difenoconazole and were assessed separately from the parent compound. Acute dietary risk estimates for the triazole metabolites ranged from 20% to 70% of the aPAD and were not of concern (where risk estimates below 100% are not of concern). Chronic dietary risk estimates for the metabolites ranged from 11% to 86% of the cPAD and were also not of concern.

Cancer Classification

In accordance with EPA's 2005 Guidelines for Carcinogenic Risk Assessment, difenoconazole was re-classified as "Suggestive Evidence of Carcinogenic Potential" based on liver tumors in male and female mice. Difenoconazole is not genotoxic and there is no evidence of carcinogenicity in rats. Quantification of cancer risk was not required. The current reference dose (RfD) for chronic dietary exposure assessment would address the concern for chronic toxicity, including carcinogenicity, likely to result from exposure to difenoconazole.

Residential Assessment

There are registered uses on commercial and residential landscapes and interior plantscapes, as well as turf applications to golf courses, that would result in residential handler and post-application exposures.

There are no residential handler inhalation risks of concern. Inhalation exposures result in margins of exposures (MOEs) ranging from 3,200,000 to 340,000,000, where the LOC is 100 and risk estimates below 100 are of concern.

A quantitative residential post-application assessment was not performed because a dermal endpoint was not identified due to the lack of adverse effects attributable to a single dose. Because the use around the home is limited to ornamentals (not turf), the Agency has concluded that homeowner use of difenoconazole would not result in incidental oral exposure to children.

Aggregate Assessment

There are no aggregate (dietary + residential) risks of concern. The Agency evaluated acute, short-term, and chronic aggregate exposure. Because no acute or chronic dietary risks of concern were identified, there are no risks of concern for acute and chronic aggregate exposures.

Short-term aggregate risk was estimated by combining chronic dietary exposure (food + water) with the residential handler inhalation exposures from applications to gardens/ornamentals via hose-end sprayer. The resulting risk estimate is an MOE of 5,000 which is not of concern (LOC=100, risk estimates below 100 are potentially of concern).

No intermediate-term aggregate exposure scenarios were identified; therefore, a quantitative intermediate-term aggregate assessment was not required.

A separate cancer aggregate assessment was not required because the chronic dietary exposure assessment is adequately protective of carcinogenicity from exposure to difenoconazole.

Aggregate Assessment of Free Triazole & its Conjugates

Application of triazole-containing pesticides, such as difenoconazole, also result in exposure to free triazole and its conjugates, which are considered toxicologically different from difenoconazole and are assessed separately from the parent compound. The aggregate MOE's range from 1,700 – 12,000 (LOC = 1,000) and, therefore, are not of concern.

Non-occupational Spray Drift Assessment

A quantitative spray drift assessment for difenoconazole was performed for incidental oral exposure to children 1 to 2 years old, the highest exposed population subgroup. Risk estimates were not of concern using difenoconazole-specific turf transferable residue (TTR) data and the highest registered application rates (0.126 lb ai/A - 0.427 lb ai/A) from the assessed occupational handler scenarios and equipment combinations. MOEs ranged from 94,000 to 420,000 at the field edge and were not of concern (LOC=100, MOEs below 100 are potentially of concern).

A dermal point of departure (POD) was not selected due to the lack of an adverse effect attributable to a single dose, so a quantitative dermal assessment was not performed.

Non-occupational Bystander Post-Application Assessment

The Agency considered volatilization as a source of post-application inhalation exposure to individual near application sites. The Agency sought expert advice on issues related to volatilization of pesticides in a FIFRA Science Advisory Panel meeting in December 2009 and received the SAP's final report in March 2010.[17] The Agency developed a volatilization screening tool and a subsequent volatilization screening analysis.[18] EPA will utilize this analysis during registration review to determine if data or further analysis is required for difenoconazole.

[17] https://www.regulations.gov/document/EPA-HQ-OPP-2009-0687-0037
[18] https://www.regulations.gov/docket/EPA-HQ-OPP-2014-0219

Cumulative Assessment

EPA has not made a common mechanism of toxicity finding as to difenoconazole and any other substances. Although the triazole fungicides produce common metabolites, these metabolites do not contribute to the toxicity of the parent difenoconazole. The Agency assessed aggregate risk from the metabolites separately and did not find risks of concern. Difenoconazole does not appear to produce any other toxic metabolite produced by other substances.

Occupational Handler Assessment

The Agency assessed risk to occupational handlers who may be exposed while mixing, loading, and applying difenoconazole. No dermal endpoint was selected because the database does not show systemic effects after exposure from the dermal route at doses that would be relevant to risk assessment, so occupational handler dermal risk assessments were not conducted. Occupational handler inhalation risk estimates range from 4,300 to 45,000,000 for agricultural crop uses and were not of concern (LOC=100, MOEs below 100 are potentially of concern). For seed treatment use, risk estimates range from 11,000 to 1,100,000 and were not of concern (LOC=100, MOEs below 100 are potentially of concern). For post-harvest use, MOEs range from 77,000 to 320,000 and were not of concern (LOC=100, MOEs below 100 are potentially of concern).

Occupational Post-Application Assessment

Dermal Post-Application Risk

A quantitative post-application dermal assessment was not completed because a dermal endpoint was not identified due to the lack of adverse effects attributable to dermal exposure. The potential for post-application exposure following the planting of treated seeds was considered unlikely because sustained levels of contact with treated seed after planting would not be expected.

Restricted Entry Interval

Difenoconazole is classified as Toxicity Category III for acute dermal and oral exposures, inhalation exposures, and acute dermal toxicity and eye irritation. It is classified as Toxicity Category IV for skin irritation potential, and it is not a skin sensitizer. Under 40 CFR 156.208(c)(2) (iii), active ingredients classified as Acute III or IV for acute dermal, eye irritation and primary skin irritation are assigned a 12-hour restricted-entry interval (REI). This REI is adequate to protect agricultural workers where post-application exposures to difenoconazole are expected to occur. For products that contain difenoconazole and inert ingredients or other active ingredients, longer REIs may be required.

Inhalation Post-Application Risk

A quantitative inhalation post-application exposure assessment was not performed for workers re-entering treated fields. Handler exposure resulting from application of pesticides outdoors is

likely to result in higher exposure than post-application exposure. Since occupational handler inhalation risk estimates were not of concern for field applications, occupational post-application inhalation risk estimates are also not of concern.

A post-application inhalation exposure assessment was not required for the seed treatment uses of difenoconazole, as exposure is expected to be negligible. A post-application inhalation exposure assessment for the post-harvest uses of difenoconazole was conducted to assess risk from activities such as sorting and packing treated commodities and MOEs ranged from 990,000 to 22,000,000, which are not of concern with baseline attire composed of long-sleeved shirt, long pants, shoes, and socks (LOC=100, MOEs below 100 are potentially of concern).

2. Human Incidents and Epidemiology

On July 15, 2020, EPA reviewed difenoconazole incidents reported to both the OPP Incident Data System (IDS) and the Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health (CDC/NIOSH) Sentinel Event Notification System for Occupational Risk-Pesticides (SENSOR) databases. For the Main IDS from January 1, 2015 to June 9, 2020, there were seven incidents reported that involved difenoconazole. All difenoconazole incidents reported involved multiple active ingredients. One incident was classified as minor severity and six incidents were classified as moderate severity. For Aggregate IDS for the same time period, there were three minor severity incidents reported that involved difenoconazole. A query of SENSOR-Pesticides from 1998-2015 identified nine cases that involved difenoconazole. Of these difenoconazole cases, eight involved multiple active ingredients and one case involved difenoconazole as a single active ingredient. All nine cases were occupational and were classified as low severity. Difenoconazole is not included in the Agricultural Health Study (AHS), a federally-funded study that evaluates associations between pesticide exposures and cancer and other health outcomes, and represents a collaborative effort between the U.S. National Cancer Institute, the National Institute of Environmental Health Sciences, the Centers for Disease Control's National Institute of Occupational Safety and Health, and the U.S. EPA.

For more detail on incidents, please refer to *Difenoconazole: Tier I Update Review of Human Incidents and Epidemiology for Draft Risk Assessment*, available on the docket at https://www.regulations.gov/document/EPA-HQ-OPP-2015-0401-0020. The frequency and severity of the difenoconazole incidents are consistent with the conclusions drawn from the empirical data. The Agency intends to conduct ongoing human incident monitoring for difenoconazole and will conduct additional analyses if necessary.

3. Tolerances

Tolerances for difenoconazole are established in 40 CFR §180.475. EPA is anticipating several commodity definition revisions and some tolerance level changes consistent with OECD rounding class practice or to harmonize with established Codex and Canadian MRLs. Mexico adopts U.S. tolerances and/or Codex MRLs for its export purposes. For more detail, see Section 2.C., below.

4. Human Health Data Needs

The human health database for difenoconazole is complete. The Agency does not anticipate any further human health data needs for difenoconazole.

B. Ecological Risks

The Agency used the most current science policies and risk assessment methodologies to prepare a risk assessment in support of the registration review of difenoconazole.[19] For additional details on the 2020 ERA, see *Difenoconazole: Draft Ecological Risk Assessment for Registration Review* in EPA's public docket (EPA-HQ-OPP-2015-0401).

The EPA is currently working with its federal partners and other stakeholders to improve the consultation process for federally-listed species and their designated critical habitats. The Agency has not yet fully evaluated difenoconazole's risks to listed species. However, EPA will complete its listed-species assessment and any necessary consultation with the Services before completing the difenoconazole registration review. See Appendix C for more details. As such, potential risks for non-listed species only are described below.

1. Risk Summary and Characterization

The Agency uses a deterministic approach/risk quotient method to estimate risk to non-target taxa based on the current registered uses of difenoconazole, the available ecotoxicity data, and the environmental fate properties. The risk estimates (Risk Quotients, or RQs) are compared to the EPA's Levels of Concern (LOC). Potential risk was identified when RQs are greater than the LOC. For scenarios in which RQs could not be calculated (*e.g.,* when endpoints are nondefinitive), EPA compared the estimated environmental concentrations (EECs) of difenoconazole with the appropriate toxicological endpoint. For birds and mammals, the acute and chronic LOCs are 0.5 and 1.0 respectively; for plants the LOC is 1.0. For bees, the acute and chronic LOCs are 0.4 and 1.0, respectively.

As established in the Overview document[20], the Agency's risk assessments for all taxa rely on a surrogate species approach where one or a few tested species are used to represent the potential sensitivity for all species of a taxon. For example, fish serve as the surrogates for aquatic-phase amphibians and reptiles, daphnia and mysids serve as surrogates for aquatic invertebrates, birds as surrogates for terrestrial phase amphibians and reptiles, the laboratory rat is a surrogate for mammals, and honey bees may serve as surrogates for terrestrial invertebrates. If toxicity data

[19] The 2020 ERA only addresses potential risks to species not listed under the Endangered Species Act. EPA is working with its federal partners and other stakeholders to implement a Revised Method (EPA-HQ-OPP-2019-0185-0054) for assessing potential risk to listed species and their designated critical habitats. The Agency will complete difenoconazole's listed-species assessment once EPA has fully implemented the scientific methods necessary to complete listed species' risk assessments. For more details, see Appendix C.

[20] USEPA. 2004. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs; January 23, 2004. Environmental Fate and Effects Division. Office of Pesticide Programs. U.S. Environmental Protection Agency. Available at https://www.epa.gov/sites/production/files/2014-11/documents/ecorisk-overview.pdf)

are available in the open literature (*i.e.,* on amphibians), these data may be used instead of data on the surrogate species.

Given the uses of difenoconazole, the available ecotoxicity data, and environmental fate properties, there are potential risks of concern for fish (from chronic exposure), aquatic invertebrates (from acute and/or chronic exposure), birds (from chronic exposure), and mammals (from chronic exposure). Risks are considered low for honey bees, aquatic plants, and terrestrial plants, despite minor LOC exceedances for honey bees and aquatic plants.

Terrestrial Risks

Mammals

Foliar Applications

No acute risks of concern were identified for mammals for any registered uses of difenoconazole. Acute dose-based endpoints did not exceed mammalian non-listed species LOCs at the maximum single application rate of difenoconazole (RQs < 0.07). Subacute dietary toxicity data were not available for mammals; therefore, risks from this route of exposure could not be evaluated.

Chronic risks of concern for mammals were identified from foliar applications and seed treatment uses. The chronic endpoint for mammals was derived from a 2-generation reproduction study in rats. The most sensitive endpoint (no observable adverse effect concentration [NOAEC]=250 mg a.i./kg-diet; lowest observable adverse effect concentration [LOAEC]=2,500 mg a.i./kg-diet) was based on a decrease (≥ 10%) in body weight observed in parental females and first-generation males and females and decreased pup weight that progressed over time (6-8% at birth to 29-33% at weaning).

On a chronic basis, no dietary-based risks of concern were identified (RQs ranged from 0.004-0.96). Risks of concern were identified on a dose-basis only. Dose-based risk estimates account for the fact that different sized animals need to consume different proportions of food relative to body size. For a single application, there were risks of concern at the maximum single application rate for non-agricultural uses (turf, 0.43 lbs a.i./A) for small mammals consuming short and tall grass and broadleaf plants/small insects, and medium and large mammals consuming short grass (RQs ranged from 0.01-2.2, LOC=1). No chronic LOC exceedances were reported for single applications on agricultural use sites.

For multiple applications, at the lowest single application rate (pome fruit, 0.0684 lbs a.i./A x 3 applications x 14-day application interval), risks of concern were identified for small and medium-sized mammals consuming short grass only (RQs ranged from <0.01-1.4, LOC=1). For multiple applications at the maximum agricultural application rate (citrus, 0.125 lbs a.i./A x 4 applications x 7-day application interval), risks of concern were identified for small and medium mammals consuming short grass and broadleaf plants/small insects (RQs ranged from 0.01-2.1, LOC=1). For multiple applications at the maximum overall application rate for non-agricultural applications (turf, 0.43 lbs a.i./A x 3 applications x 14-day application interval), there were risks

of concern for small, medium and large mammals consuming short grass, tall grass, broadleaf plants/small insects and arthropods (RQs ranged from 0.03-5.2, LOC=1). Additional lines of evidence for chronic risks to mammals include: 1) LOC exceedances using mean estimates of dietary consumption for multiple applications, and 2) for single applications, dose-based LOC exceedances occurred 0-41 days after application and, for multiple applications, 0 to >56 days after application.

Seed Treatments

Chronic risks of concern were identified for mammals ingesting difenoconazole-treated seeds (all seed treatment application rates except seed potato) (RQs up to 1.7, LOC=1). The number of seeds (except seed potato) required to reach the mammalian chronic LOC for seed treatment uses based on the NOAEC ranged from 11 (small mammals consuming corn seed) to 12,893 seeds (large mammals consuming rapeseeds). Chronic exposure from difenoconazole seed treatment has some inherit uncertainties in that actual ingestion depends on the number of seeds available at the ground surface and how closely they are planted to each other. Once seeds are planted at depth (*e.g.,* potato chunks), the main exposure route would be limited to spillage. The actual difenoconazole exposure is additionally dependent on foraging behavior, seed size, and timing of consumption.

Off-Field Risks

Risks of concern to mammals from spray drift extends up to 3.3 feet from the edge of the field from the use site (the "effects distance," based on applications to turf at 0.43 lbs a.i./A x 3 applications x 14-day application interval: using fine to medium droplet sizes for aerial and ground applications). In general, applications with larger droplet sizes and lower boom heights have lower effects distances; aerial applications generally have greater drift potential than ground applications.

Birds (Surrogates for Reptiles, and Terrestrial-Phase Amphibians)

No acute risks of concern were identified for birds for the any registered use of difenoconazole. Acute oral dose-based exposure RQs were not calculated for birds, since definitive endpoints are not available. No avian mortalities were reported at concentrations tested in acute oral studies in canary and mallard ducks; LD_{50} values (lethal dose which caused death in 50% of the test animals) were greater than 2,000 mg a.i./kg body weight (bw). Based on EECs for a single application at the maximum application rate, there was no evidence that residues exceeded the tested concentrations. On a subacute dietary basis, RQs (<0.01-0.06) did not exceed the acute risk LOC (0.5). Overall, there were no risks of concern on a subacute dietary and acute dose basis for birds.

Chronic risks of concern were identified for birds from foliar application and seed treatment uses. The most sensitive chronic endpoint (NOAEC=21.8 mg a.i./kg-diet; LOAEC=108 mg a.i./kg-diet) for birds was selected from a study with bobwhite quail, in which a 4% reduction in hatchling body weight was observed.

On-field Chronic Risks from Foliar Applications

Overall, chronic dietary risks of concern were identified for birds for both single and multiple applications of difenoconazole. For single applications, chronic dietary-based RQs (0.09-4.7) exceeded the LOC of 1.0 for birds for applications to turf (birds foraging on short grass, tall grass, broadleaf plants/small insects and arthropods) and citrus (short grass only). There were no LOC exceedances for single applications to pome fruits (minimum application rate). For multiple applications, chronic dietary-based RQs (0.18-11) exceeded the LOC for applications to pome fruits, citrus and turf for all foraging groups (with the exception of fruits/pods and seeds). Additional evidence for potential chronic risk to birds included: 1) exceedances at the mean estimates of dietary consumption (RQs up to 3.9), 2) exceedances at the LOAEC for multiple applications to turf resulted in exceedances of the LOC (chronic RQs up to 2.2), and 3) exposure estimates exceeded the LOC for up to 80 days for single applications and up to 150 days for multiple applications.

Chronic Risks from Seed Treatments

Chronic risks of concern to birds from difenoconazole-treated seeds were identified for all seed treatment uses (RQs 1.1-16). The number of seeds required to reach the avian chronic LOC for seed treatment uses based on the NOAEC ranged from 4 (small birds consuming barley seed) to 2,166 seeds (large birds consuming rapeseeds). Despite an estimated consumption of only 1-3 potato seeds necessary to exceed the LOC (large birds), potatoes are planted at depth (4-8 inches) and any surface-available seed pieces would most likely be due to potential spillage. RQs also exceeded chronic LOCs at the avian LOAEC (108 mg a.i./kg-diet) for all labeled uses except potatoes.

Off-Field Risks

Risks of concern to birds from spray drift extends up to 29.5 feet from the application site (single application at 0.125 lbs a.i./A to turf; assuming fine to medium droplets with aerial application).

Terrestrial Invertebrates

Difenoconazole is registered for foliar application use on a suite of crops that are pollinator-attractive (*e.g.*, tree nuts, ornamentals, citrus, and fruiting vegetables) including several that require the use of managed pollinators. To understand the risks posed by pesticides, EPA relies on data about honey bees as a surrogate for terrestrial invertebrate species. Despite its use on pollinator-attractive crops, based on the available data and representative exposure scenarios, EPA has concluded that difenoconazole uses do not present risks of concern to honey bees.

Difenoconazole is absorbed by leaves and is then distributed within plant tissue by translaminar movement. Acute and chronic Tier I data on technical grade active ingredient (TGAI) difenoconazole are available for adult honey bees; however, for larval honey bees only chronic toxicity data are available. Two additional chronic toxicity studies were conducted on honey bee adults and larvae using a typical end-use product (TEP) of difenoconazole. For adult honey bees, there were no acute or chronic risks of concern at both the minimum and maximum

difenoconazole application rates for TEP- and TGAI-based endpoints. No larval acute toxicity data are available for TGAI; however, the 8-day LD_{50} from the 22-day larval chronic study is approximately 10 times greater than the estimated maximum dietary dose and, therefore, results in an RQ which is below the acute risk LOC of 0.4 and is not of concern. For the TEP data, acute RQs for larvae are of concern (RQs up to 0.99; LOC=0.4). On a chronic exposure basis for larval bees, there are no risks of concern for the TGAI-based NOAEC; however, for TEP-based endpoints, there are LOC exceedances (RQ=1.35; LOC=0.4) at the maximum difenoconazole application rate.

Honey bee larvae may be exposed to the TEP following applications made during bloom and when pollen and nectar resources are collected soon after application and fed directly to larvae. It is assumed that this is not a common exposure scenario, when considering the likely timing of difenoconazole applications relative to bloom and that adult bees are likely to process nectar and pollen prior to feeding to bees. Since the proportion of the components of the formulation are expected to change by the time difenoconazole reaches larvae, it is assumed that the TGAI toxicity data are more representative of the exposure larvae will encounter. Therefore, risks to honey bee larvae are considered low. Given the lack of acute or chronic risks of concern for difenoconazole based on Tier I data and current registered uses, additional higher-tier toxicity and/or exposure data are not needed at this time.

Terrestrial Plants

There are no risks of concern for terrestrial plants.

Aquatic Risks

Difenoconazole is persistent in terrestrial and aquatic environments. The Agency has concluded that repeated use of difenoconazole can increase chronic risks significantly over time.

Freshwater and Estuarine/Marine Fish (Surrogates for Aquatic-Phase Amphibians)

Difenoconazole has similar acute toxicity to both freshwater and estuarine/marine fish. There were no acute risk concerns identified for fish (RQs $\leq$ 0.14, acute LOC=0.5). Due to the lack of available chronic toxicity studies on the most acutely sensitive freshwater fish species (rainbow trout), chronic fish endpoints were estimated using an ACR (acute to chronic ratio). Chronic risks to freshwater fish were identified at the ACR-based NOAEC (0.86 µg a.i./L; (RQs ranged from 0.7-22.2, chronic LOC=1). Additional evidence for potential chronic risks of concern included LOC exceedances based on empirical data for the less sensitive fish species (fathead minnow; NOAEC=1.9 µg a.i./L and LOAEC=3.7 µg a.i./L based on a 5% reduction in male length in F0 generation post-hatch) at both the NOAEC (RQs ranged from 0.4 to 16) and LOAEC (RQs ranged from 0.2-8.1). No empirical data were available for estuarine/marine fish; however, the ACR estimated-NOAEC for estuarine/marine fish was identical to freshwater fish; therefore, both RQs and risk conclusions were the same.

Freshwater and Estuarine/Marine Invertebrates

Risks of concern for water column invertebrates were estimated using the most sensitive acute and chronic endpoints for freshwater invertebrates (daphnid acute LC_{50}=770 µg a.i./L; chronic daphnid NOAEC = 5.6 µg a.i./L) and estuarine/marine invertebrates (acute mysid LC_{50} = 150 µg a.i./L and chronic mysid NOAEC= 4.8 µg a.i./L) respectively. There are no acute risks of concern associated with the terrestrial uses of difenoconazole for freshwater water-column aquatic invertebrates (RQs ranged from <0.01-0.14; acute LOC =0.5). However, for estuarine/marine water-column invertebrates there was an acute risk LOC exceedance identified for rice applications (RQ=0.74; acute LOC = 0.5).

Chronic risks of concern were identified for aquatic invertebrates. Chronic RQs based on the NOAEC for both freshwater and estuarine/marine invertebrates exceeded the LOC of 1.0 Chronic RQs for freshwater invertebrates ranged from 0.12-5.4, with the rice use at the high-end of that range. In addition, chronic risks of concern were identified for freshwater invertebrates for a suite of uses including potatoes, cabbage, peppers, cucumbers, citrus, tomatoes, melon, grapes, nurseries and turf. There were chronic risk LOC exceedances for the scenarios with the highest EECs (*i.e.,* potatoes, rice and grapes). For estuarine/marine invertebrates in the water column, RQs followed the same pattern, with rice at the high-end (RQs ranged from 0.12-6.3, chronic LOC=1). Chronic risks of concern were identified for estuarine/marine invertebrates for the aforementioned crop groups and apples. There were limited LOC exceedances at the LOAECs for aquatic invertebrates in the water column for those scenarios with the highest EECs (including rice, potatoes, grapes, and cherries) with RQs ranging up to 3.0.

Chronic endpoints for benthic invertebrates were based on reductions in the number of offspring (up to a 47% reduction). For benthic invertebrates, there were no sediment-based exposures resulting in risks of concern (RQs ranged from <0.01- 0.8; LOC = 1.0). Risks of concern for freshwater benthic invertebrates from pore water exposure were identified for a subset of uses including potatoes, tomatoes, cherries, grapes, and rice (RQs ranged from 0.08-1.9; LOC=1). There were no risks of concern identified for estuarine/marine benthic invertebrates.

Aquatic Vascular and Non-Vascular Plants

No risks of concern were identified for aquatic non-vascular plants for terrestrial agricultural and non-agricultural uses (RQs ranged from < 0.01-0.15; LOC=1.0). However, RQs for the semi-aquatic agricultural use on rice (with the exception of rice in Mississippi) exceeded the LOC (rice RQs ranged from 0.78-1.1). Overall, risk to non-vascular plants is considered low.

No toxicity studies were available for vascular aquatic plants with difenoconazole TGAI. Therefore, risks to vascular aquatic plants remain uncertain. Given the generally low risks for non-vascular aquatic plants and terrestrial plants, it is likely that there is a low-risk potential to aquatic vascular plants.

2. Ecological Incidents

The Incident Data System (IDS) and the Agency's Aggregated Incidents Reports database were reviewed for difenoconazole incidents on May 19, 2020. The search excluded incidents classified as "unlikely" or "unrelated" and only included incidents with the certainty categories of

"possible" and "probable." Overall, there were 4 possible incidents, 4 unlikely incidents, and one that was unclassified. Three incidents are reported in IDS for damage (necrotic browning and death) to wine grapes in New York, all with a certainty of "possible" for difenoconazole as a causal agent. Two of the three incidents involved application of a fungicide containing both mandipropamid and difenoconazole and the other incident involved a fungicide containing difenoconazole and cyprodinil.

In addition to the incidents recorded in IDS, additional incidents are reported to the Agency in aggregated form. For difenoconazole, there were 10 minor plant incidents associated with a fungicide containing mandipropamid and difenoconazole and one minor plant incident associated with a fungicide containing azoxystrobin and difenoconazole. A single aggregate minor wildlife incident was reported and was associated with a formulation containing sedaxane, difenoconazole, fludioxonil, mefenoxam, and thiamethoxam. The frequency and severity of the difenoconazole incidents are consistent with the conclusions drawn from the laboratory data. The Agency intends to conduct ongoing ecological incident monitoring for difenoconazole.

3. Ecological and Environmental Fate Data Needs

Both the environmental fate and ecological effects datasets are considered complete. Given the lack of acute or chronic risks of concern to honey bees from difenoconazole based on Tier I data and current registered uses, the Agency does not plan to require additional honey bee higher-tier toxicity and/or exposure data at this time.

C. Benefits Assessment

Difenoconazole is a systemic fungicide with protective, curative, and eradicative properties against plant diseases (FRAC, 2021). Preventative properties inhibit fungal spore germination and establishment of fungal pest infection whereas curative and eradicative properties kill fungal pathogens that have already infected plant tissues.

Foliar Applications to Agricultural Crops

Difenoconazole foliar applications on soybean control leaf frogeye spot and *Cercospora* spot (Kynetec, 2020). On almonds, difenoconazole is used for controlling brown blossom rot, blossom blight, shot hole, anthracnose (Kynetec, 2020, Gemperle *et al.*, 2018; Adaskaveg *et al.*, 2017); and on sugar beet, difenoconazole co-formulated with propiconazole has excellent activity against *Cercospora* leaf spot and powdery mildew (Kynetec, 2020; Tedford *et al.*, 2011).

Difenoconazole also is used for controlling early blight disease on potato, scab disease and powdery mildew on apple, and various fungal pests on crops such as cucumber, grapes, pumpkin, squash, tomato, and watermelon.

Post-Harvest Use

Difenoconazole is used for post-harvest treatments of apples and pears for fungal pests such as blue mold caused and gray mold causing fruit rot during storage (Amiri, 2021). Infected fruits

may cause reductions in marketable yields of apple and pears if fruits are not treated with difenoconazole.

Seed Treatment

Difenoconazole provides broad-spectrum and systemic control of important seed and soil-borne diseases of many crops. Prior to planting, small grain seeds (barley, oats, rye, triticale and wheat) and other crop seeds (such as cotton, potato, canola, corn) are treated with difenoconazole to control seed and soil-borne fungal pests (such as *Fusarium* spp., *Tilletia tritici*, *Ustilago* spp., *Urocystis* spp.) (Smiley *et al.*, 2002). Seed potatoes also are treated with difenoconazole for controlling soil-borne fungal diseases (Johnson, 2019).

Ornamentals and Turf

On roses and other ornamentals (such as gerbera, ranunculus, petunia, and hydrangea), difenoconazole is used to control powdery mildew (Minuto *et al.*, 2018; Kumar, 2018). On turf, difenoconazole is used against multiple fungal pests (such as *Rhizoctonia* spp., *Sclerotinia homoeocarpa*, *Bipolaris* spp., *Lycoperdon* spp., *Agrocybe pediades*, *Bovistra plumbea*, *Puccinia* spp., *Erysiphe graminis*) (Clarke, 2020; Syngenta, 2018). Fungal pests can reduce yields and quality and decrease aesthetic value of ornamentals and turf.

IV. INTERIM REGISTRATION REVIEW DECISION

A. Risk Mitigation and Regulatory Rationale

The Agency has reviewed the risks, benefits, and uses of difenoconazole in formulating its decision. As discussed in Section II of this document, no human health risks of concern have been identified for difenoconazole when used as directed on current labels. Difenoconazole poses potential ecological risks of concern from its labeled uses. EPA identified potential risks of concern to mammals, birds, terrestrial invertebrates, freshwater and estuarine/marine fish, and aquatic invertebrates.

While no risks of concern have been identified for occupational handlers, the Agency is requiring that labels of difenoconazole products that already require the use of gloves be amended to specify the appropriate glove type. The Agency is adding soil incorporation instructions for treated seeds and new labeling for treated seed containers to prevent the ingestion of treated seeds by nontarget organisms. The Agency is also adding use restrictions for foliar rice applications in order to prevent potential exposure to aquatic organisms. Additionally, the Agency is also implementing consistent advisory spray drift management and pesticide resistance management labeling and updated advisory and environmental hazard language aimed at reducing the potential exposure of difenoconazole in the environment.

4. Updated Glove Label Language

The Agency is updating the glove statements currently on labels that already require the use of gloves by handlers, to be consistent with the Label Review Manual. In particular, EPA is removing any references to specific categories in EPA's chemical-resistance category selection chart and specifying the appropriate types of gloves.[21] This clarification does not fundamentally change the personal protective equipment (PPE) that workers currently must use, and therefore should impose no impacts on users.

5. Soil Incorporation of Treated Seed

The Agency identified potential chronic avian and mammalian risks of concern from ingestion of difenoconazole-treated seeds. Birds and mammals may have increased access to treated seeds on the soil surface relative to seed that is covered or incorporated into the soil. Most seed treatment products containing difenoconazole are co-formulated with other pesticides that require that seeds be planted at depth. Currently, some labels of seed treatment products for barley, canola, cotton, oats, potato, rye, sweet corn, wheat, and triticale in which difenoconazole is the only active ingredient do not require soil incorporation of treated seeds. The Agency is adding soil-incorporation instructions for all difenoconazole-treated seed products, and other measures to reduce access to spilled seed.

The Agency has determined it is necessary that difenoconazole-treated seed be planted to specific depths depending on seed type to remove the need for workers to follow the default REI after treated seeds are planted and to reduce potential exposure to non-target birds and mammals. The depth of planting for each seed type is consistent with established agronomic practice and is not expected to impose new impacts on users. The REI and the WPS exceptions will remain on the product labels.

- For canola and rapeseed crop subgroup 20A (borage, crambe, cuphea, echium, flax seed, gold of pleasure, hare's ear mustard, lesquerella, lunaria, meadowfoam, milkweed, mustard seed, oil radish, poppy seed, rapeseed, sesame, and sweet rocket): Treated seed must be planted into the soil at a depth of at least ½ inch.
- For cotton, small grain cereals (barley, oats, rye, triticale, and wheat), and sweet corn: Treated seed must be planted into the soil at a depth of at least 1 inch.
- For potato: Treated seed must be planted into the soil at a depth of at least 2 inches.

6. Seed Treatment Label Instructions

In addition to the soil incorporation instructions above, the Agency is standardizing seed treatment label instructions to include a number of elements designed to:

- identify the pesticide used to treat the seed and clarify the rate at which the seed has been treated,
- limit access to or potential contamination from the treated seed,
- implement PPE when handling treated seed, and
- address the disposition of spilled and excess seed and waste from the planting process.

[21] For specific label language, see Appendix B.

The new seed treatment labeling is consistent with good management practices and is not expected to impart economic impacts on users.

On-Farm Treated Seed that are Not Sold or Distributed

The new seed treatment labeling is as follows for pesticide products intended to be used on-farm to treat seeds:

- "Store treated seed away from food and feedstuffs.
- Do not allow children, pets, or livestock to have access to treated seeds.
- When loading/pouring the treated seed/seed-pieces or covering/collecting spilled seed during loading and planting, wear long-sleeved shirt, long pants, shoes, socks, and chemical resistant gloves [list specific glove types].
- Treated seeds exposed on the soil surface may be hazardous to wildlife. Cover or collect treated seeds spilled during loading and planting (such as in row ends).
- Dispose of all excess treated seed by burying seed away from bodies of water.
- Do not contaminate bodies of water when disposing of planting equipment wash water.
- Plant treated seed into the soil to the recommended minimum depth or greater to minimize exposure.
- DO NOT plant treated seed by broadcasting to the soil surface. Ensure that all planted seeds are thoroughly incorporated by the planter during planting, additional incorporation may be required to thoroughly cover exposed seeds.
- Dispose of seed packaging or containers in accordance with local requirements."

Commercial Seed Treatments and On-Farm Treated Seeds that are Distributed or Sold

The Agency has determined it is necessary that labels of products containing difenoconazole that are registered for commercial seed treatment or on-farm treated seeds that are distributed or sold are required to add comporting statements directing seed treatment facilities to include the following language on containers of treated seed:

"Seed treated with [PRODUCT NAME] must be labeled in accordance with all applicable requirements of the Federal Seed Act. All bags containing treated seed must be labeled or tagged as follows. Any seed treated with difenoconazole that is sold or distributed without these statements is an unregistered pesticide, in violation of FIFRA section 12."

"The Federal Seed Act requires that bags containing treated seeds shall be labeled with the following statement(s):

- This seed has been treated with (insert name of active ingredient of pesticide).
- Do not use for food, feed or oil purposes."

"The U.S. Environmental Protection Agency requires that bags containing treated seeds shall be labeled with the following statements:

- This seed has been treated with [INSERT PRODUCT NAME(s), EPA REG. NO(s)), a [INSERT CHEMICAL TYPE(S)] containing [INSERT ACTIVE INGREDIENT(S)].
- This seed is treated with [number] lbs of difenoconazole per 100 lbs of seed.
- Do not use treated seed for feed, food, or oil purposes.
- Store treated seed away from food and feedstuffs.
- Do not allow children, pets, or livestock to have access to treated seeds.
- When loading/pouring the treated seed/seed-pieces or covering/collecting spilled seed during loading and planting, wear long-sleeved shirt, long pants, shoes, socks, and chemical-resistant gloves [list specific glove types].
- Treated seeds exposed on the soil surface may be hazardous to wildlife. Cover or collect treated seeds spilled during loading and planting (such as in row ends).
- Dispose of all excess treated seed by burying seed away from bodies of water.
- Do not contaminate bodies of water when disposing of planting equipment wash water.
- Plant treated seed into the soil to the recommended minimum depth or greater to minimize exposure.
- Do not plant treated seed by broadcasting to the soil surface. Ensure that all planted seeds are thoroughly incorporated by the planter during planting, additional incorporation may be required to thoroughly cover exposed seeds.
- Dispose of seed packaging or containers in accordance with local requirements.
- Excess treated seed may be used for ethanol production it (1) by-products are not used for livestock feed and (2) no measurable residues of pesticide remain in ethanol by-products that are used in agronomic practice."

"All other requirements regarding the use of the treated seed, which include, but are not limited to, instructions relating to endangered species protection, environmental hazard statements, maximum use rates, plant back intervals, personal protective equipment, and storage and disposal statements, remain and must be listed on the bag tag."

7. Use Restrictions for Foliar Rice Application

In order to address potential chronic risks to aquatic organisms, the Agency is adding use restrictions for foliar rice applications to flooded fields by adding the following instructions on product labels:

- "Do not treat fields simultaneously used for aquaculture of fish or crustaceans.
- Do not drain water from treated rice fields into ponds used for aquaculture of fish and crustaceans.
- Do not use water drained from treated fields to irrigate other crops.
- Do not allow release of irrigation or flood water for at least 7 days after the last application.
- Do not apply when weather conditions favor drift from treated areas to non-target aquatic habitat."

These restrictions are consistent with good management practices and are not expected to impose economic impacts on users.

8. Standardize Environmental Hazard Language

The Agency is updating and standardizing existing environmental hazard warnings to be consistent across all product labels and to warn users of potential exposure and risk to aquatic organisms. The Agency is requiring that the following statements be included on all product labels under the Environmental Hazard section:

> *Drift and Runoff Warning:*
> "Drift and runoff may be hazardous to aquatic organisms in water adjacent to treated areas."
>
> *Terrestrial Use Environmental Hazard Statement:*
> "Do not apply directly to water or to areas where surface water is present or to intertidal areas below the mean high-water mark. Do not contaminate water when disposing of equipment washwater or rinsate."

The Agency is adding the following surface water advisory on all agricultural product labels under the Environmental Hazard section:

> "This product may impact surface water quality due to runoff of rain water. This is especially true for poorly draining soils and soils with shallow groundwater. This product is classified as having a high potential for reaching surface water via runoff for several months or more after application.
>
> A level, well-maintained vegetative buffer strip between areas to which this product is applied and surface water features such as ponds, streams, and springs will reduce the potential loading of difenoconazole from runoff water and sediment. Runoff of this product will be reduced by avoiding applications when rainfall or irrigation is expected to occur within 48 hours. Sound erosion control practices will reduce this product's potential to reach aquatic sediment via runoff."

The Agency is adding the following statement on all non-agricultural product labels under the Environmental Hazard section:

> "This product may impact surface water quality due to runoff of rain water. This is especially true for poorly draining soils and soils with shallow groundwater. This product is classified as having a high potential for reaching surface water via runoff for several months or more after application."

These updates are advisory and are not expected to impose economic impacts on users.

9. Fungicide Resistance Management

The Agency is implementing resistance-management language to difenoconazole labels[22] to address fungicide resistance.[23] Fungicide resistance occurs when genetic or behavioral changes enable a portion of a pest population to tolerate or survive what would otherwise be lethal doses of a given pesticide. The development of such resistance is influenced by a number of factors. One important factor is the repeated use of pesticides with the same mode (or mechanism) of action. This practice kills sensitive pest individuals but allows less susceptible ones in the targeted population to survive and reproduce, thus increasing in numbers. These individuals will eventually be unaffected by the repeated pesticide applications and may become a substantial portion of the pest population. An alternative approach, recommended by resistance management experts as part of integrated pest management (IPM) programs, is to use pesticides with different chemical modes (or mechanisms) of action against the same target pest population. This approach may delay and/or prevent the development of resistance to a particular mode (or mechanism) of action without resorting to increased rates and frequency of application, possibly prolonging the useful life of pesticides. EPA is adding resistance-management labeling, as listed in Appendix B, for products containing difenoconazole, in order to provide pesticide users with easy access to important information to help maintain the effectiveness of useful pesticides. Additional information on EPA's guidance for resistance management can be found at the following website: https://www.epa.gov/pesticide-registration/prn-2017-1-guidance-pesticide-registrants-pesticide-resistance-management. Adding this language will provide pesticide users with easy access to important information on maintaining the effectiveness of pesticides—including difenoconazole—thereby preserving the benefits of difenoconazole and other useful pesticides.[24]

The measures are consistent with best management practices and are not expected to impose economic impacts on users.

10. Advisory Spray Drift Management

The Agency is adding advisory spray drift management labeling to reduce off-target spray drift and establish a baseline level of protection against spray drift that is consistent across all difenoconazole products.[25] Reducing spray drift will reduce the extent of environmental exposure and risk to non-target plants and animals.

These updates are advisory and are not expected to impose economic impacts on users.

---

[22] For specific label language, see Appendix B.

[23] Pesticide resistance is the ability of portions of a pest population to tolerate or survive otherwise lethal doses of a pesticide through genetic or behavioral changes. EPA considers increased pesticide resistance an adverse effect that can drive increased use of pesticides. For more details, see PRN 2017-1 and PRN 2017-2, available at https://www.epa.gov/pesticide-registration/pesticide-registration-notices-year.

[24] For a detailed discussion of difenoconazole's benefits, see Section III.C, above.

[25] Reducing spray drift will decrease environmental exposure and risk to nontarget organisms. Although EPA is not making a listed species finding at this time, these label changes are expected to reduce the extent of exposure and may reduce risk to listed species whose range and/or critical habitat co-occur with the use of difenoconazole. For specific label language, see Appendix B.

B. Environmental Justice

EPA seeks to achieve environmental justice, the fair treatment and meaningful involvement of all people, regardless of race, color, national origin, or income, in the development, implementation, and enforcement of environmental laws, regulations, and policies. Throughout the registration review process, EPA has sought to include all communities and persons across the Nation, including minority, low-income, and indigenous populations who may be disproportionately overburdened by the use of difenoconazole.

One community which may experience disproportionate exposure to pesticides is agricultural farmworkers. EPA has conducted assessments of risks to farmworkers who handle difenoconazole or may be exposed to difenoconazole when working in treated fields and have found no risks of concern. EPA has also evaluated the risks to people living adjacent to treated fields, which may include many farmworker families, and found no risks of concern for difenoconazole. EPA has also evaluated risk to residential handlers (such as homeowners) and adults/children that may be exposed to residues after pesticide application and has not found risks of concern.

The Agency sought information during the public comment periods throughout registration review on any other groups or segments of the population who, as a result of their proximity and exposure to pesticides, unique exposure pathway (e.g., as a result of cultural practices), location relative to physical infrastructure, exposure to multiple stressors and cumulative impacts, lower capacity to participate in decision making, or other factors, may have unusually high exposure to difenoconazole compared to the general population or who may otherwise be disproportionately affected by the use of difenoconazole as a pesticide. EPA requested but did not receive any comments concerning environmental justice.

C. Tolerance Actions

As outlined in the ID, the Agency anticipates making several commodity definition revisions, removing trailing zeros to be consistent with OECD rounding class practice, and making changes to harmonize with established Canadian and Codex MRLs. Only the anticipated commodity definition and tolerance level revisions are presented below.

Summary of Anticipated Tolerance Revisions for Difenoconazole (40 CFR §180.475).			
Commodity/ **Correct Commodity Definition**	Established Tolerance (ppm)	Anticipated Revised Tolerance (ppm)	Comments
40 CFR §180.475(a)(1)			
Almond, hulls	7.0	7	Correct level to be consistent with OECD Rounding Class Practice.
Apple, wet pomace	--	30	Tolerance level increase to correct level to be consistent with OECD Rounding Class Practice.
Apple, wet pomace	25	Remove	
Beet, sugar, dried pulp	--	2	
Beet, sugar, dried pulp	1.9	Remove	
Bushberry subgroup 13-07B	4.0	4	Correct level to be consistent with OECD Rounding Class Practice.
Cottonseed subgroup 20C	0.40	0.4	

Summary of Anticipated Tolerance Revisions for Difenoconazole (40 CFR §180.475).			
Commodity/ **Correct Commodity Definition**	Established Tolerance (ppm)	Anticipated Revised Tolerance (ppm)	Comments
Cranberry	0.60	0.6	
Fruit, citrus, group 10-10	0.60	0.6	
Fruit, citrus, group 10-10, dried pulp	--	2	Commodity definition revision. Correct level to be consistent with OECD Rounding Class Practice.
Citrus, dried pulp	2.0	remove	
Fruit, citrus, group 10-10, oil	--	25	Commodity definition revision. Level not consistent with OECD Rounding Class Practice; however, retained due to harmonization with Canadian MRL.
Citrus, oil	25	remove	
Fruit, pome, group 11-10	5.0	5	Correct level to be consistent with OECD Rounding Class Practice.
Fruit, small, vine climbing, except fuzzy kiwifruit, subgroup 13-07F	3.0	3	
Grain, aspirated fractions	--	100	Commodity definition revision. Tolerance level increase to correct level to be consistent with OECD Rounding Class Practice.
Aspirated grain fractions	95	remove	
Grape, raisin	6.0	6	Correct level to be consistent with OECD Rounding Class Practice.
Guava	3.0	3	
Kohlrabi	2.0	2	
Onion, bulb, subgroup 3-07A	0.20	0.2	
Onion, green, subgroup 3-07B	6.0	6	
Papaya	0.60	0.6	
Pea and bean, dried shelled, except soybean, subgroup 6C	0.20	0.2	
Pea, field, forage	--	10	Commodity definition revision.
Pea, field, vines	10	remove	
Potato, wet peel	--	8	Tolerance level increase to correct level to be consistent with OECD Rounding Class Practice.
Potato, wet peel	7.3	remove	
Rapeseed subgroup 20A	0.10	0.1	Correct level to be consistent with OECD Rounding Class Practice.
Rice, grain	--	8	Tolerance level increase to harmonize with Codex MRL which is based on same data used for U.S. tolerance.
Rice, grain	7.0	remove	
Rice, wild, grain	7.0	7	Correct level to be consistent with OECD Rounding Class Practice.
Soybean, hulls	0.20	0.2	
Vegetable, *Brassica*, head and stem, group 5-16	2.0	2	
Vegetable, cucurbit, group 9	0.70	0.7	
Vegetable, fruiting, group 8-10	0.60	0.6	
Vegetable, tuberous and corm, subgroup 1C	4.0	4	
40 CFR §180.475(a)(2)			
Cattle, fat	0.10	0.1	Correct level to be consistent with OECD Rounding Class Practice.
Cattle, meat byproduct	--	1.5	Commodity definition revision.

Summary of Anticipated Tolerance Revisions for Difenoconazole (40 CFR §180.475).			
Commodity/ **Correct Commodity Definition**	Established Tolerance (ppm)	Anticipated Revised Tolerance (ppm)	Comments
Cattle, liver	0.7	remove	Tolerance level increase to harmonize with Codex MRL for edible offal (mammalian).
Cattle, meat byproduct (except liver)	0.10	remove	
Egg	0.02	0.03	Tolerance level increase to harmonize with Codex MRL
Goat, fat	0.10	0.1	Correct level to be consistent with OECD Rounding Class Practice.
Goat, meat byproduct	--	1.5	Commodity definition revision. Tolerance level increase to harmonize with Codex MRL for edible offal (mammalian).
Goat, liver	0.7	remove	
Goat, meat byproduct (except liver)	0.10	remove	
Hog, fat	0.10	0.1	Correct level to be consistent with OECD Rounding Class Practice.
Hog, meat byproduct	--	1.5	Commodity definition revision. Tolerance level increase to harmonize with Codex MRL for edible offal (mammalian).
Hog, liver	0.40	remove	
Hog, meat byproduct (except liver)	0.10	remove	
Horse, fat	0.10	0.1	Correct level to be consistent with OECD Rounding Class Practice.
Horse, meat byproduct	--	1.5	Commodity definition revision. Tolerance level increase to harmonize with Codex MRL for edible offal (mammalian).
Horse, liver	0.7	remove	
Horse, meat byproduct (except liver)	0.10	remove	
Sheep, fat	0.10	0.1	Correct level to be consistent with OECD Rounding Class Practice.
Sheep, meat byproduct	--	1.5	Commodity definition revision. Tolerance level increase to harmonize with Codex MRL for edible offal (mammalian).
Sheep, liver	0.7	Remove	
Sheep, meat byproduct (except liver)	0.10	Remove	

D. Interim Registration Review Decision

The Agency is issuing this ID in accordance with 40 C.F.R. §§ 155.56 and 155.58. The Agency has made the following interim decision: (1) no additional data are required at this time; and (2) EPA has determined that difenoconazole does not meet the registration standard without changes to the affected registrations and their labeling. EPA has determined that the mitigation specified in Sections IV.A-C and Appendices A and B are sufficient to address certain concerns.

The Agency conducted detailed human health and ecological risk assessments. EPA did not identify any human health risks of concern from use of difenoconazole. EPA identified potential risk to fish, aquatic invertebrates, mammals, birds, and terrestrial invertebrates from use of difenoconazole. To address potential risks to birds and mammals from ingestion of treated seed, the Agency is adding soil incorporation labeling and directions to limit access to spilled seed and address the disposition of spilled seed for seed treatment products. To warn users of potential risk to aquatic animals, the Agency is adding water advisories on product labels. Additional label updates include pesticide resistance management labeling and updated environmental hazard

statements aimed at educating the user of potential exposure of difenoconazole to the environment.

EPA also determined that continuing to register difenoconazole provides benefits when used as a foliar application to agricultural crops, ornamentals, and turf, during post-harvest processing, as a seed treatment. As a foliar application, difenoconazole is used for controlling various fungal pests on soybean, almonds, sugar beets, potato, apple, and other crops. Use of difenoconazole on roses and other ornamentals, as well as turf, controls fungal pests that can otherwise reduce yields, quality, and decrease aesthetic value. Difenoconazole is used for post-harvest treatments of apples and pears to avoid fruit rot during storage. Infected fruit may cause a reduction in marketable yields. As a seed treatment, difenoconazole prevents fungal seed decay and seedling infections that may result in yield loss for crops such as barley, oats, rye, cotton, potato, corn.

During registration review, EPA considers whether a pesticide registration "continues to satisfy the FIFRA standard for registration."[26] Here, EPA has determined that difenoconazole does not meet the FIFRA registration standard without the changes to the affected registrations and their labeling described in Section IV.A and Appendices A and B.

EPA has determined that there is no human health dietary risk from registered uses of difenoconazole that is inconsistent with the FFDCA safety standard. Taking into consideration the available information on toxicity and exposure, EPA assessed difenoconazole's potential aggregate risks, including dietary (food and water) and non-occupational residential exposures, and found no risks exceeding the Agency's levels of concern.[2]

EPA has determined that there is a reasonable certainty that no harm will result from aggregate exposure to difenoconazole, including all anticipated dietary exposures and all other exposures for which there is reliable information. Therefore, difenoconazole residues do not present human dietary risk. EPA intends to revise certain commodity definitions, remove trailing zeros to be consistent with current rounding class practices, and modify certain tolerances to harmonize with established Canadian and Codex MRLs, as EPA's analysis indicates that such modifications would also be safe.

In this Interim Registration Review Decision, the Agency is making no human health or environmental safety findings associated with the EDSP screening of difenoconazole, nor is it making a complete endangered species finding. Although the Agency is not making a complete endangered species finding at this time, the mitigation described in this document is expected to reduce the extent of environmental exposure and may reduce risk to listed species whose range and/or designated critical habitat co-occur with the use of difenoconazole. The Agency's final registration review decision for difenoconazole will be dependent upon the result of the

---

[26] 40 C.F.R. § 155.40(a); 7 U.S.C. § 136a(c)(5); *see also* 7 U.S.C. §§ 136(bb) (defining "unreasonable adverse effects on the environment" as encompassing both "any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide" [FIFRA's risk-benefit standard] **and** "a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the [FFDCA safety standard]").

[2] *Difenoconazole: Human Health Risk Assessment for Registration Review.* Can be found in the public docket (EPA-HQ-OPP-2015-0401).

Agency's ESA assessment and any needed § 7 consultation with the Services and an EDSP FFDCA § 408(p) determination. For more information, see Appendices C and D.

E. Data Requirements

A Generic Data Call-In (GDCI-128847-1602) was issued for difenoconazole for data needed to conduct the registration review risk assessments. All data requirements from the registration review DCI have been satisfied and no additional data are required at this time.

V. NEXT STEPS AND TIMELINE

A. Interim Registration Review Decision

A Federal Register Notice will announce the availability of this Interim Registration Review Decision for difenoconazole. A final decision on the difenoconazole registration review case will occur after: (1) an EDSP FFDCA § 408(p) determination, and (2) an endangered species determination under the ESA and any needed § 7 consultation with the Services.

Implementation of Label Changes

Registrants must submit a cover letter, a completed Application for Registration (EPA form 8570-1) and electronic copies of the amended product labels within 60 days after the announcement of this ID in the *Federal Register*. Two copies for each label must be submitted, a clean copy and an annotated copy with changes. In order for the application to be processed, registrants must include the following statement on the Application for Registration (EPA form 8570-1):

"I certify that this amendment satisfies the requirements of the *Difenoconazole Interim Registration Review Decision* and EPA regulations at 40 CFR Section 152.44, and no other changes have been made to the labeling of this product. I understand that it is a violation of 18 U.S.C. Section 1001 to willfully make any false statement to EPA. I further understand that if this amendment is found not to satisfy the requirements of the *Difenoconazole Interim Registration Review Decision* and 40 CFR Section 152.44, this product may be in violation of FIFRA and may be subject to regulatory and/or enforcement action and penalties under FIFRA."

Within the required timeframe, registrants must submit the required documents to the Registration Review section of the EPA's Pesticide Submission Portal (PSP), which can be accessed through the EPA's Central Data Exchange (CDX) using the following link: https://cdx.epa.gov/. Registrants may instead send paper copies of their amended product labels, with an application for a fast-track, Agency-initiated non-PRIA label amendment to Lauren Weissenborn at the following address, so long as the labels and application are submitted within the required timeframe:

VIA US Mail

VI. REFERENCES

Adaskaveg, JE., Gubler, WD., Duncan, RA., Stapleton, JJ., Holtz, BA and Trouillas, FP. 2017. US-IPM Almond shot hole. Accessed on April 21, 2021. https://www2.ipm.ucanr.edu/agriculture/almond/shot-hole/

Amiri, A, 2021. Strategies to minimize postharvest rots of apple and pear. Accessed on April 23, 2021. http://treefruit.wsu.edu/article/strategies-to-minimize-postharvest-rots-of-apple-and-pear/

FRAC. 2021. Fungicide Resistance Action Committee (FRAC) Code List. Accessed on April 14, 2021. https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2021--final.pdf?sfvrsn=f7ec499a_2

Gemperle, C., Leahy, B., Kimmelshue, J. and Ludwig, G. 2018. Almond Conference. Accessed on March 8, 2021. https://www.almonds.com/sites/default/files/content/attachments/Almond%20Orchard%202025%20Responsible%20Pest%20Management.pdf

Johnson, SB. 2019. Seed potato treatments. Accessed on May 12, 2021. https://extension.umaine.edu/potatoes/wp-content/uploads/sites/49/2019/06/Potato-Seed-Treatments-2019.pdf

Kumar, V. 2018. Effect of different fungicides against *Podosphaera pannosa* causing rose powdery mildew under greenhouse conditions. Accessed on April 23, 2021. https://www.researchgate.net/publication/327866635_Effect_of_different_fungicides_against_Podosphaera_pannosa_causing_rose_powdery_mildew_under_greenhouse_conditions

Kynetec USA, Inc. 2020. "The AgroTrak® Study from Kynetec USA, Inc." Access Database. Database Subset: 2015-2019. [Accessed April 2021].

Minuto, A., Cerchiai, S., Vinotti, P., Bogliolo and Garrone, M. 2019. Control of powdery mildew on ornamentals with a mixture of azoxystrobin and difenoconazole. Accessed on April 23, 2021. https://www.cabdirect.org/cabdirect/abstract/20193521170

NCERA. 2017. North Central Regional Committee on soybean diseases ((NCERA). Foliar fungicide efficacy for control of foliar soybean diseases. Accessed on April 21, 2021. https://fyi.extension.wisc.edu/fieldcroppathology/files/2015/04/Soybean-Fungicide-efficacy-table_2017_Final.pdf

Smiley, R., Cook, RJ and Paulitz, T. 2002. Seed treatments for small grain cereals. Accessed on April 23, 2021. https://northerngraingrowers.org/wp-content/uploads/Seed-Treatment.pdf

Syngenta. 2018. Briskway Fungicide. Accessed on April 23, 2021. https://www.syngentaturf.sg/sites/g/files/zhg946/f/briskway_sg_lr_0.pdf?token=1591086706

Tedford, E., Lengkeek, V and Johnson, J. 2011. Inspire XT fungicide provides Michigan sugarbeet growers consistent *Cercospora* leaf control. Accessed on April 21, 2021. http://michiganbeets.com/doc/Cercospora_Michigan_Inspire_XT_Clean.pdf

United States Environmental Protection Agency (USEPA). Biological and Economic Analysis Branch (BEAD). Science Information and Analysis Branch (SIAB). 2019a. Difenoconazole (PC Code: 128847) Pesticide Label Use Summary (PLUS) Line by Line Report dated: 30/09/2019.

Docket Number EPA-HQ-OPP-2015-0401
www.regulations.gov

United States Environmental Protection Agency (USEPA). Biological and Economic Analysis Branch (BEAD). Science Information and Analysis Branch (SIAB). 2019b. Difenoconazole (PC Code: 128847) Summary Use and Usage Matrix dated: 28/08/2019.

Recore, S. and Evans, E. July 15, 2020. D457338. Difenoconazole: Tier I Update Review of Human Incidents and EIDemiology for Draft Risk Assessment.

Appendix A: Summary of Labeling Actions for Difenoconazole

Registration Review Case #: 7014 PC Code: 128847 Chemical Type: Fungicide Chemical Family: Triazoles Mode of Action: Sterol demethylation inhibitor; disrupts cell membrane ergosterol biosynthesis					
Affected Population(s)	Source of Exposure	Route of Exposure	Duration of Exposure	Potential Risk(s) of Concern	Labeling Actions
• Fish	• Runoff • Drift • Aquatic residues	• Ingestion • Respiration	• Chronic	• Body length reduction	• Drift and Runoff Environmental Hazards • Advisory Spray Drift Language • Rice (flooded field) Use Restrictions
• Aquatic Invertebrates	• Runoff • Drift • Aquatic residues	• Ingestion • Respiration	• Chronic	• Reduced # of young • Growth reduction • Survival reduction	
• Mammals	• Dietary	• Ingestion	• Chronic	• Effects on weight	• Terrestrial Outdoor Use Environmental Hazards • Advisory Spray Drift Language • Treated Seed Soil Incorporation • Management of wastes from treated seed
• Birds	• Dietary	• Ingestion	• Chronic	• Hatchling weight reduction	

Appendix B: Labeling Changes for Difenoconazole Products

Description	Label Language for Difenoconazole Products	Placement on Label
	End Use Products	
Mode of Action Group Number	**Note to registrant:** • Include the name of the **ACTIVE INGREDIENT** in the first column • Include the word "**GROUP**" in the second column • Include the **MODE/MECHANISM/SITE OF ACTION CODE** in the third column (for fungicides this is the FRAC Code, and for insecticides this is the Primary Site of Action; for Herbicides this is **SITE OF ACTION)** • Include the type of pesticide (**FUNGICIDE)** in the fourth column. **DIFENOCONAZOLE** | **GROUP** | **3** | **FUNGICIDE**	Front Panel, upper right quadrant. All text should be black, bold face and all caps on a white background, except the mode of action code, which should be white, bold face and all caps on a black background; all text and columns should be surrounded by a black rectangle.
Updated Gloves Statement	Update the gloves statements to be consistent with Chapter 10 of the Label Review Manual. In particular, remove reference to specific categories in EPA's chemical-resistance category selection chart and list the appropriate chemical-resistant glove types to use.	In Personal Protective Equipment (PPE) within Precautionary Statements and Agricultural Use Requirements, if applicable
Resistance-management for fungicides and bactericides	Include resistance management label language for fungicides/bactericides from PRN 2017-1 (https://www.epa.gov/pesticide-registration/pesticide-registration-notices-year)	Directions for Use, prior to directions for specific crops
Drift and Runoff Warning	"Drift and runoff may be hazardous to aquatic organisms in water adjacent to treated areas."	Environmental Hazards

Description	Label Language for Difenoconazole Products	Placement on Label
(for products with outdoor, terrestrial uses)		
Surface Water Advisory (outdoor agricultural use products only, such as fruits, nuts, vegetables, cereal grains, and other crops, and for products labeled for seed treatment)	"This product may impact surface water quality due to runoff of rain water. This is especially true for poorly draining soils and soils with shallow groundwater. This product is classified as having a high potential for reaching surface water via runoff for several months or more after application. A level, well-maintained vegetative buffer strip between areas to which this product is applied and surface water features such as ponds, streams, and springs will reduce the potential loading of difenoconazole from runoff water and sediment. Runoff of this product will be reduced by avoiding applications when rainfall or irrigation is expected to occur within 48 hours. Sound erosion control practices will reduce this product's potential to reach aquatic sediment via runoff."	Environmental Hazards (for difenoconazole products and for the seed container/bag tag)
Surface Water Advisory (outdoor non-agricultural use products only, such as ornamental turf, golf course turf, and nursery/greenhouse ornamentals)	"This product may impact surface water quality due to runoff of rain water. This is especially true for poorly draining soils and soils with shallow groundwater. This product is classified as having a high potential for reaching surface water via runoff for several months or more after application."	Environmental Hazards
Terrestrial Use Environmental Hazard (for products with outdoor, terrestrial uses, including products labeled for seed treatment)	"Do not apply directly to water or to areas where surface water is present or to intertidal areas below the mean high-water mark. Do not contaminate water when disposing of equipment washwater or rinsate."	Environmental Hazards (for difenoconazole products and for the seed container/bag tag)
Additional Required Labelling Action **Applies to all products delivered**	Remove information about volumetric mean diameter from all labels where such information currently appears.	Directions for Use

Description	Label Language for Difenoconazole Products	Placement on Label
via liquid spray applications		
Treated Seed Soil Incorporation	**Note to registrant**: include the following soil incorporation instructions, based on seed type: • For Canola and Rapeseed Crop Subgroup 20A (borage, crambe, cuphea, echium, flax seed, gold of pleasure, hare's ear mustard, lesquerella, lunaria, meadowfoam, milkweed, mustard seed, oil radish, poppy seed, rapeseed, sesame, sweet rocket): "Treated seed must be planted into the soil at a depth of at least ½ inch." • For Cotton, Small Grain Cereals (Barley, Oats, Rye, Triticale, and Wheat), and Sweet Corn: "Treated seed must be planted into the soil at a depth of at least 1 inch." • For Potato: "Treated seed pieces must be planted into the soil at a depth of at least 2 inches."	Directions for Use, for specific crops Directions for Use on Seed Container/Bag
Seed Treatment – For products allowed for on-farm seed treatment (not for distribution or sale of the seed)	• "Store treated seed away from food and feedstuffs. • Do not allow children, pets, or livestock to have access to treated seeds. • When loading/pouring the treated seed/seed-pieces, or covering/collecting spilled seed during loading and planting, wear long-sleeved shirt, long pants, shoes, socks, and chemical-resistant gloves [list specific glove types]. • Treated seeds exposed on the soil surface may be hazardous to wildlife. Cover or collect treated seeds spilled during loading and planting (such as in row ends). • Dispose of all excess treated seed by burying seed away from bodies of water. • Do not contaminate bodies of water when disposing of planting equipment wash water. • Plant treated seed into the soil to the recommended minimum depth or greater to minimize exposure. • DO NOT plant treated seed by broadcasting to the soil surface. Ensure that all planted seeds are thoroughly incorporated by the planter during planting, additional incorporation may be required to thoroughly cover exposed seeds. • Dispose of seed packaging or containers in accordance with local requirements."	Directions for Use, prior to directions for specific crops under the heading "On-farm Seed Treatment Directions"
Seed Bag/Container Labeling—for Products that Allow Commercial Seed Treatment and On-Farm Treated Seeds that are Distributed or Sold	"Seed Bag Labeling Requirements" "Seed treated with [PRODUCT NAME] must be labeled in accordance with all applicable requirements of the Federal Seed Act. All bags containing treated seed must be labeled or tagged as follows. Any seed treated with difenoconazole that is sold or distributed without these statements is an unregistered pesticide, in violation of FIFRA section 12."	In Directions for Use under the heading "Commercial Seed Container Labeling Requirements"

Description	Label Language for Difenoconazole Products	Placement on Label
(to Appear on Bag Tags for Treated Seed when Seed is to be Distributed)	**"The Federal Seed Act requires that bags containing treated seeds shall be labeled with the following statements:** • This seed has been treated with (insert name of active ingredient of pesticide). • Do not use for food, feed or oil purposes." **"The U.S. Environmental Protection Agency requires that bags containing treated seeds shall be labeled with the following statements:** • "This seed has been treated with [INSERT PRODUCT NAME(s), EPA REG. NO(s)), a [INSERT CHEMICAL TYPE(S)] containing [INSERT ACTIVE INGREDIENT(S)]. • This seed is treated with [number] lbs of difenoconazole per 100 lbs of seed. • Do not use treated seed for feed, food, or oil purposes. • Store treated seed away from food and feedstuffs. • Do not allow children, pets, or livestock to have access to treated seeds. • When loading/pouring the treated seed/seed-pieces or covering/collecting spilled seed during loading and planting, wear long-sleeved shirt, long pants, shoes, socks, and chemical-resistant gloves [list specific glove types]. • Treated seeds exposed on the soil surface may be hazardous to wildlife. Cover or collect treated seeds spilled during loading and planting (such as in row ends). • Dispose of all excess treated seed by burying seed away from bodies of water. • Do not contaminate bodies of water when disposing of planting equipment wash water. • Plant treated seed into the soil to the recommended minimum depth or greater to minimize exposure. • Do not plant treated seed by broadcasting to the soil surface. Ensure that all planted seeds are thoroughly incorporated by the planter during planting, additional incorporation may be required to thoroughly cover exposed seeds. • Dispose of seed packaging or containers in accordance with local requirements. • Excess treated seed may be used for ethanol production if (1) by-products are not used for livestock feed and (2) no measurable residues of pesticide remain in ethanol by-products that are used in agronomic practice." "All other requirements regarding the use of the treated seed, which include, but are not limited to, instructions relating to endangered species protection, environmental hazard statements, maximum use rates, plant back intervals, personal protective equipment, and storage and disposal statements, remain and must be listed on the bag tag."	

Description	Label Language for Difenoconazole Products	Placement on Label
Advisory Spray Drift Management Language for all products delivered via liquid spray application	**"SPRAY DRIFT ADVISORIES** THE APPLICATOR IS RESPONSIBLE FOR AVOIDING OFF-SITE SPRAY DRIFT. BE AWARE OF NEARBY NON-TARGET SITES AND ENVIRONMENTAL CONDITIONS. **IMPORTANCE OF DROPLET SIZE** An effective way to reduce spray drift is to apply large droplets. Use the largest droplets that provide target pest control. While applying larger droplets will reduce spray drift, the potential for drift will be greater if applications are made improperly or under unfavorable environmental conditions. **Controlling Droplet Size – Ground Boom** ***(note to registrants:*** *remove if ground boom is prohibited on product labels)* • Volume - Increasing the spray volume so that larger droplets are produced will reduce spray drift. Use the highest practical spray volume for the application. If a greater spray volume is needed, consider using a nozzle with a higher flow rate. • Pressure - Use the lowest spray pressure recommended for the nozzle to produce the target spray volume and droplet size. • Spray Nozzle - Use a spray nozzle that is designed for the intended application. Consider using nozzles designed to reduce drift. **Controlling Droplet Size – Aircraft** ***(note to registrants:*** *remove if aerial application is prohibited on product labels)* • Adjust Nozzles - Follow nozzle manufacturers' recommendations for setting up nozzles. Generally, to reduce fine droplets, nozzles should be oriented parallel with the airflow in flight. **BOOM HEIGHT – Ground Boom** ***(note to registrants:*** *remove if ground boom is prohibited on product labels)* For ground equipment, the boom should remain level with the crop and have minimal bounce. **RELEASE HEIGHT - Aircraft** ***(note to registrants:*** *remove if aerial application is prohibited on product labels)* Higher release heights increase the potential for spray drift. **SHIELDED SPRAYERS** Shielding the boom or individual nozzles can reduce spray drift. Consider using shielded sprayers. Verify that the shields are not interfering with the uniform deposition of the spray on the target area. **TEMPERATURE AND HUMIDITY** When making applications in hot and dry conditions, use larger droplets to reduce effects of evaporation.	Directions for Use, just below the Spray Drift box, under the heading "Spray Drift Advisories"

Description	Label Language for Difenoconazole Products	Placement on Label
	TEMPERATURE INVERSIONS Drift potential is high during a temperature inversion. Temperature inversions are characterized by increasing temperature with altitude and are common on nights with limited cloud cover and light to no wind. The presence of an inversion can be indicated by ground fog or by the movement of smoke from a ground source or an aircraft smoke generator. Smoke that layers and moves laterally in a concentrated cloud (under low wind conditions) indicates an inversion, while smoke that moves upward and rapidly dissipates indicates good vertical air mixing. Avoid applications during temperature inversions. **WIND** Drift potential generally increases with wind speed. o Applicators need to be familiar with local wind patterns and terrain that could affect spray drift."	
Advisory Spray Drift Management Language for products that are applied as liquids and allow boom-less ground sprayer applications	**"SPRAY DRIFT ADVISORIES** **Boomless Ground Applications:** o Setting nozzles at the lowest effective height will help to reduce the potential for spray drift."	Directions for Use, just below the Spray Drift box, under the heading "Spray Drift Advisories"
Advisory Spray Drift Management Language for all products that allow liquid applications with handheld technologies	**"SPRAY DRIFT ADVISORIES** **Handheld Technology Applications:** • Take precautions to minimize spray drift."	Directions for Use, just below the Spray Drift box, under the heading "Spray Drift Advisories"

Appendix C: Listed-Species Assessment

In 2015, EPA, along with the Services—the Fish and Wildlife Service (FWS) and the National Marine Fisheries Service (NMFS)—and the United States Department of Agriculture (USDA) released their joint Interim Approaches for assessing risks to listed species from pesticides. The agencies jointly developed these Interim Approaches in response to the 2013 National Academy of Sciences' recommendations that discussed specific scientific and technical issues related to the development of pesticide risk assessments conducted on listed species. Since that time, the agencies have been continuing to work to improve the consultation process.

EPA initially conducted biological evaluations (BEs) using the interim method on three pilot chemicals representing the first nationwide pesticide consultations (final pilot BEs for chlorpyrifos, malathion, and diazinon were completed in January 2017). These initial pilot consultations were envisioned as the start of an iterative process. Later that year, NMFS issued a final biological opinion for these three pesticides. In 2019, EPA requested to reinitiate formal consultation with NMFS on malathion, chlorpyrifos and diazinon to consider new information that was not available when NMFS issued its 2017 biological opinion. EPA recently received a draft revised biological opinion on these pesticides from NMFS and posted it for public comment.[27] In February 2022, EPA also received a final malathion biological opinion[28] from FWS, which the Agency plans to implement according to the 18-month timeframe specified in the opinion.

After receiving input from the Services and USDA on proposed revisions to the pilot interim method and after consideration of public comments received, EPA released an updated *Revised Method for National Level Listed Species Biological Evaluations of Conventional Pesticides* ("Revised Method") in March 2020.[29] During the same timeframe, EPA also released draft BEs for carbaryl and methomyl, which were the first to be conducted using the Revised Method. To date, EPA has used the Revised Method to complete final BEs for carbaryl, methomyl, atrazine, simazine, and glyphosate.

The 2018 Farm Bill established a FIFRA Interagency Working Group (IWG) to recommend improvements to the ESA section 7 consultation process for FIFRA actions and to increase opportunities for stakeholder input. This group is led by EPA and includes representatives from NMFS, FWS, USDA, and the Council on Environmental Quality (CEQ). The IWG outlines its recommendations and progress on implementing those recommendations in reports to Congress.[30] The agencies continue to work collaboratively, consistent with Congress's intent in creating the IWG.

In January 2022, EPA announced a policy[31] to evaluate potential effects of new conventional pesticide active ingredients to listed species and their designated critical habitat and initiate consultation with the Services, as appropriate, before registering these new pesticides. Before the Agency registers new uses of pesticides for use on pesticide-tolerant crops, EPA will also continue to make effects determinations.

---

[27] https://www.epa.gov/endangered-species/biological-opinions-available-public-comment-and-links-final-opinions
[28] https://www.epa.gov/endangered-species/biological-opinions-available-public-comment-and-links-final-opinions
[29] https://www.regulations.gov/document?D=EPA-HQ-OPP-2019-0185-0084
[30] https://www.epa.gov/endangered-species/reports-congress-improving-consultation-process-under-endangered-species-act
[31] https://www.epa.gov/newsreleases/epa-announces-endangered-species-act-protection-policy-new-pesticides

If these determinations are likely to adversely affect determinations, the Agency will not register the use unless it can predict that registering the new use will not jeopardize listed species or adversely modify their designated critical habitats. EPA will also initiate consultation with the Services as appropriate.

Appendix D: Endocrine Disruptor Screening Program

As required by FIFRA and FFDCA, EPA reviews numerous studies to assess potential adverse outcomes from exposure to chemicals. Collectively, these studies include acute, sub-chronic and chronic toxicity, including assessments of carcinogenicity, neurotoxicity, developmental, reproductive, and general or systemic toxicity. These studies include endpoints which may be susceptible to endocrine influence, including effects on endocrine target organ histopathology, organ weights, estrus cyclicity, sexual maturation, fertility, pregnancy rates, reproductive loss, and sex ratios in offspring. For ecological hazard assessments, EPA evaluates acute tests and chronic studies that assess growth, developmental and reproductive effects in different taxonomic groups. As part of its most recent registration decision for difenoconazole, the EPA reviewed these data and selected the most sensitive endpoints for relevant risk assessment scenarios from the existing hazard database. However, as required by FFDCA § 408(p), difenoconazole is subject to the endocrine screening part of the Endocrine Disruptor Screening Program (EDSP).

EPA has developed the EDSP to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." The EDSP employs a two-tiered approach to making the statutorily required determinations. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid (E, A, or T) hormonal systems. Chemicals that go through Tier 1 screening and are found to have the potential to interact with E, A, or T hormonal systems will proceed to the next stage of the EDSP where EPA will determine which, if any, of the Tier 2 tests are necessary based on the available data. Tier 2 testing is designed to identify any adverse endocrine-related effects caused by the substance, and establish a dose-response relationship between the dose and the E, A, or T effect.

Under FFDCA § 408(p), the Agency must screen all pesticide chemicals. Between October 2009 and February 2010, EPA issued test orders/data call-ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. The Agency has reviewed all of the assay data received for the List 1 chemicals and the conclusions of those reviews are available in the chemical-specific public dockets. A second list of chemicals identified for EDSP screening was published on June 14, 2013,32 and includes some pesticides scheduled for Registration Review and chemicals found in water. Neither of these lists should be construed as a list of known or likely endocrine disruptors. Difenoconazole is not on either list. For further information on the status of the EDSP, the policies and procedures, the lists of chemicals, future lists, the test guidelines and the Tier 1 screening battery, visit EPA website.33

[32] See http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0477-0074 for the final second list of chemicals.
[33] https://www.epa.gov/endocrine-disruption

EPA's EDSP is actively pursuing the application of new approach methods (NAMs) to create a more efficient and robust screening program. In October 2020, EPA underwent a reorganization and the EDSP was moved to the Office of Pesticide Programs. This reorganization provides better alignment of the EDSP with the procedures and methods used by the program offices. On July 28, 2021, the Office of Inspector General (OIG) released its new report on the EDSP and made ten recommendations. EPA is also developing a strategic planning document for EDSP which will be available for public comment in 2022. EPA expects additional documents for public release in 2021-2023 that address aspects of EDSP chemical determinations. EPA looks forward to working with stakeholders and the scientific community to accelerate the implementation of this important program into pesticide risk assessments and decision making.

In this ID, EPA is making no human health or environmental safety findings associated with the EDSP screening of difenoconazole. Before completing this registration review, the Agency will make an EDSP FFDCA § 408(p) determination."

Attachment 2

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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

September 16, 2020

PC Code: 128847
DP Barcode: 454125

MEMORANDUM

SUBJECT: **Difenoconazole:** Draft Ecological Risk Assessment for Registration Review

FROM: Faruque Khan, Ph.D., Senior Scientist
Rebecca Lazarus, Ph.D., Biologist
Environmental Risk Branch I
Environmental Fate and Effects Division (7507P)

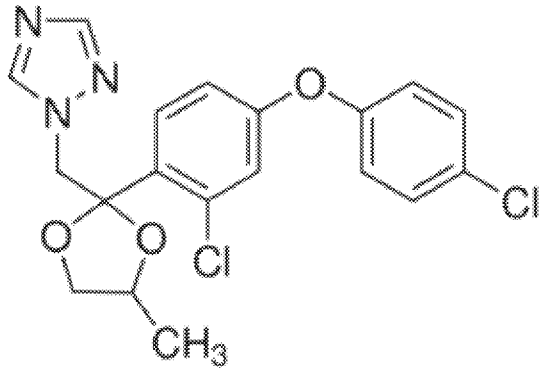
REBECCA LAZARUS Digitally signed by REBECCA LAZARUS Date: 2020.09.16 10:23:12 -04'00'

THRU: Sujatha Sankula, Ph.D., Branch Chief 2020.09.16 11:51:54 -04'00'
Gregory Orrick, Risk Assessment Process Leader 2020.09.16 11:38:30 -04'00'
Environmental Risk Branch I
Environmental Fate and Effects Division (7507P)

TO: Jordan Page, Chemical Review Manager
Jill Bloom, Team Leader
Cathryn Britton, Branch Chief
Risk Management and Implementation Branch V
Pesticide Re-evaluation Division (7508P)

This memorandum transmits the attached draft ecological risk assessment (DRA) for the Registration Review of the fungicide difenoconazole (CAS No. 119446-68-3; PC 128847).

Draft Ecological Risk Assessment for the Registration Review of Difenoconazole



Difenoconazole; CAS No. 119446-68-3
USEPA PC Code: 128847

Prepared by:
Rebecca Lazarus, Ph.D., Biologist
Faruque Khan, Ph.D., Senior Scientist

Reviewed by:
Frank Farruggia, Ph.D., Senior Effects Scientist
Christopher M. Koper, M.S., Chemist

Approved by:
Sujatha Sankula, Ph.D., Branch Chief
Gregory Orrick, Risk Assessment Process Leader
Environmental Risk Branch I
Environmental Fate and Effects Division
Office of Pesticide Programs
United States Environmental Protection Agency

Table of Contents

1.0 Executive Summary 5
1.1 Overview 5
1.2 Risk Conclusions Summary 5
1.3 Environmental Fate and Exposure Summary 7
1.4 Ecological Effects Summary 8
1.5 Identification of Data Needs 9
2.0 Introduction 13
3.0 Problem Formulation Update 14
3.1 Mode of Action 15
3.2 Label Use and Characterization 15
3.2.1 Label Summary 15
3.2.2 Usage Summary 16
4.0 Residues of Concern 22
5.0 Environmental Fate Summary 23
6.0 Ecotoxicity Summary 29
6.1 Aquatic Toxicity 29
6.2 Terrestrial Toxicity 35
6.3 Degradate Toxicity 40
6.4 Incident Data 41
7.0 Analysis Plan 42
7.1 Overall Process 42
7.2 Modeling 43
8.0 Aquatic Organisms Risk Assessment 44
8.1 Aquatic Exposure Assessment 44
8.1.1 Modeling 44
8.1.2 PWC Input Parameters for Difenoconazole Uses 44
8.1.3 Aquatic Modeling Results 47
8.1.4 Monitoring Data (non-targeted sampling) 53
8.2 Aquatic Organism Risk Characterization 54
8.2.1 Aquatic Vertebrates (Fish) 54
8.2.2 Aquatic Invertebrates 54
8.2.3 Aquatic Plants 64
9.0 Terrestrial Vertebrate Risk Assessment 65
9.1 Terrestrial Vertebrate Exposure Assessment 65
9.1.1 Dietary Items on Treated Field 65
9.1.2 Spray Drift 67
9.1.3 Seed Treatment Application 67
9.1.4 KABAM Inputs 68
9.2 Terrestrial Vertebrate Risk Characterization 70
9.2.1 Foliar Applications On-Field Dietary Risk 70
9.2.2 Spray Drift Risk 76

9.2.3 Seed Treatments 79
9.2.4 Bioaccumulation in Birds and Mammals 81
10.0 Terrestrial Invertebrate Risk Assessment 82
10.1 Bee Exposure Assessment 82
10.2 Bee Risk Characterization (Tier I) 82
11.0 Terrestrial Plant Risk Assessment 85
12.0 Conclusions 85
13.0 Literature Cited 86
13.1 New Environmental Fate Studies Since the Problem Formulation 88
13.2 New Ecological Effects Studies Since the Problem Formulation 89
Appendix A. Summary of Difenoconazole Degradate Toxicity Endpoints 91
Appendix B. ECOSAR Estimated Toxicity Endpoints for Difenoconazole and Degradates 93
Appendix C. Names and Structures of Difenoconazole and its Degradates 94
Appendix D. Example of Summary of PWC and PFAM modeling Outputs 98
Appendix E. Endocrine Disruptor Screening Program (EDSP) 101
Appendix F. T-Rex Inputs for Difenoconazole Applications 102
Appendix G. KABAM Inputs and Outputs for Difenoconazole 103
Appendix H. Bee Rex Inputs and Outputs for Difenoconazole Applications 106

1.0 Executive Summary

1.1 Overview

This Draft Risk Assessment (DRA) examines potential ecological risks associated with the labeled uses of difenoconazole on non-listed non-target organisms. Difenoconazole was first registered in the U.S. in 1994 (PC Code: 128847). It is a systemic triazole fungicide, which upon application works by inhibiting the demethylation and other processes in sterol biosynthesis. Difenoconazole is rapidly absorbed by the leaves and then distributed within the plant tissue by translaminar movement.

Difenoconazole is registered for use on many fruits, vegetables, cereals (seed treatment), field crops, golf course turf grass, and ornamental plants. It is formulated as a flowable concentrate, emulsifiable concentrate, ready-to-use product, and liquid soluble concentrate. It is applied as a ground and aerial spray, chemigation, dip/drench, or as a seed treatment. The maximum single foliar spray application rates range from 0.07 to 0.43 lbs a.i./A and maximum annual application rates range from 0.11 to 1.29 lbs a.i./A. Maximum annual rates for seed treatment range from 0.007 to 0.17 lbs a.i./A. Post-harvest dip/drench treatments use rates range from 0.00000352 lb a.i/lbs for tuberous and corm vegetables to 0.00258 a.i. lb/gallon for pome fruits.

The aquatic and terrestrial exposure assessments are streamlined and estimate exposure from difenoconazole uses with the highest usage, highest application rates, and highest percent crop treated area. The estimated environmental concentrations (EECs) for all other currently registered but not modeled use sites are expected to be lower, and thus those presented in this document are considered protective of all registered use patterns.

This assessment considers the conclusions drawn from new data submissions and several Section 3 New Use Risk Assessments conducted since the Problem Formulation for Registration Review (USEPA, 2015). The new use assessments assessed uses on cotton, rice and wild rice (USEPA, 2016), uses on guava, papaya, cranberry and watercress (USEPA, 2017), expansions of labels for seed treatment uses to include rapeseed (USEPA, 2017b), and rate increases on turf (USEPA, 2019).

1.2 Risk Conclusions Summary

Given the uses of difenoconazole, the available ecotoxicity data, and environmental fate properties, there are Level of Concern (LOC) exceedances for non-listed fish (chronic), estuarine/marine invertebrates (acute), aquatic invertebrates (chronic), freshwater benthic invertebrates (chronic), birds (chronic), mammals (chronic), honeybees (larval acute and chronic), and aquatic plants. However, risks are considered low for honeybees (adults and larvae) and aquatic plants. There were no LOC exceedances for terrestrial plants. **Table 1-1** summarizes the risks associated with the registered uses of difenoconazole.

Chronic LOC exceedances were identified for all size classes of birds on and off the treated field for all uses (except a single application of 0.0684 lbs a.i./A for pome fruits) Several lines of evidence supported chronic avian risk including: 1) chronic LOC exceedances at the LOAEC, 2) exceedances at the mean Kenaga and 3) exceedances of the LOC for up to 150 days after application (0.43 lbs a.i./A, 3 applications, 14-day application interval; short grass). In addition, avian chronic risk concerns for difenoconazole treated seeds were identified for all seed treatments. For small and medium sized birds, it would take only the consumption of 4-5 seeds (small birds; barley seeds) to 2166 for larger birds consuming corn seeds (large birds) to exceed the LOC. Despite an estimated consumption of only 1-3 seed potatoes[1] (tuber) necessary to exceed LOC (large birds), potatoes are planted at depth (~4" below surface) and any surface availability to larger birds would most likely be due to potential spillage.

Chronic risk concerns for mammals were identified for all difenoconazole application rates across multiple applications and at the maximum non-agricultural application rate for a single application for small and medium mammals consuming short grass, tall grass and broadleaf plants/small insects. Exceedances at the LOAEC and mean Kenaga only supported risk concerns at the maximum application rates. Dose based chronic risks exceeded the LOAEC for up to 41 (single application) to 56 days (multiple applications) post-treatment. Chronic risk concerns for difenoconazole treated seeds were identified for mammals at all seed treatment application rates (except seed potato[1]) and it would take between 11 to over 1,000 seeds to trigger a LOC exceedance.

Due to spray drift, the exceedance of the chronic avian and mammalian LOC extends up to 112 feet from the use site for birds (single application 0.125 lbs a.i./A; aerial application; very fine to fine droplets) and only 7 feet for mammals (0.125 lbs a.i./A, 4 applications, 7-day application interval; aerial application; very fine to fine droplets).

With a log K_{ow} of 4.2, difenoconazole has the potential to bioaccumulate in the aquatic food web and be of an exposure concern to piscivorous birds and mammals. Based on minimum and maximum annual EECs and a refined metabolism elimination rate in the aquatic food-web based model (KABAM), there were no acute or chronic LOC exceedances for piscivorous birds and mammals.

For freshwater fish and invertebrates and estuarine/marine fish, there were no acute LOC exceedances. Acute RQs for estuarine/marine water-column invertebrates exceeded the LOC for aquatic agricultural uses (*i.e.,* rice). On a chronic basis, fish endpoints at both the NOAEC and LOAEC were exceeded by EECs for the majority of difenoconazole uses, therefore resulting in LOC exceedances and a conclusion of risk. There were also chronic LOC exceedances for the uses at the NOAEC for all tested water-column invertebrates. For both fish and invertebrates, the few scenarios that did not exceed the NOAEC/LOAEC were California based (*e.g.,* CA tomatoes, CA grapes, CA citrus, and CA stone fruit) and tended to have lower EECs due to lower rainfall in the region. Overall, chronic LOC exceedances included crops that have some of the

[1]A seed potato is a potato grown to be replanted to produce a potato plant via asexual reproduction (tuber).

highest poundage of difenoconazole applied annually. Due to the persistence of difenoconazole, repeated use can considerably increase these risks over time.

There are no risks to benthic invertebrates via the sediment exposure route. However, there were LOC exceedances for the most sensitive freshwater benthic invertebrate species (*Hyalella azteca)* when compared to pore-water EECs.

For plants, the LOC was exceeded based on the most sensitive non-vascular aquatic plant species as compared to the highest EECs (rice). There is some uncertainty regarding vascular aquatic plants because of a lack of available data. There were no LOC exceedances for terrestrial plants, and no reported incidents. Therefore, risks to aquatic and terrestrial plants are considered low overall.

There were no acute or chronic risk concerns for adult honeybees and risk was considered low for larval honeybees. Based on an 8-day repeat dose toxicity test with the difenoconazole EC formulation (A7402T), there were acute risk LOC exceedances for larval bees at both the minimum and maximum difenoconazole application rates for pollinator-attractive crops. No larval bee acute toxicity studies were conducted with the TGAI; however, the day 8 LD_{50} from the 22-day larval chronic study of >20.9 µg a.i./L was approximately 10 times greater than the estimated maximum dietary dose. Chronic larval studies were available and conducted with both the formulation and TGAI. However, the chronic LOC was only exceeded at the maximum application rate for the formulation-based study endpoints. Larvae may be exposed to the formulation following applications made during bloom and when pollen and nectar resources are collected soon after application and fed directly to larvae. It is assumed that this is not a common exposure scenario, when considering the likely timing of difenoconazole applications relative to bloom and that adult bees are likely to process nectar and pollen prior to feeding to larval bees. Since the proportion of the components of the formulation are expected to change by the time difenoconazole reaches larvae, it is assumed that the TGAI toxicity data is more representative of the exposure larvae will encounter. Therefore, risks to larvae are considered low.

1.3 Environmental Fate and Exposure Summary

Difenoconazole is persistent, non-volatile, and slightly (to hardly) mobile in soil. Due to its low vapor pressure of 2.5×10^{-10} mm Hg and solubility limit in water of 3.3 mg/L, difenoconazole has a low propensity to volatilize from soil and water. Difenoconazole was hydrolytically stable in buffered sterile solutions of pH 5, 7 and 9. In aerobic soil, half-lives of difenoconazole ranged from 104 to 754 days, which is moderately persistent to persistent based on the Goring persistence scale (Goring *et al.*, 1975). The compound degrades slowly to the ketone CGA-205374, which in turn is reduced to the alcohol CGA-205375, the cleavage product 1,2,4-triazole, carbon dioxide, and minor compounds. CGA-205375 and 1,2,4-triazole were also identified as degradates in aerobic and anaerobic metabolism studies. The half-lives of difenoconazole under aerobic and anaerobic aquatic conditions ranged from 301 to 799 days, which also suggests persistence in aquatic environments. The unextracted residues (8.1% to

38.9%) in soil and sediment studies strongly bind with organic matter fractions (*i.e.,* fulvic, humic and humin) of soils/sediments and are considered a major sink for the applied difenoconazole. In the environment, the release of organic matter sequestered difenoconazole fractions will depend on the turnover/degradation rate of fulvic acid, humic acid, and humin in soil.

Difenoconazole is expected to be slightly to hardly mobile based upon K_{FOC} values of 3,471 to 11,202 L/kg. Submitted terrestrial field dissipation studies showed that difenoconazole and its degradates did not leach below the 30 cm soil depth except in one site. Difenoconazole dissipated in the field with half-lives ranging from 139 to 462 days, which is consistent with laboratory study results. Because of its high affinity for soil, difenoconazole has the potential to runoff into adjacent bodies of surface water via erosion of soil particles onto which it is sorbed. The overall stability/persistence profile for difenoconazole suggests that it has potential to accumulate in soil and aquatic environments with each successive application.

1.4 Ecological Effects Summary

In general, difenoconazole has similar acute toxicity to both freshwater fish and estuarine/marine fish with LC_{50} values of 810 and 819 µg a.i./L, respectively. On a chronic basis there were reductions in male body length for fathead minnow (NOAEC=1.9 and LOAEC=3.7 µg a.i./L). Since there were no chronic data for the most acutely sensitive freshwater fish, an ACR estimated NOAEC (0.86 µg a.i./L) was used to represent the most sensitive freshwater fish species on a chronic basis. No chronic data were available for estuarine/marine fish; however, the ACR estimated NOAEC was identical to freshwater fish due to similar acute toxicity values. Acute toxicity studies for water-column invertebrates reported a LC_{50} for the water flea of 770 µg a.i./L and 150 µg a.i./L for mysid shrimp. On a chronic basis, there were reductions in the number of young per adult and adult body length in water fleas at the LOAEC of 13.0 µg a.i./L (NOAEC=5.6 µg a.i./L). Estuarine/marine invertebrates were similarly sensitive with reductions in mysid survival reported at 10 µg a.i./L (NOAEC=4.8 µg a.i./L). Three non-guideline life cycle studies were conducted with benthic invertebrates. The most sensitive endpoint was based on a decrease in reproduction and growth (NOAEC = 1.80 mg a.i./kg-bulk sediment; 0.0074 mg a.i./L) in the freshwater amphipod (*Hyalella azteca).* The most sensitive aquatic plant was the nonvascular freshwater diatom (*Navicula pelliculosa*) with an EC_{50} of 98 µg a.i./L.

Difenoconazole is practically non-toxic to birds (LD_{50} > 2000 mg a.i./kg-bw) and mammals (LD_{50}=1453 mg a.i./kg-bw) on an acute basis. On a subacute dietary basis, effects on body weight and feed consumption were observed (LC_{50}=4760 mg a.i./kg-diet) for bobwhite quail. The most sensitive chronic avian effects endpoint was based on a reduction in hatchling body weight in bobwhite quail (NOAEC=21.9 mg a.i./kg-diet). On a chronic basis, decreases in body weight in adult females and males and pup weight were reported at 2500 mg a.i./kg-diet (NOAEC=250 mg a.i./kg-diet) in rats.

Difenoconazole is practically non-toxic to adult honey bees on both an acute contact and oral basis (LC_{50} > 100 and LD_{50} > 71.3 µg a.i./bee respectively). Two chronic adult studies were

submitted. One was conducted with the TGAI and did not report any effects on adult honeybees on a chronic basis (NOAEL=13 µg a.i./bee-day). However, the adult chronic toxicity test conducted with the difenoconazole formulation estimated a NOAEC of 10.6 µg a.i./bee-day based on a 53% increase in day 10 mortality. In a similar manner, the TGAI chronic study for honey bee larvae estimated a NOAEC of 9.28 µg a.i./larva/day based on 55% mortality at the LOAEC of 20.9 µg a.i./larva-day, while the chronic study conducted with difenoconazole formulation, estimated a NOAEC of 1.31 µg a.i./larva-day based on 97% mortality at 3.28 µg a.i./larvae on day 8. A second chronic larval study conducted with difenoconazole formulation, failed to define a NOAEC for later developmental endpoints and was terminated on day 8 due to significant larval mortality in the two highest test concentrations (LD_{50} = 1.79 µg a.i./larva-day).

Seedling emergence and vegetative vigor studies were conducted with TEP (Difenoconazole EC A7402T). For seedling emergence, the most sensitive dicot species was cabbage and the EC_{25} of 0.25 lbs a.i./A was based on reduced cabbage survival and emergence. For monocots, there were no effects reported (EC_{25} > 0.25 lbs a.i./A). For vegetative vigor, the most sensitive dicot was lettuce with an EC_{25} of 0.299 lbs a.i./A based on a reduction in dry weight and the most sensitive monocot was ryegrass with an EC_{25} of 0.204 lbs a.i./A based on a reduction in dry weight.

1.5 Identification of Data Needs

Both the environmental fate and effects datasets are complete. The problem formulation (see USEPA, 2015) identified data gaps for the higher Tier honeybee studies; however, given the lack of acute or chronic risk concerns of difenoconazole based on Tier I data and current registered uses, additional higher-tier toxicity and/or exposure data are not recommended at this time.

Table 1-1. Summary of Risk Quotients for Taxonomic Groups from Current Uses of Difenoconazole

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species[2]	Additional Information/ Lines of Evidence
Freshwater Fish	Acute	0.00-0.14	No	No risk concerns.
	Chronic	0.7-22.2	**Yes**	ACR-based chronic risks across all application rates except for CA based crops (including citrus, stone fruits, and grapes). Evidence of chronic toxicity also based on exceedances at the LOAEC as well. RQs continued to exceed at empirically based NOAEC/LOAEC for less sensitive freshwater fish species (0.2-15.8). Due to the persistence of difenoconazole, repeated use can considerably increase these risks over time.

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species[2]	Additional Information/ Lines of Evidence
Estuarine/ Marine Fish	Acute	0.00-0.14	No	-
	Chronic	0.7-22.2	**Yes**	No chronic empirical data available for estuarine/marine fish. LOC exceedances at both the ACR based NOAEC and LOAEC.
Freshwater Invertebrates (Water-Column)	Acute	0.00-0.14	No	-
	Chronic	0.12-5.4	**Yes**	Chronic risk concerns for scenarios wth EECs > 5.6 µg a.i./L (ME potatoes (ME and ID), cabbage, peppers, and cucumbers, and citrus (FL), tomatoes (FL and PA), melon (MI MO and NH), cherries (MI), grapes (NY), sugarbeets (MN), nurseries (MI, NJ, OR, TN), turf (PA) and all rice scenarios. Exceedances at the LOAEC for worst-case scenarios only (aquatic agricultural uses (rice), ME potatoes, and, NY grapes).
Estuarine/ Marine Invertebrates (Water-Column)	Acute	0.01-0.74	**Yes**	EECs exceeded LOC for rice use only based on mysid LC_{50}. No LOC exceedances for oysters on an acute basis (RQs<0.26)
	Chronic	0.14-6.3	**Yes**	Chronic risk concerns for scenarios with EECs > 4.8 µg a.i./L. Instances in which there were no LOC exceedances were for the CA based scenarios with lower EECs. LOCs were exceeded for the aforementioned scenarios as freshwater invertebrates, but also included some additional geographic scenarios for nurseries, some additional crop groups (apples), and other application methods (aerial and ground). Exceedances at the LOAEC for worst-case scenarios only (aquatic agricultural uses (rice), potatoes, grapes and cherries.
Benthic Invertebrates	Chronic	Sediment: 0.00-0.8	No	No NOAEC based RQ exceedances for freshwater or estuarine/marine benthic invertebrates.
		Pore Water: 0.08-1.9	**Yes**	LOC exceeded at pore-water NOAECs for freshwater benthic invertebrates for a subset of scenarios and risks concerns at the max pore water scenarios only (ME potato, PA tomato, MI cherries, NY grapes, and rice uses). No risk concerns for estuarine/marine benthic invertebrates.

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species[2]	Additional Information/ Lines of Evidence
Mammals	Acute	≤ 0.07[5]	No	-
	Chronic	Foliar: Single: 0.00-2.24 Multiple: 0.01-5.22	**Yes**	NOAEC/LOAEC based on a reduction in body weight at 2500 mg a.i./kg-diet. Dose based chronic risks exceeded LOC for single applications at max rate (0.43 lbs a.i./A) only, and for all multiple applications. Risks were for mammals consuming short and tall grasses, and broadleaf plants/small insects for smaller size classes. Limited chronic risk based on (1) worst-case scenario only exceedances at the LOAEC and mean Kenaga (2) Drift footprint for ground applications ranged from 3.28-16.4 ft and 3.28-6.56 ft for aerial applications. For single applications dose-based exceedances occurred from 0-41 d post application and multiple applications 0 to >56 days.
		Seed Treatment: 0.12-1.70		Chronic LOC exceeded for mammals at all applications rates (except for potato seed). It would take a minimum of 11 seeds for crops including corn to well over 1,000 seeds for rapeseed, barley and wheat.
		KABAM: 0.024-0.39		-
Birds[3]	Acute Oral	Not calculable	Not applicable	EECs are 17 times lower than non-definitive LD_{50} values
	Subacute Dietary	0.00-0.02	No	-
	Chronic	Foliar: Single Applications: 0.05-4.71 Multiple Applications: 0.18-10.99	**Yes**	NOAEC/LOAEC was based on a reduction in hatchling weight at 108 mg a.i./kg-diet. Multiple lines of evidence support chronic avian risk: (1) LOC exceeded at the LOAEC for max app rates (2) Mean Kenaga >LOC at max app rates (3) exposures exceeded LOC for 20-150 days post application (4) Drift footprint for single applications and multiple applications ranged from 3.28-25.6 feet for ground applications and 36.1-112 ft for aerial applications.

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species[2]	Additional Information/ Lines of Evidence
		Seed Treatment: 1.11-16.13		Chronic non-listed LOC exceeded for birds at all labeled seed treatment application rates. It would take a minimum of a single seed to trigger risk concerns for crops including potatoes[4] and over 1000 seeds for crops including barely and rapeseed.
		KABAM: 0.27-0.73		-
Terrestrial Invertebrates[5]	Adult Acute Oral	Not calculable	Not applicable	RQs not calculated due to non-definitive endpoint – no effects in study. EECs were over 16 times lower than the non-definitive for oral endpoints and over 250 times for contact-based endpoints.
	Adult Acute Contact	Not calculable	Not applicable	
	Larval Acute Oral	Formulation Based 0.0685 lbs a.i./A: 0.52 0.43 lbs a.i./A: 0.99	**Yes**	Based on 8-day repeated dose toxicity test with difenoconazole formulation, acute based exceedances at both min and max single application rates. No acute larval studies were conducted w/ TGAI; however, 8-d LD_{50} from 22-day TGAI larval study of > 20.9 µg a.i./L was 10 times greater than the maximum estimated dietary EECs. So, risks are considered low.
	Adult Chronic Oral	<0.39	No	TGAI based NOAEC was non-definitive (>13 µg a.i./bee). Compared to EECs at maximum application rate there were no exceedances for TGAI. NOAEC would have to be over three times more sensitive. Formulation based NOAEC did not exceed chronic Los for risks to adults (RQs <0.39).
	Larval Chronic Oral	TGAI 0.0684 lbs a.i./A: 0.10 0.13 lbs a.i./A[6]: 0.19	**Yes**	No LOC exceedances based on TGAI endpoints. However, formulation-based endpoints were 7 times more toxic. RQs exceed LOC for maximum application rate based on NOAEC only. Since the proportion of the components of the formulation are expected to change by the time difenoconazole reaches larvae, it is assumed that the TGAI toxicity data is more representative of the exposure larvae will encounter; therefore, risks are considered low.
		Formulation 0.0684 lbs a.i./A: 0.71 0.13 lbs a.i./A[6]: 1.35		

Taxa	Exposure Duration	Risk Quotient (RQ) Range[1]	RQ Exceeding the LOC for Non-listed Species[2]	Additional Information/ Lines of Evidence
	Adult & Larval Bees	Seed treatment ≤ 0.02	No	No acute oral dietary concerns (EECs < LD50). Chronic risk evaluated with TGAI based studies since no systemic transport of formulation. No chronic risk concerns to adult or larval bees.
Aquatic Plants	Not applicable	Vascular N/A	Uncertain	No data available for vascular plants. However, based on evidence from non-vascular plants, terrestrial (vascular) plants and other conazole fungicides, risks for terrestrial uses remain low. However, risks for aquatic uses (rice) continue to remain uncertain.
		Non-Vascular 0.00-1.1	**Yes**	Only LOC exceedances are for agricultural aquatic uses on rice. Risks are considered low overall.
Terrestrial Plants	Not applicable	≤0.36[7]	No	-

[1]For aquatic taxa, EECs and RQs were based on parent, difenoconazole
[2]Level of Concern (LOC) Definitions: Terrestrial Animals: Acute=0.5; Chronic=1.0; Terrestrial invertebrates=0.4; Aquatic Animals: Acute=0.5; Chronic=1.0; Terrestrial Plants: 1.0
[3]RQs for birds are used as a surrogate for toxicity to terrestrial-phase amphibians and reptiles.
[4]A seed potato is a potato grown to be replanted to produce a potato plant via asexual reproduction (tuber).
[5]RQs for terrestrial invertebrates are applicable to honey bees, which are also a surrogate for other species of bees. Risks to other terrestrial invertebrates (*e.g.*, earthworms, beneficial arthropods) are only characterized when toxicity data are available.
[6]Maximum application rate for pollinator attractive crop
[7]Risk conclusions have not changed since the last Section 3 New Use Assessment (USEPA, 2019a) on turf at 0.43 lbs a.i./A

2.0 Introduction

This Draft Risk Assessment (DRA) examines the potential ecological risks associated with labeled uses of difenoconazole on non-listed non-target organisms. Federally listed threatened and endangered species are not evaluated in the document. The DRA uses the best available scientific information on the use, environmental fate and transport, and ecological effects of difenoconazole. The general risk assessment methodology is described in the *Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs* ("Overview Document") (USEPA, 2004). Additionally, the process is consistent with other guidance produced by the Environmental Fate and Effects Division (EFED). Relevant guidance is cited when it is used. When necessary, risks identified through standard risk assessment methods are further refined using available models and data.

3.0 Problem Formulation Update

The purpose of problem formulation is to provide the foundation for the environmental fate and ecological risk assessment being conducted for the labeled uses of difenoconazole. The problem formulation identifies the objectives for the risk assessment and provides a plan for analyzing the data and characterizing the risk. As part of the Registration Review (RR) process, a detailed Problem Formulation (USEPA, 2015; DP428500) for this DRA was published to the docket in January 2016 (Review Docket EPA-HQ-OPP-2015-0401). The following sections summarize the key points of the Problem Formulation and discuss any differences between the planned analysis outlined there and the final analysis conducted in this DRA.

The following fate, exposure, and ecotoxicity data were submitted in response to Registration Review Data Call-In (DCI GDCI-031402-1406) following the completion of the problem formulation.

Fate and Exposure Data

- OCSPP 835.6200: Aquatic Field Dissipation (MRID 49804601- 49804602; Acceptable), 50213601-50213602; Acceptable)
- OCSPP 850.6100: Analytical Methods in Soil and Water (ECM/ILV) (MRIDs 46950128 & 46950214 (for soil-Unacceptable), 49862301-9862302 and 50213602 (for water-Acceptable)
- OCSPP 835.6100: Terrestrial Field Dissipation (MRIDs 50807401-50807403) (Acceptable)
- Non-guideline: Sample Storage Stability Study (MRID 50949401) (Supplemental)

Ecotoxicity Data

Aquatic Taxa

- OCSPP 850.4550: A 96-hour Toxicity Test with the Cyanobacteria (*Anabaena flos-aquae*) (MRID 4985601)-Supplemental
- OCSPP 850.1770: A Life Cycle Toxicity Test with the Freshwater Amphipod (*Hyalella azteca*) Using Spiked Sediment: (MRID 50213701)-Acceptable
- OCSPP 850.1780: A Life Cycle Toxicity Test with the Marine Amphipod (*Leptocheirus plumulosus*) Using Spiked Sediment (MRID 50213702)-Acceptable
- OCSPP 850.1760: A Life Cycle Toxicity Test with the Midge (*Chironomus dilutus*) Using Spiked Sediment (MRID 50213703)-Supplemental

Terrestrial Taxa

- OCSPP 850.4100: Toxicity Effects on the Seedling Emergence of Ten Species of Plants: (MRID 49858602)-Acceptable

- OCSPP 850.4150: Toxicity Effects on the Vegetative Vigor of Ten Species of Plants (MRID 49858603)-Acceptable
- Non-Guideline/OCSPP 850.3020/OECD 213: Acute Oral and Contact Toxicity to the Honey Bee (*Apis mellifera*) under Laboratory Conditions (MRID 50367601)-Acceptable
- Non-Guideline: Honey Bee (*Apis mellifera*) Chronic Oral Toxicity 10-day Feeding Test in the Laboratory with TGAI (MRID 50727701)-Acceptable
- Non-Guideline: Chronic Toxicity to Honey Bee (*Apis mellifera*) in a 10-day Continuous Laboratory Feeding Study with Difenoconazole EC (A7402T) (MRID 50212903)-Acceptable
- Non-Guideline: Chronic Toxicity to Honey Bee Larvae Under Laboratory Conditions (in vitro) with Difenoconazole EC (A7402T) (MRID 50212904)-Supplemental
- Non-Guideline: Honey bee (Apis mellifera L.) 22-Day Larval Toxicity Test (Repeated Exposure) with TGAI (MRID 50449801)-Acceptable
- OCSPP 850.2100: Acute Toxicity (single dose) to Japanese Quail (*Coturnix coturnix japonica*) with Difenoconazole STK 20 (Difenoconazole + Tea Tree Oil) (MRID 50804618)-Supplemental
- Non-Guideline/OECD 213: Acute Oral Toxicity of Difenoconazole SKT 20 (Difenoconazole + Tea Tree Oil) in Bees (*Apis mellifera*) (MRID 50804619)-Supplemental
- OCSPP 850.1075: Acute Toxicity (96hrs) of STK 20 (Difenoconazole + Tea Tree Oil) in Guppies (*Poecilia reticulata*) (MRID 5084617)-Supplemental

These ecotoxicity studies were reviewed and are incorporated into this DRA as appropriate. More specific information on these new data is available in **Section 6.0.** In addition, after further review of ECOSAR toxicity and environmental fate data, degradate, CGA-205375 was excluded from Residues of Concern (ROC) due to its low toxicity to aquatic organisms as compared to difenoconazole and its low formation in terrestrial and aquatic environments. The detailed rationale for excluding CGA-205375 can be found in **Section 4.0**.

3.1 Mode of Action

Difenoconazole is a broad-spectrum triazole fungicide that works by inhibiting demethylation and other processes in sterol biosynthesis. Difenoconazole is rapidly absorbed by leaves and is then distributed within plant tissue by translaminar movement (USEPA, 2015b).

3.2 Label Use and Characterization

3.2.1 Label Summary

Difenoconazole end use products are formulated as emulsifiable concentrate, soluble concentrate, emulsion [oil] in water, flowable suspension, and ready-to-use formulations. It is currently registered in the U.S. for use as a seed treatment on cereal grain crops, cotton, canola, and potato seed (tuber) pieces; for post-harvest use on members of tuberous and corm vegetables subgroup 1C and pome fruit group 11-10. It is also registered for multiple foliar

applications on numerous crops including tuberous and corm vegetables subgroup 1C, cucurbit group 9, stone fruit group 12, tree nut group 14, pome fruits group 11-10, rapeseed and cotton, subgroups 20A and 20C, fruiting vegetables subgroups of various subgroups of berry and small fruits, soybean, carrot and many other crops and various vegetables listed in **Table 3-1**. Difenoconazole is also registered for use on golf course turf and ornamental plants in commercial and residential landscapes. As a seed treatment, it is applied with commercial grade seed treatment equipment. As a foliar treatment, it is applied to field and vegetable crops, and landscape ornamentals using aerial, ground (ground boom and airblast), and chemigation application methods and equipment. Only ground foliar application is applicable for golf course turf. The maximum single application rates range from 0.07 to 0.43 lbs a.i./A and maximum annual application rates range from 0.11 to 1.29 lbs a.i./A for foliar spray. Maximum annual rates for seed treatment range from 0.007 to 0.17 lbs a.i./A. Post-harvest dip/drench treatments use rates ranged from 0.00000352 lb a.i/lbs for 1C Crop Group to 0.00258 a.i. lb/gallon for 11-10 Crop subgroup. However, post-harvested treated products are intended for the market and not for replanting and thus the environmental exposure would be considered limited. Application information for foliar spray, seed and dip/drench treatments uses are provided in **Tables 3-2 to 3-3** as reported in the Pesticide Label Use Summary (PLUS) report for difenoconazole (USEPA, 2019a).

It should be noted that many labels do not specify one or more of the following parameters: maximum number of applications per crop cycle or year; maximum application rate per crop cycle or year; and/or minimum application interval. Conservative assumptions were considered when label directions are missing.

3.2.2 Usage Summary

The Biological and Economic Analysis Division (BEAD) provided a Screening Level Usage Analysis (SLUA) that estimated the usage of difenoconazole on agricultural crops (USEPA, 2019). According to the SLUA, in recent years (2008-2017) approximately 300,000 lbs. of difenoconazole were used per year. The maximum percent crop treated amounts are for tomato (50%), grapes (45%), almond, apple and pears (40%), and grape raisin, cabbage, grapefruit, peppers, potatoes, watermelon and wheat-spring (20 to 35%). The predominant agricultural uses in terms of average annual pounds of difenoconazole applied are soybeans (100,000), wheat-winter (40,000), potatoes and sugar beet (30,000), and almond and wheat-spring (20,000 each).

Table 3-1. Current Maximum Use Patterns for Registered Foliar Treatment Uses of Difenoconazole

Crop group/subgroup Use Site	Application Method[A]	Maximum Single Use Rate (lbs a.i./A)	Maximum Annual Use Rate (lbs a.i./A/Year)	Maximum Number of Applications/ Year	Minimum Application Interval (days)	Reg. #	Geo. Restrictions	Comment[A]
Agricultural uses								
10-10 Citrus fruits	Aerial & Ground	0.125	0.50	Not specified	7	100-1313 100-1554	Not Specified	Max of sequential apps: 2
11-10 Pome Fruits	Aerial & Ground	0.068	0.34	Not specified	7	100-1317	Not Specified	--
12-12 Stone Fruits	Aerial & Ground	0.115	0.46	Not specified	7	100-1262 100-1313 100-1554	Not Specified	--
13-07B Bushberry	Aerial & Ground	0.115	0.46	Not specified	7	100-1262 100-1312	Not Specified	Max of sequential apps: 2
13-07F Small fruit vine	Aerial & Ground	0.115	0.46	Not specified	10	100-1262 100-1313 100-1554	Not Specified	Max of sequential apps: 2
13-07G Low growing berry	Aerial & Ground	0.115	0.46	Not specified	7	100-1262 100-1313 100-1554	Not Specified	Max of sequential apps: 2
14-12 Tree nuts	Aerial & Ground	0.115	0.46	Not specified	14	100-1262 100-1313 100-1554	Not Specified	Max of sequential apps: 2
1C Tuberous and corm vegetables	Aerial & Ground	0.115	0.46	Not specified	7	100-1317	Not Specified	Max of sequential apps: 2
20A Rapeseed	Aerial	0.102	0.102	1	--	100-1476	Not Specified	--
	Chemigation	0.114	0.114	1	--			
20C Cottonseed	Aerial, ground boom and chemigation	0.113	0.339	Not specified	14	100-1313 100-1554	--	Max of sequential apps: 2

Crop group/subgroup Use Site	Application Method[A]	Maximum Single Use Rate (lbs a.i./A)	Maximum Annual Use Rate (lbs a.i./A/Year)	Maximum Number of Applications/ Year	Minimum Application Interval (days)	Reg. #	Geo. Restrictions	Comment[A]
3-07A Orion Bulb	Aerial, ground boom and chemigation	0.115	0.46	Not specified	7	100-1313 100-1554	--	Max of sequential apps: 2
3-07B Orion Green	Aerial, ground boom and chemigation	0.102	0.409	4	7	100-1476	--	Max of sequential apps: 2
5 Brassica (cole) leafy vegetables	Aerial, ground boom and chemigation	0.115	0.46	Not specified	7	100-1313 100-1554	--	--
5-16 Brassica Head and Stem vegetables	Aerial, ground boom and chemigation	0.115	0.46	Not specified	7	100-1262	--	Max of sequential apps: 2
6C. Dried shelled pea and bean (except soybean)	Aerial ground boom and chemigation	0.115	0.46	Not specified	14	100-1313	--	Max of sequential apps: 2
8-10A Tomato	Aerial & ground boom	0.115	0.46	Not specified	7	100-1313	--	Max of sequential apps: 2
8-10 Fruiting vegetables	Aerial & ground boom	0.115	0.46	Not specified	7	100-1317	--	Max of sequential apps: 2
9 Cucurbit vegetables	Aerial, ground boom and chemigation	0.115	0.46	Not specified	7	100-1313 100-1554	--	--
Carrot	Aerial & ground boom	0.115	0.46	Not specified	7	100-1262 100-1313 100-1554	--	Max of sequential apps: 2
Cranberry	Aerial, ground boom and chemigation	0.115	0.345	3	7	100-1262 100-1313	--	Max of sequential apps: 2
Ginseng	Aerial, ground boom and chemigation	0.115	0.46	Not specified	7	100-1262 100-1313	--	Max of sequential apps: 2

Crop group/subgroup Use Site	Application Method[A]	Maximum Single Use Rate (lbs a.i./A)	Maximum Annual Use Rate (lbs a.i./A/Year)	Maximum Number of Applications/ Year	Minimum Application Interval (days)	Reg. #	Geo. Restrictions	Comment[A]
Guava	Aerial & ground boom	0.115	0.46	Not specified	10	100-1262 100-1313 100-1554	--	Max of sequential apps: 2
Papaya	Aerial, ground boom and chemigation	0.115	0.46	Not specified	10	100-1262 100-1313 100-1554	--	Max of sequential apps: 2
Rice and wild rice	Aerial & ground boom	0.123	0.246	2	14	100-1262 100-1313	--	--
Soybean	Aerial ground boom and chemigation	0.111	0.22	2	14	100-1476 100-1602	--	Max of sequential apps: 2
Sugar beet	Aerial, ground boom and chemigation	0.115	0.46	Not specified	7	100-1262	--	--
Watercress	Aerial, ground boom and chemigation	0.115	0.46	Not specified	7	100-1262 100-1313 100-1554	--	Max of sequential apps: 2
Non-Agricultural Uses								
Ornamental	Aerial, ground boom and chemigation	0.13	0.52	Not specified	7	100-1506	--	Max of sequential apps: 2
Turf/golf courses[B]	Ground boom	0.43	1.29	3	14	100-1433	--	--

[A] Different mode of action fungicide should be alternated after two sequential applications of difenoconazole
[B] Application rate for turf is based on recent label amendment to increase the use rate on turf/golf course (USEPA, 2019a, DP452414)

Table 3-2. Current Maximum Use Patterns for Registered Seed Treatment Uses of Difenoconazole [A,B]

Use Site	Application Method & Seeding rate (lbs/A)	Maximum Single Application Rate (lbs a.i./lb seed)	Maximum Annual Application Rate[A] (lbs a.i./A/Year)	Fl oz/100 lb seed	Maximum Number of Applications/ (Year)	Minimum Application Interval (days)	Reg. #[B]	Geo. Restrictions
Barley	Seed treatment (138.6)	0.000242	0.034	1.0	Not applicable		100-740	Not Specified
Corn (sweet)	Seed treatment (33.2)	0.0003	0.010	1.0	Not applicable		100-740	Not Specified
Cotton	Seed treatment (18.8)	0.000351	0.007	1.45	Not applicable		100-740	Not Specified
Oats	Seed treatment (90)	0.000242	0.022	1.0	Not applicable		100-740	Not Specified
Potatoes	Seed Potato[C]	0.0000242	0.17	0.1	Not applicable		100-740	Not Specified
20A Rapeseed	Seed treatment (8.2)	0.0000242	0.0020	1.24	Not applicable		100-740	Not Specified
Rye	Seed treatment (109)	0.000242	0.026	1.0	Not applicable		100-740	Not Specified
Triticale	Seed treatment (156)	0.000242	0.038	1.0	Not applicable		100-740	Not Specified
Wheat	Seed treatment (156)	0.000242	0.038	1.0	Not applicable		100-740	Not Specified

[A] Application rates of difenoconazole treated seeds for various uses were calculated using maximum application rate (lbs a.i. /lb seed) x maximum seeding rates (lbs/A) of TREX model (e.g. barley: 0.000242 lbs a.i/lb seed X 138.6 lbs seeds/A = 0.034 lbs a.i/A)
[B] Maximum annual application rate is based on 32.8% a.i. and a product density 9.52 lbs gal
[C] A tuber (chunk) rather than a true seed typically used for potato as a propagative unit

Use Site	Application Method & Seeding rate (lbs/A)	Maximum Single Application Rate (lbs a.i./lb seed)	Maximum Annual Application Rate^ (lbs a.i./A/Year)	Fl oz/100 lb seed	Maximum Number of Applications/ (Year)	Minimum Application Interval (days)	Reg. #[8]	Geo. Restrictions
A seed potato is a potato grown to be replanted to produce a potato plant via asexual reproduction (tuber).								

Table 3-3. Current Maximum Use Patterns for Registered Post-harvest Treatment Uses of Difenoconazole

Crop group /subgroup Use Site	Application Method	Maximum Single Application Rate	Application Target	Maximum Number of Applications/ Season (Year)	Minimum Application Interval (days)	Reg. #	Geo. Restrictions	Comment
1C Tuberous and corm vegetable	Dip/spray drench	0.00000352 (lbs a.i./lbs vegetables	Vegetables	Not applicable		100-1386	Not Specified	Post-harvest treatment
11-10 Pome Fruits	Dip	0.00258 (lbs a.i./gallon	Fruits	Not applicable		100-1529	Not Specified	Post-harvest treatment

4.0 Residues of Concern

The Residue of Concern (ROC) in this assessment is difenoconazole. Three major degradates (1,2,4-triazole, triazole acetic acid and CGA-205375) were detected in environmental fate studies. The Problem Formulation (USEPA, 2015) forecast a parent compound + CGA-20375 approach. However, several lines of evidence support difenoconazole only, including low formation of CGA-203375 and low toxicity from ECOSAR predicted endpoints. The selection of the parent only as the residue of concern (ROC) in this assessment is also based on additional lines of evidence including chemical structure/fate properties, ecological toxicity data, and estimated toxicity values from the Ecological Structure Activity Relationship (ECOSAR) model[2].

Aquatic

Several lines of evidence support the exclusion of the three major degradates from the ROCs for aquatic organisms. 1,2,4-trizole (13.4%) and triazole acetic acid (48.8%) were detected as major degradates in an aqueous photolysis study. 1,2,4-triazole (<36%) is also formed through microbial degradation in aerobic soils and aquatic environment. 1,2,4-triazole and its conjugates (triazole alanine and triazole acetic acid) are common metabolites to the class of compounds known as the triazole-derivative fungicides (T-D fungicides, conazoles). Available empirical toxicity data indicate that the degradates 1,2,4-triazole and triazole acetic acid are less toxic to aquatic organisms than difenoconazole (see **Appendix A**). A comparison of ECOSAR estimates to experimentally derived difenoconazole toxicity values (**Appendix B**) provides evidence that the 1,2,4-triazole and triazole acetic acid are less toxic than difenoconazole to aquatic organisms. ECOSAR estimates for 1,2,4-triazole, triazole acetic acid (and its conjugates) are several orders of magnitude higher than difenoconazole, therefore, 1,2,4-trizole and its derivatives were not included as ROC.

No empirical effects data were available for aquatic taxa for the degradate CGA-205375. In general, ECOSAR toxicity data suggest that CGA-205375 is less toxic to aquatic organisms than difenoconazole (**Appendix B**, **Table B-1**). For example, CGA-205375 is predicted by ECOSAR to be 9 to 23X times less toxic on a chronic basis to freshwater fish and invertebrates as compared to parent. CGA-205375 is a minor degradate (4.4 to 9.4%) in six aerobic soils, detected at 14.8% in CA loamy sand soil, and detected at 12.6% in an anaerobic aquatic environment. Therefore, CGA-205375 is not expected to contribute substantially to exposure, in part, because difenoconazole is a persistent chemical in the environment.

Terrestrial

For terrestrial taxa, limited empirical degradate data were available for birds and mammals (**Appendix A**). A study with 1,2,4-triazole indicated a LD_{50} of 770 mg a.i/kg-bw for birds.

---

[2]USEPA. 2018 *Guidance for Using ECOSAR as a line of evidence for identifying residues of toxicological concern.* September 26, 2018. Environmental Fate and Effects Division. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency.

However, compared to the parent compound avian LD_{50} (>2000 mg a.i./kg-bw), triazole acetic acid is more acutely toxic to birds than difenoconazole parent. Overall, both parent and 1,2,4-triazole are practically non-toxic. No data were available for birds on the other two main difenoconazole degradates. No chronic degradate data were available for birds.

Effects data were available for mammals for all three major difenoconazole degradates. Acute data were available for triazole acetic acid (LD_{50} > 5000 mg a.i./kg-bw) and CGA-205375 (LD_{50}=2309 mg a.i./kg-bw). Based on these studies there was no evidence that these degradates were more toxic than difenoconazole (LD_{50} = 1453 mg a.i./kg-bw). No acute studies were available with 1,2,4-triazole; however, a chronic study was available with this degradate. There was uncertainty about the relative chronic toxicity between 1,2,4-triazole and difenoconazole to mammals. At 250 mg a.i./kg-diet, there were no effects on mammals based on difenoconazole; however, there were significant degradate effects at this concentration in the 1,2,4-triazole study (LOAEC=250 mg a.i./kg-diet). Since 250 mg a.i/kg-diet was the lowest test concentration, it was unclear where the no effects concentration was for this degradate.

The primary exposure pathway for birds and mammals to difenoconazole is via diet; however, there is not enough data to discount potential chronic risks to birds and mammals based on the degradates. Overall, based on the exposure pathways and low percent formation (**Appendix C**), exposure of birds, mammals, and other terrestrial taxa (including plants and terrestrial invertebrates) to degradates was considered low, and the main terrestrial exposure concerns are driven by difenoconazole alone.

5.0 Environmental Fate Summary

Table 5-1 summarizes the physical and chemical properties of difenoconazole. Difenoconazole is a water soluble (3.3 mg/L) chemical. It has a relatively low vapor pressure (2.5×10^{-10} mm Hg) and Henry's Law constant (8.9×10^{-12} atm·m^3/mol), which suggest that volatilization is not expected to be a major route of dissipation from soil and water.

Difenoconazole appears to be slightly mobile to hardly mobile in soils (FAO, 2000). Freundlich adsorption coefficient (K_F) values were 11.6, 22.9, 182, and 201 mL/g for the Madera loamy sand, Visalia sandy loam, North Dakota clay loam, and Florida sand soils, respectively; corresponding K_{Foc} values were 3870, 4587, 4799, and 11202 mL/g. In another study (MRID 42245135), the K_F values ranged from 12.8 to 63 mL/g and the corresponding K_{Foc} values ranged from 3471 to 7734 mL/g. However, this study was conducted with an autoclaved soil, potentially distorting the mobility characteristics of difenoconazole. Freundlich adsorption coefficient (K_F) values for the major degradate, CGA-205375 were available. The K_F values ranged from 9.9 to 145 mL/g, and the corresponding K_{Foc} values ranged from 2470 to 6432 mL/g. CGA-205375 has the potential to be slightly more mobile (FAO, 2000) in the soil than the parent compound. For both difenoconazole and CGA-205375, K_{FOC} is a better predictor of sorption than K_F based on a lower CV. Also, for both compounds, sorption to soil was dependent on soil concentration.

The octanol water partition coefficient (log Kow of 4.2) suggests that difenoconazole has a potential to bioaccumulate. Difenoconazole accumulated rapidly in edible and non-edible bluegill sunfish tissues with bioconcentration factors of 170x for edible tissues, 570x for non-edible tissues, and 330x for whole body. Depuration was also rapid with a depuration half-life of approximately 1 day and 96-98% clearance after 14 days of depuration. One metabolite, CGA-205375, was recovered from both edible and non-edible tissues, accounting for 51-64% of the applied difenoconazole.

Table 5-1. Summary of Physical-Chemical, Sorption, and Bioconcentration Properties of difenoconazole and sorption properties of major degradate, CGA 205375

Parameter	Value[1]				Source/Study Classification/Comment
Molecular Weight (g/mole)	406.27				---
Water Solubility Limit (mg/L)	3.3, 20°C				MRIDs 49577502/49577504 Acceptable
Vapor Pressure (torr)	2.5×10^{-10}, 25 °C				MRIDs 49577502/49577504 Acceptable
Henry's Law Constant (atm-m3/mole)	8.9^{-12}				Estimated value EPIWeb Version 4.1
Log Dissociation Constant (pKa)	None				MRIDs 49577502/49577504. Acceptable. Not expected to ionize in natural waters
Octanol-water Partition Coefficient (log K_{ow}) (unitless)	4.2, 25°C				MRIDs 49577502/49577504 Acceptable
	Parent (Difenoconazole)				
Soil-Water Distribution Coefficients (K_F in L/kg-soil or sediment) Organic Carbon-Normalized Distribution Coefficients ($K_{F}oc$ in L/kg-organic carbon)	**Soil/Sediment**	**K_F**	**K_{foc}**	**1/N**	MRID 469501-21 & -22 Acceptable. slightly mobile to hardly mobile in soils (FAO classification system); K_{foc} better predictor of sorption based on lower CV.
	Madera loamy sand (pH 6.8)	11.61	3870	0.80	
	Visalia sandy loam (pH 6.1)	22.94	4587	0.84	
	North Dakota clay loam (pH 7.9)	182.4	4799	0.86	
	Florida sand (pH 5.5)	201.6	11202	0.91	
	Mean	105	6115	--	
	CV (%)	0.97	0.56	---	

Parameter	Value[1]				Source/Study Classification/Comment
	Degradate (CGA-295375)				
	Soil/Sediment	K_F	K_{FOC}	1/N	
Soil-Water Distribution Coefficients (K_F in L/kg-soil or sediment)	Madera loamy sand (pH 6.8)	9.64	3214	0.83	MRID 469501-23 & -24 Supplemental slightly mobile to hardly mobile in soils (FAO classification system); K_{FOC} better predictor of sorption based on lower CV.
	Visalia sandy loam (pH 6.1)	12.35	2470	0.83	
Organic Carbon-Normalized Distribution Coefficients (K_{FOC} in L/kg-organic carbon)	North Dakota clay loam (pH 7.9)	145.32	3824	0.73	
	Florida sand (pH 5.5)	115.77	6432	0.84	
	Mean	70.77	3985	--	
	CV (%)	0.99	0.43	--	
Fish Bioconcentration Factor (BCF)	Species	BCF			MRID 42245142 Acceptable
	Bluegill sunfish (*Lepomis macrochirus*)	170x in edible tissues 570x nonedible tissues 330x for whole body Depuration Half-life: 1 day			

CV=Coefficient of Variation
[1]All estimated values were calculated according to "*Guidance for Reporting on the Environmental Fate and Transport of the Stressors of Concern in Problem Formulations for Registration Review, Registration Review Risk Assessments, Listed Species Litigation Assessments, New Chemical Risk Assessments, and Other Relevant Risk Assessments*" (USEPA, 2010a).

Table 5-2 summarizes representative half-life values from laboratory degradation studies of difenoconazole that were used to describe the environmental fate in the environment and in exposure modeling. There are additional ancillary environmental fate data available, as described in the Problem Formulation document (USEPA, 2015). Difenoconazole is stable to hydrolysis at pH 5, 7, and 9 and essentially stable to soil photolysis. In aerobic soil, the half-lives of difenoconazole ranged from 104 to 754 days at 20°C in seven soils, which is moderately persistent to persistent based on the Goring persistence scale (Goring et al., 1975).[3] The half-life of difenoconazole under anaerobic soil conditions was 947 days, which also suggests persistence.

In an aquatic environment, the main route of difenoconazole dissipation is partitioning into the bottom sediment. Difenoconazole undergoes relatively slow aqueous photolytic degradation in clear, shallow, well-lit water; breaking down to triazolyl acetic acid and further to triazole methanol and triazole. Under aerobic conditions, difenoconazole degraded with half-lives ranging from 301 to 799 days at 20°C. Under anaerobic conditions, difenoconazole degraded with a half-life of 612 days at 20°C. The overall stability/persistence profile for difenoconazole

[3] *Goring et al. (1975) provides the following persistence scale for aerobic soil metabolism half-lives:*
- *Non-persistent less than 15 days*
- *Slightly persistent for 15-45 days*
- *Moderately persistent for 45-180 days, and*
- *Persistent for greater than 180 days.*

suggests that it has potential to accumulate in soil and aquatic environments with each successive application.

Table 5-2. Summary of Environmental Degradation Data for Difenoconazole

Study	System Details	Parent Compound Half-lives (days)	Source/Study Classification/Comment
Abiotic Hydrolysis	pH 5, 7, 9	Stable	MRID 42245127, Acceptable.
Atmospheric Degradation	Hydroxyl Radical	0.5	Estimated value EPIWeb Version 4.1
Aqueous Photolysis	pH 7, 25°C 40°N sunlight	228 (LN-SFO)	MRID 46950105 Supplemental. Study was terminated before 30 days
Soil Photolysis	KY Sandy Loam, 25°C, PH 7 37° N sunlight	349 - 823	MRIDs 469501- 06 & 07, Supplemental.
Aerobic Soil Metabolism	Swiss Loam, 20°C	120 (SFO)	MRID 46950109 Supplemental.
	Swiss Loam, 20°C	104 (SFO)	MRID 46950110 Supplemental.
	Swiss Sandy loam/loamy sand, 20°C	187 (SFO)	MRID 46950111, Supplemental.
	Swiss Sandy loam, 20°C	158 (SFO)	
	Swiss Silty Clay Loam, 20°C	198 (SFO)	
	CA Loamy Sand, 25°C	408 (SFO) (*577 @ 20°C*)[3]	MRID 469501-12 & 13, Acceptable.
	CA Loamy Sand, 25°C	533 (SFO) (*754 @ 20°C*)[3]	MRID 469501-14 & 15, Supplemental.
Anaerobic Soil Metabolism	TX Loam Soil, 25°C	947	MRID 42245132, Supplemental.
Aerobic Aquatic Metabolism	Swiss Pond Silty Clay Loam, 20°C	330 (SFO)	MRID 46950116, Supplemental.
	Swiss River Sandy Loam, 20°C	301 (SFO)	
	CA River Loamy Sand, 25°C	565 (SFO-LN) (*799 @ 20°C*)[3]	MRID 469501-17& 18, Supplemental.
Anaerobic Aquatic Metabolism	CA River Loamy Sand, 25°C	433 (SFO) (*612 @ 20°C*)[3]	MRID 469501-19 & 20, Supplemental.

[1] The value used to estimate a model input value is the calculated SFO DT_{50}, T_{IORE}, or the DFOP slow DT_{50} from the DFOP equation. The model chosen is consistent with that recommended using the, *Guidance for Evaluating and Calculating Degradation Kinetics in Environmental Media* (NAFTA, 2012).
[2] SFO=single first order and SFO-LN=SFO calculated using natural log transformed data
[3] Since most of the reported DT_{50} values were based on 20°C for fate data except for few aerobic soil and aquatic metabolisms data, the reported half-lives were adjusted to 20°C based on a Q_{10} of 2.0.

In aerobic soil, difenoconazole degrades slowly to the ketone CGA-205374, which in turn is reduced to the alcohol CGA-205375, the cleavage product 1,2,4-triazole, carbon dioxide, and minor compounds. CGA-205375 and 1,2,4-triazole were also identified as degradates in aerobic and anaerobic metabolism studies. CGA-205375 consistently reached a maximum of 4.4% to 14.8% in biodegradation studies. The greatest formation of 1,2,4-triazole was observed in the anaerobic aquatic metabolism study (35.9% at study termination; 350 days). The unextracted

residues in studies with soil or sediment (8.1% to 38.9%) were strongly bound with organic matter fractions (*i.e.,* fulvic, humic and humin) of soils/sediments and were considered a major sink for the applied difenoconazole. In the environment, the release of organic matter sequestered pesticide fractions will depend on the turnover/degradation rate of fulvic acid, humic acid and humin fractions in soil. Since the turnover of organic fractions in soil is less than 1% (Barraclogh et. al., 2005), the release of sequestered fractions will be small to negligible over years. Mineralization accounted for 0.6% to 23.4% in laboratory aerobic and anaerobic metabolism studies. Chemical names, structures, and formation of major and minor degradates are reported in **Appendix D**.

Major degradates (CGA-205375, 1,2,4 triazole, and triazole acetic acid)

Freundlich adsorption coefficient (K_F) values were the only available fate data available for the major degradate, CGA-205375 (values are provided in the **Table 5-1**). The environmental fate properties of 1,2,4-triazole and triazole acetic acid were provided in a recent Registration Review problem formulation for myclobutanil (USEPA, 2015). Abiotic hydrolysis half-lives were 99 to 421 days; it was stable to aqueous photolysis; and biotic degradation was not a major route of 1,2,4-triazole degradation. Microbial degradation of 1,2,4-triazole in aerobic soils was highly varied (half-lives = 20 days to stable). 1,2,4-triazole was moderately persistent in anaerobic soil (half-life = 81 days). The major transformation products of 1,2,4-triazole are triazole acetic acid and hydroxy triazole. Both transformation products are formed through microbial degradation in aerobic soils. 1,2,4-Triazole and its conjugates (triazole alanine and triazole acetic acid) are common metabolites to the class of compounds known as the triazole-derivative fungicides (T-D fungicides, conazoles).

A summary of terrestrial field dissipation data is provided in **Table 5-3**. Dissipation half-lives in reviewed terrestrial field dissipation studies ranged from 139 to 539 days at 5 bare soil sites in the United States. Submitted terrestrial field dissipation studies showed that difenoconazole and its degradates did not leach below 30 cm of soil depth except in two studies where leaching was noted up to 60 cm of the cropped plots soil in NC (turf) and ND (potato).

Overall, terrestrial field dissipation study results indicate that the persistence of difenoconazole is highly dependent on the environmental conditions. While field dissipation studies are designed to capture a range of loss processes; laboratory studies are designed to capture loss from one process (*e.g.,* hydrolysis, aerobic metabolism). Thus, the values from laboratory studies are not directly comparable to the values from the field studies; however, it is informative to have some understanding of how the laboratory data compares to the loss rates in the field dissipation studies.

Table 5-3. Summary of Terrestrial Field Dissipation Data for Difenoconazole

System Details	Half-life (days)	Deepest Core where Difenoconazole is Found (cm)	Source/Study Classification/Comment
GA, Loamy Sand, Bare plot, pH 7.1, 0.5 %OC	231 (SFO-LN)	15-30	MRID 46950126 Acceptable.
CA, Loam, Bare plot, pH 8.2, 0.8 %OC	139 (SFO-LN)	15 -30	MRID 46950127 Acceptable.
ND, Loam, Bare plot/potato, pH 6.0, 2.0 %OC	462 (SFO-LN)	45 -60	MRID 46950129 Supplemental.
CA Loamy sand, Bare plot, pH 7.9, 0.8 %OC	251 (SFO)	7.5-15	MRID 50807401[N] Acceptable.
CA Loamy sand, Turf plot, pH 7.9, 0.8 %OC	Grass: 3 (SFO) Soil: 47 (IORE) Total system (Grass +soil): 22 (IORE)		
NY Sandy loam, Turf plot, pH 6.4, 2.9% OC	Grass: 6.7 (DFOP) Soil: 336 (DFOP) Total system (Grass +Soil): 125 (IORE)	15-30	MRID 50807402[N] Acceptable.
NC Loamy sand, Bare plot, pH 5.8, 0.6 %OC	539 (DFOP)	45 -60	MRID 50807403[N] Acceptable.
NC Loamy sand, Turf plot, pH 6.2, 4.13%OC	Grass: 5.9 (SFO) Soil: 205 (DFOP) Total system (Grass +Soil): 119 (IORE)		

SFO=single first order; DFOP=double first order in parallel; IORE=indeterminate order (IORE); SFO DT_{50}=single first order half-life; T_{IORE}=the half-life of a SFO model that passes through a hypothetical DT_{90} of the IORE fit; DFOP slow DT_{50}=slow rate half-life of the DFOP fit, --=not available or applicable; SFO-LN=SFO calculated using natural log transformed data

[N] Studies submitted since the Problem Formulation was completed are designated with an N associated with the MRID number.

In aquatic field dissipation studies conducted in Louisiana clay and California clay loam soils, difenoconazole dissipated from total systems with half-lives of 212 and 136 days, respectively, which are shorter than laboratory aerobic and anaerobic aquatic metabolism half-lives. While aquatic field dissipation studies are designed to capture a range of loss processes; laboratory studies are designed to capture loss from one process (*e.g.*, hydrolysis, aerobic/anaerobic metabolism). Thus, the values from laboratory studies are not directly comparable to the values from the field studies. Difenoconazole dissipated from rice plants with a half-life value of 17 days following the final application. The major routes of dissipation of difenoconazole under aquatic field conditions were plant uptake and transformation to the minor transformation products CGA71019, CGA205375, and CGA142856.

Table 5-4. Summary of Aquatic Field Dissipation Data for Difenoconazole

System Details	Half-life in paddy (days)[1]	Half-life in plant (days)[2]	Source/Study Classification/Comment
LA, Moreland, Rice plot, pH 7.2, 2.1 %OC	212 (DFOP)	17.2 (IORE)	MRIDs 49804601 & 50213601[N] Acceptable.
CA, Centerville Loam, Rice plot, pH 7.8, 1.3 %OC	136 (DFOP)	17 (SFO)	MRIDs 49804602 & 50213602[N]. Acceptable.

SFO=single first order; DFOP=double first order in parallel; IORE=indeterminate order (IORE); SFO DT_{50}=single first order half-life; T_{IORE}=the half-life of a SFO model that passes through a hypothetical DT_{90} of the IORE fit; DFOP slow DT_{50}=slow rate half-life of the DFOP fit.
[1] Half-life values are based on final application of difenoconazole and in total system of rice paddy.
[2] Half-life values are based on plant residues after final difenoconazole application.
[N] Studies submitted since the Problem Formulation was completed are designated with an N associated with the MRID number.

6.0 Ecotoxicity Summary

Ecological effects data are used to estimate the toxicity of difenoconazole to target species. The ecotoxicity data for difenoconazole and its degradates have been previously reviewed in the Problem Formulation for Registration Review (USEPA 2015). The data cited in the Problem Formulation are summarized briefly in **Sections 6.1** and **6.2.** In addition, the 11 studies received since the DCI was issued in 2015 are discussed briefly in these sections.

In addition to registrant-submitted data, a search of the EFED ECOTOX refresh report, yielded no additional new data from suitable studies with more sensitive (lower) toxicity endpoints than those previously used in risk assessments. **Tables 6-1** and **6-2** summarize the measured toxicity endpoints available across taxa. Non-definitive endpoints are designated with a greater than or less than value. All studies in these tables are classified as acceptable or supplemental.

The Food Quality Protection Act (FQPA) requires EPA to screen pesticide chemicals for their potential to produce effects similar to those produced by estrogen in humans and gives EPA the authority to screen certain other chemicals and to include other endocrine effects. In response, EPA developed the Endocrine Disruptor Screening Program (EDSP). Additional information on the EDSP is available in **Appendix E.**

6.1 Aquatic Toxicity

The following sections summarize the most sensitive acute and chronic toxicity effects of fish and invertebrates, acute toxicity of mollusks, and toxicity of aquatic plants exposed to difenoconazole.

Fish

No new studies with more sensitive endpoints were submitted for fish on an acute or chronic exposure basis since the problem formulation (USEPA, 2015). A summary of the current available fish toxicity studies is provided in **Table 6-1**. Difenoconazole has similar acute toxicity to both freshwater and estuarine/marine fish with LC_{50} values of 810 (rainbow trout) and 819 (sheepshead minnow) µg a.i./L. Due to the lack of availability of chronic toxicity studies on the most acutely sensitive freshwater fish species (rainbow trout), chronic fish endpoints were estimated using an ACR (acute to chronic ratio). The ACR based NOAEC was estimated to be 0.86 µg a.i./L. Due to similar acute toxicity endpoints, ACR-based endpoints for freshwater fish were identical to those for estuarine/marine fish. ACR-based endpoints were used for risk quantification. An early life stage study (MRID 42245115) was available for fathead minnows; however, this study was classified as supplemental due to potential contamination in control replicates. Compared to the solvent control there was 4% reduction in 30-day length at the NOAEC (8.7 µg a.i./L) while only a 2% reduction in length when compared to the negative control. At the LOAEC (19 µg a.i./L) there was a 7% reduction in larval length compared to the solvent control; however, due to control contamination these values are uncertain. In a full life cycle study on fathead minnows (MRID 48453205) a 5% reduction in length was reported at the LOAEC 93.7 µg a.i./L. Therefore, this supports the selection of the life cycle study length endpoint on a chemical effect versus contamination in control.

An additional acute study conducted on guppies (*Poecilia reticulata)* on difenoconazole STK 20 (Difenoconazole + Tea Tree Oil) was also available and established an LC_{50} of 599 µg a.i./L (MRID 5084617). This study was classified as supplemental (test concentrations were not verified), and will be used in combination rainbow trout for assessing acute risk to fish.

Water Column Invertebrates

Since the problem formulation, no new studies were submitted for freshwater or estuarine/marine water column invertebrates. A summary of the available water column invertebrate toxicity studies is provided in **Table 6-1**. The most sensitive acute endpoints for freshwater and estuarine/marine invertebrates are 770 µg a.i./L (*Daphnia magna*) and 150 µg a.i./L (*Americamysis bahia*) respectively. Two acute studies on the Eastern oyster (*Crassostrea virginica*), were also submitted. Acute endpoints were estimated to be 424 µg a.i./L and >300 µg a.i./L respectively (MRID 42906701 & 42245113) and were less sensitive compared to mysid shrimp.

The available daphnid chronic study resulted in a 21-d NOAEC of 5.6 µg a.i./L based on a 21% reduction in the number of young/adult/reproductive day and 5% reduction in adult length (LOAEC = 13.0 µg a.i./L; MRID 42245114).

Two additional chronic studies were available for estuarine/marine invertebrates (MRIDs 49322901 & 49387801). The first study identified a NOAEC of 4.8 µg a.i./L based on a 44% reduction in offspring at 10 µg a.i./L. The second chronic study on mysids, identified a NOAEC <

0.31 µg a.i./L (MRID 47648603). However, this study is classified as supplemental due to no clear dose response pattern and low replication. The low replication led to high variability across most endpoints and decreased statistical power. Upon inspection of this study, the significance of the reduction in the number of young/adult/reproductive day is the only endpoint impacted (NOAEC set to the maximum tested concentration for all other endpoints) and may be an artifact of low replication rather than a chemical effect.

Benthic Invertebrates

In response to the problem formulation, non-guideline chronic life-cycle toxicity studies were submitted on three species of benthic invertebrates (*Hyalella azteca, Chironomus dilutus, and Leptocheirus plumulosus*). The most sensitive endpoint was for the freshwater amphipod (*Hyalella azteca*), with a 21.8% reduction in offspring at the LOAEC of 0.258 mg a.i./g-OC (NOAEC=0.099 mg a.i./g-OC; MRID 50213701). In the *Leptocheirus plumulosus* study, there was a 47% reduction in offspring at the LOAEC of 9.1 mg a.i./g-OC (NOAEC=3.8 mg a.i./g-OC; MRID 50213702). The study on *Chironomus dilutus* (MRID 50213703) was classified as supplemental due to solvent effects which led to uncertainty in interpretation of the male:female ratio endpoint. Compared to the solvent control there was a 50% decrease in male:female LOAEC (31.3 mg a.i./kg-dw); there was only a 25% decrease compared to the negative control; therefore, this endpoint was interpreted with caution (**Table 6-1**).

Aquatic Plants

Available difenoconazole studies with aquatic plants are listed in **Table 6-1.** Since the problem formulation, a newly submitted study was conducted on cyanobacteria (MRID 4985601). This study did not establish an EC_{50} (> 4850 µg a.i/L) and no effects were reported (MRID 4985601). This study was classified as supplemental due to a 27% increase in cell density between the negative and solvent controls, and variability in the negative control; potential solvent effects reduced confidence in the results. No studies were available for aquatic vascular plants (e.g., *Lemna*).

Table 6-1. Summary of the endpoints used for aquatic organism risk quantification and characterization

Type of Study	Test Substance (% a.i.)	Test Species	Toxicity Value (µg a.i./L)	MRID/ Classification
Fish				
Acute - Freshwater Fish 850.1075	TGAI (96% a.i.)	Rainbow trout (*Oncorhynchus mykiss*)	**96-hr LC_{50}=810 µg a.i/L**	**42245107 Acceptable**
	TGAI (96.1% a.i.)		96-hr LC_{50}=1060 µg a.i/L	42245108 Acceptable
	TGAI (96.1% a.i.)	Bluegill Sunfish (*Lepomis macrochirus*)	96-hr LC_{50}=1200 µg a.i/L	42245109 Acceptable

	TGAI (97.3% a.i.)	Fathead Minnow (*Pimephales promelas*)	96-hr LC_{50}=1800 µg a.i/L	48453201 Acceptable
	STK 20 21.0% difenoconazole 38.8% Tea Tree Oil	Guppies (*Poecilia reticulata*)	96-hr LC_{50}: 599 µg a.i./L (difenoconazole) 96-hr LC_{50}: 1,106 µg a.i./L (tea tree oil)	50804617* Supplemental-Test concentrations were not verified
Acute - Estuarine/Marine Fish 850.1075	TGAI (96.1% a.i.)	Sheepshead minnow (*Cyprinodon variegates*)	**96-hr LC_{50}=819 µg a.i/L**	**42245112 Acceptable**
	TGAI (96% a.i.)		96-hr LC_{50}=1100 µg a.i/L	42906702 Acceptable
Chronic-Freshwater Fish 850.1400	TGAI (99.2% a.i.)	Fathead Minnow (*Pimephales promelas*)	*Early Life Stage* NOAEC=8.7 µg a.i/L LOAEC=19 µg a.i/L Based on reduced larval length by 7% (30-days post hatch)	42245115 Supplemental-Contamination was observed in two control replicates and raw data on water. The relative standard deviation for fish weight in one of the control replicates (53%) was unacceptable.
	TGAI (97.4% a.i.)		*Life Cycle* **NOAEC=1.9 µg a.i/L** LOAEC=3.7 µg a.i/L Based on 5% reduced male length of F0 generation 12 weeks post hatch	48453205 Acceptable
	NA	Rainbow Trout (*Oncorhynchus mykiss*)	**NOAEC=0.86** Based on ACR (acute-to-chronic ratio) of fathead minnow data to rainbow trout data (the most acutely sensitive species)[1]	NA
Chronic-Estuarine/Marine fish 850.1400	NA	Sheepshead minnow (*Cyprinodon variegates*)	**NOAEC=0.86** Based on ACR of fathead minnow to sheepshead minnow data[2]	NA
Water-Column Invertebrates				
Acute-Freshwater Invertebrate 850.1010	TGAI (96.1% a.i.)	Water flea (*Daphnia magna*)	**48-hr EC_{50} =770 µg a.i/L**	42245110 Acceptable
Acute-Estuarine/ Marine Invertebrate 850.1035	TGAI (95.0% a.i.)	Mysid shrimp (*Americamysis bahia*)	**48-hr EC_{50} =150 µg a.i/L**	42245111 Acceptable
Acute-Estuarine /Marine Mollusk 850.1025	TGAI (96.0%)	Eastern oyster (*Crassostrea virginica*)	96-hr EC_{50} =424 µg a.i/L	42906701 Acceptable
	TGAI (95.0% a.i.)		96-hr EC_{50}>300 µg a.i/L	42245113 Acceptable

Chronic-Freshwater Invertebrate	TGAI (96.1% a.i.)	Water flea (*Daphnia magna*)	NOAEC=5.6 µg a.i/L LOAEC=13.0 µg a.i/L Based on 21% reduction in number of young/adult/ reproductive day and 5% reduction in adult length	42245114 Supplemental- Daphnid Weight Not recorded
Chronic-Estuarine/ Marine Invertebrate 850.1300	TGAI (94.4% a.i.)	Mysid shrimp (*Americamysis bahia*)	NOAEC < 0.31 µg a.i/L LOAEC ≤ 0.31 µg a.i/L Based on reduced number of young/adult/reproductive/day	47648603 Supplemental- Definitive NOAECs for most sensitive endpoints could not be determined.
	TGAI (96.6% a.i.)		**NOAEC = 4.8 µg a.i/L** **LOAEC = 10 µg a.i/L** Based on a 44% reduction in offspring/female, 18% reduction in F0 post-pairing survival, offspring/female and 5% increase in time to first brood	49322901 & 49387801 Acceptable
Benthic Invertebrates				
Non-Guideline Freshwater Benthic Invertebrate (amphipod) life cycle (spiked sediment)	TGAI (93.9% a.i.)	Freshwater Amphipod (*Hyalella azteca*)	**Pore Water** **NOAEC=0.0074 mg a.i./L** **LOAEC=0.0207 mg a.i./L** Sediment (bulk) NOAEC=1.80 mg a.i./kg dw LOAEC=4.64 mg a.i./kg dw **Sediment (OC-normalized)** **NOAEC=0.099 mg a.i./g OC** **LOAEC=0.258 mg a.i./g OC** Overlying Water NOAEC=0.000224 mg a.i./L LOAEC=0.000582 mg a.i./L *Most sensitive endpoint:* 21.8% decrease reproduction (# offspring per surviving female day 35 and 42) *Other endpoints affected:* Growth (length on day 28)	50213701* Acceptable
Non-Guideline Marine Benthic Invertebrate (amphipod) life cycle (spiked sediment)	TGAI (93.9% a.i.)	Marine Amphipod (*Leptocheirus plumulosus*)	Pore Water NOAEC=0.427 mg a.i./L LOAEC=0.949 mg a.i./L Sediment (bulk) NOAEC=10.2 mg a.i./kg dw LOAEC=24.4 mg a.i./kg dw Sediment (OC-normalized) NOAEC=3.8 mg a.i./g OC	50213702* Acceptable

			LOAEC=9.1 mg a.i./g OC Overlying Water NOAEC=0.015 mg a.i./L LOAEC=0.050 mg a.i./L *Most sensitive endpoint:* 47% decrease in reproduction (# offspring per surviving female day 35 and 42) *Other endpoints affected:* Male and female growth	
Non-Guideline Freshwater Benthic Invertebrate (midge) life cycle (spiked sediment)	TGAI (93.9% a.i.)	Non-biting midge (*Chironomus dilutus*)	Pore Water NOAEC=0.0713 mg a.i./L LOAEC=0.150 mg a.i./L Sediment (bulk) NOAEC=11.9 mg a.i./kg dw LOAEC=31.3 mg a.i./kg dw Sediment (OC-normalized) NOAEC=0.663 mg a.i./g OC LOAEC=1.739 mg a.i./g OC Overlying Water NOAEC=0.00286 mg a.i./L LOAEC=0.00973 mg a.i./L *Most sensitive endpoint:* male:female ratio[A] *Other endpoints affected:* percent emergence and growth male development rate) [A] There is a high degree of uncertainty about this endpoint due to potential solvent effects and an ambiguous response	50213703* Supplemental-Due to potential solvent effects and uncertainty in the interpretation of the results
Aquatic Plants				
Algal Toxicity 850.4500	TGAI (94.4% a.i.)	Freshwater Diatom (*Navicula pelliculosa*)	**96-hr EC_{50} = 98 µg a.i/L** **NOAEC=3 µg a.i/L** **LOAEC=150 µg a.i/L** **Based on 93% reduction in cell density at the LOAEC**	46950208 Acceptable
	TGAI (94.4% a.i.)	Freshwater Algae (*Pseudokirchneriella subcapitata*)	96-hr EC_{50} = 300 µg a.i/L NOAEC=150 µg a.i/L LOAEC=360 µg a.i/L Based on a 56% reduction in cell density	46950212 Acceptable
	TGAI (94.4% a.i.)	Marine diatom	96-hr EC_{50} = 430 µg a.i/L NOAEC<6.3 µg a.i/L	46950210 Acceptable

		(*Skeletonema costatum*)	LOAEC≤6.3 µg a.i/L Reductions in cell density observed across all treatment levels	
Algae 850.4550	TGAI (93.9% a.i.)	Cyanobacteria (*Anabaena flos-aquae*)	IC_{50} > 4850 µg a.i/L NOAEC ≥ 4850 µg a.i/L LOAEC> 4850 µg a.i/L No effects	4985601* Supplemental (Quantitative)-due to potential solvent effects and excessive variability in the negative control
Aquatic Plant Toxicity Using Lemna 850.4400	No data available			

Notes: *Newly submitted studies since the problem formulation (USEPA, 2015); **BOLD**=most sensitive endpoint

[1] Acute toxicity to fathead minnow LC_{50} = 1800 (MRID 48453201); acute toxicity to rainbow trout: LC_{50} = 810 (MRID 42245107), and chronic toxicity to fathead minnow: NOAEC = 1.9 (MRID 48453205)

[2] Acute toxicity to fathead minnow: LC_{50} = 1800 (MRID 48453201); acute toxicity to sheepshead minnow: LC_{50} = 819 µg ai/L (MRID 42245112), and chronic toxicity to fathead minnow: NOAEC = 1.9 µg ai/L (MRID 48453205)

6.2 Terrestrial Toxicity

Birds

No new data were submitted for birds since the problem formulation (USEPA, 2015). Acute oral toxicity tests of TGAI with mallard duck (*Anas platyrhynchos*; MRID 42245105), and canary (*Serinus canaria*; MRID 48453202) reported no effects at concentrations > 2000 mg a.i./kg-bw. Based on these data, difenoconazole is classified as practically non-toxic to birds on an acute oral exposure basis (**Table 6-2**). A subacute dietary study was conducted with bobwhite quail (*Colinus virginianus*; MRID 42245103). The LC_{50} was 4760 mg a.i./kg-diet and sublethal effects included reductions in body weight and food consumption.

The most sensitive chronic endpoint was based on a study with bobwhite quail (MRID 46950202). Reproductive effects (4% reduction in hatchling body weight) were observed at 108 mg a.i./kg-diet (NOAEC=21.9 mg a.i./kg-diet). A chronic mallard study (MRID 42245106) was also available but was classified as supplemental due to the inability to verify statistics and data were not provided on a per pen basis. This study identified a 5% reduction in eggshell thinning at 429.9 mg a.i./kg diet (NOAEC=110.8 mg a.i./kg-diet).

An additional acute study on Japanese Quail (*Coturnix coturnix japonica*) on difenoconazole STK 20 (Difenoconazole + Tea Tree Oil) was recently submitted and estimated a 96-hr LD_{50} > 420 mg a.i./L. This study was classified as supplemental since test concentrations were not verified.

Mammals

No new data were submitted for mammals. However, since the problem formulation, updated 2-generation mammalian toxicity endpoints were provided (personal communication with Minerva Mercado, HED, ToxSAC, June 9, 2020). On a chronic basis, the most sensitive mammalian NOAEC was 250 mg a.i./kg-diet (20 mg a.i./kg-day) and was based on decreased body weight (≥10%) in P (parent) and F1 (first initial generation) females and F1 males and decreased pup weight (6-8% at birth to 29-33% at weaning) at 2500 mg a.i./kg-diet (192 mg a.i./kg-day) (MRID 42090018; **Table 6-2**).

Terrestrial Invertebrates

Five new effects studies were submitted on honey bees. First, an acute oral and contact study (MRID 50367601) conducted with TGAI indicated that difenoconazole is practically non-toxic to honeybees (contact LD_{50}>188 µg a.i./bee; oral LD_{50} > 188 µg a.i./bee). Two chronic adult studies were also submitted. One was conducted with the TGAI (MRID 50727701) and did not report any effects on adult honeybees on a chronic basis (NOAEL > 13 µg a.i./bee-day). However, the adult chronic toxicity test conducted on the difenoconazole EC formulation (MRID 50212903) estimated a NOAEL of 10.6 µg a.i./bee-day based on a 53% increase in day-10 mortality at 21.3 µg a.i./bee-day. In a similar manner, tThe TGAI chronic study for honey bee larvae (MRID 50449801) estimated a 22-day NOAEL of 9.28 µg a.i./larva/day based on 55% reduction in emergence at the LOAEL of 20.9 µg a.i./larva-day. The 8-day NOAEL in this study based on mortality was 9.28 µg a.i./larva/day based on 37.5% mortality. In comparison, the chronic study conducted with difenoconazole EC formulation, estimated an 8-day NOAEL of 3.28 µg a.i./larva-day based on 97% mortality at 3.28 µg a.i./larvae on day 8. The chronic larval study with TGAI failed to define a NOAEL for later developmental endpoints and was terminated on day 8 due to significant larval mortality in the two highest test concentrations.

An additional acute study on adult honeybees with difenoconazole STK 20 (Difenoconazole + Tea Tree Oil) was recently submitted and estimated a 96-hr LD_{50} > 21.0 µg a.i./bee. This study was classified as supplemental since test concentrations were not verified.

Terrestrial Plants

No new data were submitted for terrestrial plants since the last Section 3 New Use Assessment (USEPA, 2019a). Overall, there were no effects to monocot species in seedling emergence studies (MRID 49858602), with the NOAEC and EC_{25} > 0.25 lbs a.i./A respectively. For dicots, there was a 27% reduction in cabbage survival and a 30% reduction in emergence at the highest tested concentration (0.25 lbs a.i./A) and the EC_{25} was approximated at 0.25 lbs a.i./A (**Table 6-2**). In vegetative vigor studies (MRID 49858603), the most sensitive monocot species was ryegrass (EC_{25}-0.368 lbs a.i./A) based on a 16% reduction in dry weight at the LOAEC (0.27 lbs a.i./A) and the most sensitive dicto was lettuce (EC_{25}=0.299 lbs a.i./A) based on a 19 % reduction in dry weight at the LOAEC (0.26 lbs a.i./A). Additional studies were available for terrestrial plants; however, they were classified as supplemental since they had poor

emergence and the highest tested concentration was lower than the maximum single application rate.

Table 6-2. Summary of the endpoints used for terrestrial organism risk quantification and/or characterization

Type of Study	Test Substance (% a.i.)	Test Species	Toxicity Value (µg a.i./L)	MRID/ Classification
Birds				
Acute-Avian Oral Dose	TGAI (97.3% a.i.)	Canary (*Serinus canaria*)	**LD_{50} > 2000 mg a.i./kg-bw**	48453202 Acceptable
	TGAI (96.1% a.i.)	Mallard duck (*Anas platyrhynchos*)	LD_{50} > 2150 mg a.i./kg-bw	42245105 Acceptable
	STK 20 21.0% difenoconazole 38.8% Tea Tree Oil	Japanese Quail (*Coturnix coturnix japonica*)	96-hr LD_{50}: 420 mg a.i./kg bw (difenoconazole) 96-hr LD_{50} > 776 mg a.i./kg bw (tea tree oil)	50804618* Supplemental-test concentrations were not verified
Acute-Avian Dietary	TGAI (95.2%)	Bobwhite Quail (*Colinus virginianus*)	**LD_{50}=4760 mg a.i./kg-diet** NOAEC=625 mg a.i./kg-diet LOAEC=1250 mg a.i./kg-diet Based on a 37% reduction in body weight and 68% reduction in feed consumption	42245103 Acceptable
	TGAI (96.1% a.i.)	Mallard duck (*Anas platyrhynchos*)	LC_{50}>5000 mg a.i./kg-diet NOAEC=625 mg a.i./kg-diet LOAEC=1250 mg a.i./kg-diet Based on mortality *Other sublethal effects:* reduced body weight and feeding	42245104 Acceptable
Chronic-Avian Dietary	TGAI (94.3% a.i.)	Bobwhite Quail (*Colinus virginianus*)	**NOAEC=21.9 mg a.i./kg-diet** **LOAEC=108 mg a.i./kg-diet** **Based on a 4% reduction in hatchling body weight**	46950202 Acceptable
	TGAI (91.9% a.i.)	Mallard duck (*Anas platyrhynchos*)	NOAEC=110.8 mg a.i./kg-diet LOAEC=492.9 mg a.i./kg-diet Based on a 5% increase in eggshell thinning	42245106 Supplemental-statistical analyses could not be verified; report did not provide data on a per pen basis
Mammals				
Acute-Mammalian Oral Dose		Laboratory rat (*Rattus norvegicus*)	**LD_{50}=1453 mg a.i./kg-bw**	42090006 Acceptable
Two Generation Reproduction-Mammalian			**NOAEC=250 mg a.i./kg-diet (20 mg a.i./kg-day)** **LOAEC=2500 mg a.i./kg-diet (192 mg a.i./kg-day)** Based on decreased body weight (≥10%) in P and F1 females and F1	42090018[1] Acceptable

			males; decreased mean pup weight that progressed over time (6-8% at birth to 29-33% at weaning)	
Honey Bees				
Acute Contact-Adult	TGAI (91.1% a.i.)	Honey Bee (*Apis mellifera*)	**LD_{50} > 100 µg a.i./bee**	42245124 Acceptable
	TGAI (93.9% a.i.)		LD_{50}>188 µg a.i./bee	50367601* Acceptable
Acute Oral-Adult 850.3020	TGAI (93.9% a.i.)		**LD_{50} >71.3 µg a.i./bee**	
	STK 20 21.0% difenoconazole 38.8% Tea Tree Oil		96-hr LC_{50} > 21.0 µg a.i./bee (difenoconazole) 96-hr LD_{50} > 38.8 µg a.i./bee (tea tree oil)	50804618* Supplemental-test concentrations were not verified
Chronic Feeding Study-Adult Non-Guideline	TGAI (93.9% a.i.)		*Diet Concentration* 10-d LC_{50} >459 mg a.i./kg-diet 10-d NOAEC=459 mg a.i./kg-diet 10-d LOAEC> 459 mg a.i./kg-diet *Dietary Dose* 10-d LD_{50} >13 µg a.i./bee/day **10-d NOAEL=13 µg a.i./bee/day** 10-d LOAEL>13 µg a.i./bee/day	50727701* Acceptable
	Difenoconazole EC (A7402T) (24.8% a.i.)		*Diet Concentration* 10-d LC_{50} >0.834 g a.i./kg-diet 10-d NOAE= 0.272 g a.i/kg-diet 10-d LOAEC= 0.544 g a.i./kg-diet *Dietary Dose* 10-d LD_{50} = 29.3 µg a.i./bee/day **10-d NOAEL=10.6 µg a.i./bee/day** 10-d LOAEL=21.3 µg a.i./bee/day Based on a 53% increase in day 10 mortality	50212903* Acceptable
Chronic-22-d Repeat Dose Larval Non-Guideline	TGAI (93.9% a.i.)		*Dietary Concentration* Day 8 Mortality 8-d LC_{50}>542 mg a.i./kg-diet 8-d NOAEC=242 mg a.i./kg-diet 8-d LOAEC = 542 mg a.i./kg-diet Day 15 Mortality 15-d LC_{50}=532 mg a.i./kg-diet 15-d NOAEC=242 mg a.i./kg-diet 15-d LOAEC = 542 mg a.i./kg-diet Adult Emergence Rate 22-d EC_{50}= 501 mg a.i./kg-diet 22-d NOAEC=242 mg a.i./kg-diet 22-d LOAEC= 542 mg a.i./kg-diet	50449801* Acceptable

			Dietary Dose Day 8 Mortality **8-d LC_{50}>20.9 µg a.i./larva/day** 8-d NOAEL=9.28 µg a.i./larva/day 8-d LOAEL = 20.9 µg a.i./larva/day Day 15 Mortality 15-d LD_{50}=20.5 µg a.i./larva/day 15-d NOAEL=9.28 µg a.i./larva/day 15-d LOAEL = 20.9 µg a.i./larva/day Adult Emergence Rate 22-d ED_{50}=19.3 µg a.i./larva/day **22-d NOAEL=9.28 µg a.i./larva/day** 22-d LOAEL=20.9 µg a.i./larva/day Based on a 55% reduction in adult emergence	
Chronic 8-d-Repeated Dose toxicity Test Larval	Difenoconazole EC (A7402T) (24.8% a.i.)		*Dietary Concentration* 8-d LC_{50}=45 mg a.i./kg-diet 8-d NOAEC=32.7 mg a.i./kg-diet 8-d LOAEC=83.2 mg a.i./kg-diet *Dietary Dose* **8-d LD_{50}=1.79 µg a.i./larva/day** **8-d NOAEL=1.31 µg a.i./larva/day** 8-d LOAEL= 3.28 µg a.i./larva/day Based on 97% mortality on day 8	50212904* Supplemental-Failed to provide NOAECs for later developmental endpoints, study terminated on day 8 due to significant larval mortality at the highest two test concentrations
Other Terrestrial Invertebrates				
Acute Contact-Terrestrial Invertebrate	TGAI (96.1% a.i.)	Earthworm (*Eisenia fetida*)	14-day LC_{50} > 610 mg a.i./kg-dw soil	42245125 Supplemental-Duration of the test (14 days) was shorter than the recommended 28 days
Terrestrial Plants				
Terrestrial Vascular Plants-Vegetative Vigor Tier II 850.4150	Difenoconazole EC250 (23.6% a.i.)	Monocots: Corn, Onion, Sorghum, and Wheat Dicots: Cabbage, Lettuce, Oilseed rape, Soybean, Sugar Beet, Tomato	Most sensitive monocot: Ryegrass **EC_{25}=0.368 lb a.i./A** EC_{05}=0.204 lb a.i./A **NOAEC=0.13 lb a.i./A** LOAEC= 0.27 lb a.i./A Based on a 16% reduction dry weight Most sensitive dicot: Lettuce **EC_{25}=0.299 lb a.i./A** EC_{05}=0.156 lb a.i./A **NOAEC=0.13 lb a.i./A** LOAEC= 0.26 lb a.i./A	49858603 Acceptable

			Based on a 19% reduction dry weight	
Terrestrial Vascular Plants-Seedling Emergence Tier II 850.4100			Monocot: **EC_{25}>0.25/0.26[2] lb a.i./A** **NOAEC ≥0.25/0.26 lb a.i./A** LOAEC > 0.25/0.26 lb a.i./A No observed effects (all tested species) Dicot **EC_{25}=0.25 lb a.i./A*** **NOAEC =0.13 lb a.i./A** LOAEC > 0.25 lb a.i./A Based on reduced cabbage survival (27% reduction) and emergence (30% reduction). No effects on other species. *A sound EC_{25} could not be calculated therefore the IC25 is an approximation	49858602 Acceptable
Terrestrial Vascular Plants-Vegetative Vigor 850.4100	Inspire (24% a.i.)	Monocots: Corn, Ryegrass, Wheat, Onion *Dicots:* Radish, Cabbage, Lettuce, Sugar Beet, Soybean, Tomato	Monocot and Dicot IC_{25}> 0.123 lb a.i./A No observed effects (all tested species)	48453204 Supplemental – Highest test concentration lower than maximum single application rate & did not establish a NOAEC for all test species.
Terrestrial Vascular Plants-Seedling Emergence 850.4150	Inspire (24% a.i.)		Monocot and Dicot EC_{25}>0.111/0.112 lbs a.i./A[3] NOAEC/LOAEC=0.11/0.112	48453203 Supplemental-Highest test concentration lower than maximum single application rate & poor emergence for sugarbeet control seedlings.

Notes: *Newly submitted studies since the problem formulation (USEPA, 2015); **BOLD**=most sensitive endpoint
[1]Mammalian endpoints updated since the last S3 New Use (USEPA, 2019a); (e-mail communication with Minerva Mercado May 29, 2020)
[2]Corn, ryegrass and wheat were exposed to 0.25 lb a.i./A and onion was exposed to 0.26 lbs a.i./A
[3]Effects at 0.11 lb a.i./A on lettuce, sugar beet and soybean were biologically significant. Lettuce showed reduced emergence (21%), survival (17%), shoot length (26%) and dry weight (24%). Soybean showed reduced shoot length (23%). Sugar beet showed reduced survival (18%).

6.3 Degradate Toxicity

The three major degradates of difenoconazole include CGA-205375, 1,2,4-triazole and triazole acetic acid. Available empirical toxicity data indicate that the degradates 1,2,4-triazole and

triazole acetic acid are less toxic to aquatic organisms than difenoconazole (see **Appendix A**). No empirical aquatic effects data were available for CGA-205375.

A summary of the available toxicity data for non-target terrestrial (birds and mammals) organisms is provided in **Appendix A** as well. No new degradate toxicity studies have been submitted since the problem formulation. A study with 1,2,4-triazole indicated an LD_{50} of 700 mg a.i/kg-bw for birds. However, compared to the parent avian LD_{50} (>2000 mg a.i./kg-bw), it is uncertain if triazole acetic acid is more or less acutely toxic to birds than difenoconazole because none of the available studies for either chemical showed treatment related mortality up to the highest doses tested. Overall, both parent and 1,2,4-triazole are practically non-toxic. No data were available for birds on the other two main difenoconazole degradates. No chronic degradate data were available for birds.

Effects data were available for mammals for all three major difenoconazole degradates. Acute data were available for triazole acetic acid (LD_{50} > 5000 mg a.i./kg-bw) and CGA-205375 (LD_{50}=2309 mg a.i./kg-bw). Based on these studies there was no evidence that these degradates were more toxic than difenoconazole parent (LD_{50} = 1453 mg a.i./kg-bw). No acute studies were available with 1,2,4-triazole; however, a chronic study was available with this degradate. There was uncertainty about the relative chronic toxicity between 1,2,4-triazole and difenoconazole parent to mammals. At 250 mg a.i./kg-diet there were no effects on mammals based on difenoconazole; however, there were significant effects at this concentration in the 1,2,4-triazole study (LOAEC=250 mg a.i./kg-diet). Since 250 mg a.i/kg-diet was the lowest test concentration it was unclear where the no effects concentration was for this degradate. This uncertainty is discussed in the risk characterization section.

6.4 Incident Data

The Incident Data System (IDS) and the Agency's Aggregated Incidents Reports database were reviewed for difenoconazole incidents on May 19, 2020. This search excluded incidents classified as 'unlikely' or 'unrelated' and only includes incidents with the certainty categories of 'possible', 'probable'. Overall, there were 4 possible incidents (**Table 6-3**), 4 unlikely incidents, and 1 unclassified. There were no new reported incidents since the last Section 3 New Use Assessment on Turf (USEPA, 2019a).

Three incidents are reported in IDS for damage (necrotic browning and death) to wine grapes, all with a certainty of "possible" for difenoconazole as a causal agent. Two of the incidents involve application of Revus Top (a dual a.i. fungicide containing 21.9% mandipropamid and 21.9% difenoconazole) and the other incident involved Inspire Super (another dual a.i. fungicide containing 8.4% difenoconazole and 24.1% cyprodinil).

In addition to the incidents recorded in IDS, additional incidents are reported to the Agency in aggregated form. Pesticide registrants report certain types of incidents to the Agency as aggregate counts of incidents occurring per product per quarter. Ecological incidents reported in aggregate reports include those categorized as 'minor fish and wildlife' (W-B), 'minor plant'

(P-B), and 'other non-target' (ONT) incidents. 'Other non-target' incidents include reports of adverse effects to insects and other terrestrial invertebrates. For difenoconazole, there were 10 minor plant incidents associated with the dual a.i. fungicide Revus Top (containing mandipropamid and difenoconazole) and one minor plant incident associated with Quadris Top (another dual a.i. fungicide containing azoxystrobin and difenoconazole). A single aggregate minor wildlife incident was reported and was associated with Cruiser Maxx Vibrance Quattro (a multiple a.i. formulation containing 1.44% sedaxane, 3.45% difenoconazole, 0.72% fludioxonil, 0.86% mefenoxam, and 5.75% thiamethoxam).

The number of actual incidents associated with difenoconazole may be higher than what is reported to the Agency. Incidents may go unreported since side effects may not be immediately apparent or readily attributed to the use of a chemical. Although incident reporting is required under FIFRA Section 6(a)(2), the absence of reports in IDS does not indicate that the chemical has no effects on wildlife; rather, it is possible that incidents are unnoticed and unreported.

Table 6-3. Ecological Incidents Associated with Difenoconazole[1]

Incident/Year	State	Legality	Certainty[2]	Use Site	Species (# Affected)	Distance	Effects
I028250-00037/2015	NY	Registered Use	Possible	Agricultural/ Orchard	wine grapes (40 acres)	Treated Directly	Browning necrosis/death
I028250-00038/2015	NY	Registered Use	Possible	Agricultural/ Orchard	wine grapes (12 acres)	Treated Directly	Browning necrosis/death
028250-0039/2015	NY	Registered Use	Possible	Agricultural/ Orchard	wine grapes (72 acres)	Treated Directly	Browning necrosis/death
I029650-00001/2016	MN	Misuse (accidental)	Possible	Residential/ Suburban	Unknown vegetable/ herbaceous plant	Drift/Spray adjacent	Mortality
I030862-00001/2017	MN	Not specified	Not Specified	Agricultural/ Cropland	Honeybee (40 colonies)	Drift/ Adjacent	Mortality

[1] 'Minor' incidents aggregately reported by the registrant(s) are not included in this table.
[2] Incidents classified as 'unrelated' and 'unlikely' are excluded.

7.0 Analysis Plan

7.1 Overall Process

This assessment uses a weight of evidence approach that relies heavily, but not exclusively, on a risk quotient (RQ) method. RQs are calculated by dividing an Estimated Environmental concentration (EEC) by a toxicity endpoint (*i.e.*, EEC/toxicity endpoint). This is a way to determine if an estimated concentration is expected to be above or below the concentration associated with the effects endpoint. The RQs are compared to regulatory Levels of Concern (LOCs). For acute and chronic risks to animals (vertebrates and invertebrates) the LOCs are 0.5 and 1.0, respectively, with the exception of acute risks to bees (the LOC is 0.4). For plants, the LOC is 1.0. In addition to RQs, other available data (*e.g.*, incident data) can be used to help

understand the potential risks associated with the use of the pesticide. Risk presumptions, along with the corresponding RQs and LOCs are summarized in **Table 7-1**.

Table 7-1. Risk Presumptions and LOCs

Risk Presumption	RQ	LOC
Birds[1]		
Acute Risk	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day	0.5
Chronic Risk	EEC/NOAEC	1
Wild Mammals[1]		
Acute Risk	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day	0.5
Chronic Risk	EEC/NOAEC	1
Aquatic Animals[2]		
Acute Risk	EEC/LC_{50} or EC_{50}	0.5
Chronic Risk	EEC/NOAEC	1
Terrestrial and Semi-Aquatic Plants		
Acute Risk	EEC/EC_{25} or IC_{25}	1
Terrestrial Invertebrates: Honeybees		
Acute Risk	EEC/LC_{50}	0.4
Chronic Risk	EEC/NOAEC	1

[1] LD50/sqft = (mg/sqft) / (LD50 * wt. of animal)
LD50/day = (mg of toxicant consumed/day) / (LD50 * wt. of animal)
[2] EEC = (ppm or ppb) in water

7.2 Modeling

Various models are used to calculate aquatic and terrestrial EECs, as shown in **Table 7-2**.

Table 7-2. List of the Models Used to Assess Risk

Environment	Taxa of Concern	Exposure Media	Exposure Pathway	Model(s) or Pathway
Aquatic	Vertebrates & invertebrates (including sediment dwelling)	Surface water and sediment	Runoff and spray drift to water and sediment	PRZM-VVWM with PWC version 1.52[1] PFAM[3]
	Aquatic Plants (vascular and nonvascular)			
Terrestrial	Vertebrates	Dietary items	Ingestion of residues in/on dietary items because of direct foliar application and ingestion of treated seeds	T-REX version 1.5.2[2]
	Bees and other terrestrial invertebrates	Contact Dietary items	Spray contact and ingestion of residues in/on dietary items as a result of direct application	BeeREX version 1.0

Environment	Taxa of Concern	Exposure Media	Exposure Pathway	Model(s) or Pathway
Bioaccumulation	Birds and mammals	Movement of pesticide through trophic levels	Estimate bioaccumulation of hydrophobic organic pesticides in aquatic food webs and risks to mammals and birds via consumption of contaminated aquatic prey	KABAM (K_{ow}) Based Aquatic BioAccumlation Model version 1.0
All Environments	All	Movement through air to aquatic and terrestrial media	Spray drift	AgDRIFT version 2.1.1 (Spray drift)

[1] The Pesticide in Water Calculator (PWC) is a Graphic User Interface (GUI) that estimates pesticide concentration in water using the Pesticide Root Zone Model (PRZM) and the Variable Volume Water Model (VVWM). PRZM-VVWM.
[2] The Terrestrial Residue Exposure (T-REX) Model is used to estimate pesticide concentration on avian and mammalian food items.
[3]Pesticides in Flooded Applications Model (PFAM) is used to simulate EECs when pesticides are applied to flooded or intermittently flooded ares.

8.0 Aquatic Organisms Risk Assessment

8.1 Aquatic Exposure Assessment

8.1.1 Modeling

Surface water aquatic modeling was simulated using the Pesticide in Water Calculator (PWC version 1.52) for use patterns to terrestrial crops and the Pesticides in Flooded Applications Model (PFAM; version 2.0 dated September 27, 2016) for use on rice. Difenoconazole exposure modeling was performed using the PWC and PFAM models for selected uses. The information concerning the models can be found on the EPA Water Models web-page[4].

Previous aquatic exposure assessments were completed before 2016, and the current version of the PWC model (1.52) is different from the versions used in assessments prior to 2016. It is also now recommended that the daily average value be used to calculate acute risk quotients for aquatic organisms rather than the peak value used in previous risk assessments (USEPA, 2017a). Therefore, revised EECs are warranted to reflect current aquatic exposure models and aquatic modeling guidance.

8.1.2 PWC Input Parameters for Difenoconazole Uses

Input parameters were selected in accordance with EFED's guidance documents (USEPA, 2009; USEPA, 2010a; USEPA, 2010b; USEPA, 2012a; USEPA, 2012b; USEPA, 2013). Since application

[4] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment

rates for foliar application are one to two orders of magnitude higher than that for seed treatment (**Table 3-2**) and post-harvest treatments (**Table 3-3**), high-end aquatic exposure was based on foliar application rates. However, it is unlikely aquatic exposure from post-harvest treatment. In addition, selected crop groups/crop subgroups and individual crops were modeled based on the following criteria:

- Maximum percent (≥ 20%) crop treated based on SLUA report (BEAD, 2019)
- Dominant uses in terms of average annual amounts (≥ 20,000 lbs) of difenoconazole applied, based on the SLUA report (BEAD, 2019)
- Major aquatic use of difenoconazole (*i.e.*, rice) and
- Maximum application rates for non-agricultural uses.

Table 8-1 lists modeled foliar use patterns for crop groups from **Table 3-1,** as well as spray efficiency, spray drift fractions, and model scenarios that were considered in the aquatic exposure assessment. An initial application at 30-days post-emergence was used for all PWC as well as PFAM modeling.

In cases where the difenoconazole labels reported numerous possible variations of application rates, application methods, or formulations for modeled crop scenarios, EFED modeled the rates, methods and formulations that would likely result in the highest Estimated Environmental Concentrations (EECs).

Table 8-1 PWC/PFAM Input Parameters for Maximum Use Patterns of Difenoconazole Foliar Treatments

Use Site	PWC/PFAM Crop Scenarios[A]	Application Method	Maximum Single Application Rate (lbs a.i./A), [*kg a.i./HA*]	Number of Applications per Year (Reapplication Interval; days)[B]	Maximum Annual Application Rate (lbs a.i./A)	Application Efficiency (Spray Drift Fraction)[C]
Agricultural Uses						
1C Tuberous and corm vegetables	IDNpotato_WirrigSTD MEpotatoSTD	Aerial	0.115 [0.13]	4(7) 2 sequential applications	0.46	0.95 (0.125)
		Ground				0.99 (0.062)
5 Brassica (cole) leafy vegetables	FLcabbageSTD	Aerial	0.115 [0.13]	4(7) 2 sequential applications	0.46	0.95 (0.125)
		Ground				0.99 (0.062)
8-10 Fruiting vegetables	FLpeppersSTD FLtomatoSTD_V2 PAtomatoSTD	Aerial	0.115 [0.13]	4(7) 2 sequential applications	0.46	0.95 (0.125)
		Ground				0.99 (0.062)
9 Cucurbit vegetables	FLcucumberSTD MImelonStd MOmelonStd NJmelonStd	Aerial	0.115 [0.13]	4(7) 2 sequential applications	0.46	0.95 (0.125)
		Ground				0.99 (0.062)
	CAcitrus_WirrigSTD	Aerial	0.125	4 (7)	0.50	0.95 (0.125)

Use Site	PWC/PFAM Crop Scenarios[A]	Application Method	Maximum Single Application Rate (lbs a.i./A), [*kg a.i./HA*]	Number of Applications per Year (Reapplication Interval; days)[B]	Maximum Annual Application Rate (lbs a.i./A)	Application Efficiency (Spray Drift Fraction)[C]
10-10 Citrus fruits	FLcitrusSTD	Airblast	[0.14]	2 sequential applications		0.99 (0.022)
11-10 Pome Fruits	NCappleSTD ORappleSTD PAappleSTD_V2	Aerial	0.068 [0.08]	5 (7)	0.34	0.95 (0.125)
		Airblast				0.99 (0.022)
12-12 Stone Fruits	CAfruit_WirrigSTD GAPeachesSTD MICherriesSTD	Aerial	0.115 [0.13]	4 (7) 2 sequential applications	0.46	0.95 (0.125)
		Ground				0.99 (0.062)
13-07F Small fruit vine	CAgrapes_WirrigSTD NYGrapesSTD	Aerial	0.115 [0.13]	4 (10) 2 sequential applications	0.46	0.95 (0.125)
		Airblast				0.99 (0.022)
14-12 Tree nuts	CAalmond_WirrigSTD GAPecansSTD	Aerial	0.115 [0.13]	4 (14) 2 sequential applications	0.46	0.95 (0.125)
		Airblast				0.99 (0.022)
Soybean	MSsoybeanSTD	Aerial	0.11 [0.12]	2	0.22	0.95 (0.125)
		Ground				0.99 (0.062)
Sugar beet	MNsugarbeetSTD	Aerial	0.115 [0.13]	4 (7)	0.46	0.95 (0.125)
		Ground				0.99 (0.062)
Rice	EcoCAWinter.PFS EcoCAWinter.PFS EcoMOnoWinter.PFS EcoMSnoWinter.PFS EcoTXnoWinter.PFS	Aerial	0.123 [0.14]	2 (14)	0.246	Not Applicable[D]
		Ground				
Non-Agricultural Uses						
Ornamental	CAnurserySTD_V2 FLnurserySTD_V2 MInurserySTD_V2 NJnurserySTD_V2 ORnurserySTD_V2 TNnurserySTD_V2	Aerial	0.13 [0.15]	4 (7) 2 sequential applications	0.52	0.95 (0.125)
		Ground				0.99 (0.062)
Turf	FLturfSTD PAturfSTD	Ground	0.43 [0.48]	3 (14) 2 sequential applications	1.29	0.99 (0.062)

[A] All crop scenarios are for PWC model except Rice. Various PFAM scenarios were used for rice
[B] Labels require different mode of action fungicide should be alternated after two sequential applications of difenoconazole or various crops. After two sequential applications of difenoconazole, a period of 7 to 28 days was paused based on reapplication intervals of difenoconazole and label requirement to accommodate application of different mode of action fungicide.
[C] USEPA, 2013b. Guidance on modeling off-site deposition of pesticides via spray drift for ecological and drinking water assessments.
[D] USEPA, 2016. PFAM model Input Guidance Version, 2.1.

Environmental fate input parameter values for difenoconazole are presented in **Table 8-2.** Additional input parameters were selected in accordance with EFED's "Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides," Version 2.1 (USEPA, 2009) and "Guidance for Selecting Input Parameters for Modeling Pesticide Concentrations in Groundwater Using the Pesticide Root Zone Model," Version 1 (USEPA 2013b). Example outputs from the model runs are provided in **Appendix D.**

Table 8-2. PWC Input Parameters for Difenoconazole Physical, Chemical and Environmental Fate Properties

Parameter	Input Value and Unit	Comment	Source
Hydrolysis ($t_{1/2}$) @25°C	0	@ pH 7	MRID 42245127
Aerobic soil metabolism ($t_{1/2}$) @20°C	440 d	Represents the 90th %ile upper confidence limit on the mean of seven half-lives (Table 5-2) of difenoconazole	Input Guidance[1] MRIDs 46950109, 46950110, 46950111, 46950112, 46950113, 46950113 & 46950114
Aerobic aquatic metabolism ($t_{1/2}$) @ 20°C	**Total Water/Sediment System** 781 d	Represents the 90th %ile upper confidence limit on the mean of three half-lives of difenoconazole	Input Guidance[1] MRIDs 46950116, 46950117 & 46950117
Anaerobic aquatic metabolism ($t_{1/2}$) @20°C	**Total Water/Sediment System** 1836 d	One difenoconazole value (612 d) multiplied by 3	Input Guidance[1] MRIDs 46950119 & 46950119
Aquatic photolysis (40°N latitude)	228 d	@pH 7	MRID 46950105
Vapor pressure @ 25°C	2.5 E-10 Torr	--	MRIDs 49577502/49577504
Solubility in water @20°C	3.3 mg/L	--	
Henry's Law constant	1.66E-09 (Unitless)	Estimated value	PWC model
Heat of Henry @ 25°C	54872 J/mol		
Molecular weight	406.3 g/mol	--	--
Partition coefficient K_{FOC}	6115 mL/g	Average of four K_{FOC} values for difenoconazole[2]	MRIDs 46950123 & 46950124

[1] USEPA, 2009. http://www.epa.gov/oppefed1/models/water/input_parameter_guidance.htm
[2] The coefficient of variance (CV) of difenoconazole K_F and K_{FOC} values are 0.97 and 0.56 for respectively, indicating K_{FOC} values (lower CV) are the appropriate input for PWC modeling.

8.1.3 Aquatic Modeling Results

Surface water, pore water, and sediment EECs for difenoconazole are summarized in **Table 8-3**. The maximum 1-in-10-year EECs of **111 µg/L** for the 1-day mean, **30.0 µg/L** for the 21-day **mean**, and **13.8 µg/L** for the 60-day mean were estimated based on the maximum annual application rate of 0.246 lbs/A for rice use. The maximum 1-in-10-year pore water EEC was **13.8 µg/L** for both the 1-day mean and 21-day mean. The 1-in-10-year EEC in bulk sediment was

74,800 µg/Kg for both the 1-day mean and 21-day mean, which was estimated based on the maximum annual use of 0.46 lbs/A for 1C tuberous and corm vegetables. Since the annual application rate of 0.17 lbs/A for seed treatment can be applied in combination with subsequent foliar application on 1C tuberous and corm vegetables, an additional PWC modeling was performed to describe exposure from combined applications of seed treatment and foliar applications. The maximum 1-in-10-year EECs of 14.5 µg/L for the 1-day mean, 13.8 µg/L for the 21-day mean, and 13.1 µg/L for the 60-day mean **are similar** for combined seed and foliar applications of difenoconazole using the MI potato scenario **(Table 8-3)**.

Table 8-3. PWC-generated Surface Water, Pore water, and Sediment EECs for Difenoconazole Uses

Crop/Use	Annual App. Rate (lbs a.i./A)	Application Method	PWC Scenario	1-in-10 year Mean EECs[A]								
				Water Column (µg/L)			Pore Water (µg/L)		Dry Bulk Sediment (µg/kg)[B]		Bulk Sediment-Organic Carbon (µg/kg)[C]	
				1-day	21-day	60-day	1-day	21-day	1-day	21-day	1-day	21-day
Terrestrial Agricultural Uses												
IC Tuberous and corm vegetables	0.46	Aerial	IDpotatoSTD	6.6	6.1	6.0	5.7	5.7	1394	1394	34851	34851
		Aerial	MEpotatoSTD	14.5	13.4	13.1	12.8	12.8	**3136**	**3136**	**78400**	**78400**
		Ground	IDpotatoSTD	4.3	4.1	4.1	3.9	3.9	946	946	23643	23643
		Ground	MEpotatoSTD	12.5	11.5	11.2	11.0	11.0	2695	2695	67375	67375
5 Brassica	0.46	Aerial	FLcabbageSTD	6.5	5.6	5.3	5.6	5.3	1367	1306	34178	32646
		Ground	FLcabbageSTD	5.7	4.6	4.5	4.6	4.5	1137	1105	28420	27624
8-10 Fruiting vegetables	0.46	Aerial	FLpeppersSTD	8.2	6.7	6.5	6.1	6.1	1487	1487	37179	37179
		Aerial	CAtomato_WirrigSTD	3.7	3.1	3.0	2.7	2.7	664	662	16599	16538
		Aerial	FLtomatoSTD_V2	7.8	6.8	6.6	6.2	6.2	1524	1521	38098	38036
		Aerial	PAtomatoSTD	10.1	9.3	8.7	8.4	8.4	2048	2046	51205	51144
		Ground	FLpeppersSTD	7.5	5.9	5.7	5.4	5.4	1321	1321	33014	33014
		Ground	CAtomato_WirrigSTD	2.0	1.8	1.7	1.5	1.5	375	372	9371	9310
		Ground	FLtomatoSTD_V2	7.2	6.1	5.9	5.5	5.5	1355	1352	33871	33810
		Ground	PAtomatoSTD	8.6	7.8	7.2	7.0	7.0	1710	1708	42753	42691
9 Cucurbit	0.46	Aerial	FLcucumberSTD	8.1	6.6	6.4	6.1	6.1	1482	1482	37056	37056
		Aerial	MImelonStd	6.4	5.8	5.6	5.3	5.3	1308	1306	32708	32646
		Aerial	MOmelonStd	7.3	6.5	6.3	6.0	6.0	1468	1465	36689	36628
		Aerial	NJmelonStd	7.1	6.5	6.0	5.7	5.7	1399	1397	34974	34913
		Ground	FLcucumberSTD	7.1	5.9	5.7	5.4	5.4	1311	1311	32769	32769
		Ground	MImelonStd	4.3	3.9	3.8	3.6	3.6	892	892	22295	22295
		Ground	MOmelonStd	6.2	5.4	5.2	5.0	5.0	1218	1215	30441	30380
		Ground	NJmelonStd	5.8	4.8	4.6	4.3	4.3	1061	1058	26521	26460
10-10 Citrus fruits	0.5	Aerial	CAcitrus_WirrigSTD	3.5	3.0	2.8	2.5	2.5	620	620	15496	15496
		Aerial	FLcitrusSTD	6.7	5.9	5.7	5.4	5.4	1325	1325	33136	33136
		Airblast	CAcitrus_WirrigSTD	0.8	0.7	0.6	0.6	0.6	146	146	3644	3644

Crop/Use	Annual App. Rate (lbs a.i./A)	Application Method	PWC Scenario	1-in-10 year Mean EECs[A]								
				Water Column (µg/L)			Pore Water (µg/L)		Dry Bulk Sediment (µg/kg)[B]		Bulk Sediment-Organic Carbon (µg/kg)[C]	
				1-day	21-day	60-day	1-day	21-day	1-day	21-day	1-day	21-day
		Airblast	FLcitrusSTD	5.2	4.3	4.1	4.0	4.0	982	980	24561	24500
11-10 Pome Fruits	0.34	Aerial	NCappleSTD	6.2	5.4	5.2	5.1	5.0	1237	1235	30931	30870
		Aerial	ORappleSTD	4.8	4.5	4.4	4.2	4.2	1027	1027	25664	25664
		Aerial	PAappleSTD_V2	6.1	5.5	5.3	5.0	5.0	1235	1235	30870	30870
		Airblast	NCappleSTD	4.4	3.6	3.4	3.2	3.2	786	786	19661	19661
		Airblast	ORappleSTD	2.3	2.1	2.1	2.0	2.0	490	490	12250	12250
		Airblast	PAappleSTD_V2	4.1	3.7	3.5	3.4	3.4	826	826	20641	20641
12-12 Stone Fruits	0.46	Aerial	CAfruit_WirrigSTD	3.5	3.0	2.8	2.5	2.5	622	622	15558	15558
		Aerial	GAPeachesSTD	4.3	3.5	3.3	3.1	3.1	757	755	18926	18865
		Aerial	MICherriesSTD	10.8	10.0	9.7	9.4	9.4	2303	2301	57575	57514
		Airblast	CAfruit_WirrigSTD	0.8	0.7	0.6	0.6	0.6	142	142	3559	3553
		Airblast	GAPeachesSTD	2.5	1.6	1.4	1.2	1.2	299	299	7473	7473
		Airblast	MICherriesSTD	7.4	6.7	6.5	6.4	6.4	1573	1570	39323	39261
13-07F Small fruit vine	0.46	Aerial	CAgrapes_WirrigSTD	3.5	3.0	2.8	2.6	2.6	625	625	15619	15619
		Aerial	NYGrapesSTD	15.1	13.5	13.1	12.8	12.8	**3136**	**3136**	**78400**	**78400**
		Airblast	Agrapes_WirrigSTD	0.8	0.7	0.7	0.6	0.6	149	149	3724	3718
		Airblast	NYGrapesSTD	12.3	10.8	10.5	10.2	10.2	2499	2499	62475	62475
14-10 Tree nuts	0.46	Aerial	CAalmond_WirrigSTD	4.6	4.1	3.9	3.6	3.6	887	887	22173	22173
		Aerial	GAPecansSTD	7.4	6.1	5.7	5.2	5.2	1281	1281	32034	32034
		Airblast	CAalmond_WirrigSTD	1.6	1.5	1.4	1.3	1.3	328	328	8208	8208
		Airblast	GAPecansSTD	5.7	4.3	3.9	3.6	3.6	877	877	21928	21928
Sugar beet	0.46	Aerial	MNsugarbeetSTD	6.9	6.4	6.2	6.0	6.0	1460	1458	36505	36444
		Ground	MNsugarbeetSTD	4.6	4.3	4.2	4.1	4.1	1000	997	24990	24929
Soybean	0.22	Aerial	MSsoybeanSTD	4.2	3.7	3.5	3.3	3.3	818	816	20458	20396
		Ground	MSsoybeanSTD	3.9	3.4	3.2	3.1	3.1	767	764	19171	19110

Crop/Use	Annual App. Rate (lbs a.i./A)	Application Method	PWC Scenario	1-in-10 year Mean EECs[A]								
				Water Column (µg/L)			Pore Water (µg/L)		Dry Bulk Sediment (µg/kg)[B]		Bulk Sediment-Organic Carbon (µg/kg)[C]	
				1-day	21-day	60-day	1-day	21-day	1-day	21-day	1-day	21-day
Terrestrial Non-Agricultural Use												
Ornamentals	0.52	Aerial	CAnurserySTD_V2	5.4	4.8	4.5	4.2	4.2	1039	1039	25970	25970
		Aerial	FLnurserySTD_V2	6.3	5.0	4.8	4.5	4.5	1093	1090	27318	27256
		Aerial	MInurserySTD_V2	8.3	7.7	7.5	7.2	7.2	1769	1769	44223	44223
		Aerial	NJnurserySTD_V2	8.1	7.4	7.2	6.9	6.9	1686	1686	42140	42140
		Aerial	ORnurserySTD_V2	6.2	5.7	5.4	5.1	5.1	1259	1257	31483	31421
		Aerial	TNnurserySTD_V2	7.5	6.9	6.6	6.3	6.3	1536	1534	38404	38343
		Ground	CAnurserySTD_V2	3.6	3.1	3.0	2.8	2.8	693	691	17334	17273
		Ground	FLnurserySTD_V2	5.5	4.2	3.9	3.6	3.6	884	884	22111	22111
		Ground	MInurserySTD_V2	5.7	5.4	5.3	5.2	5.2	1267	1267	31666	31666
		Ground	NJnurserySTD_V2	6.3	5.6	5.4	5.2	5.2	1276	1274	31911	31850
		Ground	ORnurserySTD_V2	3.7	3.4	3.3	3.2	3.2	779	779	19478	19478
		Ground	TNnurserySTD_V2	5.9	5.3	5.1	4.8	4.8	1186	1186	29645	29645
Turf	1.29	Ground	FLturfSTD	4.9	4.1	3.9	3.7	3.7	899	897	22479	22418
			PAturfSTD	8.7	7.9	7.7	7.3	7.3	1786	1784	44651	44590
Agricultural Aquatic Use												
Rice[D]	0.246	Aerial/ Ground	EcoARnoWinter.PFS	103	24.6	14.5	10.1	9.9	619	618	15475	15450
			EcoCAWinter.PFS	**111**	**30.0**	**19.1**	**13.8**	**13.8**	852	847	21300	21175
			EcoLAnoWinter.PFS	102	23.4	13.6	9.0	8.9	551	546	13775	13650
			EcoMOnoWinter.PFS	102	24.1	14.2	9.7	9.5	594	587	14850	14675
			EcoMSnoWinter.PFS	76.5	22.2	12.8	8.4	8.3	518	512	12950	12800
			EcoTXnoWinter.PFS	102	24.3	14.5	9.8	9.7	602	598	15050	14950
Seed treatment plus foliar applications												
1C Tuberous	0.63	Seed treatment +Aerial	MEpotatoSTD	6.8	6.3	6.1	5.7	5.7	1399	1399	34974	34974
			IDpotatoSTD	14.4	13.5	13.1	12.9	12.8	3161	3136	79013	78400

[A] Bolded concentration are maximum concentrations in surface water, porewater and sediment.

Crop/Use	Annual App. Rate (lbs a.i./A)	Application Method	PWC Scenario	1-in-10 year Mean EECs[A]								
				Water Column (µg/L)			Pore Water (µg/L)		Dry Bulk Sediment (µg/kg)[B]		Bulk Sediment-Organic Carbon (µg/kg)[C]	
				1-day	21-day	60-day	1-day	21-day	1-day	21-day	1-day	21-day

[B] Concentrations of pore water were multiplied by a factor of 245 to obtain dry sediment concentrations. PWC model output provided the conversion factor. The detailed equation of the conversion factor can be obtained from PWC manual
[C] Dry sediment concentrations were normalized for 4% organic carbon content.
[D] PFAM model output provides the bulk dry sediment EEC, then the dry sediment EEC was normalized for 4% organic carbon content.

8.1.4 Monitoring Data (non-targeted sampling)

Monitoring data were queried for difenoconazole and its major degradate (CGA-205375) on May 29, 2020 from the Water Quality Portal (WQP) website[5], which integrates public available water quality data from the USGS National Water Information System (NWIS), the EPA STOrage and RETrieval (STORET) Data Warehouse, and the USDA ARS Sustaining the Earth's Watersheds Agricultural Research Database System (STEWARDS). CGA-205375 has not been included as an analyte for surface water, groundwater, and sediment monitoring programs; therefore, there are no monitoring data available in the portal for this compound. Surface water and groundwater results for difenoconazole are provided in **Table 8-4.** Of the 2,666 surface water samples, only 10 samples had measurable difenoconazole detected, at concentrations which ranged from 0.0084 to 0.249 µg/L. The samples had a detection limit of 0.011 µg/L.

Groundwater samples (1,960) did not find difenoconazole at the detection limit of 0.011 µg/L. Sediment samples (497 bottom material; 381 suspended material) also did not detect difenoconazole at detection limits. Detection limits ranged from 0.6 to 1.0 µg/kg for bottom material; for 12 samples of suspended material were 0.6 µg/kg; and for the remaining 369 samples of suspended material were 0.011 µg/L.

It should be noted that monitored concentrations are not directly comparable to the modeled 1-day mean concentrations, particularly because the study design of NWIS may not account for all difenoconazole use areas, timing of application, and other factors which may more accurately represent spatially and temporally dependent variables influencing runoff vulnerability.

The USDA-Pesticide Monitoring Data (PDP)[6] were also searched on June 01, 2020 for monitoring data for raw and finished surface water samples as well as groundwater sources. Of the 2,678 surface water samples, only 1 sample from raw and 1 sample from finished water samples had a measurable concentration at 0.0006 µg/L. The samples had detection limits of 0.0003 to 0.0012 µg/L.

Table 8-4. Difenoconazole Monitoring Data

Dataset Source	Year	Sample Source	Maximum Concentration (µg/L)	Detection frequency (%) (Detects/Samples)	Sources
NWIS[7]	2009-2019	Surface Water	0.25	0.07 (5/6694)	WQP[A]
		Groundwater	No detection	0 (0/1273)	
		Bottom Sediment		0 (0/497)	
		Suspended sediment		0 (0/381)	

[5] https://www.waterqualitydata.us/
[6] https://apps.ams.usda.gov/PDP
[7] All available data in the California Department of Pesticide Regulation (CDPR) database (http://www.cdpr.ca.gov/docs/emon/surfwtr/surfcont.htm) were included in the aforementioned USGS-NWIS data set. No additional monitoring data for difenoconazole or its degradate were found

Dataset Source	Year	Sample Source	Maximum Concentration (µg/L)	Detection frequency (%) (Detects/Samples)	Sources
PDP	1994-2018	Untreated Water	0.006	< 0.0008 (1/1259)	USDA-PDP[B]
		Finished water	0.006	< 0.0007(1/1419)	
[A] Water Quality Portal (WQP) [B] USDA-Pesticide Monitoring Data					

8.2 Aquatic Organism Risk Characterization

Aquatic EECs were based on parent only concentrations in the water column and compared to the most sensitive endpoints for aquatic taxa (fish, invertebrates and aquatic plants).

8.2.1 Aquatic Vertebrates (Fish)

Difenoconazole has similar acute toxicity on both freshwater and estuarine/marine fish with LC_{50} values of 810 and 819 µg a.i./L for rainbow trout and the sheepshead minnow respectively. Overall, all RQs were less than the LOC for fish on an acute basis (RQs 0.00-0.14). An additional acute study conducted on guppies (*Poecilia reticulata)* on difenoconazole STK 20 (Difenoconazole + Tea Tree Oil) is currently in review (MRID 5084617). This study identified a preliminary LD_{50} of 599 µg a.i./L and exceeded EECs (max 1-day EEC 111 µg a.i./L).

Overall, all modeled scenarios (except CA citrus, CA fruit and CA grapes) had LOC exceedances based on the ACR estimated NOAEC for freshwater fish (RQs ranged from 0.7-22.2) (**Table 8-5**). There were multiple lines of evidence to support chronic risk to fish: 1) RQs generated at the ACR-based LOAEC of 1.7 µg a.i./L exceeded the LOC for all for all scenarios (with the exception of CA citrus, CA fruit, CA grapes and CA almond scenarios with EECs < 1.7 µg a.i./L, 2) A chronic fish toxicity study for fathead minnows (which were less sensitive on an acute basis) had a NOAEC of 1.9 µg a.i./L. Based on this endpoint, the LOC was exceeded for the majority of the modeled scenarios. At the NOAEC, RQs ranged from 0.4-15.8, and 3) RQs were also generated at the empirically based fathead minnow LOAEC of 3.7 µg a.i./L (based on a 5% reduction in male length in F0 generation post-hatch). At the LOAEC, RQs ranged from 0.2-8.1. The few scenarios that did not exceed were California-based (e.g., CA tomatoes, CA grapes, CA citrus, and CA stone fruit). Overall, California based scenarios tended to have lower EECs due to lower rainfall in the region and the drier climate. Due to the persistence of difenoconazole in terrestrial and aquatic environments, repeated use can considerably increase these chronic risks over time.

8.2.2 Aquatic Invertebrates

Water Column Invertebrates

Risks for water column invertebrates were estimated using the most sensitive acute and chronic endpoints for freshwater invertebrates (daphnid acute LC_{50}=770 µg a.i./L; daphnid

NOAEC = 5.6 µg a.i./L) and estuarine/marine invertebrates (acute mysid LC_{50} = 150 µg a.i./L and NOAEC= 4.8 µg a.i./L) respectively.

On an acute basis, there were no LOC exceedances for freshwater invertebrates. All RQs ranged from 0.00-0.14. Aquatic rice-based EECs (max EEC=111 µg a.i./L) for both terrestrial agricultural and non-agricultural applications were 7 times lower than the LC_{50} for freshwater invertebrates.

For estuarine-marine invertebrates, there were no LOC exceedances for both agricultural and non-agricultural terrestrial uses (RQs for these scenarios ranged from 0-0.1. However, EECs for all aquatic uses (e.g., rice) exceeded LOCs based on estuarine/marine acute LC_{50} values and RQs for rice ranged from 0.51-0.74. Oysters were less sensitive to difenoconazole on an acute basis. No RQs based on the oyster IC_{50} of 424 µg a.i./L exceeded LOC and RQs were all less than 0.26.

RQs exceeded chronic LOCs based on the NOAEC for both freshwater and estuarine/marine invertebrates. For freshwater invertebrates, RQs ranged from 0.12-5.36. More specifically, there were no LOC exceedances for all scenarios in which 60-day EECs were less than 5.6 µg a.i./L (**Table 8-6**). In a similar manner, estuarine/marine invertebrate RQs ranged from 0.14-6.25. EECs less than 4.8 µg a.i./L did not exceed the LOC. For terrestrial agricultural and non-agricultural uses, the LOC for freshwater invertebrates on a chronic basis was exceeded for worst case scenarios including potatoes (ID, ME aerial and ME ground), peppers (FL aerial and ground), tomatoes (FL, PA aerial and ground), cucumber (FL aerial and ground), melon (MI, MO and NJ aerial), citrus (FL aerial), cherries (MI aerial and airblast), grapes (NY aerial and Airblast), pecans (GA aerial), sugar beet (MN aerial), nurseries (FL, MI, NJ, OR and TN aerial), and turf (PA ground). Estuarine/marine invertebrates were slightly more sensitive to difenoconazole on a chronic basis compared to freshwater invertebrates. Freshwater invertebrate LOCs also were exceeded in the aforementioned scenarios, and for ground applications to melons (MI and MO) and nurseries (MI, NJ and TN). For estuarine/marine invertebrates there were exceedances that included CA and FL nurseries (aerial applications) and NC and PA apples (aerial applications). There were limited LOC exceedances at the LOAECs for aquatic invertebrates and RQs ranged from 0.05-1.34. At the LOAEC, only the maximum terrestrial agricultural and non-agricultural scenarios had RQs that exceeded the LOC and included potatoes (ME aerial and ground), cherries (MI aerial), and grapes (NY airblast).

All agricultural aquatic scenarios (*e.g.,* rice) for both estuarine/marine invertebrates and freshwater invertebrates exceeded LOCs at both the NOAEC (RQs ranged from 4.0-6.3) and LOAEC (RQs ranged from 1.7- 3.0).

Crops for which chronic risks to aquatic invertebrates were identified included rice, ornamentals, soybeans, sugar beets, tree nuts, small vine fruits (e.g., grapes), potatoes, cabbage, tomato, apples and cucurbits. These crops encompass some of the highest difenoconazole use rates in terms of lbs a.i. applied annually (**Section 3-2**). Like fish, due to the persistence of difenoconazole, repeated use can considerably increase these risks over time.

Table 8-5. Chronic RQs for Fish for ACR and Empirical Based Endpoints

Crop Use	Application Rate (lbs a.i./A)	Application Method	PWC Scenario	1-in-10 yr 60-day Mean EECs µg a.i./L	ACR-Based Fish Chronic Endpoints[1]		Empirical Freshwater Fish Data	
					60-d Mean EEC/ ACR-Based NOAEC= 0.86 µg a.i./L	60-d Mean EEC/ ACR-Based LOAEC= 1.7 µg a.i./L	60-d Mean EEC/Fathead Minnow NOAEC= 1.9 µg a.i./L	60-d Mean EEC/ Fathead Minnow LOAEC= 3.7 µg a.i./L
Terrestrial Agricultural Uses								
IC Tuberous and corm vegetables	0.46	Aerial	IDpotatoSTD	6.0	6.7	3.6	3.2	1.7
		Aerial	MEpotatoSTD	13.1	15.2	7.9	7.1	3.6
		Ground	IDpotatoSTD	4.1	4.7	2.4	2.2	1.1
		Ground	MEpotatoSTD	11.2	13.0	6.8	6.1	3.1
5 Brassica	0.46	Aerial	FLcabbageSTD	5.3	6.2	3.3	2.9	1.5
		Ground	FLcabbageSTD	4.5	5.2	2.7	2.4	1.3
8-10 Fruiting	0.46	Aerial	FLpeppersSTD	6.5	7.5	4.0	3.5	1.8
		Aerial	CAtomato_WirrigSTD	3.0	3.4	1.8	1.7	0.8
		Aerial	FLtomatoSTD_V2	6.6	7.6	4.0	3.6	1.8
		Aerial	PAtomatoSTD	8.7	10.2	5.5	4.9	2.5
		Ground	FLpeppersSTD	5.7	6.7	3.5	3.1	1.6
		Ground	CAtomato_WirrigSTD	1.7	1.9	1.0	0.9	0.5
		Ground	FLtomatoSTD_V2	5.9	6.8	3.6	3.2	1.7
		Ground	PAtomatoSTD	7.2	8.4	4.6	4.1	2.1
9 Cucurbit	0.46	Aerial	FLcucumberSTD	6.4	7.5	3.9	3.5	1.8
		Aerial	MImelonStd	5.6	6.5	3.4	3.1	1.6
		Aerial	MOmelonStd	6.3	7.4	3.8	3.4	1.8
		Aerial	NJmelonStd	6.0	7.0	3.8	3.4	1.7
		Ground	FLcucumberSTD	5.7	6.6	3.5	3.1	1.6
		Ground	MImelonStd	3.8	4.4	2.3	2.1	1.1
		Ground	MOmelonStd	5.2	6.1	3.2	2.8	1.5
		Ground	NJmelonStd	4.6	5.4	2.8	2.5	1.3
10-10 Citrus fruits	0.5	Aerial	CAcitrus_WirrigSTD	2.8	3.2	1.8	1.6	0.8
		Aerial	FLcitrusSTD	5.7	6.6	3.4	3.1	1.6
		Airblast	CAcitrus_WirrigSTD	0.6	0.7	0.4	0.4	0.2
		Airblast	FLcitrusSTD	4.1	4.8	2.6	2.3	1.2
	0.34	Aerial	NCappleSTD	5.2	6.1	3.2	2.9	1.5

Crop Use	Application Rate (lbs a.i./A)	Application Method	PWC Scenario	1-in-10 yr 60-day Mean EECs µg a.i./L	ACR-Based Fish Chronic Endpoints[i]		Empirical Freshwater Fish Data	
					60-d Mean EEC/ ACR-Based NOAEC= 0.86 µg a.i./L	60-d Mean EEC/ ACR-Based LOAEC= 1.7 µg a.i./L	60-d Mean EEC/Fathead Minnow NOAEC= 1.9 µg a.i./L	60-d Mean EEC/ Fathead Minnow LOAEC= 3.7 µg a.i./L
11-10 Pome Fruits		Aerial	ORappleSTD	4.4	5.1	2.7	2.4	1.2
		Aerial	PAappleSTD_V2	5.3	6.1	3.2	2.9	1.5
		Airblast	NCappleSTD	3.4	3.9	2.1	1.9	1.0
		Airblast	ORappleSTD	2.1	2.4	1.2	1.1	0.6
		Airblast	PAappleSTD_V2	3.5	4.0	2.2	1.9	1.0
12-12 Stone Fruits	0.46	Aerial	CAfruit_WirrigSTD	2.8	3.2	1.7	1.6	0.8
		Aerial	GAPeachesSTD	3.3	3.9	2.1	1.9	1.0
		Aerial	MiCherriesSTD	9.7	11.3	5.9	5.3	2.7
		Airblast	CAfruit_WirrigSTD	0.6	0.7	0.4	0.4	0.2
		Airblast	GAPeachesSTD	1.4	1.6	1.0	0.9	0.4
		Airblast	MiCherriesSTD	6.5	7.6	4.0	3.5	1.8
13-07F Small fruit vine	0.46	Aerial	CAgrapes_WirrigSTD	2.8	3.3	1.8	1.6	0.8
		Aerial	NYGrapesSTD	13.1	15.2	7.9	7.1	3.6
		Airblast	CAgrapes_WirrigSTD	0.7	0.8	0.4	0.4	0.2
		Airblast	NYGrapesSTD	10.5	12.0	6.4	5.7	2.9
14-10 Tree nuts	0.46	Aerial	CAalmond_WirrigSTD	3.9	4.5	2.4	2.1	1.1
		Aerial	GAPecansSTD	5.7	6.6	3.6	3.2	1.6
		Airblast	CAalmond_WirrigSTD	1.4	1.6	0.9	0.8	0.4
		Airblast	GAPecansSTD	3.9	4.5	2.5	2.3	1.2
Sugar beet	0.46	Aerial	MNsugarbeetSTD	6.2	7.2	3.8	3.4	1.7
		Ground	MNsugarbeetSTD	4.2	4.9	2.5	2.3	1.2
Soybean	0.22	Aerial	MSsoybeanSTD	3.5	4.1	2.2	1.9	1.0
		Ground	MSsoybeanSTD	3.2	3.7	2.0	1.8	0.9
Terrestrial Non-Agricultural Uses								
Ornamentals	0.52	Aerial	CAnurserySTD_V2	4.5	5.2	2.8	2.5	1.3
		Aerial	FLnurserySTD_V2	4.8	5.6	2.9	2.6	1.4
		Aerial	MInurserySTD_V2	7.5	8.7	4.5	4.1	2.1
		Aerial	NJnurserySTD_V2	7.2	8.3	4.3	3.9	2.0
		Aerial	ORnurserySTD_V2	5.4	6.3	3.3	3.0	1.5

Crop Use	Application Rate (lbs a.i./A)	Application Method	PWC Scenario	1-in-10 yr 60-day Mean EECs µg a.i./L	ACR-Based Fish Chronic Endpoints[1]		Empirical Freshwater Fish Data	
					60-d Mean EEC/ ACR-Based NOAEC= 0.86 µg a.i./L	60-d Mean EEC/ ACR-Based LOAEC= 1.7 µg a.i./L	60-d Mean EEC/Fathead Minnow NOAEC= 1.9 µg a.i./L	60-d Mean EEC/ Fathead Minnow LOAEC= 3.7 µg a.i./L
		Aerial	TNnurserySTD_V2	6.6	**7.6**	**4.1**	**3.6**	**1.9**
		Ground	CAnurserySTD_V2	3.0	**3.5**	**1.8**	**1.6**	0.8
		Ground	FLnurserySTD_V2	3.9	**4.5**	**2.4**	**2.2**	**1.1**
		Ground	MInurserySTD_V2	5.3	**6.2**	**3.2**	**2.8**	**1.5**
		Ground	NJnurserySTD_V2	5.4	**6.2**	**3.3**	**2.9**	**1.5**
		Ground	ORnurserySTD_V2	3.3	**3.9**	**2.0**	**1.8**	0.9
		Ground	TNnurserySTD_V2	5.1	**5.9**	**3.1**	**2.8**	**1.4**
Turf	1.29	Ground	FLturfSTD	3.9	**4.6**	**2.4**	**2.1**	**1.1**
			PAturfSTD	7.7	**8.9**	**4.7**	**4.2**	**2.1**
Agricultural Aquatic Uses								
Rice	0.246	Aerial/ Ground	EcoARnoWinter.PFS	14.5	**16.9**	**14.5**	**12.9**	**6.6**
			EcoCAWinter.PFS	19.1	**22.2**	**17.6**	**15.8**	**8.1**
			EcoLAnoWinter.PFS	13.6	**15.8**	**13.8**	**12.3**	**6.3**
			EcoMOnoWinter.PFS	14.2	**16.5**	**14.2**	**12.7**	**6.5**
			EcoMSnoWinter.PFS	12.8	**14.9**	**13.1**	**11.7**	**6.0**
			EcoTXnoWinter.PFS	14.5	**16.7**	**14.3**	**12.8**	**6.6**

Bold = chronic LOC exceedances (chronic LOC = 1.0)

[1]ACR Based endpoints identical for both freshwater and estuarine/marine fish due to similar acute toxicity endpoints

Table 8-6. Chronic RQs for Water Column Invertebrates

Crop Use	Application Rate (lbs a.i./A)	Application Method	PWC Scenario	1-in-10 yr 21-day Mean EECs µg a.i./L	Freshwater Invertebrates		Estuarine/Marine Invertebrates	
					21-d Mean EEC/ NOAEC= 5.6 µg a.i./L	21-d Mean EEC/ LOAEC= 13 µg a.i./L	21-d Mean EEC/ NOAEC = 4.8 µg a.i./L	21-d Mean EEC/ LOAEC = 10 µg a.i./L
Terrestrial Agricultural Uses								
IC Tuberous and corm vegetables	0.46	Aerial	IDpotatoSTD	6.1	**1.1**	0.47	1.28	0.61
		Aerial	MEpotatoSTD	13.4	**2.34**	**1.0**	**2.8**	**1.3**
		Ground	IDpotatoSTD	4.1	0.74	0.32	0.86	0.41
		Ground	MEpotatoSTD	11.5	**2.1**	0.88	**2.4**	**1.2**
5 Brassica	0.46	Aerial	FLcabbageSTD	5.6	**1.0**	0.43	**1.2**	0.56
		Ground	FLcabbageSTD	4.6	0.83	0.36	0.97	0.46
8-10 Fruiting	0.46	Aerial	FLpeppersSTD	6.7	**1.2**	0.52	**1.4**	0.67
		Aerial	CAtomato_WirrigSTD	3.1	0.56	0.24	0.65	0.31
		Aerial	FLtomatoSTD_V2	6.8	**1.2**	0.52	**1.4**	0.68
		Aerial	PAtomatoSTD	9.3	**1.7**	0.72	**1.9**	0.93
		Ground	FLpeppersSTD	5.9	**1.1**	0.46	**1.2**	0.59
		Ground	CAtomato_WirrigSTD	1.8	0.31	0.14	0.37	0.18
		Ground	FLtomatoSTD_V2	6.1	**1.1**	0.47	**1.3**	0.61
		Ground	PAtomatoSTD	7.8	**1.4**	0.60	**1.6**	0.78
9 Cucurbit	0.46	Aerial	FLcucumberSTD	6.6	**1.2**	0.51	**1.4**	0.66
		Aerial	MImelonStd	5.8	**1.0**	0.45	**1.2**	0.58
		Aerial	MOmelonStd	6.5	**1.2**	0.50	**1.4**	0.65
		Aerial	NJmelonStd	6.5	**1.2**	0.50	**1.4**	0.65
		Ground	FLcucumberSTD	5.9	**1.1**	0.46	**1.2**	0.59
		Ground	MImelonStd	3.9	0.70	0.30	0.82	0.39
		Ground	MOmelonStd	5.4	0.96	0.42	**1.1**	0.54
		Ground	NJmelonStd	4.8	0.86	0.37	**1.0**	0.48
10-10 Citrus fruits	0.5	Aerial	CAcitrus_WirrigSTD	3.0	0.53	0.23	0.62	0.30
		Aerial	FLcitrusSTD	5.9	**1.1**	0.45	**1.2**	0.59
		Airblast	CAcitrus_WirrigSTD	0.7	0.12	0.05	0.14	0.07
		Airblast	FLcitrusSTD	4.3	0.78	0.33	0.90	0.43
11-10 Pome Fruits	0.34	Aerial	NCappleSTD	5.4	0.97	0.42	**1.1**	0.54
		Aerial	ORappleSTD	4.5	0.81	0.35	0.95	0.45

		Aerial	PAappleSTD_V2	5.5	0.98	0.42	**1.2**	0.55
		Airblast	NCappleSTD	3.6	0.65	0.28	0.76	0.36
		Airblast	ORappleSTD	2.1	0.37	0.16	0.44	0.21
		Airblast	PAappleSTD_V2	3.7	0.66	0.28	0.76	0.37
12-12 Stone Fruits	0.46	Aerial	CAfruit_WirrigSTD	3.0	0.53	0.23	0.62	0.30
		Aerial	GAPeachesSTD	3.5	0.63	0.27	0.73	0.35
		Aerial	MiCherriesSTD	10.0	**1.8**	0.77	**2.1**	**1.0**
		Airblast	CAfruit_WirrigSTD	0.7	0.12	0.05	0.14	0.07
		Airblast	GAPeachesSTD	1.6	0.29	0.13	0.34	0.16
		Airblast	MiCherriesSTD	6.7	**1.2**	0.52	**1.4**	0.67
13-07F Small fruit vine	0.46	Aerial	CAgrapes_WirrigSTD	3.0	0.53	0.23	0.62	0.30
		Aerial	NYGrapesSTD	13.5	**2.4**	**1.0**	2.81	1.35
		Airblast	CAgrapes_WirrigSTD	0.7	0.12	0.05	0.14	0.07
		Airblast	NYGrapesSTD	10.8	**1.9**	0.83	**2.3**	**1.1**
14-10 Tree nuts	0.46	Aerial	CAalmond_WirrigSTD	4.1	0.73	0.31	0.85	0.41
		Aerial	GAPecansSTD	6.1	**1.1**	0.47	**1.3**	0.61
		Airblast	CAalmond_WirrigSTD	1.5	0.26	0.11	0.30	0.15
		Airblast	GAPecansSTD	4.3	0.77	0.33	0.90	0.43
Sugar beet	0.46	Aerial	MNsugarbeetSTD	6.4	**1.1**	0.49	**1.3**	0.64
		Ground	MNsugarbeetSTD	4.3	0.77	0.33	0.90	0.43
Soybean	0.22	Aerial	MSsoybeanSTD	3.7	0.66	0.28	0.77	0.37
		Ground	MSsoybeanSTD	3.4	0.60	0.26	0.70	0.34
Terrestrial Non-Agricultural Uses								
Ornamentals	0.52	Aerial	CAnurserySTD_V2	4.8	0.86	0.37	**1.0**	0.48
		Aerial	FLnurserySTD_V2	5.0	0.89	0.38	**1.0**	0.50
		Aerial	MInurserySTD_V2	7.7	**1.4**	0.59	**1.6**	0.77
		Aerial	NJnurserySTD_V2	7.4	**1.3**	0.57	**1.6**	0.74
		Aerial	ORnurserySTD_V2	5.7	**1.0**	0.43	**1.2**	0.57
		Aerial	TNnurserySTD_V2	6.9	**1.2**	0.53	**1.4**	0.69
		Ground	CAnurserySTD_V2	3.1	0.56	0.24	0.65	0.31
		Ground	FLnurserySTD_V2	4.2	0.74	0.32	0.86	0.42
		Ground	MInurserySTD_V2	5.4	0.96	0.41	**1.1**	0.54
		Ground	NJnurserySTD_V2	5.6	0.99	0.43	**1.2**	0.56

		Ground	ORnurserySTD_V2	3.4	0.61	0.26	0.71	0.34
		Ground	TNnurserySTD_V2	5.3	0.95	0.41	**1.1**	0.53
Turf	1.29	Ground	FLturfSTD	4.1	0.73	0.31	0.85	0.41
			PAturfSTD	7.9	**1.4**	0.61	**1.7**	0.79
Aquatic Agricultural Uses								
Rice[D]	0.246	Aerial/ Ground	EcoARnoWinter.PFS	24.6	**4.4**	**1.9**	**5.1**	**2.5**
			EcoCAWinter.PFS	30.0	**5.4**	**2.3**	**6.3**	**3.0**
			EcoLAnoWinter.PFS	23.4	**4.2**	**1.8**	**4.9**	**2.3**
			EcoMOnoWinter.PFS	24.1	**4.3**	**1.9**	**5.0**	**2.4**
			EcoMSnoWinter.PFS	22.2	**4.0**	**1.7**	**4.6**	**2.2**
			EcoTXnoWinter.PFS	24.3	**4.3**	**1.9**	**5.1**	**2.4**

Bold = chronic LOC exceedances (chronic LOC = 1.0)

Invertebrates in Benthic Sediment and Pore Water

Three non-guideline benthic invertebrate life cycle studies were available for difenoconazole. The most sensitive endpoints for freshwater and estuarine/marine invertebrates were based on a NOAEC of **0.0997 mg a.i./g-OC (99,700 µg a.i./kg-OC;** *Hyalella azteca*) and a NOAEC of **3.8 mg a.i./g-OC (3,800,000 µg a.i./kg-OC;** *Leptocheirus plumulosus*). For both life-cycle studies endpoints were based on reductions in the number of offspring. RQs were generated using organic carbon normalized sediment EECs and pore-water EECs based on parent-only measured concentrations.

For freshwater invertebrates, RQs for sediment exposure scenarios ranged from 0.03-0.8 based on the NOAEC. There were no LOC (1.0) exceedances on an organic carbon sediment basis at the NOAEC. However, based on pore-water endpoints for *H. azteca* (NOAEC=7.37 µg a.i./L), RQs ranged from 0.08-1.8. Use sites for which RQs for the pore water EECs exceeded the LOC at the NOAEC included potatoes (ME aerial and ground), tomatoes (PA aerial), cherries (MI aerial), grapes (NY aerial and airblast). There were LOC exceedances for all aquatic agricultural rice scenarios. No LOC exceedances were identified for *H. azteca* based on the LOAEC of 20.7 µg a.i./L in pore water. Another benthic invertebrate life cycle study was available on the midge (*Chironomus dilutus*); however, the results are less sensitive than the study with *H. azteca*. The midge study was classified as supplemental due to solvent effects which led to uncertainty in the interpretation of the male:female ratio endpoint (the most sensitive endpoint in the test) (**Table 6-1**). Therefore, this endpoint should be interpreted with caution.

For the most sensitive estuarine/marine amphipod, *L. plumulosus*, RQs for sediment-based exposure scenarios were less than 0.02 at the NOAEC. In addition, there were no pore water-based LOC exceedances for estuarine/marine amphipods (pore water NOAEC=427 µg a.i./L).

Table 8-7. Chronic RQs for Freshwater Benthic Invertebrates

Crop Use	Application Rate (lbs a.i./A)	Application Method	PWC Scenario	21-day Pore Water EECs (µg a.i./L)	21-day OC-Based Bulk Sediment EECs (µg a.i./kg-OC)	Freshwater Benthic *H.azteca* 21-d Pore Water EEC/ NOAEC=7.4 µg a.i./L
Terrestrial Agricultural Uses						
IC Tuberous and corm vegetables	0.46	Aerial	IDpotatoSTD	5.7	34851	0.77
		Aerial	MEpotatoSTD	12.8	78400	**1.7**
		Ground	IDpotatoSTD	3.9	23643	0.52
		Ground	MEpotatoSTD	11.0	67375	**1.5**
5 Brassica	0.46	Aerial	FLcabbageSTD	5.3	32646	0.72
		Ground	FLcabbageSTD	4.5	27624	0.61
8-10 Fruiting	0.46	Aerial	FLpeppersSTD	6.1	37179	0.82
		Aerial	CAtomato_WirrigSTD	2.7	16538	0.36
		Aerial	FLtomatoSTD_V2	6.2	38036	0.84
		Aerial	PAtomatoSTD	8.4	51144	**1.1**
		Ground	FLpeppersSTD	5.4	33014	0.73

		Ground	CAtomato_WirrigSTD	1.5	9310	0.21
		Ground	FLtomatoSTD_V2	5.5	33810	0.75
		Ground	PAtomatoSTD	7.0	42691	0.94
9 Cucurbit	0.46	Aerial	FLcucumberSTD	6.1	37056	0.82
		Aerial	MImelonStd	5.3	32646	0.72
		Aerial	MOmelonStd	6.0	36628	0.81
		Aerial	NJmelonStd	5.7	34913	0.77
		Ground	FLcucumberSTD	5.4	32769	0.72
		Ground	MImelonStd	3.6	22295	0.49
		Ground	MOmelonStd	5.0	30380	0.67
		Ground	NJmelonStd	4.3	26460	0.58
10-10 Citrus fruits	0.5	Aerial	CAcitrus_WirrigSTD	2.5	15496	0.34
		Aerial	FLcitrusSTD	5.4	33136	0.73
		Airblast	CAcitrus_WirrigSTD	0.6	3644	0.08
		Airblast	FLcitrusSTD	4.0	24500	0.54
11-10 Pome Fruits	0.34	Aerial	NCappleSTD	5.0	30870	0.68
		Aerial	ORappleSTD	4.2	25664	0.57
		Aerial	PAappleSTD_V2	5.0	30870	0.68
		Airblast	NCappleSTD	3.2	19661	0.43
		Airblast	ORappleSTD	2.0	12250	0.27
		Airblast	PAappleSTD_V2	3.4	20641	0.46
12-12 Stone Fruits	0.46	Aerial	CAfruit_WirrigSTD	2.5	15558	0.34
		Aerial	GAPeachesSTD	3.1	18865	0.42
		Aerial	MICherriesSTD	9.4	57514	**1.3**
		Airblast	CAfruit_WirrigSTD	0.6	3553	0.08
		Airblast	GAPeachesSTD	1.2	7473	0.16
		Airblast	MICherriesSTD	6.4	39261	0.87
13-07F Small fruit vine	0.46	Aerial	CAgrapes_WirrigSTD	2.6	15619	0.34
		Aerial	NYGrapesSTD	12.8	78400	**1.7**
		Airblast	CAgrapes_WirrigSTD	0.6	3718	0.08
		Airblast	NYGrapesSTD	10.2	62475	**1.4**
14-10 Tree nuts	0.46	Aerial	CAalmond_WirrigSTD	3.6	22173	0.49
		Aerial	GAPecansSTD	5.2	32034	0.71
		Airblast	CAalmond_WirrigSTD	1.3	8208	0.18
		Airblast	GAPecansSTD	3.6	21928	0.48
Sugar beet	0.46	Aerial	MNsugarbeetSTD	6.0	36444	0.80
		Ground	MNsugarbeetSTD	4.1	24929	0.55
Soybean	0.22	Aerial	MSsoybeanSTD	3.3	20396	0.45
		Ground	MSsoybeanSTD	3.1	19110	0.42
Terrestrial Non-Agricultural						
Ornamentals	0.52	Aerial	CAnurserySTD_V2	4.2	25970	0.57
		Aerial	FLnurserySTD_V2	4.5	27256	0.60
		Aerial	MInurserySTD_V2	7.2	44223	0.98
		Aerial	NJnurserySTD_V2	6.9	42140	0.93
		Aerial	ORnurserySTD_V2	5.1	31421	0.69
		Aerial	TNnurserySTD_V2	6.3	38343	0.85

		Ground	CAnurserySTD_V2	2.8	17273	0.38
		Ground	FLnurserySTD_V2	3.6	22111	0.49
		Ground	MInurserySTD_V2	5.2	31666	0.70
		Ground	NJnurserySTD_V2	5.2	31850	0.70
		Ground	ORnurserySTD_V2	3.2	19478	0.43
		Ground	TNnurserySTD_V2	4.8	29645	0.65
Turf	1.29	Ground	FLturfSTD	3.7	22418	0.49
			PAturfSTD	7.3	44590	0.98
Aquatic Agricultural Uses						
Rice[D]	0.246	Aerial/ Ground	EcoARnoWinter.PFS	9.9	15450	**1.3**
			EcoCAWinter.PFS	13.8	21175	**1.8**
			EcoLAnoWinter.PFS	8.9	13650	**1.2**
			EcoMOnoWinter.PFS	9.5	14675	**1.3**
			EcoMSnoWinter.PFS	8.3	12800	**1.1**
			EcoTXnoWinter.PFS	9.7	14950	**1.2**

Bold = chronic LOC exceedances (chronic LOC = 1.0)

8.2.3 Aquatic Plants

Non-Vascular

For evaluating risks to non-listed species, the highest one-day surface water EEC (**Table 8-2**) was compared to the most sensitive EC_{50} value (freshwater diatom *Navicula pelliculosa*, EC_{50} = 98 µg a.i./L) and the resulting RQ was compared to the LOC of 1.0. RQs for all terrestrial agricultural and non-agricultural uses remained below the LOC of 1.0 and ranged from 0.00-0.15. However, all RQs for the aquatic agricultural use on rice (with the exception of MS Rice) exceeded the LOC of 1.0 and RQs ranged from 0.87-1.40 (**Table 8-8**). The EC_{50} of 98 µg a.i./L is based on a steep dose response curve in which there was a 93% reduction in cell density between the NOAEC (3 µg a.i./L and LOAEC 150 µg a.i./L). Due to the steepness of the dose response relationship, any minor flux in EEC values may dramatically impact the risk profile (from huge impacts to none at all).

EC_{50} values for the less sensitive non-vascular freshwater algae (*Pseudokirchneriella subcapitata*; 300 µg a.i./L) and marine diatom (*Skeletonema costatum;* 430 µg a.i./L) were well above the maximum EEC (1-in-10 year daily average) of 111 µg a.i./L (0.246 lbs a.i./A aerial/ground application CA winter rice). A single study on cyanobacteria was available. EC_{50} was determined to be > 4.85 and no effects were reported. This study was classified as supplemental quantitative due to potential solvent effects. The non-definitive no-effects endpoints does not test up to the highest environmentally relevant concentration and therefore risks at EECs greater than 4.85 remain uncertain. Overall, there are low risk concerns to non-vascular freshwater plants, with the exception of the rice use.

Vascular

No studies were available for vascular aquatic plants with difenoconazole TGAI and risk remains uncertain. There are low risks for non-vascular aquatic plants (rice use only) and the terrestrial plants (all vascular) do not have risk concerns from direct difenoconazole application. Therefore, it is likely that there is a low risk potential (although uncertain) to aquatic vascular plants. Endpoints for other conazole fungicides including metconazole (*Lemna gibba;* EC_{50}=22 µg a.i./L) and prothioconazole (*Lemna gibba*; EC_{50}=350 µg a.i./L), exceeded the EECs for all terrestrial non-agricultural and agricultural difenoconazole uses supporting the potential for low risk to vascular aquatic plants. The metconazole EC_{50} value for *L. gibba* was exceeded by EECs for aquatic agricultural uses (*i.e.,* rice); therefore, risks are expected based on rice use.

Table 8-8. Aquatic Plant RQs for Difenoconazole EECs

Crop Use	Application Rate (lbs a.i./A)	Application Method	PWC Scenario	1-day Mean EECs µg a.i./L	Aquatic Non-Vascular Plants
					1-d Mean EEC/ EC_{50}=98 µg a.i./L
Rice	0.246	Aerial/ Ground	EcoARnoWinter.PFS	103	**1.1**
			EcoCAWinter.PFS	111	**1.1**
			EcoLAnoWinter.PFS	102	**1.0**
			EcoMOnoWinter.PFS	102	**1.0**
			EcoMSnoWinter.PFS	76.5	0.78
			EcoTXnoWinter.PFS	102	**1.0**

Bold = LOC exceedances (LOC = 1.0 for aquatic plants)

9.0 Terrestrial Vertebrate Risk Assessment

9.1 Terrestrial Vertebrate Exposure Assessment

Terrestrial wildlife exposure estimates are calculated for birds and mammals by emphasizing the dietary exposure route of uptake of pesticide active ingredients. These exposures were considered as surrogates for exposures to terrestrial-phase amphibians and reptiles. For exposures to terrestrial organisms, such as birds and mammals, pesticide residues on food items are estimated based on the assumption that organisms are exposed to pesticide residues as a function of the pesticide use pattern. For difenoconazole, terrestrial exposure has been modeled for two methods of application: 1) applied sprays aerial and ground and 2) seed treatments.

9.1.1 Dietary Items on Treated Field

T-REX (v. 1.5.2) is used to calculate dietary- and dose-based EECs of difenoconazole residues on food items for mammals and birds generated for the labeled spray uses. Upper-bound Kenaga nomogram values are used to derive EECs for difenoconazole exposures to terrestrial mammals and birds, based on a 1-year period. Mean Kenaga values (when presented) are used for

additional characterization. Consideration is given to different types of feeding strategies for mammals, including herbivores, insectivores and granivores. Dose-based exposures are estimated for three weight classes of birds (20 g, 100 g, and 1000 g) and three weight classes of mammals (15 g, 35 g, and 1000 g). Terrestrial modeling scenarios were evaluated for single and multiple applications at the minimum (Pome fruit: 0.0684 lbs a.i./A, 5 applications, 7-day application interval) and maximum (Citrus: 0.125 lbs a.i./A, 4 applications, 7-day application interval) agricultural application rates and at the maximum non-agricultural application rate for single and multiple applications (turf: 0.43 lbs a.i./A, 3 applications, 14-day application interval). The 35-day default half-life was used for risk quotient calculations (**Table 9-1**).

Table 9-1. Summary of Dietary- and Dose-based EECs as Food Residues for Birds, Reptiles, Terrestrial-Phase Amphibians and Mammals (T-REX v. 1.5.2, Upper Bound Kenaga)

Food Type	Dietary-Based EEC (mg/kg-diet)	Dose-Based EEC (mg/kg-body weight)					
		Birds			Mammals		
		Small (20 g)	Medium (100 g)	Large (1000 g)	Small (15 g)	Medium (35 g)	Large (1000 g)
Broadcast application of difenoconazole to turf at 0.43 lbs a.i./A for a single application-Maximum Non-Agricultural Rate							
Short grass	103.2	117.5	67.0	30.0	98.4	68.0	15.77
Tall grass	47.3	53.9	30.7	13.7	45.0	31.2	7.23
Broadleaf plants/small insects	58.05	66.1	37.7	16.9	55.4	38.3	8.87
Fruits/pods/ (seeds, dietary only)	6.45	7.35	4.19	1.88	6.15	4.25	0.99
Arthropods	40.4	46.0	26.2	11.8	38.54	26.6	6.18
Seeds (granivore)	NA	1.63	0.93	0.42	1.37	0.94	0.22
Broadcast application of difenoconazole to citrus at 0.125 lbs a.i./A for a single application-Maximum Agricultural Rate							
Short grass	30.0	34.2	19.5	8.72	28.6	19.8	4.58
Tall grass	13.8	15.7	8.93	4.00	13.1	9.06	2.10
Broadleaf plants/small insects	16.9	19.2	10.9	4.91	16.1	11.1	2.58
Fruits/pods/ (seeds, dietary only)	1.89	2.14	1.22	0.55	1.79	1.24	0.29
Arthropods	11.75	13.4	7.63	3.42	11.20	7.74	1.80
Seeds (granivore)	NA	0.47	0.27	0.12	0.4	0.27	0.06
Broadcast application of difenoconazole to pome fruits at 0.0684 lbs a.i./A for a single application-Minimum Agricultural Rate							
Short grass	16.4	18.7	10.6	4.77	15.6	10.8	2.51
Tall grass	7.5	8.57	4.89	2.19	7.17	4.96	1.15
Broadleaf plants/small insects	9.23	10.5	6.0	2.68	8.80	6.08	1.41
Fruits/pods/ (seeds, dietary only)	1.03	1.17	0.67	0.30	0.98	0.68	0.16
Arthropods	6.43	7.32	4.18	1.87	6.13	4.24	0.98
Seeds (granivore)	NA	0.26	0.15	0.07	0.22	0.15	0.03
Broadcast application of difenoconazole to turf at 0.43 lbs a.i./A for turf, 3 applications, 14-day application interval[1]							
Short grass	240.7	274.1	156.3	69.9	229.5	158.6	36.8
Tall grass	110.3	125.6	71.6	32.1	105.2	72.7	16.9

Broadleaf plants/small insects	135.4	154.2	87.9	39.4	129.1	89.2	20.7
Fruits/pods/ (seeds, dietary only)	15.0	17.1	9.77	4.37	14.4	9.91	2.30
Arthropods	94.3	107.4	61.2	27.4	89.9	62.1	14.4
Seeds (granivore)	NA	3.81	2.17	0.97	3.19	2.20	0.51
Broadcast application of difenoconazole to citrus at 0.125 lbs a.i./A, 4 applications, 7-day application interval[2]							
Short grass	98.6	112.3	64.1	28.7	94.1	65.0	1501
Tall grass	45.2	51.5	29.4	13.2	43.1	29.8	6.91
Broadleaf plants/small insects	55.5	63.2	36.0	16.1	52.9	35.6	8.48
Fruits/pods/ (seeds, dietary only)	6.17	7.02	4.00	1.79	5.88	4.06	0.94
Arthropods	38.6	44.0	25.1	11.2	36.8	25.5	5.90
Seeds (granivore)	NA	1.56	0.89	0.40	1.31	0.90	0.21
Broadcast application of difenoconazole to pome fruit at 0.0684 lbs a.i./A, 5 applications, 7-day application interval[3]							
Short grass	63.4	72.2	41.2	18.4	60.5	41.8	9.69
Tall grass	29.1	33.1	18.9	8.45	27.7	19.2	4.44
Broadleaf plants/small insects	35.7	40.6	23.2	10.4	34.0	23.5	5.45
Fruits/pods/ (seeds, dietary only)	3.69	4.51	2.57	1.15	3.78	2.61	0.61
Arthropods	24.8	28.3	16.1	7.22	23.7	16.4	3.79
Seeds (granivore)	NA	1.00	0.57	0.26	0.84	0.58	0.13

[1]Maximum difenoconazole application rate for turf is 1.29 lbs a.i./A. If applied at the maximum single application rate, If applied at the maximum single application rate it can be applied no more than 3 times.
[2]Maximum difenoconazole application rate for citrus is 0.5 lbs a.i./A. If applied at the maximum single application rate it can be applied no more than 3 times.
[3]Maximum difenoconazole application rate for pome fruit is 0.342 lbs a.i./A. If applied at the maximum single application rate, it can be applied no more than 5 times.

9.1.2 Spray Drift

Spray drift footprint modeling considered off-field risk in which birds and mammals are exposed to single and multiple difenoconazole spray applications. No on-field acute risks were identified; therefore, the spray drift assessment was only applied to chronic exposures. The AgDRIFT model (v. 2.1.1) and the RQs generated with TREX were used to estimate the potential distances from the edge of the use area to the chronic LOCs as a result of spray drift deposition off-field.

9.1.3 Seed Treatment Application

In addition to spray applications, difenoconazole is applied as a seed treatment (see **Table 3-2**). Therefore, potential dietary exposure for terrestrial wildlife in this assessment is also based on possible dietary ingestion of difenoconazole residues on treated seeds. For treated seeds, the label lists all application rates in terms of lbs a.i./lb seed, while T-Rex input values are in terms of fl oz/cwt. Different seed sizes and planting rates could also result in ranges of exposure due to variability in numbers of seeds planted per acre. In order to account for variability in seeding

rates (built into T-Rex) and seed size to get the amount of a.i./A, the application rate was converted to fl oz/cwt using the product density of 9.51 lbs a.i./gallon (as stated on the labels).

Maximum application rate (fl oz/cwt) =

$$\frac{maximum\ application\ rate\ (lb\ a.i./A)\ /\ (\%\ a.i.\ in\ formulation/100))\ *\ 128\ fl\ oz/gal\ *\ 100\ lb\ seed/cwt}{maximum\ seeding\ rate\ (lb\ seed/A)\ *\ product\ density\ (lb\ a.i./gallon)}$$

For assessing acute risk related to treated seeds, a dose-based RQ is calculated, where the exposure metric is an estimated ingested dose (mg a.i./kg-bw) based on the pesticide concentration on the treated seed and the food ingestion rate. An area-based RQ, analogous to an LD50/ft^2 is also calculated based on the mass of active ingredient per unit area (square foot). This method simply compares the amount pesticide expected to be present in a square foot to the acute LD_{50} and does not include any specific estimation of pesticide ingested doses. Chronic risks are estimated using a "diet-based" approach by comparing the concentration of pesticide on the treated seed divided by the chronic diet-based NOAEC Dietary-based (pesticide concentration on seed/toxicity endpoint) and acute area-based (LD50/ft^2) RQs were generated following the equations below. Risk Quotient calculations were grouped based on crop group seed treatment application rate and crop types.

Acute #1 = [(seed application rate (mg a.i./kg-seed)*daily food intake (g/day)*0.001 kg/g/bodyweight of animal (kg)/Adjusted body weight toxicity endpoint

Acute #2= [(Application Rate (lbs a.i./A)*100,000 mg/kg)/343,560 ft2*2.2 lb/kg)]/
Adjusted LD50

Chronic Bird RQ=mg/kg-seed/NOAEC

Chronic Mammalian mg a.i./kg-bw-day/adjusted NOAEL

Characterization of the risk posed by seed treatments followed the methodology of USEPA, 2016 with the following modifications: a) calculation of the number of seeds to reach an acute threshold of concern was modified to reflect the LOC (0.5), b) foraging time equations were modified to reflect the equations originally presented in Benkman and Pulliam (1988) with modifications to accurately represent passerine consumption rates for known dietary items (*e.g.* removal of chipping sparrow data that gave unrealistically large foraging times for known seed dietary items under the original equations) and c) minimum and maximum bounds around the foraging area and foraging time of concern were used, replacing the previous mean estimates.

9.1.4 KABAM Inputs

The consumption of aquatic organisms that have accumulated difenoconazole may serve as an additional exposure route for higher trophic level organisms. Risk through consumption of contaminated food was evaluated and modeled using the KABAM model (v. 1.0).

Bioaccumulation is a pathway of concern because of difenoconazole's high bioconcentration factor of up to 330X in whole bluegill sunfish (MRID 42245142) and octanol-water partition coefficient (Log Kow = 4.2). When KABAM is run with the assumption that difenoconazole does not metabolize, the estimated BCF in fish (i.e.,762X) is 2X higher than the empirical fish BCF for whole fish. The elimination rate constant value (k_T = 60 d^{-1}) from the laboratory BCF study is also much higher than the sum of the KABAM estimated loss rate constants (*i.e.*, $k_2 + k_G + k_E$ = 0.21 d^{-1}; based on fish weight (bluegill, average weight of 1.38g) , lipid content (default value) and water temperature (17°C) from the BCF study). This information suggests that metabolism may be influencing the potential bioaccumulation of difenoconazole. Therefore, metabolism of difenoconazole by the fish was estimated as outlined in the KABAM guidance. The metabolic rate of difenoconazole in fish was refined to take this into account by taking the rate constant estimated by the study (0.81 d^{-1}) and subtracting the model generated k_T of 0.21 d^{-1} to produce a refined rate constant of 0.60 d^{-1}.

The difenoconazole EEC pore water and water column values were selected for difenoconazole reflecting the time closest to the steady state. These EECs will reflect difenoconazole low solubility and high K_{FOC} values. A suite of scenarios was run using the 21-day pore and water column EECs (steady state) for difenoconazole. The scenarios modeled the minimum and maximum agricultural EECs across applications. Inputs to the model are summarized below **(Table 9-2 and 9-3)**.

Table 9-2. KABAM Model Inputs

Characteristic	Value	Comments/Guidance
Pesticide Name	Difenoconazole	
Log Kow	4.2	Enter value from acceptable or supplemental study submitted by registrant or available in scientific literature.
K_{FOC} (L/kg OC)	6115	Input value used in PRZM/EXAMS to derive EECs. Follow input parameter guidance for deriving this parameter value (USEPA 2002).
Time to steady state (TS; days)	7	No input necessary. This value is calculated automatically from the Log Kow value entered above.
Pore water EEC (µg/L)	See Table 9-3	21-day value
Water Column EEC (µg/L)	See Table 9-3	21-day value
Difenoconazole Most sensitive Effects Endpoints		
Avian	LD50 (mg/kg-bw)	2000[1]
	LC50 (mg/kg-diet)	4760
	NOAEC (mg/kg-diet)	21.9
Mammalian	LD50 (mg/kg-bw)	1453
	LC50 (mg/kg-diet)	NA
	NOAEC (mg/kg-diet)	250

[1]Non-definitive endpoint for birds

Table 9-3. KABAM EEC inputs

Scenario	Crop	Application Rate, Method	PWC Scenario/ Concentration
Min 21-day Water Column Concentration (µg/L)[1]	10-10 Citrus fruits Airblast 0.5 lbs a.i./A	0.7 µg/L	CAcitrus_WirrigSTD CAfruit_WirrigSTD
Min 21-day Pore Water Concentration (µg/L)[1]	10-10 Citrus fruits Airblast 0.5 lbs a.i./A	0.6 µg/L	CAcitrus_WirrigSTD
Max 21-day Water Column Concentration	Rice Aerial/Ground246 lbs a.i./A	30.0 µg/L	EcoCAWinter.PFS
Max 21-day Pore Water Concentration	Rice Aerial/Ground, 0.246 lbs a.i./A	13.8 µg/L	MEpotato_STD

[1]Three scenarios (CA citrus, fruits and grapes provide a minimum 21-D EECs of 0.7 µg/L for water column and 0.6 µg/L for pore water.

9.2 Terrestrial Vertebrate Risk Characterization

Residues found on dietary food items and treated seeds following difenoconazole application are compared to effects endpoints.

9.2.1 Foliar Applications On-Field Dietary Risk

Birds

Acute and Subacute Dietary

Acute dose-based exposure RQ values were not calculated for birds, since definitive endpoints are not available. No avian mortalities were reported at concentrations tested for acute oral studies in canary and mallard studies; LD_{50} values were greater than 2000 mg a.i./kg bw. Based on EECs for a single difenoconazole application at the maximum rate of 0.43 lb/A, there was no evidence that residues exceeded the tested concentrations. The maximum EEC for a single application at the maximum application rate was 117.5 mg/kg-bw (small birds consuming shortgrass), which is lower than the non-definitive acute oral endpoint for all species. At the highest application scenario, the LD_{50} would have to be over 17 times more sensitive than the non-definitive endpoints presented in order to exceed the non-listed species LOC at the maximum single difenoconazole application rate. In combination with acute data for difenoconazole degradates 1,2,4-triazole (LD_{50} = 770 mg a.i./kg-bw) and triazole acetic acid (LD_{50} > 1926 mg a.i./kg-bw), no additional risk concerns were identified and were well below EECs. As difenoconazole applications increased from single to multiple application scenarios EECs remained well below acute endpoints. On a subacute dietary basis, RQs (0.00-0.06) did

not exceed the non-listed acute LOC based on the LC_{50} of 4760 mg a.i./kg-diet (bobwhite quail). The subacute dietary based LC_{50} would have to be approximately 20 times more sensitive in order to trigger risk concerns. Overall there were no risks on a subacute dietary basis and acute dose basis for non-listed avian exposure to difenoconazole.

An additional acute study on Japanese Quail (*Coturnix coturnix japonica*) on difenoconazole STK 20 (Difenoconazole + Tea Tree Oil) is currently in review (MRID 50804618). This study identified a preliminary LD_{50} estimate of >420 mg a.i./kg-bw and was no more sensitive than the current endpoints identified for risk assessment.

Chronic

Overall, using the NOAEC (21.9 mg a.i./kg-diet) RQs started to exceed chronic LOCs when difenoconazole was applied at a single application of 0.125 lbs a.i./A (birds consuming short grass RQ=1.37). At the maximum non-agricultural application rate 0.43 lbs a.i./A for a single application there were chronic LOC exceedances across all foraging groups with the exception of birds consuming fruits/pods and seeds (RQs 0.29-4.71). EECs following all multiple application scenarios exceeded the NOAEC for all scenarios (with the exception of birds consuming fruits/pods). Additional evidence for the exceedances was also observed with the mean Kenaga values following a similar pattern of LOC exceedances. (**Table 9-4**). Exceedances at the LOAEC of 108 mg a.i./kg-diet (based on a 4 % reduction in hatchling body weight; MRID 46950202), only exceeded LOCs at the maximum non-agricultural application rate (turf 0.43 lbs a.i./A) for a single application for birds consuming short and tall grass as well as broadleaf plants and small insects. No mean Kenaga RQs exceeded LOCs at the LOAEC. According to data from BEAD presented in **Section 3**, the highest amount of active ingredient is used on major crops including soybeans (100,000 lbs a.i., single application rate 0.11 lbs a.i./A), sugar beets (30,000 lbs a.i.; single application rate 0.115 lbs a.i./a) and almonds (20,000 lbs a.i.; single application rate 0.115 lbs a.i./A). For all of the major crops, difenoconazole is applied at application rates that trigger risk concerns for birds on a chronic basis.

In order to further characterize chronic avian risk, the duration of exposure to residues on avian dietary items that result in potential risks of concern (short grass, arthropods, tall grass, broadleaf plants and fruits/pods/seeds) was assessed (**Table 9-5).** For single applications, exposure estimates exceeded chronic LOCs for up to 80 days after application (applications at 0.43 lbs a.i./A; residues on short grass). For multiple application scenarios chronic LOCs were generally exceeded after the 1st application at the lowest assessed rate (0.0684 lbs a.i./Ax 5 apps) for birds feeding on short grass. At the maximum rate, after the first application, chronic LOC exceedances did not occur for birds consuming fruits/pods/seeds but remained for all other food sources, with the longest duration for birds foraging on short grass--up to 150 days.

Chronic avian exposure estimates exceeded test concentrations that elicited effects on hatchling body weight. Thus, if the parents are exposed at any time during this exposure window, there is a potential for effects in the F_1 generation including reduced chick weights. The duration of time where exposure exceeded effect levels is similar to duration of exposure in mammalian toxicity

study prior to the observed effect. Given that the effects were observed in the F_1 generation, the parental exposure may have resulted in these effects.

Risks of concern are indicated for small birds consuming short grass, tall grass, broadleaf plants and arthropods (**Table 9-4**). Since birds are surrogates for terrestrial-phase amphibians and reptiles, it would be assumed that the risk conclusions might impact only herbivorous amphibians and reptiles and the arthropods but not those that consume fruits/pods/seeds.

Table 9-4. Chronic Dietary-Based RQ Values for Birds from Labeled Uses of Difenoconazole

Food Type	Avian Chronic Dietary-Based RQ NOAEC =21.9 mg a.i./kg-diet Upper Kenaga (Mean Kenaga)	Avian Chronic Dietary-Based RQ LOAEC =108 mg a.i./kg-diet[4] Upper Kenaga
Broadcast application of difenoconazole to turf at 0.43 lbs a.i./A for a single application-Maximum Non-Agricultural Rate		
Short grass	**4.71 (1.67)**	0.96
Tall grass	**2.16** (0.71)	0.44
Broadleaf plants/small insects	**2.65** (0.88)	0.54
Fruits/pods/ (seeds, dietary only)	0.29 (0.14)	0.06
Arthropods	**1.85 (1.28)**	0.37
Broadcast application of difenoconazole to citrus at 0.125 lbs a.i./A for a single application-Maximum Agricultural Rate		
Short grass	**1.37** (0.49)	0.28
Tall grass	0.63 (0.21)	0.13
Broadleaf plants/small insects	0.77 (0.26)	0.16
Fruits/pods/ (seeds, dietary only)	0.09 (0.04)	0.02
Arthropods	0.54 (0.37)	0.11
Broadcast application of difenoconazole to pome fruits at 0.0684 lbs a.i./A for a single application-Minimum Agricultural Rate		
Short grass	0.75 (0.27)	0.15
Tall grass	0.34 (0.11)	0.07
Broadleaf plants/small insects	0.42 (0.14)	0.09
Fruits/pods/ (seeds, dietary only)	0.05 (0.02)	0.01
Arthropods	0.29 (0.20)	0.06
Broadcast application of difenoconazole to turf at 0.43 lbs a.i./A for turf, 3 applications, 14-day application interval[1]		
Short grass	**10.99 (3.89)**	**2.23**
Tall grass	**5.04 (1.65)**	**1.02**
Broadleaf plants/small insects	**6.18 (2.06)**	**1.25**
Fruits/pods/ (seeds, dietary only)	0.69 (0.32)	0.14
Arthropods	**4.30 (2.98)**	0.87
Broadcast application of difenoconazole to citrus at 0.125 lbs a.i./A, 4 applications, 7-day application interval[2]		
Short grass	**4.50 (1.60)**	0.91
Tall grass	**2.06 (0.68)**	0.42
Broadleaf plants/small insects	**2.53 (0.84)**	0.51
Fruits/pods/ (seeds, dietary only)	0.28 (0.13)	0.06
Arthropods	**1.76 (1.22)**	0.36
Broadcast application of difenoconazole to pome fruit at 0.0684 lbs a.i./A, 5 applications, 7-day application interval[3]		
Short grass	**2.90 (1.03)**	0.59

Food Type	Avian Chronic Dietary-Based RQ NOAEC =21.9 mg a.i./kg-diet Upper Kenaga (Mean Kenaga)	Avian Chronic Dietary-Based RQ LOAEC =108 mg a.i./kg-diet[4] Upper Kenaga
Tall grass	**1.33** (0.43)	0.27
Broadleaf plants/small insects	**1.63** (0.54)	0.33
Fruits/pods/ (seeds, dietary only)	0.18 (0.08)	0.04
Arthropods	**1.13** (0.78)	0.23

Bold text indicates the chronic LOC of 1 was exceeded for birds.
[1]Maximum difenoconazole application rate for turf is 1.29 lbs a.i./A. If applied at the maximum single application rate, If applied at the maximum single application rate it can be applied no more than 3 times.
[2]Maximum difenoconazole application rate for citrus is 0.5 lbs a.i./A. If applied at the maximum single application rate it can be applied no more than 3 times.
[3]Maximum difenoconazole application rate for pome fruit is 0.342 lbs a.i./A. If applied at the maximum single application rate, it can be applied no more than 5 times.
[4]Exceedances at the LOAEC only occurred at the upper Kenaga.

Table 9-5. Estimate of Days Exceeding the Avian Risk LOC by Foraging Group and Application Rate for Dietary Based RQs

Application Rate	Days Exceeding the LOC				
	Short Grass	Tall Grass	Broadleaf Plants	Arthropods	Fruits/Pods/Seeds
Single application at 0.0684 lbs a.i./A	Does not exceed for this foraging group	Does not exceed for this foraging group	Does not exceed for this foraging group	Does not exceed for this foraging group	Does not exceed for this foraging group
Single application at 0.125 lbs a.i./A	20	Does not exceed for this foraging group	Does not exceed for this foraging group	Does not exceed for this foraging group	Does not exceed for this foraging group
Single application at 0.43 lbs a.i./A	80	40	50	30	Does not exceed for this foraging group
0.43 lbs a.i./A, 3 applications, 14-day application interval	150	115	120	100	Does not exceed for this foraging group
0.125 lbs a.i./A, 4 applications, 7-day application interval	100	Day 7-60 (only after 2nd application)	Day 7-70 (only after 2nd application)	Day 14-50 (only after 3rd application)	Does not exceed for this foraging group
0.0684 lbs a.i./A, 5 applications, 7-day application interval	Day 7-90 (after 1st application)	Day 28-45 (only after 4th application)	Day 21-60 (only after 3rd application)	Day 28-40 (only after 5th application)	Does not exceed for this foraging group

Mammals

Acute and Subacute Dietary

Acute exposure based RQ values were calculated for mammals based on the most sensitive LD_{50} of 1453 mg a.i./kg-bw for mortality in male rats. Acute dose-based endpoints did not exceed

mammalian non-listed species LOCs for risk concerns at the maximum difenoconazole single application rates and across multiple application scenarios (all dose based RQs < 0.07). Subacute dietary toxicity data were not available for mammals; therefore, risks from this route of exposure could not be evaluated.

Chronic

Overall, using the mammalian chronic endpoints (NOAEC=250 mg a.i./kg-diet, NOAEL=20 mg a.i./kg-bw-day) there were no dietary-based chronic LOC exceedances across all application scenarios. Dietary based RQs ranged from 0.004-0.96. However, on a dose basis there were LOC exceedances for the maximum single application rate for small mammals consuming short and tall grass and broadleaf plants/small insects, and medium and large mammals consuming short grass (RQs ranged from 0.002-2.24) (**Table 9-6**). For multiple application scenarios at the lowest application rate (0.0684 lbs a.i./A x 3 applications x 14-day application interval), RQs exceeded the LOC for small, medium and large mammals consuming short grass. For multiple applications at the maximum agricultural application rate (0.125 lbs a.i./A x 4 applications x 7-day application interval), risk concerns were identified for small and medium mammals consuming short grass and broadleaf plants/small insects. Finally, for multiple applications at the maximum overall application rate (turf--0.43 lbs a.i./A x 3 applications x, 14-day application interval), there were LOC exceedances for small and medium mammals consuming short, tall grass and broadleaf plants/small insects (**Table 9-6**). Mean Kenaga values for the RQs which exceeded LOCs were also evaluated. Overall, no LOC exceedances were calculated for the mean Kenaga with the exception of the small and medium mammals consuming short grass at the turf application rate. Overall, there were no exceedances at the LOAEC at the upper bound and mean Kenaga (192 mg a.i./kg-bwt/2500 mg a.i./kg-diet). According to data from BEAD presented in **Section 3**, the highest amount of active ingredient is used on major crops including soybeans (100,000 lbs a.i., single application rate 0.11 lbs a.i./A), sugar beets (30,000 lbs a.i.; single application rate 0.115 lbs a.i./a) and almonds (20,000 lbs a.i.; single application rate 0.115 lbs a.i./A). For all the major crops for which difenoconazole is registered, the maximum application rates trigger risk concerns for mammals on a chronic basis.

Overall, there was uncertainty regarding the relative chronic toxicity between 1,2,4-triazole and difenoconazole to mammals. At 250 mg difenoconazole/kg-diet, no adverse effects were observed in mammals; however, there were significant effects at this concentration in the 1,2,4-triazole study (LOAEC=250 mg 1,2,4-triazole/kg-diet); since this was the lowest test concentration, the no effects concentration is uncertain. Chronic risk concerns were identified for difenoconazole for several application rates. Chronic risks from exposure from 1,2,4-triazole may also result in risk, and this would likely manifest in and extend the duration (i.e., extended time of exposure to toxicant) and the magnitude of the risk identified with the parent.

In order to further characterize chronic mammalian risk, the duration of exposure of residues on dietary items was assessed based on the most conservative EECs (small birds consuming short grass) at each application rate (all other food items have lower EECs). For single applications, dose-based exceedances occurred from 0 to 41 days post-application and for

multiple applications dose-based exceedances occurred from 0 to >56 days post-application depending on application rate. The majority of the LOC exceedances were associated with herbivorous mammals it can be assumed that the risk conclusions would impact only herbivorous mammals in the wild.

Table 9-6. Chronic Dietary and Dose-Based RQ Values for Mammals from Labeled Uses of Difenoconazole

Food Type	Mammalian Chronic Dietary-Based RQ NOAEC = 250 mg a.i./kg-diet Upper Kenaga	Mammalian Dose Based Chronic RQs NOAEL =20 mg a.i./kg-bw Upper Kenaga (Mean Kenaga)		
		Small (15g)	Medium (35g)	Large (1000g)
Broadcast application of difenoconazole to turf at 0.43 lbs a.i./A for a single application-Maximum Non-Agricultural Rate				
Short grass	0.41	**2.24** (0.79)	**1.91** (0.68)	**1.02** (0.36)
Tall grass	0.19	**1.03** (0.34)	0.88	0.47
Broadleaf plants/small insects	0.23	**1.26** (0.42)	1.08	0.58
Fruits/pods/ (seeds, dietary only)	0.03	0.14	0.12	0.06
Arthropods	0.16	0.88	0.75	0.40
Seeds	NA	0.03	0.03	0.01
Broadcast application of difenoconazole to citrus at 0.125 lbs a.i./A for a single application-Maximum Agricultural Rate				
Short grass	0.12	0.65	0.56	0.30
Tall grass	0.06	0.30	0.25	0.14
Broadleaf plants/small insects	0.07	0.37	0.31	0.17
Fruits/pods/ (seeds, dietary only)	0.01	0.04	0.03	0.02
Arthropods	0.02	0.25	0.22	0.12
Seeds	NA	0.01	0.01	0.004
Broadcast application of difenoconazole to pome fruits at 0.0684 lbs a.i./A for a single application-Minimum Agricultural Rate				
Short grass	0.07	0.36	0.30	0.16
Tall grass	0.03	0.16	0.14	0.07
Broadleaf plants/small insects	0.04	0.20	0.17	0.09
Fruits/pods/ (seeds, dietary only)	0.004	0.02	0.02	0.01
Arthropods	0.03	0.14	0.12	0.06
Seeds	NA	0.005	0.004	0.002
Broadcast application of difenoconazole to turf at 0.43 lbs a.i./A for turf, 3 applications, 14-day application interval[1]				
Short grass	0.96	**5.22** **(1.85)**	**4.46** **(1.58)**	**2.39** (0.85)
Tall grass	0.44	**2.39** (0.78)	**2.04** (0.67)	**1.10** (0.36)
Broadleaf plants/small insects	0.54	**2.94** (0.98)	**2.51** (0.84)	**1.34** (0.45)
Fruits/pods/ (seeds, dietary only)	0.06	0.33	0.28	0.15
Arthropods	0.38	2.04	1.75	0.94
Seeds	NA	0.07	0.06	0.03
Broadcast application of difenoconazole to citrus at 0.125 lbs a.i./A, 4 applications, 7-day application interval[2]				

Food Type	Mammalian Chronic Dietary-Based RQ NOAEC = 250 mg a.i./kg-diet Upper Kenaga	Mammalian Dose Based Chronic RQs NOAEL =20 mg a.i./kg-bw Upper Kenaga (Mean Kenaga)		
		Small (15g)	Medium (35g)	Large (1000g)
Short grass	0.39	**2.14** (0.76)	**1.83** (0.65)	0.98
Tall grass	0.18	0.98	0.84	0.45
Broadleaf plants/small insects	0.22	**1.20** (0.40)	**1.03** (0.34)	0.55
Fruits/pods/ (seeds, dietary only)	0.02	0.13	0.11	0.06
Arthropods	0.15	0.84	0.72	0.38
Seeds	NA	0.03	0.03	0.01
Broadcast application of difenoconazole to pome fruit at 0.0684 lbs a.i./A, 5 applications, 7-day application interval[3]				
Short grass	0.25	**1.38** (0.49)	**1.17** **(0.42)**	0.63
Tall grass	0.12	0.63	0.54	0.29
Broadleaf plants/small insects	0.14	0.77	0.66	0.35
Fruits/pods/ (seeds, dietary only)	0.02	0.09	0.07	0.04
Arthropods	0.10	0.54	0.46	0.25
Seeds	NA	0.02	0.02	0.01

Bold text indicates the chronic LOC of 1 was exceeded for mammals.
*Mean Kenaga Values were only presented for RQs which had LOC exceedances at the maximum Kenaga
[1]Maximum difenoconazole application rate for turf is 1.29 lbs a.i./A. If applied at the maximum single application rate, it can be applied no more than 3 times.
[2]Maximum difenoconazole application rate for citrus is 0.5 lbs a.i./A. If applied at the maximum single application rate it can be applied no more than 3 times.
[3]Maximum difenoconazole application rate for pome fruit is 0.342 lbs a.i./A. If applied at the maximum single application rate, it can be applied no more than 5 times.

9.2.2 Spray Drift Risk

Spray drift footprint modeling was conducted for taxa that had on-field LOC exceedances only (*i.e.*, chronic birds and mammals) for single and multiple difenoconazole spray applications. The AgDRIFT model (v. 2.1.1) and the RQs generated with TREX were used to estimate the potential distances to the LOC for non-listed birds as a result of spray drift deposition off-field. The footprint of off-field spray drift from the agricultural field was calculated by following the *Environmental Fate and Effects Division Offsite Transport Guidance* (USEPA, 2013c). The fraction of applied difenoconazole for terrestrial animals was calculated using risk quotients from T-REX that reflect the modeling completed for the on-field risk quantification discussed above. Single and multiple applications of either 0.0684, 0.125 and 0.43 lbs a.i./A (ground broadcast and aerial applications) were considered.

For birds there were no chronic risks at a single application of 0.0684 lbs a.i./A, so drift distances were not evaluated.
For single applications at 0.125 and 0.43 lbs a.i./A difenoconazole drift distances (i.e., the distance from the edge of the field to a distance where the RQ is equal to the LOC; within this distance RQs > LOC) ranged from 3 ft for all medium/coarse droplet sizes (ground boom

applications; see **Table 9-7**) to 7 to 13 feet for high boom very fine to fine droplets. Spray drift distances were also modeled for multiple ground applications for birds and mammals. For multiple applications at the minimum application rate, drift distances to the LOC for birds ranged from 3 ft (multiple scenarios) to 30 ft (high boom fine to very fine droplets at 0.43 lbs a.i./A, 3 applications, 14-day application interval). Drift distances were also evaluated at the minimum and maximum aerial application rates for chronic risks to birds. Drift distances for aerial applications ranged from 7ft (coarse to very coarse droplets at 0.0684 lbs a.i./A, 5 applications, 7-day application interval) to 112 ft (very fine to fine droplets at single application at 0.125 lbs a.i./A) (**Table 9-8**).

For mammals there were no chronic risks at a single application rate of 0.0684 and 0.125 lbs a.i./A, so drift distances were only evaluated at the maximum single application rate (0.43 lbs a.i./A) and all multiple ground application rate scenarios. The drift distance to exceed mammalian chronic LOC was 16 feet from the edge of the field (ground boom application at high boom, very fine to fine droplet sizes, at 0.43 lbs a.i./A, 3 applications, 14-day application interval. Aerial application drift distances were at the rates registered for aerial applications and did not exceed 6.56 feet from the edge of the field (**Table 9-8**).

Drift resulting from multiple applications may increase the probability of offsite dietary exposure to wildlife; however, consideration of a single application provides the most plausible exposure condition due to drift as wind direction and speed may vary during subsequent applications. When modeling spray drift events, the assumption is that the wind is blowing at the same speed in the same direction. For ground applications, the model is limited to assessing very fine to fine and fine to medium droplet sizes. However, low boom height, larger droplets and lower wind speed are known to reduce drift distances from the edge of the field. The magnitude and direction of winds may change during application for some locations. Therefore, the estimates provided below (**Tables 9-7 and 9-8**) represent high end estimates of risks.

Table 9-7. Spray drift distances to the non-listed chronic LOC for birds and mammals for broadcast ground spray from agricultural uses[1]

	Application Rate	Ground Application Drift Distances to the Non-Listed Chronic LOC (ft)			
		Low Boom Very Fine to Fine	Low Boom Fine to Medium/ Coarse	High Boom Very Fine to Fine	Hight Boom Fine to Medium/Coarse
Birds	Single application at 0.0684 lbs a.i./A	NA			
	Single application at 0.125 lbs a.i./A	3.28	3.28	3.28	3.28
	Single application at 0.43 lbs a.i./A	6.56	3.28	13.12	3.28
	0.0684 lbs a.i./A, 5 applications, 7-day application interval	3.28	3.28	9.84	3.28

	Application Rate	Ground Application Drift Distances to the Non-Listed Chronic LOC (ft)			
		Low Boom Very Fine to Fine	Low Boom Fine to Medium/ Coarse	High Boom Very Fine to Fine	Hight Boom Fine to Medium/Coarse
	0.125 lbs a.i./A, 4 applications, 7-day application interval	6.56	3.28	13.12	3.28
	0.43 lbs a.i./A, 3 applications, 14-day application interval	25.3	3.28	29.5	25.56
Mammals	Single application at 0.0684 lbs a.i./A	NA			
	Single application at 0.125 lbs a.i./A	NA			
	Single application at 0.43 lbs a.i./A	3.28	3.28	6.56	3.28
	0.0684 lbs a.i./A, 5 applications, 7-day application interval	3.28	3.28	3.28	3.28
	0.125 lbs a.i./A, 4 applications, 7-day application interval	3.28	3.28	6.56	3.28
	0.43 lbs a.i./A, 3 applications, 14-day application interval	6.56	3.28	16.4	3.28

[1]NA: Drift distances were not calculated due to no LOC exceedances for on-field risk.

Table 9-8. Spray drift distances to the non-listed chronic LOC for birds for aerial spray from agricultural uses[1]

	Application Rate	Aerial Application Drift Distances to the Non-Listed Chronic LOC (ft)			
		Very Fine to Fine	Fine to Medium	Medium to Coarse	Coarse to Very Coarse
Birds	Single application at 0.0684 lbs a.i./A[2]	NA	NA	NA	NA
	Single application at 0.125 lbs a.i./A	112	29.5	19.7	16.4
	0.0684 lbs a.i./A, 5 applications, 7-day application interval	36.1	9.84	9.84	6.56
	0.125 lbs a.i./A, 4 applications, 7-day application interval	105	26.3	19.7	16.4
Mammals	Single application at 0.0684 lbs a.i./A	NA			
	Single application at 0.125 lbs a.i./A	NA			
	0.0684 lbs a.i./A, 5 applications, 7-day application interval	0	0	0	0

	Application Rate	Aerial Application Drift Distances to the Non-Listed Chronic LOC (ft)			
		Very Fine to Fine	Fine to Medium	Medium to Coarse	Coarse to Very Coarse
	0.125 lbs a.i./A, 4 applications, 7-day application interval	6.56	3.28	3.28	3.28

[1]Note, the maximum application for non-agricultural uses of 0.43 lbs a.i./A is not applied aerially and therefore was not assessed. NA: Drift distances were not calculated due to no LOC exceedances for on-field risk.
[2]Drift distances were not calculated for birds at a single application of 0.32 lbs a.i./A due to no LOC exceedances for on-field risk and for mammals at a single application of 0.0684 lbs a.i./A and 0.125 lbs a.i./A due to no on-field risk.

9.2.3 Seed Treatments

Seed treatment uses were modeled in T-Rex for all labeled uses presented in **Section 3.2.** Estimations of the number of oat, rye and triticale seeds needed to consume to trigger risk are not available as the information on the number of seeds per pound was not available. However, wheat is considered to be representative of these crops.

Birds

As discussed for the foliar spray use risk characterization, acute dose based RQs were not calculated since definitive endpoints were not available. On an acute basis, using the non-definitive avian LD_{50} of 2000 mg a.i./kg-bw, there was no evidence that residues approached the acute non-listed avian toxicity non-definitive endpoint. The maximum seed treatment EEC (for cotton at 1.45 fl oz/100 lb seed) of 353.4 mg a.i./kg-bw/day for small birds was about 6 times lower than the most sensitive avian non-definitive endpoint. Chronic endpoints for birds were well defined with a NOAEC of 21.9 mg a.i/kg-diet and LOAEC of 108 mg a.i./kg-diet. There were non-listed chronic LOC exceedances for all labeled seed treatment application rates for difenoconazole (RQs 1.11-16.13; **Table 9-10**). RQs also exceeded chronic LOCs at the avian LOAEC (108 mg a.i./kg-diet) for all labeled uses except potatoes (RQs ranged from 0.23-3.27). Additional characterization was conducted to determine the number of seeds that would need to be consumed to trigger risk concerns following methodology described in USEPA 2016 and the modifications discussed above. For birds, the numbers of seeds required to reach the chronic LOC for seed treatment uses based on the NOAEC ranged from 4-5 seeds (small birds; barley seeds) to 2166 for larger birds consuming corn seeds (large birds) (**Table 9-11**). Despite an estimated consumption of only 1-3 potato seeds necessary to exceed LOC (large birds), potatoes are planted at depth (4-8 inches) and any surface availability would most likely be due to potential spillage. These larger pieces of potato chunks may be consumed by waterfowl including mallards and geese (Kear, 1962).

Mammals

On an acute basis, RQs generated based on the mammalian LD_{50} of 1453 mg a.i./kg-bw for mortality in male rats did not exceed the chronic LOC of 0.5 on an acute basis (RQs ranged from 0.00-0.06). Chronic endpoints for mammals were well defined with a NOAEC of 250 mg a.i/kg-diet. There were non-listed chronic LOC exceedances for small and medium mammals for

barley, oats, triticale, wheat, rye, corn and rapeseed, and only small mammals for cotton (RQs 0.05-1.46). Additional characterization was conducted to determine the number of seeds that would need to be consumed to trigger risk concerns following methodology in USEPA 2016 and the modifications discussed above. The number of seeds (except seed potato[8]) required to reach the mammalian chronic LOC for seed treatment uses based on the NOAEC ranged from 11 (small mammals consuming corn seed) to 12893 seeds (large mammals consuming rapeseeds).

Overall, chronic exposure from difenoconazole seed treatment has some inherent uncertainties. Actual exposure from ingesting treated seeds is dependent on the number of treated seeds available at the surface and how closely they are planted to each other. Once seeds are planted at depth (e.g., potato chunks), the main exposure route would be limited to spillage. The actual difenoconazole exposure is also dependent on foraging behavior, size and timing of consumption. Newly planted fields, which are likely open and providing little cover for smaller foraging birds and mammals, may be less likely to pose a risk to seed eating than those of the no till variety based on foraging behavior (assuming no cover = no forage). Despite these uncertainties, chronic RQs for birds and mammals exceed LOCs and there is potential for risk from consumption of difenoconazole treated seeds.

Table 9-9. EECs for Seed Treatment Application Rates for Comparison with Toxicity Endpoints

Crop	Application Rate (lbs a.i./lb seed) (fl oz/100 lbs seed)	Maximum Seed Application Rate (mg a.i./kg seed)	Avian Nagy Dose (mg a.i./kg-bw/day) (Small, Medium, Large)	Mammalian Nagy Dose (mg a.i./kg bw/day) (Small, Medium, Large)
Potatoes	0.0000242 lb a.i./lb seed 0.1 fl oz/100 lb seed	24.4	6.17	5.16
			3.52	3.57
			1.57	0.83
Barley, Oats, Rye, Triticale, Wheat, Corn	0.000242 lb a.i./lb seed 1.0 fl oz/100 lb seed	243.7	61.7	51.6
			35.2	35.7
			15.8	8.27
20A Rapeseed	0.000242 lb a.i./lb seed 1.24 fl oz/100 lb seed	302.2	76.5	64.0
			43.6	44.2
			19.5	10.3
Cotton	0.000351 lb a.i./lb seed 1.45 fl oz/100 lb seed	353.4	89.4	74.9
			51.0	51.7
			22.8	12.0

Table 9-10. Summary of seed treatment RQs and the number of seeds to trigger an effect for avian chronic risk for the labeled uses of difenoconazole

Application Rate (lbs a.i./lb seed) (fl oz/100 lbs seed)	Crop	Chronic RQ	Number of Seeds to Trigger Risk Concerns		
			Small	Medium	Large
0.00000242 lb a.i./lb seed 0.1 fl oz/100 lb seed	Potatoes	1.11	Seed Size too Big	Seed Size too Big	1-3
0.000242 lb a.i./lb seed 1.0 fl oz/100 lb seed	Barley[1]	11.13	4-5	22-106	220-1307
	Oats		NA[2]		
	Rye				
	Triticale				

	Wheat[3]		5-8	26-42	257-420
0.0003 lb a.i./lb seed 1.0 fl oz/100 lb seed	Corn[4]		Seed Size too Big	Seed Size too Big	42-105
0.000242 lb a.i./lb seed 1.24 fl oz/100 lb seed	20A Rapeseed	13.8	34-43	170-217	1695-2166
0.000351 lb a.i./lb seed 1.45 fl oz/100 lb seed	Cotton	16.13	Seed size too Big	7	72

[1]Barley (malt or beer) (2-row)
[2]NA=Reliable weight data not available for oats, rye, and triticale
[3]Estimates bounded between hard red winter and soft red winter wheat seeding rates
[4]Sweet Corn

Table 9-11. Summary of seed treatment RQs and the number of seeds to trigger an effect for mammalian chronic risk for the labeled uses of difenoconazole

Application Rate (lbs a.i./lb seed) (fl oz/100 lbs seed)	Crop	Chronic RQ			Minimum Number of Seeds to Trigger Risk Concerns		
		Small	Medium	Large	Small	Medium	Large
0.00000242 lb a.i./lb seed 0.1 fl oz/100 lb seed	Potatoes	0.12	0.10	0.05	No RQ exceedance		
0.000242 lb a.i./lb seed 1.0 fl oz/100 lb seed	Barley[1]	**1.17**	**1.00**	0.54	56-60	106-112	1307-1390
	Oats				NA[2]		
	Rye						
	Triticale						
	Wheat[3]				66-107	124-202	1529-2502
0.0003 lb a.i./lb seed 1.0 fl oz/100 lb seed	Corn[4]				11-27	20-51	250-626
0.000242 lb a.i./lb seed 1.24 fl oz/100 lb seed	20A Rapeseed	**1.46**	**1.24**	0.67	432-553	816-1043	10090-12893
0.000351 lb a.i./lb seed 1.45 fl oz/100 lb seed	Cotton	**1.70**	0.45	0.78	18	35	431

[1]Barely (malt or beer) (2-row)
[2]NA=Reliable weight data not available for oats, rye, and triticale
[3]Estimates bounded between hard red winter and soft red winter wheat seeding rates
[4]Sweet Corn

9.2.4 Bioaccumulation in Birds and Mammals

The bioaccumulation modeling did not evaluate each individual use but was conducted in a way to represent the high- and low-end scenarios. Model input EECs were first selected from PWC scenarios which produced the minimum EECs for water column and pore water applications of difenoconazole.

Overall, there were no acute-based risk concerns for birds or mammals in the aquatic food web. Based on the maximum water column and pore water values, KABAM estimated EECs for birds and mammals did not exceed non-definitive acute avian and mammalian endpoints (maximum avian and mammalian dose-based EECs ranged up to 25.6 mg a.i./kg-day). LD_{50} values of >2000

mg a.i./kg-bw and >1453 mg a.i/kg-bw for birds and mammals respectively were several orders of magnitude greater than EECs.

Based on minimum and maximum annual EECs and a refined metabolism elimination rate, there were no acute or chronic LOC exceedances in the aquatic food-web based model (KABAM)[8]. All RQs were < 0.73.

10.0 Terrestrial Invertebrate Risk Assessment

An updated bee risk analysis was conducted based on a suite of effects data recently submitted since the 2015 problem formulation and the most recent Section 3 New Use Assessment.

10.1 Bee Exposure Assessment

Difenoconazole is a systemic fungicide and expected to be taken up by the roots of the plants and move to pollen and nectar. Exposure can also occur though foliar applications of difenoconazole at the time the crop is blooming. The primary exposure routes are from direct spray onto foraging bees (*i.e.,* contact) as well as through dietary (oral) exposure of contaminated nectar and pollen. These exposure routes apply to both on field applications to blooming bee-attractive crops as well as off-site drift to bee-attractive vegetation. Difenoconazole is also registered for foliar application use on a suite of crops that are pollinator attractive (*e.g.,* tree nuts, ornamentals, citrus, and fruiting vegetables). EECs (pollen, nectar and contact) were estimated using the BeeRex v 1.0 model[9] at the minimum single application rate and the maximum single application rate for foliar applications.

Table 10-1. Oral and Contact EECs for Honeybees Estimated using BeeREX

Application Rate	Maximum Dietary Dose	Contact Exposure
Minimum difenoconazole Foliar Single Application at 0.0684 lbs a.i./A for pome fruits	2.3 µg a.i./bee	0.1841 µg a.i./bee
Maximum difenoconazole Foliar Single Application at 0.13 lbs a.i./A for ornamentals[1]	4.2 µg a.i./bee	0.351 µg a.i./bee

[1]Maximum rate of 0.43 lbs a.i./A was for turf; however, highly managed turf grass is not considered attractive to bees because it is not expected to have blooming weeds present. Therefore, the maximum difenoconazole application rate for pollinator attractive crops was used.

10.2 Bee Risk Characterization (Tier I)

Adults

There is potential for both oral and contact exposure on treated field and off-site via drift to honeybee attractive vegetation. For adult honeybees there were no acute contact or dietary risk concerns at both the minimum and maximum difenoconazole application rates. For acute

[8] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment
[9] https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/models-pesticide-risk-assessment#terrestrial

oral exposure, endpoints were non-definitive for adults (>71.3 µg a.i./bee with no mortality reported). Estimated dietary EECs at the maximum application rate for pollinator attractive crops (0.13 lbs a.i./A) were much lower than the non-definitive endpoint and adult honeybees would have to be over 16 times more sensitive to result in LOC exceedances. Likewise, on a contact basis, endpoints were non-definitive (> 100 µg a.i./bee). Estimated contact EECs were much lower and adult honeybees would have to be over 250 times more sensitive to result in LOC exceedances.

An additional acute oral honeybee study conducted with difenoconazole STK 20 (Difenoconazole + Tea Tree Oil) was submitted (MRID 50804619). This study identified a preliminary 96-hr LD_{50} estimate of >21 µg a.i./bee and was no more sensitive than the current endpoints identified for risk assessment.

On a chronic exposure basis for adult bees, the TGAI based NOAEC for difenoconazole was non-definitive (>13 µg a.i./bee). Compared to the EECs at the maximum application rate, there were no chronic LOC exceedances for difenoconazole TGAI. The NOAEC would have to be over three times lower (more sensitive) to exceed the maximum dietary EECs. In a similar manner, difenoconazole EC formulation (A7402T) based NOAEC values did not exceed chronic LOCs for risks to adult honeybees (RQs <0.39; **Table 10-2**). Adults could potentially be exposed to the formulation via contaminated nectar but there were no LOC exceedances.

Larvae

Based on an 8-day repeated dose toxicity test with the difenoconazole EC formulation (A7402T) estimated a LD_{50} of 1.79 µg a.i./larva-day, there were acute based LOC exceedances at both the minimum and maximum difenoconazole application rates. No larval acute studies were conducted with the TGAI; however, the day 8 LD_{50} from the 22-day larval chronic study of >20.9 µg a.i./L was approximately 10 times greater than the estimated maximum dietary dose.

On a chronic exposure basis for larval bees, the TGAI based NOAEC for difenoconazole was 9.28 µg a.i./larva-day based on a 55% reduction in larvae at the LOAEC (20.9 µg a.i./larva-day). At the maximum difenoconazole application rate there were no LOC exceedances for larval honeybees on a chronic basis using TGAI based endpoints (RQs < 0.19). However, difenoconazole EC (A7402T) formulation-based endpoints were 7 times more toxic sensitive to larvae compared to the TGAI. RQs exceed LOC for the maximum application rate at the larvae chronic formulation-based NOAEC only (RQ=1.35; **Table 10-3**). Larvae may be exposed to the TEP following applications made during bloom and when pollen and nectar resources are collected soon after application and fed directly to larvae. It is assumed that this is not a common exposure scenario, when considering the likely timing of difenoconazole applications relative to bloom and that adult bees are likely to process nectar and pollen prior to feeding to larval bees. Since the proportion of the components of the formulation are expected to change by the time difenoconazole reaches larvae, it is assumed that the TGAI toxicity data are more representative of the exposure larvae will encounter. Therefore, risks to bee larvae are considered low. Given the lack of acute or chronic risk concerns of difenoconazole based on

Tier I data and current registered uses, additional higher-tier toxicity and/or exposure data are not recommended at this time.

Table 10-2. Difenoconazole RQs for Adult Honey Bees

Application Rate	Adults	
	Adult Chronic RQ-TGAI NOAEC>13 µg a.i./bee	**Adult Chronic RQ-Formulation** NOAEC=10.6 µg a.i./bee LOAEC=21.3 µg a.i./bee
Minimum difenoconazole Foliar Single Application at 0.0684 lbs a.i./A for pome fruits	0.17	0.21
Maximum difenoconazole Foliar Single Application at 0.13 lbs a.i./A for ornamentals[1]	0.32	0.39

[1]Bold text indicates the acute LOC of 0.4 and the chronic LOC of 1 was exceeded for honeybees

Table 10-3. Difenoconazole RQs for Larval Honey Bees

Application Rate	Larvae		
	Larval Acute RQ-Formulation LD_{50}=1.79 µg a.i./larva	**Larval Chronic RQ-TGAI** NOAEC=9.28 µg a.i./larva	**Larval Chronic RQ-Formulation** NOAEC=1.31 µg a.i./larva LOAEC=3.28 µg a.i./larva
Minimum difenoconazole Foliar Single Application at 0.0684 lbs a.i./A for pome fruits	**0.52**	0.10	0.71
Maximum difenoconazole Foliar Single Application at 0.13 lbs a.i./A for ornamentals[1]	**0.99**	0.19	**1.35**

[1]Bold text indicates the acute LOC of 0.4 and the chronic LOC of 1 was exceeded for honeybees

Seed Treatments

For seed treatments, pesticide concentrations in pollen and nectar are based on concentrations in leaves and stems of treated plants (based on the European and Mediterranean Plant Protection Organization (EPPO) default value discussed in the White Paper, assumed to be 1 milligram per kilogram (mg/kg) or 1 part per million (ppm). Based on this exposure route, there are no acute oral dietary concerns for adult honeybees (EECs are less than the LD_{50} > 71.3 µg a.i./bee). Chronic risk for seed treatment uses was evaluated with the TGAI-based studies since there is no systemic transport of the formulation (as each component of the formulation moves and persists in its own way). Thus, using endpoints from TGAI studies, there were no chronic risk concerns to adult or larvae honeybees (RQs ≤ 0.02).

Table 10-4. Summary of labeled use information for seed treatment applications of difenoconazole (screening-level oral on-field)

Use pattern	Bee Life Stage	EEC in pollen and nectar	Difenoconazole Oral Dose (µg a.i/bee)[1]
All registered seed treatment use patterns	Adult	0.001 µg a.i./mg-diet	0.292
	Larval		0.124

[1] Source: USEPA et al. 2014. *Guidance for Assessing Pesticide Risks to Bees*.
[2]Dose estimate assumptions: Adults (0.001 ug a.i./mg-diet*0.292 g/day); Larva (0.001 ug a.i./mg-diet*0.124 g/day)

11.0 Terrestrial Plant Risk Assessment

The last Section 3 New Use Risk Assessment (USEPA, 2019) on turf applications conducted a terrestrial plant risk assessment at the maximum single difenoconazole application rate of 0.43 lbs a.i./A. Since the assessment, no use rate changes or new terrestrial plant data have become available. Therefore, the conclusions regarding low risk for terrestrial plants at the maximum application rate have not changed for this assessment.

12.0 Conclusions

Given the uses of difenoconazole, the available ecotoxicity data, and environmental fate properties there are Level of Concern (LOC) exceedances for non-listed fish (chronic), estuarine/marine invertebrates (acute), aquatic invertebrates (chronic), freshwater benthic invertebrates (chronic), birds (chronic), mammals (chronic), honeybees (acute and chronic larvae), and aquatic plants. However, risks are considered low for honeybees (adults and larvae) and aquatic plants. There were no risk concerns for terrestrial plants.

There were chronic exceedances for all size classes of birds and mainly small and medium size classes of mammals on the treated field. With a log K_{ow} of 4.2, difenoconazole has the potential to bioaccumulate in the aquatic food web and be of an exposure concern to piscivorous birds and mammals. Based on minimum and maximum annual EECs and a refined metabolism elimination rate in the aquatic food-web based model (KABAM), there were no acute or chronic LOC exceedances for piscivorous birds and mammals.

On a chronic basis, both ACR estimates and empirical data (at both NOAEC/LOAEC) supported chronic risk concerns across the majority of difenoconazole uses for freshwater and estuarine/marine fish. Due to the persistence of difenoconazole, repeated use can considerably increase these risks over time. There are no risks to benthic invertebrates via sediment exposure route; however, there were pore-water LOC exceedances for the most sensitive freshwater benthic invertebrate species (*Hyalella azteca)*. Minimal risk concerns were identified for aquatic plants. For the most sensitive non-vascular aquatic plant species, the LOC was only exceeded for the highest agricultural aquatic rice exposure scenarios. No data were available for vascular aquatic plants.

Based on an 8-day repeated dose bee toxicity test with difenoconazole formulation, there were acute based LOC exceedances for terrestrial insects at the minimum and maximum application rates for pollinator attractive crops. Chronic larval bee studies were available and conducted

with both the formulation and TGAI. However, the chronic LOC was only exceeded at the maximum application rate for the formulation-based study endpoints. However, based on the application methods of difenoconazole, it is unlikely bee larvae will be directly exposed to difenoconazole formulations. Therefore, risks to honeybees are considered low. A more in-depth summary of the risk conclusions for bees is available in **Section 10.**

Table 12-1. Potential Environmental Fate Concerns Identified for Difenoconazole

Bioconcentration/ Bioaccumulation	Groundwater Contamination	Sediment	Persistence[1]	Residues of Concern	Volatilization
Yes[2], log K_{ow}>3	No[3]	Yes[4]	Highly Persistent	Parent	No

[1] Persistence classification consistent with Goring *et al.* (1975) applied to aerobic soil metabolism studies.
[2] Difenoconazole's log K_{OW} of 4.2 indicates a potential for bioaccumulation; the submitted fish bioconcentration factor study reported rapid depuration (half-life <1 day).
[3] Fate properties indicate low potential for groundwater contamination. PWC modeling shows that incomplete throughput over 100 years of simulation for all six modeled groundwater scenarios (USEPA, 2019b, DP452444)
[4] Sediment concern indicated by the soil-water distribution coefficient (K_d) is ≥ 50 L/kg, K_{OC}s are ≥1,000 L/kg-organic carbon, and the log K_{OW} is ≥ 3 (40 CFR Part 158.630).

13.0 Literature Cited

Barraclough, D. Kearney, T. and A. Croxford. 2005. Bound Residues: Environmental Solution or Future Problem? Environ Pollut. 113: 85-90.

Benkman, C.W. and H.R. Pulliam. 1988. Comparative Feeding Ecology of North American Sparrows and Finches. *Ecology* 69: 1195-1199.

FAO. 2000. Appendix 2. Parameters of pesticides that influence processes in the soil. In FAO Information Division Editorial Group (Ed.), *Pesticide Disposal Series 8. Assessing Soil Contamination. A Reference Manual*. Rome: Food & Agriculture Organization of the United Nations (FAO). Available at http://www.fao.org/DOCREP/003/X2570E/X2570E06.htm

Goring, C. A. I., Laskowski, D. A., Hamaker, J. H., & Meikle, R. W. 1975. Principles of pesticide degradation in soil. In R. Haque & V. H. Freed (Eds.), *Environmental dynamics of pesticides.* NY: Plenum Press. Available at https://link.springer.com/chapter/10.1007%2F978-1-4684-2862-9_9

Kear, Janet. 1961-1962. The history of potato-eating by wildfowl in Britain. 14th Ann. Rept. Wildfowl Trust 1961-62. 113: 54-65.

USEPA. 2009. Guidance for Selecting Input Parameters in Modeling the Environmental Fate and Transport of Pesticides, Version 2.1. Environmental Fate and Effects Division. Office of Pesticide Programs. U.S. Environmental Protection Agency. Available at

https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-selecting-input-parameters-modeling

USEPA. 2010a. Guidance for Reporting on the Environmental Fate and Transport of the Stressors of Concern in the Problem Formulation for Registration Review, Registration Review Risk Assessments, Listed Species Litigation Assessments, New Chemical Risk Assessments, and Other Relevant Risk Assessments. January 25, 2010. Environmental Fate and Effects Division. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at http://www.epa.gov/pesticides/science/efed/policy_guidance/team_authors/endangered_species_reregistration_workgroup/esa_reporting_fate.htm

USEPA. 2010b. WQTT Advisory Note Number 9: Temperature Adjustments for Aquatic Metabolism Inputs to EXAMs and PE5. Memorandum From D. F. Young to Water Quality Tech Team. September 21, 2010. Environmental Fate and Effects Division. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency.

USEPA. 2012a. Standard Operating Procedure for Using the NAFTA Guidance to Calculate Representative Half-life Values and Characterizing Pesticide Degradation. November 30, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. U.S. Environmental Protection Agency. Available at https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-calculate-representative-half-life-values

USEPA. 2013a. Guidance for Using PRZM-GW in Drinking Water Exposure Assessments. December 11, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. U.S. Environmental Protection Agency.

USEPA. 2013b. Guidance on Modeling Offsite Deposition of Pesticides Via Spray Drift for Ecological and Drinking Water Assessment. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at http://www.regulations.gov/#!docketDetail;D=EPA-HQ-OPP-2013-0676

USEPA, 2014. Difenoconazole: Ecological Risk Assessment for Numerous Proposed New Uses and Changes to Registered Uses (Application Rate, Crop Groupings, and Additions to New Products. Environmental Fate and Effects Division, Office of Chemical Safety and Pollution Prevention. DP 417610+

USEPA, 2015. Difenoconazole: Preliminary Problem Formulation for Environmental Fate, Ecological Risk, Endangered Species, and Drinking Water Exposure Assessments in Support of Registration Review Environmental Fate and Effects Division, Office of Chemical Safety and Pollution Prevention. U. S. Environmental Protection Agency. DP 428500.

USEPA, 2016. Difenoconazole: Ecological Risk Assessment for Proposed New Uses on Cotton (including Cottonseed Crop Subgroup 20C), Rice, and Wild Rice. DP 432214.

USEPA, 2016. Refinements for Risk Assessment of Pesticide Treated Seeds – Interim Guidance. Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Environmental Fate and Effects Division. March 31, 2016.

USEPA, 2017. Difenoconazole: Ecological Risk Assessment for Proposed New Uses on Guava, Papaya, Cranberry, and Watercress. Environmental Fate and Effects Division, Office of Chemical Safety and Pollution Prevention. U. S. Environmental Protection Agency. DP 435064.

USEPA, 2019a. Difenoconazole: Ecological Risk Assessment for a Proposed Label Amendment to Increase the Use Rate on Golf Course Turfgrass. Environmental Fate and Effects Division, Office of Chemical Safety and Pollution Prevention. U. S. Environmental Protection Agency. DP 452414.

USEPA, 2019b. Difenoconazole: Drinking Water Assessment for a Proposed Label Amendment to Increase the Use Rate on Golf Course Turfgrass. Environmental Fate and Effects Division, Office of Chemical Safety and Pollution Prevention. U. S. Environmental Protection Agency. DP 452444.

13.1 New Environmental Fate Studies Since the Problem Formulation

MRID 49804601. Moore, A.; Jacobson, B.; Perez, S. (2015) Difenoconazole (A7402T) - Aquatic Dissipation of Difenoconazole Under Rice Production Conditions in Louisiana: Final Report. Project Number: TK0126939, 796/78, 2K13/901/TK0126939. Unpublished study prepared by Waterborne Environmental, Inc. (WEI), ADPEN Laboratories, Inc. and Syngenta Crop Protection, LLC.

MRID 49804602. Moore, A.; Jacobson, B.; Perez, S. (2015) Difenoconazole (A7402T) - Aquatic Dissipation of Difenoconazole Under Rice Production Conditions in California: Final Report. Project Number: TK0178871, 796/79, 2K13/901/TK0178871. Unpublished study prepared by Syngenta Crop Protection, LLC, Waterborne Environmental, Inc. (WEI) and ADPEN Laboratories, Inc

MRID 49804603. Perez, S. (2015) Stability of Difenoconazole, CGA205375, CGA142856 and CGA71019 in Sediment and Water Under Freezer Storage Conditions: Final Report. Project Number: TK0126941, TK0126941/01, 2K13/901/TK0126941. Unpublished study prepared by ADPEN Laboratories, Inc.

MRID 49862301. Perez, R.; Patel, D.; Perez, S. (2014) Difenoconazole- Independent Laboratory Validation of Residue Method (GRM066.01A) for the Determination of Difenoconazole, CGA205375, CGA142856 and CGA71019 in Water by LC-MS/MS: Final Report. Project Number: TK0180143, ADPEN/2K13/901/TK0180143, GRM066/01A. Unpublished study prepared by ADPEN Laboratories, Inc

MRID 49862302. Huang, S.; Manuli, M. (2014) Difenoconazole- Residue Method for the Determination of Difenoconazole, CGA205375, CGA142856, and CGA71019 in Water. Project Number: GRM066.01A, TK0180148, T013656/05. Unpublished study prepared by Syngenta Crop Protection, LLC.

MRID 50213601. Li, F.; Moore, A. (2016) Difenoconazole (A7402T) - Reanalysis of CGA71019 in Selected Sediment and Soil Samples Collected from an Aquatic Field Dissipation Study in Louisiana: Final Report. Project Number: TK0268914, 15/CPS/015. Unpublished study prepared by Syngenta Crop Protection, LLC.

MRID 50213602. Moore, A.; Ricko, A. (2016) Difenoconazole (A7402T) - Reanalysis of CGA71019 in Selected Sediment and Soil Samples Collected from an Aquatic Field Dissipation Study in California: Final Report. Project Number: TK0268915, 15/CPS/016. Unpublished study prepared by Critical Path Services, LLC and Syngenta Crop Protection, LLC.

MRID 50403701. Francis, P. (2017) Syngenta Response to EPA DER Dated 7/28/15 for MRID numbers 46950128 and 46950214. Unpublished study prepared by Syngenta Crop Protection, LLC. 8p. (re: ECM/ILV-soil)

13.2 New Ecological Effects Studies Since the Problem Formulation

MRID 50213701. Thomas, S.T., A.I. Siddiqui, and S.P. Gallagher. (2017). Difenoconazole – A Life Cycle Toxicity Test with the Freshwater Amphipod (*Hyalella azteca*) Using Spiked Sediment. Laboratory Report 528A-289.

MRID 50213702. Thomas, S.T., A.I. Siddiqui, and S.P. Gallagher. (2017). Difenoconazole – A Life Cycle Toxicity Test with the Marine Amphipod (*Leptocheirus plumulosus*) Using Spiked Sediment. Laboratory Report 528A-290.

MRID 50213703. Thomas, S.T., A.I. Siddiqui, and S.P. Gallagher. (2017). Difenoconazole - A Life Cycle Toxicity Test with the Midge (*Chironomus dilutus*) Using Spiked Sediment. Laboratory Report 528A-288.

MRID 49858601. Arnie, J.R., et al., A 96-Hour Toxicity Test with the Cyanobacteria (*Anabaena flos-aquae*). Study Completion Date: May 3, 2016. Wildlife International, Easton, Maryland. Project number 528P-152.

MRID 49858602. Orvos, A.R., et al., Difenoconazole EC (250) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants. Study completed date: February 19, 2016. Wildlife International, Easton, Maryland. Project number 528P-140.

MRID 4985603. Orvos, A.R. et al., Difenoconazole EC (250) - Toxicity Effects on the Vegetative Vigor of Ten Species of Plants. Study completed date: February 19, 2016. Wildlife International, Easton, Maryland. Project number 528P-14.

MRID 50727701. Kling, A. 2018. Difenoconazole – Honey Bee (*Apis mellifera* L.) Chronic Oral Toxicity Test 10 Day Feeding Test in the Laboratory. Study conducted by Eurofins Agroscience Services Ecotox GmbH. Study sponsored by Syngenta Ltd. Laboratory Report ID S18-03649. Study conducted from July 9 – Sept. 24, 2018.

MRID 50367601. Kling, A. 2017. Difenoconazole – Acute Oral and Contact Toxicity to the Honey Bee, *Apis mellifera* L. under Laboratory Conditions. Study conducted by Eurofins Agroscience Services EcoChem GmbH. Study sponsored by Syngenta Ltd. Laboratory Report ID S17-01522. Study conducted from July 3 – 6, 2017.

MRID 50212903. Ruhland, S. 2015. Difenoconazole EC (A7402T) – Chronic toxicity to the Honey Bee *Apis mellifera* L. in a 10 Day Continuous Laboratory Feeding Study. Study conducted by BioChem Labor. Study sponsored by Syngenta. Laboratory Report ID 15 10 48 024 B. Study conducted from May 27 – June 6, 2015.

MRID 50449801. Kling, A. 2017. Difenoconazole: Honey Bee (*Apis mellifera* L.) 22 Day Larval Toxicity Test (Repeated Exposure). Study conducted by Eurofins Agroscience Services Ecotox GmbH. Study sponsored by Syngenta Ltd. Laboratory Report ID S17-01517.

MRID 50212904. Kleebaum, K. 2015. Difenoconazole EC (A7402T) – Chronic Toxicity to the Honeybee Larvae *Apis mellifera* L. Under Laboratory Conditions (*in vitro*). Study conducted by BioChem Labor. Study sponsored by Syngenta Ltd. Laboratory Report ID 15 10 48 025 B. Study conducted from February 22 – 27, 2015.

MRID 50804617. Bustos, J.H. 2015. Acute Toxicity (96 hs) of STK 20 in Fish (*Poecilia reticulata*). Study conducted by Microquim Group, Buenos Aires, Argentina. Study number MAI-93802. Study sponsored by Stockton Crop Protection AG, Zug, Switzerland. Study initiated September 20, 2015 and completed October 23, 2015.

MRID 50804618. Catoyra, J.M. 2015. Avian (*Coturnix coturnix japonica*) Acute Toxicity (Single Dose) of STK 20. Unpublished study performed by Microquim S.A., Buenos Aires, Argentina. Laboratory Project ID: BI-93793. Study sponsored by Stockton Crop Protection AG, Zug, Switzerland. Study initiated May 5, 2015 and completed December 21, 2015.

MRID 50804619. Parodi, F. 2015. Acute Oral Toxicity of STK 20 in Bees (*Apis mellifera*). Unpublished study performed by Microquim S.A., Buenos Aires, Argentina. Laboratory Project ID: BI-93794. Study sponsored by Stockton Crop Protection AG, Zug, Switzerland. Study initiated May 5, 2015 and completed December 21, 2015.

Appendix A. Summary of Difenoconazole Degradate Toxicity Endpoints

1,2,4-Triazole

Available empirical guideline data for 1,2,4-triazole indicate that this degradate is less toxic than difenoconazole to freshwater fish (rainbow trout on an acute basis), freshwater invertebrates (water flea on an acute basis) and non-vascular plants (green algae). ECOSAR methods were also used to predict the toxicity of 1,2,4-triazole aquatic taxa based on its structural similarity to chemicals for which aquatic toxicity data are known. There is reasonable confidence in ECOSAR estimates for 1,2,4-triazole for both freshwater and estuarine/marine fish, freshwater invertebrates, and green algae given that ECOSAR estimates are within an order of magnitude of the available empirical values. A comparison of 1,2,4-triazole ECOSAR estimates to experimentally derived difenoconazole toxicity values suggests that 1,2,4-triazole is much less toxic (two orders of magnitude) than difenoconazole on a chronic basis to freshwater invertebrates and fish.

Triazole Acetic Acid

Available empirical guideline data for triazole acetic acid indicate that this degradate is less toxic than difenoconazole to freshwater fish (rainbow trout acute) and freshwater invertebrates (water flea acute). ECOSAR methods were also used to predict the toxicity of triazole acetic acid to aquatic taxa based on its structural similarity to chemicals for which aquatic toxicity data are known. However, there is no basis for judging confidence in the ECOSAR estimates because the ECOSAR estimates for acute toxicity to fish and invertebrates are substantially greater (less toxic) than the non-definitive endpoints observed in the available acute toxicity studies.

CGA-205375

No empirical data were available for the third degradate CGA-205375 regarding toxicity to aquatic taxa. A comparison of CGA-205375 ECOSAR estimates to experimentally derived difenoconazole toxicity values suggests that CGA-205375 is less toxic than difenoconazole to aquatic organisms. For example, freshwater fish and invertebrates are 9 to 23X less toxic on a chronic basis based on ECOSAR estimates as compared to difenoconazole. In addition, CGA - 205375 is a minor degradate (4.4 to 9.4%) in three aerobic soils, detected at up to 14.8% in CA loamy sand soil, and detected up to 12.6% in an anaerobic aquatic environment. Therefore, CGA- 205375 is not expected to contribute substantially to exposure or toxicity, in part, because difenoconazole is a persistent chemical in the environment. Hence, difenoconazole was considered the ROC in the aquatic environment in this assessment.

Table A-1. Summary of Toxicity Endpoints for 1,2,4-Triazole

Type of Study	Species	Toxicity Value (µg a.i./L)	MRID
Aquatic Endpoints			
Acute – Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	96-hr LC_{50} = 498,000	48474301
Acute – Freshwater Invertebrate	Water flea (*Daphnia magna*)	48-hr EC_{50} > 98,100	48453206
Non-vascular Plant	Freshwater Algae (*Pseudokirchneriella subcapitata*)	96-hr EC_{50} = 14,000	45880401
Terrestrial Endpoints			
Acute – Avian Oral Dose	Bobwhite quail (*Colinus virginianus*)	LD_{50} = 770 mg ai/kg-bw	49380701
Two Generation Reproduction – Mammalian	Laboratory rat (*Rattus norvegicus*)	NOAEC < 250 mg ai/kg-diet LOAEC ≤ 250 mg ai/kg-diet based on reduced body weight and body weight gain in F1 males, reduced spleen weight in F1 females and F2 female pups, and reduced body weight, body weight gain, and brain weight in F2 pups	46467304
Subchronic (28-day adult) and reproductive (additional 28-day offspring) – Terrestrial Invertebrate	Earthworm (*Eisenia fetida*)	LC_{50} > 70.81 µg/kg NOAEC ≥ 70.81 µg/kg LOAEC > 70.81 µg/kg	45880402
28 day – Terrestrial Invertebrate	Springtails (*Folsomia candida*)	28-day LC_{50} > 10 mg ai/kg soil NOAEC = 1.8 mg ai/kg soil LOAEC = 3.2 mg ai/kg soil based on reduced number of juveniles	45880404

Table A-2. Summary of Toxicity Endpoints for Triazole Acetic Acid

Type of Study	Species	Toxicity Value (µg ai/L)	MRID
Acute – Freshwater Fish	Rainbow trout (*Oncorhynchus mykiss*)	96-hr LC_{50} > 101,000	48453209
Acute – Freshwater Invertebrate	Water flea (*Daphnia magna*)	48-hr EC_{50} > 108,000	48453208
Acute – Avian Oral Dose	Bobwhite quail (*Colinus virginianus*)	LD_{50} > 1926 mg ai/kg-bw	49412601
Acute – Mammalian Oral Dose	Laboratory rat (*Rattus norvegicus*)	LD_{50} > 5000 mg ai/kg-bw	45596802

Table A-3. Summary of Toxicity Endpoints for CGA-205375

Type of Study	Species	Toxicity Value	MRID
Acute – Mammalian Oral Dose	Mouse	LD_{50} = 2309 mg ai/kg-bw[1]	46950303

[1] LD_{50} = 1289 mg ai/kg-bw scaled to laboratory rat weight (350 g) based on an average mouse body weight of 34 g in this study and the following equation: mouse LD_{50} * (mouse bw/rat bw)$^{0.25}$

Appendix B. ECOSAR Estimated Toxicity Endpoints for Difenoconazole and Degradates

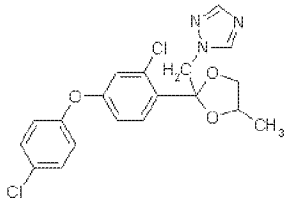
Table B-1. ECOSAR Estimate Toxicity Endpoints for Difenoconazole and Degradates (mg a.i./L)

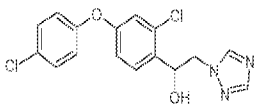
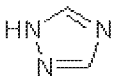
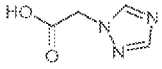
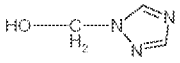
Compound (mg a.i./L)	FW fish acute LC_{50}	EM fish acute LC_{50}	FW Invert. (Daphnid) LC_{50}	EM Invert. (Mysid) LC_{50}	Green Algae EC_{50}	FW Fish Chronic	EM Fish Chronic	FW Invert. (Daphnid) Chronic	E/M Invert. (Mysid) chronic	Green Algae (NOAEC)
Difenoconazole (empirical)	0.81	0.819	0.770	0.150	0.300	0.00086[1]	0.00086[1]	0.0056	0.0048	0.150
Difenoconazole (ECOSAR)	0.31	0.58	0.75	0.06	0.26	NA	0.05	0.03	NA	0.22
1,2,4-triazole (empirical)	498	NA	>98.1	NA	14	NA	NA	NA	NA	NA
1,2,4-triazole (ECOSAR)	2264	14,361	487	1286	40	2.45	0.35	76	124,948	5.2
Triazole acetic acid (empirical)	>101	NA	>108	NA	NA	NA	NA	NA	NA	NA
Triazole acetic acid (ECOSAR)	227,531	1,750,265	33,312	151,968	2,176	169	11.69	6452	49,574,568	209
CGA-205375 (empirical)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
CGA-205375 (ECOSAR)	3.3	3.33	4.54	0.87	1.11	0.02	0.1	0.26	0.25	0.61

NA = Not applicable either due to a lack of data or unavailable toxicity estimate.
[1]acute-to-chronic ratio (ACR)

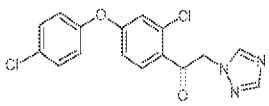
Appendix C. Names and Structures of Difenoconazole and its Degradates

Table C-1. Names and Structures of Difenoconazole and its Degradates

Code Name (Synonym)	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (days)	Final %AR (Days)
PARENT COMPOUND						
Difenoconazole (CGA-169374)	**IUPAC Name:** 3-Chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether **CAS Name:** 1-[2-[2-Chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole **CAS Number:** 119446-68-3 **Formula:** $C_{19}H_{17}Cl_2N_3O_3$ **MW:** 406.3 **SMILES:** O1CC(C)OC1(Cn2ncnc2)c3c(Cl)cc(Oc4ccc(Cl)cc4)cc3		Hydrolysis pH 5	42245127	Not applicable	stable
			Hydrolysis pH 7			stable
			Hydrolysis pH 9			stable
			Aqueous Photolysis	46950105		94.1 (15)
			Soil Photolysis	46950106 46950107		91.4 (30)
			Aerobic Soil Metabolism	46950109		18.4 (293)
				46950110		14.1 (293)
				46950111		38.7 (228)
				46950112 46950113		57.6 (360)
				46950114 46950115		59.6 (372)
			Aerobic Aquatic Metabolism	46950116		71.2 (183)
				46950117 46950118		81.53 ± 3.22 (112)
			Anaerobic Aquatic Metabolism	46950119 46950120		42.72 ± 0.88 (350)
			Terrestrial Field Dissipation	46950126		44 ppb (531)
				46950127		14 ppb (535)
				46950129		302 ppb (422)

Code Name (Synonym)	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (days)	Final %AR (Days)
MAJOR (>10%) TRANSFORMATION PRODUCTS						
CGA-205375 (CGA-179500, CGA-211391, and M1)	**CAS Name:** alpha-(2-Chloro-4-(4-chlorophenoxy)phenyl)-1H-1,2,4-triazole-1-ethanol 1-(2-Chloro-4-(4-chlorophenoxy)phenyl)-2-(1,2,4)-triazol-1-yl-ethanol **CAS Number:** 117018-19-6 **SMILES:** OC(Cn1cncn1)c2ccc(Oc3ccc(Cl)cc3)cc2Cl		Aqueous Photolysis	42245128	2.9 (5)	0.1 (30)
			Aerobic Soil metabolism	46950112 46950113	14.8 ± 1.8 (360)	14.8 ± 1.8 (360)
			Aerobic Aquatic Metabolism	46950116	6.2 (183)	6.2 (183)
			Anaerobic Aquatic Metabolism	46950119 46950120	12.60 ± 0.42 (175)	6.2 ± 0.76 (350)
			Terrestrial Field Dissipation	18ppb (364)	12.0 2ppb (535)	18ppb (364)
1,2,4-triazole (CGA-71019 and M4)	**CAS Name:** 1H-1,2,4-Triazole **CAS Number:** 288-88-0 **SMILES:** c1nnc[nH]1		Aqueous Photolysis	42245128	13.4 (9)	10.9 (30)
				46950104	12.27 ± 0.7 (30)	12.27 ± 0.7 (30)
			Aerobic Soil Metabolism	46950110	20.6 (190)	17.8 ± 2.8 (293)
			Anaerobic Aquatic Metabolism	46950119 46950120	35.92 ± 0.71 (350)	35.92 ± 0.71 (350)
			Terrestrial Field Dissipation	46950129	3.5 ppb (36)	< LOQ (535)
1,2,4-triazole acetic acid (CGA-142856)	**CAS Name:** 1H-1,2,4-Triazole-1-acetic acid **CAS Number:** 28711-29-7 **SMILES:** OC(=O)Cn1cncn1		Aqueous Photolysis	46950104	41.83 ± 0.9 (30)	41.83 ± 0.9 (30)
			Terrestrial Field Dissipation	46950126	<LOQ	11 ppb (358)
CGA-107069	**CAS Name:** 1H-1,2,4-Triazole-1-methanol **CAS Number:** 74205-82-6		Aqueous Photolysis	46950104	Reported as combined residue with 1,2,4-triazole	

Code Name (Synonym)	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (days)	Final %AR (Days)
Carbon dioxide	**CAS Number:** 124-38-9 **Formula:** CO_2 **MW:** 44.1 g/mol	O=C=O	Aqueous Photolysis	46950104	2.01 (30)	2.01 ± 2.5 (30)
			Soil Photolysis	46950106 46950107	0.02 (21)	<0.2 (30)
			Aerobic Soil Metabolism	46950109	23.4 ± 1.9 (293)	23.4 ± 1.9 (293)
			Aerobic Soil Metabolism	46950111	19.2 (228)	19.2 (228)
			Aerobic Aquatic Metabolism	46950116	3.0 (183)	3.0 (183)
			Anaerobic Aquatic Metabolism	46950119 46950120	0.55 ± 0.33 (245)	0.25 ± 0.02 (350)
Non-extractable Residues	Not identified	Not identified	Aerobic Soil Metabolism	46950109	38.9 ± 0.5 (293)	48.9 ± 0.6 (293)
			Aerobic Aquatic Metabolism	46950116	13.9 (183)	13.9 (183)
				46950117 46950118	8.87 ± 3.04 (112)	8.87 ± 3.04 (112)
			Anaerobic Aquatic Metabolism	46950119 46950120	8.06 ± 1.24 (302)	7.11 ± 0.32 (350)
Unidentified[1] Unknowns	Not identified	Not identified	Aqueous Photolysis	42245128	20.0 (15)	6.5 (30)
				46950104	29.02 ± 2.9 (30)	29.02 ± 2.9 (30)
				46950105	16.7 (15)	11.1 (15)
			Aerobic Soil Metabolism	46950111	0.4 (228)	0.4 (228)
			Aerobic Aquatic Metabolism	46950117	10.3 (183)	10.3 (183)

Code Name (Synonym)	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (days)	Final %AR (Days)
			Anaerobic Aquatic Metabolism	46950119 46950120	5.69 (320)	5.21 (350)
MINOR (<10%) TRANSFORMATION PRODUCTS						
CGA-205374 (CGA-176459 and M5)	**CAS Name:** 1-[2-Chloro-4-(4-chlorophenoxy)phenyl]-2-(1H-1,2,4-triazol-1-yl)-ethanone **CAS Number:** 136815-80-0		Aqueous Photolysis	42245128	1.5 (0)	< LOQ (30)
				46950104	1.11 ± 0.11 (14)	0.13 ± 0.1 (30)
				46950105	NA	NA
			Aerobic Soil Metabolism	46950114 46950115	2.1 ± 0.4 (272)	1.4 ± 0.1 (293)
			Anaerobic Aquatic Metabolism	46950119 46950120	0.77± 0.23 (245 day)	LOQ (350)
M2	Not identified	Not identified	Aerobic Soil Metabolism	46950109	1.5 (28)	1.4 ± 0.1 (293)
				46950110	2.1 ± 1.81 (293)	2.1 ± 1.81 (293)
				46950111	1.5 (228)	1.5 (228)
			Anaerobic Aquatic Metabolism	46950119 46950120	5.79 ± 1.42 (245)	< LOQ (350)

NA = Not Analyzed
LOQ = Limit of Quantitation (1 ppb)
[1] Unidentified unknowns consist of M3, M5, M6, and several unidentified components ranging from 0.5% to 9.7% of the applied radioactivity at study termination.

Appendix D. Example of Summary of PWC and PFAM modeling Outputs

Summary of Water Modeling of Difenoconazole_Parent and the USEPA Standard Pond

Estimated Environmental Concentrations for Difenoconazole_Parent are presented in **Table D-1** for the USEPA standard pond with the NYGrapesSTD field scenario. A graphical presentation of the year-to-year peaks is presented in Figure 1. These values were generated with the Pesticide Water Calculator (PWC), Version 1.52. Critical input values for the model are summarized in **Tables D-2 and D-3**.

This model estimates that about 4.2% of Difenoconazole_Parent applied to the field eventually reaches the water body. The main mechanism of transport from the field to the water body is by erosion (63% of the total transport), followed by spray drift (29.5%) and runoff (7.53%). In the water body, pesticide dissipates with an effective water column half-life of 1318.9 days. (This value does not include dissipation by transport to the benthic region; it includes only processes that result in removal of pesticide from the complete system.) The main source of dissipation in the water column is metabolism (effective average half-life = 1378.6 days) followed by photolysis (30615.3 days) and volatilization (6360886 days).

In the benthic region, pesticide dissipation is negligible (3240.7 days). The main source of dissipation in the benthic region is metabolism (effective average half-life = 3240.7 days). The vast majority of the pesticide in the benthic region (99.85%) is sorbed to sediment rather than in the pore water.

Table D-1. Estimated Environmental Concentrations (ppb) for Difenoconazole_Parent.

Peak (1-in-10 yr)	15.3
4-day Avg (1-in-10 yr)	14.6
21-day Avg (1-in-10 yr)	13.5
60-day Avg (1-in-10 yr)	13.1
365-day Avg (1-in-10 yr)	12.5
Entire Simulation Mean	8.43

Table D-2. Summary of Model Inputs for Difenoconazole_Parent.

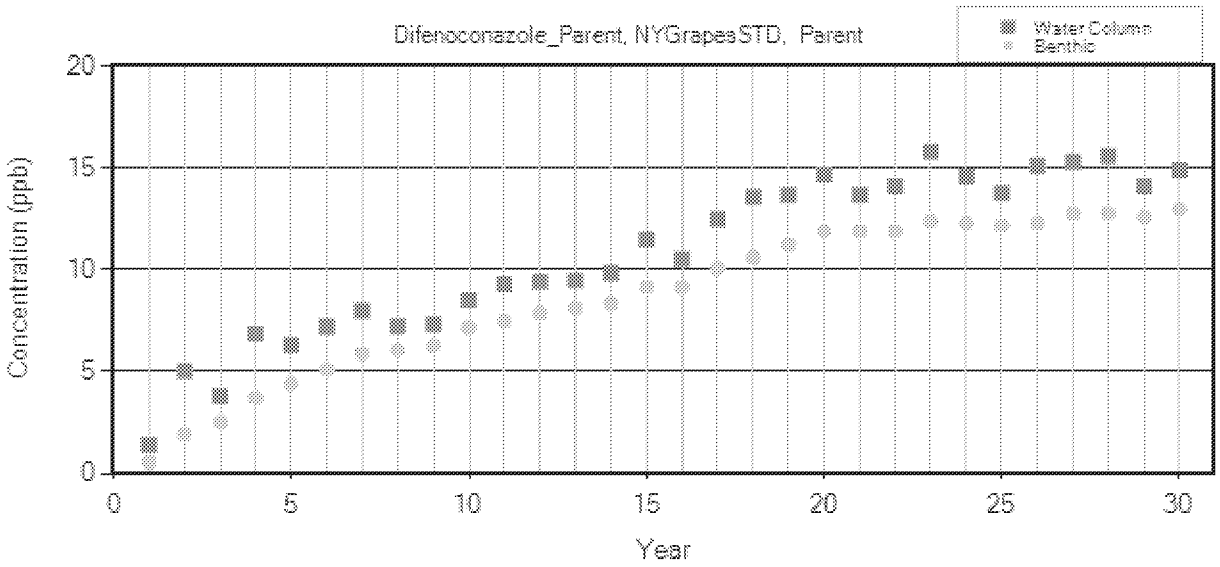
Scenario	NYGrapesSTD
Cropped Area Fraction	1
Koc (ml/g)	6115
Water Half-Life (days) @ 20 °C	781
Benthic Half-Life (days) @ 20 °C	1836
Photolysis Half-Life (days) @ 40 °Lat	228
Hydrolysis Half-Life (days)	0
Soil Half-Life (days) @ 20 °C	440
Foliar Half-Life (days)	

Molecular Weight	406.3
Vapor Pressure (torr)	2.5e-10
Solubility (mg/l)	3.3
Henry's Constant	1.66E-09

Table D-3. Application Schedule for Difenoconazole_Parent.

Date (Days Since Emergence)	Type	Amount (kg/ha)	Eff.	Drift
30	Above Crop (Foliar)	0.13	0.95	0.125
37	Above Crop (Foliar)	0.13	0.95	0.125
51	Above Crop (Foliar)	0.13	0.95	0.125
58	Above Crop (Foliar)	0.13	0.95	0.125

Figure D-1. Yearly Peak Concentrations



Example PWC Input file: NY Grape STD._Aerial

NY_Grape_A_STD.SWI

Summary of PFAM Water Modeling of Difenoconazole

Pesticide in Flooded Applications (PFAM)
Version 2
7/8/2020 10:03:39 AM
******* Summary of Paddy Concentration Rankings ********

********** Analysis for Parent **********
Max released concentration (ppb) = 0.140E+04
Index for max concentration = 9791

1-in-10 Year Return Concentrations:
********* WATER COLUMN CONCENTRATION (ug/L) **********
Water Column Peak = 154.
Water Column 1-day Avg = 111.
Water Column 4-day Avg = 58.2
Water Column 21-day Avg = 30.0
Water Column 60-day Avg = 19.1
Water Column 90-day Avg = 17.0
Water Column 365-day Avg = 8.05

****** BENTHIC PORE WATER (ug/L) Concentration *********
Benthic Pore Water Peak = 13.8
Benthic Pore Water 4-day Avg = 13.8
Benthic Pore Water 21-day Avg = 13.8
Benthic Pore Water 60-day Avg = 13.6
Benthic Pore Water 90-day Avg = 13.4
Benthic Pore Water 365-day Avg = 11.1

***** BENTHIC TOTAL CONCENTRATION (Mass/Dry Mass) ******
Benthic Total Conc. Peak = 852.
Benthic Total Conc. 4-day Avg = 852.
Benthic Total Conc. 21-day Avg = 847.
Benthic Total Conc. 60-day Avg = 834.
Benthic Total Conc. 90-day Avg = 823.
Benthic Total Conc. 365-day Avg = 681.
****

PFAM input file for CA Rice-Winter

CA_rice-winter_Parent.PFA

Appendix E. Endocrine Disruptor Screening Program (EDSP)

As required by FIFRA and the Federal Food, Drug, and Cosmetic Act (FFDCA), EPA reviews numerous studies to assess potential adverse outcomes from exposure to chemicals. Collectively, these studies include acute, subchronic and chronic toxicity, including assessments of carcinogenicity, neurotoxicity, developmental, reproductive, and general or systemic toxicity. These studies include endpoints which may be susceptible to endocrine influence, including effects on endocrine target organ histopathology, organ weights, estrus cyclicity, sexual maturation, fertility, pregnancy rates, reproductive loss, and sex ratios in offspring. For ecological hazard assessments, EPA evaluates acute tests and chronic studies that assess growth, developmental and reproductive effects in different taxonomic groups. As part of the Draft Ecological Risk Assessment for Registration Review, EPA reviewed these data and selected the most sensitive endpoints for relevant risk assessment scenarios from the existing hazard database. However, as required by FFDCA section 408(p), difenoconazole is subject to the endocrine screening part of the Endocrine Disruptor Screening Program (EDSP).

EPA has developed the EDSP to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." The EDSP employs a two-tiered approach to making the statutorily required determinations. Tier 1 consists of a battery of 11 screening assays to identify the potential of a chemical substance to interact with the estrogen, androgen, or thyroid (E, A, or T) hormonal systems. Chemicals that go through Tier 1 screening and are found to have the potential to interact with E, A, or T hormonal systems will proceed to the next stage of the EDSP where EPA will determine which, if any, of the Tier 2 tests are necessary based on the available data. Tier 2 testing is designed to identify any adverse endocrine-related effects caused by the substance and establish a dose-response relationship between the dose and the E, A, or T effect.

Under FFDCA section 408(p), the Agency must screen all pesticide chemicals. Between October 2009 and February 2010, EPA issued test orders/Data Call-Ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. A second list of chemicals identified for EDSP screening was published on June 14, 2013[10] and includes some pesticides scheduled for registration review and chemicals found in water. Neither of these lists should be construed as a list of known or likely endocrine disruptors. Difenoconazole is not on either list. For further information on the status of the EDSP, the policies and procedures, the lists of chemicals, future lists, the test guidelines and Tier 1 screening battery, please visit our website[11].

[10] See http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0477-0074 for the final second list of chemicals.
[11] Available: http://www.epa.gov/endo/

Appendix F. T-Rex Inputs for Difenoconazole Applications

Table F-1. Example T-Rex Inputs for a 0.0684 lbs a.i./A single application

% AI	100%
Application Rate	0.0684 lbs a.i./A
Half-Life	35
Application Interval	NA
Number of Applications	1
Are you assessing applications with variable rates or intervals?	No

Table F-2. Example T-Rex Inputs for a multiple application scenario

% AI	100%
Application Rate	0.0684 lbs a.i./A
Half-Life	35
Application Interval	7
Number of Applications	5
Are you assessing applications with variable rates or intervals?	Yes

Table F-3. T-Rex Toxicity Data Inputs

Avian Endpoint	Toxicity Value	Test Species
LD 50 (mg/kg-bw)	>2150*	Mallard duck
LC50 (mg/kg-diet)	4760	Bobwhite Quail
NOAEC (mg/kg-diet)	21.9	Bobwhite Quail
Mammalian Endpoints	**Toxicity Value**	**Test Species**
LD 50 (mg/kg-bw)	1453	Rat
Reported Chronic Endpoint (mg/kg-diet)	250	Rat
Is estimated daily dose (mg/kg-bw)	20	Rat

*endpoints non-definitive

Appendix G. KABAM Inputs and Outputs for Difenoconazole

Table G-1. Chemical characteristics of difenoconazole.		
Characteristic	**Value**	**Comments/Guidance**
Pesticide Name	**difenoconazole**	Required input
Log K_{OW}	**4.2**	Required input Enter value from acceptable or supplemental study submitted by registrant or available in scientific literature.
K_{OW}	**15849**	No input necessary. This value is calculated automatically from the Log K_{OW} value entered above.
K_{OC} (L/kg OC)	**6115**	Required input Input value used in PRZM/EXAMS to derive EECs. Follow input parameter guidance for deriving this parameter value (USEPA 2002).
Time to steady state (T_S; days)	**7**	No input necessary. This value is calculated automatically from the Log K_{OW} value entered above.
Pore water EEC (µg/L)	**13.8**	Required input Enter value generated by PRZM/EXAMS benthic file. PRZM/EXAMS EEC represents the freely dissolved concentration of the pesticide in the pore water of the sediment. The appropriate averaging period of the EEC is dependent on the specific pesticide being modeled and is based on the time it takes for the chemical to reach steady state. Select the EEC generated by PRZM/EXAMS which has an averaging period closest to the time to steady state calculated above. In cases where the time to steady state exceeds 365 days, the user should select the EEC representing the average of yearly averages. The peak EEC should not be used.
Water Column EEC (µg/L)	**30**	Required input Enter value generated by PRZM/EXAMS water column file. PRZM/EXAMS EEC represents the freely dissolved concentration of the pesticide in the water column. The appropriate averaging period of the EEC is dependent on the specific pesticide being modeled and is based on the time it takes for the chemical to reach steady state. The averaging period used for the water column EEC should be the same as the one selected for the pore water EEC (discussed above).

Table G-2. Input parameters for rate constants. "calculated" indicates that model will calculate rate constant.					
Trophic level	**k_1 (L/kg*d)**	**k_2 (d^{-1})**	**k_D (kg-food/kg-org/d)**	**k_E (d^{-1})**	**k_M* (d^{-1})**
phytoplankton	calculated	calculated	0*	0*	0
zooplankton	calculated	calculated	calculated	calculated	0
benthic invertebrates	calculated	calculated	calculated	calculated	0

filter feeders	calculated	calculated	calculated	calculated	0
small fish	calculated	calculated	calculated	calculated	0.6
medium fish	calculated	calculated	calculated	calculated	0.6
large fish	calculated	calculated	calculated	calculated	0.6

* Default value is 0.

k_1 and k_2 represent the uptake and elimination constants respectively, through respiration.

k_D and k_E represent the uptake and elimination constants, respectively, through diet.

k_M represents the metabolism rate constant.

Table G-3. Mammalian and avian toxicity data for difenoconazole. These are required inputs.

Animal	Measure of effect (units)	Value	Species	If selected species is "other," enter body weight (in kg) here.
Avian	LD_{50} (mg/kg-bw)	2000	mallard duck	
	LC_{50} (mg/kg-diet)	4760	Northern bobwhite quail	
	NOAEC (mg/kg-diet)	21.9	Northern bobwhite quail	
	Mineau Scaling Factor	1.15	Default value for all species is 1.15 (for chemical specific values, see Mineau et al. 1996).	
Mammalian	LD_{50} (mg/kg-bw)	1453	laboratory rat	
	LC_{50} (mg/kg-diet)	N/A	other	
	Chronic Endpoint	250	laboratory rat	
	units of chronic endpoint*	ppm		

Table G-4. Calculation of RQ values for mammals and birds consuming fish contaminated by difenoconazole.

Wildlife Species	Acute		Chronic	
	Dose Based	Dietary Based	Dose Based	Dietary Based
Mammalian				
fog/water shrew	0.003	N/A	0.390	0.070
rice rat/star-nosed mole	0.003	N/A	0.394	0.058
small mink	0.002	N/A	0.249	0.040
large mink	0.002	N/A	0.276	0.040

small river otter	0.003	N/A	0.297	0.040
large river otter	0.002	N/A	0.192	0.024
Avian				
sandpipers	0.014	0.003	N/A	0.660
cranes	0.001	0.003	N/A	0.592
rails	0.007	0.003	N/A	0.727
herons	0.001	0.003	N/A	0.627
small osprey	0.001	0.002	N/A	0.455
white pelican	0.000	0.001	N/A	0.273

Note: This is an example of the results when KABAM is run at the maximum EEC

Appendix H. Bee Rex Inputs and Outputs for Difenoconazole Applications

Table H-1. Inputs for Bee Rex

Description	Value
Application rate	0.0684 and 0.13
Units of app rate	lb a.i./A
Application method	foliar spray
Are empirical residue data available?	no

Table H-2. Toxicity data

Description	Value (µg a.i./bee)
Adult contact LD50	>100[1]
Adult oral LD50	>71.3[1]
Adult oral NOAEL	>13[1]- conducted w/ TGAI 10.6-conducted w/ Formulation
Larval LD50	>20.9-conducted w/ TGAI 1.79 conducted w/ Formulation
Larval NOAEL	9.28-conducted w/ TGAI 1.31-condcuted w/ formulation

*Endpoints are non-definitive and compared to EECs
[2]NOAEC at the maximum tested concentration

Table H-3. Estimated concentrations in pollen and nectar at 0.0684 lbs a.i./A

Application method	EECs (mg a.i./kg)	EECs (µg a.i./mg)
foliar spray	7.52	0.0075

Table H-4. Estimated concentrations in pollen and nectar at 0.134 lbs a.i./A

Application method	EECs (mg a.i./kg)	EECs (µg a.i./mg)
foliar spray	14.3	0.0143

Table H-5. RQs at 0.134 lbs a.i./A

With TGAI Studies		
Exposure	RQ Adult	RQ Larvae
Acute Contact	0.00351	NA
Acute Dietary	0.06	NA
Chronic Dietary	0.32	0.19
With Formulation Studies		
Acute Contact	0.00351	NA
Acute Dietary	0.06	**0.99**
Chronic Dietary	0.32	**1.35**

Table H-6. RQs at 0.0684 lbs a.i./A

With TGAI Studies		
Exposure	**RQ Adult**	**RQ Larvae**
Acute Contact	0.00184	NA
Acute Dietary	0.03	NA
Chronic Dietary	0.17	0.10
With Formulation Studies		
Acute Contact	0.00184	NA
Acute Dietary	0.03	**0.52**
Chronic Dietary	0.17	0.71

Attachment 3

US EPA ARCHIVE DOCUMENT

(2)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

Date: February 7, 2006

MEMORANDUM

SUBJECT: 1,2,4-Triazole, Triazole Alanine, Triazole Acetic Acid: Human Health Aggregate Risk Assessment in Support of Reregistration and Registration Actions for Triazole-derivative Fungicide Compounds.
Risk Assessment Type: Single Chemical Aggregate
PC Code: 600074 – 1,2,4-Triazole
600011 – Triazole Alanine
600082 – Triazole Acetic Acid
DP Number: 322215

FROM: Michael Doherty, Ph.D., Chemist, RAB 2;
Kathleen Raffaele, Ph.D., Toxicologist, RAB 3;
Kit Farwell, DVM, Toxicologist, RRB 1;
Steve Dapson, Ph.D., Branch Senior Scientist, RAB 3;
Kelly Schumacher, M.S., Toxicologist, RAB 2;
Jack Arthur, Environmental Health Scientist, RAB 3;
David Hrdy, Biologist, CEB;
Health Effects Division (7509C)

Iwona Maher, Chemist, ERB 1
Environmental Fate and Effects Division (7507C)

THROUGH: Richard Loranger, Ph.D., Branch Senior Scientist
Registration Action Branch 2
Health Effects Division (7509C)

R. Loranger

TO: Tamue Gibson/Cynthia Giles-Parker, FB
Registration Division (7505C)

Michael Goodis, RRB 3
Special Review and Reregistration Division (7508C)

Susan Lewis, RRB 1
Special Review and Reregistration Division (7508C)

Table of Contents

1.0 Executive Summary 5

Preface and General Information 7

2.0 Chemical Profile 8
2.1 Summary of Registered/Proposed Uses Considered in These Assessments 8
3.0 Metabolism Assessment 12
3.1 Comparative Metabolic Profile 12

1,2,4-Triazole 13

4.0 Hazard Characterization/Assessment 13
4.1 Hazard Characterization 13
4.2 FQPA Hazard Considerations 17
4.2.1 Adequacy of the Toxicity Data Base 17
4.2.2 Evidence of Neurotoxicity 17
4.2.3 Developmental Toxicity Studies 18
4.2.4 Reproductive Toxicity Study 18
4.2.5 Additional Information from Literature Sources 18
4.2.6 Pre-and/or Postnatal Toxicity 18
4.2.6.1 Determination of Susceptibility 18
4.2.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre and/or Post-natal Susceptibility 19
4.3 Recommendation for a Developmental Neurotoxicity Study 19
4.3.1 Evidence that supports requiring a Developmental Neurotoxicity study 20
4.3.2 Evidence that supports not requiring a Developmental Neurotoxicity study 20
4.3.2.1 Rationale for the UF_{DB} (when a DNT is recommended) 21
4.4 Hazard Identification and Toxicity Endpoint Selection 21
4.4.1 Acute Reference Dose (aRfD) - Females age 13-49 21
4.4.2 Acute Reference Dose (aRfD) - General Population 21
4.4.3 Chronic Reference Dose (cRfD) 21
4.4.4 Incidental Oral Exposure (Short-Term) 22
4.4.5 Incidental Oral Exposure (Intermediate-Term) 22
4.4.6 Dermal Absorption 22
4.4.7 Dermal and Inhalation Exposure (Short-Term) 23
4.4.8 Dermal and Inhalation Exposure (Intermediate- and Long-Term) 23
4.4.9 Margins of Exposure and Levels of Concern 23
4.4.10 Recommendation for Aggregate Exposure Risk Assessments 23
4.4.11 Classification of Carcinogenic Potential 24
4.5 Special FQPA Safety Factor 25
4.6 Endocrine disruption 26
5.0 Public Health Data 27
6.0 Exposure Characterization/Assessment 27
6.1 Dietary Exposure/Risk Pathway 27
6.1.1 Residue Profile 27
6.1.1.1 Residues in Food 27

6.1.1.2 Residues in Water 32
6.1.2 Acute and Chronic Dietary Exposure and Risk 32
6.2 Residential (Non-Occupational) Exposure/Risk Pathway 34
6.2.1 Home Uses 34
6.2.1.1 Application Scenarios 34
6.2.1.2 Postapplication Scenarios 37
6.2.2 Recreational Use Sites 40
6.2.3 Other (Spray Drift, etc.) 40
6.2.4 Pharmaceutical Uses 41
7.0 Aggregate Risk Assessments and Risk Characterization 41
7.1 Short-term Aggregate Risk 42
7.2 Intermediate-term Aggregate Risk 42
8.0 Cumulative Risk Characterization/Assessment 43
9.0 Occupational Exposure/Risk Pathway 44
10.0 Data Needs and Label Requirements 44
10.1 Toxicology 44
10.2 Residue Chemistry 44
10.3 Occupational and Residential Exposure 44
11.0 Hazard Characterization/Assessment 45
11.1 Hazard Characterization 45
11.1.1 Database Summary 45
11.1.1.1 Studies available and considered 45
11.1.1.2 Mode of action, metabolism, and toxicokinetic data 45
11.1.1.3 Sufficiency of studies/data 46
11.1.2 Toxicological Effects 47
11.1.2.1 Acute toxicity 47
11.1.2.2 Subchronic, Chronic, and Other Toxicity 48
11.1.3 Dose-Response 51
11.2 FQPA Hazard Considerations 61
11.2.1 Adequacy of the Toxicity Data Base 61
11.2.2 Evidence of Neurotoxicity 61
11.2.3 Developmental Toxicity Studies 62
11.2.3.1 Developmental Toxicity Study in Rats 62
11.2.3.2 Developmental Toxicity Study in Rabbits 62
11.2.4 Reproductive Toxicity Study 62
11.2.5 Additional Information from Literature Sources 63
11.2.6 Pre-and/or Postnatal Toxicity 63
11.2.6.1 Determination of Susceptibility 63
11.2.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre- and/or Postnatal Susceptibility 63
11.3 Recommendation for a Developmental Neurotoxicity Study 64
11.3.1 Evidence that supports requiring a Developmental Neurotoxicity Study 64
11.3.2 Evidence that supports not requiring a Developmental Neurotoxicity Study 64
11.3.2.1 Rationale for the UF_{DB} 64
11.4 Hazard Identification and Toxicity Endpoint Selection 64
11.4.1 Acute Reference Dose (aRfD) - Females (13 to 49 years of age) 64

11.4.2 Acute Reference Dose (aRfD) - General Population 65
11.4.3 Chronic Reference Dose (cRfD) 65
11.4.4 Incidental Oral Exposure 66
11.4.5 Dermal Absorption 66
11.4.6 Dermal Exposure (Short-, Intermediate-, and Long-term) 66
11.4.7 Inhalation Exposure (Short-, Intermediate-, and Long-term) 66
11.4.8 Margins of Exposure and Levels of Concern 66
11.4.9 Recommendation for Aggregate Exposure Risk Assessments 67
11.4.10 Classification of Carcinogenic Potential 67
11.5 Special FQPA Safety Factor 67
11.6 Endocrine Disruption 69
12.0 Public Health Data 69
13.0 Exposure Characterization/Assessment 69
13.1 Dietary Exposure/Risk Pathway 69
13.1.1 Residue Profile 69
13.1.1.1 Residues in Food 69
13.1.1.2 Residues in Drinking Water 71
13.1.2 Acute and Chronic Dietary Exposure and Risk 72
13.2 Residential (Non-Occupational) Exposure/Risk Pathway 72
14.0 Aggregate Risk Assessments and Risk Characterization 72
15.0 Cumulative Risk Characterization/Assessment 72
16.0 Occupational Exposure/Risk Pathway 73
17.0 Data Needs and Label Requirements 73
17.1 Toxicology 73
17.2 Residue Chemistry 73
17.3 Occupational and Residential Exposure 73

References 73

Attachment 1: Report of the *Ad Hoc* Triazole HED Peer Review Committee 75

Attachment 2: Second Report of the *Ad Hoc* Triazole HED Peer Review Committee 82

Attachment 3: Triazole Summaries - Additional Data Summaries/Literature Articles 87

1.0 Executive Summary

The Office of Pesticide Program's Health Effects Division (HED) has conducted aggregate human health risk assessments for 1,2,4-triazole and triazole conjugates (triazole alanine and triazole acetic acid). These assessments include evaluation of risks for various population subgroups, including those comprised of infants and children. 1,2,4-Triazole and its conjugates are common metabolites to the class of compounds know as the triazoles (a.k.a. triazole-derivative fungicides, T-D fungicides, conazoles). These compounds all have a triazole ring with nitrogen atoms at the 1, 2, and 4 positions. In 2000, the Agency delayed granting registration of any new triazole pesticides or new uses of already registered triazole pesticides pending resolution of a number of toxicological, occupational/residential, and dietary exposure concerns, and issued data call ins for a number of studies. At this time sufficient data are available to support a risk assessment for these metabolites. Based on the toxicological information available at this time, HED has conducted two assessments: one for 1,2,4-triazole (1,2,4-T) and one for combined exposure to triazole alanine (TA) and triazole acetic acid (TAA). Both assessments are highly conservative, screening-level evaluations in terms of the hazards associated with these compounds (e.g., use of the maximum combination of uncertainty factors) and potential dietary and non-dietary exposures (i.e., high-end estimates of both dietary and non-dietary exposures).

Triazole alanine and triazole acetic acid residues are primarily associated with plant commodities whereas 1,2,4-triazole is associated with rats and livestock, with lesser amounts being found in plants. All three metabolites may occur in the environment, with their relative proportions depending on a variety of environmental conditions. The degree of formation of these metabolites in plants, animals, and the environment is highly dependent on the properties of the various parent triazole pesticides.

Available studies indicate that 1,2,4-triazole affects the central and peripheral nervous systems, reproductive tissues of both sexes, and the hematological system. Developmental and reproductive effects have been noted for this compound. Based on the available metabolism data from rats and livestock, 1,2,4-triazole may form in humans following exposure to parent triazole compounds. In estimating exposures, HED has included direct exposure to residues of 1,2,4-triazole as well as indirect exposure via exposure to parent triazole pesticides and subsequent conversion to 1,2,4-triazole. Triazole-derivative fungicides (T-D fungicides) have registered or requested uses for a number of food commodities as well as registered and proposed uses for turf and ornamental plantings. HED has assessed potential dietary and non-dietary exposures. The assessments are based on health-protective assumptions regarding the toxicology of and exposure to 1,2,4-triazole. For all individual exposure pathways as well as for aggregate exposure, risk estimates fall below HED's level of concern for all population subgroups.

Relative to triazole alanine, fewer studies are available depicting the toxicological effects of the triazole conjugates. For purposes of this risk assessment, HED has assumed that the triazole conjugates are all toxicologically equivalent to triazole alanine. The available studies found developmental skeletal effects, decreased body weight and body weight gain, and decreased leukocytes and triglycerides. The triazole conjugates are generally not found in animal metabolism studies; therefore, HED has only assessed the direct exposure to these compounds. Furthermore, residues of TA and TAA are formed, and remain, within plant structures, making

them unavailable for dermal or hand-to-mouth exposures. Therefore, HED has assessed exposure to the triazole conjugates for the dietary pathway only. Exposure estimates for the triazole conjugates indicate that risk associated with these compounds is below HED's level of concern. The exposure estimates are based on health-protective assumptions regarding residues of the triazole conjugates.

Based on the available information and on conservative estimates of hazard and exposure, there are no human health risk issues associated with 1,2,4-triazole or its metabolites that would preclude re-registration of the triazole-derivative fungicides registered to date or conditional registration of the triazole-derivative fungicide uses that have been proposed as of September 1, 2005. HED recommends that resolution of the following issues be a condition of registration for new uses and new active ingredients.

- Chemistry:
  - Final two-year storage stability study with 1,2,4-triazole;
  - Resolution of concerns regarding the prevalence of conjugated residues of TA and the ability of the analytical method to quantify them.
- Toxicology:
  - Free triazole:
    - Developmental neurotoxicity study in rats;
    - Chronic toxicity/oncogenicity study in male rats and female mice [This study, included in the original data call-in, has not been submitted. A previous waiver request for this study was denied; a new waiver request submitted in August, 2005, is under review];
    - Acute neurotoxicity study in rats [This study, included in the original data call-in, was placed in reserve pending the results of the combined subchronic/neurotoxicity study, in response to a previous waiver request. A new waiver request for this study was submitted in August 2005, and is under review.];
  - Triazole alanine:
    - Developmental toxicity study in rabbits;
    - Chronic toxicity study in rats, conducted according to current guidelines that include neurobehavioral assessments, with additional neuropathology evaluations conducted according to the neurotoxicity guidelines;
  - Triazole acetic acid:
    - Developmental toxicity study in rabbits;
    - Combined 90-day feeding/neurotoxicity study in rats;
- Occupational/Residential Assessment:
  - None. HED has agreed to waive previous requirements for dislodgeable foliar and turf-transferable residue studies.

New uses for triazole pesticides should also be examined in terms of potential residues of 1,2,4-triazole and its conjugates. This assessment may require revision if new uses are for sites not already addressed by the current list of registered or proposed uses, if the formation of the metabolites exceeds the estimates used herein, or if required toxicity data raise concerns not addressed by the current risk assessment.

Preface and General Information

This document addresses human health risks associated with metabolites that are common to the class of compounds know as triazoles. These compounds all contain a triazole ring with nitrogen atoms at the 1,2, and 4 positions (Figure 1.1). The HED has identified 1,2,4-triazole (1,2,4-T) and its conjugates, triazole alanine (TA), triazole acetic acid (TAA), triazole pyruvic acid, and triazole lactic acid as compounds with potentially significant toxicological properties. The occurrence of triazole pyruvic acid and triazole lactic acid in metabolism studies is low; therefore, those two metabolites have not been explicitly included in this risk assessment. In 2000 the Agency delayed granting the registration of any new triazole-derivative fungicides (T-D fungicides) or new uses of registered T-D fungicides pending resolution of issues regarding toxicology, dietary exposure, and non-dietary exposure. At this time, the HED has enough information to proceed with risk assessments for these compounds, conditional on submission of additional toxicological and residue chemistry data as discussed in Sections 10 and 17.

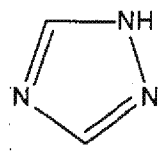
This risk assessment is intended to serve as a support document for registration and reregistration activities of the triazole-derivative fungicides. There are large number of new active ingredients and new uses that are pending for this class of compounds due to the five-year period that has elapsed since the Agency noted 1,2,4-triazole and its conjugates as residues of concern. This assessment takes into consideration the compounds and use sites that are being evaluated for reregistration, as well as the new compounds and new use sites that have been submitted to the Agency (as of 9/1/05). OPP is expecting to receive a significant number of additional new active ingredient and new use requests. HED has designed the assessments presented herein to be extremely conservative so that the assumptions associated with the risk estimates will remain valid for the majority of these expected requests. Reexamination of the risk estimates for 1,2,4-triazole, triazole alanine, and triazole acetic acid will be required:

- For crops or use sites that have not been included in the current established/requested set of uses (e.g., potatoes)
- Upon review of metabolic information for a new compound or a new use showing formation of 1,2,4-triazole, triazole alanine, triazole acetic acid, and/or labile conjugates that exceeds the maxima assumed in this assessment.
- Upon review of the required toxicology data for 1,2,4-triazole and its conjugates as discussed below.

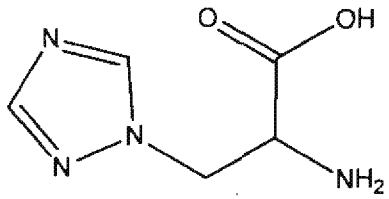
In examining exposure to 1,2,4-triazole, HED has considered both direct exposure to this compound and indirect exposure resulting from the *in-vivo* formation of 1,2,4-triazole following exposure to parent triazole-derivative compounds. At this time, the degree to which the pharamcokinetics (PK) and pharamacodynamics (PD) of exogenous 1,2,4-triazole are the same as the PK and PD of 1,2,4-triazole formed following *in vivo* metabolism is unknown. Specifically, this analysis assumes that the target tissue, the dose at the target tissue, and timing of exposure and toxic effect are similar between the exogenous and internally generated forms of 1,2,4-triazole. As the purpose of the current risk assessment is to provide high-end, screening level risk estimates, HED has made the conservative assumption that the direct and indirect exposures can occur simultaneously and are toxicologically equivalent.

2.0 Chemical Profile

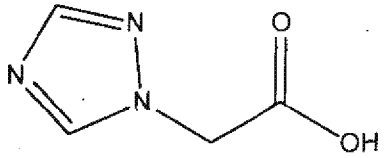
Following application of a triazole-derivative fungicide, biological and/or chemical processes may cause the triazole ring to be released from the parent compound. In rats and livestock, 1,2,4-triazole is relatively stable and is the terminal form of the triazole ring. In plants, the 1,2,4-triazole molecule may become conjugated to serine. The resulting compound, triazole alanine, may be oxidized to form triazole acetic acid. Triazole alanine and triazole acetic acid are the primary terminal forms of the triazole ring in plants, though some 1,2,4-triazole may remain. All three compounds were identified in environmental fate studies and there is evidence that in the environment there is significant conversion between all three forms. The degree of formation of any given form of the triazole ring is highly dependent on the nature and properties of the parent compound. Although other triazole conjugates such as triazole lactic acid and triazole pyruvate have been observed in plant metabolism studies, HED has concluded that TA and TAA are the predominant conjugates that need to be included in the dietary risk assessment.



1,2,4-Triazole
(a.ka. 1,2,4-T; free triazole)
CAS No. 288-88-0
PC Code: 600074
Molecular wgt.: 69.07



Triazole Alanine
(a.ka. TA)
CAS No. 86362-20-1
PC Code: 600011
Molecular wgt.: 156.15



Triazole Acetic Acid
(a.k.a. TAA)
CAS No. 28711-29-7
PC Code: 600082
Molecular wgt.: 127.10

Figure 1.1. Chemical structures for 1,2,4-triazole, triazole alanine, and triazole acetic acid

Based on the currently available toxicology data, the toxicological effects of 1,2,4-T are sufficiently different from those of the conjugates (TA, TAA) that it is appropriate to consider their risks separately. As such, this document consists of two risk assessments, one for 1,2,4-T (Sections 4 – 10) and one for combined residues of TA and TAA (Sections 11 – 17).

We note that in conducting these assessments we have used maximum metabolic conversion factors and have not made an effort to maintain mass balance with respect to residues of 1,2,4-T versus TA/TAA. As a result, a summation of the residue estimates used in these assessments results in a total residue estimate that may be greater than physically possible based on conservation of mass.

2.1 Summary of Registered/Proposed Uses Considered in These Assessments

At this time, there are 13 registered triazole-derivative fungicides with 53 uses on foods and 11 uses on non-foods (i.e., ornamental plants, turf, etc.). Additionally, the Agency has been petitioned to establish registrations for 14 triazole-derivative fungicides with 69 food uses and 4 non-food uses. This risk assessment addresses all registered and requested uses as of September 1, 2005. These uses are summarized in Table 2.1.

Table 2.1. Summary of Triazole-derivative Fungicides and Uses Addressed in these Risk Assessments.

Sorted by Active Ingredient		Sorted by Use Site	
Active Ingredient	Use Site	Use Site	Active Ingredient
Bitertanol	Banana	Almond	Fenbuconazole
Bromuconazole	Banana		Myclobutanil
	Ornamentals		Propiconazole
	Turf	Apple	Difenoconazole
Cyproconazole	Coffee	Artichoke	Myclobutanil
	Soybean		Triadimefon
	Turf	Asparagus	Myclobutanil
Difenoconazole	Apple		Tebuconazole
	Banana		Triadimefon
	Barley	Banana	Bitertanol
	Canola		Bromuconazole
	Grape		Difenoconazole
	Sweet Corn		Epoxiconazole
	Wheat		Fenbuconazole
Epoxiconazole	Banana		Hexaconazole
Fenbuconazole	Almond		Metconazole
	Banana		Myclobutanil
	Blueberry		Propiconazole
	Citrus Fruit Group		Tebuconazole
	Cranberry		Tetraconazole
	Grape		Triadimenol
	Ornamentals	Barley	Difenoconazole
	Peanut		Prothioconazole
	Pecan		Tebuconazole
	Pome Fruit Group		Triticonazole
	Stone Fruit Group	Berry Group	Propiconazole
	Sugarbeat	Blueberry	Fenbuconazole
	Turf	Bulb Vegetable Group	Propiconazole
	Wheat		Tebuconazole
Flusilazole	Soybean	Caneberry	Myclobutanil
Hexaconazole	Banana		Triadimefon
Ipconazole	Cucurbit Vegetable Group	Canola	Difenoconazole
	Ornamentals		Prothioconazole
	Sweet Corn	Carrot	Propiconazole
	Turf	Celery	Propiconazole
Metconazole	Banana	Cereal Grain Group	Propiconazole
	Soybean		Triadimenol
Myclobutanil	Almond	Citrus Fruit Group	Fenbuconazole
	Artichoke		Propiconazole
	Asparagus	Coffee	Cyproconazole
	Banana		Tebuconazole

Table 2.1. Summary of Triazole-derivative Fungicides and Uses Addressed in these Risk Assessments.

Sorted by Active Ingredient		Sorted by Use Site	
Active Ingredient	Use Site	Use Site	Active Ingredient
	Caneberry		Triadimefon
	Cotton	Corn	Propiconazole
	Cucurbit Vegetable Group		Tebuconazole
	Currant/Gooseberry		Triadimenol
	Grape	Cotton	Myclobutanil
	Hops		Tebuconazole
	Mayhaw		Triadimenol
	Ornamentals	Cranberry	Fenbuconazole
	Peppermint/Spearmint		Propiconazole
	Peppers	Cucurbit Vegetable Group	Ipconazole
	Pome Fruit Group		Myclobutanil
	Snap Bean		Tebuconazole
	Stone Fruit Group	Currant/Gooseberry	Myclobutanil
	Strawberry		Propiconazole
	Sugar Beet	Dry Bean and Pea Group	Prothioconazole
	Tomato		Tebuconazole
	Turf	Grape	Difenoconazole
Paclobutrazol	Ornamentals		Fenbuconazole
	Turf		Myclobutanil
Propiconazole	Almond		Tebuconazole
	Banana		Triadimefon
	Berry Group	Grass Grown for Seed	Propiconazole
	Bulb Vegetable Group		Tebuconazole
	Carrot	Hops	Myclobutanil
	Celery		Tebuconazole
	Cereal Grain Group	Lychee	Tebuconazole
	Citrus Fruit Group	Mango	Tebuconazole
	Corn	Mayhaw	Myclobutanil
	Cranberry		Triadimefon
	Currant/Gooseberry	Oats	Tebuconazole
	Grass Grown for Seed	Okra	Tebuconazole
	Ornamentals	Ornamentals	Bromuconazole
	Peanut		Fenbuconazole
	Pecan		Ipconazole
	Pineapple		Myclobutanil
	Pistachio		Paclobutrazole
	Sorghum		Propiconazole
	Soybean		Tebuconazole
	Stone Fruit Group		Triadimefon
	Strawberry	Peanut	Fenbuconazole
	Sugar Beet		Propiconazole
	Sugarcane		Prothioconazole

Table 2.1. Summary of Triazole-derivative Fungicides and Uses Addressed in these Risk Assessments.

Sorted by Active Ingredient		Sorted by Use Site	
Active Ingredient	Use Site	Use Site	Active Ingredient
	Turf		Tebuconazole
	Wild Rice		Tetraconazole
Prothioconazole	Barley	Pecan	Fenbuconazole
	Canola		Propiconazole
	Dry Bean and Pea Group	Peppermint/Spearmint	Myclobutanil
	Peanut	Peppers	Myclobutanil
	Rice	Pineapple	Propiconazole
	Soybean		Triadimefon
	Wheat	Pistachio	Propiconazole
Tebuconazole	Asparagus		Tebuconazole
	Banana	Pome Fruit Group	Fenbuconazole
	Barley		Myclobutanil
	Bulb Vegetable Group		Tebuconazole
	Coffee		Triadimefon
	Corn	Rice	Prothioconazole
	Cotton	Snap Bean	Myclobutanil
	Cucurbit Vegetable Group	Sorghum	Propiconazole
	Dry Bean and Pea Group		Triadimenol
	Grape	Soybean	Cyproconazole
	Grass Grown for Seed		Flusilazole
	Hops		Metconazole
	Lychee		Propiconazole
	Mango		Prothioconazole
	Oats		Tebuconazole
	Okra		Tetraconazole
	Ornamentals	Stone Fruit Group	Fenbuconazole
	Peanut		Myclobutanil
	Pistachio		Propiconazole
	Pome Fruit Group		Tebuconazole
	Soybean	Strawberry	Myclobutanil
	Stone Fruit Group		Propiconazole
	Sunflower	Sugar Beet	Myclobutanil
	Tree Nuts Group		Propiconazole
	Turf		Tetraconazole
	Turnip		Fenbuconazole
	Wheat	Sugarcane	Propiconazole
Tetraconazole	Banana	Sunflower	Tebuconazole
	Peanut	Sweet Corn	Difenoconazole
	Soybean		Ipconazole
	Sugar Beet	Tomato	Myclobutanil
	Turf	Tree Nuts Group	Tebuconazole
Triadimefon	Artichoke	Turf	Bromuconazole

Table 2.1. Summary of Triazole-derivative Fungicides and Uses Addressed in these Risk Assessments.			
Sorted by Active Ingredient		Sorted by Use Site	
Active Ingredient	Use Site	Use Site	Active Ingredient
	Asparagus		Cyproconazole
	Caneberry		Fenbuconazole
	Coffee		Ipconazole
	Grape		Myclobutanil
	Mayhaw		Paclobutrazole
	Ornamentals		Propiconazole
	Pineapple		Tebuconazole
	Pome Fruit Group		Tetraconazole
	Turf		Triadimefon
Triadimenol	Banana		Triticonazole
	Cereal Grain Group	Turnip	Tebuconazole
	Corn	Wheat	Difenoconazole
	Cotton		Fenbuconazole
	Sorghum		Prothioconazole
Triticonazole	Barley		Tebuconazole
	Turf		Triticonazole
	Wheat	Wild Rice	Propiconazole

3.0 Metabolism Assessment

3.1 Comparative Metabolic Profile

1,2,4-Triazole; triazole alanine; and triazole acetic acid are common metabolites to the class of compounds known as triazoles. In plants, 1,2,4-T is rapidly conjugated with serine to form TA. The TA can then be oxidized to form TAA. The extent to which 1,2,4-T, TA, and/or TAA forms in a given plant or animal is primarily dependent on the parent triazole-derivative fungicide. Across the various parent compounds, maximum formation of 1,2 4-T in plants ranges from 0% of the total radioactive residue (TRR) to 17% TRR, with the majority of compounds yielding 1,2,4-T at around 5-10% TRR. In rats, goats, and hens, maximum 1,2,4-T formation ranges from 0 to 77% TRR. Formation of 1,2,4-T in the rat is less than 20% TRR for the majority (approximately 80%) of the triazole-derivative fungicides for which data are available. Formation of TA ranges from 0 to 89% TRR in plants. Triazole acetic acid formation in plants ranges from 0 to 76% TRR. Triazole alanine and triazole acetic acid have generally not been found to be significant metabolites in rats, lactating goats, or laying hens. The exception to this appears to be fenbuconazole. In studies with radio-labeled fenbuconazole fed to goats and hens, TA formation ranges from 0 to 35% TRR; formation of TA from fenbuconazole was not noted in the rat metabolism studies. There is evidence from toxicological studies that there can be limited reduction of TA to 1,2,4-T following oral exposure to TA.

In environmental fate studies, all three forms of triazole (1,2,4-T, TA, and TAA) have been found and there is evidence that the three can inter-convert in soil and aquatic systems. At this time, there are not reliable data available for making general descriptions about the relative rates of formation of these compounds or any steady-state equilibria that may occur.

1,2,4-Triazole

4.0 Hazard Characterization/Assessment

4.1 Hazard Characterization

1,2,4-triazole (free triazole) is a metabolite common to a number of triazole-derivative pesticides, and is found in both mammalian (rat) and plant metabolism studies. Although for most pesticides, mammals convert only a small proportion to free triazole (less than 25%), two compounds (tetraconazole and flusilazole) demonstrate relatively high conversion (68-77%) in rat metabolism studies. As a plant metabolite, and given the wide use of triazole-derivative pesticides (used as fungicides on many crops as well as on turf) free triazole is found in a variety of food commodities, including animal byproducts. 1,2,4-triazole appears to be relatively stable in the environment, and may be found in rotational crops as well as in water.

The available toxicology database for 1,2,4-triazole consists of 2 developmental toxicity studies (in rats and rabbits), a reproductive toxicity study (in rats), a combined subchronic/neurotoxicity study (in rats), and 28- and 90-day toxicity studies in mice. Two additional non-guideline studies in rats (a 30-day gavage study and an unacceptable 90-day subchronic study) provide limited additional information. No reliable data are available for dogs (an incomplete study examining ocular toxicity only was submitted, but included very limited procedural information), and no chronic or oncogenicity studies have been submitted. Although limited chronic toxicity data (chronic toxicity/oncogenicity studies in male rats and female mice) were called in by RD in 2002 (see TXR #0052011), these studies have not been conducted and a new request to waive those studies has been recently submitted (August 2005). HED has not yet completed an evaluation of the request and, to date, the data requirement remains. A previous waiver request, submitted by USTTF in March 2003 [DP Barcode 289197], was reviewed by an HED peer review committee in June 2003, and was denied [see attached memorandum, August 5, 2003, K. Raffaele to Bob Tomerlin, TXR#0052012].

Available unacceptable metabolism studies indicate that 1,2,4-triazole is rapidly absorbed after oral administration and widely distributed in all evaluated tissues. Excretion occurs mostly via the urine, largely as unchanged parent (80-95%), although biliary canulation results suggest the possibility of some enterohepatic recirculation. With an estimated half-life of 8-10 hours, excretion is largely completed within 48 hours of administration of a single dose.

Limited acute toxicity data for 1,2,4-triazole are available, but no complete guideline studies have been submitted (Table 4.1). Available acute data indicate that 1,2,4-triazole is slightly toxic by the oral route (Category III, with oral LD50 values ranging from 666 mg/kg in rabbits to 3650 mg/kg in mice) and slightly to moderately toxic by the dermal route (dermal LD50s were less than 2000 mg/kg in rabbits, and 3000-4000 mg/kg in rats). Limited available information indicates that 1,2,4-triazole is slightly irritating or non-irritating to the skin, but severely irritating to the eye. Based on the limited acute toxicity data, as well as the available developmental toxicity data (see below), it appears that rabbits may be substantially more susceptible to 1,2,4-triazole than are rats or mice.

In spite of the limitations of the available database, a number of target organs and critical effects have been identified. 1,2,4-triazole targets the nervous system, both central and peripheral, as brain lesions (most notably in the cerebellum) were seen in both rats and mice, and peripheral nerve degeneration was also seen in the subchronic neurotoxicity study in rats. In addition, brain weight decreases were seen in several studies, including in the offspring in the reproductive toxicity study. In the subchronic/neurotoxicity study, there is evidence that effects progress over time, with an increase in incidence of clinical signs (including tremors and muscle fasciculations) during weeks 8 and 13 that were not seen during earlier evaluations. Effects were also seen on reproductive organs in both sexes, most notably ovaries (in rats) and testes (in rats and mice), in both the reproductive toxicity and subchronic toxicity studies. Hematological changes, including slightly decreased hemoglobin and/or hematocrit, have also been seen in multiple studies and species (in rats at doses of 33 mg/kg/day and above, and in mice at doses of 487 mg/kg/day and above). Studies depicting the effects of chronic exposure to free triazole or its conjugates are not currently available. A request to waive chronic/oncogenicity studies has been received by the Agency and is currently under review.

1,2,4-triazole also causes developmental toxicity in both rats and rabbits, including malformations, at doses similar to those inducing maternal toxicity (decreased body weight gain in rats and clinical signs and mortality in rabbits). Developmental toxicity was also seen in the reproductive toxicity study, with offspring showing adverse effects on multiple endpoints (including decreased brain and body weight) at doses lower than those at which effects were seen in parents. In addition, reproductive toxicity was seen in both sexes: at the highest dose (3000 ppm), only two F1 litters (one pup/litter) were produced, and neither survived to adulthood.

No data are available to directly evaluate the potential for carcinogenicity of 1,2,4-triazole. Available mutagenicity data are limited (salmonella assays submitted by the USTTF and a Russian literature report (MRID 45284011) of chromosomal aberrations in rat marrow cells), but negative. A large number of parent triazole-derivative pesticides have been classified as carcinogens (most also non-mutagenic), but the relevance of that finding to expected effects of free triazole may be limited. The types of tumors associated with exposure to the parent chemicals are most commonly hepatocellular adenomas/carcinomas in mice. Other tumor types vary considerably (including liver tumors, thyroid tumors, ovarian tumors, testicular tumors, and bladder tumors). None of the tumor types are clearly associated with the proportion of free triazole formed in available rat metabolism studies. The previous HED peer review committee concluded that it was not possible to predict toxicity of free triazole based on toxicity seen with parent compounds: these conclusions and their rationale are discussed in the memoranda from those meetings (see TXR Nos. 0052011 and 0052012, attached). This conclusion is supported by the recently submitted subchronic and reproductive toxicity studies for free triazole, identifying effects not consistently seen in toxicity studies with parent compound (including pathologic lesions in the nervous system and reproductive failure seen at the high dose in the reproductive toxicity study).

Table 4.1. Acute Toxicity Profile – 1,2,4-Triazole. Note that values on this table are based on submitted summary data; full study reports are not available.

Guideline No.	Study Type	MRID(s)	Results	Toxicity Category
870.1100	Acute oral [rat]	45284004, 45284001	LD_{50} = 1648-3080 mg/kg	III
870.1100	Acute oral [mice]	45284001	LD_{50} = 3650 mg/kg	III
870.1100	Acute oral [rabbit]	45324301	LD_{50} = 666 mg/kg	III
870.1200	Acute dermal [rat]	45284004	LD_{50} = 3129-4200 mg/kg	III
870.1200	Acute dermal [rabbit]	45324301, 45284006	LD_{50} = <2000 mg/kg	II
870.1300	Acute inhalation [mice]	45284011	LC_{50} 4 h =2200 mg/m^3	
870.1300	Acute inhalation [rats]	45284011	LC_{50} 4 h = 2050 mg/m^3	
870.2400	Acute eye irritation [rabbit]	45284004, 45324301, 45284006	severe	
870.2500	Acute dermal irritation [rabbit]	45284004, 45324301, 45284006	not irritating to slightly irritating	IV
870.2600	Skin sensitization [species]		No studies available	unknown

TABLE 4.2 Toxicity Profile for 1,2,4 triazole.

Gdln	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.3050	*28-Day oral toxicity in mice Acceptable/ non-guideline*	46467301	0, 50,250, 500, 2000 ppm **M:** 9, 47, 90, 356 mkd **F:** 12, 60, 120, 479 mkd	**NOAEL:** 90 mg/kg/day **LOAEL:** 356 mg/kg/day (male) based on testicular degeneration
870.3100	90-Day oral toxicity in mice *Acceptable/ guideline*	46467302	0, 500, 1000, 3000, 6000 ppm **M:** 80, 161, 487, 988 mkd **F:** 105, 215, 663, 1346 mkd	**NOAEL:** 80 mg/kg/day **LOAEL:** 161 mg/kg/day based on ↓testicular wt and microscopic testicular changes At 487 mg/kg/day, also tremors, ↓brain wt, slight hematology changes. At 988 mg/kg/day, also cerebellar degeneration.
870.3050	30-Day oral toxicity in rats - gavage *Acceptable/ non-guideline*	45537401	0, 8, 57, 400 mg/kg/day	**NOAEL:** <8 mg/kg/day **LOAEL:** 8 mg/kg/day based on ↓adrenal wt At 57 mg/kg/day, also slight hematology changes. At 400 mg/kg/day, also clinical signs (staggering, tremors, hunched) ↓BW
870.3100	90-Day oral toxicity in rats *Unacceptable*	45284007	0, 100, 500, 2500 ppm **M:** 8, 38, 212 mg/kg/day **F:** 10, 54, 267 mg/kg/day	**NOAEL:** 38 mg/kg/day **LOAEL:** 212 mg/kg/day based on ↓BW, convulsions, fatty infiltration of liver in males
870.3100 870.6200	90-Day oral toxicity/ neurotoxicity in rats. *Acceptable/ guideline*	46467303	0, 250, 500, 3000, 1000/ 4000 ppm **M:** 16, 33, 183, 210 mkd **F:** 19, 41, 234, 275 mkd	**NOAEL:** 16 mg/kg/day **LOAEL:** 33 mg/kg/day (male) based on ↓TSH Also at 183 mg/kg/day and above: ↓BW, tremors and other FOB, ↓brain wt, neuropath in peripheral nerves and brain (most prominently cerebellum); also slight ↓hematology, sl ↑CL.
870.3150	90-day oral toxicity study in nonrodents	---	---	No study available

TABLE 4.2 Toxicity Profile for 1,2,4 triazole.

Gdln	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.3200	21/28-Day dermal toxicity (species)	---	---	No study available
870.3250	90-Day dermal toxicity (species)	---	---	No study available
870.3465	90-Day inhalation toxicity (species)	---	---	No study available
870.3700	Developmental toxicity in rats *Acceptable/ guideline*	45223401 45223402	0, 100, 200 mg/kg/day 0, 10, 30, 100 mg/kg/day	**Maternal NOAEL:** 30 mg/kg/day **Maternal LOAEL**: 100 mg/kg/day based on ↓BW gain **Developmental NOAEL**: 30 mg/kg/day **Developmental LOAEL:** 100 mg/kg/day based on ↓fetal BW, skeletal variations, undescended testes Also at 200, increased resorptions and decreased number of viable fetuses, cleft palate, hydronephrosis, increased incidence of major malformations
870.3700	Developmental toxicity in rabbits *Acceptable/ guideline*	46492903	0, 5, 15, 30, 45 mg/kg/day	**Maternal NOAEL:** 30 mg/kg/day **Maternal LOAEL**: 45 mg/kg/day based on mortality and clinical signs (↓motor activity, head tilt, lacrimation, drooping eyelids, diarrhea, salivation) **Developmental NOAEL**: 30 mg/kg/day **Developmental LOAEL:** 45 mg/kg/day based on ↓fetal wt and urinary tract malformations
870.3800	Reproduction and fertility effects *Acceptable*	46467304	0, 250, 500, 3000 ppm **M:** 15, 31, 189 mkd **F:** 18, 36, 218 mkd	**Parental NOAEL:** <15 mg/kg/day **Parental LOAEL**: 15 mg/kg/day (male) based on ↓BW and BWG in F1 males, ↓spleen weight in F1 females At 218 mg/kg/day: cerebellar lesions, ↓brain weight, ↓thyroid weight, **Offspring NOAEL:** <19 mg/kg/day **Offspring LOAEL:** 19 mg/kg/day based on ↓BW, BWG and brain wt in F2 pups, ↓spleen weight in F2 female pups. **Repro NOAEL**: 15 mg/kg/day **Repro LOAEL**: 31 mg/kg/day based on abnormal sperm and ↓# of CL in F1 females At 218 mg/kg/day, reproductive failure (no viable offspring), ↑CL in F0 parental females
870.4100a	Chronic toxicity (rodent)	---	---	No study available
870.4100b	Chronic toxicity (dog)	---	---	No study available
870.4200	Carcinogenicity (rat)	---	---	No study available
870.4300	Carcinogenicity (mouse)	---	---	No study available
870.[ ]	Gene Mutation	---	---	See attached summaries

TABLE 4.2 Toxicity Profile for 1,2,4 triazole.

Gdln	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.[ ]	Cytogenetics	---	---	No study available
870.[ ]	Other Effects	---	---	No study available
870.6200a	Acute neurotoxicity screening battery	---	---	No study available
870.6200b	Subchronic neurotoxicity screening battery	---	---	See combined subchronic/neurotoxicity study (above)
870.6300	Developmental neurotoxicity	---	---	No study available
870.7485	Metabolism and pharmacokinetics - rat *Unacceptable/ guideline*	45284018	intraduodenal 1 mg/kg *i.v.* 0.1 - 100 mg/kg oral 0.4 - 866 mg/kg	Oral absorption of 80-95%. Excreted in urine (80-95%) and feces (<15%), biliary excretion of 10% with enterohepatic recirculation. Unchanged triazole accounted for ~95% of radioactivity.
870.7600	Dermal penetration (species)	---	---	No study available
Special studies	---	---	---	See Attachment 3

4.2 FQPA Hazard Considerations

4.2.1 Adequacy of the Toxicity Data Base

The available toxicity data base, including developmental toxicity studies in two species, a rat 2-generation reproductive toxicity study, and a subchronic/neurotoxicity study in rats, is adequate to evaluate the potential for increased toxicity in infants and children.

4.2.2 Evidence of Neurotoxicity

The available toxicity data base includes substantial evidence that exposure to 1,2,4-triazole causes neurotoxicity, including: neuropathological lesions in the brain (seen in the 90-day subchronic toxicity studies in both mice and rats, and in the reproductive toxicity study in rats [at doses of 183 mg/kg/day and above]); neuropathological lesions in the peripheral nervous system (seen in the subchronic neurotoxicity study in rats at doses similar to those causing brain lesions); and decreases in brain weight in several studies in both rats and mice (including decreases in brain weight of offspring in the reproductive toxicity study at doses not causing similar effects in F0 parents [see below]). In addition, effects indicating potential neurotoxicity were seen in the FOB and motor activity evaluations in the subchronic neurotoxicity study in rats (including tremors, muscle fasciculations, decreased arousal, decreased rearing, and decreased motor activity [at doses similar to those causing brain lesions]), and in the developmental toxicity study in rabbits (decreased motor activity, excessive salivation, hyperpnea, lacrimation, and head tilt [seen in does at doses of 45 mg/kg/day and above]).

4.2.3 Developmental Toxicity Studies

There is evidence of developmental toxicity in available studies in rats and rabbits. In rats, reduced fetal body weight, an increased incidence of runts, an increase in skeletal variations and an increase in incidence of undescended testes were seen at the LOAEL of 100 mg/kg/day, a dose also causing decreased body weight gain in dams. At 200 mg/kg/day in rats, there was an increase in malformations, including cleft palate and hydronephrosis, accompanied by an increase in post-implantation loss. In rabbits, there was a decrease in fetal weight and an increase in incidence of urinary tract malformations at doses causing severe effects in does (weight loss, multiple clinical signs, and increased mortality). The dose-response in rabbits appears to be very steep, with no effects seen at 30 mg/kg/day, and mortality seen at 45 mg/kg/day (only 15 mg/kg/day higher). In summary, there was no increase in quantitative severity in either species. There was an increase in qualitative sensitivity (more severe effects) in rats, but not in rabbits.

4.2.4 Reproductive Toxicity Study

There is evidence of increased offspring sensitivity, both quantitative and qualitative, in the reproductive toxicity study in rats. In adult (F1) male offspring, decreases in body weight and brain weight were seen at doses of 15-16 mg/kg/day and 36 mg/kg/day, respectively. Similar effects were seen in parental (F0) animals only at the highest dose of 189 mg/kg/day. Similarly, decreased brain weight and body weight were seen at (parental) doses of 18.9 mg/kg/day in F2 pups, doses below those causing similar effects in F0 animals (189 mg/kg/day). Decreases in corpora lutea were seen in F1 females at 36 mg/kg/day; similar effects were not seen in F0 females (increases in corpora lutea were seen at the high dose of 218 mg/kg/day; no changes were seen in mid-dose F0 females).

4.2.5 Additional Information from Literature Sources

None.

4.2.6 Pre-and/or Postnatal Toxicity

Toxicity was seen in offspring in all studies in which they were evaluated. Pre-natal findings include malformations as well as decreases in body weight. Findings in the reproductive toxicity study included effects on multiple organ systems, including decreases in body and brain weight and changes in reproductive organs (including testes and ovaries). It is not possible to determine whether the effects seen in the reproductive toxicity study were due to pre- or post-natal exposure.

4.2.6.1 Determination of Susceptibility

Available data indicate evidence of increased qualitative sensitivity in the rat developmental toxicity study (malformations seen at doses causing only decreased body weight gain in dams), and of quantitative sensitivity in the rat reproductive toxicity study (similar effects seen at lower

doses in offspring). No increase in qualitative or quantitative sensitivity was seen in the rabbit, with malformations occurring at doses causing severe maternal toxicity.

4.2.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre and/or Post-natal Susceptibility

Although a developmental neurotoxicity study is unavailable, available data (including nervous system lesions seen in adults and decreased brain weight in offspring) raise concern regarding developmental effects on the nervous system. In an attempt to address this concern, additional brain evaluations, including morphometric measures in weanlings, were performed in the available reproductive toxicity study. Although no effects were seen in either the qualitative or quantitative evaluations performed in pups, similar evaluations were not conducted in adult animals, in spite of evidence (decreases in brain weight in F1 adults) of persistent effects on the central nervous system. It is also important to note that the highest dose evaluated in offspring, approximately 32 mg/kg/day, was substantially lower than that causing nervous system lesions in adults not developmentally dosed (approximately 183 mg/kg/day in males in the subchronic/neurotoxicity study and 189 mg/kg/day in F0 parents in the reproductive toxicity study). Although lesions were found in the peripheral nervous system (PNS) in adults in the subchronic neurotoxicity study, there has been no evaluation of PNS tissues in offspring. In addition, behavioral evaluations of pups are not available; in the subchronic/neurotoxicity study in adult rats, behavioral effects were detected during FOB and motor activity evaluations that were not seen in routine clinical observations. It is possible similar effects could occur in offspring during development, that would not have been detected in the reproductive toxicity study. Although the toxicity seen in offspring in the reproductive toxicity study indicates that offspring were exposed, the degree of lactational transfer, and thus the relative exposure of pups and adults, is unknown.

In addition to the nervous system lesions, 1,2,4-triazole also appears to impact the function of the endocrine system, based on reproductive failure at the high dose and delays in sexual maturation seen in the reproductive toxicity study, and on lesions in reproductive organs and changes in corpora lutea counts seen in various studies in rats and/or mice.

The HED Risk Assessment Review Committee (RARC) found the residual concern for the above-described effects to be low, since the endpoint of concern (effects in the offspring at the LOAEL in the reproductive toxicity study) is being used as the basis for the current risk assessment, and the appropriate uncertainty factors are being used to account for the residual uncertainty (RARC Meeting 10/4/05; see below).

4.3 Recommendation for a Developmental Neurotoxicity Study

The triazole toxicology team determined that a Developmental Neurotoxicity study is <u>required</u> based on multiple nervous system and endocrine-related findings in available studies, including effects seen in offspring at doses not causing toxicity in F0 parental animals.

4.3.1 Evidence that supports requiring a Developmental Neurotoxicity study

Pathological lesions of the nervous system have been seen in all evaluated species, in multiple studies:

- Cerebellar lesions in the 90-day study in mice at 487/663 and 988/1346 mg/kg/day (males/females).
- Brain and peripheral nervous system lesions and behavioral effects in rats at 183/234 and 210/275 mg/kg/day in the subchronic/neurotoxicity study.
- Decreases in brain weight and cerebellar lesions in both sexes of F0 adults in the rat reproductive toxicity study at 188/218 mg/kg/day (M/F).
- Decreases in brain weight in adult offspring and weanlings in the rat reproductive toxicity study at doses of 16/19 and 33/41 mg/kg/day (M/F).
- Slight increases in incidence of retinal degeneration in the 90-day subchronic/neurotoxicity study in rats, at 183/234 and 210/275 mg/kg/day (M/F).
- Possible changes in lens biochemistry were seen in an ocular toxicity study in dogs.

Clinical signs, indicative of neurobehavioral effects have been seen in multiple studies:

- Effects were seen during FOB evaluations (including tremors, muscle fasciculations, and decreases in rearing) and motor activity evaluations in the subchronic/neurotoxicity study in rats, at 183/234 and 210/275 mg/kg/day (M/F).
- Clinical signs, including gait and postural changes, tremors, and slight convulsions, were seen in two older rat toxicity studies (30-day gavage and 90-day dietary).
- Clinical signs indicative of neurotoxicity were seen in does in the rabbit developmental toxicity study.

Possible endocrine-mediated effects have been seen in multiple studies and species:

- Testicular changes in mice (28- and 90-day mouse studies), sperm abnormalities in rats (reproductive toxicity study), decreases in testes weight in old rat 30- and 90-day toxicity studies.
- Ovarian changes in rats (90-day subchronic rat and reproductive toxicity study in rats).
- Delays in sexual maturation in rat reproductive toxicity study.
- Reproductive failure at high dose in rat reproductive study (only two litters produced, with no pups surviving lactation).
- Increased incidence of undescended testicle in rat developmental toxicity study.
- Dose-related decreases in TSH at 33 mg/kg/day and above in male rats in the subchronic/neurotoxicity study.

4.3.2 Evidence that supports not requiring a Developmental Neurotoxicity study

- No effects were seen in morphometric evaluation of weanling rats in the reproductive toxicity study.

- No CNS malformations were seen in available developmental toxicity studies in rats and rabbits.

4.3.2.1 Rationale for the UF_{DB} (when a DNT is recommended)

Based on the evidence described in Section 4.3.1 regarding the concern for neurotoxic effects in developing offspring, as well as other data gaps (lack of chronic studies, oncogenicity studies, non-rodent toxicity studies), the triazole toxicology team determined that a database UF should be set at 10x. The need for this factor will be re-evaluated upon receipt of the additional required toxicology data (see Section 10.1). A larger factor will be used for some endpoints due to failure to identify a NOAEL in the reproductive toxicity study (see below).

4.4 Hazard Identification and Toxicity Endpoint Selection

4.4.1 Acute Reference Dose (aRfD) - Females age 13-49

Study Selected: Developmental Toxicity study in rabbits
MRID Number: MRID 46492903
Dose and Endpoint for Establishing aRfD: 30 mg/kg/day, based on urinary tract malformations seen at the LOAEL of 45 mg/kg/day
Uncertainty Factor(s): UF=1000 (10x for inter-species variability, 10x for intra-species variability, and 10x for data gaps and FQPA concerns)
Comments about Study/Endpoint/Uncertainty Factor: Identified developmental toxicity suitable for this exposure duration. Although changes in birth weight and in weanling brain weight were seen in offspring from the reproductive toxicity study, these were seen at the LOAEL in the F2 generation only, and thus are unlikely to occur following a single exposure.

Acute RfD = 30 mg/kg/day (NOAEL) ÷ 1000 (UF) = 0.03 mg/kg/day

4.4.2 Acute Reference Dose (aRfD) - General Population

Study Selected: Developmental Toxicity study in rabbits
MRID Number: MRID 46492903
Dose and Endpoint for Establishing aRfD: NOAEL=30 mg/kg/day, based on clinical signs and mortality in does seen at the LOAEL of 45 mg/kg/day,
Uncertainty Factor(s): UF=1000 (10x for inter-species variability, 10x for intra-species variability, and 10x for data gaps and FQPA concerns)
Comments about Study/Endpoint/Uncertainty Factor: Effects in does were seen starting on Gestation Day 6 or 7, and are therefore suitable for this exposure duration.

Acute RfD = 30 mg/kg/day (NOAEL) ÷ 1000 (UF) = 0.03 mg/kg/day

4.4.3 Chronic Reference Dose (cRfD)

Study Selected: Reproductive toxicity study in rats (MRID 46467304)
MRID Number: MRID 46467304
Dose and Endpoint for Establishing cRfD: LOAEL=15 mg/kg/day, based on decreased body weight and body weight gain in F1 males, decreased body weight, body weight gain, and brain

weight in F2 pups. A NOAEL was not established for this study (NOAEL=less than 15 mg/kg/day).

Uncertainty Factor(s): UF=3000 (10x for intraspecies, 10x for interspecies, 10x for data gaps (lack of DNT, chronic studies, oncogenicity studies, non-rodent toxicity studies), 3x for extrapolation from LOAEL to NOAEL).

Comments about Study/Endpoint/Uncertainty Factor: Longest duration study available, with lowest effect level, in available database.

Chronic RfD = 15 mg/kg/day (LOAEL) ÷ 3000 (UF) = 0.005 mg/kg/day

4.4.4 Incidental Oral Exposure (Short-Term)

Study Selected: Developmental Toxicity study in rabbits
MRID Number: MRID 46492903
Dose and Endpoint: NOAEL=30 mg/kg/day, based on clinical signs and mortality in does seen at the LOAEL of 45 mg/kg/day,
Uncertainty Factor(s): UF=1000 (10x for inter-species variability, 10x for intra-species variability, and 10x for data gaps and FQPA concerns)

Comments about Study/Endpoint/MOE: Duration appropriate for this time frame (up to 28 days). MOE=1000 (10x for intraspecies, 10x for interspecies, 10x for data gaps and FQPA concerns).

4.4.5 Incidental Oral Exposure (Intermediate-Term)

Study Selected: Reproductive toxicity study in rats
MRID Number: 46467304
Dose and Endpoint: LOAEL=15 mg/kg/day, based on decreased body weight and body weight gain in F1 males, decreased body weight, body weight gain, and brain weight in F2 pups. A NOAEL was not established in this study (less than 15 mg/kg/day).

Comments about Study/Endpoint/MOE: Duration appropriate for this time frame (28 days to 6 months). MOE=3000 (10x for intraspecies, 10x for interspecies, 10x for data gaps (lack of DNT, chronic studies, oncogenicity studies, non-rodent toxicity studies), 3x for extrapolation from LOAEL to NOAEL).

4.4.6 Dermal Absorption

No data are available regarding dermal absorption of 1,2,4-triazole, therefore if direct dermal exposure to 1,2,4-triazole is anticipated, a default assumption of 100% dermal absorption should be used. For evaluation of risk from estimated exposure to 1,2,4-triazole occurring as a result of metabolism following dermal exposure to parent conazole compounds, dermal absorption estimates for parent compounds should be used.

4.4.7 Dermal and Inhalation Exposure (Short-Term)

Study Selected: Developmental Toxicity study in rabbits
MRID Number: MRID 46492903
Dose and Endpoint: NOAEL=30 mg/kg/day, based on clinical signs and mortality in does seen at the LOAEL of 45 mg/kg/day.
Uncertainty Factor(s): UF=1000 (10x for inter-species variability, 10x for intra-species variability, and 10x for data gaps and FQPA concerns)

Comments about Study/Endpoint/MOE: Duration appropriate for this time frame (up to 28 days). MOE=1000 (10x for intraspecies, 10x for interspecies, 10x for data gaps and FQPA concerns).

4.4.8 Dermal and Inhalation Exposure (Intermediate- and Long-Term)

Study Selected: Reproductive toxicity study in rats
MRID Number: 46467304
Dose and Endpoint: LOAEL=15 mg/kg/day, based on decreased body weight and body weight gain in F1 males, decreased body weight, body weight gain, and brain weight in F2 pups. NOAEL was not established in this study (less than 15 mg/kg/day).

Comments about Study/Endpoint/MOE: Duration appropriate for this time frame (28 days to lifetime). No longer-term study is available. MOE=3000 (10x for intraspecies, 10x for interspecies, 10x for data gaps (lack of DNT, chronic studies, oncogenicity studies, non-rodent toxicity studies), 3x for extrapolation from LOAEL to NOAEL)

4.4.9 Margins of Exposure and Levels of Concern

The levels of concern (LOC) for residential and occupational risk assessment are as follows:

Table 4.3. Summary of Levels of Concern for Residential and Occupational Risk Assessments for 1,2,4-triazole.

Route of Exposure	Duration of Exposure		
	Short-Term (1-30 Days)	Intermediate-Term (1-6 Months)	Long-Term (>6 Months)
Occupational Exposure			
Dermal	1000	3000	3000
Inhalation	1000	3000	3000
Residential Exposure			
Incidental Oral	1000	3000	3000
Dermal	1000	3000	3000
Inhalation	1000	3000	3000

4.4.10 Recommendation for Aggregate Exposure Risk Assessments

For a given duration of exposure, the same study and endpoint is being used for all exposure pathways; therefore, HED is assuming that the toxicological effects following exposure via those routes are the same. Exposure from all routes should be aggregated.

4.4.11 Classification of Carcinogenic Potential

There are no available cancer bioassay studies on 1,2,4-triazole. 1,2,4-triazole and its conjugate (triazole alanine), however, are not mutagenic. The mutagenicity data on the parent triazole fungicides (i.e., the conazoles) indicate that this class of compounds also generally lacks genotoxic potential. Some parent triazole fungicides have been shown to be carcinogenic in rodents, in particular some have produced mouse liver tumors while other have produced rat thyroid tumors, and a few produced both tumor types. Other tumor types noted with several individual triazoles include ovarian tumors, testicular tumors, and bladder tumors. For the last several years, the EPA's National Health and Environmental Effects Research Laboratory (NHEERL) has been conducting research on the potential mode of carcinogenic action underlying the rodent hepatic and thyroid follicular cell tumor responses found for the parent triazole fungicides. The NHEERL cancer mechanistic studies on fluconazole, myclobutanil, propiconazole, and triadimefon are just beginning to appear in the scientific literature (Sun et al., 2004; Wolf et al., 2005; Allen et al., 2005; Goetz et al., 2005; Hester et al., 2005; Ren et al., 2005; Ward et al., 2005). NHEERL used both traditional toxicology endpoints and DNA microarrays to profile gene expression in their investigations on potential modes of carcinogenic action. This recent evidence indicates that the parent triazole compounds appear to result in a tumor response subsequent to perturbation of liver metabolism, specifically xenobiotic and fatty acid metabolic pathways. Some of the altered pathways are regulated by CAR, PXR, and other nuclear receptors. In addition the thyroid response appears to be secondary to perturbation of thyroid homeostasis. Thus, the conazoles appear to drive a tumor response secondary to epigenetic effects and not from direct interaction with the DNA. An epigenetic mode of action would be consistent with a nonlinear process.

The toxicity profile of 1,2,4-triazole is not similar to those of related conazole pesticides showing a high rate of metabolic conversion to 1,2,4-triazole (see Appendix). Unlike the conazoles currently being studied by NHEERL, 1,2,4-triazole did not alter thyroid hormones and did not cause any of the pre-neoplastic changes in liver or thyroid typically observed following subchronic exposures. There were decreases in liver weights reported in the 30-day and 2-generation reproductive rat studies but these effects were not associated with preneoplastic changes. Also, a decrease in serum TSH was reported in a subchronic neurotoxicity study which is inconsistent with the mode of thyroid carcinogenic action which requires increased circulating TSH and subsequent thyroid follicular cell hyperplasia. The available toxicity studies on 1,2,4-triazole indicate that the nervous system and reproductive tissue are primary targets and thus those toxicities should be considered in the risk assessment.

In summary, because a chronic cancer study is not available, it is recommended that a margin of exposure or RfD approach based on the most sensitive toxicity endpoint with the inclusion of a database uncertainty factor to account for the absence of chronic toxicity studies should be used. This approach should be adequately protective of human health and would be consistent with the recently reported cancer-related findings for a series of triazole-derivative fungicides.

4.5 Special FQPA Safety Factor

Based on the hazard data, the RARC recommended the special FQPA SF be reduced to 1x because there are low residual concerns and no residual uncertainties with regard to pre- and/or postnatal toxicity. The 1,2,4-triazole risk assessment team evaluated the quality of the exposure data and, based on these data, recommended that the special FQPA SF be reduced to 1x. The recommendation is based on the following:

- The dietary exposure assessment is based on 100% CT information for all food commodities and conservatively estimated anticipated residue levels. Estimates for drinking water were generated by models and associated modeling parameters which are designed to provide conservative, health protective, high-end estimates of water concentrations. By using these screening-level assessments, dietary exposures/risks will not be underestimated.

- The residential exposure assessment is based on high-end assumptions regarding incidental oral and dermal exposures to 1,2,4-T.

Table 4.4. Summary of Toxicological Doses and Endpoints for Chemical for Use in Human Risk Assessments.

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (females 13-49)	NOAEL = 30 mg/kg UF=1000 **Acute RfD**= 0.03 mg/kg	FQPA SF = 1 **aPAD** = acute RfD / FQPA SF = 0.03 mg/kg/day	**Developmental Toxicity study in rabbits** LOAEL=45 mg/kg based on urinary tract malformations in fetuses
Acute Dietary (general population)	NOAEL = 30 mg/kg UF=1000 **Acute RfD**= 0.03 mg/kg	FQPA SF = 1 **aPAD** = acute RfD / FQPA SF = 0.03 mg/kg/day	**Developmental Toxicity study in rabbits** LOAEL=45 mg/kg based on clinical signs and mortality in does starting on Gestation Day 6 or 7
Chronic Dietary (all populations)	LOAEL = 15 mg/kg/day UF =3000 **Chronic RfD** = 0.005 mg/kg/day	FQPA SF =1 **cPAD** = chronic RfD / FQPA SF =0.005 mg/kg/day	**Reproductive Toxicity study in rats** LOAEL = 15 mg/kg/day based on decreased body weight in adult males, decreased body weight and brain weight in offspring
Incidental Oral Short-term (1-30 days)	NOAEL = 30 mg/kg/day	FQPA SF =1 LOC=1000	**Developmental Toxicity study in rabbits** LOAEL=45 mg/kg/day based on clinical signs and mortality in does starting on Gestation Day 6 or 7

Table 4.4. Summary of Toxicological Doses and Endpoints for Chemical for Use in Human Risk Assessments.

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Incidental Oral Intermediate- or Long-term (30 days to 6 months)	LOAEL = 15 mg/kg/day	FQPA SF =1 LOC=3000	**Reproductive Toxicity study in rats** LOAEL = 15 mg/kg/day based on decreased body weight in adult males, decreased body weight and brain weight in offspring
Dermal Short-term (1-30 days)	NOAEL = 30 mg/kg/day	LOC=1000	**Developmental Toxicity study in rabbits** LOAEL=45 mg/kg/day based on clinical signs and mortality in does starting on Gestation Day 6 or 7
Dermal Intermediate- or Long-term (30 days to 6 months)	LOAEL = 15 mg/kg/day	LOC=3000	**Reproductive Toxicity study in rats** LOAEL = 15 mg/kg/day based on decreased body weight in adult males, decreased body weight and brain weight in offspring
Inhalation Short-term (1 - 30 days)	NOAEL = 30 mg/kg/day	LOC=1000	**Developmental Toxicity study in rabbits** LOAEL = 45 mg/kg/day based on clinical signs and mortality in does starting on Gestation Day 6 or 7
Inhalation Intermediate- or Long-term (30 days to 6 months)	LOAEL = 15 mg/kg/day	LOC=3000	**Reproductive Toxicity study in rats** LOAEL = 15 mg/kg/day based on decreased body weight in adult males, decreased body weight and brain weight in offspring
Cancer (oral, dermal, inhalation)	**Classification:** Not determined. Evaluate by RfD approach.		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable
* Refer to Section 4.5

4.6 Endocrine disruption

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following recommendations of its Endocrine Disruptor and Testing Advisory Committee (EDSTAC), EPA determined that there was a scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the

wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

Effects were observed in tests which indicated potential estrogen, androgen, and/or thyroid mediated toxicity.

- Testicular changes in mice (28- and 90-day mouse studies), sperm abnormalities in rats (reproductive toxicity study), decreases in testes weight in old rat 30- and 90-day toxicity studies.
- Ovarian changes in rats (90-day subchronic rat and reproductive toxicity study in rats).
- Delays in sexual maturation in rat reproductive toxicity study.
- Reproductive failure at high dose in rat reproductive study (only two litters produced, with no pups surviving lactation).
- Increased incidence of undescended testicle in rat developmental toxicity study.
- Dose-related decreases in TSH at 33 mg/kg/day and above in male rats in the subchronic/neurotoxicity study.

When additional appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, 1,2,4-triazole may be subjected to further screening and/or testing to better characterize effects related to endocrine disruption.

5.0 Public Health Data

No incidents involving 1,2,4-triazole were found from a search of the Incident Data System (1992 to present), the Poison Control Center Data (1993-2003), California (1982-2003, or NIOSH (1998-2003).

6.0 Exposure Characterization/Assessment

6.1 Dietary Exposure/Risk Pathway

(M. Doherty, DP 322238, 11/1/05)

6.1.1 Residue Profile

6.1.1.1 Residues in Food

Exposure to 1,2,4-triazole may occur through direct or indirect means. For purposes of this assessment, direct exposure is defined as exposure to residues of 1,2,4-triazole, *per se*. Indirect exposure to 1,2,4-triazole may occur when there is exposure to a triazole-derivative fungicide. Indirect exposure is defined as exposure to 1,2,4-triazole that occurs from exposure to a triazole fungicide and subsequent metabolic conversion of the parent compound to 1,2,4-triazole within the human system. For assessing direct and indirect dietary exposure, residue levels of 1,2,4-triazole were estimated from parent triazole-derivative fungicide tolerances. For each food/parent fungicide tolerance combination, a residue estimate was calculated by multiplying the tolerance by a metabolic conversion factor and by a molecular weight conversion factor. Direct-exposure residue levels used the metabolic factors from plant metabolism studies for crop

commodities and from livestock metabolism studies for livestock commodities. Indirect-exposure residue levels used metabolic factors from rat metabolism studies. For some compounds metabolic conversion factors were not readily available and the maximum factors from the entire class of compounds were used (20% for plants, 77% for livestock).

Through a joint effort by the U.S. Triazole Task Force (USTTF) and USDA's Pesticide Data Program (PDP), monitoring data depicting residues of 1,2,4-triazole are available for apples, peaches, wheat flour, bananas, eggs, peanut butter, soybeans, finished water, strawberry, milk, grapes, and tomato. Most of the monitoring data for 1,2,4-triazole were reported as being below the level of quantification.

The following rules were used to select residues for the dietary exposure analysis:

1. If no monitoring data were available for the crop, or from a representative commodity, the calculated residue value was used.
2. If the monitoring dataset contains values that are greater than the LOQ, the monitoring data were used.
3. If the monitoring data are all less than the reported LOQ and the calculated residue value is less than ½ LOQ, the calculated residue values were used.
4. If the monitoring data are less than the reported LOQ and the calculated residue value is greater than the LOQ, a value of ½ LOQ was used.
5. Average residue estimates from the indirect exposure pathway were added to either the maximum direct residue estimate for acute assessments or the average direct residue estimate for chronic assessments.

For the indirect-exposure part of the analysis, average indirect-residue estimates were used for both the acute and chronic assessments. The average value was selected due to the fact that this indirect exposure is addressed, at least in part, by the toxicology studies and risk assessment for the various triazole-derivative parent fungicides. A summary of the values used in the assessments and their sources is shown in Table 6.1. Field trial studies for some triazole-derivative fungicides include analyses for 1,2,4-triazole. These data could be used to refine the exposure and risk estimates; however, due to timing issues, resource constraints, and the various stages of review of the submissions, they were not considered for these assessments.

1,2,4-Triazole is not currently listed in 40 CFR Section 180, nor is it included in the tolerance expression for any triazole-derivative fungicide. Due to the multiple potential sources of 1,2,4-triazole and its occurrence in the environment, HED believes that 1,2,4-triazole is not an appropriate analyte for tolerance-enforcement purposes and is not recommending that tolerances for this compound be established.

Table 6.1. Summary of Direct and Indirect Residue Estimates Used to Assess Dietary Exposure to 1,2,4-Triazole.

Item	Dietary Model Input, ppm [1]		Direct Exposure [2]									Average Indirect Residue Estimate, ppm [6]
			Selected Residue Estimate, ppm [3]				Calculated Data, ppm [4]		Monitoring Data, ppm [5]			
	Acute	Chronic	Acute	Acute Source	Chronic	Chronic Source	Max.	Avg.	LOQ	Max.	Avg.	
Pome Fruit (Apple)	0.0253	0.0253	0.0100	1/2 LOQ	0.0100	1/2 LOQ	0.0470	0.0182	0.02000	0.02000	0.02000	0.0153
Artichoke	0.0753	0.0655	0.0478	Max. Calc'd	0.0380	Avg. Calc'd	0.0478	0.0380	---	---	---	0.0275
Asparagus	0.0099	0.0056	0.0071	Max. Calc'd	0.0028	Avg. Calc'd	0.0071	0.0028	---	---	---	0.0028
Banana	0.0238	0.0238	0.0050	1/2 LOQ	0.0050	1/2 LOQ	0.1913	0.0279	0.01000	0.00315	0.00036	0.0188
Dry Bean	0.0230	0.0137	0.0202	Max. Calc'd	0.0109	Avg. Calc'd	0.0202	0.0109	---	---	---	0.0028
Succulent Bean	0.0568	0.0291	0.0478	Max. Calc'd	0.0201	Avg. Calc'd	0.0478	0.0201	---	---	---	0.0090
Blueberry	0.0462	0.0321	0.0404	Max. Calc'd	0.0263	Avg. Calc'd	0.0404	0.0263	---	---	---	0.0058
Caneberry	0.1495	0.1305	0.0957	Max. Calc'd	0.0767	Avg. Calc'd	0.0957	0.0767	---	---	---	0.0538
Canola	0.0100	0.0082	0.0040	Max. Calc'd	0.0022	Avg. Calc'd	0.0040	0.0022	---	---	---	0.0060
Carrot	0.0101	0.0101	0.0081	Max. Calc'd	0.0081	Avg. Calc'd	0.0081	0.0081	---	---	---	0.0020
Leafy Petioles	0.2523	0.2523	0.2018	Max. Calc'd	0.2018	Avg. Calc'd	0.2018	0.2018	---	---	---	0.0505
Barley	0.0055	0.0043	0.0040	Max. Calc'd	0.0028	Avg. Calc'd	0.0040	0.0028	---	---	---	0.0015
Oats	0.0056	0.0046	0.0040	Max. Calc'd	0.0030	Avg. Calc'd	0.0040	0.0030	---	---	---	0.0016
Rice	0.3073	0.1236	0.2826	Max. Calc'd	0.0989	Avg. Calc'd	0.2826	0.0989	---	---	---	0.0247
Rye	0.0058	0.0050	0.0040	Max. Calc'd	0.0032	Avg. Calc'd	0.0040	0.0032	---	---	---	0.0018
Wheat	0.0053	0.0040	0.0040	Max. Calc'd	0.0027	Avg. Calc'd	0.0040	0.0027	---	---	---	0.0013
Wheat Flour	0.0053	0.0040	0.0040	Max. Calc'd	0.0027	Avg. Calc'd	0.0040	0.0027	0.01000	0.01000	0.01000	0.0013
Wild Rice	0.0252	0.0252	0.0202	Max. Calc'd	0.0202	Avg. Calc'd	0.0202	0.0202	---	---	---	0.0050
Citrus Group	0.0436	0.0436	0.0410	Max. Calc'd	0.0410	Avg. Calc'd	0.0410	0.0410	---	---	---	0.0026
Coffee	0.0411	0.0005	0.0410	Max. Calc'd	0.0004	Avg. Calc'd	0.0410	0.0004	---	---	---	0.0001
Field Corn	0.0052	0.0035	0.0040	Max. Calc'd	0.0023	Avg. Calc'd	0.0040	0.0023	---	---	---	0.0012
Sweet Corn	0.0051	0.0033	0.0040	Max. Calc'd	0.0022	Avg. Calc'd	0.0040	0.0022	---	---	---	0.0011
Cotton	0.0965	0.0300	0.0898	Max. Calc'd	0.0233	Avg. Calc'd	0.0898	0.0233	---	---	---	0.0067
Cranberry	0.0460	0.0266	0.0404	Max. Calc'd	0.0210	Avg. Calc'd	0.0404	0.0210	---	---	---	0.0056
Cucurbits	0.0126	0.0100	0.0096	Max. Calc'd	0.0070	Avg. Calc'd	0.0096	0.0070	---	---	---	0.0030
Curant	0.1844	0.1328	0.1435	Max. Calc'd	0.0919	Avg. Calc'd	0.1435	0.0919	---	---	---	0.0409
Elderberry	0.0505	0.0505	0.0404	Max. Calc'd	0.0404	Avg. Calc'd	0.0404	0.0404	---	---	---	0.0101
Grape	0.0500	0.0337	0.0213	Max. Mon.	0.0050	Avg. Mon.	0.2244	0.0727	0.01000	0.02130	0.00150	0.0287

Table 6.1. Summary of Direct and Indirect Residue Estimates Used to Assess Dietary Exposure to 1,2,4-Triazole.

Item	Dietary Model Input, ppm [1]		Direct Exposure [2]									Average Indirect Residue Estimate, ppm [6]
			Selected Residue Estimate, ppm [3]				Calculated Data, ppm [4]		Monitoring Data, ppm [5]			
	Acute	Chronic	Acute	Acute Source	Chronic	Chronic Source	Max.	Avg.	LOQ	Max.	Avg.	
Raisin	0.5932	0.5132	0.4800	Max. Calc'd	0.4000	Avg. Calc'd	0.4800	0.4000	---	---	---	0.1132
Hops	1.5278	1.5278	1.3460	Max. Calc'd	1.3460	Avg. Calc'd	1.3460	1.3460	---	---	---	0.1818
Lychee	0.0855	0.0855	0.0673	Max. Calc'd	0.0673	Avg. Calc'd	0.0673	0.0673	---	---	---	0.0182
Mango	0.0114	0.0114	0.0090	Max. Calc'd	0.0090	Avg. Calc'd	0.0090	0.0090	---	---	---	0.0024
Mayhaw	0.0419	0.0419	0.0335	Max. Calc'd	0.0335	Avg. Calc'd	0.0335	0.0335	---	---	---	0.0084
Bulb Vegetables	0.0142	0.0104	0.0121	Max. Calc'd	0.0083	Avg. Calc'd	0.0121	0.0083	---	---	---	0.0021
Green Onion	0.3350	0.3350	0.3229	Max. Calc'd	0.3229	Avg. Calc'd	0.3229	0.3229	---	---	---	0.0121
Okra	0.0570	0.0570	0.0449	Max. Calc'd	0.0449	Avg. Calc'd	0.0449	0.0449	---	---	---	0.0121
Peanut	0.0097	0.0053	0.0081	Max. Calc'd	0.0037	Avg. Calc'd	0.0081	0.0037	---	---	---	0.0016
Peanut Butter	0.0779	0.0266	0.0763	Max. Mon.	0.0250	Avg. Mon.	0.0081	0.0037	0.01000	0.07630	0.02500	0.0016
Peppers	0.0717	0.0717	0.0478	Max. Calc'd	0.0478	Avg. Calc'd	0.0478	0.0478	---	---	---	0.0239
Peppermint	0.2153	0.2153	0.1435	Max. Calc'd	0.1435	Avg. Calc'd	0.1435	0.1435	---	---	---	0.0718
Pineapple	0.2192	0.1507	0.1411	Max. Calc'd	0.0726	Avg. Calc'd	0.1411	0.0726	---	---	---	0.0781
Sorghum	0.0094	0.0056	0.0081	Max. Calc'd	0.0043	Avg. Calc'd	0.0081	0.0043	---	---	---	0.0013
Soybean	0.0145	0.0145	0.0050	1/2 LOQ	0.0050	1/2 LOQ	0.0807	0.0290	0.01000	0.00290	0.00060	0.0095
Spearmint	0.2153	0.2153	0.1435	Max. Calc'd	0.1435	Avg. Calc'd	0.1435	0.1435	---	---	---	0.0718
Stone Fruit-no cherry	0.1158	0.0858	0.0957	Max. Calc'd	0.0657	Avg. Calc'd	0.0957	0.0657	0.21000	0.21000	0.21000	0.0201
Cherry	0.2863	0.1824	0.2392	Max. Calc'd	0.1353	Avg. Calc'd	0.2392	0.1353	---	---	---	0.0471
Strawberry	0.0315	0.0315	0.0180	1/2 LOQ	0.0180	1/2 LOQ	0.0605	0.0422	0.03600	0.03600	0.03600	0.0135
Sugar Beet	0.0152	0.0092	0.0121	Max. Calc'd	0.0061	Avg. Calc'd	0.0121	0.0061	---	---	---	0.0031
Sugar Beet Molasses	0.1520	0.0920	0.1210	Max. Calc'd	0.0610	Avg. Calc'd	0.1210	0.0610	---	---	---	0.0310
Sunflower	0.0028	0.0028	0.0022	Max. Calc'd	0.0022	Avg. Calc'd	0.0022	0.0022	---	---	---	0.0006
Tomato	0.0122	0.0122	0.0050	1/2 LOQ	0.0050	1/2 LOQ	0.0144	0.0144	0.01000	0.01000	0.01000	0.0072
Turnip	0.0228	0.0228	0.0180	Max. Calc'd	0.0180	Avg. Calc'd	0.0180	0.0180	---	---	---	0.0048
Turnip Greens	0.3638	0.3638	0.3590	Max. Calc'd	0.3590	Avg. Calc'd	0.3590	0.3590	---	---	---	0.0048
Tree Nuts	0.0048	0.0039	0.0040	Max. Calc'd	0.0031	Avg. Calc'd	0.0040	0.0031	---	---	---	0.0008
Almond	0.0186	0.0120	0.0164	Max. Calc'd	0.0098	Avg. Calc'd	0.0164	0.0098	---	---	---	0.0022
Pecan	0.0049	0.0049	0.0041	Max. Calc'd	0.0041	Avg. Calc'd	0.0041	0.0041	---	---	---	0.0008
Pistachio	0.0048	0.0039	0.0040	Max. Calc'd	0.0031	Avg. Calc'd	0.0040	0.0031	---	---	---	0.0008

HED Records Center Series 361 Science Reviews - File R124628 - Page 30 of 95

Table 6.1. Summary of Direct and Indirect Residue Estimates Used to Assess Dietary Exposure to 1,2,4-Triazole.

Item	Dietary Model Input, ppm [1]		Direct Exposure [2]									Average Indirect Residue Estimate, ppm [6]
			Selected Residue Estimate, ppm [3]				Calculated Data, ppm [4]		Monitoring Data, ppm [5]			
	Acute	Chronic	Acute	Acute Source	Chronic	Chronic Source	Max.	Avg.	LOQ	Max.	Avg.	
Poultry Meat	0.0073	0.0051	0.0045	Max. Calc'd	0.0023	Avg. Calc'd	0.0045	0.0023	---	---	---	0.0028
Poultry Fat	0.0064	0.0038	0.0045	Max. Calc'd	0.0019	Avg. Calc'd	0.0045	0.0019	---	---	---	0.0019
Poultry Meat Byprod.	0.0063	0.0036	0.0045	Max. Calc'd	0.0018	Avg. Calc'd	0.0045	0.0018	---	---	---	0.0018
Poultry Liver	0.0073	0.0056	0.0045	Max. Calc'd	0.0028	Avg. Calc'd	0.0045	0.0028	---	---	---	0.0028
Egg	0.0073	0.0056	0.0045	Max. Calc'd	0.0028	Avg. Calc'd	0.0045	0.0028	0.01000	0.00370	0.00050	0.0028
Cattle Meat	0.0644	0.0254	0.0517	Max. Calc'd	0.0127	Avg. Calc'd	0.0517	0.0127	---	---	---	0.0127
Cattle Fat	0.0644	0.0323	0.0517	Max. Calc'd	0.0196	Avg. Calc'd	0.0517	0.0196	---	---	---	0.0127
Cattle Meat Byprod.	0.0644	0.0253	0.0517	Max. Calc'd	0.0126	Avg. Calc'd	0.0517	0.0126	---	---	---	0.0127
Cattle Liver	0.6834	0.2236	0.5716	Max. Calc'd	0.1118	Avg. Calc'd	0.5716	0.1118	---	---	---	0.1118
Cattle Kidney	0.0675	0.0316	0.0517	Max. Calc'd	0.0158	Avg. Calc'd	0.0517	0.0158	---	---	---	0.0158
Milk	0.0080	0.0052	0.0053	Max. Mon.	0.0025	Avg. Mon.	0.0071	0.0027	0.00500	0.00530	0.00140	0.0027

[1] Values used in the dietary exposure assessment are a sum of the selected acute or chronic residue estimate and the average indirect residue estimate.
[2] Values used to estimate direct ingestion of residues of 1,2,4-triazole. Values were selected from calculated data or monitoring data as indicated.
[3] Residue estimates and sources selected from calculated data or monitoring data using the criteria discussed in Section 6.1.1.1.
[4] Maximum or average residue estimates based on parent triazole-derivative fungicide tolerances after compensating for metabolic and molecular weight factors.
[5] Residue data for 1,2,4-triazole from USDA Pesticide Data Program or U.S. Triazole Task Force monitoring data.
[6] Average indirect residue estimates based on parent triazole-derivative fungicide tolerances after compensating for rat metabolic and molecular weight factors.

HED Records Center Series 361 Science Reviews - File R124628 - Page 31 of 95

ED_014083_00002613-00227

6.1.1.2 Residues in Water

(I. Maher, DP 320682, In Preparation)

Residues of 1,2,4-triazole in drinking water were provided to HED by the Environmental Fate and Effects Division. The Tier II PRZM/EXAMS (surface water) and SCIGROW (groundwater) residue estimates are summarized in Table 6.2. The estimated surface water concentrations were used directly in the acute and chronic dietary exposure model. A small-scale prospective groundwater monitoring study was conducted in New Jersey for parent triadimefon (1997). In that study, the average maximum concentration (663-day mean) of 1,2,4-T in pore water at a depth of 9 ft was 0.0167 ppm. That level is significantly greater than the SCIGROW estimate. HED notes that there were no detections of 1,2,4-triazole in any of the 271 water samples analyzed by PDP. The limit of quantification for those analyses is 730 parts-per-trillion (0.73 ppb, 0.00073 ppm), more than two orders of magnitude less than the modeled surface water residue estimates.

Table 6.2. Summary of Tier II Modeled Concentrations of Triazole Metabolites in Drinking Water.

Exposure Duration	Surface Water Concentration, ppm	Groundwater Concentration, ppm
Acute	0.041	0.001
Chronic	0.011	0.001

6.1.2 Acute and Chronic Dietary Exposure and Risk

Acute and chronic dietary risk assessments were conducted using the Dietary Exposure Evaluation Model (DEEM-FCID, Version 2.03), which uses food consumption data from the USDA's Continuing Surveys of Food Intakes by Individuals (CSFII) from 1994-1996 and 1998. A separate cancer dietary assessment was not conducted. Although there is some concern regarding the carcinogenicity of 1,2,4-T, HED believes that the chronic dietary exposure assessment is sufficiently protective of any cancer-related effects.

The acute and chronic assessments include direct dietary exposure to 1,2,4-triazole as well as indirect exposure that may occur due to food-borne residues of parent triazole-derivative fungicide and the subsequent *in-vivo* conversion of parent compound to 1,2,4-trizole within the human system. For both direct and indirect exposures, residue estimates were derived from parent-compound tolerance values, taking into consideration any monitoring data that were available for 1,2,4-triazole. Both acute and chronic assessments make the conservative assumption that 100% of foods with triazole-derivative fungicide registrations (active, pending, and requested) were treated with a triazole-derivative fungicide. Both assessments are deterministic and include residue estimates for water.

Acute exposure estimates for 1,2,4-triazole range from 0.0025 to 0.0096 mg/kg/day at the 95th percentile of exposure. The maximum exposure estimate is for infants less than 1 year of age and corresponds to a risk estimate of 32% of the acute Population-adjusted Dose (aPAD). Chronic exposure estimates range from 0.0005 to 0.00196 mg/kg/day. For the chronic exposure estimates, the maximum is for children aged 1 to 2 years. The risk estimate associated with that group is 39% of the chronic PAD (cPAD). HED is generally concerned when risk estimates for any representative population subgroup exceed 100% of the PAD. The risk estimates associated with 1,2,4-triazole are below HED's level of concern. These assessments are considered to be

conservative because (1) the food residue estimates are derived from parent-compound tolerances or from high-end residues in monitoring data, (2) the residue in drinking water is based on conservatively modeled estimates, (3) all food and water in the analysis are assumed to have high-end levels of residue, and (4) the inclusion of the indirect exposure pathway is already partially addressed by the dietary exposure and risk analyses for the various parent compounds. As shown in Table 6.3, risk estimates for all population subgroups are below HED's level of concern at all presented percentiles of exposure. Based on the conservative nature of this assessment, the 95th percentile is the most appropriate for regulatory purposes.

Although these dietary exposure assessments are principally based on anticipated residues, the data, selection criteria, and assumptions that serve as the source of those residues are conservative in nature. HED has assumed that application of multiple triazole-derivative fungicides will not occur for any given crop during a single growing season. Although such a scenario is not currently prohibited via product labeling, HED believes that such practices will be unlikely due to economic and resistance-management issues. Overall, this assessment likely overestimates actual direct and indirect dietary exposure to 1,2,4-triazole. Even with the conservatisms in the dietary assessments, risk estimates for the general U.S. population and all representative population subgroups, including those of infants and children, are well below HED's level of concern. Reviewed field trial data depicting measured residues of 1,2,4-triazole in foods are likely to be available as HED progresses with evaluation of new-use and new-active-ingredient petitions. Use of these data, as well as incorporation of the entire distribution of monitoring data, will result in more realistic dietary exposure and risk estimates and may be useful if exposure and risk estimates require refinement.

Table 6.3. Dietary (Food + Water) Direct and Indirect Exposure and Risk Estimates for 1,2,4-Triazole.

Population Subgroup	aPAD, mg/kg/day	Exposure Estimate, mg/kg/day			Risk Estimate, %aPAD[a]		
		95th %ile	99th %ile	99.9th %ile	95th %ile	99th %ile	99.9th %ile
Acute							
U.S. Population (total)	0.03	0.0036	0.0060	0.0108	12	20	36
All infants (< 1 year)	0.03	**0.0096**	**0.0130**	**0.0212**	**32**	**44**	**71**
Children 1-2 yrs	0.03	0.0072	0.0107	0.0145	24	36	48
Children 3-5 yrs	0.03	0.0059	0.0085	0.0144	20	28	48
Children 6-12 yrs	0.03	0.0038	0.0055	0.0082	13	18	27
Youth 13-19 yrs	0.03	0.0027	0.0044	0.0067	9	15	22
Adults 20-49 yrs	0.03	0.0028	0.0044	0.0069	9	15	23
Adults 50+ yrs	0.03	0.0025	0.0034	0.0056	8	11	19
Females 13-49 yrs	0.03	0.0028	0.0043	0.0067	9	14	22
Chronic							
Population Subgroup	cPAD, mg/kg/day	Exposure Estimate, mg/kg/day			Risk Estimate, % cPAD[a]		
U.S. Population (total)	0.005	0.00069			14		
All infants (< 1 year)	0.005	0.00156			31		
Children 1-2 yrs	0.005	**0.00196**			**39**		
Children 3-5 yrs	0.005	0.00149			30		
Children 6-12 yrs	0.005	0.00088			18		
Youth 13-19 yrs	0.005	0.00054			11		
Adults 20-49 yrs	0.005	0.00055			11		
Adults 50+ yrs	0.005	0.00055			11		
Females 13-49 yrs	0.005	0.00054			11		

The values for the population with the highest risk for each type of risk assessment are bolded.
[a] Reported to 2 significant figures.

6.2 Residential (Non-Occupational) Exposure/Risk Pathway

(J. Arthur, DP 322240, 12/9/05)

6.2.1 Home Uses

There is a potential for exposure to 1,2,4-T for homeowners in residential settings during the application of T-D fungicide products on lawns (turf) and from subsequent contact during activities in such treated areas. As a result, risk assessments have been completed for both residential handler and postapplication scenarios.

Toddlers can be exposed directly to 1,2,4-T by ingesting soil where T-D fungicides have been applied due to environmental degradation of the parent compound to 1,2,4-T. Indirect exposure to 1,2,4-T for toddlers can occur from dermal contact and absorption of T-D fungicide residues on treated turf, with subsequent internal metabolism to 1,2,4-T. Similarly, indirect exposure also can occur through incidental ingestion of parent residues from hand-to-mouth and object-to-mouth activities on T-D fungicide-treated turf. Indirect 1,2,4-T exposure can occur for adults following direct dermal and inhalation exposure to T-D fungicides while applying these products to home lawns. Indirect exposure may also occur after application via the dermal route from subsequent contact with treated lawns. HED believes that formation of 1,2,4-T in plants will occur within the structures of the plant or result from uptake from soil. Therefore surface, dislodgable residues will not be available and direct hand-to mouth, object-to-mouth, or dermal exposure to 1,2,4-T are unlikely.

1,2,4-T exposure is determined by certain key characteristics specific to the parent T-D fungicide from which it is formed. These characteristics can be different for each parent T-D fungicide and include application rate, environmental and metabolic conversion rates, molecular weight, and dermal absorption factors. HED has based its assessment of residential exposure on the currently registered turf-use T-D fungicide that results in the highest 1,2,4-T exposure (i.e., triadimefon)

6.2.1.1 Application Scenarios

Non-occupational exposure is likely during the handling of T-D fungicides in the treatment of residential lawns. The major residential exposure scenarios are mixer/loader/applicators using a hose-end sprayer or a low-pressure hand wand.

The following assumptions and factors are specific to the residential assessment:

- Residential handler exposure scenarios are limited to short-term duration due to the episodic uses associated with homeowner products.
- Homeowner handler assessments are based on individuals wearing shorts and short-sleeved shirts.
- Homeowner handlers are expected to complete all tasks associated with the use of a pesticide product including mixing/loading, if needed, as well as the application.
- The Agency always considers the maximum application rates allowed by labels in its risk

assessments to consider what is legally possible based on the label.

- The Agency based calculations on what would reasonably be treated by homeowners such as the size of a lawn, or the size of a garden.
- A 70-kg body weight is used for adults because the toxicity endpoint is not gender-specific.

Residential handlers may be exposed dermally and by inhalation during mixing, loading and application of T-D fungicides for short-term durations. Because a common toxicity endpoint was identified for both dermal and inhalation routes, a combined risk from both routes of exposure is assessed. Results from these risk calculations for residential handlers are seen in Table 6.4 for triadimefon.

Table 6.4. Non-occupational Handler Exposure and Risk Estimates for 1,2,4-Triazole from Triadimefon Application to Turf.

Exposure Scenario	Personal Protective Equipment	Exposure Route	Applic. Rate, lb ai/A	Absorption Rate, %	Amount Treated per day, acre	Unit Exposure, mg/lb ai	Data Confidence	Daily Dose,[1] mg/kg/day	MOE[2]	Total Daily Dose, mg/kg/day	Total MOE[3]
M/L/A Liquids: hose-end sprayer	short sleeves short pants no gloves	Dermal	2.75	8	0.5	11[4]	High	0.00091	33,000	0.00093	32,000
		Inhalation		100		0.016[4]	High	0.000017	1.8E+6		
M/L/A Liquids: low-pressure hand wand	short sleeves short pants no gloves	Dermal	2.75	8	0.023 (1000 ft^2)	56[5]	Low	0.00021	140,000	0.00021	140,000
		Inhalation		100		0.0038[5]	Medium	0.00000018	1.7E+8		

[1] Daily Dose = [Application Rate * MW Ratio (0.24) * Metabolic Rate (0.22) * Absorption Rate * Amount Treated * Unit Exposure)]/Body Weight (70 kg)
[2] MOE = NOAEL/Daily Dose. The dermal and inhalation NOAEL = 30 mg/kg/day, was used for all calculations. The LOC = 1000.
[3] Total MOE = NOAEL/(dermal daily dose + inhalation daily dose)
[4] Unit exposure values taken from ORETF study (OMA004), "Mixer/Loader/Applicator: Hose-end Sprayer. Mix your own."
[5] Unit exposure values taken from ORETF study (OMA005), "Resident Mixer/loader/applicator - Handheld Pump Sprayer: Fruit Trees and Ornamentals."

HED Records Center Series 361 Science Reviews - File R124628 - Page 36 of 95

Residential handler risk estimates from exposure to 1,2,4-T do not exceed HED's LOC for lawn use of triadimefon. Dermal absorption factors used in this assessment were taken from the May 2005 assessment (MRID 46553701) provided by the U.S. Triazole Task Force (USTTF).

6.2.1.2 Postapplication Scenarios

Individuals of varying ages can potentially be exposed from activities on treated turf. Potential routes of exposure include dermal and incidental ingestion (toddlers only). Residential uses of T-D fungicides may result in short-term (1 to 30 days) postapplication exposures. Available data for 1,2,4-T and various parent T-D fungicides indicates that residues of these compounds may persist long enough to warrant assessment of intermediate-term exposures (> 30 days to 180 days).

The HED Standard Operating Procedures for Residential Exposure Assessments (Draft, December 18, 1997) were used as a guideline for performing the residential postapplication assessment. Also used in the assessment were interim changes to these SOPs which were adopted by the HED Exposure Science Advisory Council regarding standard values, including, for turf transferrable residues, turf transfer coefficients and hand-to-mouth activities (Policy 11, February 22, 2001). The exposure and risk estimates for the four residential exposure scenarios are assessed for the day of application (day "0") because it is assumed that adults and toddlers could contact the lawn immediately after application. On the day of application, it was assumed that 5 percent of the application rate is available from the turf grass as transferable residue (20 percent for object-to-mouth activities).

Assessment of residential postapplication exposure was performed using the same approach as was used for handler exposure above using the T-D fungicide triadimefon.

A summary of the estimated exposures and risks, along with the algorithms used for each turf exposure scenario are presented below in Tables 6.5 – 6.8 for triadimefon.

Table 6.5. Dermal Exposure and Risk for Adults and Children from Treated Lawns (Triadimefon)

Subgroup exposed	Application Rate, lb ai/A	Fraction of ai Available	Turf Transferrable Residue at Day "0" (ug/cm^2)[1]	Dermal Transfer Coefficient, cm^2/hr	Exposure Time, hrs/day	Absorption Factor	Body Weight, kg	Daily Dose,[2] mg/kg/day	MOE[3]
Adult	5.4	0.05	2.94	14,500	2	8%	70	0.0051	5,900
Children	5.4	0.05	2.94	5200	2	8%	15	0.0086	3,500

[1] Turf Transferrable Residue (ug/cm^2) = Application rate (lb ai/A) x Fraction of ai Available x 4.54E+8 ug/lb x 2.47E-8 A/cm^2
[2] Daily Dose = (Turf Transferrable Residue x Absorption Factor x 1E-3 mg/ug x Dermal Transfer Coefficient x Exposure Time x MW ratio (0.24) x Metab. Conv. Rate (0.22))/Body weight
[3] MOE = Dermal NOAEL (30 mg/kg/day) /Daily Dose. LOC = 1000.

Table 6.6. Oral Hand-to-mouth Exposure and Risk for Children from Treated Lawns (Triadimefon)

Application Rate, lb ai/A	Fraction of ai Available	Turf Transferrable Residue at Day "0", (ug/cm^2)[1]	Exposure Time, hrs/day	Extraction by saliva	Hand Surface Area, cm^2/event	Frequency, events/ hr	Body Weight, kg	Daily Dose,[2] mg/kg/day	MOE[3]
5.4	0.05	2.94	2	0.5	20	20	15	0.0041	7,300

[1] Turf Transferrable Residue (ug/cm^2) = Application rate (lb ai/A) x Fraction of ai Available x 4.54E+8 ug/lb x 2.47E-8 A/cm^2
[2] Daily Dose = (Turf Transferrable Residue x Extraction by Saliva x Hand Surface Area x Frequency x 1E-3 mg/ ug x Exposure Time x MW ratio x Metab. Conv. Rate)/Body Weight.
[3] MOE = Oral NOAEL (30 mg/kg/day) /Daily Dose. LOC = 1000.

Table 6.7. Oral Object-to-mouth (Turfgrass) Exposure and Risk for Children from Treated Lawns (Triadimefon)

Application Rate, lb ai/A	Fraction of ai Available	Grass Residue at Day "0", (ug/cm^2)[1]	Surface Area Mouthed, cm^2/day	Body Weight, kg	Daily Dose,[2] mg/kg/day	MOE[3]
5.4	0.2	11.6	25	15	0.001	30,000

[1] Grass Residue (ug/cm^2) = Application rate (lb ai/A) x Fraction of ai Available x 4.54E+8 ug/lb x 2.47E-8 A/cm^2
[2] Daily Dose = (Grass residue x Surface Area Mouthed x 1E-3 mg/ug x MW ratio (0.24) x Metab. Conv. Rate (0.22))/Body Weight.
[3] MOE = Oral NOAEL (30 mg/kg/day) /Daily Dose. LOC = 1000.

HED Records Center Series 361 Science Reviews - File R124628 - Page 38 of 95

ED_014083_00002613-00234

Table 6.8 Exposure and Risk for Children from Ingestion of Soil from Treated Lawns (Triadimefon)

Application Rate, lb ai/A	Fraction of ai Available	Soil Residue at Day "0", (ug/g)[1]	1,2,4-T to Parent MW Ratio	Max. Soil Conversion Rate to 1,2,4-T, %	Ingestion Rate, mg/day	Body Weight, kg	Daily Dose,[2] mg/kg/day	MOE[3]
5.4	1	39.4	0.24	30.7	100	15	0.000019	1,600,000

[1] Soil residue (ug/g) = [Application Rate (lbs ai/A) x Fraction of ai Available x 4.54E+8 ug/lb x 2.47E-8 A/cm^2 x 0.67 cm^3/g soil]
[2] Daily Dose = [Soil residue (ug/g) x Ingestion rate (mg/day) x 1E-6 g/ug] x Soil Conversion Rate x MW ratio / [Body Weight (kg)]
[3] MOE = Oral NOAEL (30 mg/kg/day) /Daily Dose. LOC = 1000.

HED Records Center Series 361 Science Reviews - File R124628 - Page 39 of 95

The MOEs for postapplication exposure to the T-D fungicide turf product, triadimefon are all > 1000 for individual routes of exposure, and therefore do not exceed HED's level of concern. FQPA requires residential exposures that could reasonably be expected to occur on the same day be combined and compared to the appropriate toxicity endpoint. Toddler dermal and incidental ingestion by hand-to-mouth, object-to-mouth and soil ingestion activities may co-occur. When the exposure estimates from these routes are combined, the MOE is 2200. As with the individual route MOEs, the aggregate MOE does not exceed HED's LOC.

HED has considered only ingestion of soil-borne residues in assessing intermediate-term risk. Although residues of parent T-D fungicides may persist on turf (environmental half-lives are on the order of 1-3 months), the act of mowing the lawn removes residues from the lawn surface and makes them unavailable for hand-to-mouth, object-to-mouth, and dermal exposures. The exposure estimate of 0.00013 mg/kg/day from short-term soil ingestion is being used as a highly conservative estimate for intermediate-term exposure as well. Applying the intermediate-term NOAEL of 15 mg/kg/day gives a MOE of approximately 120,000, which is significantly greater than the LOC of 3000. Therefore, the estimated intermediate-term risk is below HED's level of concern.

The exposure estimates in this assessment are based on some upper-percentile (i.e., maximum application rate) and some central tendency (i.e., surface area, hand-to-mouth activity, and body weight) assumptions and are, therefore, considered to be representative of central to high-end exposures. The uncertainties associated with this assessment stem from the use of an assumed amount of pesticide available from turf, and assumptions regarding transfer of chemical residues, and hand-to-mouth activity. Dermal absorption factors used in the assessments were taken from the May 2005 assessment (MRID 46553701) provided by the USTTF.

6.2.2 Recreational Use Sites

T-D fungicides may be used on turf at recreational use sites and, therefore may result in postapplication exposure to adults and children involved in recreational activities. Exposures to adults and children from the use of T-D fungicides at recreational use sites are assumed to be the same as those assessed for residential use sites, and therefore, a separate recreational exposure assessment was not included. Results from the residential turf exposure assessment are considered upper percentile risk estimates. Therefore, it is not expected that the high-end residential exposure scenario would occur on the same day as a high-end recreational exposure scenario. Exposures from the residential and recreational scenarios are not aggregated. Rather, the residential risk estimate should serve as a high-end estimate for both residential and recreational exposure.

6.2.3 Other (Spray Drift, etc.)

While the drifting of agricultural spray applications of T-D fungicides to nearby residential settings is possible, the T-D funcgicide turf uses addressed in the above residential risk assessment are considered to be conservative, worst case scenarios, that would cover any potential 1,2,4-T risks from agricultural spraying operations.

Spray drift is always a potential source of exposure to residents nearby to spraying operations. This is particularly the case with aerial application, but, to a lesser extent, could also be a potential source of exposure from the groundboom application. The Agency has been working with the Spray Drift Task Force, EPA Regional Offices and State Lead Agencies for pesticide regulation and other parties to develop the best spray drift management practices. The Agency is now requiring interim mitigation measures for aerial applications that must be placed on product labels/labeling. The Agency has completed its evaluation of the new data base submitted by the Spray Drift Task Force, a membership of U.S. pesticide registrants, and is developing a policy on how to appropriately apply the data and the AgDRIFT computer model to its risk assessments for pesticides applied by air, orchard airblast and ground hydraulic methods. After the policy is in place, the Agency may impose further refinements in spray drift management practices to reduce off-target drift and risks associated with aerial as well as other application types where appropriate.

6.2.4 Pharmaceutical Uses

Triazole fungicides have proved to be useful tools in combating systemic fungal infections in humans. Examples of these compounds include fluconazole, itraconazole, voriconazole, and posaconazole. The Food and Drug Administration (FDA) approves uses of pharmaceutical products under FFDCA. EPA is currently working with FDA to derive appropriate exposure assessment methodology to determine how the pharmaceutical uses of triazole fungicides should be considered in the aggregate risk assessments for 1,2,4-triazole and the triazole conjugates (triazole alanine and triazole acetic acid). A supplementary exposure analysis will be completed, and the incremental impact of exposure to pharmaceutical uses of the triazole fungicides will be considered in the Agency's reregistration decisions for propiconazole, triadimefon, and triadimenol. For additional information, please refer to the EPA memorandum to FDA regarding the pharmaceutical uses of triazole fungicides (included in the dockets for propiconazole, triadimefon, and triadimenol).

7.0 Aggregate Risk Assessments and Risk Characterization

In assessing acute and chronic risk from aggregate exposure to 1,2,4-triazole, the only scenarios appropriate for consideration are dietary exposure (all non-dietary exposures are short- to intermediate-term in duration). Water was included in the dietary exposure estimates; therefore, the acute and chronic aggregate risk estimates are the same as those presented in Section 6.1.2. Risk estimates for those durations were all less than HED's level of concern. As previously noted, the chronic risk assessment is believed to be protective of any carcinogenic effects attributable to 1,2,4-T and a separate cancer risk assessment has not been completed. Risk estimates for short- and intermediate-term aggregate exposure are presented below. Evaluation of short- and intermediate-term risk includes exposures from non-dietary sources coupled with chronic dietary exposure estimates, as an approximation of background-level exposure by the dietary route. HED notes that this approach implicitly assumes that the non-dietary and dietary exposures are constantly co-occurring during the short- or intermediate-term duration being evaluated. The approach does not take into account the temporal variability inherent in exposures from these different pathways. Triadimefon use on turf is being used to cover non-dietary exposures for all T-D fungicides.

7.1 Short-term Aggregate Risk

In assessing short-term aggregate risk, HED has combined estimates of dietary exposure (direct and indirect), dermal exposure (indirect), hand-to-mouth exposure (indirect, toddlers only), object-to-mouth exposure (indirect, toddlers only) and soil ingestion (direct, toddlers only). These short-term aggregate risk estimates are considered to be very conservative. The pathway-specific exposure estimates are derived from high-end, health-protective assumptions. In aggregating these exposure estimates, HED has made the further conservative assumption that the exposures co-occur for the duration of the exposure interval (1-30 days). The exposure estimates and resulting aggregate MOE are summarized in Table 7.1. Aggregate MOEs are greater than the LOC of 1000 for all population subgroups. Therefore, the estimated risks are not of concern.

Table 7.1. Short-term Aggregate Exposure and Risk Estimates for 1,2,4-Triazole.

Population Subgroup	Exposure Estimate, mg/kg/day							Aggregate MOE[1]
	Dietary	Dermal (M/L/A)	Dermal (Post-Applic.)	Hand-to-Mouth	Object-to-Mouth	Soil Ingestion	Aggregate	
U.S. Population (total)	0.00069	0.00183	0.0051	N/A	N/A	N/A	0.00762	3,900
All infants (< 1 year)	0.00156	N/A	0.0086	0.0041	0.0010	0.000019	0.01528	2,000
Children 1-2 yrs	0.00196	N/A	0.0086	0.0041	0.0010	0.000019	0.01568	1,900
Children 3-5 yrs	0.00149	N/A	0.0086	0.0041	0.0010	0.000019	0.01521	2,000
Children 6-12 yrs	0.00088	N/A	0.0086	N/A	N/A	N/A	0.00948	3,200
Youth 13-19 yrs	0.00054	0.00183	0.0051	N/A	N/A	N/A	0.00747	4,000
Adults 20-49 yrs	0.00055	0.00183	0.0051	N/A	N/A	N/A	0.00748	4,000
Adults 50+ yrs	0.00055	0.00183	0.0051	N/A	N/A	N/A	0.00748	4,000
Females 13-49 yrs	0.00054	0.00183	0.0051	N/A	N/A	N/A	0.00747	4,000

[1] Aggregate MOE = NOAEL (30 mg/kg/day) ÷ Aggregate Exposure Estimate (mg/kg/day). LOC = 1000.

7.2 Intermediate-term Aggregate Risk

In assessing intermediate-term aggregate risk, HED has combined dietary exposure (direct and indirect) with soil ingestion (direct, toddlers only). Other sources of non-dietary exposure have not been included. As noted in Section 6.2, many T-D fungicides are relatively stable (half-lives on the order of 1-3 months). Although residues may persist into an intermediate-term timeframe, they will not be available for hand-to-mouth, object-to-mouth, or dermal exposure. Therefore, intermediate-term aggregate risk estimates only include pathways of dietary exposure and soil ingestion. Aggregate MOEs for infants and children ages 1 to 2 years are well above the intermediate-term LOC of 3000 and, therefore, represent risks that are below HED's level of

concern. Aggregate risk estimates for older populations, where soil ingestion is not an issue, are equivalent to the dietary risk estimates presented in Section 6.1.

Table 7.2. Intermediate-term Aggregate Exposure and Risk Estimates for 1,2,4-Triazole.

Population Subgroup	Exposure Estimate, mg/kg/day			Aggregate MOE[1]
	Dietary	Soil Ingestion	Aggregate	
U.S. Population (total)	0.00069	N/A	0.00069	22,000
All infants (< 1 year)	0.00156	0.000019	0.00158	9,500
Children 1-2 yrs	0.00196	0.000019	0.00198	7,600
Children 3-5 yrs	0.00149	0.000019	0.00151	9,900
Children 6-12 yrs	0.00088	N/A	0.00088	17,000
Youth 13-19 yrs	0.00054	N/A	0.00054	28,000
Adults 20-49 yrs	0.00055	N/A	0.00055	27,000
Adults 50+ yrs	0.00055	N/A	0.00055	27,000
Females 13-49 yrs	0.00054	N/A	0.00054	28,000

[1] Aggregate MOE = NOAEL (15 mg/kg/day) ÷ Aggregate Exposure Estimate (mg/kg/day). LOC = 3000.

8.0 Cumulative Risk Characterization/Assessment

FQPA (1996) stipulates that when determining the safety of a pesticide chemical, EPA shall base its assessment of the risk posed by the chemical on, among other things, available information concerning the cumulative effects to human health that may result from dietary, residential, or other non-occupational exposure to other substances that have a common mechanism of toxicity. The reason for consideration of other substances is due to the possibility that low-level exposures to multiple chemical substances that cause a common toxic effect by a common mechanism could lead to the same adverse health effect as would a higher level of exposure to any of the other substances individually. A person exposed to a pesticide at a level that is considered safe may in fact experience harm if that person is also exposed to other substances that cause a common toxic effect by a mechanism common with that of the subject pesticide, even if the individual exposure levels to the other substances are also considered safe.

HED did not perform a cumulative risk assessment as part of this assessment for the triazole metabolites. Investigations into the potential cumulative effects of 1,2,4-triazole, triazole alanine and triazole acetic acid, and parent triazole-derivative fungicides are currently being undertaken by the Office of Research and Development. Additionally, EPA has not made a common mechanism of toxicity finding regarding the triazole metabolites and any other substances. For purposes of this assessment, EPA has assumed that 1,2,4-triazole and triazole alanine/triazole acetic acid do not have a common mechanism of toxicity and that the triazole metabolites do not have a common mechanism of toxicity with other substances.

Before undertaking a cumulative risk assessment, HED will follow procedures for identifying chemicals that have a common mechanism of toxicity as set forth in the "*Guidance for Identifying Pesticide Chemicals and Other Substances that Have a Common Mechanism of Toxicity*" (64 FR 5795-5796, February 5, 1999). For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for

cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

On this basis, the Registrant must submit, upon EPA's request and according to a schedule determined by the Agency, such information as the Agency directs to be submitted in order to evaluate issues related to whether the triazole metabolites share a common mechanism of toxicity with any other substances. If HED identifies other substances that share a common mechanism of toxicity with the triazole metabolites, HED will perform aggregate exposure assessments on each chemical and will begin to conduct a cumulative risk assessment.

As previously noted, this assessment does take into consideration the multiple parent triazole-derivative fungicides that may lead to dietary and non-dietary exposure to the triazole metabolites.

9.0 Occupational Exposure/Risk Pathway

Occupational exposure to 1,2,4-triazole is likely to occur as a result of activities associated with a single T-D compound. The risks associated with occupational exposure to this compound are addressed in the risk assessments for the parent compounds. Those risk estimates inherently include risk associated with occupational exposure to 1,2,4-triazole. Therefore, an occupational exposure and risk assessment for 1,2,4-triazole, *per se*, is not warranted. This is unlike the case for dietary exposure where it is likely that multiple T-D fungicides simultaneously lead to 1,2,4-triazole exposure.

10.0 Data Needs and Label Requirements

10.1 Toxicology

- Developmental neurotoxicity study in rats.
- Chronic toxicity/oncogenicity study in male rats and female mice [This study, included in the original data call-in, has not been submitted. As noted above, a previous waiver request for this study was denied. A new waiver request submitted in August, 2005, is under review.]
- Acute neurotoxicity study in rats [This study, included in the original data call-in, was placed in reserve pending the results of the combined subchronic/neurotoxicity study, in response to a previous waiver request. A new waiver request for this study was submitted in August 2005, and is under review.]

10.2 Residue Chemistry

- A final two-year storage stability study with 1,2,4-triazole remains outstanding. An interim report, depicting residues following one year of storage indicates that 1,2,4-T is stable in frozen food commodities.

10.3 Occupational and Residential Exposure

- A study examining dislodgeable foliar residues of the T-D fungicide metabolites was requested. However, the USTTF has requested that the Agency waive the study, arguing that any residues of metabolites would be contained within the leaf surface and therefore not dislodgeable. The waiver request has been reviewed by HED and recommended to be granted (Jack Arthur, D319566, 12/22/05).

Triazole Alanine and Triazole Acetic Acid

11.0 Hazard Characterization/Assessment

11.1 Hazard Characterization

11.1.1 Database Summary

11.1.1.1 Studies available and considered

For triazole alanine, acute toxicity was evaluated using acute oral toxicity studies and a non-guideline acute intraperitoneal toxicity study. Subchronic studies available for hazard characterization included 14-day, 28-day, and 90-day oral toxicity studies in the rat and a 90-day oral toxicity study in the dog. No chronic studies were submitted. Developmental toxicity and two-generation reproduction studies in the rat were evaluated; a developmental toxicity study in the rabbit was not available. Multiple mutagenicity and metabolism studies were considered.

For triazole acetic acid, five acceptable studies were available for hazard characterization: an acute oral toxicity study, a non-guideline 14-day oral toxicity study in the rat, a bacterial reverse mutation assay, and two non-guideline metabolism studies.

For triazole lactic acid and triazole pyruvate, no mammalian toxicology studies were available.

11.1.1.2 Mode of action, metabolism, and toxicokinetic data

Mode of action. The parent triazole fungicides act by inhibiting sterol synthesis, particularly by preventing the 14-demethylation of the sterols. The sterols, which are similar to cholesterol in mammals, are important for fungi membrane structure and function. The mode(s) of action for mammalian toxicity associated with 1,2,4-triazole and the triazole conjugates is currently unknown.

Metabolism and toxicokinetic data. Pharmacokinetic studies showed that triazole alanine is rapidly absorbed and excreted following single oral gavage doses of 0.5, 0.56, 5, 54, 56, or 994 mg/kg, following five daily oral gavage doses of approximately 48-49 mg/kg/day, and following a single dose of 5 mg/kg by i.v. injection. Excretion occurred mostly in the urine, accounting for 81-85% to >90% of the administered radioactivity in three single oral and i.v. injection studies and ~55-63% in the multiple oral dose study at 168 hours. Much less of the radioactivity was excreted in the feces, which accounted for ~2-5% of the administered radioactivity in the single dose studies and ~11-27% in the multiple oral dose study. Volatiles accounted for <1% of the

radioactivity. Tissue residues were low in all studies, with the majority of the radiolabel found in tissues located in the liver and kidneys.

The two single oral dose triazole alanine metabolism studies are consistent, since both found high amounts of unchanged triazole alanine (72-93% of the radioactivity) recovered in the urine 24 hours after exposure to 0.56-994 mg/kg, lesser amounts of N-acetyl triazole alanine (8-30%), and a small percentage of unknown metabolites. There was no mention that triazole acetic acid or 1,2,4-triazole were detected in these urinary samples. (Note that N-acetyl triazole alanine and triazole acetic acid are structurally different.) From the fecal samples taken 24 hours following oral exposure to 56 mg/kg, a high proportion of the recovered radioactivity was unchanged triazole alanine (50%), with less N-acetyl triazole alanine identified (16%), similar to what was found in the urine. However, 30% of the radioactivity found in the fecal samples consisted of an unknown component that did not appear in the urinary samples.

In the repeated oral dose metabolism study, a large amount (63%) of the radioactivity recovered in the urine samples taken 168 hours following 5 doses of ~48-49 mg/kg/day triazole alanine was identified as unchanged triazole alanine, similar to what was seen 24 hours after a single dose. However, 30% of the urinary radioactivity was identified as triazole acetic acid, and 1.6% was identified as 1,2,4-triazole. These compounds were not seen in the single dose studies; furthermore, N-acetyl triazole alanine, which was seen in the single dose studies, was not seen in the repeated dose study. This study also showed that the same three components seen in the urine were found in the feces. However, a much larger percentage of the radioactivity recovered in the feces was identified as triazole acetic acid (69%) and 1,2,4-triazole (11%), with significantly less excreted as unchanged triazole alanine (2%). The remaining radioactivity in the feces was made up of three unknown conjugates, none of which was greater than 7%.

For triazole acetic acid, two metabolism and pharmacokinetic studies showed rapid absorption and excretion following a single oral dose of approximately 0.6, 59, or 1035 mg/kg or five daily oral doses of approximately 50-51 mg/kg/day. Excretion occurred mainly in the urine, as it did for triazole alanine, accounting for ~90-104% of the administered radiolabel in the single dose study and ~66-75% of the radiolabel in the multiple dose study at 168 hours. The feces accounted for much less of the administered radiolabel: ~1-7% in the single dose study and ~11-32% in the multiple dose study. In both studies, tissue burdens were very low. Unlike triazole alanine, the sole component identified in both the urinary and fecal extracts from these two studies was triazole acetic acid, indicating that triazole acetic acid administered to the rat is excreted intact without metabolic conversion.

11.1.1.3 Sufficiency of studies/data

Triazole alanine. The partial toxicology database available for triazole alanine is insufficient to fully characterize the hazards associated with this conjugate. However, it was possible to categorize the acute toxicity of triazole alanine, to identify effects following subchronic exposure, to begin examination of the differences in toxicity associated with longer duration of exposure and across species (i.e., rat and dog), to examine potential reproductive toxicity, to evaluate the differences in susceptibility between rodent parents and offspring, and to examine the potential of triazole alanine to cause mutagenicity.

The toxicity resulting from exposures to triazole alanine longer than 90 days was not determined because no chronic studies were available.

Interspecies differences were not fully examined because no triazole alanine studies were conducted in rabbits or mice. The lack of a developmental toxicity study in rabbits is a particularly important data gap, because mortality and clinical signs were seen in adult rabbits following a single dose of 45 mg/kg 1,2,4-triazole in a rabbit developmental study (MRID 46492903). For free triazole, the rabbit was shown to be the most sensitive species; in the absence of data to suggest otherwise, it is likely that rabbits are also more sensitive to triazole alanine.

Triazole acetic acid. The toxicology database for triazole acetic acid consists of five acceptable studies, including three non-guideline studies. In the absence of additional data, the studies on triazole acetic acid were compared to corresponding studies on triazole alanine to determine potential differences in potency between these two conjugates.

Other triazole conjugates. No mammalian toxicology studies are available for triazole lactic acid or triazole pyruvate. These compounds occur only infrequently in plant metabolism studies and have not been included in the quantitative risk assessment.

11.1.2 Toxicological Effects

11.1.2.1 Acute toxicity

Triazole alanine. The acute toxicity of triazole alanine is low, based on acute oral toxicity studies in the the rat and mouse (Category IV) and an acute intraperitoneal toxicity study in the rat (LD50 > 5000 mg/kg). Although triazole alanine was classified as Category III according to results from one acute oral toxicity study (MRID 00138119), this was because inadequate dose levels were tested. Note that although the acute toxicity of 1,2,4-triazole was greater in the rabbit than in the rat or mouse, a similar comparison of the acute toxicity of triazole alanine across species could not be made since acute rabbit studies were not submitted for the conjugate.

Triazole acetic acid. The acute toxicity of triazole acetic acid is also low, based on an acute oral toxicity study in the rat (Category IV).

Table 11.1. Acute Toxicity Profile for Triazole Alanine

Test Material [% ai]	Guideline Number	Study Type	MRID Number	Results	Toxicity Category
Triazole alanine [not specified]	870.1100	Acute oral - rat	00138119 (TXR 004766 Acc. 252132) 00133356	LD_{50} (♂+♀) > 2000 mg/kg Unacceptable/supplementary	III
Triazole alanine ["analytically pure"]	870.1100	Acute oral - rat	00138120 (TXR 004766 Acc. 252132) 00133357	LD_{50} (♂+♀) > 5000 mg/kg Acceptable/guideline	IV

Table 11.1. Acute Toxicity Profile for Triazole Alanine

Test Material [% ai]	Guideline Number	Study Type	MRID Number	Results	Toxicity Category
Triazole alanine ["analytically pure"]	870.1100	Acute oral - mouse	00138120 (TXR 004766 Acc. 252132) 00133357	LD_{50} (♂+♀) > 5000 mg/kg Acceptable/guideline	IV
Triazole alanine [99%]	870.1100	Acute oral - dog	00138130 (TXR 004766 Acc. 252132)	Both dogs vomited a portion of the test material (5000 mg/kg) within 4 hours of dosing. No other signs were observed in the male. Slight salivation, slightly unsteady gait, and decreased food consumption and body weight were seen in the female. Unacceptable	Not determined
Triazole alanine ["analytical pure"]	Non-GDLN	Acute intraperitoneal - rat	00138120 (TXR 004766 Acc. 252132) 00133357	LD_{50} (♂+♀) > 5000 mg/kg Acceptable/non-guideline	N/A
Triazole alanine	870.1200	Acute dermal - rat	ND	ND	ND
Triazole alanine	870.1300	Acute inhalation - rat	ND	ND	ND
Triazole alanine	870.2400	Acute eye irritation - rabbit	ND	ND	ND
Triazole alanine	870.2500	Acute dermal irritation - rabbit	ND	ND	ND
Triazole alanine	870.2600	Skin sensitization - guinea pig	ND	ND	ND

ND = Study not done

Table 11.2. Acute Toxicity Profile for Triazole Acetic Acid

Test Material [% ai]	Guideline Number	Study Type	MRID Number	Results	Toxicity Category
Triazole acetic acid [>99%]	870.1100	Acute oral - rat	45596802	LD_{50} (♂+♀) > 5000 mg/kg	IV
Triazole acetic acid	870.1200	Acute dermal - rat	ND	ND	ND
Triazole acetic acid	870.1300	Acute inhalation - rat	ND	ND	ND
Triazole acetic acid	870.2400	Acute eye irritation - rabbit	ND	ND	ND
Triazole acetic acid	870.2500	Acute dermal irritation - rabbit	ND	ND	ND
Triazole acetic acid	870.2600	Skin sensitization - guinea pig	ND	ND	ND

ND = Study not done

11.1.2.2 Subchronic, Chronic, and Other Toxicity

Triazole alanine. The toxicity of triazole alanine in the rat was examined in 14-, 28-, and 90-day oral toxicity studies, a developmental toxicity study, and a two-generation reproduction study. Of these five studies, adverse effects in adults were only observed in the 90-day feeding study.

These effects included decreased leukocytes in males and decreased triglycerides at the LOAEL (370/400 mg/kg/day, males/females), with decreased body weight and body weight gain (males), decreased leukocytes (both sexes), and decreased triglycerides (both sexes) observed at 1510/1680 mg/kg/day (males/females).

No treatment-related effects were seen in the 14-day drinking water study, in which male rats were exposed to triazole alanine at dose levels up to ~1500 mg/kg/day. Because hematological and clinical chemistry parameters (including those decreased in the 90-day study) were not measured in this study, it is unknown if they would be affected by two weeks exposure to triazole alanine. The decreases in body weight and body weight gain seen at the high dose in the 90-day rat study were not observed until week 3, so it is not unexpected that body weight measurements remained comparable to controls in this two week study.

No treatment-related effects were seen in the 28-day oral toxicity study, in which rats were exposed to triazole alanine via oral gavage at dose levels up to 400 mg/kg/day. This study was classified unacceptable due to the lack of analytical data on the test compound and incomplete hematological, clinical chemistry, and histopathological analyses. Leukocyte counts, which were measured in this study, were unaffected following 28 days exposure to triazole alanine by gavage (vehicle = Cremophor EL), although they were slightly decreased after one month of dietary exposure in the 90-day feeding study. Triglycerides, which were decreased in the 90-day study, were not evaluated in this study. The decreased body weights and body weight gains observed on week 3 of the 90-day study were not seen in this study, which is not unexpected given that the highest dose tested in the 28-day study was just 400 mg/kg/day vs. 1510 mg/kg/day in the 90-day study.

Systemic maternal/paternal toxicity was not seen in the developmental toxicity or two-generation reproduction studies in rats at dose levels up to ~1000 mg/kg/day. Reproductive toxicity was also not noted. Because hematology and clinical chemistry are not examined in developmental or reproduction studies, it is unknown if they were affected as in the 90-day feeding study. Changes in body weights observed in the 90-day study were not seen in either of these studies; however, the highest dose levels tested in the developmental and reproduction studies were lower than those which produced body weight effects in the 90-day study.

Differences in toxicity due to increased duration of exposure to triazole alanine could not be confirmed since treatment-related effects were only seen in the 90-day study and since hematological and clinical chemistry parameters were not measured in the shorter duration studies.

Differences between routes of exposure to triazole alanine could not be examined because dermal and inhalation studies were not available.

Differences in the toxicity of triazole alanine across mammalian species were examined by comparing the 90-day feeding studies in the rat and the dog. (Studies using the mouse or rabbit were not available. The lack of a rabbit study is a particularly important data gap because the rabbit is the most sensitive species to free triazole. Mortality and clinical signs of neurotoxicity were seen in adult rabbits following a single gavage dose of 45 mg/kg 1,2,4-triazole in a rabbit

developmental study [MRID 46492903]). As stated previously, treatment-related effects in the 90-day rat study include decreased leukocytes (males) and triglycerides (females) at the LOAEL (370/400 mg/kg/day in males/females), as well as decreased leukocytes and triglycerides in both sexes and decreased body weights and body weight gains in males at the highest dose tested (1510/1680 mg/kg/day in males). Triazole alanine is less toxic in dogs. The only treatment-related effect seen in the 90-day dog study was a 10% decrease in female food consumption near the limit dose (902 mg/kg/day), with no effects seen in males.

Increased quantitative and qualitative susceptibility of the offspring was seen in the developmental toxicity rat study, and increased quantitative susceptibility was seen in the two-generation reproduction study. In the developmental toxicity study, increased incidences of skeletal findings were seen in the offspring at the mid and high doses, while no treatment-related effects were seen in the dams up to the limit dose. These skeletal findings include unossified odontoid processes at 300 and 1000 mg/kg/day, with partially ossified transverse processes of the 7th cervical vertebra (bilateral), unossified 5th sternebra, and partially ossified 13th thoracic centrum observed only at 1000 mg/kg/day. In the reproduction study, mean litter weights were decreased approximately 10-20% for both generations at ~1000 mg/kg/day, compared to controls, but parental body weights were unaffected at all treatment levels.

No mutagenic potential was noted in the following acceptable mutagenicity studies:

1. bacterial reverse mutation assays,
2. *in vitro* mammalian cell gene mutation tests conducted in BALB/3T3 and CHO cells,
3. mammalian erythrocyte micronucleus tests conducted using mice and Chinese hamsters, and
4. bacterial DNA damage and repair tests.

An increased number of transformed colonies was seen in an *in vitro* mammalian cell gene mutation test conducted in baby hamster kidney cells (MRID 00132914); however, this study was classified unacceptable/inconclusive due to severe toxicity, excessive concentrations, and lack of information regarding test material purity. Mutagenic potential was also seen in a mammalian erythrocyte micronucleus test conducted using mice (MRID 0013912); however, this study was classified unacceptable/inconclusive because effects were seen only at 24 hours and not at 48 or 72 hours. Finally, triazole alanine inhibited protein biosynthesis in microorganisms (TXR 005841); however, this study was classifed unacceptable due to inconsistent results, a lack of individual data, not enough dose levels tested, and no explanation of incubation times used.

Triazole acetic acid. In a 14-day toxicity feeding study in rats, no treatment-related effects were observed up to the highest dose tested (788/703 mg/kg/day in males/females). No other studies are available to determine the target organs and critical effects of triazole acetic acid; to compare toxicity across species, routes of exposure, or duration of exposure; or to examine potential developmental, reproductive, or neurological toxicity. This short-term study is comparable to the 14-day oral triazole alanine study, in which no effects were seen in male rats up to 1491 mg/kg/day, and to the 28-day oral triazole alanine study, in which no effects were seen in male or female rats up to 400 mg/kg/day. However, since no effects were seen in the available short-term studies, a difference in toxicity between triazole alanine and triazole acetic acid could not be evaluated.

Mutagenicity was not seen in an acceptable/guideline bacterial reverse mutation assay using triazole acetic acid or in the available mutagenicity studies for triazole alanine. Therefore, neither compound appears mutagenic.

11.1.3 Dose-Response

For triazole alanine, greater toxicity was seen at higher doses. At the mid dose (LOAEL) in the 90-day feeding study, decreased leukocytes were seen in males only and decreased triglycerides were seen only in females (370/400 mg/kg/day, males/females). However, at the high dose, decreased leukocytes and triglycerides were each seen in both sexes, along with decreased body weight and body weight gain in males (1510/1680 mg/kg/day, males/females). In the rat developmental toxicity study, more types of skeletal findings were observed in offspring at the high dose (1000 mg/kg/day), compared to the mid dose (300 mg/kg/day).

For triazole acetic acid, dose-response could not be evaluated because no treatment-related effects were observed in the available toxicity studies.

Table 11.3. Subchronic, Chronic, and Other Toxicity Profile for Triazole Alanine

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
Non-guideline	*14-Day drinking water toxicity in rodents - rat* *Acceptable/non-guideline*	00138121 (TXR 004766 Accession # 252132) 00133358	0, 3000, 10000 ppm M: 0, 448, 1491 mg/kg/day F: not tested	NOAEL = 1491 mg/kg/day (M) LOAEL = >1491 mg/kg/day (M)
870.3050	28-Day oral toxicity in rodents - rat *Unacceptable*	00138122 (TXR 004766 Accession # 252132) 00133359	0, 25, 100, 400 mg/kg/day	NOAEL = 400 mg/kg/day (M/F) LOAEL = >400 mg/kg/day (M/F) Many of the required hematology, clinical chemistry, and microscopic pathology parameters were not examined. Dosages administered to the test animals were not analytically quantitated.

Table 11.3. Subchronic, Chronic, and Other Toxicity Profile for Triazole Alanine

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.3100	90-Day oral toxicity in rodents - rat *Acceptable/non-guideline*	00164107 (TXR 005532 005094)	0, 1250, 5000, 20000 ppm M: 0, 90, 370, 1510 mg/kg/day F: 0, 160, 400, 1680 mg/kg/day	NOAEL = 90/160 mg/kg/day (M/F) LOAEL = 370/400 mg/kg/day (M/F) based on decreased leukocyte counts in males and decreased triglycerides in females. At 1510/1680 mg/kg/day (M/F), decreased body weight (M), body weight gain (M), leukocytes (M&F), and triglycerides (M&F) were seen. There was no assessment of motor activity, grip strength, or sensory reactivity of the test animals. There was no indication of how often fresh batches of food containing the test material were given to the animals.
870.3100	90-Day oral toxicity in rodents - mouse	ND	ND	ND
870.3150	90-Day oral toxicity in nonrodents - dog *Acceptable/guideline*	00164106 00154946 (TXR 004469 005841 Accession # 256058)	0, 3200, 8000, 20000 ppm M: 0, 144, 322, 850 mg/kg/day F: 0, 150, 345, 902 mg/kg/day	NOAEL = 850/345 mg/kg/day (M/F) LOAEL = >850/902 mg/kg/day (M/F) based on 10% decreased food consumption in females only. No treatment-related effects were seen in males.
870.3200	21/28-Day dermal toxicity	ND	ND	ND
870.3250	90-Day dermal toxicity	ND	ND	ND
870.3465	90-Day inhalation toxicity	ND	ND	ND
870.3700	Prenatal developmental toxicity in rodents - rat *Acceptable/guideline*	00138128 (TXR 004766 & 005155 Accession # 252132) 00132915 00147889 (TXR 0005155 Accession # 252132)	0, 100, 300, 1000 mg/kg/day	Maternal NOAEL = 1000 mg/kg/day LOAEL = >1000 mg/kg/day Developmental NOAEL = 100 mg/kg/day LOAEL = 300 mg/kg/day based on increased incidence of skeletal findings (unossified odontoid process). At 1000 mg/kg/day, increased incidences of partially ossified transverse processes of the 7th cervical vertebra (bilateral), unossified 5th sternebra, and partially ossified 13th thoracic centrum were also seen.

Table 11.3. Subchronic, Chronic, and Other Toxicity Profile for Triazole Alanine

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.3700	Prenatal developmental toxicity in nonrodents	ND	ND	ND
870.3800	Reproduction and fertility effects - rat *Acceptable/guideline*	00164112 (TXR 005841 & 008292 Accession # 252132) 41326803 (TXR 008292) 41326804 (TXR 008292)	0, 500, 2000, 10000 ppm M (F0/F1): 0, 50/47, 213/192, 1098/929 mg/kg/day F (F0/F1): 0, 51/49, 223/199, 1109/988 mg/kg/day	Parental/Systemic NOAEL = 929/988 mg/kg/day (M/F) LOAEL = >929/>988 mg/kg/day (M/F) Reproductive NOAEL = 929/988 mg/kg/day (M/F) LOAEL = >929/>988 mg/kg/day (M/F) Offspring NOAEL = 192/199 mg/kg/day (M/F) LOAEL = 929/988 mg/kg/day (M/F) based on reduced mean litter weights in both generations.
870.4100	Chronic toxicity	ND	ND	ND
870.4200	Carcinogenicity	ND	ND	ND
870.4300	Combined chronic toxicity/ carcinogenicity	ND	ND	ND
870.5100	Bacterial reverse mutation assay *Acceptable/guideline*	00138123 00132911 (TXR 004469 004562 004766 Accession #252132)	20, 100, 500, 2500, 12500 ug/plate for *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 (±S9)	Not mutagenic in bacteria (*Salmonella typhimurium*,±S9) under conditions of this assay.
870.5100	Bacterial reverse mutation assay *Acceptable*	00164111 (TXR 005841)	20-5000 µg/plate for *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 (±S9).	Not mutagenic.

Table 11.3. Subchronic, Chronic, and Other Toxicity Profile for Triazole Alanine

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.5300	*In vitro* mammalian cell gene mutation test (Baby hamster kidney cells) *Unacceptable*	00132914 00139576 (TXR 004562 004766 Accession #252132)	-S9: 0.5, 1, 2, 4, 8 mg/ml +S9: 1, 2, 4, 8, 16 mg/ml	Inconclusive. -S9: LD_{39} = 8mg/ml (HDT). Transformation frequency was 39 vs 10 for control. An increase in number of transformed colonies. +S9: LD_{50} = 5.2 mg/ml. Transformation frequency was 28 vs 0 for the control at 16 mg/ml. Increase in number of transformed colonies, but in the presence of severe toxicity and excessive concentrations. In addition, the lack of information on the test material purity raises concerns regarding the validity of the response.
870.5300	*In vitro* mammalian cell gene mutation test (BALB/3T3 cells) *Acceptable*	00147892 (TXR 005155 Accession # 257997)	62.5, 125, 250, 500, 1000 ug/ml (±S9)	Not mutagenic. Triazole alanine did not induce cell transformations at levels up to 1000 µg/ml. The HDT of 1000 µg/ml is the highest level recommended for this test system. The increase in cell transformation frequency in the presence of S9 was not significant.
870.5300	*In vitro* mammalian cell gene mutation test (CHO cells) *Acceptable*	00164108 41326801 (TXR 005841 008292)	500, 1000, 2000, 4000, 6000, 8000, 10000 µg/ml	Not mutagenic with or without S9 activation. Viability of cells at 10000 µg/ml was 66-67% relative to control. At 10000 µg/ml, a 3-fold increase was within the accepted spontaneous range for this cell line. Stability of compound was confirmed.
870.5375	*In vitro* mammalian chromosome aberration test	ND	ND	ND
870.5395	Mammalian erythrocyte micronucleus test (mice) *Unacceptable*	00132912 00138125 00138131 (TXR 004562 004766 Accession #252131)	8000 mg/kg, single oral dose to 15 mice/sex	Inconclusive because effects were only seen at 24 hours. No clinical or cytotoxice effects. TA caused slight induction of micronuclei in bone marrow PCEs at 24 hrs, but not at 48 or 72 hrs. No data for the negative control at these two intervals.

Table 11.3. Subchronic, Chronic, and Other Toxicity Profile for Triazole Alanine

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.5395	Mammalian erythrocyte micronucleus test (mice) *Acceptable/guideline*	00133361 00138124 (TXR 004562 004766 Accession # 252132)	2500 or 5000 mg/kg, by intraperitoneal injection	Not mutagenic. Under the conditions of this test, triazole alanine did not cause induction of micronuclei in mouse bone marrow PCE stem cells when administered by i.p. injection at 2500 or 5000 mg/kg. The effects of the positive control were clearly demonstrated.
870.5395	Mammalian erythrocyte micronucleus test (Chinese hamster) *Acceptable*	00164110 41326802 (TXR 005841 008292)	5000 mg/kg (limit dose) Preliminary test: 200, 1000, 5000 mg/kg	Not mutagenic. Based on scoring 1000 polychromatic erythrocytes per animal for the incidence of micronuclei, TA was comparable to the control. The best 5 slides per sex were scored.
870.5500	Bacterial DNA damage test *Acceptable/guideline*	00132913 00138126 (TXR 004469 004562 004766 Accession #252131)	62.5, 125, 250, 500, 1000 µg/plate (±S9) E. coli p3478 (polA-) & E. coli W3110 (polA+)	Not mutagenic. No measurable inhibition areolae were found for E. coli polA- or polA+ strains (±S9). Under the conditions of this assay, triazole alanine (±S9) did not elicit measurable DNA damage.
870.5550	Bacterial DNA repair test (rat hepatocytes) *Acceptable/guideline*	00164109 (TXR 005841)	0.08, 0.4, 2, 10 mg/ml Induced with Arochlor 1254	Not mutagenic. No evidence that unscheduled DNA synthesis was induced.
Non-guideline	Potential inhibition of protein biosynthesis in microorganisms *Unacceptable*	No MRID TXR 005841 Accession # 265203-265209	1, 10, 100, 500 ppm for *E. coli*, *S. cerevisiae*, and *A. flavus*	TA inhibited protein synthesis in all three microorganisms in the presence of both radiolabeled phenylalanine and alanine after 1 hr of incubation. Growth curve of E. coli indicated that maximum period of protein synthesis was ~1 hr of incubation. Unacceptable due to lack of individual data, not enough doses of TA tested, no explanation of incubation times used, inconsistent results.

Table 11.3. Subchronic, Chronic, and Other Toxicity Profile for Triazole Alanine

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/non-guideline*	45607001	~10.1 mg/day* radiolabeled, 5 days by oral gavage, to 1 rat/sex *Approx. 48-49 mg/kg/day, based on given body weights	Triazole alanine is readily absorbed and excreted within 48 hours following 5 daily doses of ~10 mg/day of either compound. At 168 hours, radioactivity recovery was ~92-98% of the administered dose. Absorption of the administered doses (urine and cage wash) at 168 hours was ~71-81%. Urinary excretion accounted for ~55-63% of the administered radioactivity; fecal excretion accounted for ~11-27% of the administered radioactivity. Minor gender-related quantitative differences were observed, but because only 1 rat/sex/compound was tested, these are likely due to individual variability. Tissue burdens were negligible at 72 hrs post-dosing. Triazole alanine, triazole acetic acid, free triazole, plus three additional unknown metabolites were detected in the urine and feces. The investigators propose that one of these unknown degradates is a pyruvic acid derivative.
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/non-guideline*	00138118 (TXR 004766 Accession # 252132) 00133354	0.5 or 56 mg/kg ^{14}C-triazole alanine, single dose by oral gavage, to 4 rats/sex/dose	For both dose levels, >90% of the administered dose was excreted in urine at 168 hours, ~4-5% in feces, and <1% in volatiles, with most excretion in the first 24 hours. At the low dose, no radioactivity was detected in tissues. At the high dose, 0.002-0.02 ppm of TA equivalents were detected in tissues, mostly in the liver and kidneys. TA made up 72% and 80.3%, and N-acetyl TA made up 19.4% and 12.7% of the radioactivity recovered from the urine of low dose males and females, respectively. TA made up 83.4% and 86.4%, and N-acetyl TA made up 10.6% and 7.7% of the radioactivity recovered from the urine of high dose males and females, respectively.

Table 11.3. Subchronic, Chronic, and Other Toxicity Profile for Triazole Alanine

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/non-guideline* Continuation of MRID 00138118	00139575 (TXR 004766 Accession # 252132) 00133355	56 mg/kg ^{14}C-triazole alanine (TA), single dose by oral gavage, to 4 rats/sex/dose Urine and feces samples included the first 24 hours after exposure.	Triazole alanine is completely absorbed and rapidly excreted. The major route of excretion is urine. Most of the radiolabel recovered in the urine sample from the first 24 hours was unchanged TA (72-86%), with 8-19% of the urinary radioactivity as N-acetyl TA. Two or three minor unknown metabolites (<3% each) were also found in the various urine samples. About 50% of the radiolabel recovered in the feces was TA, 16% was recovered as N-acetyl TA, and 30% was not identified but wasn't found in the urine.
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/non-guideline*	00164114 (TXR 005841)	0.56, 54.4 or 993.7 mg/kg ^{14}C triazole alanine, single dose by oral gavage, to 2 rats/sex/dose	Radioactivity recoveries at 168 hours were 101.2%, 90.7%, and 92.1% for the low, mid, and high doses, respectively. There were no major sex differences in excretion patterns. The major route of excretion was urine: 81-82% of the administered dose for males and 84-85% for females. Absorption and excretion was rapid: within 24 hours after dosing, 76-82% of the administered dose in males and 71-91% in females was eliminated in the feces and urine. Tissue residues were low (<1% for both sexes).
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/non-guideline* Continuation of MRID 00164114.	00164115 (TXR 005841)	0.56, 54.4 or 993.7 mg/kg ^{14}C triazole alanine, single dose by oral gavage, to 2 rats/sex/dose Urine samples included the first 24 hours after exposure.	Triazole alanine made up 82-93% of the radioactivity recovered in the first 24 hours urine sample, and N-acetyl triazole alanine made up 13-30%. The significance of an apparent decrease in the percentage of N-acetylation at the high dose is questionable.

Table 11.3. Subchronic, Chronic, and Other Toxicity Profile for Triazole Alanine

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/non-guideline*	00138132 (TXR 004766 Accession # 252132)	Metabolism: 5 mg/kg ^{14}C-triazole alanine, by oral gavage or i.v. injection, to 5 males/exposure route Autoradiography: 10 mg/kg ^{14}C-triazole alanine, by i.v. injection, to 6 males	Absorption and excretion was rapid, with most excretion in the urine. At 48 hours after oral exposure, 94.5% and 3.5% of the administered radiolabel was recovered in the urine and feces, respectively. At 48 hours after i.v. exposure, 92.4% and 2.1% of the administered radiolabel was recovered in the urine and feces, respectively. Tissue residues were low, with the highest residues found in the kidney and liver. Following oral administration, radioactivity increased in the plasma and a maximum was attained within 40 minutes. Radioactivity declined in a biphasic manner with half lives of 3 and 8 hours. Following iv administration, radioactivity decreased in triphasic manner with half lives of 15 minutes, 4.4 hours, and 12.4 hours. Whole body autoradiography indicated radioactivity distribution in all tissues and organs except the compacts of the bone.

ND = Study not done

Table 11.4. Subchronic, Chronic, and Other Toxicity Profile for Triazole Acetic Acid

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
Non-guideline	*14-Day oral toxicity in rodents - rat* *Acceptable/non-guideline*	45596801	0, 100, 1000, 8000 ppm M: 0, 10.6, 102.8, 788.3 mg/kg/day F: 0, 10.1, 97.2, 703.5 mg/kg/day	NOAEL = 788.3/703.5 mg/kg/day (M/F) LOAEL = >788.3/>703.5 mg/kg/day (M/F)
870.3050	28-Day oral toxicity in rodents	ND	ND	ND
870.3100	90-Day oral toxicity in rodents	ND	ND	ND
870.3150	90-Day oral toxicity in nonrodents	ND	ND	ND
870.3200	21/28-Day dermal toxicity	ND	ND	ND
870.3250	90-Day dermal toxicity	ND	ND	ND
870.3465	90-Day inhalation toxicity	ND	ND	ND
870.3700	Prenatal developmental toxicity in rodents	ND	ND	ND

Table 11.4. Subchronic, Chronic, and Other Toxicity Profile for Triazole Acetic Acid

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.3700	Prenatal developmental toxicity in nonrodents	ND	ND	ND
870.3800	Reproduction and fertility effects	ND	ND	ND
870.4100	Chronic toxicity	ND	ND	ND
870.4200	Carcinogenicity	ND	ND	ND
870.4300	Combined chronic toxicity/ carcinogenicity	ND	ND	ND
870.5100	Bacterial reverse mutation assay *Acceptable/guideline*	45596803	20, 80, 320, 1280 and 5120 ug/plate for *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 (his⁻)	No mutagenic activity in bacteria (*Salmonella typhimurium*) in the presence or absence of S9 activation.
870.5300	*In vitro* mammalian cell gene mutation test	ND	ND	ND
870.5375	*In vitro* mammalian cell chromosome aberration test	ND	ND	ND
870.5395	Mammalian erythrocyte micronucleus test	ND	ND	ND
870.5550	Unscheduled DNA synthesis in mammalian cells in culture	ND	ND	ND

Table 11.4. Subchronic, Chronic, and Other Toxicity Profile for Triazole Acetic Acid

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/non-guideline*	45596804 45596805	0.58, 58.63, or 1034.69 mg/kg radiolabeled, single dose by oral gavage.	Triazole acetic acid was readily absorbed and excreted within 48 hours following a single oral dose of 0.58, 58.63, or 1034.69 mg/kg. Average absorption at 168 hours was nearly complete (~93-103%, ~102-104%, and ~92-101% of the administered dose for the males and females in the low-, mid-, and high-dose groups, respectively) and did not appear to be saturated at the highest dose. Urinary excretion accounted for ~90-104% of the administered radioactivity, whereas fecal excretion accounted for only ~1-7% of the administered radioactivity in all treatment groups. Excretory patterns did not exhibit gender-related variability. Because radioactivity in tissues was very low, neither triazole acetic acid nor its metabolites appear to undergo significant sequestration. The urinary metabolite was identified as triazole acetic acid, indicating that the parent compound was excreted intact without being metabolized in the rat.

Table 11.4. Subchronic, Chronic, and Other Toxicity Profile for Triazole Acetic Acid

Guideline Number	Study Type/ *Classification*	*MRID Number*	*Doses*	*Results*
870.7485	Metabolism and pharmacokinetics - rat *Acceptable/non-guideline*	45607001	~10.5 mg/day* radiolabeled, 5 days by oral gavage, to 1 rat/sex *Approx. 50-51 mg/kg/day, based on given body weights	Triazole acetic acid is readily absorbed and excreted within 48 hours following 5 daily doses of ~10.5 mg/day. At 168 hours, radioactivity recovery was ~101-102% of the administered dose. Absorption of the administered doses (urine and cage wash) at 168 hours was ~69-91%. Urinary excretion accounted for ~66-75% of the administered radioactivity. Fecal excretion accounted for ~11-32% of the administered radioactivity. Minor gender-related quantitative differences were observed, but because only 1 rat/sex/compound was tested, these are likely due to individual variability. Tissue burdens were negligible at 72 hrs post-dosing. The sole component identified in extracts of feces and urine from radiolabeled-triazole acetic acid-fed rats was triazole acetic acid, which indicates excretion without metabolic conversion of triazole acetic acid.

ND = Study not done

11.2 FQPA Hazard Considerations

11.2.1 Adequacy of the Toxicity Data Base

The toxicology database for triazole alanine is considered incomplete in terms of endpoint studies and dose-response information to characterize potential pre- and/or post-natal risk for infants and children. Acceptable developmental toxicity and two-generation reproduction studies in the rat were evaluated, but a developmental toxicity study in the rabbit was not available.

No studies on triazole acetic acid, including rat or rabbit developmental toxicity studies or a two-generation reproduction study in the rat, were available to characterize any potential pre- and/or post-natal risk for infants and children.

11.2.2 Evidence of Neurotoxicity

There is no evidence that exposure to triazole alanine results in neurotoxicity. No clinical signs of neurotoxicity, changes in brain weights, changes in brain gross or microscopic pathology, or any other neurotoxic effects were observed in the short-term rat studies, the subchronic rat and

dog feeding studies, the rat developmental toxicity study, or the two-generation reproduction study. Slight salivation and a slightly unsteady gait were seen in the female dog tested in an acute oral dog study; however, the neurotoxicological significance of this effect is questionable, since the effects were seen at an extremely high dose (5000 mg/kg), and similar effects were not seen in the male dog. Spastic gait, piloerection, lethargy, and diarrhea were initially seen in rats tested in an acute intraperitoneal study; however, the significance of these effects is also questionable because the rats were exposed to an extremely high dose (5000 mg/kg), and similar effects were not seen at 5000 mg/kg in three acute oral studies of triazole alanine in rats.

While the available repeated dose studies show no evidence for triazole alanine to adversely affect the nervous system, there is some concern that this is due to an incomplete database, rather than a true inability of the compound to produce neurotoxicity. For instance, no acute or subchronic neurotoxicity tests or an evaluation of perfused brain tissue are available for triazole alanine. Tremors, decreased brain weight, and cerebellar degeneration were seen in the 90-day mouse study on 1,2,4-triazole; however, no mouse studies on triazole alanine are available for comparison. Clinical signs of neurotoxicity were seen in adults in the developmental rabbit study on 1,2,4-triazole, including decreased motor activity, head tilt, lacrimation, drooping eyelids, diarrhea, salivation; however no rabbit studies are available on triazole alanine. Cerebellar lesions and decreased brain weights were seen at the highest dose tested in the two-generation reproduction study on free triazole; however, brains were not microscopically examined in the triazole alanine reproduction study.

11.2.3 Developmental Toxicity Studies

11.2.3.1 Developmental Toxicity Study in Rats

For triazole alanine, increased quantitative and qualitative susceptibility was seen in the developmental toxicity study in rats. (See section A-1.3.1 of the appendix for the executive summary of MRIDs 00138128 and 00147889). In this study, increased incidences of skeletal findings were seen in the offspring at the mid and high doses, while no treatment-related effects were seen in the dams up to the limit dose. The skeletal findings included unossified odontoid processes at 300 and 1000 mg/kg/day, with partially ossified transverse processes of the 7th cervical vertebra (bilateral), unossified 5th sternebra, and partially ossified 13th thoracic centrum observed only at 1000 mg/kg/day.

A developmental toxicity study in rats was not submitted for triazole acetic acid.

11.2.3.2 Developmental Toxicity Study in Rabbits

A developmental toxicity study in rabbits was not submitted for either triazole alanine or triazole acetic acid.

11.2.4 Reproductive Toxicity Study

For triazole alanine, increased quantitative susceptibility was seen in the two-generation reproduction study in rats. (See section A-1.4.1 of the appendix for the executive summary of

MRIDs 164112, 41326803, and 41236804). In this study, mean litter weights were decreased approximately 10-20% for both generations at ~1000 mg/kg/day, compared to controls, but parental body weights were unaffected at all treatment levels tested. The lowest dose associated with decreased body weights and body weight gains in adult rats was 1510 mg/kg/day, from the 90-day toxicity study.

A two-generation reproduction study in rats was not submitted for triazole acetic acid.

11.2.5 Additional Information from Literature Sources

No relevant specific information on the toxicity of triazole alanine or triazole acetic acid was noted in the open scientific literature.

11.2.6 Pre-and/or Postnatal Toxicity

11.2.6.1 Determination of Susceptibility

Increased quantitative and qualitative susceptibility of the offspring was seen in the developmental toxicity rat study, and increased quantitative susceptibility was seen in the two-generation reproduction study. In the developmental toxicity study, increased incidences of skeletal findings were seen in the offspring at the mid and high doses, while no treatment-related effects were seen in the dams up to the limit dose. These skeletal findings include unossified odontoid processes at 300 and 1000 mg/kg/day, with partially ossified transverse processes of the 7th cervical vertebra (bilateral), unossified 5th sternebra, and partially ossified 13th thoracic centrum observed only at 1000 mg/kg/day. In the reproduction study, mean litter weights were decreased approximately 10-20% for both generations at ~1000 mg/kg/day, compared to controls, but parental body weights were unaffected at all treatment levels.

11.2.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre- and/or Postnatal Susceptibility

The toxicology database for triazole alanine is incomplete with respect to pre-and post-natal toxicity because a rabbit developmental toxicity study was not available, and the existing two-generation reproduction study, completed in 1986, did not meet the current guideline protocol. Evidence of increased qualitative and quantitative susceptibility in the offspring was seen in the rat developmental toxicity study in the form of skeletal findings observed in the offspring at dose levels that produced no treatment-related effects in the dams. Evidence of increased quantitative susceptibility was also seen in the two-generation reproduction study as a reduction in mean litter weights for both generations at dose levels that produced no treatment-related effects in the parents. However, the concern is low for the increased susceptibilities seen in these studies, as well as for the residual uncertainties regarding the data gaps, because the doses currently used for dietary, residential, and occupational exposure risk assessments are similar or lower than the doses that caused the effects.

For triazole acetic acid, the toxicology database is incomplete with respect to pre-and post-natal toxicity because no rat or rabbit developmental toxicity studies or a two-generation reproduction

study in the rat are available. Increased qualitative and quantitative susceptibility was seen for both triazole alanine and 1,2,4-triazole. Therefore, there is residual uncertainty for increased pre- and/or postnatal susceptibility following exposure to triazole acetic acid, but this uncertainty is addressed by retaining the 10X database uncertainty factor.

11.3 Recommendation for a Developmental Neurotoxicity Study

At this time, a developmental neurotoxicity study on the triazole conjugates is not required.

11.3.1 Evidence that supports requiring a Developmental Neurotoxicity Study

At present, there is no evidence to support requiring a Developmental Neurotoxicity Study.

11.3.2 Evidence that supports not requiring a Developmental Neurotoxicity Study

There was no evidence of neurotoxicity in any of the studies available in the toxicology database for triazole alanine or triazole acetic acid, including the short-term rat studies, the subchronic rat and dog feeding studies, the rat developmental toxicity study, and the two-generation reproduction study.

11.3.2.1 Rationale for the UF_{DB}

The database uncertainty factor is 10X, based on the lack of a developmental toxicity study in rabbits for both triazole alanine and triazole acetic acid, lack of a chronic study in rats (including additional neuropathological and neurobehavioral measurements) with triazole alanine, and lack of a combined 90-day/subchronic neurotoxicity study in rats for triazole acetic acid.

11.4 Hazard Identification and Toxicity Endpoint Selection

Because no effects were seen in the available acute oral toxicity studies (14-day oral studies in rats and a bacterial reverse mutation assay), a difference in toxicity between triazole alanine and triazole acetic acid could not be evaluated. In the absence of data to suggest otherwise, the endpoint selection discussed in the following sections applies to all triazole conjugates (i.e., triazole alanine, triazole acetic acid, triazole lactic acid, and triazole pyruvate).

11.4.1 Acute Reference Dose (aRfD) - Females (13 to 49 years of age)

Study Selected: Prenatal developmental toxicity in rodents - rat
MRID Number: 00138128 and 00147889
Dose and Endpoint for Establishing aRfD: 100 mg/kg/day (NOAEL), based on increased incidence of skeletal findings (unossified odontoid process) at 300 mg/kg/day (LOAEL).
Uncertainty Factor(s): 1000 (10X for interspecies extrapolation, 10X for intraspecies variations, and 10X for database uncertainty).
Comments about Study/Endpoint/Uncertainty Factor: Adverse effects seen in offspring from an oral developmental study are appropriate for setting an acute dietary endpoint for women of

childbearing age. There is some uncertainty as to whether effects on ossification are due to a single dose. Delays in ossification can indicate an effect on growth, which would be seen with repeated exposure, but no change in fetal body weights was observed in this study. However, in the absence of a developmental rabbit study, which is the most sensitive species for free triazole, this dose and study were selected for this risk assessment. In accordance with current FQPA policy, a 1000X uncertainty factor is applied, which includes a 10X database UF to account for the lack of developmental toxicity studies on triazole alanine and triazole acetic acid in rabbits.

Acute RfD = 100 mg/kg/day (NOAEL) ÷ 1000 (UF) = 0.1 mg/kg/day

11.4.2 Acute Reference Dose (aRfD) - General Population

In 2003, the Triazole Peer Review Committee (PRC) determined that the acute endpoint for the triazole conjugates should be based on the developmental toxicity study in rats discussed previously, with an uncertainty factor of 300X to include a 3X database UF (TXR No. 0052011). However, reevaluation of the triazole alanine database showed that no appropriate endpoints (*i.e.*, effects seen in adult animals following a single, oral dose) were available to set an acute reference dose for the general population.

11.4.3 Chronic Reference Dose (cRfD)

Study Selected: 90-Day oral toxicity in rodents - rat
MRID Number: 00164107
Dose and Endpoint for Establishing cRfD: 90 mg/kg/day (NOAEL), based on decreased leukocyte counts in males at 370 mg/kg/day (LOAEL).
Uncertainty Factor(s): 1000 (10X for interspecies extrapolation, 10X for intraspecies variations, and 10X for database uncertainty).
Comments about Study/Endpoint/Uncertainty Factor: For females, the NOAEL is 160 mg/kg/day, and the LOAEL is 400 mg/kg/day, based on decreased triglycerides. Out of the available toxicology studies on the triazole conjugates, the two-generation reproduction study and the 90-day studies were the longest in duration. Chronic studies in rats, mice, or dogs were not available. Additionally, rabbits, which are more sensitive than rats to free triazole, were not used in any of the available toxicology studies on the triazole conjugates. Although no adverse effects were seen in adults in the reproduction study, fewer endpoints are measured in this study compared to the subchronic feeding studies. Treatment-related effects were seen in both the 90-day dog and rat studies, but the 90-day rat study was chosen because rats were more sensitive to triazole alanine than dogs. The 10X database uncertainty factor used for the chronic endpoint is to account for the lack of a developmental toxicity study in rabbits for both triazole alanine and triazole acetic acid, the lack of a chronic study in rats (including additional neuropathological and neurobehavioral measurements) with triazole alanine, and a lack of a combined 90-day/subchronic neurotoxicity bridging study on triazole acetic acid.

In 2003, the Triazole Peer Review Committee (PRC) determined that the chronic endpoint for the triazole conjugates should be based on the developmental toxicity study in rats discussed above, with an uncertainty factor of 300X to include a 3X database UF (TXR No. 0052011). This decision was made prior to the current FQPA policy; to the receipt of the 90-day/subchronic

neurotoxicity rat study, developmental rabbit toxicity study, and reproduction study on 1,2,4-triazole; and to the reevaluation of the triazole alanine studies. The reanalysis of the triazole alanine studies resulted in a change in chronic dose and endpoint selection. The subchronic rat study is a more appropriate basis for a chronic endpoint than a developmental study. The change in FQPA policy and receipt of additional free triazole studies resulted in an increased database UF.

Chronic RfD = 90 mg/kg/day (NOAEL) ÷ 1000 (UF) = 0.09 mg/kg/day

11.4.4 Incidental Oral Exposure

The endpoint for short- and intermediate-term incidental oral exposure is based on changes in hematology observed in the 90-day oral feeding study in rats (MRID 00164107), which is discussed earlier in section 11.4.3. The oral route of exposure used in this study and the type of effects seen are considered appropriate for short- or intermediate-term incidental oral endpoints. Additionally, the NOAEL from the 90-day study is protective of the offspring toxicity observed in the developmental rat study. The 90-day duration of the study is most appropriate for an intermediate term endpoint. However, due to the lack of studies and of parameters measured in the available studies and because the effects seen in the 90-day rat study could occur with short- to long-term exposure, this study was used as the basis for residential and occupational endpoints for all durations.

11.4.5 Dermal Absorption

No dermal absorption studies on triazole alanine or triazole acetic acid are available in the database. Therefore, there are no data to support a dermal absorption factor other than 100%.

11.4.6 Dermal Exposure (Short-, Intermediate-, and Long-term)

In the absence of dermal studies, the endpoint for short-, intermediate-, and long-term dermal exposure is the same as for short and intermediate term incidental oral exposure. See section 11.4.4 for more details.

11.4.7 Inhalation Exposure (Short-, Intermediate-, and Long-term)

In the absence of inhalation studies, the endpoint for short-, intermediate-, and long-term inhalation exposure is the same as for short- and intermediate-term incidental oral exposure. See section 11.4.4 for more details.

11.4.8 Margins of Exposure and Levels of Concern

The levels of concern (LOCs) for residential and occupational exposure and risk assessment are given below. Each LOC is based on the conventional uncertainty factor of 100X (10X for intraspecies variation and 10X for interspecies extrapolation), as well as a 10X database uncertainty factor for the lack of a developmental toxicity study in rabbits for both triazole alanine and triazole acetic acid, the lack of a chronic study in rats (including additional

neuropathological and neurobehavioral measurements) with triazole alanine, and a lack of a combined 90-day/subchronic neurotoxicity study in rats for triazole acetic acid.

Table 11.5. Summary of Levels of Concern for Residential and Occupational Risk Assessments for Triazole Alanine and Triazole Acetic Acid.

Route of Exposure	Duration of Exposure		
	Short-Term (1-30 Days)	Intermediate-Term (1-6 Months)	Long-Term (>6 Months)
Occupational Exposure			
Dermal	1000	1000	1000
Inhalation	1000	1000	1000
Residential Exposure			
Incidental Oral	1000	1000	1000
Dermal	1000	1000	1000
Inhalation	1000	1000	1000

11.4.9 Recommendation for Aggregate Exposure Risk Assessments

Because the same endpoints are used for all exposure routes, oral, dermal, and inhalation exposures can be aggregated.

11.4.10 Classification of Carcinogenic Potential

There are no available cancer bioassay studies on 1,2,4-triazole. 1,2,4-triazole and its conjugate (triazole alanine), however, are not mutagenic. For a further discussion of carcinogenicity and triazole metabolites, see Section 4.4.11.

11.5 Special FQPA Safety Factor

The special FQPA safety factor has been removed (i.e., reduced to 1X) for the current risk assessment on the triazole conjugates because the currently selected endpoints, together with a 10X database uncertainty factor, are protective of increased susceptibility of infants and children seen in the available studies. Although increased qualitative and quantitative susceptibility of the offspring was seen in the developmental toxicity and two-generation reproduction studies in rats (see section 11.2.6), the currently selected dietary, residential, and occupational endpoints are all based on NOAELs that are protective of these adverse effects. Additionally, no evidence of neurotoxicity was seen in the available toxicology database, so a developmental neurotoxicity study is not recommended at this time. Finally, while the current recommendation is to reduce the special FQPA safety factor to 1X, residual uncertainty is accounted for by a 10X database uncertainty factor. This 10X database uncertainty factor is retained for the lack of developmental toxicity (rabbit) studies with triazole alanine and triazole acetic acid, lack of a chronic rat study (with additional neuropathological and neurobehavioral endpoints) with triazole alanine, and lack of a combined 90-day/subchronic neurotoxicity rat study for triazole acetic acid.

Future receipt of additional, required toxicology studies on triazole alanine (or the other triazole conjugates) may warrant removal of the database uncertainty factor (i.e., reduction to 1X). At

that time, the need for a 10X special FQPA safety factor based on the newly available toxicity data will be re-evaluated.

Table 11.6. Summary of Toxicological Doses and Endpoints for Triazole Conjugates to be Used in Human Health Risk Assessments.

Exposure Scenario	Dose Used in Risk Assessment and UF	Special FQPA SF * and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (females 13-49)	NOAEL = 100 mg/kg/day UF = 1000 Acute RfD = 0.1 mg/kg/day	FQPA SF =1 aPAD = acute RfD / FQPA SF = 0.1 mg/kg/day	Prenatal developmental toxicity in rodents - rat LOAEL = 300 mg/kg/day based on increased incidence of skeletal findings (unossified odontoid process).
Acute Dietary (general population, including infants and children)	None	None	No appropriate dose and endpoint could be identified for these population groups.
Chronic Dietary (all populations)	NOAEL = 90 mg/kg/day UF = 1000 Chronic RfD = 0.09 mg/kg/day	FQPA SF =1 cPAD = chronic RfD / FQPA SF = 0.09 mg/kg/day	90-Day oral toxicity in rodents - rat LOAEL = 370/400 mg/kg/day (M/F) based on decreased leukocyte counts in males and decreased triglycerides in females.
Incidental Oral (all durations)	NOAEL = 90 mg/kg/day	Residential LOC for MOE = 1000[a] Occupational = NA	90-Day oral toxicity in rodents - rat LOAEL = 370/400 mg/kg/day (M/F) based on decreased leukocyte counts in males and decreased triglycerides in females.
Dermal (all durations)	NOAEL = 90 mg/kg/day (dermal absorption rate = 100%)	Residential LOC for MOE = 1000[a] Occupational LOC for MOE = 1000[a]	90-Day oral toxicity in rodents - rat LOAEL = 370/400 mg/kg/day (M/F) based on decreased leukocyte counts in males and decreased triglycerides in females.
Inhalation (all durations)	NOAEL = 90 mg/kg/day (inhalation absorption rate = 100%)	Residential LOC for MOE = 1000[a] Occupational LOC for MOE = 1000[a]	90-Day oral toxicity in rodents - rat LOAEL = 370/400 mg/kg/day (M/F) based on decreased leukocyte counts in males and decreased triglycerides in females.
Cancer (oral, dermal, inhalation)	**Classification:** Not determined. Evaluate by RfD approach.		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

* Refer to Section 11.5

[a] Additional 10x database uncertainty factor for lack of developmental toxicity (rabbit) studies with triazole alanine and triazole acetic acid, a chronic rat study (with additional neuropathological and neurobehavioral endpoints) with triazole alanine, and a combined 90-day/subchronic neurotoxicity rat study for triazole acetic acid.

11.6 Endocrine Disruption

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following recommendations of its Endocrine Disruptor and Testing Advisory Committee (EDSTAC), EPA determined that there was a scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

In the available toxicity studies on triazole alanine and triazole acetic acid, no estrogen-, androgen-, and/or thyroid-mediated toxicity was observed.

When additional appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, triazole conjugates may be subjected to further screening and/or testing to better characterize effects related to endocrine disruption.

12.0 Public Health Data

None.

13.0 Exposure Characterization/Assessment

13.1 Dietary Exposure/Risk Pathway
(M. Doherty, D322239, 12/20/05)

13.1.1 Residue Profile

13.1.1.1 Residues in Food

Through a joint effort by the U.S. Triazole Task Force (USTTF) and USDA's Pesticide Data Program (PDP), monitoring data depicting residues of TA and TAA are available for apples, peaches, wheat flour, bananas, eggs, peanut butter, soybeans, finished water, strawberry, milk, grapes, and tomato. For all foods addressed by these assessments, including those with monitoring data, an anticipated residue was derived by converting the tolerance value for each parent triazole-derivative fungicides to its TA equivalent using molecular weight conversion factors. For the acute assessment, the highest anticipated residue was used for a given food; for the chronic assessment, the average anticipated residue was used. For foods with monitoring data, the greater of the maximum monitoring data (maximum TA plus maximum TAA) or the anticipated residue was used in the assessment. For all commodities except those of peanut and cereal grain, the anticipated residues were used. For peanut commodities the maximum monitored residue value from peanut butter was used. For cereal grain commodities, the

maximum monitored residue value from wheat flour was used. The assessments include default processing factors from DEEM Version 7.81. The Agency was recently made aware of an issue with the analytical method for TA in soybeans in which the method underestimates residues by 4 to 12 fold. That issue is still being resolved. In order to ensure that this assessment does not underestimate exposure to TA/TAA via soybean, all inputs for soybean were multiplied by 10 in both the acute and chronic analyses. This is a very conservative, higher-end deterministic assessment.

Table 13.1. Summary of Input Residue Values for the Acute and Chronic Dietary Analyses of Triazole Alanine and Triazole Acetic Acid.

Food	DEEM Input Value, ppm		Anticipated Residue, ppm		Max. Monitored Residue, ppm[1]	Remarks
	Acute	Chronic	Acute	Chronic		
Pome Fruit (Apple)	0.53	0.23	0.53	0.21	0.23	–
Artichoke	0.54	0.43	0.54	0.43	–	–
Asparagus	0.80	0.03	0.80	0.03	–	–
Banana	2.16	0.57	2.16	0.32	0.57	–
Dry Bean/Pea	0.23	0.12	0.23	0.12	–	–
Succulent Bean/Pea	0.54	0.22	0.54	0.22	–	–
Blueberry	0.46	0.30	0.46	0.30	–	–
Caneberry	1.08	0.87	1.08	0.87	–	–
Canola	0.05	0.02	0.05	0.02	–	–
Carrot	0.09	0.09	0.09	0.09	–	–
Leafy Petioles	2.28	2.28	2.28	2.28	–	–
Barley	0.55	0.55	0.046	0.31	–	From wheat flour
Oats	0.55	0.55	0.05	0.03	–	From wheat flour
Rice	3.19	1.11	3.19	1.11	–	–
Rye	0.55	0.55	0.05	0.04	–	From wheat flour
Wheat	0.55	0.55	0.05	0.03	–	From wheat flour
Wheat Flour	0.55	0.55	0.05	0.03	0.55	–
Wild Rice	3.19	1.11	0.23	0.23	–	From rice
Citrus Group	0.46	0.23	0.46	0.23	–	–
Coffee	0.005	0.005	0.005	0.005	–	–
Field Corn	0.55	0.55	0.05	0.03	–	From wheat flour
Sweet Corn	0.55	0.55	0.05	0.03	–	From wheat flour
Cotton	1.01	0.26	1.01	0.26	–	–
Cranberry	0.46	0.24	0.46	0.24	–	–
Cucurbits	0.11	0.08	0.11	0.08	–	–
Currant	1.62	1.04	1.62	1.04	–	–
Elderberry	0.46	0.46	0.46	0.46	–	–
Grape	2.53	0.82	2.53	0.82	0.29	–
Raisin	2.53	0.82	2.53	0.82	–	–
Hops	15.20	7.60	15.20	7.60	–	–
Lychee	0.76	0.76	0.76	0.76	–	–
Mango	0.10	0.10	0.10	0.10	–	–
Mayhaw	0.38	0.19	0.38	0.19	–	–
Bulb Vegetables	0.14	0.09	0.14	0.09	–	–
Okra	0.51	0.51	0.51	0.51	–	–
Peanut	2.32	2.32	0.09	0.04	–	From peanut butter
Peanut Butter	2.32	2.32	0.09	0.04	2.32	–
Peppers	0.54	0.54	0.54	0.54	–	–
Peppermint	1.62	1.62	1.62	1.62	–	–

Table 13.1. Summary of Input Residue Values for the Acute and Chronic Dietary Analyses of Triazole Alanine and Triazole Acetic Acid.

Food	DEEM Input Value, ppm		Anticipated Residue, ppm		Max. Monitored Residue, ppm[1]	Remarks
	Acute	Chronic	Acute	Chronic		
Pineapple	1.59	0.82	1.59	0.82	–	–
Sorghum	0.55	0.55	0.09	0.05	–	From wheat flour
Soybean	9.1	3.3	0.91	0.33	0.12	10X the anticipated residue to account for method issue.
Spearmint	1.62	1.62	1.62	1.62	–	–
Stone Fruit-no cherry	1.08	0.95	1.08	0.74	0.95	–
Cherry	2.70	1.53	2.70	1.53	–	–
Strawberry	0.68	0.48	0.68	0.48	0.13	–
Sugar Beet	0.14	0.07	0.14	0.07	–	–
Sugar Beet Molasses	0.14	0.07	0.14	0.07	–	–
Sunflower	0.03	0.03	0.03	0.03	–	–
Tomato	0.16	0.16	0.16	0.16	0.09	–
Turnip	0.20	0.20	0.20	0.20	–	–
Turnip Greens	2.28	2.28	2.28	0.04	–	From Leafy petiole
Tree Nuts	0.19	0.11	0.19	0.11	–	–
Almond	0.19	0.11	0.19	0.11	–	–
Pecan	0.05	0.05	0.05	0.05	–	–
Pistachio	0.05	0.04	0.05	0.04	–	–
Poultry Meat	0.03	0.02	0.03	0.02	–	–
Poultry Fat	0.03	0.02	0.03	0.02	–	–
Poultry Meat Byprod.	0.03	0.02	0.03	0.02	–	–
Poultry Liver	0.03	0.02	0.03	0.02	–	–
Egg	0.03	0.02	0.03	0.02	0.016	–
Cattle Meat	0.53	0.13	0.53	0.13	–	–
Cattle Fat	0.53	0.15	0.53	0.15	–	–
Cattle Meat Byprod.	0.53	0.14	0.53	0.14	–	–
Cattle Liver	1.68	0.63	1.68	0.63	–	–
Cattle Kidney	0.91	0.29	0.91	0.29	–	–
Milk	0.11	0.04	0.11	0.04	0.01	–

[1] Residue data for TA/TAA from USDA Pesticide Data Program or U.S. Triazole Task Force monitoring data. Values are the combined maxima for TA and TAA for monitored foods and do not reflect the maximum combined residue of TA and TAA for a particular sample.

13.1.1.2 Residues in Drinking Water

As noted in Section 6, there is not sufficient information available to model potential residues of the triazole conjugates in drinking water. As a surrogate for modeled concentrations of TA and TAA, HED has used the modeled estimates for 1,2,4-T (Table 6.2) in the TA/TAA dietary exposure assessment, multiplying the values by 2.26 to correct for differences in molecular weight. Given the infrequent and very low residue levels reported in the monitoring data (Table 13.1), the use of 1,2,4-T residue estimates in drinking water is highly conservative.

13.1.2 Acute and Chronic Dietary Exposure and Risk

Table 13.2. Dietary (Food + Water) Exposure and Risk Estimates for Triazole Alanine and Triazole Acetic Acid.

Population Subgroup	aPAD, mg/kg/day	Exposure Estimate, mg/kg/day			Risk Estimate, %aPAD[a]		
		95th %ile	99th %ile	99.9th %ile	95th %ile	99th %ile	99.9th %ile
Acute							
Females 13-49 yrs	0.1	0.0274	27	0.0412	41	0.0827	83

Chronic			
Population Subgroup	cPAD, mg/kg/day	Exposure Estimate, mg/kg/day	Risk Estimate, % cPAD[a]
U.S. Population (total)	0.09	0.0080	9
All infants (< 1 year)	0.09	0.0160	18
Children 1-2 yrs	0.09	0.0239	27
Children 3-5 yrs	0.09	0.0196	22
Children 6-12 yrs	0.09	0.0121	13
Youth 13-19 yrs	0.09	0.0073	8
Adults 20-49 yrs	0.09	0.0062	7
Adults 50+ yrs	0.09	0.0054	6
Females 13-49 yrs	0.09	0.0060	7

The values for the population with the highest risk for each type of risk assessment are bolded.
[a] Reported to 2 significant figures.

Due to the conservatism of the assessment inputs, HED believes that it is most appropriate to base regulatory recommendations on results at the 95th percentile of exposure for the acute assessment. The results indicate that for both acute and chronic exposure durations and for all population subgroups, risk estimates are well below HED's level of concern.

13.2 Residential (Non-Occupational) Exposure/Risk Pathway

Triazole alanine is formed in plants by the conjugation of 1,2,4-T to serine. The TA may then be further oxidized to form TAA. Because of the nature of this process, HED has assumed that it occurs within the plant itself on not on leaf surfaces. Therefore, the residues are not available for dermal, hand-to-mouth, or object-to-mouth exposures and HED has not conducted a residential exposure assessment for the triazole conjugates. Residues of TA and TAA may occur in soil. 1,2,4-Triazole is more toxic than TA/TAA and exposures to TA/TAA via soil ingestion are unlikely to exceed those of 1,2,4-T. The assessment for soil ingestion of 1,2,4-T shows that risk estimates are below HED's level of concern; therefore, risk estimates for soil ingestion of TA/TAA will also be below HED's level of concern.

14.0 Aggregate Risk Assessments and Risk Characterization

Dietary exposure is the only pathway of concern for TA/TAA; therefore, aggregate risk estimates are equivalent to those discussed for dietary risk (Section 13.1.2). All aggregate risk estimates are below HED's level of concern.

15.0 Cumulative Risk Characterization/Assessment

See Section 8.0.

16.0 Occupational Exposure/Risk Pathway

Triazole alanine and triazole acetic acid are not present in pesticide formulations; therefore, there is no risk from exposure to these chemicals that results from mixing, loading, or application activities. Although TA and TAA are significant plant metabolites, the residues occur inside the plant structures and are not available for dermal contact (see Section 13.2). Therefore, there is no significant exposure as a result of post-application activities and quantitative occupational exposure and risk assessments are not needed for TA and TAA.

17.0 Data Needs and Label Requirements

17.1 Toxicology

A 10X database uncertainty factor is retained for the lack of the following studies:

Triazole alanine

- Developmental toxicity study in rabbits
- Chronic toxicity study in rats, conducted according to current guidelines that include neurobehavioral assessments, with additional neuropathology evaluations conducted according to the neurotoxicity guidelines

Triazole acetic acid

- Developmental toxicity study in rabbits
- Combined 90-day feeding/ neurotoxicity study in rats

17.2 Residue Chemistry

- Resolution of concerns regarding the prevalence of conjugated residues of TA and the ability of the analytical method to quantify them.

17.3 Occupational and Residential Exposure

- None.

References

Allen, J., Wolf, D., George, M. Hester, S., Sun G., Thai, S-F., Delker, D., Nelson, G., Winkfield, E. Roop, B., Leavitt, S., Ward, W., and Nesnow, S. (2005) Comparable Liver P450 Enzyme Activity and Histopathology in Mice Treated With the Conazole Fungicides: Myclobutanil, Propiconazole and Triadimefon. *The Toxicologist*. Abstract. No, 2146.

Goetz, A.K., Blystone, C., Ren, H., Schmid, J.E., Nichols, H.P., Bao, W., Best, D.S., Narotsky, M.G., Wolf, D.C., Rockett, J.C., and Dix, D.J. (2005) Reproductive Effects of Exposure to Conazole Fungicides in the Male Rat. *The Toxicologist*. Abstract No 2148.

Hester, S., Thai, S.-F., Wolf, D., Ward, W., and Nesnow, S. (2005) Comparison of Gene Expression Profiles from Rats Fed Acute Levels of Three Toxicologically Different Conazoles. *The Toxicologist*. Abstract No. 2149.

Ren, H., Schmid, J.E., Retief, J., Turpaz, Y., Zhang, X., Jones, P.D., Newsted, J.L., Giesy, J.P., Wolf, D.C., Wood, C.R., Bao, W., and Dix, D.J. (2005) Profiling Gene Expression in Human H295R Adrenocortical Carcinoma Cells and Rat Testes to Identify Pathways of Toxicity for Conazole Fungicides. *The Toxicologist*. Abstract No. 2150.

Sun G, Thai SF, Tully DB, Lambert GR, Goetz AK, Wolf DC, Dix DJ, Nesnow S: Propiconazole-induced cytochrome P450 gene expression and enzymatic activities in rat and mouse liver. Tox Lett 155: 277-287, 2004; and abstracts from the 2005 SOT meeting,

Ward, W., Delker, D., Hester, S., Thai, S-F., Allen, J., Jones, C., Wolf, D., and Nesnow, S. (2005) Comparison of Hepatic Gene Expression Profiles from Mice Exposed to Three Toxicologically Different Conazoles. *The Toxicologist*. Abstract No. 2147.

Wolf, D.C., Allen, J., Sun, G., Thibodeaux, J., George, M., Hester, S.D., Thai, S.-F., Delker, D., Nelson, G., Winkfield, E., Roop, B., Leavitt, S., Ward, W., and Nesnow, S. (2005) Triadimefon Induces Rat Thyroid Tumors Through a Non-TSH Mediated Mode of Action. *The Toxicologist*. Abstract No. 2144.

MRID 45284011. Rakhmatov, R.M., V.B. Danilov, U.A. Madzhidov, and S.A. Gamiyants. (1991). Basis data for setting the limiting allowed concentration (LAC) of 1,2,4-triazole in workplace air. Gigiena i Sanitariya 2:30-1. [Abstract only].

MRID 46553701. Heard, N.E. (2005). 1,2,4-Triazole Aggregate Exposure Assessment, Syngenta Study T022748-04, May 20, 2005

Attachments:

1. TXR 0052011. Report of the *Ad Hoc* Triazole HED Peer Review Committee

2. TXR 0052012. Second Report of the *Ad Hoc* Triazole HED Peer Review Committee

3. Triazole Summaries - Additional Data Summaries/Literature Articles

Attachment 1: Report of the *Ad Hoc* Triazole HED Peer Review Committee

TXR NO. 0052011

DATE: August 5, 2003

MEMORANDUM

SUBJECT: ***TRIAZOLES*** - Report of the *Ad Hoc* HED Peer Review Committee.

FROM: Kathleen Raffaele, Toxicologist
Registration Action Branch 3
Health Effects Division (7509C)

THROUGH: Stephen Dapson
Senior Scientist, Registration Action Branch 3
Health Effects Division (7509C)

TO: Bob Tomerlin
Registration Division (7505C)

cc: Margaret Stasikowski, HED
Elizabeth Doyle, OW

PC Codes (include but are not limited to): 004401; 120503; 128993; 128847; 123909; 129011; 128835; 128925; 128857; 125601; 122101; 120603; 128997; 109901; 127201; 128976

On 11/4/02, an *ad hoc* HED committee conducted an internal Peer Review of the OPP Triazole Team's 7/8/02 analysis of the Triazolylalanine Group's (TAG) 1/9/02 document entitled "Profile of the Triazole-derivative Fungicide Compounds and their Common Metabolites"(MRID# 45575501). The committee, led by Elizabeth Doyle, addressed toxicology and dietary exposure issues associated with this class of fungicides and its metabolites/degradates of concern. The conclusions of the committee with respect to these issues are presented in this report.

Ad Hoc HED Peer Review Committee Members in Attendance

Elizabeth Doyle (Chair), Karl Baetcke, William Burnam, Vicki Dellarco, Steve Knizner, Nancy McCarroll, Alberto Protzel, Jess Rowland, and Brenda Tarplee (Executive Secretary).

OPP Triazole Team Members in Attendance

Steve Dapson, William Hazel, Rick Loranger, Kathleen Raffaele, Clark Swentzel, and Jean Holmes (EFED).

Also in attendance were: Rick Keigwin (RD), Timothy McMahon (AD)

Chemistry Data Evaluation / Report Presentation: _____
Rick Loranger,
Chemist

_____
William Hazel,
Chemist

Toxicology Data Evaluation / Report Presentation: _____
Kathleen Raffaele,
Toxicologist

INTRODUCTION

On 11/4/02, an *ad hoc* HED committee conducted an internal Peer Review of the OPP Triazole Team's 7/8/02 analysis of the Triazolylalanine Group's (TAG) 1/9/02 document entitled "Profile of the Triazole-derivative Fungicide Compounds and their Common Metabolites" (MRID# 45575501). The committee, led by Elizabeth Doyle, addressed toxicology and dietary exposure issues associated with this class of fungicides and its metabolites/degradates of concern.

I. DIETARY EXPOSURE CONCLUSIONS

The conclusions/recommendations of the Peer Review Committee (PRC) related to dietary exposure are as follows:

1. The PRC agreed with the OPP Triazole Team that there will be dietary exposure to 1,2,4-triazole and its conjugates, that such exposure could be significant, and that reliable estimates of dietary exposure cannot be made based upon available data.

2. The PRC had no issues with the dietary exposure components of the 7/8/02 OPP Triazole Team's evaluation of the TAG's 1/9/02 risk assessment including responses to TAG conclusions 3 and 4. The OPP Triazole Team's responses were as follows (the last four bullets are responses to TAG conclusions 3 and 4):

    3. The TAG continues to exclude 1,2,4-triazole conjugates from dietary exposure which the Agency currently considers to be residues of concern for the triazole fungicides.

    4. Exclusion of the triazole conjugates results in a great underestimate of dietary exposure.

    5. Even if found to be less toxic than free 1,2,4-triazole, the much higher exposure to the conjugates is expected to result in significant risk.

    6. Calculation of free triazole residues based on the amount of parent fungicide at harvest also is expected to greatly underestimate dietary exposure (the soil serves as a reservoir).

    7. Exposure to free and conjugated residues in rotational crops, often with some of the highest residues, is expected to result in significant dietary exposure underestimation.

    8. Use of 100% crop treated and 95th percentile of exposure are appropriate assumptions for a Tier 1 assessment but they may not be that conservative due to crop rotation and the soil serving as a reservoir of triazole degradates/metabolites.

9. With all of these exposure uncertainties, hazard uncertainties, and sources of potentially great exposure underestimation, risk due to exposure to free plus conjugated triazole residues is likely to be much higher than the TAG's estimates for free triazole alone.

10. The TAG states that free triazole formation in livestock commodities and water is compound-specific and should be considered only in the context of each individual triazole fungicide's risk. The first statement is likely correct, but the law requires all sources of exposure be considered in aggregate risk assessment.

11. The TAG states that residues of free triazole in plant commodities are very low and occur in ≤15% of samples. We agree there are low, infrequent residues in raw crops, but effects of processing on triazole formation are unknown.

12. The TAG further states that plant residues have a low contribution to total dietary load. We believe that insufficient data exist to determine relative exposure contributions of plant, livestock and water residues.

13. The TAG also believes that there is no need to include free triazole in crop residue definitions. We agree that free triazole should not be part of the residue definition for tolerance enforcement purposes due to its numerous sources. However, from a risk assessment perspective, both free triazole and its conjugates need to be included based on toxicological properties.

3. The PRC agreed that monitoring of 1,2,4-triazole and its conjugates in the U.S. food supply would provide representative and rapid data to permit assessment of human dietary risk associated with this class of fungicides needed for risk management decisions on numerous registration actions and several reregistration eligibility decisions. Based on their significance as major food consumption items (particularly for children), based on properties of free triazole and its conjugates, and based on the need to represent livestock commodities and extensive crop rotation practices, it was highly recommended that survey/monitoring data be generated on **milk, eggs, and soybean protein isolate**.

4. The PRC agreed that the TAG be requested to perform cooking and processing studies (including baking studies to assess flour residues) to determine the potential for, and extent of, conversion of parent fungicides and triazole conjugates to free 1,2,4-triazole at elevated pressure, temperature, and at the pHs of various processed products as well as the fractionation of the various residues in different processed products. In addition, the PRC recommended that the TAG be requested to perform studies in which livestock are fed triazole conjugates to determine distribution of the various residues in tissues and milk and to determine

the likelihood and extent of conversion of triazole conjugates to free 1,2,4-triazole in livestock (metabolism may be more rapid in the rumen).

II. TOXICOLOGY CONCLUSIONS

The conclusions/recommendations of the Peer Review Committee (PRC) related to toxicology are as follows:

1. The PRC concluded that, based on LD_{50} values, the acute toxicity of free triazole and triazole alanine are comparable to the parent compounds.

2. The PRC concluded that the subchronic toxicity data indicate that triazole alanine is less toxic than free triazole; however, there is no data available to evaluate neurotoxic potential.

3. The PRC concluded that there is no need for additional mutagenicity data; available data indicate that free triazole and triazole alanine are not mutagenic.

4. The PRC agreed with the OPP Triazole Team that there is concern for toxicity of free triazole, triazole alanine, and other triazole conjugates

5. The PRC concurred with the OPP Triazole Team that free triazole has not been adequately tested in toxicity studies with the parent triazole fungicides.

6. The PRC determined that no analogies could be made between toxicity of parents and expected toxicity of free triazole and triazole conjugates, based on the following considerations:

   - Target tissues and toxicological effects vary across parent compounds, with some overlap but no consistent pattern.

   - Carcinogenicity also varies across parent compounds, as does tumor site for carcinogenic parents.

   - The relationship between toxicity of free triazole, triazole conjugates, and parent compounds (based on available limited data), is not consistent; e.g. some parent compounds exhibit toxicity at lower doses than free triazole, others at higher doses.

7. The PRC determined that separate risk assessments should be conducted for free triazole and for triazole conjugates, and selected endpoints and Uncertainty Factors:

- For free triazole, the developmental toxicity study, with a NOAEL of 30 mg/kg should be used for both acute and chronic risk assessments; UF=1000 (10x intraspecies, 10x interspecies, 10x database).

- For triazole alanine and other triazole conjugates, the developmental toxicity study, with a NOAEL of 100 mg/kg, should be used for both acute and chronic risk assessments; UF=300 (10x intraspecies, 10x interspecies, 3x database).

- The developmental endpoints from the rat developmental toxicity studies for free triazole and triazole alanine were selected for use based on the available limited database, and are considered to be protective for all risk assessment scenarios, in the absence of reliable alternative endpoints.

- The use of a smaller database factor (3x) for triazole alanine and other triazole conjugates is based on the following considerations:
  - Available studies for triazole alanine showed minimal toxicity in adults at doses approaching the limit dose;
  - There is no indication of neurotoxicity in the available triazole alanine studies;
  - If additional studies were requested for triazole alanine, they would likely also be conducted at doses approaching the limit dose, leading to endpoints higher than that selected above from the developmental toxicity study;
  - The absence of a developmental toxicity study in rabbits, given the findings in the developmental toxicity study in rats, supports the use of an additional 3x database factor.

8. The PRC determined toxicology data needs for free triazole and triazole alanine (following evaluation of requested studies, it is possible that additional data may be needed):

   - For free triazole, the following studies should be required:
     Acute neurotoxicity study in rat
     Combined 90-day/subchronic neurotoxicity study in rat
     Developmental toxicity study in rabbit
     Reproductive toxicity study in rat (2-generation)
     Two-year chronic toxicity/oncogenicity study in female mice and male rats

   - For triazole alanine, requirements for additional studies should be reserved pending re-review of previously submitted studies or results of monitoring studies.

III. REFERENCES

Triazolylalanine Group (TAG). January 9, 2002. Profile of the Triazole-derivative Fungicide Compounds and their Common Metabolites. MRID# 45575501.

Review of "Profile of the Triazole-derivative Fungicide Compounds and their Common Metabolites". *Memorandum* from C. Swentzel to R. Keigwin dated July 8, 2002.

Attachment 2: Second Report of the *Ad Hoc* Triazole HED Peer Review Committee

TXR NO. 0052012

DATE: August 5, 2003

MEMORANDUM

SUBJECT: ***TRIAZOLES*** - 2nd Report of the *Ad Hoc* HED Peer Review Committee.

FROM: Kathleen Raffaele, Toxicologist
Registration Action Branch 3
Health Effects Division (7509C)

THROUGH: Stephen Dapson
Senior Scientist, Registration Action Branch 3
Health Effects Division (7509C)

TO: Bob Tomerlin
Registration Division (7505C)

PC Codes (include but are not limited to): 004401; 120503; 128993; 128847; 123909; 129011; 128835; 128925; 128857; 125601; 122101; 120603; 128997; 109901; 127201; 128976

On 11/4/02, an *ad hoc* HED committee conducted an internal Peer Review of the OPP Triazole Team's 7/8/02 analysis of the Triazolylalanine Group's (TAG) 1/9/02 document entitled "Profile of the Triazole-derivative Fungicide Compounds and their Common Metabolites"(MRID# 45575501). The committee, led by Elizabeth Doyle, addressed toxicology and dietary exposure issues associated with this class of fungicides and its metabolites/degradates of concern, including toxicology data needs (see Memo dated July 10, 2003, TXR# 0052011, K. Raffaele to Bob Tomerlin). On June 16, 2003, the Committee reconvened, to address a data waiver request submitted by the US Triazole Task Force (USTTF, DP Barcode 289197), requesting that data requirements for the acute neurotoxicity study, the rabbit developmental study, and the chronic/oncogenicity studies on male rats and female mice be waived.

Ad Hoc HED Peer Review Committee Members in Attendance

Elizabeth Doyle, Karl Baetcke, William Burnam, Vicki Dellarco, George Herndon (substituting for Steve Knizner), Alberto Protzel, Jess Rowland, and Brenda Tarplee (Executive Secretary).

OPP Triazole Team Members in Attendance

Kathleen Raffaele, Paula Deschamp, and Mike Metzger.

Also in attendance were: Timothy McMahon (AD)

Toxicology Data Evaluation / Report Presentation: _____

Kathleen Raffaele,
Toxicologist

INTRODUCTION

On 11/4/02, an *ad hoc* HED committee conducted an internal Peer Review of the OPP Triazole Team's 7/8/02 analysis of the Triazolylalanine Group's (TAG) 1/9/02 document entitled "Profile of the Triazole-derivative Fungicide Compounds and their Common Metabolites" (MRID# 45575501). The committee, led by Elizabeth Doyle, addressed toxicology and dietary exposure issues associated with this class of fungicides and its metabolites/degradates of concern, including toxicology data needs (for conclusions from this meeting, see TXR#0052011). On June 16, 2003, the Committee reconvened, to address a data waiver request submitted by the US Triazole Task Force (USTTF, DP Barcode 289197), proposing that data requirements for the acute neurotoxicity study, the rabbit developmental toxicity study, and the chronic/oncogenicity studies on male rats and female mice be waived.

BACKGROUND

In their meeting on 11/4/02, the *ad hoc* Triazole Peer Review Committee (PRC) determined toxicology data needs for free triazole. These data needs were transmitted to the US Triazole Task Force (USTTF) in November, 2002. The OPP Triazole Team met with the USTTF to discuss toxicology data needs on February 20, 2003, and a detailed proposal for generation of toxicology data, including a rationale for waiver of several studies, was submitted by the USTTF on March 28, 2003. This proposal was reviewed by the OPP Triazole Team, and the PRC was reconvened on June 16, 2003 to evaluate the proposed waiver.

CONCLUSIONS

The conclusions/recommendations of the Peer Review Committee (PRC) regarding the toxicology study waiver requests are as follows:

1. Acute Neurotoxicity Study

The PRC determined that the data requirement for the acute neurotoxicity study should be placed in reserve, pending the outcome of the combined subchronic/neurotoxicity study in rats. Upon receipt of the combined subchronic/neurotoxicity study, the need for the acute neurotoxicity study will be reassessed. In the absence of data from an acute neurotoxicity study, the PRC concluded that the use of the developmental endpoint from the developmental toxicity study in rats, when used for acute risk assessments in all populations, would be protective for effects that might have been seen in the acute neurotoxicity study.

2. Developmental Toxicity Study in Rabbits

The PRC reaffirmed their previous decision that the developmental toxicity study in rabbits should be required. Given the developmental effects seen in the rat study, the potential for developmental toxicity cannot be fully evaluated in the absence of a study in rabbits (to assess the possibility that the rabbit may be more sensitive than the rat). Although the USTTF argued,

based on results from parent compounds, that the rat is more sensitive to developmental toxicity from triazole-derivative compounds, the PRC reaffirmed its previous decision that the toxicity of free triazole cannot be predicted based on results from parent compounds. The PRC also noted that the results from studies in parent compounds were mixed with respect to most sensitive species for developmental effects (for some compounds, lower NOAELs were seen in rabbit studies).

3. Chronic toxicity/oncogenicity studies in male rats and female mice

The previous recommendation for chronic toxicity/oncogenicity studies in male rats and female mice was based on the widespread presence and persistance of free triazole in the environment and concerns regarding possible carcinogenicity of free triazole. To perform an adequate assessment of carcinogenicity, the PRC determined that both rat and mouse studies were needed.

The USTTF argued that chronic toxicity studies for free triazole are not needed; they suggested that chronic endpoints could be extrapolated based on subchronic data, since subchronic and chronic endpoints are within an order of magnitude for parent compounds. As mentioned above, the PRC previously concluded that toxicity of free triazole could not be predicted based on results of studies conducted in parent compounds. In addition, the PRC noted that the relationship between subchronic and chronic NOAELs/LOAELs has much to do with dose selection, so comparisons of NOAELs/LOAELS between subchronic and chronic studies provides limited information. The PRC also noted that subchronic studies do not evaluate the potential for carcinogenicity, which is seen with many triazole-derivative compounds.

The USTTF stated that the parent triazole compounds are not mutagenic or genotoxic, and that available mutagenicity data for free triazole are also negative, mitigating the need for carcinogenicity data. The PRC agreed that available mutagenicity data are negative, but also noted that many triazole-derivative compounds are carcinogenic, in the absence of mutagenicity or genotoxicity, limiting the reliance that can be placed on these negative data.

The USTTF stated that there was no indication of increased toxicity over time in the available 30- and 90-day studies with free triazole, and no accumulation of free triazole was seen in available metabolism studies. The PRC concluded that differences in the route of administration (gavage vs. dietary) and dose selection, and limitations on the endpoints evaluated, preclude direct comparison of results across the 30- and 90-day studies (both of which were unacceptable, with multiple deficiencies). The PRC also noted that the length of the cited studies was not substantially different, when compared to the duration of chronic studies. Regarding the metabolism data, the PRC noted that cumulative toxicity can occur due to repeated insult, without need for bioaccumulation, therefore the presence or absence of bioaccumulation is not definitive. In addition, the estimated half-life of 10-12 h from these available metabolism studies does not preclude the possibility of bioaccumulation. The PRC also noted that the available metabolism studies for free triazole are limited (single exposure only), and have been classified as Unacceptable, based on multiple deficiencies (including inadequate methodological information, inadequate characterization of test substance, etc.); the reliability of these data is therefore questionable.

The USTTF indicated that additional endpoints would be included in subchronic rat and mouse studies to address possible cancer issues. Based on review of submitted protocols by the Triazole team, the added endpoints were limited (retention of liver tissue for possible enzyme analyses [criteria for performance of analyses was not provided] and analysis of thyroid hormone levels in the subchronic rat study). No mechanistic data were proposed or provided, nor was there any discussion of how proposed enzyme analyses (if performed) or thyroid hormone analyses would be predictive of carcinogenicity.

In conclusion, the PRC determined that the subchronic studies do not assess carcinogenicity, increased severity of effects, or changes in dose/response curve, which may occur with long term continuous exposure. In the absence of chronic toxicity/oncogenicity studies, long term risk from exposure to free triazole, including possible carcinogenic effects, cannot be evaluated. The requirement for chronic toxicity/oncogenicity studies in male rats and female mice was reaffirmed.

Attachment 3: Triazole Summaries - Additional Data Summaries/Literature Articles

1. O. Hockwin and A. Wegener. (1989). Final Expert Opinion on the In-Vivo examination of the lens using slit-lamp microscope and Schiempflug photography and post-mortem biochemistry of the lenses from Bayer Study T 3 027 392 in Beagle Dogs. Department of Exp. Ophthalmology of the Rheinische Friedrich-Wilhelm University Bonn, Sigmund-Freud Street 25, 5300 Bonn 1, Germany. Laboratory Project ID AC Report No. 109988. MRID 45284013. Unpublished.

This document contains information regarding a study evaluating ocular effects in Beagle dogs during exposure to several Bayer compounds, including tebuconazole (referred to in the document as HWG 1608) and triazole (1,2,4-Triazole). The document contains no information regarding the study protocol, exposure levels, or clinical signs in the exposed animals. Administered doses are not described in the document, although a listing on one page seems to indicate doses of 100 mg/kg for both triazole and tebuconazole. The report does contain detailed information regarding the ocular findings, including in vivo examinations and in vitro studies of changes in lens biochemistry. Statistically significant changes were found on some parameters for both tebuconazole and triazole. Given the lack of procedural information, the results are difficult to interpret.

Unless additional information can be provided for this study (for example, clinical signs or necropsy data, and a more adequate explanation of the procedures), the data available do not provide information usable in assessing risk from triazole exposure.

2. J. Thyssen and G. Kimmerle. (1976). 1,2,4-Triazole Occupational Toxicology Study. Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. Bayer AG Report No. 5926. MRID 45284004. Unpublished.

This document contains short summaries of several acute toxicity studies on 1,2,4-triazole (studies are described separately below). Very little information was provided on the specific procedures used, nor was there analytical data on the test substance.

Acute oral toxicity: 1,2,4-triazole was administered by gavage to Wister rats (size of most dose groups was 15/sex, but there was some variation among doses). Doses ranged from 250-2500 mg/kg for males, 100-2500 mg/kg for females; there were 9 dose levels/sex, with dose intervals usually varying by 250 mg/kg. Results were presented as the number of animals/group who died or showed clinical signs, as well as a range of days on which death occurred for each group. An LD50 was also calculated separately for each sex.

For males, clinical signs were not seen at 250 mg/kg, but were seen in all animals for all other dose groups (starting at 500 mg/kg). For females, clinical signs were not seen at 100 mg/kg, but were seen in all animals for all dose groups starting at 250 mg/kg. Clinical signs were not listed for individual animals, but the summary listed the following clinical signs: reduction in general well-being, sedation, and breathing disorders; at higher doses, lying in the abdominal or side position (doses at which these occurred was not stated, time of occurrence was given as "within

an hour of administration and ... for a maximum of up to 13 days after administration" [study report, p. 6]). The lowest dose at which death occurred was 1250 mg/kg for both sexes, with the death occurring on the day of dosing. The oral LD50 was similar for both sexes (1650 mg/kg for males, 1648 mg/kg for females).

Dermal toxicity: 1,2,4-triazole (moistened with Cremophor EL) was applied to the shaven dorsal skin of male and female Wistar rats. After 24 h, substance was washed off (with soap and water). Animals were observed for 14 days. The summary stated that studies were carried out using the occlusive dressing method (not further described, although a citation was provided). Group size varied from 5 to 20, with most groups including 10 animals/sex. The dose range was 1000-5000 mg/kg for males (4 dose groups) and for females (6 dose groups). Clinical signs were seen in all animals from all treatment groups. Again, individual data were not provided but the summary stated that the symptoms were similar to those seen in the oral studies. Death was seen at the 2500 mg/kg dose in both sexes; time of death varied from 1-9 days after treatment, with earlier deaths occurring after exposure to higher dose levels. The calculated dermal LD50 was somewhat lower for females (3129 mg/kg) than for males (4200 mg/kg).

Inhalation: No effects were observed in the inhalation studies (using 5 male rats and 5 male mice), but the summary also states that "no substance vaporized or atomized in the 4 and 6-hr. experiments", so it is not clear that any exposure actually occurred.

Dermal irritation: 1,2,4-triazole was applied to cellulose patches (1.5 cm2, 500 mg/patch). The patches were applied for 24 h to the hairless skin of the ears of 2 rabbits. Treated skin was observed for 7 days following removal of patches. The summary stated that the 'skin revealed no changes'.

A similar study was also performed using similar treatment on the human forearm (specific procedure and dose not provided), with exposure from 2-8 h and 7 day observation periods (a total of six subjects, 1 female and 5 male). The summary stated that treated skin was 'physiologically normal' after removal of the dressing and for the remainder of the observation period.

Ocular irritation: 1,2,4-triazole was applied to the conjuctival sac of the left eye (50 mg/animal) of 2 rabbits. Intense reddening and very intense swelling of the eye and conjuctivae were observed immediately after application and at 24 h. After 5 days, one animal had returned to normal but the other still had some redness and swelling of the conjuctivae. Effects on the cornea and iris were also observed during the first 2 days after application. The test substance was classified as a severe eye irritant.

Summary: The report concluded that 1,2,4-triazole was moderately acutely toxic by the oral and dermal route, and that the central nervous system was affected at high oral and dermal doses.

3. P.K. Chan, P.M. Fisher, and R.D. Morrison. (1981). 1,2,4-Triazole Acute range-finding studies. Rohm and Haas Toxicology Department, 727 Norristown Rd., Spring House, PA 19477. Rohm and Haas Report No. 81R 0057. MRID No. 45284006. Unpublished.

and

K.R. Procopio and J.D. Hamilton. (1992). 1,2,4-Triazole Acute range-finding studies. Rohm and Haas Company, Toxicology Department, 727 Norristown Rd., Spring House, PA 19477. Rohm and Haas Report No. 81R-057A. MRID No. 45324301 (Supplemental submission to EPA MRID No. 45284006). Unpublished.

Both of the above-cited documents provide information regarding the same series of acute toxicity studies on 1,2,4-triazole, with the second submission including additional information not provided in the first submission.

Acute oral toxicity: 1,2,4-triazole (Sample No. TD 81-112, Lot No. 113296, 92.8% pure), in 0.5% methylcellulose, was administered by gavage to male rats (Crl:CDBR, 3/group) at 0.5 or 5 mg/kg. At 5.0 mg/kg, all rats died within 10 min. of dosing; at 0.5 mg/kg, all rats survived and no clinical signs were noted.

Acute dermal toxicity: 1,2,4-triazole (lot number as above), was moistened with saline and applied to shaven intact skin of rabbits (2 males/dose level) at 0.2, 2.0, and 5.0 g/kg. Application sites were occluded. After 24 hours, covers were removed and site was wiped with paper towels. Animals were observed for 14 days; skin irritation was also recorded.

All animals treated with 2.0 or 5.0 g/kg died; the higher dose animals died on days 1 and 2, the lower dose animals on day 3 and 4. Observed clinical signs at those doses included passiveness, scant droppings, soft feces, tremor, ataxia, salivation, abdominal breathing, gasping, and nasal discharges. No clinical signs were seen in animals treated at 0.2 g/kg. Skin irritation was noted as well defined erythema and very slight edema. It was not clear at which dose(s) these irritant effects were observed.

Skin irritation: 1,2,4-triazole, 0.5 g/patch, was moistened with saline and applied to the skin of 2 male rabbits. Two patches were applied to each rabbit, 1 on intact skin and 1 on abraded skin. Application sites were occluded, under a gauze lined patch and an impervious cuff, for 24 h, after which the patches were removed and the site wiped with paper towels.

No edema was observed for either site on either rabbit. Erythema, scored 1 or 2, was observed on one or both sites for both rabbits. Based on these results, 1,2,4-triazole was rated as 'slightly irritating' to skin.

Eye irritation: 1,2,4-triazole, 0.1 g/eye, was applied to the conjunctival sac of the left eye of two male rabbits. Eye irritation was evaluated according to the method of Draize, for up to 14 days. Effects were seen on the cornea, iris, and conjunctivae starting at 4 h. By day 7, corneal and conjunctival effects had cleared. Iridal effects had cleared by day 14. Based on these results, 1,2,4-triazole was rated as 'substantially irritating' to the eye.

Summary: Findings in this series of studies were similar to those in the previous report. The only notable difference was that there were no clinical signs reported in the current acute oral

study at 0.5 g/kg, while clinical signs were seen in both sexes in the previous acute oral study at 500 mg/kg (the same dose). We also note that only males were tested in the current study, and that females were found to be more sensitive in the previous report.

4. Wickramaratne, G.A. de S. (1987). The Chernoff-Kavlock Assay: Its validation and application in rats. Teratogenesis, Carcinogenesis, and Mutagenesis 7:73-83. MRID No. 45284010.

This document consists of a copy of a literature article reporting on a study intended to validate the use of an abbreviated Chernoff-Kavlock assay in rats to predict chemicals likely to cause teratogenic effects (testing was conducted at Central Toxicology Laboratory, Imperial Chemical Industries PLC, Cheshire, UK). Test substance was administered to pregnant rats (Wistar-derived) from gestation days 7-17. Maternal observations consisted of maternal bodyweights on days 1, 7-17, and 22 of gestation; offspring observations included only litter weights of live pups on PNDs 1 and 5, and number of live and dead pups on PNDs 1 and 5.

Triazole, at dose of 25 and 100 mg/kg, was included as one of the many substances tested in this assay. Authors reported no differences between triazole-treated animals and control animals.

The parameters evaluated and reported in this study were very limited; data collected in the rat developmental studies conducted by Bayer are much more complete and would supercede those reported in this study.

5. Wickings, E.J., M.C. Middleton,. and S.G. Hillier. (1987). Non-Steroidal Inhibition of Granulosa Cell Aromatase Activity in vitro. J. Steroid Biochem. 26 (6):641-646. MRID No. 45284014.

In this published article, a substituted triazole (R151885 [1,1-di(4-fluorophenyl)-2-1,2,4-triazol-1-yl)-ethanol) was evaluated in several in vitro assays for effects on steroid metabolism/synthesis (including granulosa cell estradiol production, progesterone production, and testosterone aromatization). A series of structurally related compounds were then evaluated, to determine which structual components of the compound were responsible for aromatase inhibition. Results indicated that imidazolyl or pyridyl moieties, when replacing the triazolyl substituent, produced more potent and specific inhibition.

These results appear to indicate that the triazole moiety was not the active component of this compound, with respect to inhibition of the aromatase enzyme in the assay used in this study.

6. Flucke, W. (1978). 1,2,4-Triazole. Determination of Acute Toxicity (LD50). Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. AC Report No. 109972. MRID No. 45284008. Unpublished.

This document consists of a one page report, with no procedural information or explanation of the data. It appears to report on an acute oral LD50 study; doses were administered to male rats only, 10/dose. Doses ranged from 850-2500 mg/kg (8 dose levels tested). It appears that clinical signs (not described) were seen in all animals at all doses. The lowest dose at which death

occurred was 1200 mg/kg, all animals died at 2000 and 2500. The LD50 was calculated to be 1375 mg/kg (range 1273-1485 mg/kg).

The findings from this study appear to be similar to those described above.

7. Rakhmatov, R.M., V.B. Danilov, U.A. Madzhidov, and S.A. Gamiyants. (1991). Basis data for setting the limiting allowed concentration (LAC) of 1,2,4-triazole in workplace air. Gigiena i Sanitariya 2:30-1. [Abstract only]. MRID No. 45284011.

This submission consists of a translated Russian abstract that describes very briefly a series of studies conducted for use in setting safe exposure levels (in air) for 1,2,4-triazole. Little or no procedural information was provided for the various studies. The results reported are described below.

-Oral LD50s for mice (3650 mg/kg), rats (3080 mg/kg) and rabbits (666 mg/kg). Signs reported were lethargy and ataxia, with death on the 'first day of observation.'

-Inhalation LC50 (4 hour) for mice (2200 mg/m3) and rats (2050 mg/m3).

-Skin irritation was not seen upon dermal exposure to rats.

-Eye irritation (slight) was seen in rabbits.

-Subacute exposure (dose, route, and duration not specified) to rats demonstrated some potential for cumulative toxicity. Effects seen after repeated oral exposure were described as 'macrovesicular fatty dystrophy of the liver, plethora and acirculatory expansion of vessels in the kidneys with filling of canal loops of glomerulus capillaries by blood, and shallow hemorrhaging.'

-Threshold concentration following single inhalation exposure (duration not specified) was stated as 226.6±10.5 mg/m3, with findings of 'increased methemoglobin concentration and decreased cholinesterase activity in the blood.'

-Effects of aerosol exposure were evaluated in rats and rabbits at doses of 19.8 ±0.33 mg/m3 and 4.9±0.08 mg/m3. Following 3 months exposure (apparently at the higher dose), decreased levels of urea in urine were seen; following 4 months, decreased cholinesterase and increased methemoglobin were seen in blood. No effects were noted at the lower dose.

-Mutagenic (as represented by chromosome aberrations in rat marrow cells) or 'embryotropic' activity (not further described) were not found (studies not described but conducted using inhalation exposure at the above doses).

The abstract concluded that toxic effects of the compound included 'primarily effects on the liver, kidneys, and CNS.' A LAC of 5 mg/m3 in workplace air was proposed.

8. Saratikov, A.S., E.M. Trofimovich, T.P. Novozheeva, T.A. Zimina, M.R. Ozhegina, and E.L. Morokova. (1986). Toxicological and Health Evaluation of 1,2,4-triazole for health standards in aquifers. Gigiena i Sanitariya 51 (11):65. [Abstract only]. MRID 45284012.

This submission also consists of a translated abstract, describing briefly a series of studies conducted for use in evaluating safe levels of 1,2,4-triazole in aquifers. It was stated that the triazole used for studies was 98% pure. In addition to toxicity, several properties of triazole in aqueous solutions were evaluated.

Acute toxicity in mice and rats was evaluated following intraperitoneal administration of an aqueous solution. Average toxic dose was stated to be 1350 mg/kg for mice and 1750 mg/kg for rats, with clinical signs listed as decreased motor activity, excitability, muscle cramp, and (in some cases) tremors and difficulty breathing.

Irritancy to skin was evaluated using a 25% solution, eye irritation using a 12.5% solution; no irritant effects were noted.

Cumulative properties were also evaluated, and it was stated that the compound has moderatively cumulative properties, but insufficient information was provided to interpret the stated results.

A six-month study was performed in male white rats (strain not specified), using i.p. doses of 0.02, 0.20, 2.0 mg/kg. Evaluated parameters included body weights, blood cell counts, and a variety of clinical chemistry parameters; apparently no histopathology or necropsy data were collected. Stated results included an increase in urine protein content, changes in leukocytes, and changes in alkaline phosphatase activity at the high dose (no other description provided). No changes were noted at the two lower doses. An LAC for aquifers was recommended at 4 mg/dm3.

9. Holzum, B. (2000). Developmental toxicity of triazole-fungicides, Characterization and risk assessment. Bayer AG, Department of Toxicology, Friedrich-Ebert-Strasse 217-333, D-42096 Wuppertal, Germany. AC Report No. 109973. MRID No. 45284009. Unpublished.

This submission consists of a copy of a presentation by a Bayer scientist, regarding common developmental toxicity of triazole fungicides, and discussion of classification of developmental effects under the European classification and labelling requirements (used within the European Community). Several of the slide copies include tables listing the specific types of developmental toxicity seen following exposure to triazole fungicides (including the doses at which effects were seen maternally and in offspring), with the rat and rabbit studies compared separately.

In rats, the summary notes that common effects generally seen include embryolethality, retarded development, and supernumerary ribs, with urinary tract variations and malformations seen in a subgroup of compounds. In rabbits, the only general effect noted was embryolethality, with a variety of malformations again seen in a subgroup of compounds.

Included in the summary is a reference to developmental toxicity studies using triazole; these studies have been reviewed separately and the results will not be discussed here.

With respect to common toxicity across triazoles, the report authors concluded "we may conclude that, to a certain extent, a typical pattern exists for triazole developmental toxicity in animals." (p. 9), although they did point out some differences in the patterns of abnormalities across studies. The report author, however, attributes developmental effects to maternal toxicity for this group of compounds (perhaps acting via maternal hypoxia or adrenal effects); this attribution of causation appears to be relevant in the European system of classification.

10. Menegola, E., M.L. Broccia, F. DiRenzo, and E. Giavini. (date not provided). Poster Presentation: In Vitro Comparative Study of the Teratogenic Activity of Some Triazoles. University of Milan, Dept. of Biology, Milan-Italy. [Copy of a poster, presentation meeting not specified.] MRID No. 45344602. Unpublished.

This submission consists of a one page copy of a poster. Two substituted triazoles (Flusilazole and fluconazole) and triazole were evaluated for teratogenic potential in vitro in 48 h whole embryo cultures of 9.5 day old rat embryos. Methodological details regarding the culture conditions (including the number of embryos evaluated in each condition) and analytical procedures were not provided. The results stated that flusilazole (6.25-250 μM) and fluconazole (125-500 μM) "showed abnormalities at the branchial apparatus level and increased cell death at the branchial mesenchymes" (hypoplasia of I and II branchial arches and fusion of I-II branchial arches) without indication of developmental delays. Exposure to triazole (2500-5000 μM) caused slight developmental delays and severe anemia of the visceral yolk sac. Authors concluded that both mono-triazole derivatives showed teratogenic potential, in particular for cranio-facial abnormalities, but that triazole was not teratogenic.

We note that insufficient information was provided for us to evaluate the conclusions stated in this document, and that the conditions of exposure for this in vitro study may not represent those that would occur in vivo. We also note that the results from the available developmental toxicity study in rats would supercede findings from this study with respect to evaluating teratogenic effects of triazole.

Old metabolism studies

In a series of metabolism/disposition studies (MRID 45284018, 45284019, and 45297202), groups of male and female Sprague-Dawley rats were given a single intraduodenal (1 mg/kg), intravenous (0.1-100 mg/kg), or oral dose (0.4-866 mg/kg) of ^{14}C-labeled triazole (MRID 45284018 – Lot No. not reported, sp. act. 470 μCi/mg, purity ≈97%; MRID 45284019 – Lot No. CL-V-70, sp. act. >67 μCi/mg, purity >98%; MRID 45297202, Lot No. CFQ 2458, sp. act. 103 μCi/mg, purity ≥99%). The test material is a common moiety found in several compounds under development by Ciba-Geigy at the time these studies were conducted. Urine and feces were collected up to 7 days postdose and expired air was monitored for residual activity. Bile samples were collected up to 24 hours after treatment with the radiolabel. Tissue/carcass burdens were investigated at selected time intervals up to 7 days postdose.

No treatment-related signs of toxicity among any of the test animals were reported. Overall recovery of administered radioactivity ranged from 97-105%. Based upon urinary and biliary excretion and tissue/carcass burden data, 80% - 95% of the administered dose was absorbed. Elimination of administered radioactivity was primarily via the urine, accounting for 80-95% of the dose, and was ~95% complete within 48 hours after treatment. Elimination via expired air was negligible (<0.1%). Fecal excretion typically accounted for <15% of the administered radioactivity and was essentially complete within 48 hours. Biliary excretion accounted for approximately 10% of the dose during the 24 hr study period, and the results were suggestive of enterohepatic circulation and diffusion/secretion by the stomach mucosa.

The only compound identified in the urine was the unchanged parent which constituted ~95% of the radioactivity. There were possibly three other potential polar metabolites in the urine, each constituted <3% of the administered dose. Their structures were not elucidated, but all three seem to be more polar than triazole as judged by their delayed migration relative to triazole under normal-phase TLC separation conditions. Based on the study results, triazole was nearly completely absorbed and rapidly excreted mostly unchanged in the urine.

These metabolism/disposition studies in rats are Unacceptable/Guideline and do not satisfy the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)]. None of the studies were described in detail and gave the impression they were more laboratory notes than metabolic study reports. Neither the care and treatment of the animals nor the test material were adequately described. Details concerning dose preparation, such as stability and homogeneity were also lacking. Finally, the potential metabolites in the feces were not identified, particularly in those instances when the feces contributed >5% of the total isotope recovery (MRID 45284019). The study can be upgraded to acceptable if it can be shown that the feces also contained unchanged parent compound and if more information were provided for the test material (description, purity, stability, homogeneity, etc.).

13544

R124628

Chemical: 1H-1,2,4-Triazole (A metabolite of tebuconazole & metabolite of acaricidal & fungicidal compounds)
1H-1,2,4-Triazole-1-propanoic acid, .alpha.-amino-
Triazolyl acetic acid (A metabolite)

PC Code:
600074
600011
600082

HED File Code: 14000 Risk Reviews
Memo Date: 2/7/2006
File ID: DPD322215
Accession #: 412-06-0013

HED Records Reference Center
3/30/2006

Attachment 4

October 4, 2021

Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, DC 20460–0001.

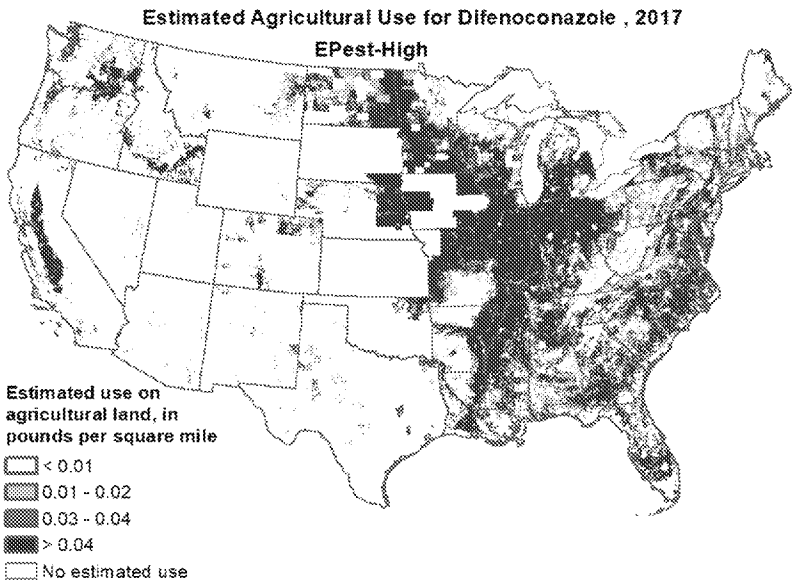
RE: Docket EPA-HQ-OPP-2015-0401
Comments on proposed interim registration decision for difenoconazole

Center for Food Safety appreciates the opportunity to comment on EPA's proposed interim registration decision for the fungicide difenoconazole, on behalf of itself and its 970,000 members and supporters. Center for Food Safety (CFS) is a public interest, nonprofit membership organization with offices in Washington, D.C., San Francisco, California, and Portland, Oregon. CFS's mission is to empower people, support farmers, and protect the earth from the harmful impacts of industrial agriculture. Through groundbreaking legal, scientific, and grassroots action, CFS protects and promotes the public's right to safe food and the environment. CFS has consistently supported comprehensive EPA review of registered pesticides and individual inert ingredients.

Introduction

First registered by EPA in 1994, difenoconazole is a broad-spectrum fungicide registered for use on many fruits, vegetables, cereals (seed treatment), field crops as well as on golf course turf grass and ornamental plants. Difenoconazole kills fungi by blocking the synthesis of sterols, which are key components of fungal cell walls. It belongs to the triazole class of demethylase inhibitor (DMI) fungicides, which block ergosterol synthesis by inhibiting the CYP51 enzyme, which catalyzes the 14 alpha demethylase step in ergosterol synthesis.

WASHINGTON D.C. OFFICE: 660 Pennsylvania Ave, SE, #402, Washington, DC 20003, T (202) 547-9359, F (202) 547-9429
CALIFORNIA OFFICE: 303 Sacramento St, 2nd floor, San Francisco, CA 94111, T (415) 826-2770, F (415) 826-0507
PACIFIC NORTHWEST OFFICE: 2009 NE Alberta St, Suite 207, Portland, OR 97211, T (971) 271-7372
www.centerforfoodsafety.org office@centerforfoodsafety.org



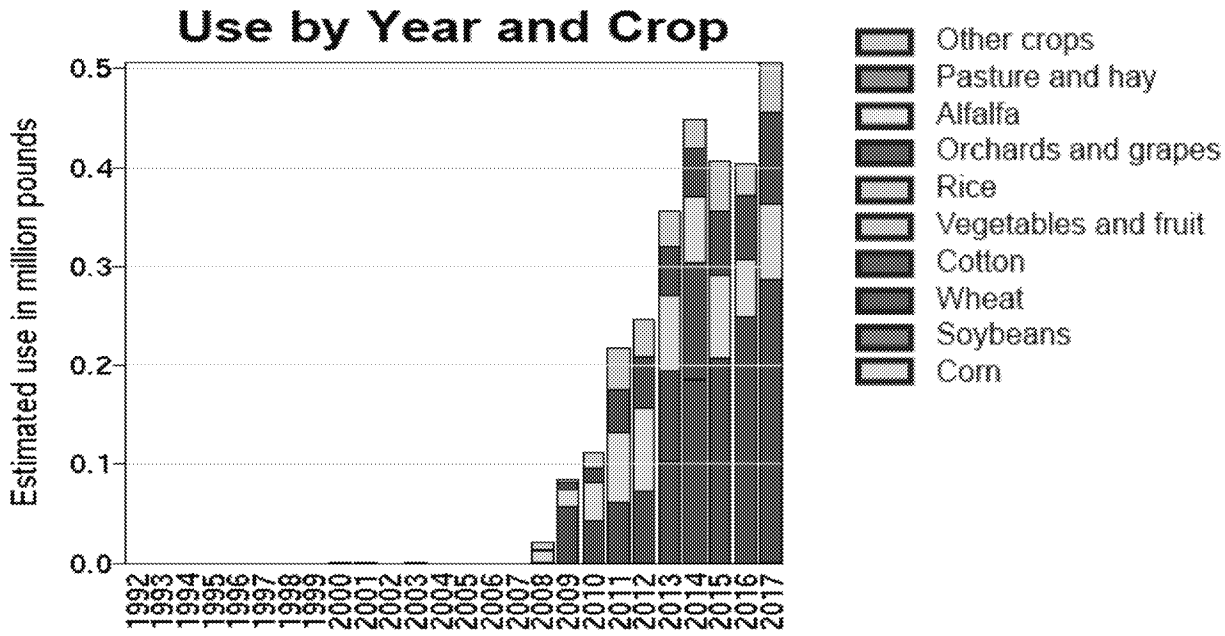


Figure 1: Difenoconazole Use, from: US Geological Survey, Pesticide National Synthesis Project, Difenoconazole, Epest-High. https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2017&map=DIFENOCONAZOLE&hilo=L&disp=Difenoconazole. Note: Seed treatment uses excluded beginning in 2015.

Difenoconazole was little used for a dozen years after it was first registered. Agricultural use first registers in 2008, and when one excludes seed treatments, has increased by roughly 20-fold over the decade from 2008 to 2017: from 25,000 to 500,000 lbs. per year (Figure 1). Usage is increasing on many crops, as shown by comparing figures in EPA's 2014 Screening Level Usage Analysis (average 2004 to 2012) and the Proposed Interim Decision (PID), averaging use over 2015 to 2019. For instance, percent acres treated has increased in tomatoes (25 to 45%), almonds (5 to 30%), sugar beets and apples (both 15 to 30%), grapes (5 to 30%) and

watermelon (5 to 20%).[1] Soybeans constitute half or more of difenoconazole's foliar use (Fig. 1); and because this represents treatment of just 2% of soybean acres, and soybean use is rising, there is huge potential for much greater spraying of soybeans with this fungicide. Finally, seed treatment use on wheat is likely on the order of 240,000 lbs./year, making overall agricultural use at least 750,000 lbs./year.[2]

Several features of difenoconazole and its use deserve particular consideration. First, because difenoconazole is one of many DMI/triazole fungicides with the same mode of action in fungi, and similar effects on non-target organisms, its putative benefits and impacts must be viewed in the broader context of its class. Second, triazole use overall is dramatically increasing. There are at least 15 DMI/triazole fungicides used in the U.S., and their collective use as of 2016 (excluding seed treatments) is nearly 7-fold greater than in 1992, and over 5-fold (434%) greater since just 2006 (Toda et al. 2021). Finally, difenoconazole in particular and other members of its class are quite persistent in the environment.

RELEVANT LEGAL STANDARDS

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

FIFRA authorizes EPA to regulate the registration, use, sale, and distribution of pesticides in the United States. Pursuant to FIFIRA, EPA oversees both initial registration of an active ingredient as well as any new uses of the registered active ingredient.

Section 3(c) of FIFRA states that a manufacturer must submit an application to register the use of a pesticide.[3] Under Section 3(c)(5) of FIFRA, EPA shall register a pesticide only if the agency determines that the pesticide "will perform its intended function without unreasonable adverse effects on the environment" and that "when used in accordance with widespread and commonly recognized practice[,] it will not generally cause unreasonable adverse effects on the environment."[4] FIFRA defines "unreasonable adverse effects on the environment" as "any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide."[5] Alternatively, where there are data gaps and missing information, EPA can register a pesticide with conditions (conditional registration) under Section 3(c)(7) of FIFRA "for a period reasonably sufficient for the generation and submission of required data," but only if EPA also determines that the conditional registration of the pesticide during that time period "will not cause any

[1] Compare figures in EPA (10/2/14), the SLUA, and the PID, p. 10.
[2] EPA's Biological and Economics Analysis Division (BEAD) has conflicting figures on wheat seed treatment usage. See EPA 12/2/15, Table 3 for 240,000 lbs. on 27.3 million acres of wheat, roughly 50% of national wheat acreage over the designated period; however, BEAD also says only 10% of wheat seed is treated (p. 7). No figures are reported for seed treatment use on other crops.
[3] 7 U.S.C. § 136a(c)(1); 40 C.F.R. § 152.42.
[4] 7 U.S.C. § 136a(c)(5).
[5] 7 U.S.C. §136(bb).

unreasonable adverse effect on the environment, and that use of the pesticide is in the public interest."[6]

The culmination of the registration process is EPA's approval of a label for the pesticide, including use directions and appropriate warnings on safety and environmental risks. It is a violation of the FIFRA for any person to sell or distribute a "misbranded" pesticide.[7] A pesticide is misbranded if the "labeling accompanying it does not contain directions for use which...if complied with ...are adequate to protect health and the environment."[8]

Endangered Species Act

As recognized by the Supreme Court, the Endangered Species Act (ESA) is "the most comprehensive legislation for the preservation of endangered species ever enacted by any nation."[9] The ESA's statutory scheme "reveals a conscious decision by Congress to give endangered species priority over the 'primary missions' of federal agencies."[10] Federal agencies are obliged "to afford first priority to the declared national policy of saving endangered species."[11]

Section 7(a)(2) of the ESA requires every federal agency to consult the appropriate federal fish and wildlife agency—the U.S. Fish and Wildlife Service (FWS), in the case of land and freshwater species and the National Marine Fisheries Service (NMFS) in the case of marine species—to "insure" that the agency's actions are not likely "to jeopardize the continued existence" of any listed species or "result in the destruction or adverse modification" of critical habitat.[12] The ESA's implementing regulations broadly define agency action to include "all activities or programs of any kind authorized, funded or carried out ... by federal agencies," including the granting of permits and "actions directly <u>or indirectly</u> causing modifications to the land, water or air."[13] A species' "critical habitat" includes those areas identified as "essential to the conservation of the species" and "which may require special management considerations or protection."[14]

EPA is required to review its actions "at the earliest possible time" to determine whether the action may affect listed species or critical habitat.[15] To facilitate compliance with Section 7(a)(2)'s prohibitions on jeopardy and adverse modification, the ESA requires each federal agency that plans to undertake an action to request information from the expert agency "whether any species which is listed or proposed to be listed [as an endangered species or a

---

[6] 7 U.S.C. §136a(c)(7)(C).
[7] 7 U.S.C. § 136j(a)(1)(E).
[8] 7 U.S.C. § 136(q)(1)(F).
[9] *Tenn. Valley Authority v. Hill*, 437 U.S. 153, 180 (1978).
[10] *Id.* at 185.
[11] *Id.*
[12] 16 U.S.C. § 1536(a)(2); *see also* 50 C.F.R. § 402.01(b).
[13] 50 C.F.R. § 402.02 (emphasis added).
[14] 16 U.S.C. § 1532(5)(A).
[15] 50 C.F.R. § 402.14(a).

threatened species] may be present in the area of such proposed action."[16] If FWS/NMFS advises the agency that listed species or species proposed to be listed may be present, the agency must then prepare a biological assessment for the purpose of identifying any such species that are likely to be affected by the proposed agency action.[17]

If, based on a biological assessment, an agency determines that its proposed action may affect any listed species and/or their critical habitat, the agency generally must engage in formal consultation with FWS/NMFS.[18] At the end of the formal consultation, FWS/NMFS must provide the agency with a "biological opinion" detailing how the proposed action will affect the threatened and endangered species and/or critical habitats.[19] If FWS/NMFS concludes that the proposed action will jeopardize the continued existence of a listed species or result in the destruction or adverse modification of critical habitat, the biological opinion must outline "reasonable and prudent alternatives" to the proposed action that would avoid violating ESA section 7(a)(2).[20]

Pending the completion of formal consultation with the expert agency, an agency is prohibited from making any "irreversible or irretrievable commitment of resources with respect to the agency action which has the effect of foreclosing the formulation or implementation of any reasonable and prudent alternative measures."[21]

Human Health Concerns and Assessment Deficiencies

Liver Toxicity Endpoint and Chronic Reference Dose

The major target organ of difenoconazole is the liver, as demonstrated in both chronic and subchronic mouse and rat feeding trials conducted by registrants. Adverse hepatic effects included hepatocellular hypertrophy, hepatic vacuolation, cell necrosis, increased liver weight, fatty changes, bile stasis, increased serum levels of enzymes indicative of liver injury – alanine aminotransferase (ALA), serum alkaline phosphatase (SAP), and sorbitol dehydrogenase (SDH) – and an increased albumin/globulin ratio (EPA 9/18/20, pp. 5-6, 19; EPA 7/27/94).

In 1994, EPA established a chronic reference dose of 0.01 mg/kg/day based on hepatoxicity, in particular hepatocellular hypertrophy, in males in a chronic rat study (NOAEL = 0.96 mg/kg/day) (EPA 2/24/94; 7/27/94, p. 18). By 2015, EPA had retained the same chronic reference dose, based on the same rat study, but changed the endpoint from hepatocellular hypertrophy to cumulative decreases in body weight gains in both sexes (EPA 2/24/15). In EPA's latest human health assessment, the proposed chronic reference dose has been increased five-fold to 0.05 mg/kg/day, and based on a mouse rather than rat study, in which liver lesions were observed in male mice and hepatocellular hypertrophy in both sexes of mice, with an NOAEL of 4.7 mg/kg/day (EPA 9/18/20).

---

[16] 16 U.S.C. § 1536(c)(1); *see also* 50 C.F.R. § 402.12(c).
[17] *Id.*
[18] 50 C.F.R. § 402.14.
[19] 16 U.S.C. § 1536(b); 50 C.F.R. § 402.14.
[20] 16 U.S.C. § 1536(b)(3)(A).
[21] 16 U.S.C. § 1536(d).

EPA's dismissal of hepatocellular hypertrophy in male rats and the associated NOAEL and reference dose (0.96 and 0.01 mg/kg/day, respectively) in favor of the five-fold higher mouse study endpoint is incorrect and should be reversed. First, hepatocellular hypertrophy in rats is properly regarded as an adverse effect when accompanied by other adverse liver effects, which is the case here: the rats in this chronic study also exhibited increased liver weight and an increased albumin/globulin ratio (EPA 7/27/94, p. 18). Second, subchronic (13-week) rat and mouse studies also demonstrated low-dose adverse hepatic effects. The mouse study's effects included histopathologic alterations in the liver at an LOEL of 30 mg/kg/day and NOEL of 3 mg/kg/day. Rats in the subchronic study exhibited increased liver weights at the LOEL of 15.5 mg/kg/day (F), with an NOEL of 1.43 mg/kg/day (F) (EPA 7/27/94, pp. 12-13).

By illegitimately dismissing the chronic rat NOAEL and reference dose (0.96 and 0.01 mg/kg/day), and by ignoring the subchronic rat and mouse NOEL's, all three of which are below EPA's new chronic mouse study endpoint, EPA enables 5-fold greater chronic exposure to this hepatotoxic fungicide.

We would also note that the European Food Safety Authority continues to base its NOAEL of 1 mg/kg/day on hepatotoxicity (increased incidence and severity of hepatocellular hypertrophy), as EPA once did (EFSA 2009, p. 109).

Carcinogenicity

EPA originally classified difenoconazole as a Group C Possible Human Carcinogen in 1994, based on clear inducement of hepatocellular adenomas and carcinomas in a mouse study (EPA 7/27/94), then subsequently re-classified it under the descriptor Suggestive Evidence of Carcinogenicity (EPA 9/18/20, p. 6).[22]

Toxicity of Metabolite Unknown

EPA has identified a major metabolite of difenoconazole that is present in humans, livestock and fish – CGA-205375 – yet has practically no toxicological information on it (EPA 9/18/20). EPA should demand toxicity studies on this and other metabolites rather than rely on guesstimates based on unreliable, *in silica* structure-activity modeling.

Dermal Absorption

An *in vivo* dermal absorption study in rats found dermal absorption of 48% of the applied dose after 24 hours in rats exposed to 0.5 ug/cm^2 of difenoconazole (EPA 9/18/20, p. 18). Rather than use this value as the dermal absorption factor, EPA reduced it to 6% based on two *in vitro* dermal absorption tests, one with human and one with rat skin. EPA multiplied the ratio of the *in vitro* absorption results (human/rat = 0.12) by the *in vivo* rat result of 48% to arrive at a human dermal absorption factor of 6%. There are multiple flaws that Invalidate EPA's 6% estimate, utilizing the so-called "triple-pack" approach.

---

[22] In the executive summary of EPA's health assessment, the Agency wrongly limits the evidence for carcinogenicity of difenoconazole to benign tumors – "liver tumors (adenomas)" – when in fact some of the observed tumors were malignant (carcinomas) (see EPA 9/18/20, compare statements on pp. 6 and 19).

First, the relevant EPA regulations for the dermal penetration assay (40 CFS, Part 158.500, Guideline No. 870.7600) prescribe an *in vivo* rat study, and provide no support for EPA's manipulation of this figure by applying *in vitro* results as EPA implies (EPA 9/18/20, pp 49-50, citing Guideline No. 870.7600 for *in vitro* as well as *in vivo* tests). Guideline No. 870.7600 (see EPA 1998) details a test protocol involving live rats to determine dermal absorption, not *in vitro* tests, much less the use of *in vitro* tests to reduce the dermal absorption factor derived from the *in vivo* rat study.

Second, even if one accepts the triple pack method as acceptable in principle, its use was entirely inappropriate with difenoconazole because the *in vitro* human/rat dermal absorption ratio was derived from tests employing far higher doses (10, 100, 1000 ug/cm^2) than the *in vivo* study (0.5, 1.3, 2.5 ug/cm^2), rendering them incompatible, especially given the large differences in absorption that were observed as a function of dermal dose (EPA 9/18/20, pp. 49-50). Indeed, the 20-40-fold difference in dosage between the *in vivo* and *in vitro* tests violated EPA's precondition that the protocols and doses be the same in all three studies in order to utilize the triple pack approach (see EPA 10/29/13, dermal absorption of glufosinate; see also EPA 6/2/10, pp. 2-3, dermal absorption of thiabendazole, likewise citing "identical protocols ... in both the *in vivo* and *in vitro* studies" as a precondition for use of the triple pack approach). The difenononazole tests also failed a second EPA criterion for application of the triple pack approach – that the dermal absorption factors from the *in vitro* and *in vivo* animal tests be roughly equal (i.e. their ratio approximately equal to 1) (see EPA 6/2/10; see also EPA 10/29/13, where EPA rejects the triple pack method for estimating dermal absorption of glufosinate because the rat *in vitro* and rat *in vivo* results diverged substantially, among other reasons). In the case of difenoconazole, the *in vivo* and *in vitro* rat absorption factors from the tests carried out at the same doses differed by 3- to 4-fold (10 and 100 ug/cm^2), violating this criterion as well.

Finally, the test substance used in these assays was not specified, and if it is the technical active ingredient, this would likely lead to an underestimate of dermal absorption relative to use of real-world formulations with absorption-enhancing surfactants. Even use of a particular difenoconazole formulation in this test would not be predictive of absorption with other formulations.

EPA should demand full dermal absorption data for various difenoconazole formulations. Until then, it should conduct residential and occupational exposure assessments that incorporate dermal absorption based on a dermal absorption factor of 48%, based on a test that most closely follows the EPA (1998) protocol, rather than the 6% from illegitimate use of the triple-pack approach.

Need for Cumulative Exposure and Risk Assessment of Triazole Fungicides

Triazole fungicides clearly meet EPA's criteria for designation as a common mechanism group (CMG), for which a cumulative risk assessment must be carried out, as mandated by the Food Quality Protection Act (EPA 1/29/99, 1/14/02). They have a similar chemical structure, the liver is their primary target organ, they exert similar toxic effects on the liver, and do so via

common mechanisms of toxicity. In more modern language, they share a mode of action and adverse outcome pathways for several endpoints (MOA/AOP) (EPA 4/12/16). The European Food Safety Authority conducted a cumulative assessment of triazoles over a decade ago, forming cumulative assessment groups for developmental effects observed following acute exposure (cranio-facial malformations), and for hepatotoxicity as the chronic endpoint (EFSA 2009).

A review of registrant studies submitted to European regulators found that difenoconazole and all or most of 10 other triazole fungicides that were reviewed induced hepatocellular hypertrophy, hepatic cell degeneration or death, fatty changes, inflammation and hepatocellular tumors (Nielsen et al. 2012). As discussed further below, they exert these effects by activating nuclear receptors that induce the production of cytochrome P_{450} detoxification enzymes in the liver, causing an increase in cellular organelles (endoplasmic reticulum, peroxisomes and mitochondria) that is responsible for hepatic cell enlargement (hypertrophy). Hypertrophy is sometimes regarded as an adaptive effect, but persistent hypertrophy is adverse, particularly when it progresses to other adverse liver impacts as it does with triazoles (Nielsen et al. 2012). There are at least two endpoints, shared by most triazoles, that should be the focus of a cumulative assessment: fatty changes and carcinogenicity.

Fatty changes

The liver is the body's primary detoxification organ, and many industrial chemicals and pesticides are hepatotoxic. The most common hepatic pathology induced by chemicals is fatty liver (Al-Eryani et al. 2015) – the accumulation of lipids in liver cells – which can progress to more serious conditions, steatohepatitis and cirrhosis, which in turn are the most important risk factors for liver cancer (Wahlang et al. 2013). According to EPA scientists, fatty liver disease is "a growing epidemic" that affects 20-30% of the U.S. population (Angrish et al. 2016), while the incidence of liver cancer it predisposes to tripled from 1975 to 2005 (Altekruse et al. 2009).

In a review of chemical exposure and rodent toxicology databases maintained by the EPA and the National Toxicology Program, Al-Eryani et al. (2015) found that 54 pesticides, including 22 fungicides, caused fatty changes in the liver, many of them triazoles. In a similar review of registrant submissions to the European Union, 10 triazole fungicides induced fatty changes in the liver (Nielsen et al 2012). Altogether, at least 15 triazole fungicides induce lipid accumulation in liver cells (Table 1).

Table 1: Triazole Fungicides That Induce Fatty Changes in the Liver

Fungicide	Regulatory Authority (US, EU)	Comments
Bromuconazole	US	
Cyproconazole	US	
Difenoconazole	US, EU	
Epoxiconazole	EU	
Flusilazole	US, EU	

Hexaconazole	US	
Metconazole	EU	
Paclobutrazole	US	
Propiconazole	US, EU	
Prothioconazole	EU	
Tebuconazole	EU	
Tetraconazole	EU	
Triadimefon	US	
Triadimenol	US, EU	Primary metabolite of triadimefon
Triticonazole	EU	

Sources: Al-Eryani et al. (2005) for US; Nielsen et al. (2012) for EU. US = United States, EU = European Union. Listings in one rather than both jurisdictions does not necessarily mean differing assessments of this endpoint. Rather, it may be that particular triazoles are registered in only the US or the EU, or were at the time of the source publications.

Difenoconazole was shown to induce hepatic lipid accumulation as well as hepatocyte vacuolation and oxidative stress in male mice treated with the fungicide, and a molecular analysis supported these findings by revealing the transcriptomic signature of perturbed energy and especially glycolipid metabolism (Zhang H et al. 2021). Difenoconazole and two other triazoles, propiconazole and tebuconazole, were shown to promote accumulation of triglycerides in human HepaRG cell culture, with all three activating the pregnane-X-receptor (PXR) (Lasch et al. 2021). The critical role of PXR was demonstrated by a second study of propiconazole and tebuconazole (Knebel et al. 2019). Both triazoles induced expression of steatosis-related genes and triglyceride accumulation in HepaRG cells via interactions with several nuclear receptors – the constitutive androstane receptor (CAR), peroxisome proliferator-activated receptor alpha (PPAR α), and PXR. But in experiments with HepaRG subclones with knockouts of either PXR or CAR, triazole-induced triglyceride accumulation was abolished only with the PXR, not the CAR, knockout, demonstrating the critical role of PXR in mediating lipid accumulation triggered by triazoles.

Other studies provide still more supporting evidence. In a 28-day rat feeding trial with cyproconazole, epoxiconazole and prochloraz (an azole but not triazole fungicide), Heise et al. (2005) found hepatocellular hypertrophy and occasional necrosis of liver cells for all three compounds, increased absolute and relative liver weights for the two triazoles, and hepatic cell vacuolization with cyproconazole. A gene expression analysis found that triazoles induced expression of more than 30% of the genes in four toxicity pathways, including two involved in lipid metabolism: steatosis and phospholipidosis. Linkages between gene expression and histopathology were also found: vacuolization of hepatic cells is associated with steatosis; while cyproconazole also upregulated fatty acid synthase and transporter genes. Heise et al. (2007) tested combination of the same three fungicides in rats, and found similar effects as for the individual compounds, with dose additivity sufficient to account for combined effects. In 28-day rat feeding trials, Kwon et al. (2021) found that still another triazole, flutriafol, induced fatty infiltration of the liver by impairing liver metabolism and inducing apoptosis.

In a review article on the hepatic impacts of triazole fungicides, Marx-Stoelting et al. (2020) lay out adverse outcome pathways for liver hypertrophy and liver steatosis that link the molecular, cellular and tissue/organ level changes wrought by triazole exposure (see below). For hypertrophy, the molecular initiating events are triazole activation of the aryl hydrocarbon (AHR), CAR and PXR nuclear receptors, followed by four key events that mediate the adverse outcome on the tissue/organ level: hypertrophy of the liver:

1) Increased expression of CYP genes, with AHR, CAR and PXR preferentially but not exclusively inducing CYP families CPY1A1 and 1A2, CYP2B and CYP3A, respectively;
2) Increased expression of the corresponding CYP enzymes;
3) Proliferation of endoplasmic reticulum and other organelles to produce the additional CYP enzymes; and
4) Increased size of hepatic cells ensuing from the additional organelles.

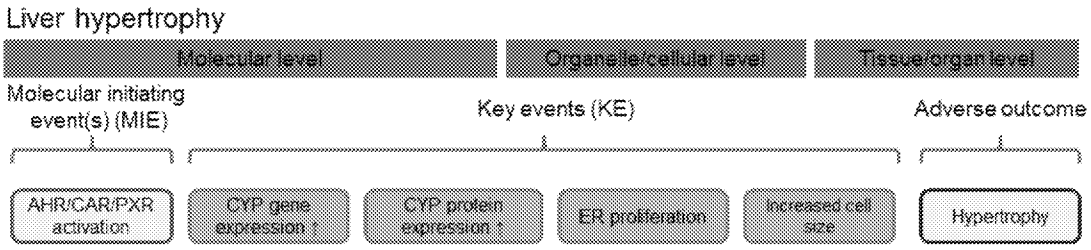


Figure 2. Schematic delineation of a nuclear receptor-dependent molecular pathway leading to hepatocellular hypertrophy. Nuclear receptor activation functions as molecular initiating event. Abbreviation: ER, endoplasmic reticulum.

Figure 2. Adverse Outcome Pathway for Liver Hypertrophy. Source: Marx-Stoelting et al. (2020).

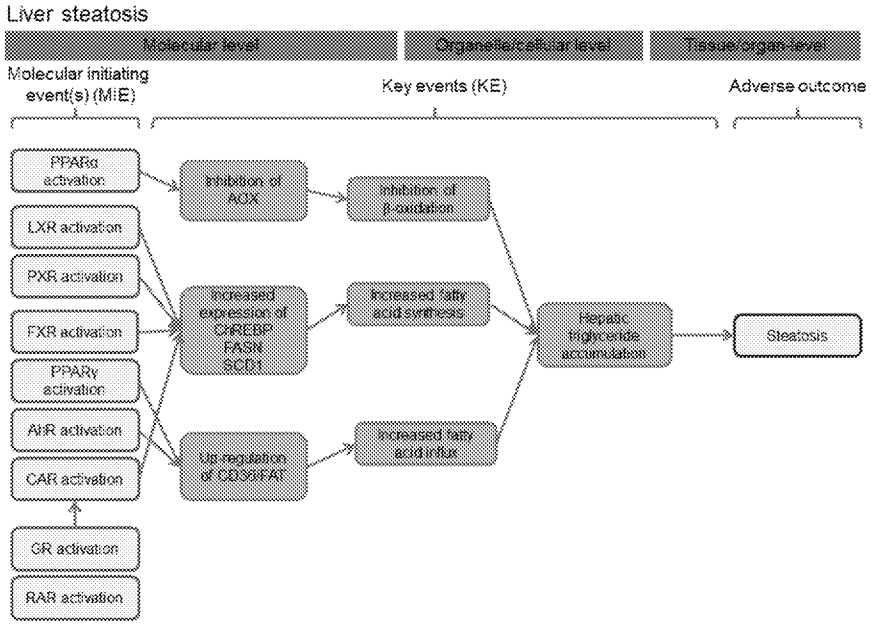


Figure 3. Schematic delineation of the AOP for hepatocellular steatosis. The figure was adapted from [58]. Abbreviations: FXR, farnesoid-X-receptor, GR, glucocorticoid receptor.

Figure 3. Adverse Outcome Pathway for Liver Steatosis. Source: Marx-Stoelting et al. (2020).

Hypertrophy of hepatic cells and the liver is a sensitive indicator of liver damage, for instance lipid accumulation. The adverse outcome pathway for hepatic steatosis is more complicated than that for hypertrophy, in that it involves multiple molecular initiating events, each activating a different toxic pathway with different key events, the cumulative outcome of which is steatosis (see Fig. 3).

Not every triazole fungicide will initiate each of these pathways in the same way on the molecular level, nor is it reasonable to demand that they do, in order to find that triazoles constitute a common mechanism group. Each pathway contributes to the same outcome, steatosis, whether through inhibition of fatty acid degradation via activation of PPRA α, increased fatty acid synthesis through upregulation of fatty acid synthase genes, and/or via increased influx of fatty acids into hepatic cells via increased expression of the corresponding transport gene.

The fact that at least 15 triazoles trigger fatty changes in the liver (Table 1), coupled with abundant evidence that they activate nuclear receptors (particularly PRX) in ways that lead to this outcome, is more than enough scientific justification to require EPA to conduct a cumulative exposure and risk assessment of triazole fungicides for this endpoint.

Carcinogenicity

A second endpoint for which EPA must cumulatively assess triazoles is carcinogenicity. EPA itself recognized the need for this in 1994, when difenoconazole was first registered. The Agency's Carcinogenicity Peer Review Committee noted that "Difenaconazole is a member of a class of chemicals, many of which have been associated with liver tumors in CD-1 mice" (EPA 7/27/94, p. 3). EPA then noted that eight structurally related triazole compounds have also been found to induce hepatocellular tumors (EPA 7/27/94, pp. 14-15), while a review of EU regulatory submissions identified seven triazoles that induced neoplasms (Nielsen et al 2012), for a total of 13 (Table 2).

Table 2: Triazole Fungicides That Induce Tumors

Fungicide	Regulatory Authority (US, EU)	Comments
Cyproconazole	US	
Difenoconazole	US, EU	
Epoxiconazole	EU	
Etaconazole	US	
Fenbuconazole	US	
Flusilazole	EU	
Metconazole	EU	
Propiconazole	US, EU	
Tebuconazole	US, EU	
Tetraconazole	EU	
Triadimefon	US	Also referred as Bayleton
Triadimenol	US	Primary metabolite of triadimefon, aka Baytan

Uniconazole	US	

Sources: EPA (7/27/94) for US; Nielsen et al. (2012) for EU. US = United States, EU = European Union. Listings in one rather than both jurisdictions does not necessarily mean differing assessments of this endpoint. Rather, it may be that particular triazoles are registered in only the US or the EU, or were at the time of the source publications.

Pesticide industry scientists tend to discount the carcinogenic effects of non-genotoxic, nuclear receptor-activating compounds (such as triazoles) in rodents as not relevant to humans (Elcombe et al. 2014). They do this by defining the mode of action of such compounds as equivalent to that of phenobarbital (PB), a model CAR activator that induces tumors in mice, but which epidemiology suggests may not induce tumors in humans. However, EPA Office of Research and Development scientists dispute this simplistic branding of rodent carcinogens that elicit some of the same hepatic toxicological responses as phenobarbital as then automatically irrelevant to humans (Nesnow et al. 2009). They showed that propiconazole and triadimefon, for instance, have gene expression profiles that differ substantially from phenobarbital's, their mechanisms of tumorigenic action are likely to differ, and hence the triazoles' induction of liver tumors in mice might well be relevant to humans.

Finally, the fact that so many triazoles induce hypertrophy, as well as steatosis, which is a risk factor for liver cancer, argues for the necessity of conducting a cumulative assessment of triazoles for liver cancer as well.

Cumulative Risk Assessment of 1,2,4-Triazole and its Conjugates

Triazole fungicides share an eponymous structural feature, 1,2,4-triazole, a five-membered aromatic ring comprising 3 nitrogen and 2 carbon atoms. 1,2,4-triazole and its conjugates (triazole-alanine and triazole acetic acid, TA and TAA, respectively) are common metabolites of these fungicides (EPA 2/7/06). Due to concerns over the toxicity of these metabolites, in the year 2000 EPA delayed granting any new triazole registrations pending more toxicology and exposure data for the metabolites (Ibid.).

To fill the data gaps, EPA issued a data call-in for studies on the developmental neurotoxicity, acute neurotoxicity, and carcinogenicity of free 1,2,4-triazole, and for a developmental toxicity study (rabbits) for both TA and TAA; a chronic rat study with neurological evaluations for TA; and a combined 90-day feeding/neurotoxicity study (rat) for TAA (Ibid., p. 6). The registrant group US Triazole Task Force (USTTF) did not respond to the 2002 call-in, and requested waivers from EPA in 2003 that EPA denied. The studies were still outstanding in 2005, when USTTF submitted renewed waiver requests (Ibid.).

To our knowledge, registrants to this day have not submitted the studies EPA demanded 15 years ago as a condition for any further registrations of triazoles (Ibid., p. 6).

Developmental Neurotoxicity (DNT) Study

The developmental neurotoxicity (DNT) study is designed to capture adverse neurological impacts of a pesticide when a fetus's or infant's developing nervous system is exposed, an exposure window when incredibly low doses can have profoundly destabilizing effects on nervous system architecture. Lifelong adverse impacts such as

reduced IQ, developmental delays and attention-deficit hyperactivity disorder have been linked to fetal/infant exposure to extremely low levels of chlorpyrifos, for instance. The DNT study was called for due to substantial evidence of 1,2,4-triazole's neurotoxicity in other animal trials, including:

- Neuropathological lesions in the brain and peripheral nervous system;
- Decreases in brain weight, including in offspring at doses that did not cause the same effect in adults in the rat reproduction study;
- Tremors, muscle fasciculations, decreased arousal, decreased rearing, decreased motor activity in rats, and excessive salivation, hyperpnea, lacrimation and head tilt in rabbits (Ibid., pp. 17, 20).

Registrants apparently decided to ignore EPA's demands, because the DNT study has still not been submitted (EPA 5/16/18, p. 22600). Neither did EPA cease registration of new uses and new triazoles until it had received this study, as it had demanded in 2006 (EPA 2/7/06, p. 6).

Chronic toxicity/carcinogenicity study

EPA had also required a chronic toxicity/oncogenicity study on 1,2,4-triazole in male rats and female mice to determine whether this metabolite was the common cause of liver tumors found with so many triazoles (Ibid., p. 6). We find no record this study has been submitted either.

Developmental toxicity study in rabbits

EPA demanded this study to fulfill "a particularly important data gap" for both TA and TAA because there were no rabbit tests with either of these compounds, the rabbit was the most sensitive species to 1,2,4-triazole, and because of the gravity of the adverse impact (mortality) ensuing from just a single dose of 1,2,4-triazole (45 mg/kg) in rabbits (Ibid., p. 47). We see no evidence these studies on TA or TAA have been submitted.

EPA applied arbitrary safety factors in an attempt to compensate for the missing studies, but has no way of knowing whether they are adequate. In any case, these safety factors are intended only as a temporary stopgap until the relevant studies are submitted, permitting a data-based assessment. Here, the relevant studies have been outstanding for at least 15 years, a period during which EPA has issued numerous registrations for new uses of triazoles.

Agricultural Triazole Use Likely Breeds Resistance to Triazole Antifungal Drugs in Human Pathogens

Fungal diseases are spiraling worldwide, with the global mortality rate from fungal infections now exceeding that from malaria or breast cancer, and rivalling deaths from tuberculosis and HIV (Fisher et al. 2018). There are nine times more antifungal compounds for crop disease than for animal infections, and just four classes of antifungals licensed for human

use (Ibid.). Triazoles are the dominant compounds used to treat crops, animals and humans; are the only class used in both medicine and agriculture (ibid.).

Drivers of resistance in plant and human pathogens share some similarities. In modern industrial agriculture, breeding has long been primarily concerned with increasing yield, and conducted with use of pesticides to eliminate pest and disease pressure. These factors lead to loss of disease resistance, and increasing dependence on fungicides accompanied by accelerating resistance. Ever more people are at risk of fungal infection due to age, medical interventions or HIV infections. Immune suppression with chemotherapy or organ transplantation increases susceptibility to opportunistic fungi, leading to greater use of antifungal drugs and pathogens resistant to them. Global movement of people and goods promotes rapid spread of fungal pathogens of crops and people (Ibid.).

Candida auris was first described in 2009 in Japan, and has spread worldwide primarily as a nosocomial pathogen resistant to all clinical antifungal medications (Ibid., Richtel and Jacobs 2019), one of several fungal pathogens on the rise (Fisher et al 2018).

Invasive aspergillosis is a serious and frequently fatal lung disease that mainly affects people who are immunocompromised: for instance, those recovering from tuberculosis, with pulmonary disease, or in conjunction with organ transplantation (for this discussion generally, see Toda et al. 2021 unless otherwise cited). It also afflicts millions of asthmatics worldwide, greatly exacerbating their disease, with conditions known as allergic bronchopulmonary aspergillosis and severe asthma with sensitization (Bowyer and Denning 2014).

The major pathogen of this disease is *Aspergillus fumigatus*, which is commonly found in the environment (e.g. decaying plant matter), has unusually high tolerance to heat and so propagates quite well in the human body, and is not known to cause plant disease. The major medications (and only ones available in oral form) used to treat this disease are triazole antifungal medicines such as itraconazole, voriconazole and posoconazole.

Over the past several decades, there has been an extremely concerning rise in invasive aspergillosis caused by *A. fumigatus* that is resistant to triazole antifungals; in such virtually untreatable infections, the mortality rate rises to 42-88%.

Resistant *A. fumigatus* has been reported in patients with aspergilloma undergoing long-term therapy with triazoles antifungals. In this disease, a fungal mass grows in a lung cavity, where it can reproduce. These resistant strains induced by medical antifungal use are characterized by a great diversity of resistance mechanisms (Snelders et al. 2012). However, there is a large and growing body of scientific literature demonstrating that agricultural use of triazole fungicides is another source of this growing resistance problem.

First, resistant strains of *A. fumigatus* have been isolated from triazole-naïve patients around the world, infections that cannot be due to treatment of these individuals with the antifungals. In addition, a disproportionate number of resistant strains isolated from patients in the Netherlands, an early site for emergence of this problem, have a particular resistance mechanism – a tandem repeat of 34 base pairs in the *cyp51* promoter region and a leucine to histidine substitution at codon 98 in the coding region (TR_{34}/L98H) – that is also commonly found in the environment. This TR34/L98H strain was first cultured from a patient in the

Netherlands in 1998, following close on the heels of a ramping up of agricultural triazole use there and in Europe generally from 1990-1996 (Snelders et al. 2012).

Moreover, the first medical antifungal (itraconazole) was only licensed in 1997 (Zhang J et al. 2017), very little time for it to have driven selection of the resistant strain noted above, even assuming the first TR34/L98H strain ***discovered*** in a patient were the first such to ***emerge,*** which appears unlikely. Additional reasons to doubt that medical use is responsible for all or even most resistance are, first, the miniscule amounts used to treat human disease relative to agricultural use; and the fact that itraconazole is excreted from the body in non-active form, making selection for resistance in sewage or receiving waters unlikely (Bowyer and Denning 2014).

The agricultural triazoles that most resemble their medical counterparts – both structurally and in terms of their docking at the CYP51 binding site – are difenoconazole, bromuconazole, epoxiconazole, propiconazole and tebuconazole. In susceptibility testing, these five triazoles (as well as metconazole and imazalil) showed the greatest dissimilarity in activity on wild-type versus resistant L98H isolates, as measured by minimum inhibitory concentration (Snelders et al. 2012). Moreover, these same five triazoles selected for *A. fumigatus* strains with cross-resistance to the medical antifungals – particularly itraconazole – after seven weeks of exposure. Interestingly, "difenoconazole imposes the strongest induction of cross-resistance to medical triazoles..." (Zhang J et al. 2017).

Resistance could arise in any environment where triazole fungicides are used and decaying plant matter provides habitat for *A. fumigatus.* Several studies have assessed stockpiles of plant waste for *A. fumigatus* populations and for presence of agricultural triazoles and their breakdown products. Schoustra et al. (2019) examined stockpiles of dead flower bulbs, green materials, and wood chips, finding substantial populations of *A. fumigatus* in each, ranging from roughly 10^3 to 10^5 colony-forming units (CFUs)/gram. Triazoles and their degradation products were found in most (78%) of 41 samples, at concentrations ranging from 0.001 to 6.4 ppm. Another study by the same team similarly found on average 10^5 CFUs/gram plant waste inn 114 samples, and estimated a plant waste stockpile just 50 x 50 x 10 meters would contain 2.5 quadrillion (10^{15}) spores. Roughly half of the isolates were triazole-resistant, with 90% resistant to both itraconazole (medical) and tebuconazole (agricultural). They also found a variety of resistance mechanisms (Zhang J et al. 2021).

A. fumigatus is a common component of bioaerosols, and it is estimated that an average person inhales 200 spores (conidia) each day (Dagenais and Keller 2009). Inhalation of *A. fumigatus* spores in the air is thought to be the major route of infection. Aerial dispersal of A. fumigatus from compost piles has been demonstrated, with a surge in release when the piles are turned, and substantial quantities then found in the downwind air (Millner et al. 1977, 1980).

EPA must assess the public health threats posed by continued and expanding use of difenoconazole and other agricultural triazoles in terms of increasing resistance of human fungal pathogens such as *A. fumigatus* and *C. auris* to medical antifungal compounds.

Environmental Concerns and Assessment Deficiencies

The rapidly rising use of difenoconazole is having unacceptable environmental impacts, including but not limited to threatened and endangered species. A key aspect of this fungicide's threat is its extreme persistence in the environment, which as described below EPA has not sufficiently accounted for in its environmental assessments to date. Unless otherwise noted, the following discussion is based on EPA (9/16/20).

Difenoconazole's Environmental Persistence

Difenoconazole is extremely persistent in multiple laboratory and field tests, in soil and water. It is stable to abiotic hydrolysis at pH values of 5, 7 or 9; it has a half-life of 228 days in an aqueous photolysis test; and half-lives ranging from 349-823 days in soil photolysis tests. Aerobic soil metabolism half-lives range from over 100 days to over 500 days, depending on soil type, while the anaerobic soil metabolism half-life is nearly three years (947 days). Aerobic aquatic metabolism half-lives are 300-565 days; anaerobic 433 days (EPA 9/16/20, p. 26). Terrestrial field dissipation data also show considerable persistence, with most half-lives in various soil types, bare plot vs. vegetative cover, ranging from over 100 to 535 days (Ibid, p. 28).

It is no wonder that EPA scientists warn repeatedly of the potential for difenoconazole to accumulate in soils and water with repeat applications:

> "The overall stability/persistence profile for difenoconazole suggests that it has potential to accumulate in soil and aquatic environments with each successive application" (Ibid., pp. 25-26).

Critically, EPA's exposure and risk assessments do not appear to account for the accumulation of difenoconazole over a single season or over years. This is a huge data gap that in itself invalidates EPA's latest risk assessments and argues strongly against the proposed interim registration decision, and certainly against approval of ANY new uses – particularly on a crop so widely grown as corn, as Syngenta recently proposed.

Risks to Terrestrial Organisms

Risk quotients exceed levels of concern for a host of different taxa. This is true even though For instance, chronic risks to mammals reach risk quotients up to 5.2 from consumption of grass and other forage with difenoconazole residues. Similarly, birds are chronically threatened by both foliar applications (risk quotients up to 10.99) and via consumption of treated seeds (risk quotients up to 16.13). These risks are exacerbated by difenoconazole's persistence. EPA has identified chronic risk LOC exceedances for birds for ***up to 150 days after application in some scenarios***, and after 56 days for mammals. Risks in some scenarios persist even when EPA utilizes mean rather than maximum Kenaga difenoconazole residues in the

assessment.[23] Moreover, spray drift of difenoconazole also poses chronic risks to birds up to 112 feet from the application site, when applied aerially to turf at just 0.125 lb/acre (PID at 15).

Honeybees are also likely threatened by difenoconazole, particularly its formulations, which are more toxic than the active ingredient alone. Acute and chronic risk quotients for larval bees, 0.99 and 1.35, exceed the respective levels of concern, 0.4 and 1.0, for one difenoconazole formulation that was tested. Additionally, difenoconazole's extreme persistence and potential for build-up in soil over a single season or years pose potential risks to ground-dwelling bees and a host of other soil-borne invertebrates that are not well-represented by the honeybee. This points up the importance of EPA expanding its required testing to include effects on soil organisms.

Risks to Aquatic Organisms

Difenoconazole also threatens aquatic organisms. EPA scientists identified risk quotients up to 22 for chronic risks to aquatic vertebrates, and noted that: "Overall, chronic LOC exceedances included crops that have some of the highest poundage of difenoconazole applied annually. ***Due to the persistence of difenoconazole, repeated use can considerably increase these risks over time."*** (EPA 9/16/20, pp. 6-7, 9, emphasis added).

Nearly all usage scenarios posed chronic risks to fish, but even so, risks are still greater than represented by these risk quotients due to difenoconazole's persistence. EPA scientists stated this clearly: "Due to the persistence of difenoconazole in terrestrial and aquatic environments, repeated use can ***considerably increase these chronic risks*** over time." (emphasis added).

Fish and other aquatic organisms are also threatened by bioaccumulation of difenoconazole and its metabolites. EPA documented a 330x bioconcentration factor for whole fish. Over half of the applied dose was detected in the fish in the form of the metabolite, CGA-205375, for which EPA has next to no toxicity data (setting aside unreliable ECOSAR structure-activity guesstimates).

Aquatic invertebrates are likewise at risk, with chronic risk quotients up to 6.3, far exceeding the LOC of 1. As with fish, EPA's risk assessment methodologies do not appear to encompass risks arising from accumulating levels of difenoconazole over years:

> "Crops for which chronic risks to aquatic invertebrates were identified included rice, ornamentals, soybeans, sugar beets, tree nuts, small vine fruits (e.g., grapes), potatoes, cabbage, tomato, apples and cucurbits. These crops encompass some of the highest difenoconazole use rates in terms of lbs a.i. applied annually (Section 3-2). ***Like fish, due to the persistence of difenoconazole, repeated use can considerably increase these risks over time***." (EPA 9/16/20, p. 55, emphasis added).

[23] Kenaga residues refers to a nomogram developed by EPA scientists in the early 1970s that purports to predict residue levels on plants after application of any pesticide, based only on the application rate of the pesticide and the type of plant (e.g. long grass, leafy crops) or plant part (fruit, pods containing seeds).

In short, difenoconazole poses seriously ecological risks to several taxa, risks exacerbated by the fungicide's extreme persistence in the environment. Even without accounting for buildup of difenoconazole in the environment over years, EPA's assessments find substantial exceedances of risk quotients. Until EPA conducts assessments that account for accumulation of this fungicide in the environment over years, it cannot gauge the true risks to wildlife or finalize this interim registration review decision. EPA must also abstain from approving any new uses of difenoconazole – particularly one with potential for such an extreme increase in usage as corn – prior to correcting the deficiencies in its ecological risk assessment and completing its registration review.

Costs and Benefits

Putative benefits

Roughly half of non-seed treatment use of difenoconazole is on soybean. Syngenta is seeking registration for use on corn as well. The following discussion will focus on these field crops.

While agronomists are disturbed by the dramatically increasing use of fungicides of all sorts, the concern is especially acute for use on field crops like corn and soybeans, which began around 2007 (see Hershman et al. 2011 and Wise and Mueller 2011 for the following discussion). These agronomists note that foliar fungicide applications were extremely rare on corn and soybeans until this time; to the small extent fungicides were used, it was for seed production or specialty corn varieties, where higher prices justified the expenditures.

Agronomists attribute the rise in fungicide use on corn and soybeans largely to marketing drives by fungicide manufacturers, who have had success selling farmers on fungicides for dubious "plant health" reasons rather than disease; to higher corn prices beginning in 2007; and to growers' prioritization of yield potential over disease-resistance in selection of corn hybrids. There is also a troubling "insurance treatment" approach to fungicide spraying that goes fundamentally against IPM principles to use a pesticide only when needed, and only when the expenditure delivers more benefit in yield than the cost of the pesticide.

There are already a number of other fungicides, including DMI/triazole fungicides, already approved for use on soybeans and corn. To the very limited circumstances in which their use might be justified, there are already sufficient control options available to growers, and no need for still another foliar fungicide for corn.

Costs

Resistance to triazole/DMI fungicides has been building steadily over years, and together with widespread resistance to strobilurin and other fungicides is a serious problem.

> "For decades, scientists have watched as fungi all over the world have become incrementally more and more resistant to DMI fungicides. The use of any

fungicide for 'plant health' reasons increases the risk of developing resistance" (Hershman et al. 2011).

Clearly, superfluous use of fungicides like difenoconazole – as for "plant health" reasons – must be avoided at all costs to stem or at least slow resistance development. In this respect, too, one must recall how difenoconazole and other triazoles are also likely fostering increased resistance to critical antifungal triazole medications and the associated costs in terms of human health and deaths (discussed above).

Difenoconazole's use on soybeans has risen dramatically since 2012 (essentially zero) to 2017 (about 200,000 lbs./year). This soybean use represents about half of (non-seed treatment) uses of difenoconazole, even though only roughly 2% of soybean acres are, at present, being sprayed. This usage will likely continue to rise, usually for no good reason.

The expansion of difenoconazole to corn would exacerbate an unhealthy trend of excessive and largely unnecessary triazole use in corn and soybeans (Toda et al. 2021, Toda et al. 2021 Supplemental). Critically, it would expand those acres that are sprayed with a triazole every year in corn-soybean rotations, intensifying selection pressure for resistant plant and human fungal pathogens across the Corn Belt, where just 15-20 years ago hardly anyone saw any need to spray fungicides on these crops. Cross-resistance among triazole herbicides is common. For instance, even the fungicide manufacturers' group Fungicide Resistance Action Committee has stated: "Generally wise to accept that cross resistance is present between DMI fungicides active against the same fungus." (FRAC 2021, p. 11).

Potential Mitigations

EPA's proposed mitigations consist largely in toothless advisory statements on spray drift, environmental hazard and surface water contamination that the Agency itself admits have no impact on the risks of concern. The other proposed label mitigations are entirely inadequate to the task of reducing any of the risks difenoconazole poses to humans and non-human organisms, or the risks of resistance in agricultural or human pathogens.

Clearly, the many costs of renewing current uses of difenoconazole with this registration review decision far outweigh any putative and highly dubious benefits. This holds in particular for use on soybeans and the proposed use on corn, which should be rejected.

Threatened and Endangered Species

EPA has not completed an assessment of difenoconazole for its impact on threatened and endangered species. EPA must comply with its duties under Section 7 of the Endangered Species Act (ESA) prior to finalizing its interim registration decision, as it is a separate, discretionary action that may affect species listed as threatened or endangered under the ESA. Because there are many acknowledged risks of concern of difenoconazole to a range of taxa, and imperiled species listed under the ESA are highly susceptive to additional threats, it is clear that listed species will continue to be put at risk with a registration review decision as EPA has proposed, and at still greater risk from registration of foliar use on corn.

Difenoconazole may affect numerous threatened and endangered species across the country including, but not limited to, the species listed below.

Fish	
Neosho madtom	*Noturus placidus*
Pallid sturgeon	*Scaphirhynchus albus*
Topeka shiner	*Notropis topeka*
Terrestial Invertebrates	
Rusty patched bumblebee	*Bombus affinis*
Mitchell's Satyr Butterfly	*Neonympha mitchellii mitchellii*
Poweshiek skipperling	*Oarisma poweshiek*
Monarch butterfly (candidate)	*Danaus plexippus plexippus*
Aquatic Invertebrates	
Rabbistfoot	*Quadrula cylindrica cylindrica*
Birds	
Least tern	*Sternula antillarum*
Whooping crane	*Grus americana*
Piping plover	*Charadrius melodus*

EPA must complete endangered species consultation to ensure the registration does not jeopardize the existence of species protected as threatened or endangered under the ESA prior to finalizing its registration decision. Without having fulfilled this duty under the ESA, in consultation with the expert wildlife agencies, EPA cannot ensure no jeopardy for protected species. EPA claims its proposed label changes "are expected to reduce the extent of exposure and may reduce risk to listed species whose range and/or critical habitat co-occur with the use of difenoconazole" even though EPA "is not making a complete endangered species finding at this time."[24] However, without a full analysis and ESA consultation EPA cannot determine the full impacts of difenoconazole on ESA-listed species and their critical habitats and ensure that it will not jeopardize any of those species. What EPA is doing here is clearly not sufficient to comply with the ESA.

Conclusion

Clearly, EPA has failed to properly assess the human health and environmental risks posed by difenoconazole in its proposed interim decision, and must revisit its assessment prior to any final decision. CFS urges EPA to reject the proposed 5-fold increase in the chronic reference dose, conduct a guideline-compliant assessment of dermal absorption with

[24] Proposed Interim Registration Decision at 23

difenoconazole formulations, and obtain toxicological data on the major metabolites, especially CG-205375. EPA must correct its ecological exposure and risk assessments by taking full account of difenoconazole's extreme persistence and likely accumulation over time. Furthermore, EPA should conduct a cumulative risk assessment of triazole fungicides because of their structural similarity, their common primary target, the liver, and common effects and adverse outcome pathways for major impacts such as hepatic steatosis and carcinogenicity. EPA must also obtain the long-missing data needed to complete its cumulative assessment of the triazole metabolites, 1,2,4-triazole and its major conjugates, particularly the developmental neurotoxicity study on 1,2,4-triazole.

EPA must also assess the role difenoconazole and other triazole fungicides used in agriculture have played in selecting for human fungal pathogens that are resistant to medical azole antifungal drugs. Pathogens exhibiting increasing resistance include *Aspergillus fumigatus* and *Candida auris*. Resistant strains of each have become a huge, global public health threat, as the few antifungal drugs that can treat diseases such as invasive aspergillosis become ineffective.

EPA must also assess the threats posed by difenoconazole to threatened and endangered species, beginning with consultation with the expert agencies.

Sincerely,

Bill Freese, Science Director
Center for Food Safety

References

Al-Eryani L. et al. (2015). Identification of environmental chemicals associated with the development of toxicant-associated fatty liver disease in rodents. Toxicologic Pathology 43: 482-497.

Altekruse SF et al. (2009). Hepatocellular carcinoma incidence, mortality, and survival trends in the United States From 1975 to 2005. Journal of Clinical Oncology 27(9): 1485-91.

Angrish MM et al. (2016). Tipping the balance: Hepatotoxicity and the four apical key events of hepatic steatosis. Toxicol. Sci. 150(2): 261-268.

Bowyer P and Denning DW (2014). Environmental fungicides and triazole resistance in *Aspergillus*. Pest Management Science 70(2): 173-178.

Dagenais TRT and Keller NP (2009). Pathogenesis of *Aspergillus fumigatus* in Invasive Aspergillosis. Clinical Microbiology Reviews 22(3): 447-64.

EFSA (2011). Conclusion on the peer review of the pesticide risk assessment of the active substance difenoconazole. European Food Safety Authority, EFSA Journal 9(1): 1967.

EFSA (2009). Scientific Opinion on Risk Assessment for a Selected Group of Pesticides from the Triazole Group to Test Possible Methodologies to Assess Cumulative Effects from Exposure through Food from these Pesticides on Human Health. European Food Safety Authority, EFSA Journal 7 (9); 1167.

Elcombe CR et al. (2014). Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit. Rev. Toxicol. 44(1): 64-82.

EPA (6/28/21). Oxyfluorfen: Proposed Interim Registration Review Decision, Case Number 2490. EPA, June 28, 2021.

EPA (9/18/20). Difenoconazole. Draft Human Health Risk Assessment for Registration Review. EPA, EPA-HQ-OPP-2015-0401-0021, September 18, 2020.

EPA (9/16/20). Difenoconazole: Draft Ecological Risk Assessment for Registration Review. EPA, September 16, 2020. EPA-HQ-OPP-2015-0401-0019.

EPA (5/16/18). Tebuconazole: Pesticide Tolerances. 83 Fed. Reg. 22595-601 at 22600 (May 16, 2018).

EPA (4/12/16). Pesticide Cumulative Risk Assessment: Framework for Screening Analysis Purpose, EPA, April 12, 2016. EPA-HQ-OPP-2015-0422-0019.

EPA (12/2/15). BEAD Chemical Profile (BCD) for Registration Review: Difenoconazole (128847). EPA, December 2, 2015. EPA-HQ-OPP-2015-0401-0002.

EPA (2/24/15). **Difenoconazole**: Human Health Risk Assessment for proposed new foliar uses on legume subgroup 6C and bushberry subgroup 13-07B; post-harvest uses on pome fruit group 11-10; and ornamental plants and vegetable transplants grown in both indoor and outdoor production facilities. EPA, February 24, 2015.

EPA (10/2/14). Difenoconazole: Screening Level Usage Analysis, EPA, October 2, 2014. EPA-HQ-OPP-2015-0401-0006.

EPA (10/29/13). Glufosinate ammonium: Response to comments on the human health risk assessment for registration review. EPA, October 29, 2013. EPA-HQ-OPP-2008-0190-0043.

EPA (6/2/10). Review of triple pack dermal absorption studies for Maxim Quattro. EPA, June 2, 2010.

EPA (2/7/06). 1,2,4-Triazole,TriazoleAlanine,TriazoleAceticAcid:HumanHealthAggregate Risk Assessment in Support of Reregistration and Registration Actions for Triazole-derivative Fungicide Compounds. EPA, February 7, 2006.

EPA (1/14/02). Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity, EPA, January 14, 2002.

EPA (1/29/99). Guidance for Identifying Pesticide Chemicals and Other Substances That Have a Common Mechanism of Toxicity, EPA, January 29, 1999.

EPA (1998). Health Effects Test Guidelines. OPPTS 870.7600: Dermal Penetration. EPA 712-C-98-350, August 1998.

EPA (7/27/94). Carcinogenicity Peer Review of Difenoconazole. EPA, July 27, 1994.

EPA (2/24/94). RfD/Peer Review Report of Difenoconazole. EPA, February 24, 1994.

Fisher MC et al. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science 360: 739-42 (May 18, 29018).

FRAC (2021). FRAC Code List 2021:Fungal control agents sorted by cross resistance pattern and mode of action (including coding for FRAC Groups on product labels). Fungicide Resistance Action Committee, 2021.

Heise T et al. (2017). Hepatotoxic combination effects of three azole fungicides in a broad dose range. Arch Toxicol 92(2): 859-872.

Heise T, Schmidt F, Knebel C et al (2015). Hepatotoxic effects of (tri)azole fungicides in a broad dose range. Arch Toxicol 89(11): 2105–2117. https://doi.org/10.1007/s00204-014-1336-1.

Hershman DE, Vincelli P and Kaiser CA (2011). Foliar Fungicide Use in Corn and Soybeans. University of Kentucky College of Agriculture, Plant Pathology Fact Sheet, PPFS-GEN-12, October 2011.

Knebel C et al (2019). Pregnane X receptor mediates steatotic effects of propiconazole and tebuconazole in human liver cell lines. Archives of Toxicology 93(5): 1311-22.

Kwon H-C et al (2021). Effects of Flutriafol Fungicide on the Lipid Accumulation in Human Liver Cells and Rat Liver. Foods 10: 1346.

Lasch A et al (2021). More than additive effects on liver triglyceride accumulation by combinations of steatotic and non-steatotic pesticides in HepaRG cells. Archives of Toxicology 95: 1397-1411.

Marx-Stoelting P et al (2020). The Connection of Azole Fungicides with Xeno-Sensing Nuclear Receptors, Drug Metabolism and Hepatotoxicity. Cells 9: 1192; doi:10.3390/cells9051192.

Millner PD et al. (1980). Dispersal of *Aspergillus fumigatus* from sewage sludge compost piles subjected to mechanical agitation in open air. Applied and Environmental Microbiology 39(5): 1000-09.

Millner PD et al. (1977). Occurrence of *Aspergillus fumigatus* during composting of sewage sludge. Applied and Environmental Microbiology 34(6): 765-772.

Nesnow et al. (2009). Discrimination of Tumorigenic Triazole Conazoles from Phenobarbital by Transcriptional Analyses of Mouse Liver Gene Expression. Toxicological Sciences 110(1): 68-83.

Nielsen E. et al. (2012). Identification of Cumulative Assessment Groups of Pesticides, External Scientific Report Submitted to EFSA [European Food Safety Authority]. National Food Institute, Technical University of Denmark, 2012

Richtel M ad Jacobs A (2019). A Mysterious Infection, Spanning the Globe in a Climate of Secrecy: The rise of Candida auris embodies a serious and growing public health threat: drug-resistant germs. The New York Times, April 6, 2019.

Schoustra SE et al. (2019). Environmental Hotspots for Azole Resistance Selection of *Aspergillus fumigatus*, the Netherlands. Emerging Infectious Diseases 25(7): 1347-53.

Snelders E et al (2012). Triazole Fungicides Can Induce Cross-Resistance to Medical Triazoles in *Aspergillus fumigatus*. PLoS One 7(3): e31801.

Toda M. et al. (2021). Trends in Agricultural Triazole Fungicide Use in the United States, 1992–2016 and Possible Implications for Antifungal-Resistant Fungi in Human Disease. Environmental Health Perspectives 129(5), May 2021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8098123/pdf/ehp7484.pdf.

Toda et al. (2021), Supplemental. Supplemental materials for paper cited above.

Wahlang B et al. (2013). Toxicant-Associated Steatohepatitis. Toxicol. Pathol. 41(2): 343-360.

Wise K and Mueller D (2011). Are Fungicides No Longer Just For Fungi? An Analysis of Foliar Fungicide Use in Corn. American Phytopathological Society, APSnet Features. doi:10.1094/APSnetFeature-2011-0531.

Zhang H et al. (2021). Insights into the effects of difenoconazole on the livers in male mice at the biochemical and transcriptomic levels, Journal of Hazardous Materials 422: 126933.

Zhang J et al. (2021). Dynamics of Aspergillus fumigatus in Azole Fungicide- Containing Plant Waste in the Netherlands (2016–2017). Applied and Environmental Microbiology 87(2): e02295-20.

Zhang J et al. (2017). Evolution of cross-resistance to medical triazoles in Aspergillus fumigatus through selection pressure of environmental fungicides. Proc Biol Sci. 284(1863): 20170635.

Case No. 22-70118

UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

CENTER FOR FOOD SAFETY,
Petitioner,

v.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, *et al.*,
Respondents,

and

SYNGENTA CROP PROTECTION, LLC,
Respondent-Intervenor.

On Petition for Review of an Order of the
United States Environmental Protection Agency

VOLUME 2 OF 2 – ATTACHMENTS 5-9

CENTER FOR FOOD SAFETY
Amy van Saun
2009 NE Alberta St., Suite 207
Portland, OR 97211
T: (971) 271-7372
avansaun@centerforfoodsafety.org

Sylvia Shih-Yau Wu
Meredith Stevenson
303 Sacramento Street, 2F
San Francisco, CA 94111
(415) 826-2770
swu@centerforfoodsafety.org
mstevenson@centerforfoodsafety.org

Counsel for Petitioners

TABLE OF CONTENTS

VOLUME 1 – ATTACHMENTS 1-4	
Attach. No.	Title
1.	Interim Registration Review Decision; Case Number 7014 (March 2022), EPA-HQ-OPP-2015-0401 (IRRD)
2.	Difenoconazole: Draft Ecological Risk Assessment for Registration Review (Sept. 16, 2020) (Ecological Risk Assessment)
3.	Human Health Aggregate Risk Assessment in Support of Reregistration and Registration Actions for Triazole-derivative Fungicide Compounds (Feb. 7, 2006) (2006 Memo)
4.	Center for Food Safety; Comments on proposed interim registration decision for difenoconazole (Oct. 4, 2021) (CFS Comment)
VOLUME 2 – ATTACHMENTS 5-9	
Attach. No.	Title
5.	Declaration of Jennifer Loda
6.	Declaration of Alice Cimino Naegele
7.	Declaration of Carrie Santulli Schudda
8.	Declaration of Sylvia Shih-Yau Wu
9.	Declaration of Kristina Sinclair (with Exhibits A-I)

Attachment 5

UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

CENTER FOR FOOD SAFETY, *Petitioner*, v. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, *et al.*, *Respondents*, and SYNGENTA CROP PROTECTION, LLC, *Respondent-Intervenor.*	**Case No. 22-70118** **DECLARATION OF JENNIFER LODA**

DECLARATION OF JENNIFER LODA IN SUPPORT OF MOTION FOR SUMMARY VACATUR

I, Jennifer Loda, declare that if called as a witness in this action I would competently testify of my own personal knowledge as follows:

1. I am a member of Petitioner Center for Food Safety (CFS). I have been a member of CFS for more than five years and have been employee of the CFS staff since June 1, 2022. In my current role I work

to expand CFS's efforts to fight against the extinction crisis and protect imperiled species from the harmful impacts of industrial agriculture. As a member, I rely on CFS to represent my interest in protecting biodiversity, including sensitive species and their habitats, from adverse impacts of industrial agriculture and pesticide use through litigation, public education, and other means.

2. I am a resident of Oakland, California which is in Alameda County. The state of California is major agricultural producer, growing a significant portion of the vegetables, fruits, and nuts in the United States.

3. I have personal and professional interests in wildlife and conservation.

4. I earned a Bachelor of Science degree in Biology with a concentration in Ecology, Behavior, and Evolution from the University of California, San Diego in 1999. During my undergraduate studies I took courses in biology, chemistry, ecology, and conservation biology, and I studied abroad in Costa Rica where I studied tropical ecology and agroecology and conducted an independent research project on the

effects of different bean cropping systems on soil nematode communities. I also conducted research with a professor on an endangered primate species at the San Diego Zoo and Wild Animal Park.

5. After receiving my bachelor's degree, I worked for about five years primarily conducting surveys and research on plants and wildlife and restoring native habitats. I also worked as an agricultural apprentice on an organic community supported agriculture farm for one season.

6. I started graduate school at Iowa State University in 2004 and earned a Master of Science Degree in Wildlife Biology in 2007, where most of my studies focused on the interactions of agricultural systems and wildlife. My master's thesis research focused on looking at the impacts of water quality, surrounding land use, and fish on wetland amphibians, primarily on or near agricultural lands. The water quality factors I looked at included a wide range of pesticides, nutrients, and heavy metals. In addition to the analysis of my field and lab research,

my thesis included an extensive review of the literature on the impacts of pesticides on amphibians.

7. After completing my master's degree, I worked at University of California, Davis assisting in the development of a research project to assess habitat suitability criteria for reproducing foothill yellow-legged frogs (*Rana boylii*) in streams and rivers in the Sierra Nevada foothills. At the time, the foothill yellow-legged frog was a species of special concern in California, but it is now protected under the California Endangered Species Act in most of its range and it has been proposed for listing under the federal Endangered Species Act (ESA). During this job I surveyed for and observed adult and juvenile foothill yellow-legged frogs and their habitats in the Sierra foothills.

8. I also worked as a biologist for various environmental consulting firms in 2008, 2009, and 2013, during which I conducted surveys for sensitive resources, such as nesting birds, wetlands and endangered species, before and during construction projects across the San Francisco Bay area and elsewhere in northern California. The ESA-listed species I monitored for included: western snowy plover

(*Charadrius alexandrinus nivosus*), giant garter snake (*Thamnophis gigas*), California tiger salamander (*Ambystoma californiense*), and California red-legged frog (*Rana draytonii*).

9. In 2012, I earned a law degree from Lewis and Clark Law School with a specialization in Environmental and Natural Resources Law.

10. From 2015 to 2020 I worked as an Amphibian and Reptile Attorney at the Center for Biological Diversity, in which I utilized my scientific and legal background to advocate for and litigate on behalf of rare amphibians and reptiles, primarily in the western United States. This work included providing comments, drafting petitions, and supporting and participating in appeals and litigation challenging projects that would be harmful for amphibians and reptiles, including the California red-legged frog and the foothill yellow-legged frog. I also advocated to the California Fish and Game Commission for protections of the foothill yellow-legged frog under the California Endangered Species Act.

11. In 2018 I served as an instructor in a four-day workshop on foothill yellow-legged frog ecology, management, and regulation, hosted by the Western Section of the Wildlife Society. In addition to contributing to the lectures and Q&A sessions in the workshop, I also assisted as an instructor for the field day portion of the workshop where we looked for foothill yellow-legged frogs in the Trinity River. During this field day we saw numerous foothill yellow-legged frog tadpoles, subadults, and adults and trained attendees in proper handling of the frogs.

12. Here is a photo I took of a foothill yellow-legged frog held by a fellow instructor during the 2018 workshop. Frog handling was done under a scientific collecting permit obtained for the workshop.

13. Here is another photo I took at the 2018 workshop, of foothill yellow-legged frog larvae in the Trinity River.

14. Because I personally enjoy searching for and observing wildlife, especially amphibians, I have also looked for and seen foothill-yellow legged frogs and California red-legged frogs in my personal time outside of my professional duties. I have looked for foothill-yellow legged frogs multiple times over the years in the streams of Sunol Regional Wilderness in Sunol, California and observed multiple egg

masses, as well as an adult, there in 2016. I also hiked to Little Carson Falls in Marin County, California in 2015, specifically to look for foothill yellow-legged frogs, with fellow members of the organization Save the Frogs. There I saw one adult male foothill yellow-legged frog and at least one egg mass. I had planned to go back to both areas this past spring (during the ideal time to spot them) but was unable to because of a sprained ankle. I plan to return to both areas in spring 2023 to look for foothill yellow-legged frogs.

15. Here is a photo of a foothill yellow-legged frog egg mass that I observed in Sunol Regional Wilderness in 2016.

16. I have seen California red-legged frogs multiple times adjacent to Sharp Park Golf Course and Mori Point (part of Golden Gate National Recreation Area) in Pacifica, California, most recently this past April 2022. I also saw California red-legged frogs on a field trip with the San Francisco Bay chapter of the Wildlife Society in 2016 in another part of Pacifica, California. I have also looked for California red-legged frogs while hiking near wetlands in other parts of the bay area. I plan to continue looking for this species, including on a return trip to Mori Point within the next year.

17. Here is a photo of the California red-legged frog I observed in April of this year, adjacent to Mori Point and Sharp Park Golf Course.

18. In summary, I have personal and professional interests in the preservation and recovery of imperiled amphibians and other wildlife, as I derive benefits from visiting their habitats, searching for and observing them in the wild, and working to conserve these species.

19. I am concerned that the United States Environmental Protection Agency (EPA) approval of the interim registration review decision for the fungicide difenoconazole puts the foothill yellow-legged frog, California red-legged frog, and other amphibians and wildlife at risk. I am especially concerned about this decision because I am aware that the EPA approved difenoconazole without first consulting on its decision with the expert wildlife agencies—the U.S. Fish and Wildlife Service and the National Marine Fisheries Service—as required by the federal Endangered Species Act (ESA). By failing to consult, EPA failed to ensure that difenoconazole use authorized by its decision will not jeopardize any ESA-listed species or destroy or adversely modify any of their critical habitats. Consultation with the expert wildlife agencies could have resulted in restrictions or more protective measures on use

of difenoconazole to ensure threatened and endangered species can be preserved and have a chance at recovery.

20. I am aware that EPA uses fish as surrogates for amphibians in its ecological risk assessments and that EPA determined that difenoconazole is harmful to fish, and therefore is also considered to be harmful to amphibians. I am also aware that amphibians are particularly vulnerable to pesticides because of their permeable skin and potential for exposures both in and out of water, and that the U.S Fish and Wildlife Service considers pesticides to be a threat contributing to the imperilment of the California red-legged frog and foothill yellow-legged frog. Both species have ranges that include agricultural areas. I am also aware the difenoconazole has been approved for use on turfgrass, and so I am particularly concerned about the population of California red-legged frogs that I regularly observe adjacent to the Sharp Park Golf Course. I also recently learned of a study on the effects of difenoconazole on European green frog tadpoles that found this fungicide caused an increase in oxidative stress for the

tadpoles, even at low concentrations.[1] As such, I am worried that EPA's difenoconazole decision will endanger the California red-legged frog and foothill yellow-legged frog and contribute to further declines and risk of extinction for these species, because EPA ignored its duty to ensure against jeopardy for these and other ESA-listed species.

21. It makes me happy to know that wildlife like the California red-legged frog and foothill yellow-legged frog exist, even though they are rare and so I may not always be able to observe them in the wild. I worry that the continued approval of toxic pesticides, including difenoconazole, without proper considerations of their risk to these frogs and other endangered species will contribute to pushing these species closer to extinction. The decline or loss of these species would deprive me of the benefits I currently receive from the existence of these unique animals.

---

1 https://www.researchgate.net/publication/355700567_Influence_of_Alpha_Cypermethrin_and_Difenoconazole_Pesticides_on_European_Green_Toad_Tadpoles_Bufotes_viridis_L

22. EPA's action injures my aesthetic, professional, recreational, conservation, and moral interests in the California red-legged frog, foothill yellow-legged frog, and other wildlife species. My interests continue to be harmed by EPA's failure to fulfill its obligations under the ESA through consultation with the expert wildlife agencies on the California red-legged frog, foothill yellow-legged frog, and other imperiled species listed or proposed for listing under the Endangered Species Act. Compliance with the ESA could result in protective measures aimed at reducing impacts of difenoconazole on California red-legged frog, foothill yellow-legged frog, and other ESA-listed species, which is important to ensuring my interests in these species are protected.

I declare under penalty of perjury under the laws of the United States that the foregoing is true and correct and that this declaration was executed on August 26, 2022 in Oakland, California.

Jennifer Loda

Attachment 6

UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

CENTER FOR FOOD SAFETY, *Petitioner*, v. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, *et al.*, *Respondents*, and SYNGENTA CROP PROTECTION, LLC, *Respondent-Intervenor*.	**Case No. 22-70118** **DECLARATION OF ALICE CIMINO NAEGELE**

DECLARATION OF ALICE CIMINO NAEGELE IN SUPPORT OF MOTION FOR SUMMARY VACATUR

I, Alice Cimino Naegele, declare that if called as a witness in this action I would competently testify of my own personal knowledge as follows:

1. I am a member of Center for Food Safety (CFS). As a member, I rely on CFS to represent my interest in protecting biodiversity, including sensitive and endangered species and their

habitats, from the adverse impacts of industrial agriculture and pesticide use though litigation, public education and other means.

2. I am a resident of Lake Park, Florida which is located in Palm Beach County. The state of Florida is one of the largest producers of citrus in the United States. Palm Beach County is the biggest farming county in the state according to the USDA. I teach violin at local schools in the area.

3. I live about a twenty-minute drive north of West Palm Beach and spend most weekends around, or near, Lake Okeechobee and the Atlantic Ocean. I often swim, canoe, or walk around the lake or other nearby waterways. The places I most frequent during the weekends are all impacted by pollution and runoff from agricultural and sugar cane production.

4. Crops for which difenoconazole is approved, such as potatoes, citrus, melons, sweet potatoes, tomatoes, peppers, snap beans, and rice, are all grown around Lake Okeechobee. Lake Okeechobee, and other nearby water sources have been severely impacted for years due to runoff and pollution from nearby farming, specifically from the citrus groves.

5. Lake Okeechobee is often referred to as "the liquid heart" of the Everglades. However, due to all of the pollution and runoff from agricultural use of pesticides and fungicides, the lake is severely polluted and is causing other waterways to become polluted as well.

6. Runoff from farms north of the lake drains into the Kissimmee River, which flows into Lake Okeechobee. Lake Okeechobee then feeds both the Everglades and other freshwater sources across Southern Florida. Okeechobee's discharge has impacted other nearby sources such as, Lake Worth and the Atlantic Ocean because water is released from Lake Okeechobee as a preventative measure to keep the lake from flooding nearby communities.

7. I regularly recreate outdoors. I canoe about every two weeks, and regularly swim, bike, and walk. I enjoy spending my weekends at the beach or around the freshwater bodies near where I live.

8. I am concerned about the impacts on the environment and wildlife due to the excessive spraying of fungicides and pesticides. I am particularly concerned about the impact of the chemicals and runoff on threatened and endangered species. I am especially concerned about the continued existence of Florida's marine and freshwater wildlife

including the endangered Everglade snail kite. I go out looking for the snail kite every couple of weeks and plan to continue looking for them in different areas around me.

9. I have seen the Everglade snail kite alive in many water sources around where I live, including in the Grassy Waters Everglade Preserve and while canoeing at Winding Waters and the Hungryland Slough Natural Areas. I enjoy seeing them in their natural habitat and feeding on snails.

10. Unfortunately, I am aware that agricultural pollution is threatening the snail kite's habitat. Excessive degradation of wetlands and surroundings areas also threaten the snail kite. It is devastating to see how all the problems with Lake Okeechobee and the dike are killing the snail kite and other wildlife. The snail kite population has only just begun to recover, and I don't want to see it go down again.

11. I plan to continue to go out looking for the snail kite during my regular outdoor activities in Palm Beach, such as canoeing and biking.

12. I am aware that in March 2022, EPA issued an interim registration review decision for the fungicide difenoconazole. As a result

of this decision, this new herbicide can be sold and used throughout the United States, including on citrus groves, and other farms, in Florida. I am aware that EPA found difenoconazole exposure to exceed its level of concern for bird species like the snail kite.

13. EPA's interim registration review decision for difenoconazole will likely negatively impact the snail kite because their range overlaps with and is adjacent to the citrus groves and other agricultural crops in Florida, which are approved for use of the fungicide. This harms me and my interest in this species, as any additional impact will further contribute to the decline of the snail kite and will make it less likely I will be able to see this species alive in the wild again, and more likely that I will suffer harm by seeing or hearing about the population continuing to decline to near extinction.

14. I am aware that it is possible that lakes, wetlands, and marine areas in the snail kite's range could or will have high levels of difenoconazole from runoff from citrus groves and other farms. This exposure may have adverse effects on the snail kite and makes me greatly concerned about their continued survival.

15. I do not believe that the risks of re-approving difenoconazole have been properly assessed by EPA. It concerns me that given the stresses the snail kite already must endure, continuing to allow this fungicide to be used in agricultural fields adjacent to their habitat will severely harm their recovery. I am injured by the fact that EPA's interim registration decision for difenoconazole makes it more likely that I will see less snail kites when I am out recreating in the local Nature Areas.

16. In summary, I have aesthetic, and recreational interests in the preservation of the snail kite and its habitat. These interests are harmed and continue to be harmed by EPA's failure to consider the impacts of the interim registration review decision for difenoconazole on the snail kite. EPA failed to consider how the already over-polluted Lake Okeechobee will become even more degraded by agricultural runoff. Specifically, I believe EPA's failure to follow the law makes the snail kite more likely to suffer further population declines. Additional threats to the snail kite make it more likely that I will suffer by not being able to see the snail kite when I am out canoeing and walking. I will suffer if the snail kite declines to the point of near extinction,

because this loss with deprive me of the benefits I currently enjoy from seeing the snail kite's existence in its natural habitat. EPA must consider the current degradation of the waterways and consult with the relevant state and federal agencies to ensure that protective measures are in place to reduce the impact of this fungicide on snail kites, which is important to ensure that my interest in this species are preserved and remain free from injury.

I declare under penalty of perjury that the forgoing is true and correct to the best of my ability.

Executed this 26th day of August 2022 in Lake Park, FL.

Alice Cimino Naegele

Attachment 7

UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

CENTER FOR FOOD SAFETY, *Petitioner*, v. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, *et al.*, *Respondents*, and SYNGENTA CROP PROTECTION, LLC, *Respondent-Intervenor*.	**Case No. 22-70118** **DECLARATION OF CARRIE SANTULLI SCHUDDA**

DECLARATION OF CARRIE SANTULLI SCHUDDA IN SUPPORT OF MOTION FOR SUMMARY VACATUR

I, Carrie Santulli Schudda, declare that if called as a witness in this action I would competently testify of my own personal knowledge as follows:

1. I am a member of Petitioner Center for Food Safety (CFS). As a member, I rely on CFS to represent my interest in protecting my

and my family's health, and to represent my interest in protecting biodiversity from adverse impacts of industrial agriculture and fungicide use through litigation, public education, and other means.

2. I am a resident of Oregon, Wisconsin which is located in Dane County. Dane County is the leading producer of soybeans in Wisconsin, and Wisconsin is one of the largest producers of soybeans in the United States. There are many soybean fields near my home in the suburbs and near the lakes in which my husband regularly fishes.

3. I have worked as an attorney for more than thirty years and am currently a solo general practitioner. I am now 62 years-old, my husband is 56 years-old, and we have a 24-year-old daughter who regularly stays with us and eats with us.

4. I am a lifelong birdwatcher and have seen the positive effects of pesticide bans on bird populations, especially the great blue heron and the bald eagle. I was a child the first time I saw a great blue heron in northern Wisconsin and remember remarking about how beautiful it was. Since DDT was banned, I have seen many more.

5. My husband and I are outdoorsy people, and we recreate in the areas around our home and in the northern part of the state

regularly. We birdwatch daily, as there are many trees and birds where we live. We also regularly observe sandhill cranes in the field behind our house and pay close attention to local crane populations. My husband also fishes weekly, and even ice fishes.

6. I am concerned about the effects of difenoconazole on the whooping cranes in Horicon Marsh nearby, because Horicon Marsh is located in the largely agricultural landscape of southeast Wisconsin, specifically in Dodge and Fond du Lac counties, which each produce tens of thousands of soybean acres annually.

7. I am also concerned about the effects of difenoconazole on the Hine's emerald dragonfly. I enjoy looking for dragonflies, including the Hine's emerald dragonfly, which has critical habitat just a half hour drive from my residence.

8. I am aware that in March 2022, EPA issued an interim registration review decision for the fungicide difenoconazole. As a result of this decision, this new fungicide can be sold and used throughout the United States, including on soybean fields in Wisconsin. I am aware EPA found difenoconazole can be toxic to birds like the whooping crane, and to humans, especially infants.

9. I am concerned that EPA's interim registration review decision for difenoconazole will likely negatively impact the whooping crane, the Hine's emerald dragonfly, and the bald eagle, because their ranges overlap with, and are adjacent to, soybean fields in Wisconsin and other parts of the Midwest, which are approved for use of the fungicide. This harms me and my interest in these species, as any additional impact will further contribute to their decline and will make it less likely I will be able to see these species alive in the wild again, and more likely that I will suffer harm by observing their declining populations.

10. I am aware that it is possible that marshes and wetlands in the whooping crane's and Hine's emerald dragonfly's range could or will have high levels of difenoconazole from runoff from soybean fields. This exposure may have adverse effects on these species and makes me greatly concerned about their survival.

11. I do not believe that the risks of re-approving difenoconazole have been properly assessed by EPA. It concerns me that given the stresses the whooping crane and the Hine's emerald dragonfly already must endure, continuing to allow this fungicide to be used in

agricultural fields adjacent to their essential habitat will be another stress that can and will severely harm their recovery. I am injured by the fact that EPA's interim registration decision for difenoconazole makes it more likely that I will see the decline of the whooping crane and previously-endangered bald eagle populations that I have personally watched grow over the course of my life.

12. I am also concerned about the effects of agricultural runoff into the lakes in which my husband fishes. The lakes near Madison, WI are a huge fishing spot, upon which some people depend for food. The lakes are already polluted largely from agricultural runoff, and we are worried that continued fungicide on soybeans nearby may adversely affect our health due to our regular consumption of these fish.

13. I have been concerned about my and my family's health for more than twenty years, going back to when I wanted to have children, and thus eat organic food whenever available. I did not and still do not want to have chemicals in my food.

14. I am aware that difenoconazole is approved for use on many of the foods that I regularly purchase and consume and is approved for use on soybean fields located near lakes from which I regularly consume

fish. Due to my family's health history, and because my husband and I are getting older and my daughter likely has an autoimmune disease, we do not want to be exposed to any harm resultant from consuming foods tainted by the toxic fungicide difenoconazole.

15. Specifically, my family and I regularly purchase and eat tomato paste and tomatoes, raisins, grapes, sweet bell peppers, blueberries, and potatoes. We try to eat seasonally and buy these organically as much as possible, although organic versions are not always readily available.

16. My daughter has not formally been diagnosed with Crohn's disease, but she has many of the symptoms, and we have autoimmune diseases on both sides of our family. My daughter also wants to have children, and we want to be sure that her children, my grandchildren, will be healthy and free from toxins in their food.

17. Recently I had a close friend die of cancer, following years of driving a potato truck. I am concerned that the cancer was related to pesticide exposure. My father also died of cancer, also likely due to chemical exposure.

18. I am aware that difenoconazole can impair the developing infant's brain and nervous system, cause cancer, disrupt hormonal symptoms, and lead to liver tumors. I do not want my potential grandchildren to be exposed to toxic substances and am myself concerned about my own exposure, due to the deaths of my father and a close friend from cancer. I am injured by the fact that EPA's interim registration decision for difenoconazole makes it more likely that I and my family will consume toxic substances and be regularly exposed to toxic substances.

19. In summary, I have aesthetic and recreational interests in the preservation of currently endangered species including the whooping crane and the Hine's emerald dragonfly, and previously-endangered species such as the bald eagle and their habitats. I have personal and family health interests in consuming healthy foods without difenoconazole residues. These interests are harmed and continue to be harmed by EPA's failure to adequately review how difenoconazole with impact species and public health.

20. Consultation with the expert wildlife agencies could result in protective measures aimed at reducing impacts of this fungicide on the

whooping crane, the Hine's emerald dragonfly, and the bald eagle, while proper review of health studies could protect my health and my family's health, which is important to ensure that my interests are preserved and remain free from injury.

I declare under penalty of perjury that the foregoing is true and correct to the best of my ability.

Executed on the 26th day of August 2022 in Manitowish Waters, Wisconsin.

Carrie Schudda

Attachment 8

UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

CENTER FOR FOOD SAFETY, *Petitioner*, v. UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, *et al.*, *Respondents*, and SYNGENTA CROP PROTECTION, LLC, *Respondent-Intervenor.*	**Case No. 22-70118** **DECLARATION OF SYLVIA SHIH-YAU WU**

DECLARATION OF SYLVIA SHIH-YAU WU IN SUPPORT OF MOTION FOR SUMMARY VACATUR

I, Sylvia Shih-Yau Wu, declare that if called as a witness in this action I would competently testify of my own personal knowledge as follows:

1. I am a Senior Attorney with Petitioner Center for Food Safety (CFS) and counsel in this case. I submit this declaration in support of Center for Food Safety in the above-captioned matter. I have

been a member of CFS for nearly 10 years.

2. CFS is a tax-exempt, nonprofit membership organization with offices in San Francisco, California; Portland, Oregon; and Washington, D.C. CFS represents more than a million farmer and consumer members, in every state throughout the country. CFS and its members are being, and will be, adversely affected by the EPA's interim registration decision of difenoconazole.

3. Since its inception in 1997, CFS's mission has been to empower people, support farmers, and protect the environment from the harmful impacts of industrial agriculture. Every day CFS staff works to address the adverse health and environmental impacts of our industrial food system, while at the same time advocating for a more sustainable future for our food. When we think of "food safety" we mean it in a broad sense: food that is safe for consumers, but also safe for those workers that produce the food, and for all species and the overall environment.

4. Accordingly, CFS's program activities cover the gamut of all aspects of the food and agriculture system including but not limited to:

foodborne illness; truth in food labeling; ocean aquaculture and fisheries issue; livestock pollution; soil protection; industrial monocultures; new and emerging food technologies; and many more. A cornerstone of this mission is to advocate for thorough, science-based safety assessments of agricultural products and technologies.

5. Of relevance here, as part of its broader mission one of CFS's flagship programs has always been addressing the adverse environmental, health, and socioeconomic impacts of pesticides. CFS has multiple staff—scientific, policy, and legal—that work on this program, including myself.

6. CFS combines multiple tools and strategies in pursuing its mission, including public and policymaker education, outreach, and campaigning. For example, CFS disseminates a wide array of informational materials to government agencies, lawmakers, nonprofits, and the general public regarding the effects of industrial food production, agricultural products, and pesticides, on human health and the environment. These educational and informational materials include, but are not limited to, news articles, policy reports, white

papers, legal briefs, press releases, newsletters, product guides, action alerts, and fact sheets. CFS often has provided expert testimony to policymakers on the issues including the adverse impacts and risks of pesticides.

7. Staff members monitor the Federal Register daily, and regularly submit comments to EPA and other regulatory agencies via the public notice-and-comment process regarding food production technologies, including pesticides. CFS also regularly sends out action alerts to its members, encouraging them to participate in the notice-and-comment process, or to submit letters to government officials related to the oversight of industrial agriculture, pesticide use, genetically engineered crops, and other issues affecting CFS's mission to build a sustainable food system.

8. CFS submitted organizational comments in 2022 to the EPA docket on EPA's proposed interim registration decision for difenoconazole.

9. When necessary, CFS also engages in public interest litigation to address the impacts of industrial food production and

pesticides on its members, the environment, and the public interest. As a party to this proceeding, CFS and its members are injured by EPA's interim registration decision. EPA's decision means that difenoconazole will continued to be sprayed and used as seed treatment on across the United States. CFS and its members are greatly concerned about the detrimental impacts of this toxic spraying and pesticide use on farmers, the environment, including on endangered species and their habitat, and on their health.

10. CFS and its members are being, and will be, adversely affected by the challenged decision. First, many members of CFS are heavily involved with maintaining a healthy environment for many species of animals for recreational, aesthetic, and personal reasons. The use of difenoconazole will negatively harm creatures they care about, such as the Hine's emerald dragonfly and California red-legged frog, and other common and endangered wildlife, injuring CFS members' recreational, vocational, environmental, and aesthetic interests.

11. Second, many of CFS's members are also farmers or gardeners and live in rural areas, where excessive amounts of pesticides

are sprayed during the farming of commodity crops such as soybean, which will include spraying of difenoconazole. These members are especially susceptible to the health and environmental risks caused by EPA's registration of difenoconazole. Moreover, the use of difenoconazole on crops compromises our farmer members' enjoyment of their local environment and injures the aesthetic and recreational interests of our members in maintaining biodiversity and protecting sensitive species.

12. Third and most relevant to this motion, CFS members' interests are injured by EPA's failure to consult under the Endangered Species Act with the expert wildlife agencies before making this decision. Many of CFS's members have significant personal, environmental, vocational, professional, aesthetic, and recreational interests in regularly observing imperiled species protected under the Endangered Species Act and in preserving their habitats. These species include , Evergalde snail kite, California red-legged frog, Hine's emerald dragonfly, and whooping crane. CFS's members' interests in biodiversity and protection of these sensitive species are injured by

EPA's decision to issue the decision without consulting with the expert wildlife agencies.

13. Finally, as a member and employee of CFS, I have personal and professional interests in the safety of the foods we consume. I am aware that EPA's interim registration decision of difenoconazole authorizes the use of the toxic pesticide on many of the foods I eat and feed my child, such as soybean, sugar beets, potatoes, and apples. I am also aware that EPA issued the interim registration without critical studies to assess difenoconazole's potential reproductive and developmental health impacts.

14. As a consumer and mother of an infant, I go out of my way to ensure that the foods I purchase and prepare for myself and my child are free of development and reproductive toxins. When shopping for my family, I purchase organic produced foods whenever possible, and I spend time and energy looking into potential reproductive and developmental toxins that may be in processed foods by regularly reviewing food blogs, literature from government agencies, and general consumer information. Even with these efforts, I am not always able to

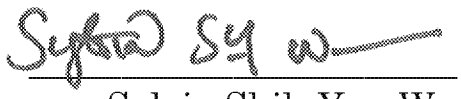
find organically produced foods and food products in the stores or on restaurant menus when we dine out.

15. EPA's interim registration decision of difenoconazole authorizes the use of the toxic pesticide on common ingredients in children's prepared meals and offered on restaurant menus, such as apples, sugar beets, and soybeans. This injures my personal health interests as a consumer and mother to feed myself and my child foods free of developmental and reproductive toxins.

16. In sum, EPA's interim registration review decision for difenoconazole injures my personal health interest, as well as CFS's organizational interests in protecting the environment and endangered species from harmful pesticides, and the vocational, environmental, aesthetic, and recreational interests of CFS's hundreds of thousands of conservationist members. CFS and its members, including me, will be redressed if and when this Court vacates the registration.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on August 31, 2021, in Berkeley, CA.



Sylvia Shih-Yau Wu

Attachment 9

UNITED STATES COURT OF APPEALS
FOR THE NINTH CIRCUIT

CENTER FOR FOOD SAFETY,

Petitioner,

v.

U.S. ENVIRONMENTAL PROTECTION AGENCY, *et al.*,

Respondents,

and

SYNGENTA CROP PROTECTION, LLC,

Respondent-Intervenor.

Case No. 22-70118

DECLARATION OF KRISTINA SINCLAIR IN SUPPORT OF PETITIONER'S MOTION FOR SUMMARY VACATUR

DECLARATION OF KRISTINA SINCLAIR IN SUPPORT OF PETITIONER'S MOTION FOR SUMMARY VACATUR

I, Kristina Sinclair, declare as follows:

1. I am an associate attorney at the Center for Food Safety (CFS) and a member in good standing of the State Bar of California.

2. I have experience using multiple Geographic Information Systems (GIS) services and applications, and I frequently use my knowledge of GIS to support CFS's legal work. For over three years, I have been using GIS to assist multiple legal advocacy organizations with factual research, data analysis, litigation, community outreach, and case development purposes.

3. I prepared the maps attached as exhibits to this declaration. These maps demonstrate the overlap between certain crops, current species ranges, and final designated critical habitat under the Endangered Species Act.

4. I used ArcGIS Online to prepare these maps.

Crop Data

5. I selected crop data for specific states from the U.S. Department of Agriculture, National Agricultural Statistics Service's

(NASS) Cropland Data Layer for 2021 (CDL 2021), https://nassgeodata.gmu.edu/CropScape.

6. For the state of **California**, I selected the following crops from the CDL 2021: Tomatoes, Almonds, Apples, and Grapes.

7. For the state of **Florida**, I selected the following crops from the CDL 2021: Cantaloupes, Citrus, Oranges, Peas, Potatoes, Rice, Sweet Potatoes, and Watermelons.

8. For the states in the **Midwest** (Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, and Wisconsin), I selected the following crops from the CDL 2021: Soybeans, Double Crop Winter Wheat/Soybeans, Double Crop Soybeans/Cotton, Double Crop Soybeans/Oats, Double Crop Corn/Soybeans, and Double Crop Barley/Soybeans.

9. I downloaded crop acreage data from NASS's 2021 CDL Attribute Table, https://www.nass.usda.gov/Research_and_Science/Cropland/docs/2021_CDL_Histogram_Summary.xlsx.

Current Range & Critical Habitat

10. I downloaded current species range and final critical habitat data for specific threatened and endangered species from the U.S. Fish

and Wildlife (FWS) and National Oceanic and Atmospheric Administration (NOAA).

11. For the endangered **California Condor** (*Gymnogyps californianus*), I downloaded the current species range data (last updated on April 14, 2021) and the final critical habitat data (last updated on September 22, 1977) from FWS's Species Profile for the California Condor, https://ecos.fws.gov/ecp/species/8193.

12. For the endangered **Conservancy Fairy Shrimp** (*Branchinecta conservatio*), I downloaded the current species range data (last updated on March 29, 2018) and the final critical habitat data (last updated on February 10, 2006) from FWS's Species Profile for the Conservancy Fairy Shrimp, https://ecos.fws.gov/ecp/species/8246.

13. For the threatened **California Red-Legged Frog** (*Rana draytonii*), I downloaded the current species range data (last updated on April 6, 2022) and the final critical habitat data (last updated on March 17, 2010) from FWS's Species Profile for the California Red-Legged Frog, https://ecos.fws.gov/ecp/species/2891.

14. For the endangered **Hine's Emerald Dragonfly** (*Somatochlora hineana*), I downloaded the current species range data

(last updated on July 30, 2021) and the final critical habitat data (last updated on April 23, 2010) from FWS's Species Profile for the Hine's Emerald Dragonfly, https://ecos.fws.gov/ecp/species/7877.

15. For the endangered **Everglade Snail Kite** (*Rostrhamus sociabilis plumbeus*), I downloaded the current species range data (last updated on January 28, 2022) and the final critical habitat data (last updated on September 22, 1977) from FWS's Species Profile for the Everglade Snail Kite, https://ecos.fws.gov/ecp/species/7713.

16. For the endangered **Smalltooth Sawfish** (Pristis pectinate), I downloaded the final critical habitat data (last updated on September 2, 2009) from NOAA's website, https://www.fisheries.noaa.gov/resource/map/smalltooth-sawfish-critical-habitat-map-and-gis-data.

Exhibits

California

17. Attached as **Exhibit A** is an accurate copy of the map that I created showing selected crops planted in California in 2021.

18. Attached as **Exhibit B** is an accurate copy of the maps that I created showing (1) the current species range and final critical habitat

for the California Condor, and (2) the overlap between this species and selected crops in California.

19. Attached as **Exhibit C** is an accurate copy of the maps that I created showing (1) the current species range and final critical habitat for the Conservancy Fairy Shrimp, and (2) the overlap between this species and selected crops in California.

20. Attached as **Exhibit D** is an accurate copy of the maps that I created showing (1) the current species range and final critical habitat for the California Red-Legged Frog, and (2) the overlap between this species and selected crops in California.

Florida

21. Attached as **Exhibit E** is an accurate copy of the map that I created showing selected crops planted in Florida in 2021.

22. Attached as **Exhibit F** is an accurate copy of the maps that I created showing (1) the current species range and final critical habitat for the Everglade Snail Kite, and (2) the overlap between this species and selected crops in Florida.

23. Attached as **Exhibit G** is an accurate copy of the maps that I created showing (1) the final critical habitat for the Smalltooth

Sawfish, and (2) the overlap between this species and selected crops in Florida.

Midwest

24. Attached as **Exhibit H** is an accurate copy of the map that I created showing selected crops planted in the Midwest in 2021.

25. Attached as **Exhibit I** is an accurate copy of the maps that I created showing (1) the current species range and final critical habitat for the Hine's Emerald Dragonfly, and (2) the overlap between this species and selected crops in the Midwest.

I declare under penalty of perjury that the foregoing is true and correct. Executed on September 1, 2022, in Santa Clara County, California.

Kristina Sinclair

EXHIBIT A

Crops Planted in California

Map Color	Category	Acreage
	Tomatoes	266,031
	Apples	11,210
	Grapes	854,691
	Almonds	1,851,192
		2,983,124

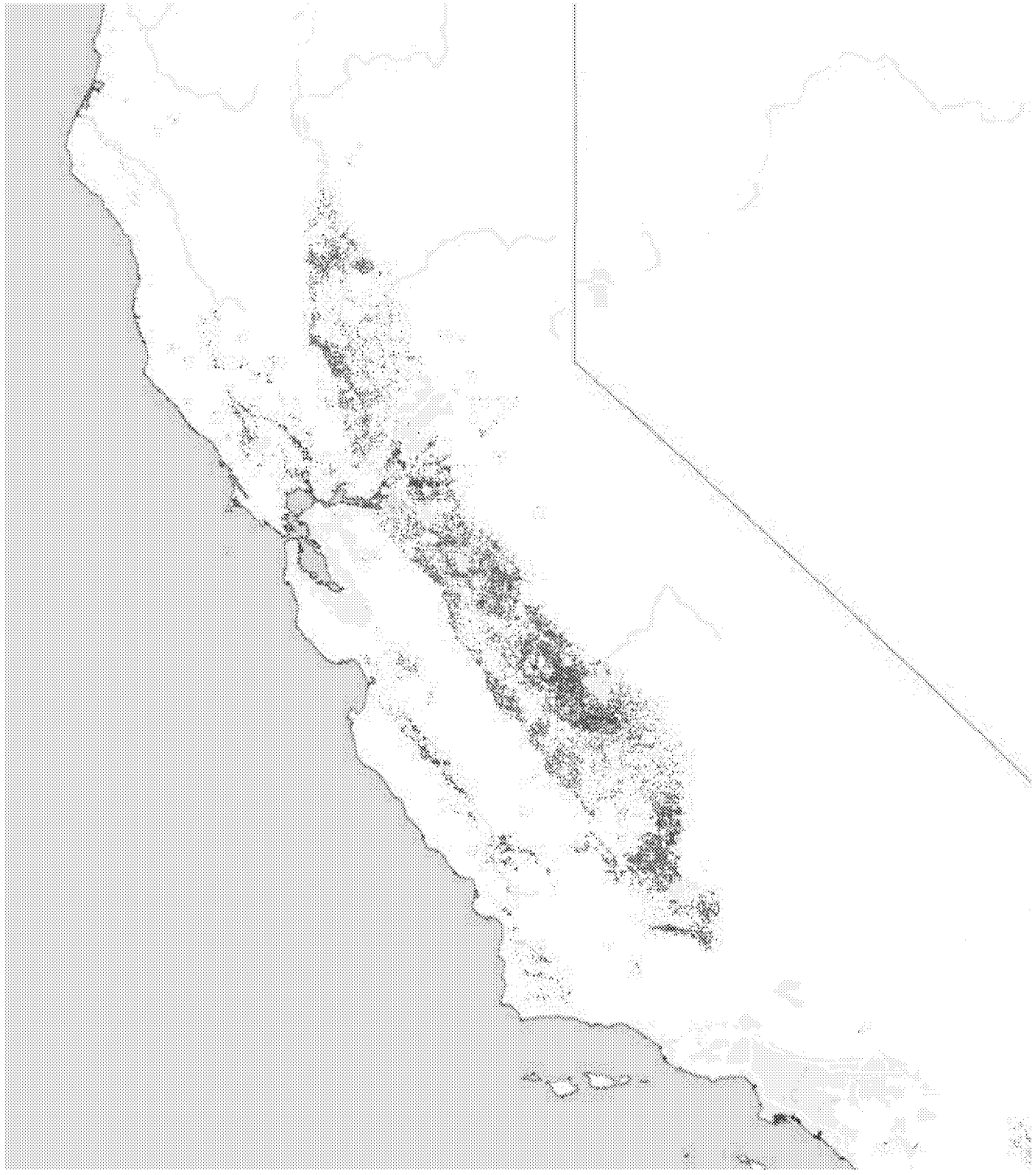
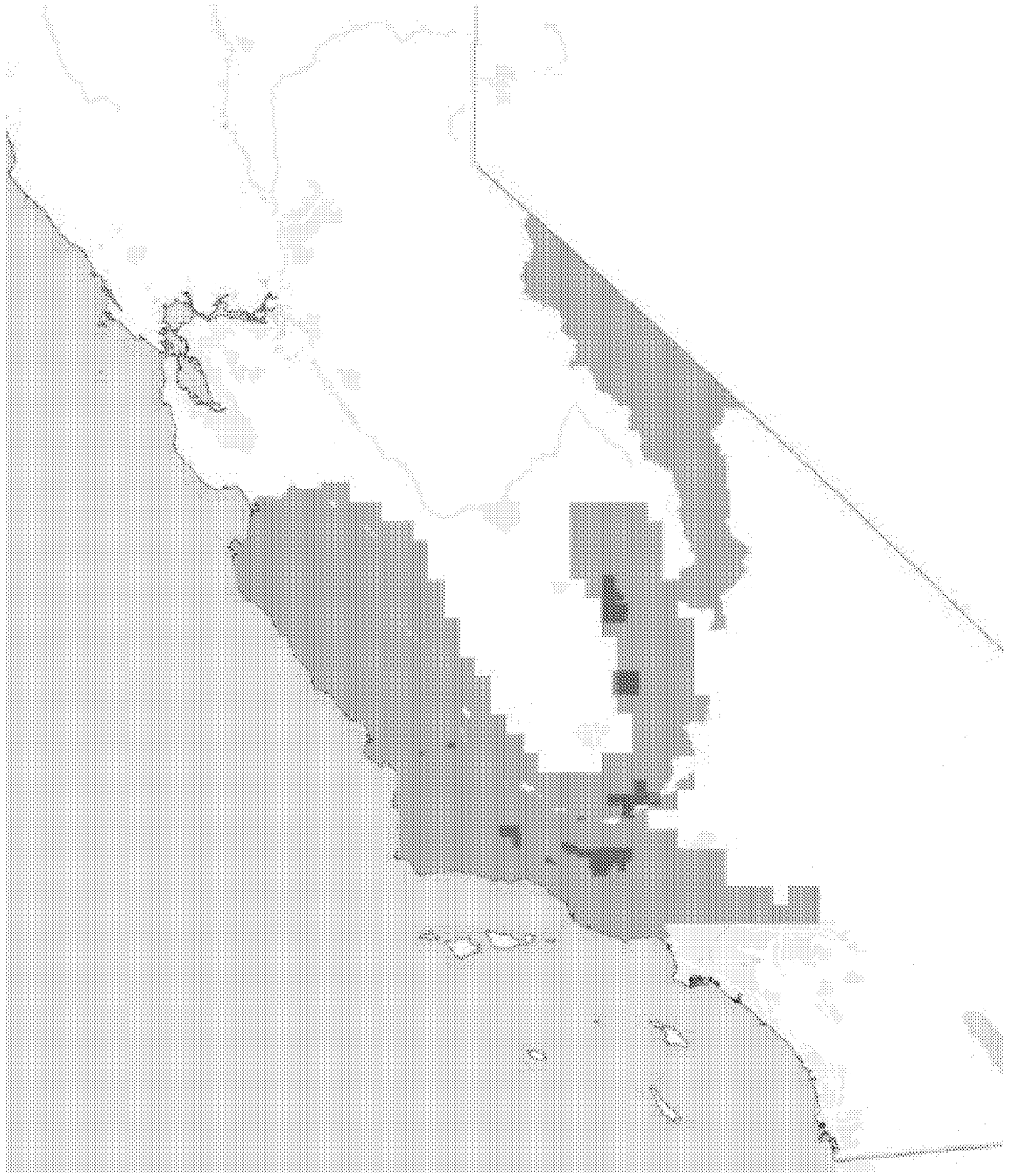


EXHIBIT B

Endangered California Condor in California

Final Critical Habitat

Current Species Range



Overlap Between Endangered California Condor and Selected Crops in California

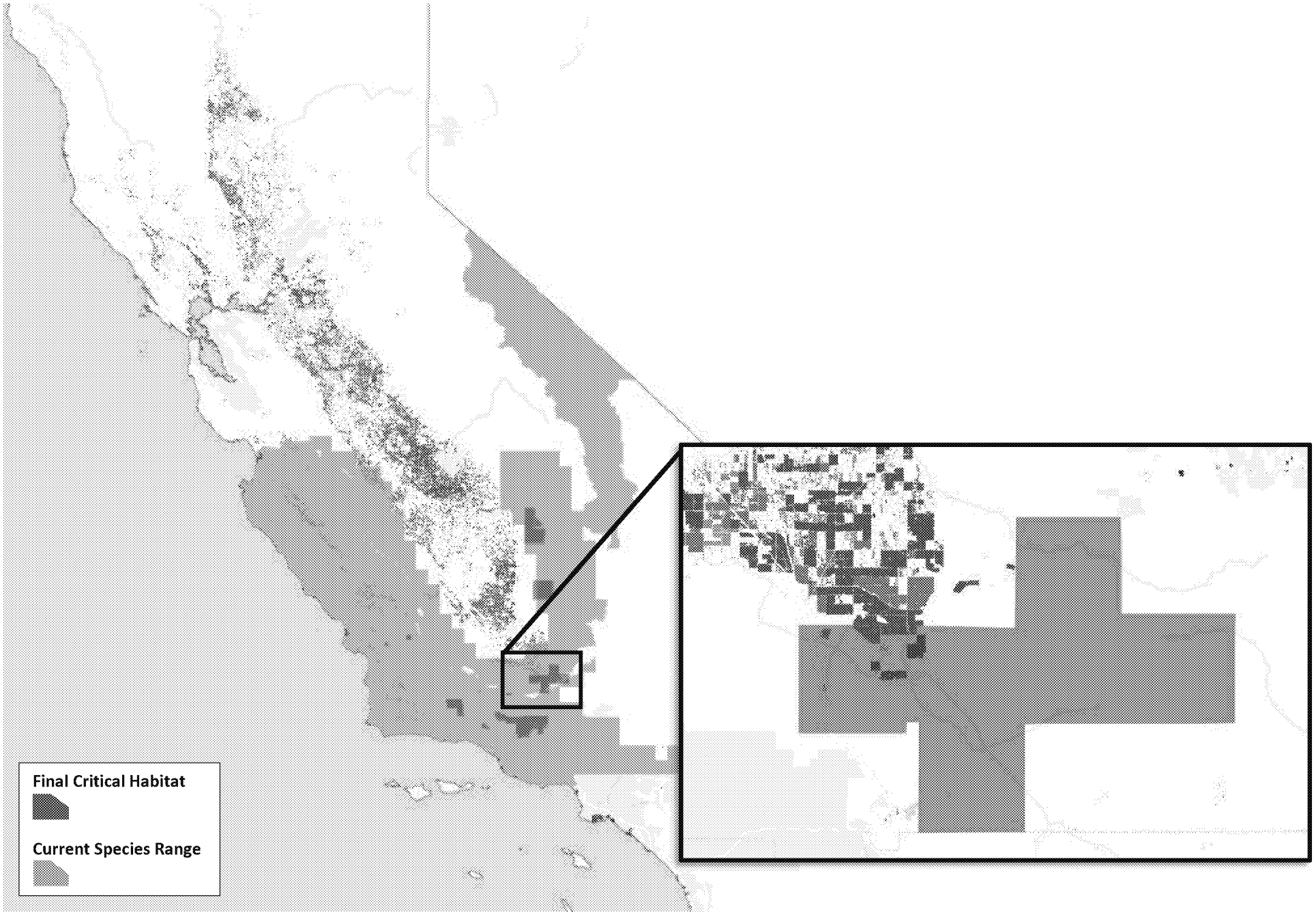
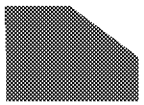


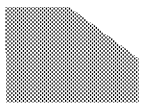
EXHIBIT C

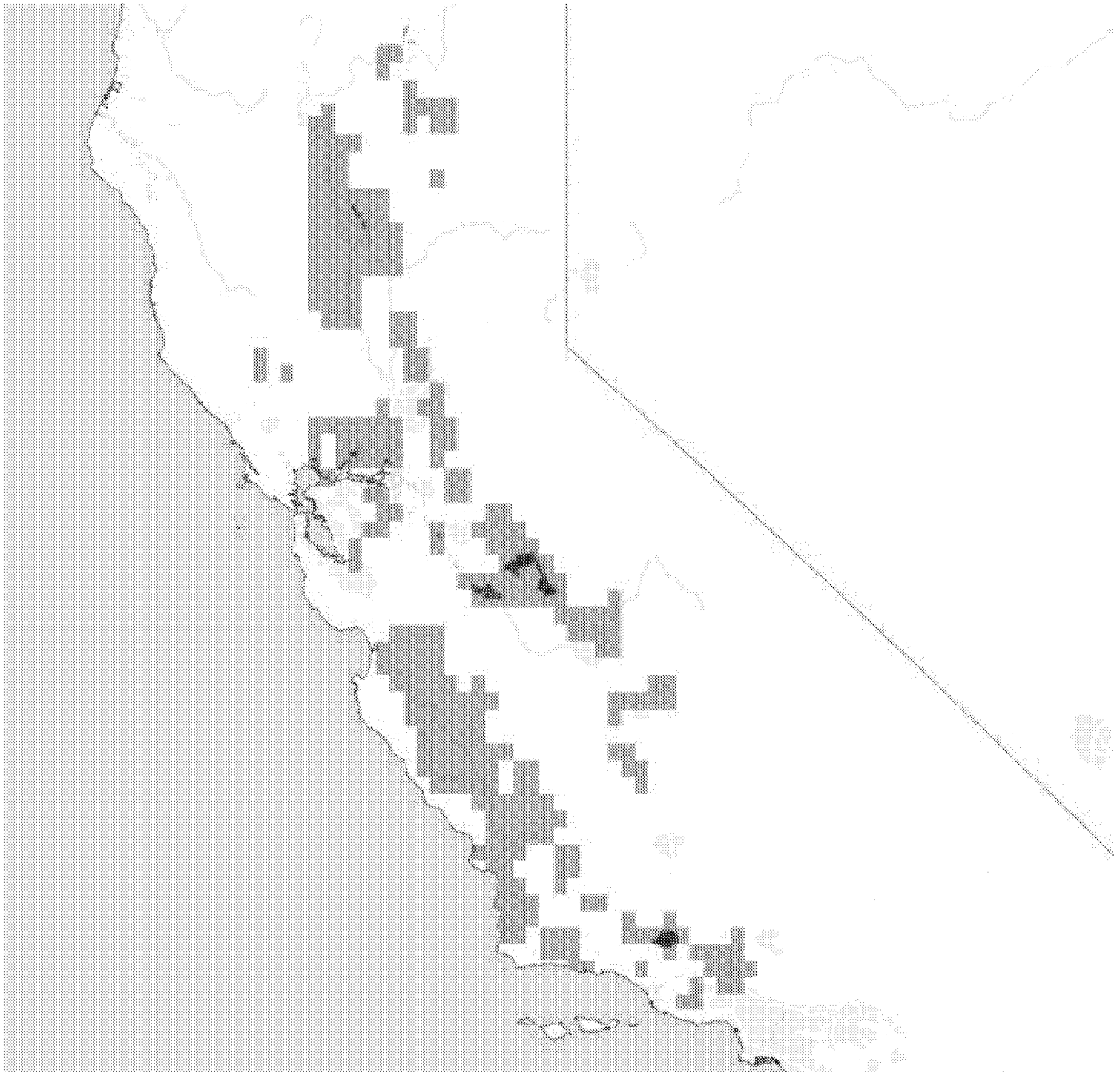
Endangered Conservatory Fairy Shrimp in California

Final Critical Habitat



Current Species Range





Overlap Between Endangered Conservatory Fairy Shrimp and Selected Crops in California

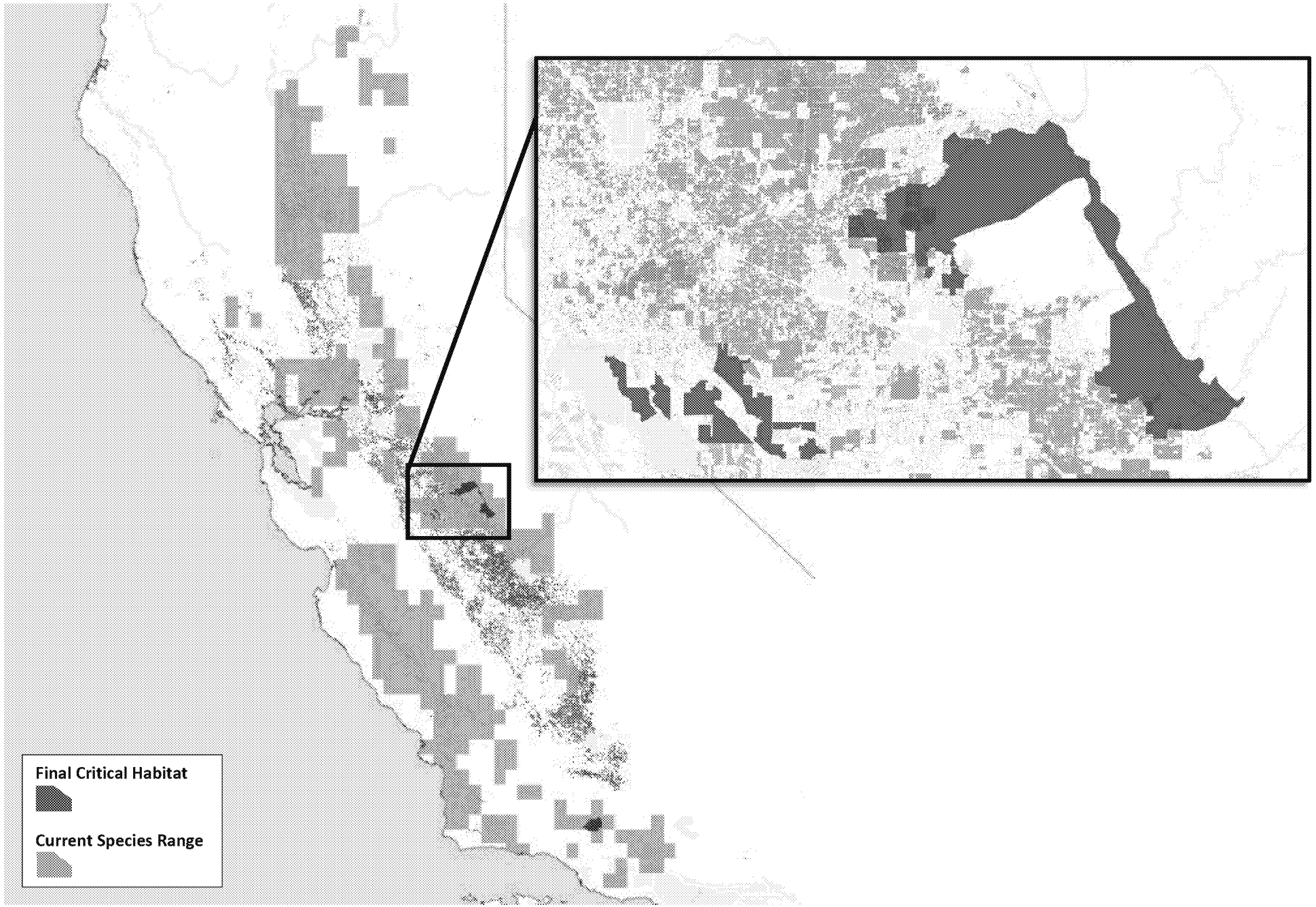
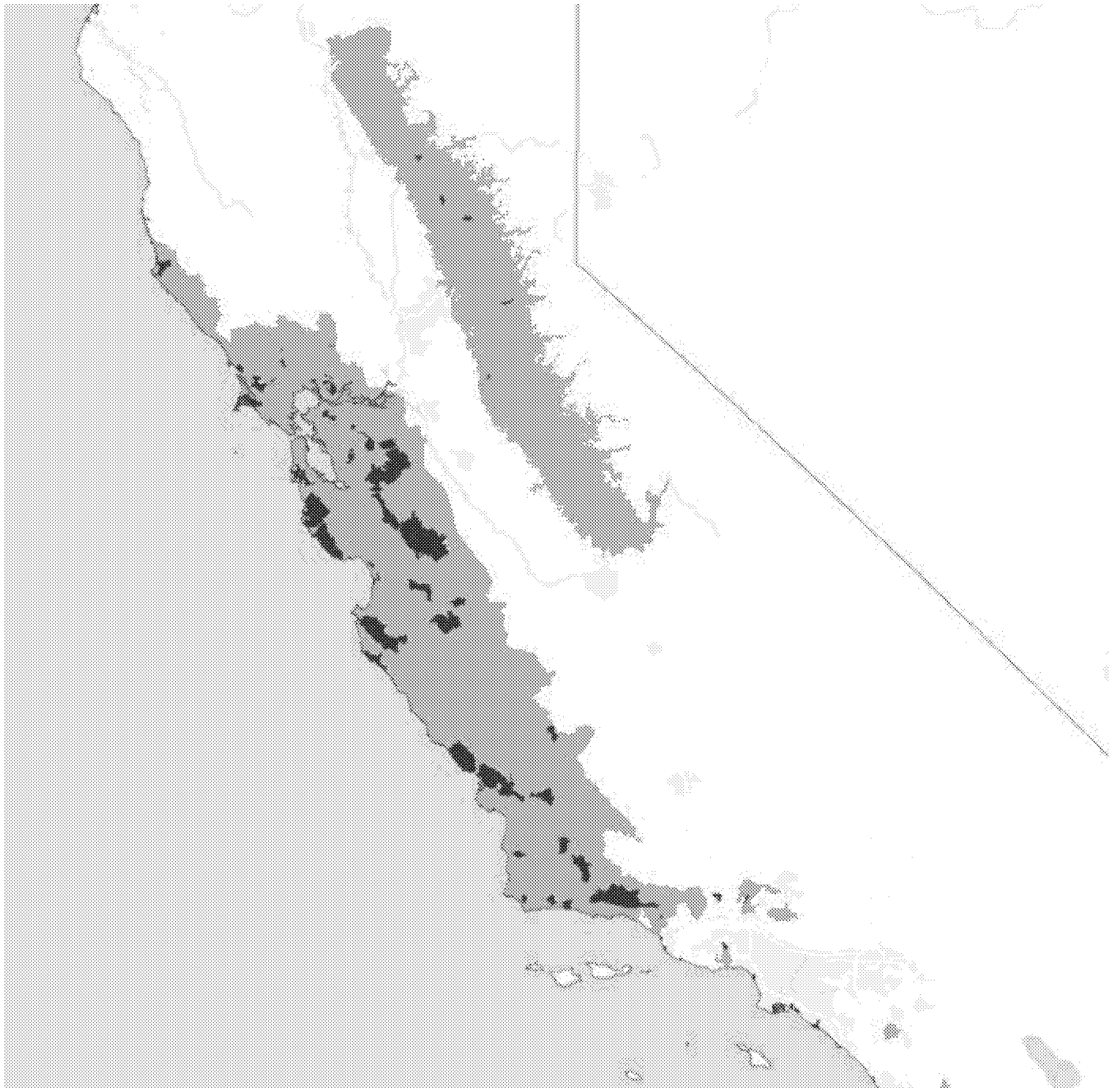


EXHIBIT D

Threatened California Red-Legged Frog in California

Final Critical Habitat

Current Species Range



Overlap Between Threatened California Red-Legged Frog and Selected Crops in California

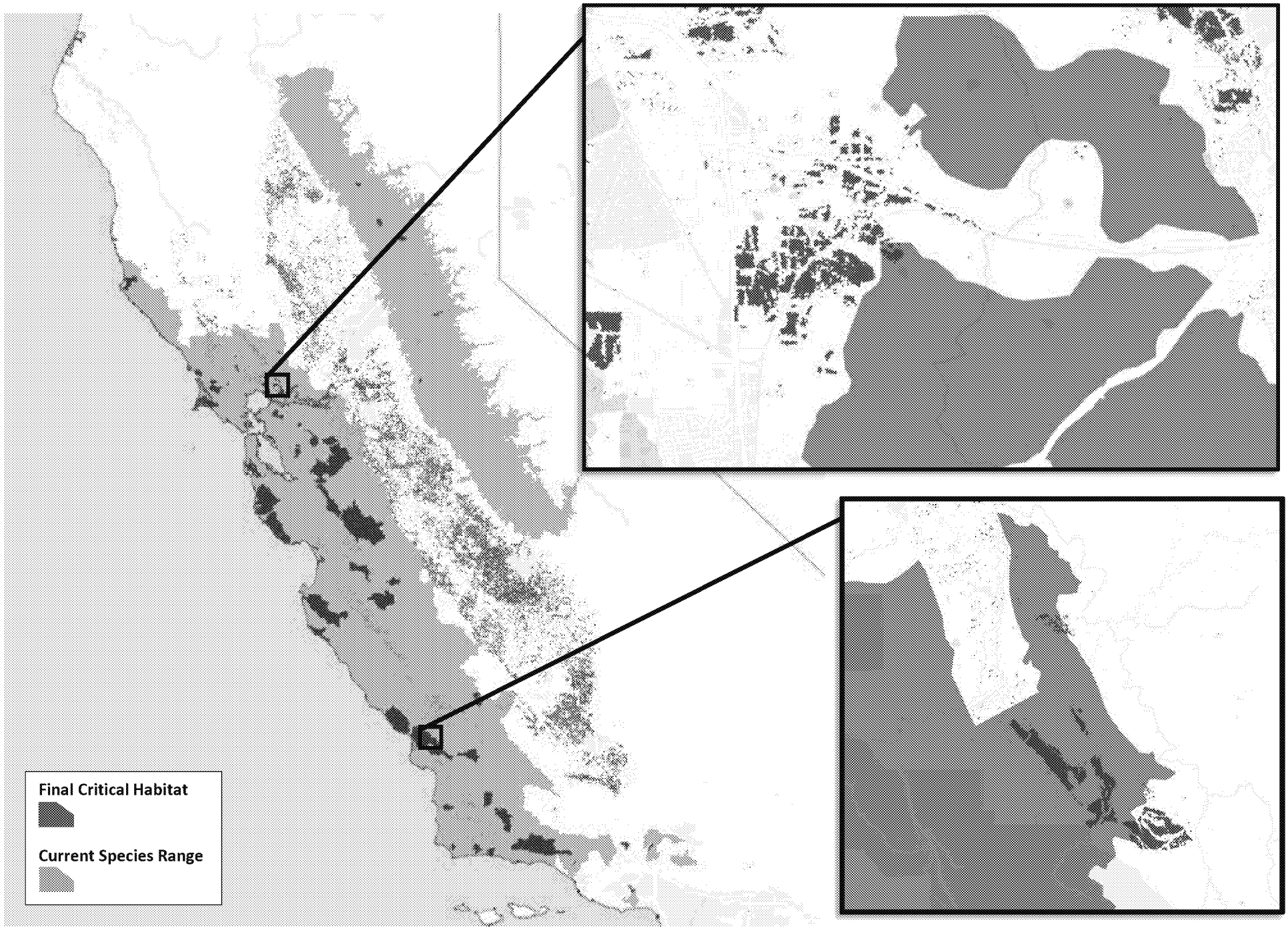


EXHIBIT E

Crops Planted in Florida

Map Color	Category	Acreage
	Cantaloupes	20
	Citrus	117,539
	Oranges	713,708
	Peas	5,415
	Potatoes	13,852
	Rice	1,440
	Sweet Potatoes	526
	Watermelons	4,912
		857,412

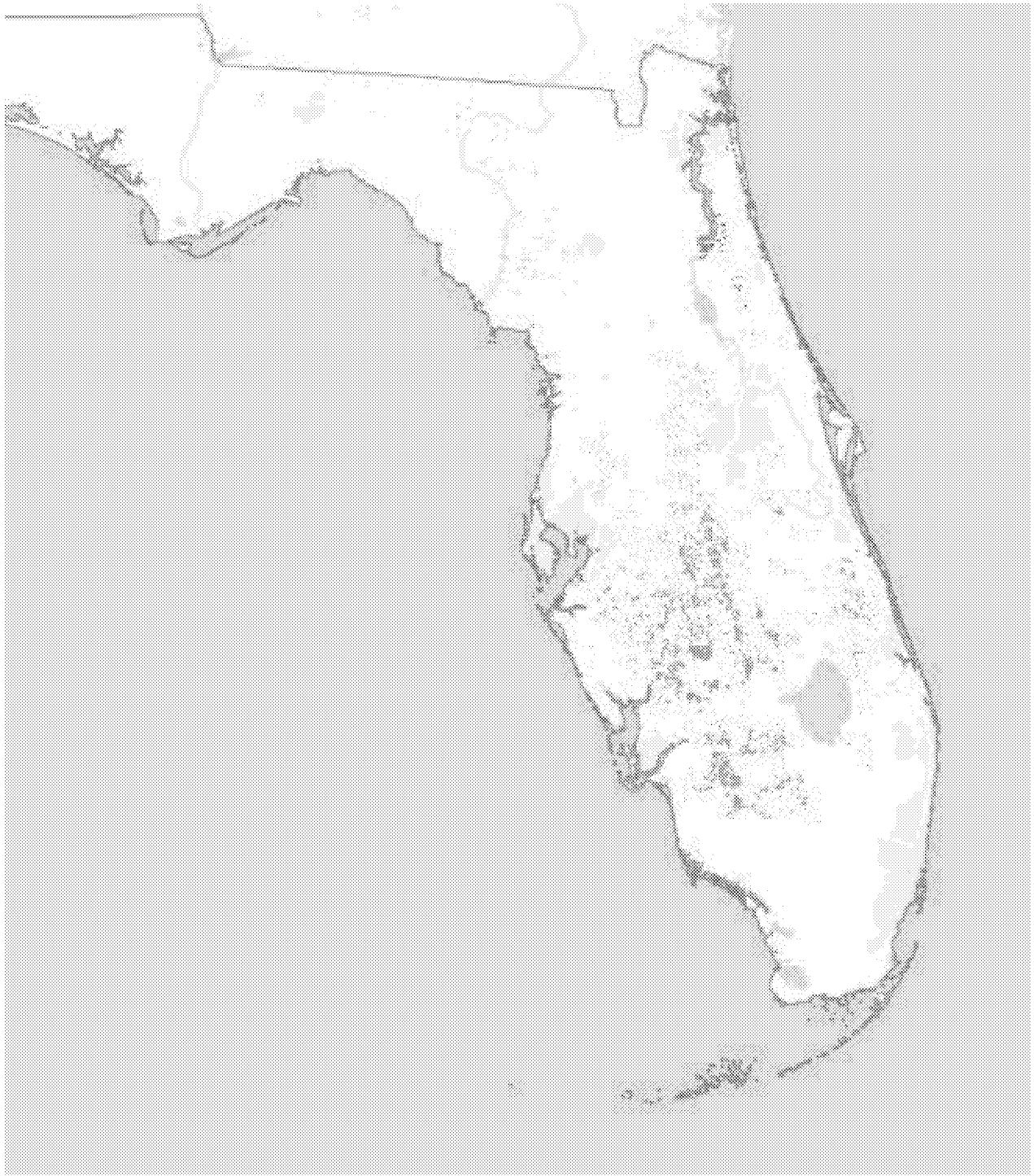
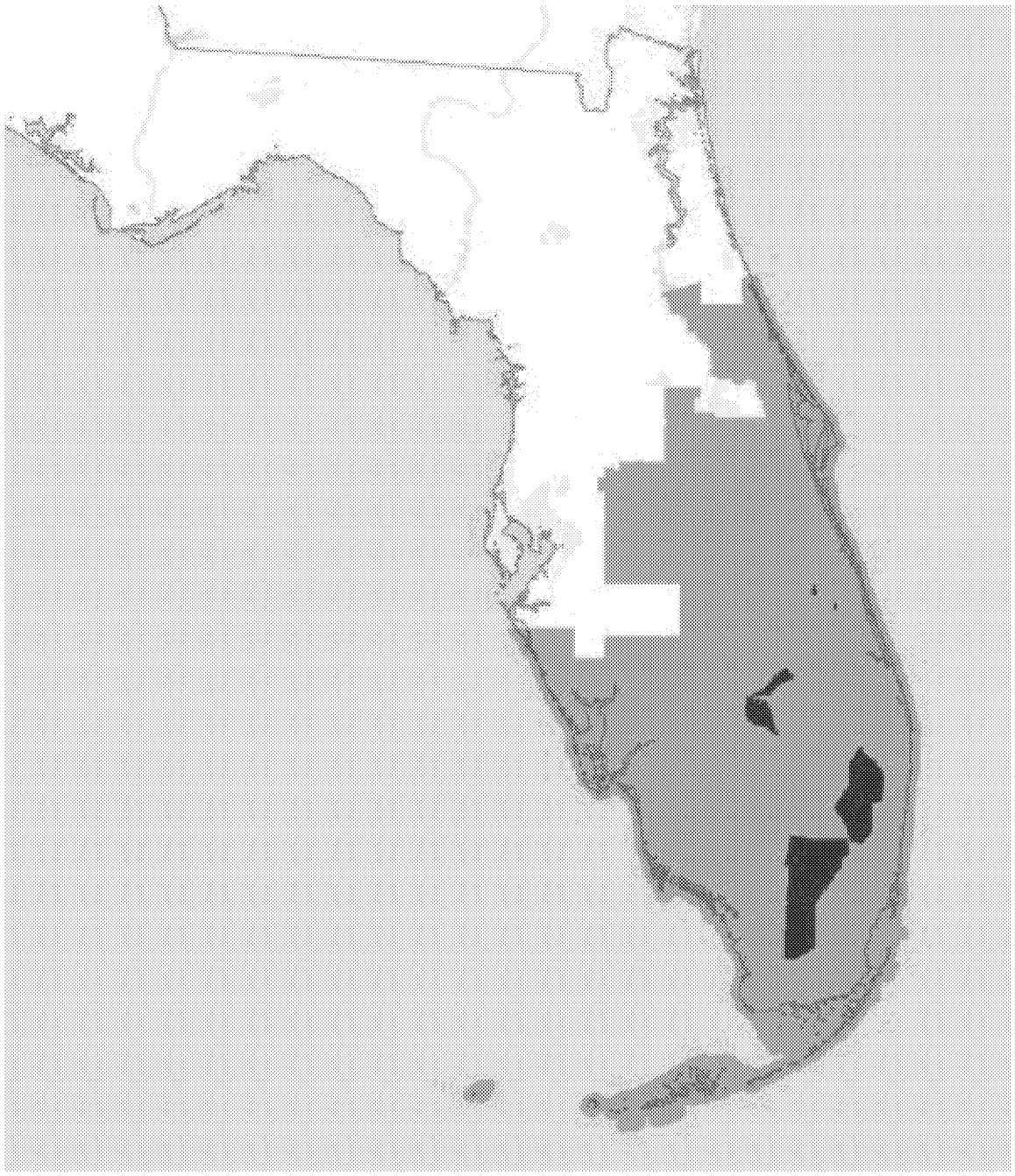


EXHIBIT F

Endangered Everglade Snail Kite in Florida

Final Critical Habitat

Current Species Range



Overlap Between Endangered Everglade Snail Kite and Selected Crops in Florida

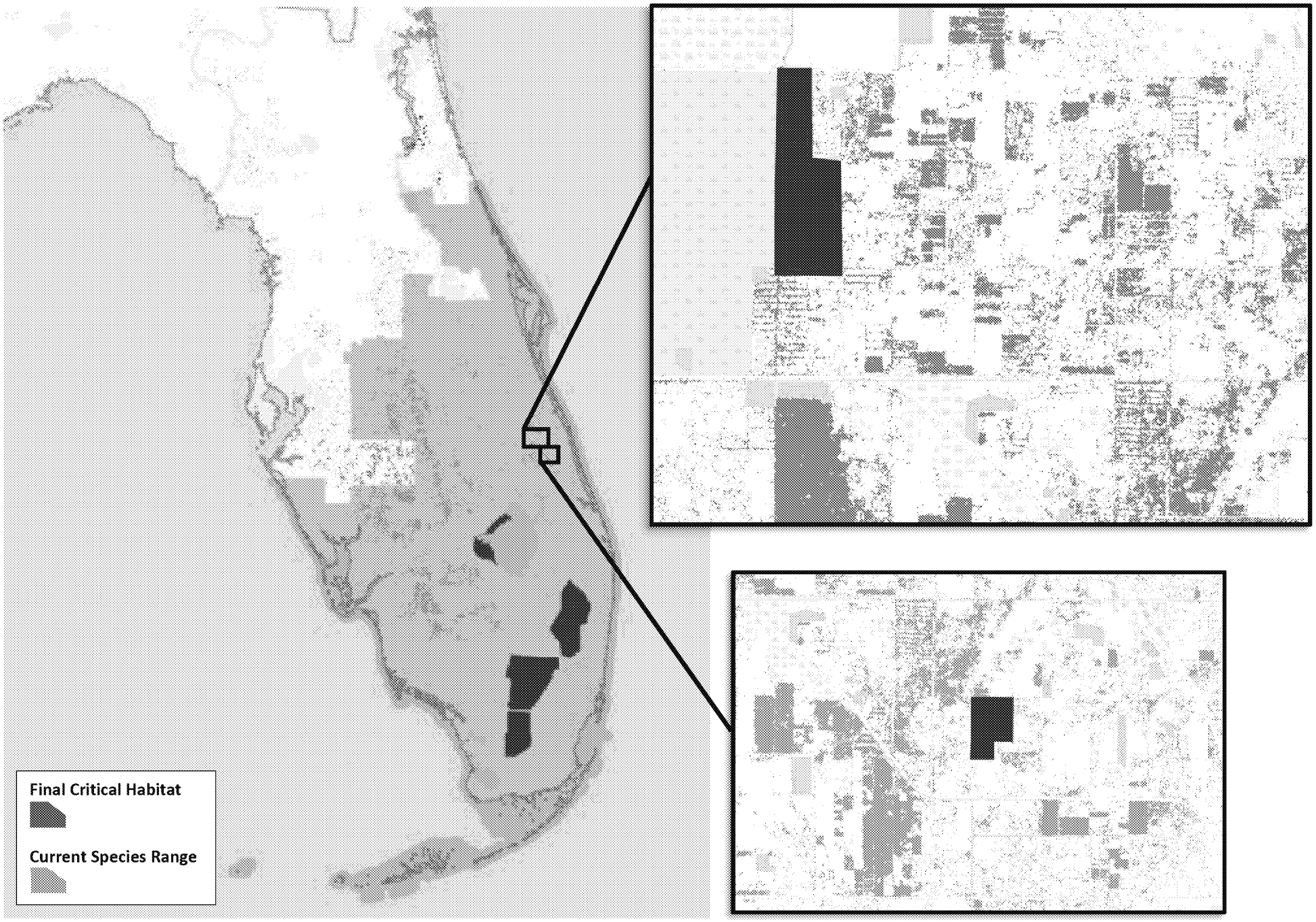
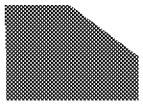
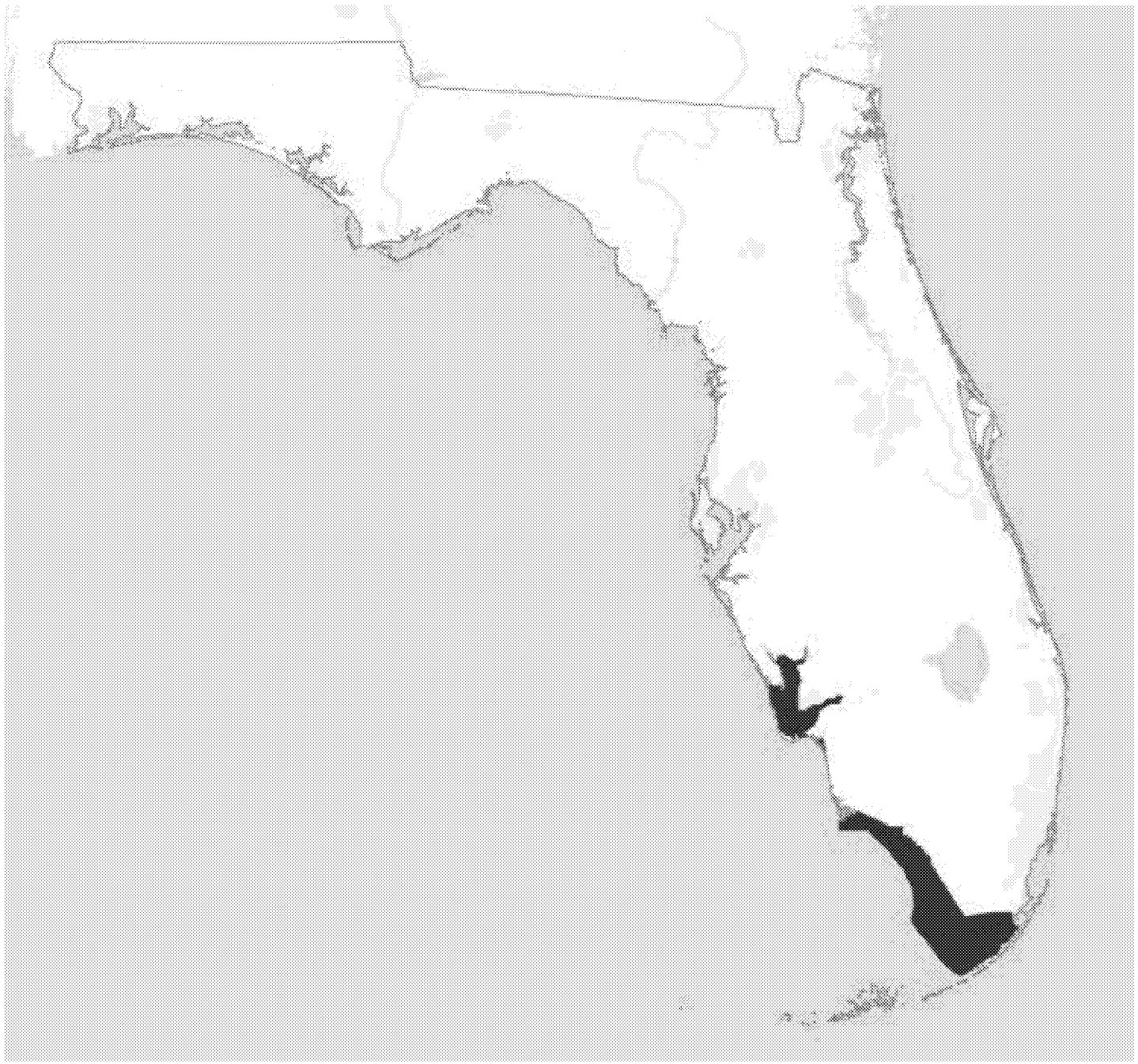


EXHIBIT G

Endangered Smalltooth Sawfish in Florida

Final Critical Habitat





Overlap Between Endangered Smalltooth Sawfish and Selected Crops in Florida

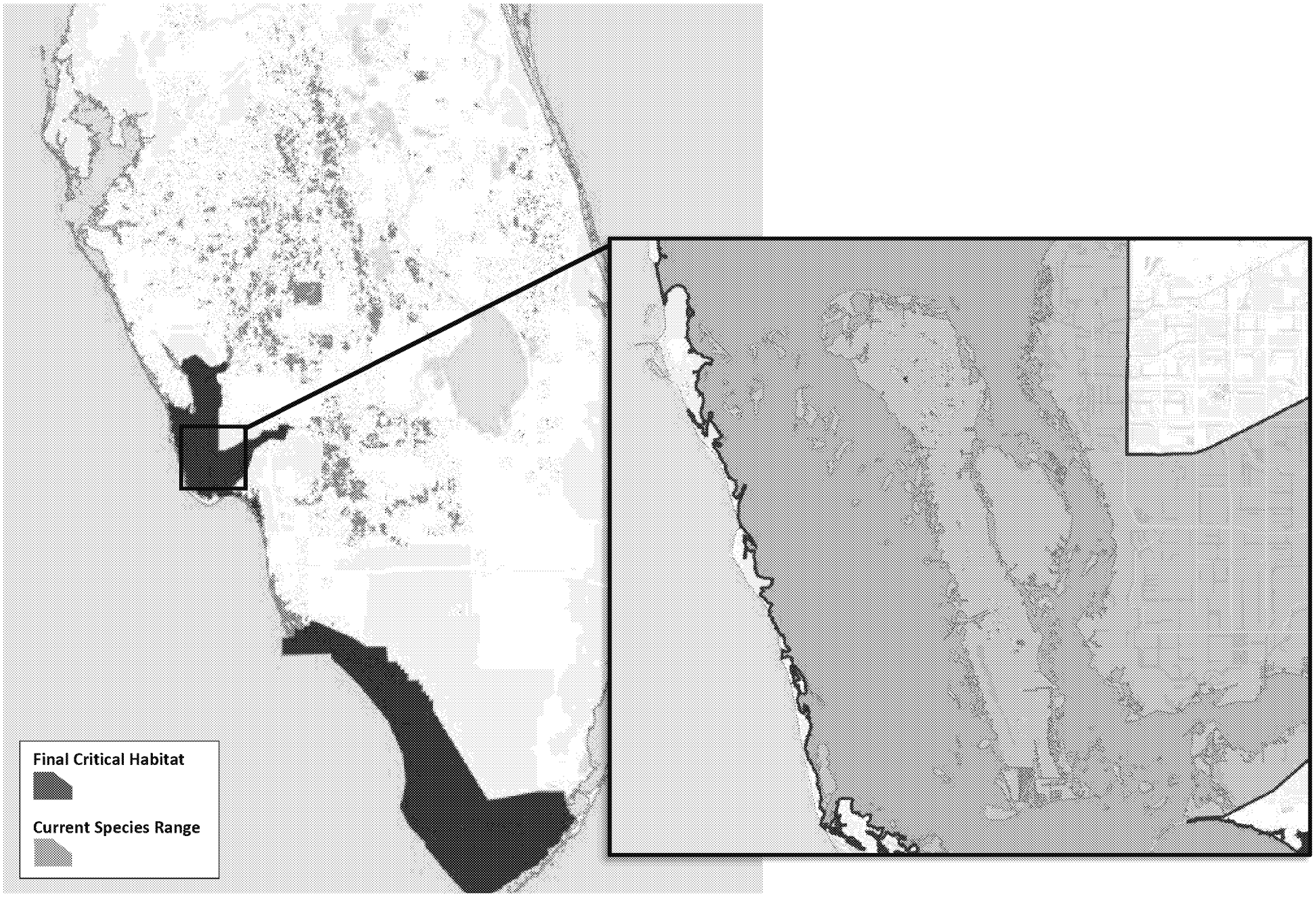


EXHIBIT H

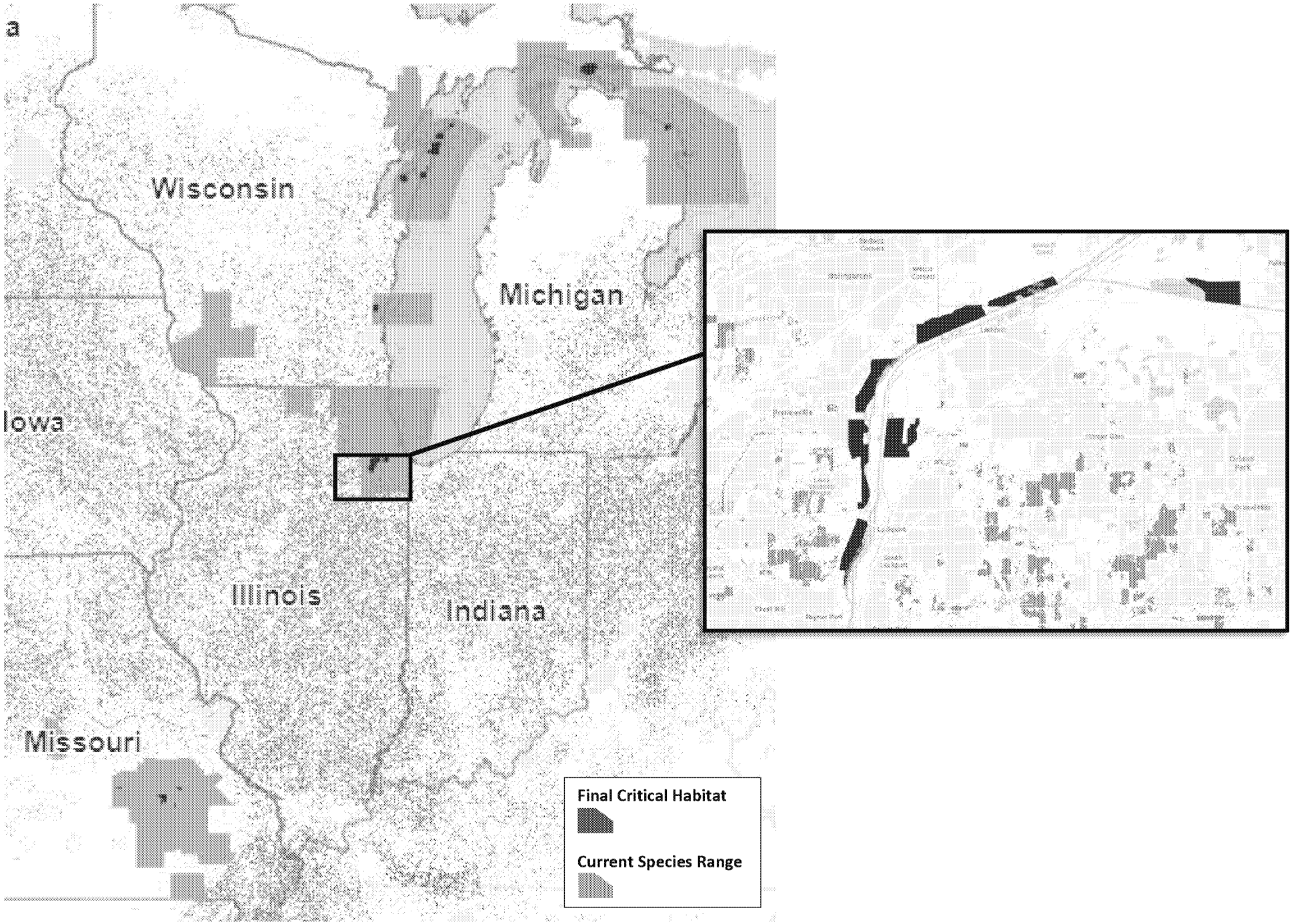
Crops Planted in Midwest

Map Color	Category
	Soybeans
	Dbl Crop WinWht/Soybeans
	Dbl Crop Soybeans/Cotton
	Dbl Crop Soybeans/Oats
	Dbl Crop Corn/Soybeans
	Dbl Crop Barley/Soybeans

EXHIBIT I

Endangered Hine's Emerald Dragonfly in Midwest

Final Critical Habitat

Current Species Range

Overlap Between Endangered Hine's Emerald Dragonfly and Selected Crops in Midwest