CENTER FOR FOOD SAFETY

December 20, 2021

Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, DC 20460–0001

RE: Docket EPA-HQ-OPP-2015-0061
Comments on proposed interim registration decision for tetraconazole

Center for Food Safety appreciates the opportunity to comment on EPA's proposed interim registration decision for the fungicide tetraconazole, 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

Tetraconazole is a broad-spectrum fungicide registered for use on many fruits, vegetables, cereals, soybeans, sugar beets, peanuts, and pecans. Tetraconazole 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.

Although not registered by EPA until 2005, tetraconazole was used on sugar beets for at least six years before that under emergency authorizations and temporary tolerances issued by EPA beginning no later than 1999, before the Agency had completed a risk assessment (EPA 5/18/00, EPA 2015, see also Fig. 1 below).

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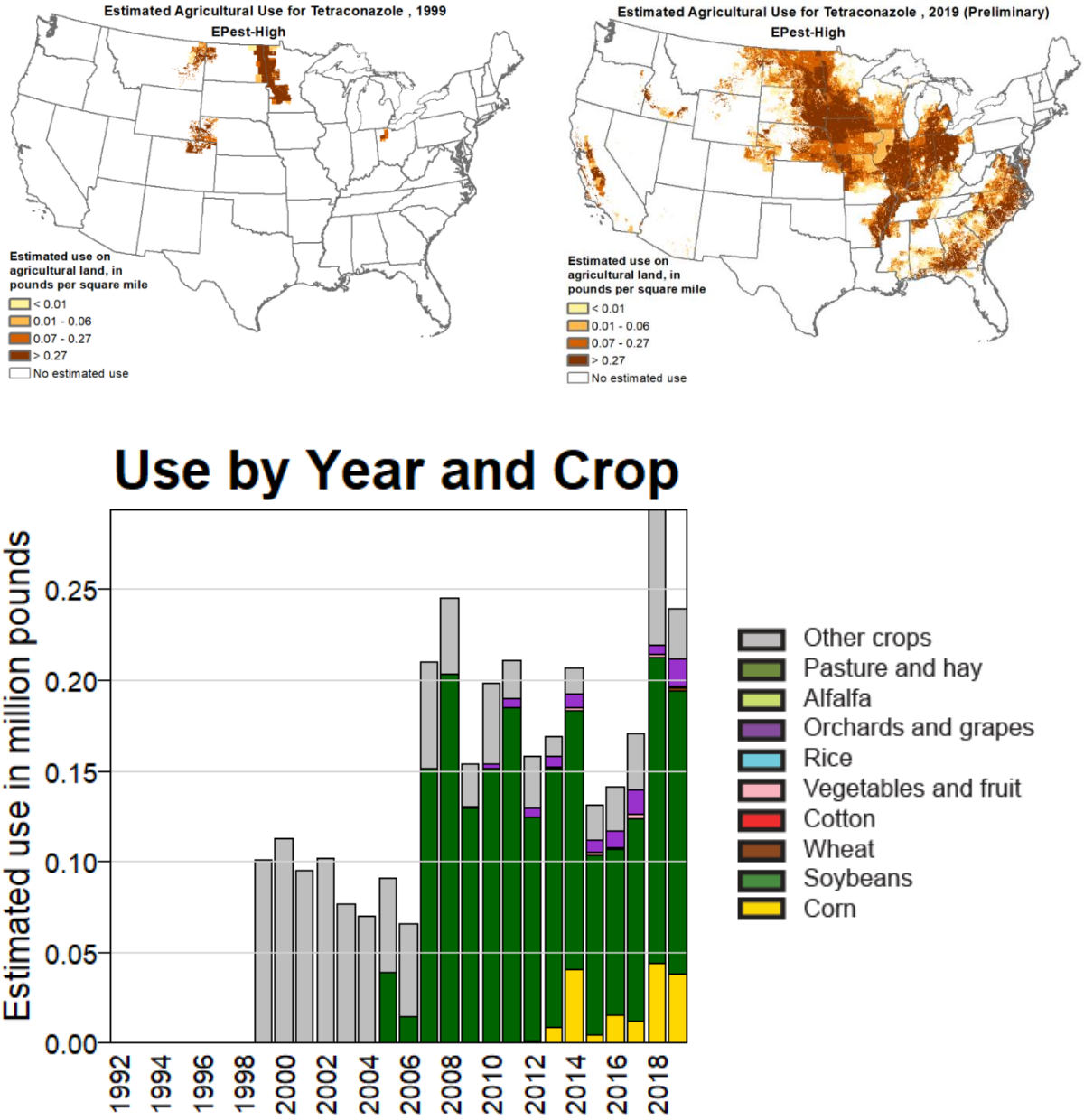

Figure 1: Tetraconazole Use, from: US Geological Survey, Pesticide National Synthesis Project, Tetraconazole, Epest-High. https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2017&map=TETRACONAZOLE&hilo=L&disp=Tetraconazole.

Once used exclusively on sugar beets, the bulk of tetraconazole is now applied to soybeans and increasingly corn. According to USDA's National Agricultural Statistics Service, use of tetraconazole on soybeans has exploded from 19,000 lbs (< 1% of acreage) in 2017 to 267,000 lbs. on 4% of soybean acreage in 2020; while much less is used on corn, USDA NASS report 24,000 and 22,000 lbs./year in 2016 and 2018, respectively.[1] These figures agree reasonably well with USGS data portrayed above, based on surveys by the private firm,

[1] From USDA NASS Agricultural Chemical Usage reports, figures cited cover Program States only; total national usage would be somewhat greater.

Kynetec.[2] Other major uses besides sugar beets include strawberries and grapes. Soybeans constitute the majority of tetraconazole's use (Fig. 1); and because this represents treatment of just 4% of soybean acres, there is huge potential for much greater spraying of soybeans with this fungicide.

Several features of tetraconazole and its use deserve particular consideration. First, because tetraconazole is one of many DMI/triazole fungicides with the same mode of action in fungi, and similar effects on human health and 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 applied 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, tetraconazole 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 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

[2] EPA vastly understates tetraconazole usage, especially on soybeans, as 30,000 lbs/year on less than 1% of acreage. EPA has also failed to make its own latest usage estimates available in the docket, as it said it would (EPA 2021, p. 8 and footnotes).
[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).
[6] 7 U.S.C. §136a(c)(7)(C).

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 *or indirectly* 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 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]

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[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).
[16] 16 U.S.C. § 1536(c)(1); *see also* 50 C.F.R. § 402.12(c).
[17] *Id*.

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]

COMMENTS

Human Health Concerns and Assessment Deficiencies

Thyroid Toxicity

Several registrant animal studies demonstrate that tetraconazole has negative impacts on the thyroid. In a 28-day inhalation study, rats exhibited follicular cell hypertrophy of the thyroid at both mid and high doses. Rats treated orally with tetraconazole for two years developed thyroid follicular cell tumors in a dose-related manner that narrowly missed the conventional measure of statistical significance (EPA 1/11/00, Table 1).[22] Despite this clear evidence of adverse, systemic thyroid effects in studies by two different routes of exposure and with vastly different durations, EPA has failed to even take the minimal step of gathering additional data to further investigate this potentially serious issue (EPA 12/11/20, p. 15).

EPA inexplicably decided against requiring a comparative thyroid assay, and has not even begun the process of requiring that tetraconazole be tested for endocrine-disrupting effects on the thyroid [or sex] hormonal systems, as required by the Food Quality Protection Act, ***a law enacted 25 years ago.***[23] The Agency has also failed to collect any data on how much

[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).
[22] The p value for trend was 0.089, signifying a better than 9 in 10 chance that the tumors were ***not*** due to chance (were due to tetraconazole), just failing to meet the conventional "statistical significance" cutoff of p = 0.05, where associations are rejected unless there is a 19 in 20 chance (or better) of the results being due to the treatment. For a critique of the p = 0.05 standard as arbitrary, see Amrhein et al. (2019) in *Nature*, the world's leading science journal.
[23] Tetraconazole is absent from both the First List and Second List of chemicals for Tier 1 Screening under the Agency's Endocrine Disruptor Screening Program, see https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-chemical-screening-and-testing-progress.

tetraconazole workers absorb into their systems via dermal contact (discussed further below), and thus has no estimate of the internal systemic dose workers receive via all routes combined.

The decision to waive the comparative thyroid assay was made by the Agency's Hazard and Science Policy Council (HASPOC). In a 2018 report that purports to detail "process improvements in the pesticide program," EPA brags that HASPOC "was very active again in 2018," with its major activity being to "review[] data waiver requests for a variety of toxicity studies, primarily for comparative thyroid assay (CTA) … [among other] … studies" – the very study waived here for tetraconazole. The measure of HASPOC's success is the number of waivers it grants – 62 of 71 waiver requests were granted in 2018 – and the associated cost savings for pesticide companies: $8.9 million (EPA 2018, p. 2). That EPA's Office of Pesticide Programs takes pride in ***actively reducing*** the amount and quality of toxicological information upon which it bases critical pesticide decisions is a sad commentary on the Agency's priorities.

Liver toxicity

The liver is tetraconazole's major target organ in dog, mouse, and rat studies. Long-term exposure in dogs caused increased liver weight, hepatocyte enlargement, marked centrilobular fat, eosinophilic intracytoplasmic inclusion in hepatocytes, centrilobular hepatocyte rarefaction, increased levels of liver enzymes and cholesterol, decreased albumin, and proteinuria. Tetraconazole in mice induced an increase in absolute and relative liver weights, hepatocellular hypertrophy, fat deposition, granulomatous inflammation, single liver cell degeneration and necrosis, bile duct hyperplasia, increased liver enzymes, and hepatocellular tumors. Tetraconazole elicits many of the same effects in rat studies: increased liver weight, enlarged livers and centrilobular hepatocytes, as well as increased liver enzyme levels.

Carcinogenicity

EPA originally classified tetraconazole as "likely to be carcinogenic to humans" based on tetraconazole's induction of liver tumors in mice; and the finding that structurally related compounds – namely ***six of nine other*** triazole fungicides – ***also*** induced hepatocellular tumors in mice (EPA 1/11/00). EPA also rejected the theory presented by the registrant – that tetraconazole only caused liver tumors to form in mice above a certain threshold dose, via induction of liver enzymes – for lack of evidence, and because rats fed tetraconazole did not develop liver tumors, despite also exhibiting elevated liver enzyme levels (EPA 1/11/00).

In calculating cancer risk, EPA rejected the threshold dose approach, and embraced instead linear low-dose extrapolation, which assumes that increased risk of cancer from exposure scales with dose, without any minimum threshold dose below which the agent (here, tetraconazole) would ***not*** induce additional cancers (EPA 1/11/00, EPA 2005).

Thirteen years later, EPA reversed its cancer designation to "Not likely to be carcinogenic to humans at levels that do not cause increased cell proliferation in the liver" (EPA 12/11/20). EPA changed its designation despite not receiving any explanation as to why elevated liver enzyme levels in rats did not induce cancer; and accepted the "threshold dose" it had previously rejected for lack of evidence.

Other adverse effects

In the rat developmental study, tetraconazole caused supernumerary ribs to form in pups at a dose lower than that causing adverse maternal animals. In the two-generation rat study, tetraconazole decreased litter weight and mean pup weight in all generations prior to weaning, and decreased mean litter size and number of pups in the F1A generation.

Toxicity of Metabolites Unknown

EPA has identified five major metabolites of tetraconazole, but has collected no toxicity data on any of them. EPA guesstimates the toxicity of degradates based on modeling with the Ecological Structure Activity Relationships (ECOSAR) model, on the grounds that ECOSAR-predicted toxicity values for parent tetraconazole were on the same order of magnitude as experimentally-derived values with respect to some aquatic organisms. EPA found that ECOSAR-guesstimated toxicity of two of the five degradates was similar to parent, but then ruled them out as residues of concern based on ECOSAR toxicity estimates and the percent of parent compound they represent.

ECOSAR has clear limitations. First, it is intended only for predicting the toxicity of compounds to aquatic, not terrestrial organisms, yet EPA excluded degradates as residues of concern in terrestrial risk assessments as well. Second, ECOSAR-predicted values often deviate from empirically-derived toxicity data, even for aquatic organisms, as in the case of trifludimoxazin. With no empirical data for any degradate, EPA's reliance on ECOSAR values to exclude them from risk assessments is unwise, and risks underestimating the risks posed by tetraconazole. Finally, EPA itself admits ECOSAR is suitable only for rough "screening-level assessments,"[24] and thus cannot substitute for quality empirical data.

Dermal Absorption and Risks to Workers

EPA nowhere considers aggregate systemic exposure to tetraconazole. Instead, the Agency's risk assessments are based on incomplete, single-route exposure scenarios. In the case of consumers, EPA assesses dietary exposure (residues in food and water). In the case of workers, EPA considers only inhalational exposure. But workers who handle, mix, spray, and otherwise utilize this fungicide will obviously get some on their skin as well as in their lungs, and thus the occupational risk assessment must aggregate systemic exposure via these two routes.

[24] https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-ecosar-predictive-model.

EPA cannot do this, because it failed to collect even one appropriate study from the registrant (as per 40 C.F.R., Part 158.500, Guideline No. 870.7600, Dermal Penetration Study). This failure on the Agency's part explains why "[n]o dermal absorption study is available in the database for tetraconazole." Instead, the Agency substituted a surrogate value for a ***different*** triazole fungicide, mefentrifluconazole, which has 32% structural ***dis***similarity to tetraconazole (EPA 12/11/20, p. 16). This is obviously illegitimate.

Moreover, even if it were legitimate to use a surrogate dermal absorption factor for a different pesticide (it is not), EPA's use of it here for tetraconazole was absurd. Instead of using it to calculate the total internal (systemic) dose a worker receives from combined inhalational + dermal exposure, and then relating that to tetraconazole's various hazards ***to assess risk to workers***, EPA bizarrely uses it to calculate the "dermal only" exposure that would cause harm to rats; and because that exposure level is high, EPA blithely assumes no human health risk, without troubling itself to conduct a quantitative risk assessment (EPA 12/11/20, p. 19).

EPA must collect dermal penetration studies for each tetraconazole formulation at issue in this proposed interim registration decision, and then use the resulting dermal absorption factors to calculate dermal dose from occupational use. That dermal dose is then added to the inhalational dose to obtain the aggregate systemic exposure needed to assess risk to workers.

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 similar chemical structures, 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 tetraconazole 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, among other adverse liver effects (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, many of them triazoles, caused fatty changes in the liver. 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	
Tetraconazole	US, EU	
Epoxiconazole	EU	
Flusilazole	US, EU	
Hexaconazole	US	
Metconazole	EU	
Paclobutrazole	US	
Propiconazole	US, EU	
Prothioconazole	EU	
Tebuconazole	EU	
Tetraconazole	US, EU	For US, see EPA (12/11/20), e.g. p. 16: "fat deposition," "marked centrilobular fat"
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.

Tetraconazole was shown to induce marked centrilobular fat and fat deposition in the livers of dogs and mice, respectively, and hepatocellular vacuolation in mice (EPA 12/11/20, pp. 16, 41-43). Difenoconazole, 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 (PPARα), 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 and 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.

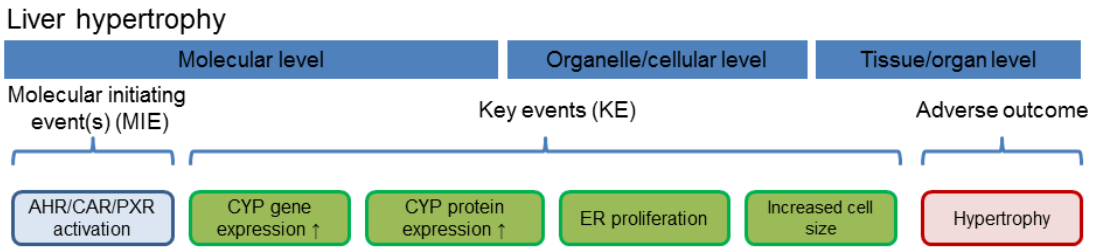

Figure 2. Schematic delineation of a nuclear receptor-dependent molecular pathway leading to hepatocellular hypertrophy. Nuclear receptor activation functions as molecular initiating event. Abbreviation: ER, endoplasmic reticulum.

Figure 2. Adverse Outcome Pathway for Liver Hypertrophy. Source: Marx-Stoelting et al. (2020).

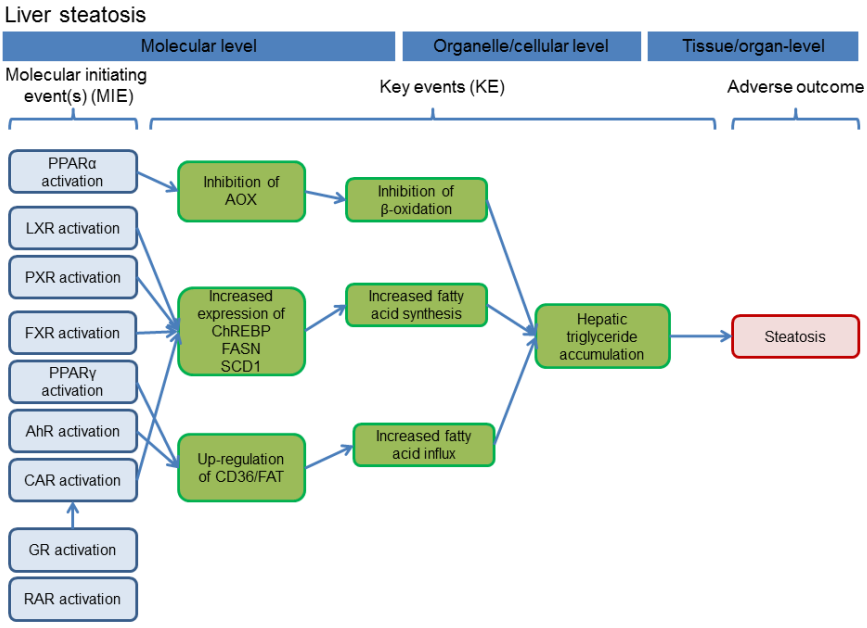

Figure 3. Schematic delineation of the AOP for hepatocellular steatosis. The figure was adapted from [58]. Abbreviations: FXR, farnesoid-X-receptor, GR, glucocorticoid receptor.

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 PPRAα, 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 2000, when tetraconazole was first registered. The Agency's Carcinogenicity Peer Review Committee noted that tetraconazole was one of the seven triazole fungicides (out of 10) that induced liver tumors in mice (EPA 1/11/00). EPA had recognized and properly assigned weight to this striking common effect of triazole herbicides as long ago as 1994, in an assessment of difenoconazole: "Difenoconazole 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). Six years later EPA made a similar argument to support its likely to be carcinogenic designation of tetraconazole (EPA 1/11/00). 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	
Tetraconazole	US, EU	
Epoxiconazole	EU	
Etaconazole	US	
Fenbuconazole	US	
Flusilazole	EU	
Metconazole	EU	
Propiconazole	US, EU	
Tebuconazole	US, EU	
Tetraconazole	US, 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.

Cumulative Risk Assessment of Tetraconazole with Other Thyroid Toxins

As described above, tetraconazole caused thyroid follicular cell hypertrophy and/or tumors in inhalation and carcinogenicity studies on rats. In a review of pesticide toxicology submissions and assessments of them by European regulators, Nielsen et al. (2012) assigned tetraconazole to a cumulative assessment group for thyroid toxicity, and to subgroups for pesticides that: 1) decreased serum levels of T3 and/or T4 hormones, 2) increased levels of thyroid-stimulating hormone (TSH), and 3) caused follicular cell hypertrophy/hyperplasia. The

European Food Safety Authority has also assigned tetraconazole to a cumulative assessment group for pesticides having adverse effects on the thyroid – namely hypothyroidism – and recommends a variety of different tests (e.g. analysis of T3, T4 and TSH hormone levels in repeat-dose animal studies) to ascertain whether liver enzyme induction is responsible for the thyroid effects, and to help determine their human relevance (EFSA 2019). As discussed above, EPA for some reason waived such additional studies to better elucidate the thyroid toxicity of tetraconazole.

While the cumulative assessments of triazole fungicides and their 1,2,4-triazole degradate discussed above are based on groups of compounds with structural similarity, it is interesting to note that the cumulative assessment groups for thyroid toxicity include quite dissimilar pesticides. European regulators are far ahead of EPA in this respect. While EPA has yet to even conduct an obviously needed cumulative assessment of triazoles (performed in 2009 by EFSA), European regulators are moving beyond structural similarity as the primary basis for constructing cumulative assessment groups, and working to base such groups on common toxic effects, and modes/mechanisms of such effects, irrespective of structure. The next necessary step would be to broaden cumulative assessments beyond pesticides, to encompass the universe of industrial chemicals to which humans are exposed.

Agricultural Triazole Use 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).

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).

A recent literature review found that 1,292 azole-resistant isolates of *A. fumigatus* had been identified worldwide, over one-third of which were from agricultural environments (Burks et al. 2021). Of the total, 57% were detected in soil, 17% in air, 11% in plant debris and 9% in compost (Ibid.). The intensity of agricultural triazole use is highest in European countries, particularly the Netherlands; it is no coincidence that this is where the majority of resistant *A. fumigatus* strains, and especially those from agricultural environments, have been found (Ibid.).

Resistance is also beginning to emerge in the United States, where *A. fumigatus* strains with environmental-origin resistance mutations have been isolated from clinics since 2015 (Hurst et al. 2017). Resistance is detected in agricultural environments as well. Hurst et al. (2017) found triazole-resistant, TR34/L98H strains of *A. fumigatus* in the crop debris, soil, and compost of Georgia peanut fields with a history of triazole exposure. Kang et al. (2020) isolated resistant strains with the other major environmental-origin mutation, TR46/Y121F/T289A, from samples taken from a strawberry field, pecan debris, and a compost pile (source plants not identified) in 56 sites in Georgia and Florida.

Importantly, Kang et al. (2020) confirmed the agricultural origin of clinically relevant, azole-resistant *A. fumigatus* strains. They did this by establishing that some strains collected from both clinical and agricultural settings had *additional* resistance to one or both of two classes of fungicide – quinone outside inhibitors (QoI's) and benzimidazoles – that are only used in agriculture.

Interestingly, the sites of agriculture-origin, azole-resistant *A. fumigatus* strains discovered in the U.S. thus far – pecans, peanuts, and strawberries – match three major uses of tetraconazole (and likely other triazole fungicides), including the two uses with the highest

permitted application rates (pecans: up to 0.126 lbs/acre, up to 4 applications per year; peanuts: up to 4 applications per season of 0.102 lbs/acre).

EPA must assess the public health threats posed by continued and expanding use of tetraconazole and other agricultural triazoles in terms of increasing resistance of human fungal pathogens

Tetraconazole's Environmental Persistence

A key aspect of this fungicide's threat is its extreme persistence in the environment, which according to one review is second only to flutriafol among triazole fungicides (Roman et al. 2021).

Tetraconazole is extremely persistent in multiple laboratory and field tests, in soil and water. It is stable to abiotic hydrolysis (half-life 8,402 days); does not break down at all in aerobic soil metabolism studies (half-life: 44,126 days); and degrades very slowly in aquatic metabolism tests, with half-lives of 320-383 and 8,123 days in aerobic and anaerobic studies, respectively (EPA 12/2/20). While photolysis half-lives in both soil and water are considerably shorter, much of the tetraconazole in the environment will be shielded from light in the soil, and at deeper depths and sediments of aquatic bodies.

Terrestrial dissipation of tetraconazole is also a slow process. Roman et al. (2021) cite DT_{50} values of 136 days to 4.6 years; and a range of DT_{90} values from over 1 to more than 15 years. EPA also finds tetraconazole dissipates slowly in the field, with half-lives ranging from 91 to 800 days, and concludes: "These results indicate that tetraconazole has the potential to accumulate in soil with successive annual applications" (EPA 12/2/20, p. 19). Tetraconazole is also persistent in aquatic environments, and will migrate to and persist in benthic sediments (Ibid., p. 60).

Critically, EPA's exposure and risk assessments do not appear to account for the accumulation of tetraconazole 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.

To the extent EPA assumes tetraconazole will bind to soil organic matter or other soil components and thereby be rendered unavailable or neutralized, it is important to understand that we cannot predict with any certainty that tetraconazole residues that are bound today will remain so in the future, particularly in the context of multiple additions of numerous long-lived compounds to the soil over time (Barraclough et al. 2005).

Environmental Impacts and Assessment Deficiencies

The rising use of tetraconazole is having unacceptable environmental impacts, including but not limited to threatened and endangered species. Unless otherwise noted, the following discussion is based on EPA (12/2/20).

Risks to Aquatic Organisms

EPA finds that tetraconazole is highly toxic to estuarine/marine invertebrates on an acute basis; and that chronic exposure shifts sex ratios in freshwater fish and reduces growth in estuarine/marine fish. Tetraconazole's lipophilicity (log K_{ow} = 3.56) means it could bioconcentrate in aquatic food webs. Interestingly, Roman et al. (2021) find that tetraconazole is the most lipophilic of 14 triazoles, with a logP value of 4.4. Assuming this partition coefficient (P) is equivalent to the n-octanol/water coefficient (K_{ow}) cited by EPA, tetraconazole might be more lipophilic than suggested by the registrant's study. This in turn would suggest a greater potential to bioaccumulate in fish than the bioconcentration factor in rainbow trout reported by the registrant of 39-42 L/kg wet weight, casting doubt on that study.

Tetraconazole exhibited synergistic acute effects on the rare minnow (*Gobiocypris rarus*) in binary mixtures with thiamethoxam or beta-cypermethrin, highlighting the importance of considering real-world situations in which aquatic organisms are exposed to multiple pesticides (Yang et al. 2021).

Risks to Terrestrial Vertebrates and Plants

EPA assessed three tetraconazole use scenarios: grapes, sugar beets, and pecans. The Agency found a likelihood of acute and chronic adverse effects on birds, reptiles, and terrestrial-phase amphibians; and chronic risks of concern for mammals (increased mortality and increased gestation time) as well. The chronic risks apply in particular to birds and mammals that feed on aquatic prey, given the potential for tetraconazole to bioaccumulate. Risks to terrestrial plants appear low based on registrant study results, but a significant 2016 incident suggests tetraconazole formulations may have the potential to harm plants as well. Sprayed together with compounds unlikely to harm plants on 97 acres of a grape vineyard, tetraconazole-based Mettle 125 may have been responsible for or contributed to 67 acres of blemished fruit (EPA 12/2/20, p. 60). Whether tetraconazole, additional ingredients in Mettle 125 ("other ingredients" comprise 88.4% of Mettle 125), or the combination, this incident illustrates the need for EPA to assess pesticides by formulation rather than by active ingredient only.

Risks to Terrestrial Invertebrates

Honey bees are also threatened by tetraconazole on a chronic basis. Adults exposed to just 0.21 ug/bee (the lowest dose tested) experienced a 10.2% decline in food consumption, while the next higher dose of 0.86 ug/bee resulted in 50% mortality. A safe level of exposure (NOAEL) was not determined in this study. Registrants submitted three chronic oral toxicity studies for honey bee larvae. Neither of the two used to characterize chronic risk established a "safe dose" (NOAEL) either, since significant adverse effects were noted at the lowest dose in each study: 1) 23% increased mortality at 32 ug/larva in one study, and 2) 22% reduction in adult emergence at 1.63 ug/larva in the other. The registrant also conducted two semi-field tunnel studies that were deficient and could not be used due to serious deficiencies in their execution.

EPA modeled exposure of adult worker honey bees to tetraconazole in nectar in various treated crops that are attractive to bees (EPA 12/2/20, Table 10-3, pp. 58-59). Based on these EECs and the chronic endpoint (LOAEL of 0.21 ug/bee, since no NOAEL was available), one can calculate pseudo risk quotients of: >2.0 for corn (pollen), >6.2 for berry vines, >9.0 for fruiting vegetables, >9.5 for cucurbit vegetables (squash family), >11.4 for soybeans, and >15.2 for canola, peas, beans and peanuts.[25]

Tetraconazole residues have been detected in pollen and in bees themselves, along with many other pesticides (Roszko et al. 2016, Chauzat et al. 2009). Tetraconazole has been shown to synergize the toxicity to honey bees of nine different pyrethroid insecticides upon 7-day oral exposure; synergism was especially pronounced in binary mixtures with either lambda-cyhalothrin or bifenthrin (Wang et al. 2020). Pilling and Jepson (1993) showed that the acute contact toxicity of lambda-cyhalothrin was synergized to varying degrees by nine different ergosterol biosynthesis inhibiting (EIB) fungicides in tests involving binary mixtures of the pyrethroid and each fungicide, with topical application of the respective mixture at typical application rates.

Azole fungicides have been shown to synergize non-pyrethroid insecticides as well. When sprayed on honey bees, a binary mixture of tetraconazole and imidacloprid synergistically increased the lethality of imidacloprid by 20% (Zhu et al. 2017). Raimets et al. (2018) found that the EIB fungicide imazalil increased the lethality to bumblebees of fipronil and thiamethoxam as well as the pyrethroid cypermethrin. The mechanism with respect to pyrethroids and perhaps the other insecticides is EIB fungicides' well-known inhibition of detoxifying cytochrome P450 enzymes in bees and other organisms (Cedergreen 2014).

In a study conducted in the United Kingdom, both neonicotinoids and fungicides were detected frequently in the pollen of oilseed rape and nearby wildflowers (David et al. 2016). They were also detected in pollen collected by honey bees and bumblebees and stored in colonies and nests, respectively, placed in the vicinity of the oilseed rape fields. Fungicides, including EIB fungicides like tebuconazole, comprised the majority of pesticide residue in pollen of both honey bees and bumble bees. However, bumblebee pollen had higher levels of pesticide residues, perhaps reflecting greater exposure to fungicides due to their ground-nesting habit, and bumblebees are thus likely even more threatened by EIB fungicides than honey bees.

Fungicides in honey bee pollen end up in bee bread, and have been shown to reduce the levels of beneficial fungi that ferment bee bread, with potentially adverse effects on larval and colony health, including reduced protection from microbial pathogens (Yoder et al. 2013).

EPA must go beyond collecting new studies on tetraconazole's toxicity to bees, and assess the impact on bees and other terrestrial invertebrates of aggregate exposure to azole fungicides, and these fungicides in combination with insecticides whose toxicity they synergize.

[25] Pseudo-risk quotients because they are derived by dividing the EEC by the LOAEL, and since the unknown NOAEL will be lower than the LOAEL, the pseudo-risk quotients will be smaller than the actual ones.

Adverse Impacts on Soil Health

EPA must assess the impacts of tetraconazole and triazoles fungicides, cumulative, on soil microbiota and soil health generally (Roman et al. 2021). For instance, there are obviously concerns that tetraconazole and other fungicides of its class could suppress the abundance and diversity of mycorrhizal fungi that are so critical for the nutrition and health of most plant species (e.g. Kjoller and Rosendahl 2000).

In short, tetraconazole poses serious ecological risks to several taxa, including mammals, birds and aquatic organisms. Its extreme persistence and lipophilicity exacerbate these threats, and mean it likely will bioaccumulate in aquatic food webs, and in sediments. Tetraconazole cannot be adequately assessed without a cumulative assessment of azole fungicides that have similar adverse effects, particularly suppression of detoxification mechanisms in bees. This suppression renders honey bees, bumblebees, and likely many other beneficial insects more susceptible to the toxic effects of pyrethroid and other insecticides. Finally, EPA should examine the effects of tetraconazole and other azole fungicides on beneficial soil microbiota, particularly mycorrhizal fungi.

Costs and Benefits

Putative benefits

Roughly 80% of tetraconazole use in the U.S. is on soybean and corn (Fig. 1). 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. Another reason is bad agronomic practice – increased planting of corn-on-corn, which increases disease risk (Robertson and Mueller 2007). 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 and its application (Robertson and Mueller 2007).

EPA also needs to factor in alternatives to tetraconazole for disease control. In fact, an array of cultural practices like crop rotations and intercropping can greatly reduce fungal disease pressure and thus reduce or eliminate the "need" for fungicide treatments (Liebman and Wallace 2019). For instance, rotating strawberries with broccoli has proven to be an effective strategy to mitigate harm from the fungal disease Verticillium wilt (Shetty et al 1999).

Costs

Resistance to triazole/DMI fungicides has been building steadily over years, and together with widespread resistance to strobilurin and other classes of fungicide 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 tetraconazole – as for "plant health" reasons – must be avoided at all costs to stem or at least slow resistance development. The costs of resistance in agricultural practice are dwarfed by the human costs (i.e. deaths) resulting from the growing resistance to antifungal drugs in fungal pathogens that is attributable in part to intensive use of tetraconazole and other triazoles (discussed above).

Tetraconazole's use on soybeans has risen dramatically since 2004 (essentially zero) to 2020 (about 267,000 lbs./year) (see Figure 1 and USDA NASS figures cited above). The fact that this large amount is applied to just 4% of soybean acres, with a rising trend, suggests the likelihood of dramatically increased use on this crop, often for no good reason.

Tetraconazole in corn first registered in 2013, and is also trending upward (Fig. 1). Thus, the area sprayed with tetraconazole or some other triazole fungicide every year in the common corn-soybean rotation is rising sharply (Toda et al. 2021, Toda et al. 2021 Supplemental). This will intensify 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 at all. 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 has admitted have no impact on the risks of concern. They are entirely inadequate to the task of reducing any of the risks tetraconazole 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 tetraconazole with this registration review decision far outweigh any putative and highly dubious benefits. This holds in particular for uses on soybeans and on corn, which should be canceled.

Threatened and Endangered Species

EPA has not completed an assessment of tetraconazole 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 tetraconazole 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.

Tetraconazole 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 tetraconazole" even though EPA "is not making a complete endangered species finding at this time."[26] However, without a full analysis and ESA consultation EPA cannot determine the full impacts of tetraconazole 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 tetraconazole in its proposed interim decision, and must revisit its assessment prior to any final decision. With regard to human health, CFS urges EPA to gather more data to assess tetraconazole's thyroid toxicity, and the toxicity of its degradates; to conduct a cumulative assessment of triazole fungicides for liver toxicity (steatosis and carcinogenicity) and adverse thyroid effects; to gather reliable data on dermal absorption of tetraconazole and use it together with inhalational exposure to assess occupational risk; and to complete its long overdue assessment of the 1,2,4-triazoles by collecting the needed studies. With regard to the environment, EPA must assess tetraconazole's effects on a variety of bee species (including ground-dwelling bumblebees) and other terrestrial invertebrates, particularly in combination with pyrethroid and other insecticides with which it exhibits synergy. EPA should investigate the impact of tetraconazole and azoles cumulatively on fungi that ferment bee bread and the consequences for honey bee colony health; and on beneficial soil microbes, particularly mycorrhizal fungi.

EPA must also assess the role tetraconazole 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 tetraconazole to threatened and endangered species, beginning with consultation with the expert agencies.

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Center for Food Safety

[26] Proposed Interim Registration Decision at 19.

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