September 12, 2022

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

RE: Docket EPA-HQ-OPP-2015-0378
Comments on proposed interim registration decision for tebuconazole

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

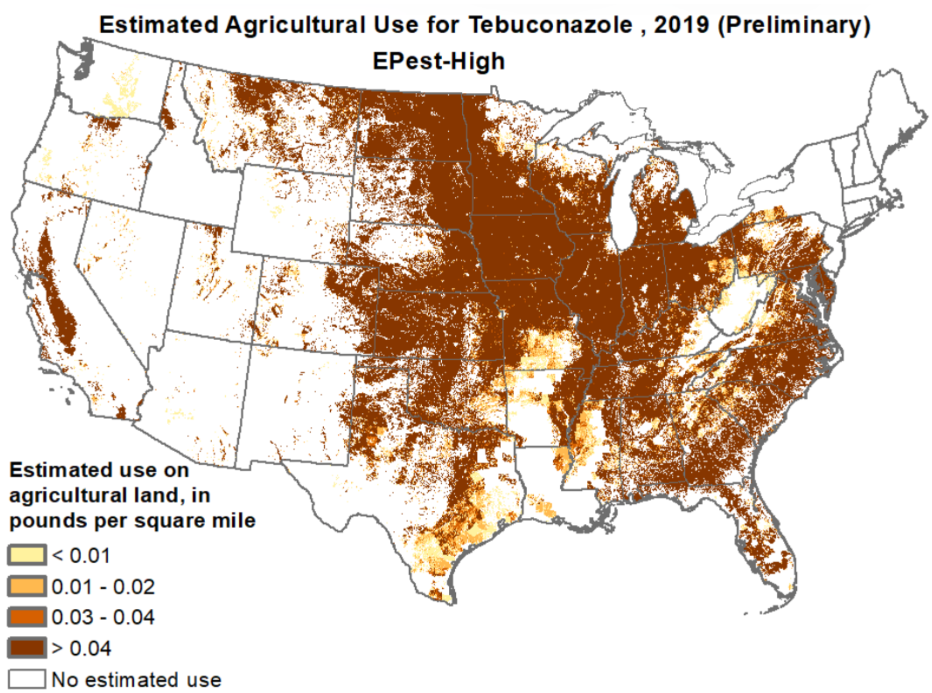
Introduction

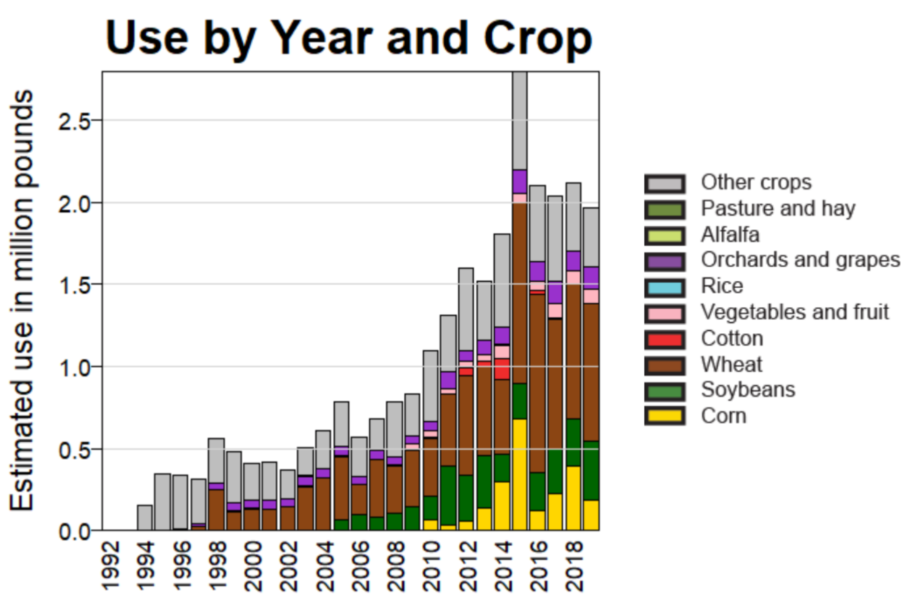
Tebuconazole is a broad-spectrum, systemic fungicide that stops the growth of fungi by blocking the synthesis of sterols, key components of fungal cell walls. It belongs to the triazole class of demethylase inhibitor (DMI) fungicides, and in particular inhibits the CYP51 enzyme.

Tebuconazole is registered for use on many fruits, vegetables, tree nuts, oilseeds and cereal grains, as well as turfgrass and ornamentals. It can be applied aerially, by ground or handheld equipment, or via tree injection or sprinkler irrigation. Tebuconazole is sprayed onto foliage; coated onto seeds of barley, dry beans, oats, rye, soybean, triticale and wheat; and applied post-harvest to fruit. Total agricultural use on crops has risen from a few hundred thousand lbs./year in the 1990s to roughly 2 million lbs/year today (excluding seed treatments). Approximately 12.5 million total acres are treated, with wheat (8 million acres) and peanuts (2.2 million acres) comprising roughly 80%.

Tebuconazole is also used extensively as an antimicrobial to treat numerous wood products as well as plastics, glues and adhesives, among other industrial uses.

Several features of tebuconazole and its use deserve particular consideration. First, because it 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, as of 2016 tebuconazole was the second-most heavily used triazole fungicides, second only to propiconazole, and triazole use overall is dramatically increasing. There are at least 15 DMI/triazole fungicides applied in the U.S., and their collective use (excluding seed treatments) was nearly 7-fold greater in 2016 than in 1992, and has risen more than 5-fold (434%) since just 2006 (Toda et al. 2021). Finally, tebuconazole and other members of its class are quite persistent in the environment.





Source: US Geological Survey, https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2019&map=TEBUCONAZOLE&hilo=L&disp=Tebuconazole.

Relevant Legal Standards

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

Under FIFRA, EPA licenses the sale, distribution, and use of pesticides, including herbicides, through the process of registration.[1] EPA can register a pesticide only upon determining that "it will perform its intended function without unreasonable adverse effect on the environment,"[2] and that "when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment."[3] 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."[4]

FIFRA's registration review process is mandated at 7 U.S.C. § 136a(g). FIFRA requires that pesticide registrations are periodically reviewed, and that EPA "shall by regulation establish a procedure for accomplishing the periodic review of registrations."[5] EPA adopted regulations pursuant to this provision in 2006, which state that each pesticide is required to be reviewed every 15 years.[6] Registration review is intended to ensure that each active ingredient's registration is based on current science, including its effects on human health and the environment. If a product "fails to satisfy the FIFRA standard for registration, the product's registration may be subject to cancellation or other remedies under FIFRA."[7]

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."[8] The ESA's statutory scheme "reveals a conscious decision by Congress to give endangered species priority over the 'primary missions' of federal agencies."[9] Federal agencies are obliged "to afford first priority to the declared national policy of saving endangered species."[10]

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.[11] The ESA's implementing regulations broadly define agency action to include "all

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[1] 7 U.S.C. § 136a(5)(D).
[2] *Id*. § 136a(c)(5)(C).
[3] *Id*. § 136a(c)(5)(D).
[4] *Id*. § 136(bb).
[5] *Id*. § 136a(g)(1)(A).
[6] 40 C.F.R. §§ 155.40-155.58.
[7] *Id*. § 155.40(a).
[8] *Tenn. Valley Authority v. Hill*, 437 U.S. 153, 180 (1978).
[9] *Id*. at 185.
[10] *Id.*
[11] 16 U.S.C. § 1536(a)(2); *see also* 50 C.F.R. § 402.01(b).

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."[12] In June 2022, the Ninth Circuit confirmed that interim registration review decisions are affirmative agency actions for which consultation is required. *Nat. Res. Def. Council v. EPA*, 2022 WL 2184936, at *18 (9th Cir. June 17, 2022). 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."[13]

EPA is required to review its actions "at the earliest possible time" to determine whether the action may affect listed species or critical habitat.[14] 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."[15] 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.[16]

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.[17] 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.[18] 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).[19]

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."[20]

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[12] 50 C.F.R. § 402.02 (emphasis added).
[13] 16 U.S.C. § 1532(5)(A).
[14] 50 C.F.R. § 402.14(a).
[15] 16 U.S.C. § 1536(c)(1); *see also* 50 C.F.R. § 402.12(c).
[16] *Id.*
[17] 50 C.F.R. § 402.14.
[18] 16 U.S.C. § 1536(b); 50 C.F.R. § 402.14.
[19] 16 U.S.C. § 1536(b)(3)(A).
[20] 16 U.S.C. § 1536(d).

Human Health Concerns and Assessment Deficiencies

Liver toxicity

The liver is a primary target organ of tebuconazole in four species of test animals: rat, mouse, dog and rabbit (EPA 3/18/21, p. 17; EPA 11/15/17, p. 30, Table A.2.2; EPA 6/29/15, pp. 29, 32). In various studies, tebuconazole was shown to induce increased liver enzyme activities, increase liver weight and the liver/body weight ratio, increase triglyceride levels as well as the severity of hepatic vacuoles, and increase lipid deposition/induce lipidoses; tebuconazole administration was also associated with pale lobular livers in mice. In a mouse carcinogenicity study, histopathology of the mouse liver revealed hepatocyte hypertrophy, eosinophilic foci of hepatocyte alteration and other microscopic alterations; rabbits treated with tebuconazole exhibited single cell necrosis in liver cells, which in females occurred in every doe examined in each of the three treatment groups (5 of 5) (EPA 3/18/21, Table A.2.2, pp. 82-84; EPA 6/29/15, pp. 29-32, 66). Most concerningly, tebuconazole induces both hepatocellular adenomas and carcinomas in mice (EPA 11/15/17, Table A.2.2, p. 31; EPA 3/18/21, Table A.2.2, p. 84). Independent studies also show tebuconazole induces hepatocellular hypertrophy as well as hepatocellular steatosis (Knebel et al. 2019).

These mammalian findings are supported by evidence in fish studies. In the fish short-term reproduction assay, dose-dependent hepatocyte necrosis occurred in the liver of females in all three treatments, and occasionally in males; both sexes exhibited microgranulomas, while in females there were increases in multinucleated hepatocytes and hepatocyte megalocytosis (EPA 6/29/15, pp. 28-29). Hepatocyte necrosis and similar lesions were also reported in the amphibian metamorphosis assay (Ibid., p. 28). In the fish sexual development test involving fathead minnows, hepatocellular hypertrophy, pleopmorphism of liver cells and nuclei, liver cell degeneration and/or significant inflammations of the liver were observed at doses ranging from 5.8, to 11.1 to 22.1 ppb (Ibid., p. 78).

Developmental toxicity

Tebuconazole is also a developmental toxin, as evidenced by developmental studies in rats (delayed ossification of several bones and increased numbers of fetuses with supernumerary ribs); mice (skull and neural tube defects, such as cleft palate, micrognathia, and parietal bone dysplasia); and rabbits (acrania, spina bifida; increased resorptions and post-implantation losses, decreased live fetuses/does, and external and skeletal abnormalities) (EPA 3/18/22, Table A.2.2, pp. 82-83).

In a rat developmental neurotoxicity (DNT) study, adverse developmental effects were observed at the lowest offspring dose of 8.8 mg/kg/day, effects that included decreases in body weights, body weight gains, and absolute brain weights in both sexes; decreases in auditory startle amplitude, decreases in motor activity (males), and decreases in the size of the anterior/posterior cerebrum (EPA 11/15/17, Table A.2.2, p. 31; EPA 6/29/15, pp. 70-72).

As recently as 2015, this rat DNT study served as the basis for a chronic reference dose (cRfD) of 0.0088 mg/kg/day, derived by applying the usual interspecies (10x) and inter-

interindividual (10x) safety factors as well as an additional 10x factor to account for lack of an NOAEL (EPA 6/29/15, p. 36).

Endocrine disruption

Tebuconazole has been repeatedly documented to be an endocrine-disrupting compound (e.g. Hass et al. 2012,). The EPA likewise concluded tebuconazole disrupts the estrogen and androgen pathways in mammals and fish, while many independent studies show tebuconazole also disrupts the thyroid endocrine system (EPA 6/29/15, Li et al. 2019a,b). EPA has apparently failed to collect a Medaka extended one-generation reproduction test, as recommended in 2015 in the Agency's assessment of tebuconazole's endocrine disrupting activity (EPA 6/29/15, pp. 3, 36).

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 a cumulative assessment group for liver toxicity as the chronic endpoint (EFSA 2009).

A review of registrant studies submitted to European regulators found that tebuconazole and all or most of 10 other triazole fungicides that were reviewed induced hepatocellular hypertrophy, hepatic cell degeneration or death, fatty changes, inflammation and foci of cellular alteration in the liver, 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 in the liver

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	
Difenoconazole	US, EU	
Epoxiconazole	EU	
Flusilazole	US, EU	
Hexaconazole	US	
Metconazole	EU	
Paclobutrazole	US	
Propiconazole	US, EU	
Prothioconazole	EU	
Tebuconazole	EU, US	See discussion above under Liver Toxicity and cited sources for evidence.
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, unless otherwise noted. 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.

Tebuconazole, along with difenoconazole and propiconazole, was 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 tebuconazole and propiconazole (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.

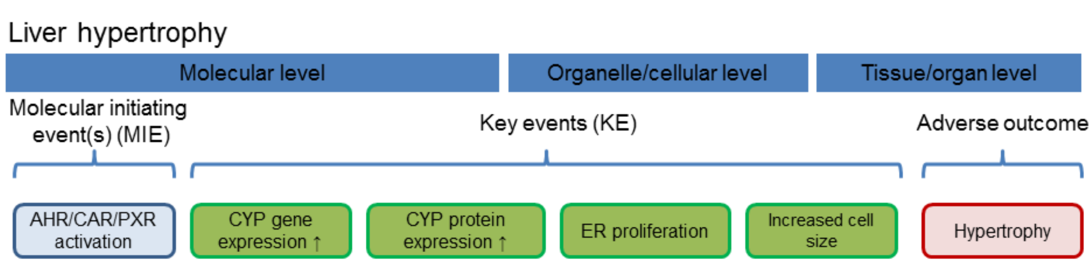

Figure 2. Schematic delineation of a nuclear receptor-dependent molecular pathway leading to hepatocellular hypertrophy. Nuclear receptor activation functions as molecular initiating event. Abbreviation: ER, endoplasmic reticulum.

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

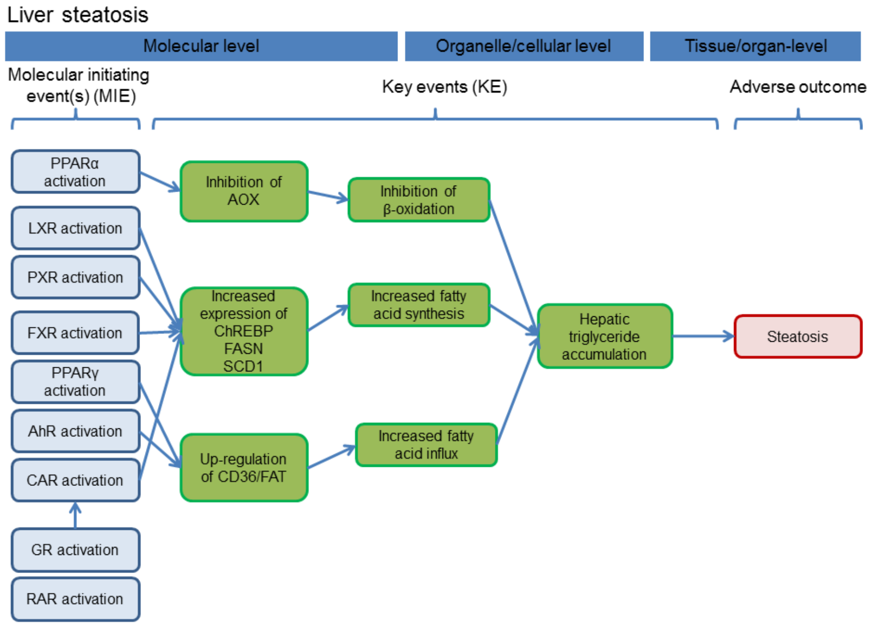

Figure 3. Schematic delineation of the AOP for hepatocellular steatosis. The figure was adapted from [58]. Abbreviations: FXR, farnesoid-X-receptor, GR, glucocorticoid receptor.

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 precisely 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.

Liver Tumors

A second endpoint for which EPA must cumulatively assess triazoles is carcinogenicity. EPA itself recognized the need for this in 1994, when another triazole fungicide, difenoconazole, was first registered. The Agency's Carcinogenicity Peer Review Committee noted that difenoconazole was one of nine triazole fungicides (out of 12) that induced liver tumors in rodents (EPA 7/27/94, p. 3 and Table 6). 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	
Difenoconazole	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.

Developmental toxicity

EPA should also establish a cumulative assessment group for the developmental effects of triazole herbicides, given their common inducement of developmental abnormalities, particularly in nervous system development, as described above. We would note that EFSA –

besides finding triazoles shared a common chronic endpoint in the liver – also conducted a cumulative assessment of triazoles for developmental effects observed following acute exposure (cranio-facial malformations) (EFSA 2009).

EPA once seemed to agree with the need for cumulative assessment of triazoles. In a 2017 human health risk assessment, the Agency identified the same two endpoints discussed above for the group:

> *"The toxicological effects of tebuconazole are consistent with those of other triazoles-derivative chemicals. In particular, developmental toxicity and hepatocellular tumors are effects common to a number of these pesticides" (EPA 11/15/17, p. 11).*

The Agency again identified these endpoints in its latest health assessment (EPA 3/18/21, p. 6), but then tried to obfuscate matters in its formal discussion of cumulative assessment by falsely suggesting that various triazole fungicides have vastly different spectra of effects (Ibid., p. 42), when in fact the commonalities are quite strong for chronic effects to the liver and birth defects upon acute or short-term exposure in prenatal developmental studies. EPA likewise pretends ignorance as to the common mechanisms of toxicity discussed above for the group's adverse hepatic effects.

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.).

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, see also EPA 3/10/22, p. 8).

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 (EPA 2/7/06, 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). We would note that the rat DNT for tebuconazole described above elicited many of the same effects as have been observed in various studies of 1,2,4-triazole, underscoring the importance of obtaining the more nuanced data only a DNT study can provide for this common metabolite of the triazoles.

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. 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 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 (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 in 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.

The sites of agriculture-origin, azole-resistant *A. fumigatus* strains discovered in the U.S. thus far include two crops – peanuts and pecans – for which tebuconazole is registered. In fact, 2.2 million acres of peanuts (51% of peanut acreage) are treated with tebuconzole, the highest percentage of any crop, while the fungicide is applied to 18% of pecan acres (EPA 2022, p. 20).

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

Environmental Fate of Tebuconazole

In laboratory testing, tebuconazole is stable to abiotic hydrolysis and to aqueous photolysis. It is very persistent in soils, with resistance to breakdown by sunlight demonstrated by a half-life of 192.5 days, while the aerobic soil metabolism half-life is over two years. Over half a given amount of tebuconazole remains in oxygenated water for one to over two years, while its anaerobic metabolism half-life in water is still longer at four years (EPA 3/31/21, p. 19, Table 5-2).

In field dissipation studies, tebuconazole exhibited 50% dissipation times (DT_{50})[21] ranging from 100 to 857 days, hence up to over two years. However, these nine studies

[21] Although EPA correctly notes that DT_{50} values are not directly comparable to the laboratory degradation data cited in the preceding paragraph, its use of the term "half-life" instead of "50% dissipation time" encourages just such misleading comparisons. The key difference is that field dissipation encompasses both the breakdown

described field dissipation of tebuconazole in only a narrow range of soil types, from sand (4) to sandy loam (4) to loamy sand (1), leaving dissipation in heavier soil types uncharacterized. Because all but one site was cropped – peanut (2), turf (3), grape (2), grass seed (1) – tebuconazole's fate in uncropped soils is also uncertain (Ibid, p. 20, Table 5-3).

Collectively, these studies demonstrate that tebuconazole is extremely resistant to the full range of breakdown processes operative in crop fields and water bodies, and hence will likely accumulate in environmental media with frequent use over a season and over years.

Environmental Impacts and Assessment Deficiencies

Aquatic Vertebrates

Fish

Tebuconazole is highly toxic to fish, but EPA has apparently nevertheless failed to demand even the basic studies from registrants that are required by its regulations at 40 CFR Part 158: Data Requirements for Pesticides. EPA lists only one acute toxicity study on one species of freshwater fish (the rainbow trout, Oncorhynchus mykiss), while the regulations call for acute toxicity studies on two freshwater (warm and cold freshwater species) fish and one saltwater species (EPA 3/31/21, Table 6-1, p. 23).[22]

Neither does EPA appear to have collected the required Fish Early Life Stage tests on either freshwater or estuarine/marine (saltwater) species, both of which are required at 40 CFR Part 158.630, Guideline No. 850.1400.[23] These tests are designed to investigate the effects of a pesticide on fish from embryonic stage to early juvenile development, critical periods in the fish's growth and development. Neither does EPA discuss or list any Fish Life Cycle test, which is required for one freshwater and one saltwater species for a persistent pesticide like tebuconazole (40 CFR Part 158.630, Guideline No. 850.1500). While EPA lists one study – MRID 48109802 – as fulfilling this guideline requirement (see EPA 3/31/21, pp. 96-97), it most decidedly does not, for at least one very important reason: because it failed to "evaluate reproductive success as measured by fecundity and fertilization success" (EPA 6/29/15, p. 79), a critical parameter that is explicitly evaluated in a Guideline-compliant Fish Life Cycle test (see EPA 1996, the Guidelines for this test, where effects to be reported and evaluated include "reproductive effects" and "detailed records of spawning, egg numbers, fertility and fecundity").

Tebuconazole is known to reduce fish reproductive performance. For instance, Li et al. (2019a) found significant reductions in egg production, among other effects, in zebrafish exposed to low, environmentally relevant levels of tebuconazole from 60 to 120 days post-

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processes measured individually in the lab tests, as well as "transport processes, such as volatilization, leaching, plant uptake, runoff or erosion," that move the intact molecule (and any degradates) into other environmental compartments (as per EPA Test Guideline OPPTS 835.6100, Terrestrial Field Dissipation, Oct 2008). EPA should clarify that field dissipation rates are ***not*** "breakdown rates under field conditions," but rather that they measure dissipation via transport processes as well as degradation.

[22] EPA lists a second acute toxicity study, but it involved the same species – rainbow trout or *Oncorhynchus mykiss* – and moreover seems to have involved testing a mixture of tebuconazole with another pesticide, fluopyram (EPA 3/31/21, p. 96, see studies under 850.1075: Fish Acute Toxicity Test, freshwater and marine).

[23] There is no Guideline 850.1400-compliant study listed in EPA's ecological assessment (EPA 3/31/21).

fertilization; Teb accumulated more in gonads than in any other tissue except for liver. Other studies suggest xenobiotics in gonads are associated with endocrine disruption and reproductive dysfunction. Tebuconazole also decreased the gonadal somatic index (weight ratio of gonads to body) in the female zebrafish of this study, and inhibited the expression of *cyp19a*, an enzyme involved in estrogen production, thereby reducing estrogen levels, the likely cause of the observed reduction in fecundity.

Other triazoles and pesticides of other classes also impair fish reproduction. In a study 10 of whose 11 authors are EPA scientists, Skolness et al. (2013) exposed fathead minnows to propiconazole at nominal concentrations of 5, 50, 500 and 1,000 ug/liter for 21 days. Among other findings, the fecundity of females as measured by cumulative egg production was reduced at all doses, significantly for the 5, 500 and 1,000 ug/liter groups. The authors also found disruption of steroidogenesis – for instance, reduced plasma estradiol and vitellogenin concentrations, and compensatory upregulation of genes encoding key steroidogenic CYP enzymes as well as STAR protein. Teng et al. (2020) exposed zebrafish to propiconazole for 120 days at doses of 0.1, 5 and 250 ug/liter: from embryonal stage 2 hours after fertilization until sexual maturity. In this study, too, fecundity of females decreased sharply at all doses, caused by disruption of steroidogenesis and alteration of DNA methylation patterns. Skolness et al. (2013) noted that conazoles as a class are known to be steroid synthesis inhibitors, and found "the overall pattern of responses" in their study to be "remarkably consistent" with the effects seen in other studies of fish exposed to conazoles – including reduced fecundity.

And fish encounter not just conazole fungicides in waterways, but many other toxic contaminants as well, some of which have similar adverse reproductive impacts. To take just one example, atrazine is an extremely persistent herbicide and common water contaminant. It has also been found to reduces egg production in Japanese medaka fish at levels as low as the barely detectable 0.5 ppb (Papoulias et al. 2014). Co-exposure to multiple pesticides/chemicals in waterways is common, ever more xenobiotics are shown to impair fish and amphibian reproduction, and many will have additive and in some cases synergistic effects.

EPA must follow through on the recommendation of its endocrine disruption team and commission an independent Medaka extended one generation reproduction test (MEOGRT) with tebuconazole (EPA 6/29/15, p. 3). EPA should enlist experienced and impartial government scientists to conduct this test, for instance those involved in the atrazine test noted above (Papoulias et al. 2014).

Full information on such aquatic effects for tebuconazole and other triazoles is urgently needed, particularly as use of these fungicides skyrockets, meaning ever more situations in which fish will be exposed to higher concentrations of multiple azoles, with additive effects likely. With safety thresholds for the 15 or more azole fungicides applied in the U.S. established separately, without regard to simultaneous exposure to others, cumulative exposure to the class will easily exceed any individual threshold, without necessarily there being any exceedance of an individual threshold. This once again points to the need for a cumulative toxicity assessment of triazole fungicides, as argued above but here for ecological effects, and as completed by the European Food Safety Authority for human health effects over a decade ago (EFSA 2009).

Finally, reductions in cumulative egg production is an impact with direct relevance to the continued existence of the impacted species, making it an extremely important parameter in both general ecological and in particular endangered species assessments.

EPA established acute and chronic safety thresholds for aquatic invertebrates in the absence of critical registrant data, and without adequately considering independent literature, some of which is discussed above. Another study that EPA should consider shows a multitude of injuries to the livers of zebrafish after incubation for 2, 4 and 8 days in water containing as little as 5 ppb tebuconazole (Macirella et al. 2022).

EPA also needs to clarify the concentrations involved in the fish sexual development test on fathead minnows and discussed at EPA (6/29/15, pp. 36-37, 77-79), which are listed as mg/l in some places and ug/l in others.

Amphibians

EPA's toxicity thresholds for aquatic vertebrates are uncertain due to the data gaps discussed above, but are in any case set too high. Amphibians are extremely sensitive to many xenobiotics, tebuconazole among them. Wrubleswski et al (2018) established a 96-hour LC_{50} value for *Physalaemus cuvieri* (Cuvier's foam froglet) of just 980 ug/l, less than half the EPA's acute toxicity threshold for freshwater fish of 2270 ppb (EPA 3/31/21, p. 21, ECOTOX Ref. 179653). EPA downgraded this study to "Supplemental" for purported inadequacies as justification for not using it to establish the toxicity threshold, but fails to note that the study it ***did*** choose to base the acute toxicity threshold on was also rated "Supplemental," and moreover lacked analytical verification of test material stability (Ibid., p. 23). The bottom line is that after years of its registration review process, EPA still has huge data gaps in aquatic toxicity database, and still refuses to use high-quality independent studies to establish quantitative safety thresholds.

And there is little doubt that amphibians are exposed and bioaccumulate tebuconazole, given its persistence in the environment and lipophilicity. Smalling et al. (2013) collected 15 Pacific chorus frogs from each of seven sites spanning the north-south extent of the Sierra Nevadas of California – once in 2009 and then again in 2010. They analyzed frog tissues for 98 pesticides and pesticide degradates. Tebuconazole was among those most frequently detected (44% of frogs), with by far the highest detection concentrations, both mean (74 ug/kg wet weight) and maximum (363 ug/kg wet weight), with multiple samples > 250 ug/kg. None of the pesticides were applied where the frogs were collected. They most likely moved via atmospheric transport, downwind, from the Central Valley. Tebuconazole was one of the only compounds consistently detected in both frog tissue and sediment collected from the same site.

In two studies of similar design, tebuconazole was shown to sharply reduce the survival of Italian tree frogs upon exposure for 78 days from Gosner stages 25 to 46 at concentrations of both 5 and 50 ppb, and it was also shown to induce deformities in tadpoles, delay development, and result in large numbers of sexually undifferentiated individuals (Bernabo et al. 2016, 2020).

Aquatic invertebrates

As EPA notes, seven open literature studies in ECOTOX provide more sensitive acute toxicity endpoints for freshwater invertebrates than those chosen by EPA for the acute safety threshold (EPA 3/31/21, pp. 21-22). For instance, Ochoa-Acuna et al. (2009) established 48-hour LC50/LC10 values for Daphnia magna of just 750/6.2 ppb, far lower than EPA's chosen acute endpoint. Two independent studies also provide lower chronic endpoints for freshwater invertebrates. Qi et al. (2015) found that both racemic and S-enantiomer tebuconazole significantly reduced reproduction and impacted the development of Daphnia magna at concentrations of 50 ppb or higher upon 21-day exposure. Qi et al. (2017) established a still more sensitive endpoint, finding that Daphnia suffered a 32% reduction in fecundity at the lowest dose tested of 25 ug/liter over a 14-day exposure period. EPA should apply an adjustment factor to this LOAEL, as it often does for registrant studies that lack an NOAEL, to derive an extrapolated NOAEL.

Mammals

Groups of pigs were fed tebuconazole for 28 days at five different rates – 0.25 to 25 mg/kg bw/day – with the dose administered in two administrations per day. The tebuconazole treatment caused collagen fibrosis in the liver, kidney and fat tissues of the the pigs (Jeong et al. 2022).

Terrestrial Invertebrates

Tebuconazole poses clear and serious risks to terrestrial invertebrates, as shown by the tests submitted by registrants, which however are grossly inadequate (EPA 3/31/21, pp. 26-28). There is particular concern for tebuconazole's chronic toxicity to honeybees and other pollinators, with LOAEL's of as little as 2 ug/bee/day over 10 days, and several tests not establishing an NOAEL, or with deficiencies that otherwise led EPA to classify them as supplemental and not fit for quantitative use. The Tier II studies also give cause for concern, with tebuconazole concentrations particularly high in pollen, up to 67.5 mg/kg, even though the tests were conducted with application rates just one-sixth to one-fifteenth the maximum rate of 1.36 lb/acre. It is difficult to see how these studies can justify any conclusions as to tebuconazole's effects on honeybees, much less on other species of bees and pollinators.

EPA should also assess the impact of tebuconazole in combination with insecticides, given that triazole fungicides are known to suppress the P450 detoxification enzymes that honeybees and other terrestrial invertebrates rely upon to detoxify insecticides. One of many examples in the literature is Wade et al. (2019), which assessed the toxicity of three insecticides and three fungicides commonly used on almond orchards in California. Chlorantraniliprole was found to increase mortality to larval bees when combined with propiconazole, but not alone.

Similarly, the combination of chlorantraniliprole and propiconazole applied topically was highly toxic to worker bees.

Pilling and Jepson (1993) showed that the acute contact toxicity of lambda-cyhalothrin was synergized to varying degrees by propiconazole and eight other 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 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).

We urge the EPA to begin the task of assessing how tebuconazole and other azole fungicides potentiate the toxicity of insecticides they are frequently applied with, either together or in close succession. EPA's long-time refrain is that it does assess pesticide mixtures in large part because there are too many possible combinations to assess. However, EPA could canvas the literature for toxic combinations such as Wade and colleagues demonstrate, and limit its assessment of mixture toxicity to those pesticides that are likely to be used together, and which demonstrate clear additive or synergistic toxicity.

In addition, it is a decade since a Scientific Advisory Panel told EPA it needed to begin collecting pesticide toxicity data on one or more additional bee species "to address the stated goal of protecting diversity," with suggestions that included the alfalfa leafcutting bee, a mason and orchard bee, or a representative bumblebee, in light of the fact that most bee species are ground-dwelling, with a very different exposure profile than the honey bee (SAP 2012).

EPA must go beyond collecting new studies on propiconazole'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.

Costs and Benefits

Putative benefits

Over half of tebuconazole use in the U.S. is on wheat, soybean and corn (see graph above). 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 tebuconazole 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 tebuconazole – 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 tebuconazole and other triazoles (discussed above).

Tebuconazole’s use on corn and soybeans has risen substantially over the past decade (see graph above). Coupled with the rise in use of other triazoles, the area sprayed with or a 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).

Mitigations

EPA's mitigations will do little to ameliorate the harms of tebuconazole to human health and the environment, as most are advisory or unenforceable in nature (or at least unenforceable in practice). EPA supplies no evidence these mitigations will be effective, and if so what degree of efficacy they might have.

Threatened and Endangered Species

EPA has not completed an assessment of tebuconazole 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. *See Nat. Res. Def. Council v. EPA*, 2022 WL 2184936, at *18 (9th Cir. June 17, 2022) (establishing that EPA must complete consultation for interim registration review decisions). Because there are many acknowledged risks of concern of tebuconazole 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.

Tebuconazole 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*

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 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.

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