CENTER FOR FOOD SAFETY

October 4, 2021

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

RE: Docket EPA-HQ-OPP-2015-0401
Comments on proposed interim registration decision for difenoconazole

Center for Food Safety appreciates the opportunity to comment on EPA's proposed interim registration decision for the fungicide difenoconazole, on behalf of itself and its 970,000 members and supporters. Center for Food Safety (CFS) is a public interest, nonprofit membership organization with offices in Washington, D.C., San Francisco, California, and Portland, Oregon. CFS's mission is to empower people, support farmers, and protect the earth from the harmful impacts of industrial agriculture. Through groundbreaking legal, scientific, and grassroots action, CFS protects and promotes the public's right to safe food and the environment. CFS has consistently supported comprehensive EPA review of registered pesticides and individual inert ingredients.

Introduction

First registered by EPA in 1994, difenoconazole is a broad-spectrum fungicide registered for use on many fruits, vegetables, cereals (seed treatment), field crops as well as on golf course turf grass and ornamental plants. Difenoconazole kills fungi by blocking the synthesis of sterols, which are key components of fungal cell walls. It belongs to the triazole class of demethylase inhibitor (DMI) fungicides, which block ergosterol synthesis by inhibiting the CYP51 enzyme, which catalyzes the 14 alpha demethylase step in ergosterol synthesis.

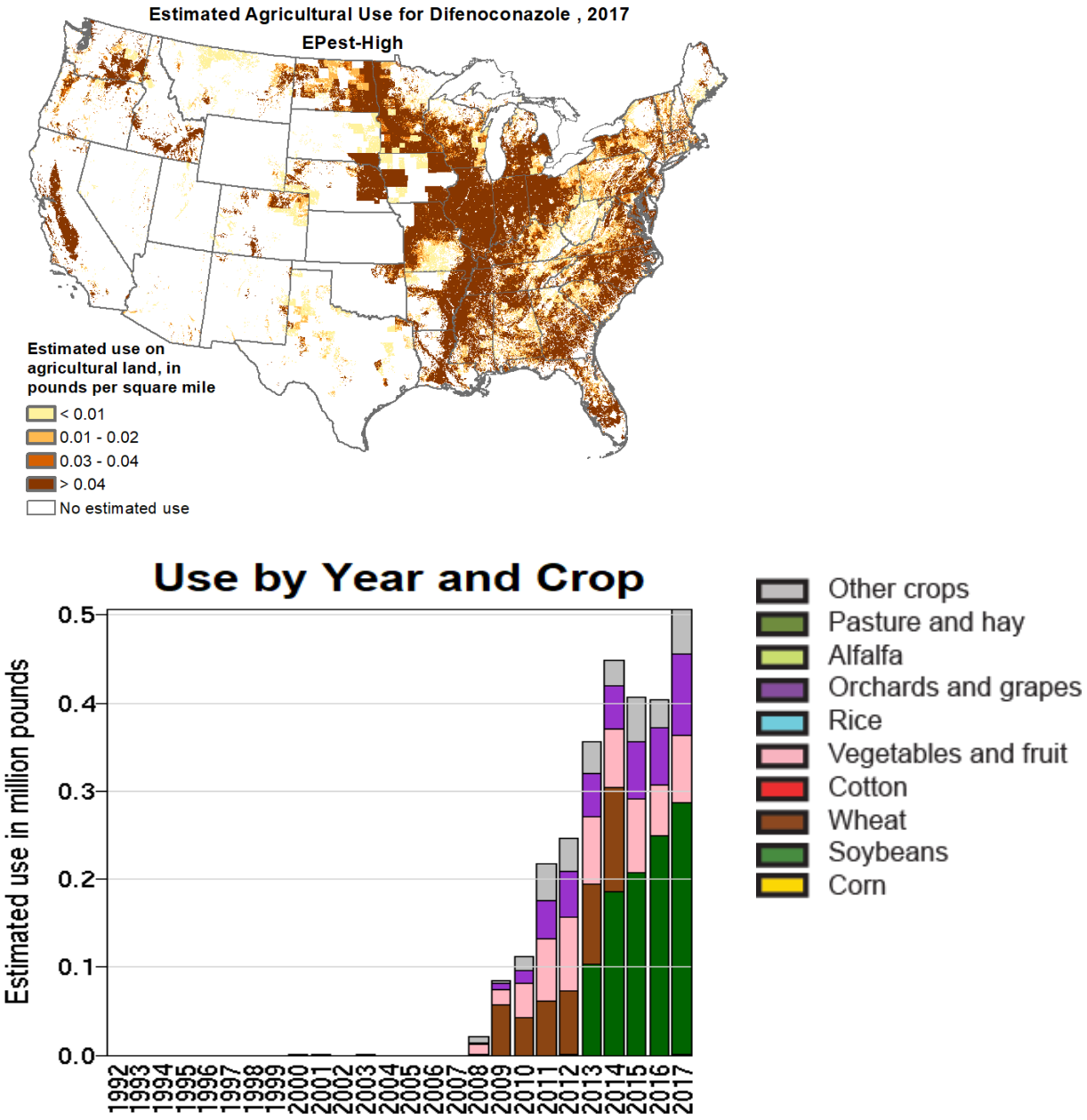

Figure 1: Difenoconazole Use, from: US Geological Survey, Pesticide National Synthesis Project, Difenoconazole, Epest-High. https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2017&map=DIFENOCONAZOLE&hilo=L&disp=Difenoconazole. Note: Seed treatment uses excluded beginning in 2015.

Difenoconazole was little used for a dozen years after it was first registered. Agricultural use first registers in 2008, and when one excludes seed treatments, has increased by roughly 20-fold over the decade from 2008 to 2017: from 25,000 to 500,000 lbs. per year (Figure 1). Usage is increasing on many crops, as shown by comparing figures in EPA's 2014 Screening Level Usage Analysis (average 2004 to 2012) and the Proposed Interim Decision (PID), averaging use over 2015 to 2019. For instance, percent acres treated has increased in tomatoes (25 to 45%), almonds (5 to 30%), sugar beets and apples (both 15 to 30%), grapes (5 to 30%) and

watermelon (5 to 20%).[1] Soybeans constitute half or more of difenoconazole's foliar use (Fig. 1); and because this represents treatment of just 2% of soybean acres, and soybean use is rising, there is huge potential for much greater spraying of soybeans with this fungicide. Finally, seed treatment use on wheat is likely on the order of 240,000 lbs./year, making overall agricultural use at least 750,000 lbs./year.[2]

Several features of difenoconazole and its use deserve particular consideration. First, because difenoconazole is one of many DMI/triazole fungicides with the same mode of action in fungi, and similar effects on non-target organisms, its putative benefits and impacts must be viewed in the broader context of its class. Second, triazole use overall is dramatically increasing. There are at least 15 DMI/triazole fungicides used in the U.S., and their collective use as of 2016 (excluding seed treatments) is nearly 7-fold greater than in 1992, and over 5-fold (434%) greater since just 2006 (Toda et al. 2021). Finally, difenoconazole in particular and other members of its class are quite persistent in the environment.

RELEVANT LEGAL STANDARDS

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

FIFRA authorizes EPA to regulate the registration, use, sale, and distribution of pesticides in the United States. Pursuant to FIFIRA, EPA oversees both initial registration of an active ingredient as well as any new uses of the registered active ingredient.

Section 3(c) of FIFRA states that a manufacturer must submit an application to register the use of a pesticide.[3] Under Section 3(c)(5) of FIFRA, EPA shall register a pesticide only if the agency determines that the pesticide "will perform its intended function without unreasonable adverse effects on the environment" and that "when used in accordance with widespread and commonly recognized practice[,] it will not generally cause unreasonable adverse effects on the environment."[4] FIFRA defines "unreasonable adverse effects on the environment" as "any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide."[5] Alternatively, where there are data gaps and missing information, EPA can register a pesticide with conditions (conditional registration) under Section 3(c)(7) of FIFRA "for a period reasonably sufficient for the generation and submission of required data," but only if EPA also determines that the conditional registration of the pesticide during that time period "will not cause any

[1] Compare figures in EPA (10/2/14), the SLUA, and the PID, p. 10.

[2] EPA's Biological and Economics Analysis Division (BEAD) has conflicting figures on wheat seed treatment usage. See EPA 12/2/15, Table 3 for 240,000 lbs. on 27.3 million acres of wheat, roughly 50% of national wheat acreage over the designated period; however, BEAD also says only 10% of wheat seed is treated (p. 7). No figures are reported for seed treatment use on other crops.

[3] 7 U.S.C. § 136a(c)(1); 40 C.F.R. § 152.42.

[4] 7 U.S.C. § 136a(c)(5).

[5] 7 U.S.C. §136(bb).

unreasonable adverse effect on the environment, and that use of the pesticide is in the public interest."[6]

The culmination of the registration process is EPA's approval of a label for the pesticide, including use directions and appropriate warnings on safety and environmental risks. It is a violation of the FIFRA for any person to sell or distribute a "misbranded" pesticide.[7] A pesticide is misbranded if the "labeling accompanying it does not contain directions for use which...if complied with …are adequate to protect health and the environment."[8]

Endangered Species Act

As recognized by the Supreme Court, the Endangered Species Act (ESA) is "the most comprehensive legislation for the preservation of endangered species ever enacted by any nation."[9] The ESA's statutory scheme "reveals a conscious decision by Congress to give endangered species priority over the 'primary missions' of federal agencies."[10] Federal agencies are obliged "to afford first priority to the declared national policy of saving endangered species."[11]

Section 7(a)(2) of the ESA requires every federal agency to consult the appropriate federal fish and wildlife agency—the U.S. Fish and Wildlife Service (FWS), in the case of land and freshwater species and the National Marine Fisheries Service (NMFS) in the case of marine species—to "insure" that the agency's actions are not likely "to jeopardize the continued existence" of any listed species or "result in the destruction or adverse modification" of critical habitat.[12] The ESA's implementing regulations broadly define agency action to include "all activities or programs of any kind authorized, funded or carried out … by federal agencies," including the granting of permits and "actions directly 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

[6] 7 U.S.C. §136a(c)(7)(C).
[7] 7 U.S.C. § 136j(a)(1)(E).
[8] 7 U.S.C. § 136(q)(1)(F).
[9] *Tenn. Valley Authority v. Hill*, 437 U.S. 153, 180 (1978).
[10] *Id*. at 185.
[11] *Id.*
[12] 16 U.S.C. § 1536(a)(2); *see also* 50 C.F.R. § 402.01(b).
[13] 50 C.F.R. § 402.02 (emphasis added).
[14] 16 U.S.C. § 1532(5)(A).
[15] 50 C.F.R. § 402.14(a).

threatened species] may be present in the area of such proposed action."[16] If FWS/NMFS advises the agency that listed species or species proposed to be listed may be present, the agency must then prepare a biological assessment for the purpose of identifying any such species that are likely to be affected by the proposed agency action.[17]

If, based on a biological assessment, an agency determines that its proposed action may affect any listed species and/or their critical habitat, the agency generally must engage in formal consultation with FWS/NMFS.[18] At the end of the formal consultation, FWS/NMFS must provide the agency with a "biological opinion" detailing how the proposed action will affect the threatened and endangered species and/or critical habitats.[19] If FWS/NMFS concludes that the proposed action will jeopardize the continued existence of a listed species or result in the destruction or adverse modification of critical habitat, the biological opinion must outline "reasonable and prudent alternatives" to the proposed action that would avoid violating ESA section 7(a)(2).[20]

Pending the completion of formal consultation with the expert agency, an agency is prohibited from making any "irreversible or irretrievable commitment of resources with respect to the agency action which has the effect of foreclosing the formulation or implementation of any reasonable and prudent alternative measures."[21]

Human Health Concerns and Assessment Deficiencies

Liver Toxicity Endpoint and Chronic Reference Dose

The major target organ of difenoconazole is the liver, as demonstrated in both chronic and subchronic mouse and rat feeding trials conducted by registrants. Adverse hepatic effects included hepatocellular hypertrophy, hepatic vacuolation, cell necrosis, increased liver weight, fatty changes, bile stasis, increased serum levels of enzymes indicative of liver injury – alanine aminotransferase (ALA), serum alkaline phosphatase (SAP), and sorbitol dehydrogenase (SDH) – and an increased albumin/globulin ratio (EPA 9/18/20, pp. 5-6, 19; EPA 7/27/94).

In 1994, EPA established a chronic reference dose of 0.01 mg/kg/day based on hepatoxicity, in particular hepatocellular hypertrophy, in males in a chronic rat study (NOAEL = 0.96 mg/kg/day) (EPA 2/24/94; 7/27/94, p. 18). By 2015, EPA had retained the same chronic reference dose, based on the same rat study, but changed the endpoint from hepatocellular hypertrophy to cumulative decreases in body weight gains in both sexes (EPA 2/24/15). In EPA's latest human health assessment, the proposed chronic reference dose has been increased five-fold to 0.05 mg/kg/day, and based on a mouse rather than rat study, in which liver lesions were observed in male mice and hepatocellular hypertrophy in both sexes of mice, with an NOAEL of 4.7 mg/kg/day (EPA 9/18/20).

---

[16] 16 U.S.C. § 1536(c)(1); *see also* 50 C.F.R. § 402.12(c).
[17] *Id.*
[18] 50 C.F.R. § 402.14.
[19] 16 U.S.C. § 1536(b); 50 C.F.R. § 402.14.
[20] 16 U.S.C. § 1536(b)(3)(A).
[21] 16 U.S.C. § 1536(d).

EPA's dismissal of hepatocellular hypertrophy in male rats and the associated NOAEL and reference dose (0.96 and 0.01 mg/kg/day, respectively) in favor of the five-fold higher mouse study endpoint is incorrect and should be reversed. First, hepatocellular hypertrophy in rats is properly regarded as an adverse effect when accompanied by other adverse liver effects, which is the case here: the rats in this chronic study also exhibited increased liver weight and an increased albumin/globulin ratio (EPA 7/27/94, p. 18). Second, subchronic (13-week) rat and mouse studies also demonstrated low-dose adverse hepatic effects. The mouse study's effects included histopathologic alterations in the liver at an LOEL of 30 mg/kg/day and NOEL of 3 mg/kg/day. Rats in the subchronic study exhibited increased liver weights at the LOEL of 15.5 mg/kg/day (F), with an NOEL of 1.43 mg/kg/day (F) (EPA 7/27/94, pp. 12-13).

By illegitimately dismissing the chronic rat NOAEL and reference dose (0.96 and 0.01 mg/kg/day), and by ignoring the subchronic rat and mouse NOEL's, all three of which are below EPA's new chronic mouse study endpoint, EPA enables 5-fold greater chronic exposure to this hepatotoxic fungicide.

We would also note that the European Food Safety Authority continues to base its NOAEL of 1 mg/kg/day on hepatotoxicity (increased incidence and severity of hepatocellular hypertrophy), as EPA once did (EFSA 2009, p. 109).

Carcinogenicity

EPA originally classified difenoconazole as a Group C Possible Human Carcinogen in 1994, based on clear inducement of hepatocellular adenomas and carcinomas in a mouse study (EPA 7/27/94), then subsequently re-classified it under the descriptor Suggestive Evidence of Carcinogenicity (EPA 9/18/20, p. 6).[22]

Toxicity of Metabolite Unknown

EPA has identified a major metabolite of difenoconazole that is present in humans, livestock and fish – CGA-205375 – yet has practically no toxicological information on it (EPA 9/18/20). EPA should demand toxicity studies on this and other metabolites rather than rely on guesstimates based on unreliable, *in silica* structure-activity modeling.

Dermal Absorption

An *in vivo* dermal absorption study in rats found dermal absorption of 48% of the applied dose after 24 hours in rats exposed to 0.5 ug/cm^2 of difenoconazole (EPA 9/18/20, p. 18). Rather than use this value as the dermal absorption factor, EPA reduced it to 6% based on two *in vitro* dermal absorption tests, one with human and one with rat skin. EPA multiplied the ratio of the *in vitro* absorption results (human/rat = 0.12) by the *in vivo* rat result of 48% to arrive at a human dermal absorption factor of 6%. There are multiple flaws that Invalidate EPA's 6% estimate, utilizing the so-called "triple-pack" approach.

[22] In the executive summary of EPA's health assessment, the Agency wrongly limits the evidence for carcinogenicity of difenoconazole to benign tumors – "liver tumors (adenomas)" – when in fact some of the observed tumors were malignant (carcinomas) (see EPA 9/18/20, compare statements on pp. 6 and 19).

First, the relevant EPA regulations for the dermal penetration assay (40 CFS, Part 158.500, Guideline No. 870.7600) prescribe an *in vivo* rat study, and provide no support for EPA's manipulation of this figure by applying *in vitro* results as EPA implies (EPA 9/18/20, pp 49-50, citing Guideline No. 870.7600 for *in vitro* as well as *in vivo* tests). Guideline No. 870.7600 (see EPA 1998) details a test protocol involving live rats to determine dermal absorption, not *in vitro* tests, much less the use of *in vitro* tests to reduce the dermal absorption factor derived from the *in vivo* rat study.

Second, even if one accepts the triple pack method as acceptable in principle, its use was entirely inappropriate with difenoconazole because the *in vitro* human/rat dermal absorption ratio was derived from tests employing far higher doses (10, 100, 1000 ug/cm^2) than the *in vivo* study (0.5, 1.3, 2.5 ug/cm^2), rendering them incompatible, especially given the large differences in absorption that were observed as a function of dermal dose (EPA 9/18/20, pp. 49-50). Indeed, the 20-40-fold difference in dosage between the *in vivo* and *in vitro* tests violated EPA's precondition that the protocols and doses be the same in all three studies in order to utilize the triple pack approach (see EPA 10/29/13, dermal absorption of glufosinate; see also EPA 6/2/10, pp. 2-3, dermal absorption of thiabendazole, likewise citing "identical protocols … in both the *in vivo* and *in vitro* studies" as a precondition for use of the triple pack approach). The difenononazole tests also failed a second EPA criterion for application of the triple pack approach – that the dermal absorption factors from the *in vitro* and *in vivo* animal tests be roughly equal (i.e. their ratio approximately equal to 1) (see EPA 6/2/10; see also EPA 10/29/13, where EPA rejects the triple pack method for estimating dermal absorption of glufosinate because the rat *in vitro* and rat *in vivo* results diverged substantially, among other reasons). In the case of difenoconazole, the *in vivo* and *in vitro* rat absorption factors from the tests carried out at the same doses differed by 3- to 4-fold (10 and 100 ug/cm^2), violating this criterion as well.

Finally, the test substance used in these assays was not specified, and if it is the technical active ingredient, this would likely lead to an underestimate of dermal absorption relative to use of real-world formulations with absorption-enhancing surfactants. Even use of a particular difenoconazole formulation in this test would not be predictive of absorption with other formulations.

EPA should demand full dermal absorption data for various difenoconazole formulations. Until then, it should conduct residential and occupational exposure assessments that incorporate dermal absorption based on a dermal absorption factor of 48%, based on a test that most closely follows the EPA (1998) protocol, rather than the 6% from illegitimate use of the triple-pack approach.

Need for Cumulative Exposure and Risk Assessment of Triazole Fungicides

Triazole fungicides clearly meet EPA's criteria for designation as a common mechanism group (CMG), for which a cumulative risk assessment must be carried out, as mandated by the Food Quality Protection Act (EPA 1/29/99, 1/14/02). They have a similar chemical structure, the liver is their primary target organ, they exert similar toxic effects on the liver, and do so via

common mechanisms of toxicity. In more modern language, they share a mode of action and adverse outcome pathways for several endpoints (MOA/AOP) (EPA 4/12/16). The European Food Safety Authority conducted a cumulative assessment of triazoles over a decade ago, forming cumulative assessment groups for developmental effects observed following acute exposure (cranio-facial malformations), and for hepatotoxicity as the chronic endpoint (EFSA 2009).

A review of registrant studies submitted to European regulators found that difenoconazole and all or most of 10 other triazole fungicides that were reviewed induced hepatocellular hypertrophy, hepatic cell degeneration or death, fatty changes, inflammation and hepatocellular tumors (Nielsen et al. 2012). As discussed further below, they exert these effects by activating nuclear receptors that induce the production of cytochrome P_{450} detoxification enzymes in the liver, causing an increase in cellular organelles (endoplasmic reticulum, peroxisomes and mitochondria) that is responsible for hepatic cell enlargement (hypertrophy). Hypertrophy is sometimes regarded as an adaptive effect, but persistent hypertrophy is adverse, particularly when it progresses to other adverse liver impacts as it does with triazoles (Nielsen et al. 2012). There are at least two endpoints, shared by most triazoles, that should be the focus of a cumulative assessment: fatty changes and carcinogenicity.

Fatty changes

The liver is the body's primary detoxification organ, and many industrial chemicals and pesticides are hepatotoxic. The most common hepatic pathology induced by chemicals is fatty liver (Al-Eryani et al. 2015) – the accumulation of lipids in liver cells – which can progress to more serious conditions, steatohepatitis and cirrhosis, which in turn are the most important risk factors for liver cancer (Wahlang et al. 2013). According to EPA scientists, fatty liver disease is "a growing epidemic" that affects 20-30% of the U.S. population (Angrish et al. 2016), while the incidence of liver cancer it predisposes to tripled from 1975 to 2005 (Altekruse et al. 2009).

In a review of chemical exposure and rodent toxicology databases maintained by the EPA and the National Toxicology Program, Al-Eryani et al. (2015) found that 54 pesticides, including 22 fungicides, caused fatty changes in the liver, many of them triazoles. In a similar review of registrant submissions to the European Union, 10 triazole fungicides induced fatty changes in the liver (Nielsen et al 2012). Altogether, at least 15 triazole fungicides induce lipid accumulation in liver cells (Table 1).

Table 1: Triazole Fungicides That Induce Fatty Changes in the Liver

Fungicide	Regulatory Authority (US, EU)	Comments
Bromuconazole	US	
Cyproconazole	US	
Difenoconazole	US, EU	
Epoxiconazole	EU	
Flusilazole	US, EU	

Hexaconazole	US	
Metconazole	EU	
Paclobutrazole	US	
Propiconazole	US, EU	
Prothioconazole	EU	
Tebuconazole	EU	
Tetraconazole	EU	
Triadimefon	US	
Triadimenol	US, EU	Primary metabolite of triadimefon
Triticonazole	EU	

Sources: Al-Eryani et al. (2005) for US; Nielsen et al. (2012) for EU. US = United States, EU = European Union. Listings in one rather than both jurisdictions does not necessarily mean differing assessments of this endpoint. Rather, it may be that particular triazoles are registered in only the US or the EU, or were at the time of the source publications.

Difenoconazole was shown to induce hepatic lipid accumulation as well as hepatocyte vacuolation and oxidative stress in male mice treated with the fungicide, and a molecular analysis supported these findings by revealing the transcriptomic signature of perturbed energy and especially glycolipid metabolism (Zhang H et al. 2021). Difenoconazole and two other triazoles, propiconazole and tebuconazole, were shown to promote accumulation of triglycerides in human HepaRG cell culture, with all three activating the pregnane-X-receptor (PXR) (Lasch et al. 2021). The critical role of PXR was demonstrated by a second study of propiconazole and tebuconazole (Knebel et al. 2019). Both triazoles induced expression of steatosis-related genes and triglyceride accumulation in HepaRG cells via interactions with several nuclear receptors – the constitutive androstane receptor (CAR), peroxisome proliferator-activated receptor alpha (PPAR α), and PXR. But in experiments with HepaRG subclones with knockouts of either PXR or CAR, triazole-induced triglyceride accumulation was abolished only with the PXR, not the CAR, knockout, demonstrating the critical role of PXR in mediating lipid accumulation triggered by triazoles.

Other studies provide still more supporting evidence. In a 28-day rat feeding trial with cyproconazole, epoxiconazole and prochloraz (an azole but not triazole fungicide), Heise et al. (2005) found hepatocellular hypertrophy and occasional necrosis of liver cells for all three compounds, increased absolute and relative liver weights for the two triazoles, and hepatic cell vacuolization with cyproconazole. A gene expression analysis found that triazoles induced expression of more than 30% of the genes in four toxicity pathways, including two involved in lipid metabolism: steatosis and phospholipidosis. Linkages between gene expression and histopathology were also found: vacuolization of hepatic cells is associated with steatosis; while cyproconazole also upregulated fatty acid synthase and transporter genes. Heise et al. (2007) tested combination of the same three fungicides in rats, and found similar effects as for the individual compounds, with dose additivity sufficient to account for combined effects. In 28-day rat feeding trials, Kwon et al. (2021) found that still another triazole, flutriafol, induced fatty infiltration of the liver by impairing liver metabolism and inducing apoptosis.

In a review article on the hepatic impacts of triazole fungicides, Marx-Stoelting et al. (2020) lay out adverse outcome pathways for liver hypertrophy and liver steatosis that link the molecular, cellular and tissue/organ level changes wrought by triazole exposure (see below). For hypertrophy, the molecular initiating events are triazole activation of the aryl hydrocarbon (AHR), CAR and PXR nuclear receptors, followed by four key events that mediate the adverse outcome on the tissue/organ level: hypertrophy of the liver:

1) Increased expression of CYP genes, with AHR, CAR and PXR preferentially but not exclusively inducing CYP families CPY1A1 and 1A2, CYP2B and CYP3A, respectively;
2) Increased expression of the corresponding CYP enzymes;
3) Proliferation of endoplasmic reticulum and other organelles to produce the additional CYP enzymes; and
4) Increased size of hepatic cells ensuing from the additional organelles.

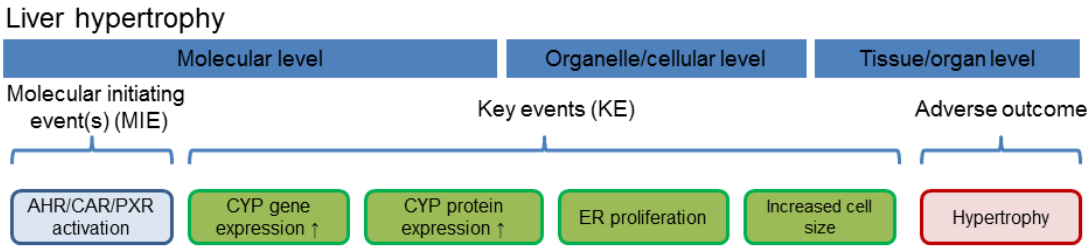

Figure 2. Schematic delineation of a nuclear receptor-dependent molecular pathway leading to hepatocellular hypertrophy. Nuclear receptor activation functions as molecular initiating event. Abbreviation: ER, endoplasmic reticulum.

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

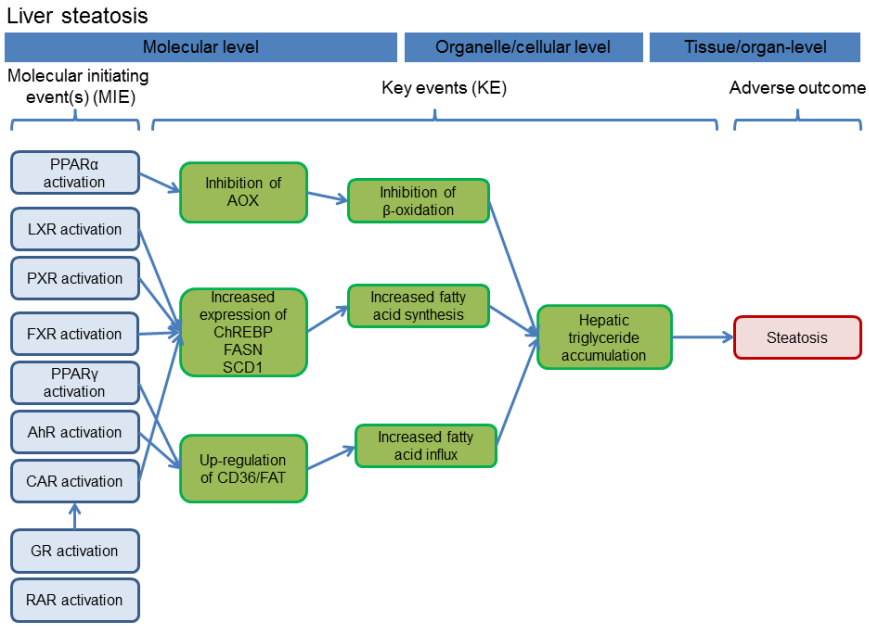

Figure 3. Schematic delineation of the AOP for hepatocellular steatosis. The figure was adapted from [58]. Abbreviations: FXR, farnesoid-X-receptor, GR, glucocorticoid receptor.

Figure 3. Adverse Outcome Pathway for Liver Steatosis. Source: Marx-Stoelting et al. (2020).

Hypertrophy of hepatic cells and the liver is a sensitive indicator of liver damage, for instance lipid accumulation. The adverse outcome pathway for hepatic steatosis is more complicated than that for hypertrophy, in that it involves multiple molecular initiating events, each activating a different toxic pathway with different key events, the cumulative outcome of which is steatosis (see Fig. 3).

Not every triazole fungicide will initiate each of these pathways in the same way on the molecular level, nor is it reasonable to demand that they do, in order to find that triazoles constitute a common mechanism group. Each pathway contributes to the same outcome, steatosis, whether through inhibition of fatty acid degradation via activation of PPRA α, increased fatty acid synthesis through upregulation of fatty acid synthase genes, and/or via increased influx of fatty acids into hepatic cells via increased expression of the corresponding transport gene.

The fact that at least 15 triazoles trigger fatty changes in the liver (Table 1), coupled with abundant evidence that they activate nuclear receptors (particularly PRX) in ways that lead to this outcome, is more than enough scientific justification to require EPA to conduct a cumulative exposure and risk assessment of triazole fungicides for this endpoint.

Carcinogenicity

A second endpoint for which EPA must cumulatively assess triazoles is carcinogenicity. EPA itself recognized the need for this in 1994, when difenoconazole was first registered. The Agency's Carcinogenicity Peer Review Committee noted that "Difenaconazole is a member of a class of chemicals, many of which have been associated with liver tumors in CD-1 mice" (EPA 7/27/94, p. 3). EPA then noted that eight structurally related triazole compounds have also been found to induce hepatocellular tumors (EPA 7/27/94, pp. 14-15), while a review of EU regulatory submissions identified seven triazoles that induced neoplasms (Nielsen et al 2012), for a total of 13 (Table 2).

Table 2: Triazole Fungicides That Induce Tumors

Fungicide	Regulatory Authority (US, EU)	Comments
Cyproconazole	US	
Difenoconazole	US, EU	
Epoxiconazole	EU	
Etaconazole	US	
Fenbuconazole	US	
Flusilazole	EU	
Metconazole	EU	
Propiconazole	US, EU	
Tebuconazole	US, EU	
Tetraconazole	EU	
Triadimefon	US	Also referred as Bayleton
Triadimenol	US	Primary metabolite of triadimefon, aka Baytan

Uniconazole	US	

Sources: EPA (7/27/94) for US; Nielsen et al. (2012) for EU. US = United States, EU = European Union. Listings in one rather than both jurisdictions does not necessarily mean differing assessments of this endpoint. Rather, it may be that particular triazoles are registered in only the US or the EU, or were at the time of the source publications.

Pesticide industry scientists tend to discount the carcinogenic effects of non-genotoxic, nuclear receptor-activating compounds (such as triazoles) in rodents as not relevant to humans (Elcombe et al. 2014). They do this by defining the mode of action of such compounds as equivalent to that of phenobarbital (PB), a model CAR activator that induces tumors in mice, but which epidemiology suggests may not induce tumors in humans. However, EPA Office of Research and Development scientists dispute this simplistic branding of rodent carcinogens that elicit some of the same hepatic toxicological responses as phenobarbital as then automatically irrelevant to humans (Nesnow et al. 2009). They showed that propiconazole and triadimefon, for instance, have gene expression profiles that differ substantially from phenobarbital's, their mechanisms of tumorigenic action are likely to differ, and hence the triazoles' induction of liver tumors in mice might well be relevant to humans.

Finally, the fact that so many triazoles induce hypertrophy, as well as steatosis, which is a risk factor for liver cancer, argues for the necessity of conducting a cumulative assessment of triazoles for liver cancer as well.

Cumulative Risk Assessment of 1,2,4-Triazole and its Conjugates

Triazole fungicides share an eponymous structural feature, 1,2,4-triazole, a five-membered aromatic ring comprising 3 nitrogen and 2 carbon atoms. 1,2,4-triazole and its conjugates (triazole-alanine and triazole acetic acid, TA and TAA, respectively) are common metabolites of these fungicides (EPA 2/7/06). Due to concerns over the toxicity of these metabolites, in the year 2000 EPA delayed granting any new triazole registrations pending more toxicology and exposure data for the metabolites (Ibid.).

To fill the data gaps, EPA issued a data call-in for studies on the developmental neurotoxicity, acute neurotoxicity, and carcinogenicity of free 1,2,4-triazole, and for a developmental toxicity study (rabbits) for both TA and TAA; a chronic rat study with neurological evaluations for TA; and a combined 90-day feeding/neurotoxicity study (rat) for TAA (Ibid., p. 6). The registrant group US Triazole Task Force (USTTF) did not respond to the 2002 call-in, and requested waivers from EPA in 2003 that EPA denied. The studies were still outstanding in 2005, when USTTF submitted renewed waiver requests (Ibid.).

To our knowledge, registrants to this day have not submitted the studies EPA demanded 15 years ago as a condition for any further registrations of triazoles (Ibid., p. 6).

Developmental Neurotoxicity (DNT) Study

The developmental neurotoxicity (DNT) study is designed to capture adverse neurological impacts of a pesticide when a fetus's or infant's developing nervous system is exposed, an exposure window when incredibly low doses can have profoundly destabilizing effects on nervous system architecture. Lifelong adverse impacts such as

reduced IQ, developmental delays and attention-deficit hyperactivity disorder have been linked to fetal/infant exposure to extremely low levels of chlorpyrifos, for instance. The DNT study was called for due to substantial evidence of 1,2,4-triazole's neurotoxicity in other animal trials, including:

- Neuropathological lesions in the brain and peripheral nervous system;
- Decreases in brain weight, including in offspring at doses that did not cause the same effect in adults in the rat reproduction study;
- Tremors, muscle fasciculations, decreased arousal, decreased rearing, decreased motor activity in rats, and excessive salivation, hyperpnea, lacrimation and head tilt in rabbits (Ibid., pp. 17, 20).

Registrants apparently decided to ignore EPA's demands, because the DNT study has still not been submitted (EPA 5/16/18, p. 22600). Neither did EPA cease registration of new uses and new triazoles until it had received this study, as it had demanded in 2006 (EPA 2/7/06, p. 6).

Chronic toxicity/carcinogenicity study

EPA had also required a chronic toxicity/oncogenicity study on 1,2,4-triazole in male rats and female mice to determine whether this metabolite was the common cause of liver tumors found with so many triazoles (Ibid., p. 6). We find no record this study has been submitted either.

Developmental toxicity study in rabbits

EPA demanded this study to fulfill "a particularly important data gap" for both TA and TAA because there were no rabbit tests with either of these compounds, the rabbit was the most sensitive species to 1,2,4-triazole, and because of the gravity of the adverse impact (mortality) ensuing from just a single dose of 1,2,4-triazole (45 mg/kg) in rabbits (Ibid., p. 47). We see no evidence these studies on TA or TAA have been submitted.

EPA applied arbitrary safety factors in an attempt to compensate for the missing studies, but has no way of knowing whether they are adequate. In any case, these safety factors are intended only as a temporary stopgap until the relevant studies are submitted, permitting a data-based assessment. Here, the relevant studies have been outstanding for at least 15 years, a period during which EPA has issued numerous registrations for new uses of triazoles.

Agricultural Triazole Use Likely Breeds Resistance to Triazole Antifungal Drugs in Human Pathogens

Fungal diseases are spiraling worldwide, with the global mortality rate from fungal infections now exceeding that from malaria or breast cancer, and rivalling deaths from tuberculosis and HIV (Fisher et al. 2018). There are nine times more antifungal compounds for crop disease than for animal infections, and just four classes of antifungals licensed for human

use (Ibid.). Triazoles are the dominant compounds used to treat crops, animals and humans; are the only class used in both medicine and agriculture (ibid.).

Drivers of resistance in plant and human pathogens share some similarities. In modern industrial agriculture, breeding has long been primarily concerned with increasing yield, and conducted with use of pesticides to eliminate pest and disease pressure. These factors lead to loss of disease resistance, and increasing dependence on fungicides accompanied by accelerating resistance. Ever more people are at risk of fungal infection due to age, medical interventions or HIV infections. Immune suppression with chemotherapy or organ transplantation increases susceptibility to opportunistic fungi, leading to greater use of antifungal drugs and pathogens resistant to them. Global movement of people and goods promotes rapid spread of fungal pathogens of crops and people (Ibid.).

Candida auris was first described in 2009 in Japan, and has spread worldwide primarily as a nosocomial pathogen resistant to all clinical antifungal medications (Ibid., Richtel and Jacobs 2019), one of several fungal pathogens on the rise (Fisher et al 2018).

Invasive aspergillosis is a serious and frequently fatal lung disease that mainly affects people who are immunocompromised: for instance, those recovering from tuberculosis, with pulmonary disease, or in conjunction with organ transplantation (for this discussion generally, see Toda et al. 2021 unless otherwise cited). It also afflicts millions of asthmatics worldwide, greatly exacerbating their disease, with conditions known as allergic bronchopulmonary aspergillosis and severe asthma with sensitization (Bowyer and Denning 2014).

The major pathogen of this disease is *Aspergillus fumigatus*, which is commonly found in the environment (e.g. decaying plant matter), has unusually high tolerance to heat and so propagates quite well in the human body, and is not known to cause plant disease. The major medications (and only ones available in oral form) used to treat this disease are triazole antifungal medicines such as itraconazole, voriconazole and posoconazole.

Over the past several decades, there has been an extremely concerning rise in invasive aspergillosis caused by *A. fumigatus* that is resistant to triazole antifungals; in such virtually untreatable infections, the mortality rate rises to 42-88%.

Resistant *A. fumigatus* has been reported in patients with aspergilloma undergoing long-term therapy with triazoles antifungals. In this disease, a fungal mass grows in a lung cavity, where it can reproduce. These resistant strains induced by medical antifungal use are characterized by a great diversity of resistance mechanisms (Snelders et al. 2012). However, there is a large and growing body of scientific literature demonstrating that agricultural use of triazole fungicides is another source of this growing resistance problem.

First, resistant strains of *A. fumigatus* have been isolated from triazole-naïve patients around the world, infections that cannot be due to treatment of these individuals with the antifungals. In addition, a disproportionate number of resistant strains isolated from patients in the Netherlands, an early site for emergence of this problem, have a particular resistance mechanism – a tandem repeat of 34 base pairs in the *cyp51* promoter region and a leucine to histidine substitution at codon 98 in the coding region (TR_{34}/L98H) – that is also commonly found in the environment. This TR34/L98H strain was first cultured from a patient in the

Netherlands in 1998, following close on the heels of a ramping up of agricultural triazole use there and in Europe generally from 1990-1996 (Snelders et al. 2012).

Moreover, the first medical antifungal (itraconazole) was only licensed in 1997 (Zhang J et al. 2017), very little time for it to have driven selection of the resistant strain noted above, even assuming the first TR34/L98H strain ***discovered*** in a patient were the first such to ***emerge,*** which appears unlikely. Additional reasons to doubt that medical use is responsible for all or even most resistance are, first, the miniscule amounts used to treat human disease relative to agricultural use; and the fact that itraconazole is excreted from the body in non-active form, making selection for resistance in sewage or receiving waters unlikely (Bowyer and Denning 2014).

The agricultural triazoles that most resemble their medical counterparts – both structurally and in terms of their docking at the CYP51 binding site – are difenoconazole, bromuconazole, epoxiconazole, propiconazole and tebuconazole. In susceptibility testing, these five triazoles (as well as metconazole and imazalil) showed the greatest dissimilarity in activity on wild-type versus resistant L98H isolates, as measured by minimum inhibitory concentration (Snelders et al. 2012). Moreover, these same five triazoles selected for *A. fumigatus* strains with cross-resistance to the medical antifungals – particularly itraconazole – after seven weeks of exposure. Interestingly, "difenoconazole imposes the strongest induction of cross-resistance to medical triazoles…" (Zhang J et al. 2017).

Resistance could arise in any environment where triazole fungicides are used and decaying plant matter provides habitat for *A. fumigatus.* Several studies have assessed stockpiles of plant waste for *A. fumigatus* populations and for presence of agricultural triazoles and their breakdown products. Schoustra et al. (2019) examined stockpiles of dead flower bulbs, green materials, and wood chips, finding substantial populations of *A. fumigatus* in each, ranging from roughly 10^3 to 10^5 colony-forming units (CFUs)/gram. Triazoles and their degradation products were found in most (78%) of 41 samples, at concentrations ranging from 0.001 to 6.4 ppm. Another study by the same team similarly found on average 10^5 CFUs/gram plant waste inn 114 samples, and estimated a plant waste stockpile just 50 x 50 x 10 meters would contain 2.5 quadrillion (10^{15}) spores. Roughly half of the isolates were triazole-resistant, with 90% resistant to both itraconazole (medical) and tebuconazole (agricultural). They also found a variety of resistance mechanisms (Zhang J et al. 2021).

A. fumigatus is a common component of bioaerosols, and it is estimated that an average person inhales 200 spores (conidia) each day (Dagenais and Keller 2009). Inhalation of *A. fumigatus* spores in the air is thought to be the major route of infection. Aerial dispersal of A. fumigatus from compost piles has been demonstrated, with a surge in release when the piles are turned, and substantial quantities then found in the downwind air (Millner et al. 1977, 1980).

EPA must assess the public health threats posed by continued and expanding use of difenoconazole and other agricultural triazoles in terms of increasing resistance of human fungal pathogens such as *A. fumigatus* and *C. auris* to medical antifungal compounds.

Environmental Concerns and Assessment Deficiencies

The rapidly rising use of difenoconazole is having unacceptable environmental impacts, including but not limited to threatened and endangered species. A key aspect of this fungicide's threat is its extreme persistence in the environment, which as described below EPA has not sufficiently accounted for in its environmental assessments to date. Unless otherwise noted, the following discussion is based on EPA (9/16/20).

Difenoconazole's Environmental Persistence

Difenoconazole is extremely persistent in multiple laboratory and field tests, in soil and water. It is stable to abiotic hydrolysis at pH values of 5, 7 or 9; it has a half-life of 228 days in an aqueous photolysis test; and half-lives ranging from 349-823 days in soil photolysis tests. Aerobic soil metabolism half-lives range from over 100 days to over 500 days, depending on soil type, while the anaerobic soil metabolism half-life is nearly three years (947 days). Aerobic aquatic metabolism half-lives are 300-565 days; anaerobic 433 days (EPA 9/16/20, p. 26). Terrestrial field dissipation data also show considerable persistence, with most half-lives in various soil types, bare plot vs. vegetative cover, ranging from over 100 to 535 days (Ibid, p. 28).

It is no wonder that EPA scientists warn repeatedly of the potential for difenoconazole to accumulate in soils and water with repeat applications:

> "The overall stability/persistence profile for difenoconazole suggests that it has potential to accumulate in soil and aquatic environments with each successive application" (Ibid., pp. 25-26).

Critically, EPA's exposure and risk assessments do not appear to account for the accumulation of difenoconazole over a single season or over years. This is a huge data gap that in itself invalidates EPA's latest risk assessments and argues strongly against the proposed interim registration decision, and certainly against approval of ANY new uses – particularly on a crop so widely grown as corn, as Syngenta recently proposed.

Risks to Terrestrial Organisms

Risk quotients exceed levels of concern for a host of different taxa. This is true even though For instance, chronic risks to mammals reach risk quotients up to 5.2 from consumption of grass and other forage with difenoconazole residues. Similarly, birds are chronically threatened by both foliar applications (risk quotients up to 10.99) and via consumption of treated seeds (risk quotients up to 16.13). These risks are exacerbated by difenoconazole's persistence. EPA has identified chronic risk LOC exceedances for birds for ***up to 150 days after application in some scenarios***, and after 56 days for mammals. Risks in some scenarios persist even when EPA utilizes mean rather than maximum Kenaga difenoconazole residues in the

assessment.[23] Moreover, spray drift of difenoconazole also poses chronic risks to birds up to 112 feet from the application site, when applied aerially to turf at just 0.125 lb/acre (PID at 15).

Honeybees are also likely threatened by difenoconazole, particularly its formulations, which are more toxic than the active ingredient alone. Acute and chronic risk quotients for larval bees, 0.99 and 1.35, exceed the respective levels of concern, 0.4 and 1.0, for one difenoconazole formulation that was tested. Additionally, difenoconazole's extreme persistence and potential for build-up in soil over a single season or years pose potential risks to ground-dwelling bees and a host of other soil-borne invertebrates that are not well-represented by the honeybee. This points up the importance of EPA expanding its required testing to include effects on soil organisms.

Risks to Aquatic Organisms

Difenoconazole also threatens aquatic organisms. EPA scientists identified risk quotients up to 22 for chronic risks to aquatic vertebrates, and noted that: "Overall, chronic LOC exceedances included crops that have some of the highest poundage of difenoconazole applied annually. ***Due to the persistence of difenoconazole, repeated use can considerably increase these risks over time."*** (EPA 9/16/20, pp. 6-7, 9, emphasis added).

Nearly all usage scenarios posed chronic risks to fish, but even so, risks are still greater than represented by these risk quotients due to difenoconazole's persistence. EPA scientists stated this clearly: "Due to the persistence of difenoconazole in terrestrial and aquatic environments, repeated use can ***considerably increase these chronic risks*** over time." (emphasis added).

Fish and other aquatic organisms are also threatened by bioaccumulation of difenoconazole and its metabolites. EPA documented a 330x bioconcentration factor for whole fish. Over half of the applied dose was detected in the fish in the form of the metabolite, CGA-205375, for which EPA has next to no toxicity data (setting aside unreliable ECOSAR structure-activity guesstimates).

Aquatic invertebrates are likewise at risk, with chronic risk quotients up to 6.3, far exceeding the LOC of 1. As with fish, EPA's risk assessment methodologies do not appear to encompass risks arising from accumulating levels of difenoconazole over years:

> "Crops for which chronic risks to aquatic invertebrates were identified included rice, ornamentals, soybeans, sugar beets, tree nuts, small vine fruits (e.g., grapes), potatoes, cabbage, tomato, apples and cucurbits. These crops encompass some of the highest difenoconazole use rates in terms of lbs a.i. applied annually (Section 3-2). ***Like fish, due to the persistence of difenoconazole, repeated use can considerably increase these risks over time***." (EPA 9/16/20, p. 55, emphasis added).

[23] Kenaga residues refers to a nomogram developed by EPA scientists in the early 1970s that purports to predict residue levels on plants after application of any pesticide, based only on the application rate of the pesticide and the type of plant (e.g. long grass, leafy crops) or plant part (fruit, pods containing seeds).

In short, difenoconazole poses seriously ecological risks to several taxa, risks exacerbated by the fungicide's extreme persistence in the environment. Even without accounting for buildup of difenoconazole in the environment over years, EPA's assessments find substantial exceedances of risk quotients. Until EPA conducts assessments that account for accumulation of this fungicide in the environment over years, it cannot gauge the true risks to wildlife or finalize this interim registration review decision. EPA must also abstain from approving any new uses of difenoconazole – particularly one with potential for such an extreme increase in usage as corn – prior to correcting the deficiencies in its ecological risk assessment and completing its registration review.

Costs and Benefits

Putative benefits

Roughly half of non-seed treatment use of difenoconazole is on soybean. Syngenta is seeking registration for use on corn as well. The following discussion will focus on these field crops.

While agronomists are disturbed by the dramatically increasing use of fungicides of all sorts, the concern is especially acute for use on field crops like corn and soybeans, which began around 2007 (see Hershman et al. 2011 and Wise and Mueller 2011 for the following discussion). These agronomists note that foliar fungicide applications were extremely rare on corn and soybeans until this time; to the small extent fungicides were used, it was for seed production or specialty corn varieties, where higher prices justified the expenditures.

Agronomists attribute the rise in fungicide use on corn and soybeans largely to marketing drives by fungicide manufacturers, who have had success selling farmers on fungicides for dubious "plant health" reasons rather than disease; to higher corn prices beginning in 2007; and to growers' prioritization of yield potential over disease-resistance in selection of corn hybrids. There is also a troubling "insurance treatment" approach to fungicide spraying that goes fundamentally against IPM principles to use a pesticide only when needed, and only when the expenditure delivers more benefit in yield than the cost of the pesticide.

There are already a number of other fungicides, including DMI/triazole fungicides, already approved for use on soybeans and corn. To the very limited circumstances in which their use might be justified, there are already sufficient control options available to growers, and no need for still another foliar fungicide for corn.

Costs

Resistance to triazole/DMI fungicides has been building steadily over years, and together with widespread resistance to strobilurin and other fungicides is a serious problem.

> "For decades, scientists have watched as fungi all over the world have become incrementally more and more resistant to DMI fungicides. The use of any

fungicide for 'plant health' reasons increases the risk of developing resistance" (Hershman et al. 2011).

Clearly, superfluous use of fungicides like difenoconazole – as for "plant health" reasons – must be avoided at all costs to stem or at least slow resistance development. In this respect, too, one must recall how difenoconazole and other triazoles are also likely fostering increased resistance to critical antifungal triazole medications and the associated costs in terms of human health and deaths (discussed above).

Difenoconazole's use on soybeans has risen dramatically since 2012 (essentially zero) to 2017 (about 200,000 lbs./year). This soybean use represents about half of (non-seed treatment) uses of difenoconazole, even though only roughly 2% of soybean acres are, at present, being sprayed. This usage will likely continue to rise, usually for no good reason.

The expansion of difenoconazole to corn would exacerbate an unhealthy trend of excessive and largely unnecessary triazole use in corn and soybeans (Toda et al. 2021, Toda et al. 2021 Supplemental). Critically, it would expand those acres that are sprayed with a triazole every year in corn-soybean rotations, intensifying selection pressure for resistant plant and human fungal pathogens across the Corn Belt, where just 15-20 years ago hardly anyone saw any need to spray fungicides on these crops. Cross-resistance among triazole herbicides is common. For instance, even the fungicide manufacturers' group Fungicide Resistance Action Committee has stated: "Generally wise to accept that cross resistance is present between DMI fungicides active against the same fungus." (FRAC 2021, p. 11).

Potential Mitigations

EPA's proposed mitigations consist largely in toothless advisory statements on spray drift, environmental hazard and surface water contamination that the Agency itself admits have no impact on the risks of concern. The other proposed label mitigations are entirely inadequate to the task of reducing any of the risks difenoconazole poses to humans and non-human organisms, or the risks of resistance in agricultural or human pathogens.

Clearly, the many costs of renewing current uses of difenoconazole with this registration review decision far outweigh any putative and highly dubious benefits. This holds in particular for use on soybeans and the proposed use on corn, which should be rejected.

Threatened and Endangered Species

EPA has not completed an assessment of difenoconazole for its impact on threatened and endangered species. EPA must comply with its duties under Section 7 of the Endangered Species Act (ESA) prior to finalizing its interim registration decision, as it is a separate, discretionary action that may affect species listed as threatened or endangered under the ESA. Because there are many acknowledged risks of concern of difenoconazole to a range of taxa, and imperiled species listed under the ESA are highly susceptive to additional threats, it is clear that listed species will continue to be put at risk with a registration review decision as EPA has proposed, and at still greater risk from registration of foliar use on corn.

Difenoconazole may affect numerous threatened and endangered species across the country including, but not limited to, the species listed below.

Fish	
Neosho madtom	*Noturus placidus*
Pallid sturgeon	*Scaphirhynchus albus*
Topeka shiner	*Notropis topeka*
Terrestial Invertebrates	
Rusty patched bumblebee	*Bombus affinis*
Mitchell's Satyr Butterfly	*Neonympha mitchellii mitchellii*
Poweshiek skipperling	*Oarisma poweshiek*
Monarch butterfly (candidate)	*Danaus plexippus plexippus*
Aquatic Invertebrates	
Rabbistfoot	*Quadrula cylindrica cylindrica*
Birds	
Least tern	*Sternula antillarum*
Whooping crane	*Grus americana*
Piping plover	*Charadrius melodus*

EPA must complete endangered species consultation to ensure the registration does not jeopardize the existence of species protected as threatened or endangered under the ESA prior to finalizing its registration decision. Without having fulfilled this duty under the ESA, in consultation with the expert wildlife agencies, EPA cannot ensure no jeopardy for protected species. EPA claims its proposed label changes "are expected to reduce the extent of exposure and may reduce risk to listed species whose range and/or critical habitat co-occur with the use of difenoconazole" even though EPA "is not making a complete endangered species finding at this time."[24] However, without a full analysis and ESA consultation EPA cannot determine the full impacts of difenoconazole on ESA-listed species and their critical habitats and ensure that it will not jeopardize any of those species. What EPA is doing here is clearly not sufficient to comply with the ESA.

Conclusion

Clearly, EPA has failed to properly assess the human health and environmental risks posed by difenoconazole in its proposed interim decision, and must revisit its assessment prior to any final decision. CFS urges EPA to reject the proposed 5-fold increase in the chronic reference dose, conduct a guideline-compliant assessment of dermal absorption with

[24] Proposed Interim Registration Decision at 23

difenoconazole formulations, and obtain toxicological data on the major metabolites, especially CG-205375. EPA must correct its ecological exposure and risk assessments by taking full account of difenoconazole's extreme persistence and likely accumulation over time. Furthermore, EPA should conduct a cumulative risk assessment of triazole fungicides because of their structural similarity, their common primary target, the liver, and common effects and adverse outcome pathways for major impacts such as hepatic steatosis and carcinogenicity. EPA must also obtain the long-missing data needed to complete its cumulative assessment of the triazole metabolites, 1,2,4-triazole and its major conjugates, particularly the developmental neurotoxicity study on 1,2,4-triazole.

EPA must also assess the role difenoconazole and other triazole fungicides used in agriculture have played in selecting for human fungal pathogens that are resistant to medical azole antifungal drugs. Pathogens exhibiting increasing resistance include *Aspergillus fumigatus* and *Candida auris*. Resistant strains of each have become a huge, global public health threat, as the few antifungal drugs that can treat diseases such as invasive aspergillosis become ineffective.

EPA must also assess the threats posed by difenoconazole to threatened and endangered species, beginning with consultation with the expert agencies.

Sincerely,

Bill Freese, Science Director
Center for Food Safety

References

Al-Eryani L. et al. (2015). Identification of environmental chemicals associated with the development of toxicant-associated fatty liver disease in rodents. Toxicologic Pathology 43: 482-497.

Altekruse SF et al. (2009). Hepatocellular carcinoma incidence, mortality, and survival trends in the United States From 1975 to 2005. Journal of Clinical Oncology 27(9): 1485-91.

Angrish MM et al. (2016). Tipping the balance: Hepatotoxicity and the four apical key events of hepatic steatosis. Toxicol. Sci. 150(2): 261-268.

Bowyer P and Denning DW (2014). Environmental fungicides and triazole resistance in *Aspergillus*. Pest Management Science 70(2): 173-178.

Dagenais TRT and Keller NP (2009). Pathogenesis of *Aspergillus fumigatus* in Invasive Aspergillosis. Clinical Microbiology Reviews 22(3): 447-64.

EFSA (2011). Conclusion on the peer review of the pesticide risk assessment of the active substance difenoconazole. European Food Safety Authority, EFSA Journal 9(1): 1967.

EFSA (2009). Scientific Opinion on Risk Assessment for a Selected Group of Pesticides from the Triazole Group to Test Possible Methodologies to Assess Cumulative Effects from Exposure through Food from these Pesticides on Human Health. European Food Safety Authority, EFSA Journal 7 (9); 1167.

Elcombe CR et al. (2014). Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit. Rev. Toxicol. 44(1): 64-82.

EPA (6/28/21). Oxyfluorfen: Proposed Interim Registration Review Decision, Case Number 2490. EPA, June 28, 2021.

EPA (9/18/20). Difenoconazole. Draft Human Health Risk Assessment for Registration Review. EPA, EPA-HQ-OPP-2015-0401-0021, September 18, 2020.

EPA (9/16/20). Difenoconazole: Draft Ecological Risk Assessment for Registration Review. EPA, September 16, 2020. EPA-HQ-OPP-2015-0401-0019.

EPA (5/16/18). Tebuconazole: Pesticide Tolerances. 83 Fed. Reg. 22595-601 at 22600 (May 16, 2018).

EPA (4/12/16). Pesticide Cumulative Risk Assessment: Framework for Screening Analysis Purpose, EPA, April 12, 2016. EPA-HQ-OPP-2015-0422-0019.

EPA (12/2/15). BEAD Chemical Profile (BCD) for Registration Review: Difenoconazole (128847). EPA, December 2, 2015. EPA-HQ-OPP-2015-0401-0002.

EPA (2/24/15). **Difenoconazole**: Human Health Risk Assessment for proposed new foliar uses on legume subgroup 6C and bushberry subgroup 13-07B; post-harvest uses on pome fruit group 11-10; and ornamental plants and vegetable transplants grown in both indoor and outdoor production facilities. EPA, February 24, 2015.

EPA (10/2/14). Difenoconazole: Screening Level Usage Analysis, EPA, October 2, 2014. EPA-HQ-OPP-2015-0401-0006.

EPA (10/29/13). Glufosinate ammonium: Response to comments on the human health risk assessment for registration review. EPA, October 29, 2013. EPA-HQ-OPP-2008-0190-0043.

EPA (6/2/10). Review of triple pack dermal absorption studies for Maxim Quattro. EPA, June 2, 2010.

EPA (2/7/06). 1,2,4-Triazole,TriazoleAlanine,TriazoleAceticAcid:HumanHealthAggregate Risk Assessment in Support of Reregistration and Registration Actions for Triazole-derivative Fungicide Compounds. EPA, February 7, 2006.

EPA (1/14/02). Guidance on Cumulative Risk Assessment of Pesticide Chemicals That Have a Common Mechanism of Toxicity, EPA, January 14, 2002.

EPA (1/29/99). Guidance for Identifying Pesticide Chemicals and Other Substances That Have a Common Mechanism of Toxicity, EPA, January 29, 1999.

EPA (1998). Health Effects Test Guidelines. OPPTS 870.7600: Dermal Penetration. EPA 712-C-98-350, August 1998.

EPA (7/27/94). Carcinogenicity Peer Review of Difenoconazole. EPA, July 27, 1994.

EPA (2/24/94). RfD/Peer Review Report of Difenoconazole. EPA, February 24, 1994.

Fisher MC et al. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science 360: 739-42 (May 18, 29018).

FRAC (2021). FRAC Code List 2021:Fungal control agents sorted by cross resistance pattern and mode of action (including coding for FRAC Groups on product labels). Fungicide Resistance Action Committee, 2021.

Heise T et al. (2017). Hepatotoxic combination effects of three azole fungicides in a broad dose range. Arch Toxicol 92(2): 859-872.

Heise T, Schmidt F, Knebel C et al (2015). Hepatotoxic effects of (tri)azole fungicides in a broad dose range. Arch Toxicol 89(11): 2105–2117. https://doi.org/10.1007/s00204-014-1336-1.

Hershman DE, Vincelli P and Kaiser CA (2011). Foliar Fungicide Use in Corn and Soybeans. University of Kentucky College of Agriculture, Plant Pathology Fact Sheet, PPFS-GEN-12, October 2011.

Knebel C et al (2019). Pregnane X receptor mediates steatotic effects of propiconazole and tebuconazole in human liver cell lines. Archives of Toxicology 93(5): 1311-22.

Kwon H-C et al (2021). Effects of Flutriafol Fungicide on the Lipid Accumulation in Human Liver Cells and Rat Liver. Foods 10: 1346.

Lasch A et al (2021). More than additive effects on liver triglyceride accumulation by combinations of steatotic and non-steatotic pesticides in HepaRG cells. Archives of Toxicology 95: 1397-1411.

Marx-Stoelting P et al (2020). The Connection of Azole Fungicides with Xeno-Sensing Nuclear Receptors, Drug Metabolism and Hepatotoxicity. Cells 9: 1192; doi:10.3390/cells9051192.

Millner PD et al. (1980). Dispersal of *Aspergillus fumigatus* from sewage sludge compost piles subjected to mechanical agitation in open air. Applied and Environmental Microbiology 39(5): 1000-09.

Millner PD et al. (1977). Occurrence of *Aspergillus fumigatus* during composting of sewage sludge. Applied and Environmental Microbiology 34(6): 765-772.

Nesnow et al. (2009). Discrimination of Tumorigenic Triazole Conazoles from Phenobarbital by Transcriptional Analyses of Mouse Liver Gene Expression. Toxicological Sciences 110(1): 68-83.

Nielsen E. et al. (2012). Identification of Cumulative Assessment Groups of Pesticides, External Scientific Report Submitted to EFSA [European Food Safety Authority]. National Food Institute, Technical University of Denmark, 2012

Richtel M ad Jacobs A (2019). A Mysterious Infection, Spanning the Globe in a Climate of Secrecy: The rise of Candida auris embodies a serious and growing public health threat: drug-resistant germs. The New York Times, April 6, 2019.

Schoustra SE et al. (2019). Environmental Hotspots for Azole Resistance Selection of *Aspergillus fumigatus*, the Netherlands. Emerging Infectious Diseases 25(7): 1347-53.

Snelders E et al (2012). Triazole Fungicides Can Induce Cross-Resistance to Medical Triazoles in *Aspergillus fumigatus*. PLoS One 7(3): e31801.

Toda M. et al. (2021). Trends in Agricultural Triazole Fungicide Use in the United States, 1992–2016 and Possible Implications for Antifungal-Resistant Fungi in Human Disease. Environmental Health Perspectives 129(5), May 2021.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8098123/pdf/ehp7484.pdf.

Toda et al. (2021), Supplemental. Supplemental materials for paper cited above.

Wahlang B et al. (2013). Toxicant-Associated Steatohepatitis. Toxicol. Pathol. 41(2): 343-360.

Wise K and Mueller D (2011). Are Fungicides No Longer Just For Fungi? An Analysis of Foliar Fungicide Use in Corn. American Phytopathological Society, APSnet Features. doi:10.1094/APSnetFeature-2011-0531.

Zhang H et al. (2021). Insights into the effects of difenoconazole on the livers in male mice at the biochemical and transcriptomic levels, Journal of Hazardous Materials 422: 126933.

Zhang J et al. (2021). Dynamics of Aspergillus fumigatus in Azole Fungicide- Containing Plant Waste in the Netherlands (2016–2017). Applied and Environmental Microbiology 87(2): e02295-20.

Zhang J et al. (2017). Evolution of cross-resistance to medical triazoles in Aspergillus fumigatus through selection pressure of environmental fungicides. Proc Biol Sci. 284(1863): 20170635.