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***Sent by email to: pmra.noo-ado.arla@hc-sc.gc.ca* and by attachment to internet form**

PMRA Notice of Objection Officer
Pest Management Regulatory Agency
Health Canada

Dear Sir/Madam:

Re: **Re-evaluation decision RVD2024-09, Tebuconazole and its associated end-use products**

This notice of objection is submitted by Ecojustice Canada, Environmental Defence Canada, Canadian Association of Physicians for the Environment, Friends of the Earth, and Safe Food Matters.

We agree that turf uses should be cancelled, however, the PMRA must cancel all uses until it has completed a full cumulative health risk assessment, an endangered species assessment and utilized increased uncertainty factors.

About tebuconazole

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 acts by inhibiting the CYP51 enzyme.

Tebuconazole is of particular interest because of high exposure levels. It is among the top ten fungicides sold in Canada since 2018, and is sold in amounts over 100,000 kg/yr (PMRA Sales Report, 2022 p.10). Internationally the use of triazole fungicides such as tebuconazole, which is among the more heavily used, is increasing.

Tebuconazole is registered in Canada for foliar uses (including large field crops, asparagus, and turf), seed treatment, and as a heavy-duty wood preservative.

A cumulative risk assessment is required

Because it is one of many demethylation inhibitor (DMI)/triazole fungicides with the same mode of action in fungi, and similar effects on human health and non-target organisms, it requires an

assessment of cumulative risk along with other triazoles to human health under s.19(2) of the *Pest Control Products Act.* No cumulative risk assessment was conducted contrary to the requirements in the Act and no uncertainty factor was added to address this. Tebuconazole is a triazole-based fungicide which belongs to a group of pesticides known as the conazole fungicides. These pesticides are structurally similar and contain a triazole moiety. As a result of these structural similarities, conazole fungicides share common metabolites including 1,2,4-triazole and triazole conjugates. It is not lawful for the PMRA to defer the cumulative risk assessment of triazole fungicides and their 1,2,4 triazole and triazole conjugates to a separate assessment.

Tebuconazole also poses risks to unborn children which need to be addressed with the full 10-fold PCPA Factor. Other changes to the risk assessment are needed to address the potential accumulation of tebuconazole in the environment and fungicide resistance which could cause serious human health problems.

Toxicity to humans

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 postimplantation 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).

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 EPA's assessment of tebuconazole's endocrine disrupting activity (EPA 6/29/15, pp. 3, 36).

PMRA cannot continue the registration of triazole fungicides without conducting a cumulative health assessment that includes food, drinking water and residential uses

The re-evaluation of tebuconazole took place as a post-market review under sections 16-21 of the *Pest Control Products Act*. Section 19 of the *Pest Control Products Act*, provides that:

- The registrant has the burden of persuading the Minister that the health risks are acceptable (s.19(1)(b))
- In evaluating health and environmental risks the Minister shall apply a scientifically-based approach (s.19(2)(a))
- In relation to health risks the Minister shall consider available information on aggregate exposure from diet, drinking water and residential/school uses and the cumulative effects with other pest control products that have common mechanisms of toxicity (s.19(2)(b)(i)).

Accordingly, the PMRA is obligated to take a scientifically based approach to the cumulative health risk assessment of tebuconazole, an analysis that must include drinking water, food residue and all non-occupational exposures. Tebuconazole has acknowledged common mechanisms of toxicity with other triazole pesticides.

Triazole fungicides clearly meet all criteria for designation as an EPA common mechanism group (CMG), (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).

There are 70 tebuconazole products registered in Canada, 32 difenoconazole products, 2 febuconazole products, 3 flutriafol products, 11 ipconazole products, 12 mefrentrifluconazole products, 20 metconazole products, 20 metconazole products, 4 myclobutanil products, 62 propiconazole products, 77 prothioconazole products, 5 tetraconazole products and 7 triticonazole products. Many of these are registered on the same crops.

In the difenoconazole proposed re-evaluation decision PVRD2021-06, it was acknowledged that all triazole fungicides share common metabolites resulting from the release of triazole ring 1,2,4 triazole from the parent compound and its subsequent conjugation to produce triazolylaacetic acid (TAA) and triazolylanine (TA). Due to their intrinsic toxicological properties residue chemistry and human health risks which result from the use of all registered triazole fungicides it was acknowledged that the risks had to be assessed but this was unlawfully deferred (PVRD 2021-06 p.10).

Difenoconazole resulted in females aged 13-49 years being exposed to 18% of the acute reference dose, with children 1-2 being the most exposed subpopulation. Exposure to difenoconazole was 80% of the chronic reference dose for children 1-2 years. Foods contributing to this were apples, potatoes, apple sauce, grapes and pears (PVRD2021-06 p.11-12) This has not been updated with post-2017 food residue or drinking water data or models.

Propiconazole is currently under a special review due to potential exposure to propiconazole's transformation products in groundwater. (Special review announcement 22, July 2022).
The PMRA must address the combined effects of the various triazoles, their transformation products and the conjugates of those transformation products together in a cumulative risk assessment. All triazole registrations should be cancelled until this is completed, as is required by s.19(2)(i) of the *Pest Control Products Act*.

A review of registrant studies submitted to European regulators found that tebuconazole and all or most of 10 other triazole fungicides induce hepatocellular hypertrophy, hepatic cell r regeneration 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 P450 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 health 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.[1]

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 (PPARα), and PXR. But in experiments with HepaRG subclones with knockouts of either PXR or CAR, triazole-induced triglyceride accumulation was abolished only with the PXR, not the CAR, knockout, demonstrating the critical role of PXR in mediating lipid accumulation triggered by triazoles.

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

[1] Al-Eryani et al. (2005) for US; Nielsen et al. (2012)

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.

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.

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 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 the PMRA to conduct a cumulative exposure and risk assessment of triazole fungicides for this endpoint.

Liver Tumors
The PMRA's cancer risk assessment for tebuconazole is not acceptable. The PMRA must revise the cancer risk assessment for tebuconazole, triazoles as a group and for metabolites and their conjugates.

In the proposed re-evaluation decision, the PMRA initially acknowledged the carcinogenicity of tebuconazole. However, no cancer endpoint was provided. The PMRA found that mechanistic studies were provided to support both mitogenic and cytotoxic modes of action (MOAs) for tebuconazole-induced liver tumour formation in mice. Additionally, evidence of cytotoxicity and regenerative proliferation was noted in short- and long-term toxicity studies in the database. Thus, the submitted data supported a threshold mode of action (MOA) for the liver tumours observed in the mouse. Although a recently published study provided some evidence of a CAR-dependant MOA for tebuconazole-induced liver tumours in mice, tebuconazole has also been shown to trigger liver hypertrophy independently of CAR activation (PMRA# 2873585).

While the proposed decision asserted that the endpoints for non-cancer risk were protective of carcinogenic potential, the absence of any cancer endpoint in appendix II p.43 of the proposed

decision makes this impossible to verify. The PMRA must identify whether there are non-threshold cytotoxic effects or alternatively identify a clear carcinogenic endpoint.

In the final re-evaluation decision, the PMRA claimed to have re-examined several of the studies. This revised assessment is found in Appendix IV of the final re-evaluation decision. There was no change to the cancer risk assessment. The PMRA concluded that: Evidence of liver tumours in mice, for which a mode of action was accepted and a threshold approach for risk assessment was considered appropriate. Equivocal increase in histiocytic sarcomas in mice results in a lack of reasonable certainty that no harm will occur, pursuant to subsection 2(2) of the *Pest Control Products Act*. The PMRA asserts that toxicological reference values selected for non-cancer risk assessment are protective of any residual concerns regarding carcinogenic potential. However no clear cancer endpoint was provided in the public risk assessment. The PMRA needs to provide a clear analysis justifying the use of a threshold for liver tumors and a clear cancer endpoint so that it is transparently comparable to the existing effects levels from the non-cancer risk assessment.

EPA recognized the need for a cancer assessment for tebuconazole 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 1/22/00).

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, points to the necessity of conducting a cumulative assessment of triazoles for liver cancer in order to establish reasonable certainty that no harm will occur. This issue should be referred to a review panel.

Developmental toxicity
PMRA should also establish a cumulative assessment group for the developmental effects of triazoles, 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).

The PMRA must also conduct a cumulative health risk assessment for 1,2,4, triazole and its metabolites

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).

It is unclear whether PMRA requested these studies and if so whether they were provided and how they were incorporated into the risk assessment.

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 for 1,2,4 triazole's was requested by the EPA 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).

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. Without this the PMRA cannot have reasonable certainty that no harm would occur. This issue should be referred to a review panel.

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 are unable to determine whether PMRA required this study and whether it was provided.

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). It is unclear from a review of the PMRA's risk assessment whether these studies were required or provided.

PMRA must address the potential indirect health impacts of fungicide resistance to medical applications of fungicides.

In order to continue the registration of tebuconazole the PRMA must find that there is reasonable certainty that no harm will occur to human health and the environment from the use of tebuconazole. Harm to health includes harm to the health of humans from fungicide resistance, which may make it extremely challenging to treat a rising tide of fungal infections in humans.

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 and are the only class used in both medicine and agriculture (Ibid.). The PMRA should cancel the registrations of triazole fungicides.

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 longterm 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 (TR34/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 103 to 105 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 105 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 (1015) 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.

The development of fungal resistance is a potential effect on human health that is covered by the definition of "health" in the *Pest Control Products Act*. The PMRA is required to address this effect in its evaluation of tebuconazole and has not done so. The PMRA lacks reasonable certainty that no harm will occur to human health from the development of fungicide resistant human pathogens from the use of triazole pesticides in Canada. This is an issue that should be referred to a review panel.

Need to apply the full 10-fold PCPA factor

We agree with the PMRA's decision to revise the proposed re-evaluation to use the 10 fold PCPA factor for assessing risk to women of reproductive age, however it should be used for developmental toxicity as well.

Section 19(2)(b)(iii) of the *Pest Control Products Act* requires the PMRA to use a 10 fold safety factor to take into account incompleteness of data on exposure and toxicity to infants and children. We emphasize that this requirement also relates to exposure, not just toxicity. The PMRA has incomplete information on exposure due to a lack of biomonitoring, water and food residue monitoring and must maintain the 10 fold safety factor. The European Union classifies tebuconazole as "suspected of damaging the unborn child," and a 2014 review by the European Food Safety Agency suggested that the chemical be classified as one that "may damage the unborn child."[2]

[2] European Food Safety Authority (EFSA). Conclusion on the peer review of the pesticide risk assessment of the active substance tebuconazole. EFSA Journal 2014; 12(1): 3485.
https://www.efsa.europa.eu/en/efsajournal/pub/3485

In the proposed re-evaluation decision the PMRA found that the toxicology database was adequate for determining the sensitivity of the young. Therefore, it was deemed "overly conservative" to retain the default 10-fold *Pest Control Products Act* factor (PCPA factor) when using these endpoints to establish a point of departure. As a result, the PCPA factor was reduced to threefold when endpoints from the developmental neurotoxicity (DNT) study, or malformations occurring in the presence of maternal toxicity in the developmental toxicity studies, are used to establish the reference dose. However, the PMRA lacks any bottled or treated water quality data for Canada, and the monitoring of tebuconazole in surface, groundwater and drinking water sources is too limited to ensure that there are no harms to children and unborn children. The estimated environmental concentrations also could not be refined due to the lack of environmental fate data (RVD p.32). Moreover, the lack of a dermal DNT study (RVD p.32) reinforces the need to use the full PCPA factor. The uncertainty factor that should be used for potential harms to fetuses is also an issue that should be referred to a review panel.

Tebuconazole accumulates in the environment

The PMRA's risk assessment does not address evidence that tebuconazole is resistant to the full range of breakdown processes in operative crop fields and water bodies and will likely accumulate in the environment. The PMRA should add significant additional uncertainty factors to address these significant gaps in environmental fate. This issue should be referred to a review panel.

In the final re-evaluation decision, the PMRA noted that one aerobic biotransformation study from 1987 was available to Health Canada, which examined the biotransformation of tebuconazole in a sandy loam soil (PMRA# 1229603). The study showed that tebuconazole is persistent under the test conditions with a DT50 value of 883 days (slower rate of the Double First Order in Parallel kinetic model, DFOP), which was used for the environmental and drinking water modelling and in risk assessment. It should be noted that the microbial activity of the soil was not reported, therefore, it is not clear if the soil was microbially active. To investigate further, Health Canada considered additional studies on the degradation of tebuconazole in soil, with different rates and different incubation intervals submitted to the European Food and Safety Association (EFSA, PMRA# 3093536). The EFSA report indicates that either it was not possible to measure the decrease of tebuconazole concentrations as a function of time in a reliable way, due to concentrations declining quite slowly, and calculated half-lives being too great to be determined under laboratory conditions, or the study was not reliable because of an uncommon application technology. The lack of aerobic soil data limits the ability of Health Canada to assess this pesticide; therefore, conservative assumptions were made to ensure the protection of Canadians. With only a single value, Health Canada has assumed that this study represents the upper 90% confidence interval bound on the mean for aerobic soil transformation. (RVD p.33) This is not a scientifically based approach and cannot result in reasonable certainty that no harm will occur pursuant to s.2(2) of the *Pest Control Products Act*. This issue should be referred to a review panel.

Failure to assess effects on endangered insects and fish

There is evidence that tebuconazole may affect numerous threatened and endangered species including sturgeon, rusty-patched bumblebees, and monarch butterflies. A jeopardy assessment should be conducted under the *Species at Risk Act* for all species that are potentially vulnerable to the chronic and acute effects of tebuconazole and other triazoles. The general and acute-only focused "representative species" assessment does not target the most ecologically or toxicologically vulnerable species and does not address potential ecological-scale impacts as required by the *Pest Control Products Act*. This issue should be referred to a review panel.

All treated seed uses should be cancelled

Tables 19 through 24 in the proposed re-evaluation decision confirm high levels of risks to birds, from treated seed uses, which can kill birds after eating only 5 treated corn seeds. These uses are not acceptable. This issue should be referred to a review panel.

Other uses should be cancelled for environmental risks

The proposed re-evaluation decision includes numerous apparently serious environmental risks for which no clear mitigation measure has been imposed in the final re-evaluation decision. All of these should be referred to a review panel.

Table 24-25 in the proposed re-evaluation shows that there is an unacceptable risk to terrestrial plants from overspray uses, these uses should be cancelled. This issue should be referred to a review panel.

Table 27 in the proposed re-evaluation shows that chronic risks to amphibians are exceeded for amphibians from aerial spraying uses, these uses should be cancelled. This issue should be referred to a review panel.

Table 32 in the proposed re-evaluation shows that soybean uses should be cancelled because they pose unacceptable risks to amphibians. This issue should be referred to a review panel.

Tables 35-37 in the proposed re-evaluation shows that treated wood uses should be cancelled due to unacceptable risks to aquatic organisms. This issue should be referred to a review panel.

Table 15 in the proposed re-evaluation shows that risks to bees from foliar uses are not acceptable. This issue should be referred to a review panel.

Table 17 in the proposed re-evaluation shows that off-field thresholds are exceeded for beneficial arthropods. The associated uses should be cancelled. This issue should be referred to a review panel.

Failure to assess the impact of tebuconazole on fungus

There were no toxicological tests on environmental fungi, including beneficial fungi in agriculture which are essential to terrestrial plant immune functions including for crops. Aquatic fungi are also entirely neglected in the risk assessment even though they play an essential ecological role. The absence of any evaluation of the ecological effects of widespread agricultural use of fungicides on fungi and their beneficial relationships with terrestrial plants creates very considerable uncertainty about whether environmental harm will result from the use of tebuconazole. All registrations of tebuconazole and other triazoles should be cancelled until a fungal risk assessment is completed. This issue should also be referred to a review panel.

The appropriate test for a review panel

As noted in the notice of objection for tiafenacil submitted by many of the same organizations submitting this letter, we dispute that the test used in the response to the notice of objection to glyphosate is the appropriate test under the *Review Panel Regulations* and s.35 of the *Pest Control Products Act* as it is inconsistent with the objectives of the Act. We assert that this objection meets any reasonable version of the test in the *Review Panel Regulations*.

As with our previous objections we note that a reasonable time for a response would be no more than 6 months. The PMRA has outstanding objections for several products that have gone on for three years or more. If in-depth analysis is required of an objection this should be conducted by a review panel, not by the PMRA. The PMRA owes a statutory obligation to respond "without delay." Further, the potential harm to the rights of Canadians under s.7 of the *Charter of Rights and Freedoms* from ongoing exposure to tebuconazole and other triazole fungicides, and the need for the Minister to consider whether to amend the registration to meet the objectives of the Act weighs against delaying a response for more than six months, regardless of the complexity of the matter.

Sincerely,

Laura Bowman
Barrister & Solicitor

cc: Cassie Barker, Environmental Defence
Mary Lou MacDonald, Safe Food Matters
Beatrice Olivastri, Friends of the Earth

Jane McArthur, Canadian Association of Physicians for the Environment

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