Pesticide Action Network Europe

Have your priorities right.

Brussels, 03-05-2024

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To: Ms. Kyriakides
European Commissioner for Health and Consumer Policy
European Commission
B-1049 Brussels.

Concerning: The pesticide difenoconazole.

Dear Health Commissioner Ms. Kyriakides,

We are writing to you regarding the delayed decision on the non-renewal of the pesticide difenoconazole. The deadline for the assessment of the active substance was December 2018 and with the help of five '*prolongations*' this is now moved to December 2026. This pesticide can cause serious harm to humans and the environment as we demonstrate in this letter and we urge you to give high priority to this matter. The overwhelming evidence in public literature on the endocrine activity and harmful endocrine effects of difenoconazole should lead, according to Art. 4.1 of the Regulation[1] to '*first establish*' if the hazard criteria are met, to the non-renewal of the substance. We regret to see that this has not been the case.

Unfortunately, difenoconazole is not the only pesticide for which we do not understand the priorities of your services at DG SANTE. Toxic to reproduction classified pesticides like dimethomorph and quinoline-8-ol, endocrine disrupting pesticides like metribuzin and thiabendazole along with other very harmful pesticides like captan, sulfoxaflor and acetamiprid remain on the market while harmful effects have been demonstrated in the applicants' dossiers and in independent studies. At the same time, we note that your services spend significant time and resources assessing substances like milk, urea, magnesium and garlic. We simply don't understand this choice, since your mission is supposed to be to protect people and the environment against harmful pesticides.

Difenoconazole is an endocrine disrupting chemical. Azole antifungal agents are designed to interfere with the heme-group of the fungal cytochrome P450 (CYP) enzyme CYP51[2]. In this way, azoles block the production of the essential fungal membrane component ergosterol, disrupting the cell membrane and ultimately inhibiting fungal growth. The downside of this fungicide use, however, is that CYP enzymes are found in all kingdoms of life (animals, plants, fungi, bacteria). Many azoles can interfere with members of the CYP superfamily of enzymes, of which there are many thousands. With respect to reproductive development, the CYP enzymes of the steroidogenic pathway are crucial and regulate sex hormone synthesis and metabolism such as CYP17 and CYP19. In fact, CYP19 (aromatase) has been shown to be a key target of many azole fungicides. In humans and other mammals, CYP19 is responsible for converting androgens to oestrogens and as such plays a critical role in sex hormone homeostasis throughout development and in adulthood. Hence, perturbing CYP19 activity can impede on sexual development and function.

Given the mode of action, which is hormone disruption, the lack of urgency of decision-taking on conazoles, such as difenoconazole, is incomprehensible. Indeed, the endocrine disruption by difenoconazole was already discussed in the consultation in 2014 between EFSA and member states[3] on 'confirmatory information' of the applicant. However, only a poorly conducted (industry performed) 'fish full-life cycle' study was discussed. This raises questions on what has happened in the past 10

[1] The assessment of the active substance shall first establish whether the approval criteria set out in points 3.6.2 to 3.6.4 and 3.7 of Annex II are satisfied. If these criteria are satisfied the assessment shall continue to establish whether the other approval criteria set out in points 2 and 3 of Annex II are satisfied.
[2] Draskau MK et al. (2022), Azole Fungicides and Their Endocrine Disrupting Properties: Perspectives on Sex Hormone-Dependent Reproductive Development. Front. Toxicology 4:883254.
[3] EFSA supporting publication 2014:EN-680

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years. Even a recent EFSA opinion[4], almost 10 years later, focusses on the same poorly conducted fish study as the sole test to assess endocrine disruption. This lack of progress is incomprehensible. We would expect that based on your commitment to protect the public you would interfere to call for a higher level of protection of human health.

Our concerns are reinforced since the data from independent literature are convincing on the endocrine effects and harms of difenoconazole. It is highly unlikely that your services at DG Sante are not aware of these studies

Studies from independent scientists consistently demonstrate the endocrine activity of difenoconazole and its related negative endocrine-related health effects. Much research has been done in (zebra)fish, and to a lesser extent in mammals, both with great relevance for other animals such as humans.
According to this research: difenoconazole,

- **inhibits aromatase** (reproductive damage in fish, decrease in androgen levels, increase in oestrogen and aromatase levels[5]), (inhibition of brain and ovarian aromatase activities[6]);
- **changes the endocrine system** (alterations in mRNA levels of brain follicle-stimulating hormone, ovarian cytochrome P450 aromatase (CYP19s), hepatic oestrogen receptors and vitellogenin[7]), (interference with growth endocrine system, sex-specifically altered expression[8]), (lowering cholesterol[9]);
- **oestrogen disruption** (estrogenic endocrine-disruption effects, transferred to offspring[10])
- **effects on thyroid hormones** (alters thyroid hormone levels and gene transcription[11]),(T3 increased, and T4 decreased[12]);
- **effects on gene regulation of hormones**, (the luteinizing hormone (lhβ) and the follicle-stimulating hormone (fshβ) genes in the brain all exhibited a significant down-regulation, the expression of androgen receptors (arα and arβ) was decreased and that of oestrogen receptor β and cytochrome P450 aromatase (cyp19B) was increased in the testes[13]), (increased expression of genes relevant to the GH/insulin-like growth factor axis (GH/IGF) axis in the brain, liver, and testis as well as increased GH levels[14]);

Studies that demonstrate harmful effects of difenoconazole on organisms:

- on **development**, (egg number produced, the hatch ability and the swim-up success in the F1 generation- all showed a U-shaped dose response; decrease of fecundity and viability of the next generation at realistic environmental concentration[15]); (impact on the spermatogenesis via the brain–pituitary–gonad pathway; less viability of F1 generation[16]), (hatching inhibition, abnormal spontaneous movement, slow heart rate, growth regression and morphological deformities[17]);

[4] EFSA Supporting publication 2023:EN-8474
[5] Dong X et al. Exposure to difenoconazole inhibits reproductive ability in male marine medaka (Oryzias melastigma). J Environ Sci (China). 2018 Jan;63:126-132.
[6] Hinfray N et al. Inhibition of rainbow trout (Oncorhynchus mykiss) P450 aromatase activities in brain and ovarian microsomes by various environmental substances. Comp Biochem Physiol C Toxicol Pharmacol. 2006 Nov;144(3):252-62.
[7] Dong X et al. Reproductive effects of life-cycle exposure to difenoconazole on female marine medaka (Oryzias melastigma). Ecotoxicology. 2017 Aug;26(6):772-781.
[8] Teng M et al. Sex-specific effects of difenoconazole on the growth hormone endocrine axis in adult zebrafish (Danio rerio). Ecotoxicol Environ Saf. 2017 Oct;144:402-408.
[9] Mu X et al. Sex specific response in cholesterol level in zebrafish (Danio rerio) after long-term exposure of difenoconazole, Environmental Pollution, Volume 197, February 2015, Pages 278-286
[10] Teng M et al. Effects of the bioconcentration and parental transfer of environmentally relevant concentrations of difenoconazole on endocrine disruption in zebrafish (Danio rerio). Environ Pollut. 2018 Feb;233:208-217.
[11] Liang X at al. Exposure to difenoconazole causes changes of thyroid hormone and gene expression levels in zebrafish larvae. Environ Toxicol Pharmacol. 2015 Nov;40(3):983-7.
[12] Chen L, et al. Environmental Hormone Effects and Bioaccumulation of Propiconazole and Difenoconazole in Procypris merus. Bull Environ Contam Toxicol. 2022 Nov;109(5):823-830.
[13] Dong X et al. Exposure to difenoconazole inhibits reproductive ability in male marine medaka (Oryzias melastigma). J Environ Sci (China). 2018 Jan;63:126-132.
[14] Teng M et al. Sex-specific effects of difenoconazole on the growth hormone endocrine axis in adult zebrafish (Danio rerio). Ecotoxicol Environ Saf. 2017 Oct;144:402-408.
[15] Dong X et al. Reproductive effects of life-cycle exposure to difenoconazole on female marine medaka (Oryzias melastigma). Ecotoxicology. 2017 Aug;26(6):772-781.
[16] Dong X et al. Exposure to difenoconazole inhibits reproductive ability in male marine medaka (Oryzias melastigma). J Environ Sci (China). 2018 Jan;63:126-132.
[17] Mu X et al. Evaluation of acute and developmental effects of difenoconazole via multiple stage zebrafish assays, Environmental Pollution, Volume 175, April 2013, Pages 147-157.

- on **reproduction** (reduced sperm counts; decrease of the fertilization success, the hatch ability and the swim-up success in the F1 generation[18]), (reproductive toxicity and transgenerational effects, reduced egg number produced, realistic levels of exposure[19]), (estrogenic endocrine-disruption effects via altering homeostasis of sex steroid hormones in the HPGL axis and the adverse effects can be transferred to the offspring[20]); (endocrine-disrupting effect on zebrafish HPG axis, reduced fertility[21]), (Sex Hormone-Dependent Reproductive Development[22]);
- on **spermatogenesis** (reduced number of sperm count[23]);
- on dysregulation and **testicular injury in mice**[24]
- on **neurotoxicity and developmental neurotoxicity** (malformations, abnormal locomotor activity[25]), (oxidative stress, neuronal necrosis[26]), (DNA damage in human neuroblastoma[27]);
- effects on **offspring,** (adverse effects transferred to offspring, realistic levels of exposure[28]), (development embryo, teratogenic effects, gene upregulation[29]), (reproductive disease in the offspring[30]), (embryotoxicity in rat embryo culture, difenoconazole most potent[31]), (steroid biosynthesis mediated malformations in F1 of parental exposure to difenoconazole[32]);

Plausible connections between endocrine activity and harmful effects do exist. For instance the changes in sex hormones and the harmful effects seen in reproduction and development, the changes in thyroid hormones and neural maturation, the decrease in mRNA and the reduction of sperm and decrease of fertilization, the U-shapes dose response that give an explanation for the effects at low dose. Moreover the exposure in most cases of these studies is at realistic concentration levels. In the face of such overwhelming evidence and a pattern of being an endocrine disruptor, difenoconazole should be banned immediately. We would welcome an explanation for the absence of such action to date.

Industry of course, despite their duty to do so, disregarded the independent literature studies in their application for difenoconazole. This is no surprise. The mission of industry is to make profit and get their products on the market. And not to protect the public. The dossier on difenoconazole is another bad example of industry trying 'to throw sand in the eyes' of the regulators. Industry didn't put forward solid tests on endocrine disruption and has disregarded a wealth of evidence from the independent studies[33]. Their suggestion that nothing is wrong with difenoconazole regarding endocrine disruption, is concerning. This is a continuing problem for the integrity of the risk assessment. To address this we propose that the Commission sends a mandate to an independent institute (or university) to maintain an up-to-date database of all independent studies of all approved (and applied for) pesticides. And truly independent, excluding studies that are done by industry or by industry consultants or scientists known to work for industry.

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[18] Dong X et al. Exposure to difenoconazole inhibits reproductive ability in male marine medaka (Oryzias melastigma). J Environ Sci (China). 2018 Jan;63:126-132

[19] Dong X et al. Reproductive effects of life-cycle exposure to difenoconazole on female marine medaka (Oryzias melastigma). Ecotoxicology. 2017 Aug;26(6):772-781

[20] Teng M et al. Effects of the bioconcentration and parental transfer of environmentally relevant concentrations of difenoconazole on endocrine disruption in zebrafish (Danio rerio). Environ Pollut. 2018 Feb;233:208-217.

[21] Chen X et al. Exposure to difenoconazole induces reproductive toxicity in zebrafish by interfering with gamete maturation and reproductive behaviour. Sci Total Environ. 2022 Sep 10;838(Pt 1):155610.

[22] Draskau MK et al. (2022), Azole Fungicides and Their Endocrine Disrupting Properties: Perspectives on Sex Hormone-Dependent Reproductive Development. Front. Toxicology 4:883254.

[23] Dong X et al. Exposure to difenoconazole inhibits reproductive ability in male marine medaka (Oryzias melastigma). J Environ Sci (China). 2018 Jan;63:126-132.

[24] Zheng X et al. Difenoconazole Exposure Induces Retinoic Acid Signalling Dysregulation and Testicular Injury in Mice Testes. Toxics. 2023 Mar 30;11(4):328.

[25] Yang et al. Developmental Neurotoxicity of Difenoconazole in Zebrafish Embryos. Toxics. 2023 Apr 8;11(4):353.

[26] Liu et al. Difenoconazole disrupts the blood-brain barrier and results in neurotoxicity in carp by inhibiting the Nrf2 pathway mediated ROS accumulation. Ecotoxicol Environ Saf. 2022 Oct 1;244:114081

[27] Wang X et al. Difenoconazole induces oxidative DNA damage and mitochondria mediated apoptosis in SH-SY5Y cells. Chemosphere. 2021 Nov;283:131160

[28] Teng M et al. Effects of the bioconcentration and parental transfer of environmentally relevant concentrations of difenoconazole on endocrine disruption in zebrafish (Danio rerio). Environ Pollut. 2018 Feb;233:208-217.

[29] Mu et al. The developmental effect of difenoconazole on zebrafish embryos: A mechanism research. Environ Pollut. 2016 May; 212:18-26.

[30] Draskau MK et al. (2022), Azole Fungicides and Their Endocrine Disrupting Properties: Perspectives on Sex Hormone-Dependent Reproductive Development. Front. Toxicology 4:883254.

[31] Dimopoulou N. et al. Embryotoxic and pharmacologic potency ranking of six azoles in the rat whole embryo culture by morphological and transcriptomic analysis, Toxicology and Applied Pharmacology 322 (2017) 15–26.

[32] Chang Y. et al. Developmental defects and potential mechanisms in F1 generation of parents exposed to difenoconazole at different life stages of zebrafish (Danio rerio), Science of The Total Environment Volume 883, 20 July 2023, 163529.

[33] Draft RAR difenoconazole 2019.

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The rapporteur Member State Spain[34], in 2019, fortunately took the initiative to have a look at the independent literature itself and discovered a lot of data reporting adverse effects, mainly on fish. This rightly put a lot of doubt on the reliability of the data submitted by the applicant. Spain concluded that "*the available information on endocrine effects in aquatic organisms (fish) should be used to investigate the mammals/birds data set with heightened scrutiny for similar effects and* to *target potential requests for the generation of further information on mammals/birds*". But still, even in 2023, EFSA didn't ask for additional studies on mammals/birds[35].
While Spain concluded that the entire endocrine assessment should be reassessed, it proposed to ask the applicant to submit '*confirmatory information*'. This is a regulatory loophole that gives the industry sector extra time to claim safety of their products. Spain already provided industry with some critique on the independent studies[36] (validation, lack of critical endpoints) and this will enable the applicant to 'dump' these independent studies in favour of their own tests produced under a major conflict of interest and thus unreliable, that might be submitted at some point in future. In the meantime, difenoconazole keeps on being sprayed in the crop fields and people and the environment continue to be exposed. While we have enough evidence for a ban. We would appreciate an justification about failing to at least initiate an Art. 21 procedure

Unfortunately, there is more. Much more.
For example, in relation to the metabolite 1,2,4-triazole.
Difenoconazole produces several metabolites, 1,2,4-triazole (1,2,4-T), triazole alanine (TA), triazole acetic acid (TAA), and triazole lactic acid (TLA). The metabolite 1,2,4-triazole specifically is a classified reprotoxic substance (R1B[37], due to adverse effects on fertility) while the conclusion on carcinogenicity and on endocrine disruption are still inconclusive. Certainly, it is a metabolite that people and particularly pregnant women and the unborn should not be exposed to, but this is not the case unfortunately in practice[38]. Regulation 1107/2009 is clear on this in Annex II and provides for '*negligible exposure*' (=no contact with humans) for this type of extremely harmful chemicals , also because an experimental safe dose cannot be established. However, EFSA made use of a loophole in the rules that seems to exclude metabolites from the rule, based on negligible exposure. From a scientific point of view this of course makes no sense. If a person is exposed to a R1B, whether it is an active substance or a metabolite, makes no difference. These will be equally harmful. And with the purpose of the Regulation to establish a "*high level of protection*" (Art. 1.3), it is impossible to exclude the metabolite from the "negligible-rule". Nevertheless, EFSA managed to derive a 'safe level' for the metabolite[39] based on an (insensitive) 12-month (industry) rat study and op top of it a default given "*the lack of a developmental neurotoxicity (DNT) study and carcinogenicity and dog studies*". This default is just fictional and there is no science behind it. We even would not be surprised if EFSA derived it by first checking the level that allows actual exposure to 1,2,4-T. Anyway, we hope you will disregard this kind of unscientific regulatory manoeuvres of EFSA and defend '*negligible*' for the metabolite.

In relation to cumulative exposure.
Difenoconazole belongs to a group of conazoles and the cumulative/synergistic (harmful) effects of an exposure to a cocktail of conazoles should be assessed. Back in 2009, EFSA did an attempt to assess the cumulative effects on food residues[40], but with a totally flawed (industry-designed[41]) computer simulation. In the simulation every consumer in a country is supposed to buy their fruit and vegetables in every supermarket in the country at random and on a daily basis. However, consumers usually buy their food from specific shops on a regular basis. Therefore, unless you aim towards a specific, desired outcome, it is incomprehensible that you accept such a blatantly wrong assessment. And also allow EFSA to assume that all "pollution " comes from one source, the food, while all other routes of exposure (chemicals in the air, cosmetics, biocides, chemicals in dust. etc.) are considered non-existent. This again is scientifically biased.
Food is one route of exposure, but what about people living in the countryside? They are not only exposed to pesticides via food, but also via house dust(198 pesticide residues was assessed in 128 indoor dust samples[42]), air[43] , etc. Residents are exposed to many dozens of pesticides every day. But

[34] Draft RAR difenoconazole 2019.
[35] EFSA Supporting publication 2023:EN-8474
[36] Draft RAR difenoconazole 2019. Page 288
[37] RAC evaluation report, march 2021.
[38] UK report on Triazole Derivative Metabolites, 2016.
[39] EFSA Journal 2018;16(7):5376
[40] EFSA Journal 2009; 7 (9); 1167
[41] https://www.pan-europe.info/sites/pan-europe.info/files/public/resources/reports/pane-2014-a-poisonous-injection.pdf
[42] Navarro I. et al. Occurrence of pesticide residues in indoor dust of farmworker households across Europe and Argentina, Science of the Total Environment 905 (2023) 167797.
[43] Silva V. et al. Pesticide residues with hazard classifications relevant to non-target species

so far there has been no cumulative assessment in your decisions. We are eager to learn about your plans for evaluating the cumulative effects on residents, including both the timeline and methodology for these assessments

Fungicide resistance.

Aspergillus fumigatus is a saprobic fungus that may cause allergic syndromes, chronic pulmonary aspergillosis (CPA), and acute invasive aspergillosis (IA). Many patients suffering from aspergillus diseases (weak immune system, risk pneumonia) benefit from antifungal therapy. Conazoles, including difenoconazole[44], have been shown to be the most effective compounds for prevention and treatment of the various aspergillus diseases. Unfortunately, the effective use of conazoles has been threatened by the emergence of resistance in *A. fumigatus*. As the number of available drug classes is already very limited, some aspergillus diseases, such as central nervous system IA, are virtually untreatable if caused by a conazole-resistant isolate[45]. Resistant varieties of *Aspergillus fumigatus* have been discovered since 1998 and it is hard to understand why the authorities have been hesitating so long to tackle this issue. The problem is increasing fast because spores of the resistant fungus, arising on agricultural fields, are transported by air[46]. Waste heaps on field where flower bulbs are growing are one of the 'hot spots' of resistant fungi spores[47]. Again, residents that are exposed greatly. And your services at DG Sante are very much aware of the problem. The Danish ministry of environment and food send to DG SANTE a letter in December 2019 expressing their concerns on fungi resistance and your lack of action. The Netherlands already in 2020 voted against a prolongation of difenoconazole because of the problem of resistance[48]. Your reaction is that resistance can be taken into account but so far no mention was made in the regulatory dossiers (except metconazole) on the potential clinical and epidemiological issue of cross-resistance to medical azoles in patients infected with *A. fumigatus.* There is a simple conclusion: we have to ban all uses of conazoles in agriculture to save human lives. On your website we can read: "*we intend to make Europe a healthier and safer place. Our mission is to protect the citizens' health and monitor their food making sure it is safe*".

We respectfully urge you Ms. Kyriakides, to put your commitment into action by initiating an Article 21 procedure to ban the conazoles, starting with difenoconazole.

May we anticipate your support in this important matter?

Thank you in advance for your prompt response.

Sincerely yours,

Hans Muilerman,

Pesticide Action Network, Brussels.

including humans are omnipresent in the environment and farmer residences, Environment International 181 (2023) 108280.

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

[45] Buil JB. et al. , (2019) The fading boundaries between patient and environmental routes of triazole resistance selection in Aspergillus fumigatus. PLoS Pathog 15(8): e1007858

[46] Royal Haskoning/DHV, Resistentieontwikkeling van Aspergillus fumigatus tegen triazolen door gebruik van biociden en gewasbeschermingsmiddelen, 2013.

[47] Leenderse PC. et al. Verkenning naar de aanwezigheid van resistente Aspergillus fumigatus in de land- en tuinbouwketen, CLM, publicatienummer 1067, juni 2021.

[48] ScoPAFF, 29 september 2020.