EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

**Director-General**

Brussels,
SANTE/E4/MW/gb(2021)8786383

**Subject: Request for a Scientific Report on the impact of the use of azole fungicides, other than as human medicines, on the development of azole-resistant *Aspergillus spp.***

Dear Dr Url,
Dear Mr Hansen,
Dear Ms Cooke,
Dear Ms Ammon,
Dear Mr Bruyninckx

*Aspergillus fumigatus, Aspergillus flavus and Aspergillus terreus* are common and naturally occurring saprophytic fungi found in soil, to which humans are commonly exposed through inhalation under normal circumstances.

In healthy individuals the spores are rapidly cleared from the airways, however a range of diseases may be caused in specific patient groups. Many *Aspergillus* infections ("aspergillosis") are treated with azoles-based medicines with alternatives being limited. However, the emergence of azole-resistant *Aspergillus spp.* is diminishing the effectiveness of medicinal azole treatments.

Resistance may develop during treatment with azole medicines (patient route), but may also develop in the environment (environmental route). There is growing evidence that *Aspergillus spp.* acquire resistance mutations when exposed to azoles in the environment. The incidence of environmental resistance has been increasing over the past decade and azole resistant *Aspergillus (*in particular *A. fumigatus)* isolates have been found in patients not previously treated with azole-based medicines (azole-naive patients).

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Other than their use as human medicines, azoles are widely used in agriculture (for the control of fungal diseases), as biocides (in particular as wood preservatives), veterinary medicines (to treat fungal diseases in companion and food-producing animals), industrial chemicals (for example as intermediates or dyes) and in cosmetics (as anti-dandruff agents or preservatives). Azoles may reach various environmental compartments through those uses. Therefore, *Aspergillus spp.* can come into contact with azole substances from various sources and through various routes of exposure other than human medicinal products.

Several Member States have carried out research or reviewed existing knowledge in this area, in particular looking at the conditions or uses which favour such resistance development (so called 'hotspots'), and have raised concerns about the emergence of environmental resistance, calling on the Commission to further investigate the matter, in view of considering any necessary regulatory actions or follow up work that may be required.

Data from Denmark and the Netherlands (Member States with national monitoring programmes), indicate that the incidence of resistant isolates originating from environmental exposure is increasing (environmental resistance mutations have been found in 77% to 84% of resistant patient *A. fumigatus* isolates in the Netherlands[1] (2017-2019) and 74% (14 out of 19 isolates) in Denmark[2], and are an important cause of triazole-resistant aspergillus disease).

Given those concerns, in March 2021, the Netherlands amended the authorisations of twelve azole-containing plant protection products used on flower bulbs and tubers, including the flowers resulting therefrom, introducing a protocol[3] to reduce the long-term storage of plant waste and reduce further development and spread of resistant *Aspergillus fumigatus*.

The European Centre for Disease Prevention and Control (ECDC) published a Technical Report[4] on this topic in 2013. Since that time, further research has been undertaken and should now be reviewed to advance knowledge and understanding and to provide the necessary information to inform on the possible actions to limit the further emergence of azole-resistant *Aspergillus* spp and its spread into humans.

The agrochemical industry has also commissioned research into azole resistance[5], and is currently establishing a Scientific Network on anti-fungal resistance to promote scientific exchange and cooperation between scientists and research institutions (public & private) from the medical and agricultural fields.

Given the importance and the One Health nature of this topic, I would like to ask the European Food Safety Authority (EFSA), the European Chemical Agency (ECHA), the

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1 See NethMap reports 2018 – 2020. The NethMap reports can be downloaded from https://swab nl/en/nethmap

2 Danish State Serum Institute. Ref. 18/01631 Monitoring of *A. fumigatus* – half-yearly report (October 2018 – March 2019).https://www.ssi.dk/aktuelt/nyheder/2019/de-forste-tal-fra-den-nye-overvagning-af-resistens-i-svampen-aspergillus

3 http://www.ctgb nl/azolenprotocol

4 European Centre for Disease Prevention and Control. Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in Aspergillus species. Stockholm: ECDC; 2013.

5 https://croplife.org/crop-protection/stewardship/azole-resistance-aspergillus-fumigatus/

European Medicines Agency (EMA), the European Centre for Disease Prevention and Control (ECDC) and the European Environment Agency (EEA) to collaborate and produce a Scientific Report addressing the Terms of Reference included in the Annex.

I note that the Joint Research Centre (JRC) has agreed to provide input and support to this activity. The agencies may also wish to involve the Member States' authorities in the task, in particular those which have already conducted and/or continue to carry out research in this area – further details can be found in the Annex.

The agencies and JRC are responsible for agreeing the distribution of tasks and the working modalities required to deliver the final Scientific Report. EFSA has agreed to act as the coordinator for this mandate.

Given the complex nature of the mandate and the need for coordination and agreement of the different tasks, as well as between the different parties involved, the Scientific Report should be delivered 30 months from the date of issue of this mandate.

My services remain at your disposal for further information. On this matter, you can contact [redacted] (DG SANTE E4) who is the coordinator for this file – contact details can be found below.

Yours sincerely,

[e-signed]
Sandra GALLINA

Contact: [redacted]

Cc.: [redacted]

[redacted] (DG SANTE)

[redacted] (JRC)

## ANNEX

## 1. BACKGROUND

In its 2013 report, the European Centre for Disease Control (ECDC) considered the burden of aspergillosis in the European region and the implications for azole resistance, concluding that more than 2.3 million patients with allergic or chronic aspergillosis could potentially benefit from long-term oral azole therapy and therefore azole resistance is highly problematic for both groups of patients. Failure of azole therapy can result in serious health outcomes including mortality, particularly in patients with invasive aspergillosis.

In addition to their use as human medicines, azole fungicides (primarily triazoles and imidazoles but also other classes e.g. thiazoles) are important and effective substances for disease control in agriculture[6]. They have been used extensively for decades to control foliar and seed-borne diseases due to their relatively low cost and effectiveness against a broad range of fungal pests; triazoles are the most widely used systemic fungicides. Over recent years, the number of substances approved in the EU for use in plant protection products has decreased because the approvals of several azole substances (e.g. epoxiconazole, propiconazole) which were used in significant quantities in the EU have not been renewed.

Azoles are also used as biocides (used in various biocidal product-types, mainly for preservative function, in particular for wood), veterinary medicines (to treat fungal diseases in companion and food-producing animals[7]), industrial chemicals and less commonly in cosmetic products.

It should also be noted that azole substances breakdown into metabolites that may be present on or in foodstuffs or in the environment. One common metabolite of triazole fungicides is 1,2,4-triazole which is also widely used as an industrial chemical (e.g. as a nitrification inhibitor in nitrogen fertilizers). However, research suggests that 1,2,4-triazole and other metabolites of azoles are unlikely drivers of resistance in *Aspergillus spp*[8].

Some important and relevant areas of completed or ongoing research are outlined below.

<u>Development of azole-resistant *Aspergillus* and identification of environments or uses of azoles that select for resistance (including so called 'hotspots')</u>

The Danish Working Group on Azole Resistance[9] has taken the view that the current literature shows that there is a correlation between the use of azole substances in the environment and the development of environmental resistance mutations observed in *A. fumigatus* isolated in patients. At the same time, the Working Group has flagged an increasing incidence of azole resistance – both because of treatment with azole medicines (patient route) and through environmental resistance (environmental route).

6 Against a wide range of fungal diseases, but not directly targeted at *Aspergillus fumigatus*

7 One central authorisation for the treatment of *Aspergillus spp* (and *Candida spp.)* in ornamental birds exists for the active substance itraconazole. National marketing authorisations exist for veterinary medicines containing azoles for the treatment of dermatophytosis in cattle, horses and companion animals, and for treatment of *Malassezia otitis* in companion animals

8 Jørgensen, K.M.; Helleberg, M.; Hare, R.K.; Jørgensen, L.N.; Arendrup, M.C. Dissection of the Activity of Agricultural Fungicides against Clinical *Aspergillus* Isolates with and without Environmentally and Medically Induced Azole Resistance. *J. Fungi* 2021, *7*, 205. https://doi.org/10.3390/jof7030205 https://www mdpi.com/2309-608X/7/3/205

9 https://www ft.dk/samling/20181/almdel/MOF/bilag/407/2016336.pdf

In 2019, a workshop on Azole resistance was held in Amsterdam, in which medical and agricultural researchers, representatives from the government, public health, fungicide producers and end-users, reviewed the current situation[10].

The Norwegian Veterinary Institute (NVI) also published a report[11] in 2019 as part of the network project "Interdisciplinary think tank to minimize the emergence and spread of antifungal resistance» (ResAzoleNet)". The report provides a short overview of the current knowledge on azole resistance in a One Health perspective and identified some major knowledge gaps from a Norwegian perspective.

Research over recent years has advanced knowledge on important aspects of environmental resistance, including its development and in particular on the identification of 'hotspots' where azole-resistant *Aspergillus spp.* could emerge and propagate.

Such research has highlighted important findings, including:

- Identification of similarities between some of the medical azoles and the azoles used in agriculture. Laboratory studies have shown that at least five azole fungicides have an effect on wild type *A. fumigatus* comparable to the medical azoles – with very similar molecular structures and similar poses while docking to the active site of *A. fumigatus*[12].
- Identification of specific mutations associated with resistance have shown that some mutations are associated with environmental exposure, rather than clinical exposure e.g. $TR_{46}$ and $TR_{92}$ mutations were recovered only from flower bulb waste, which might be caused by use of specific (combination of) azole fungicides or concentrations of azole residues (Schoustra., et al., 2019)[13].
- The hypothesis that there are certain so-called environmental 'hotspots' where azole-resistant *Aspergillus* can emerge, leading to formation of a source of azole-resistant air-borne spores. These hotspots of azole-resistant *Aspergillus* are environments favourable for the abundant growth and sporulation of *Aspergillus* in the presence of environmental azoles.
- Indication of aquatic environments as a source of human exposure to *Aspergillus spp.* and as a place for development or resistance and subsequent reservoir of resistant forms has been described in a recent review[14].

Hotspots

Three hotspots have been identified in the Netherlands - flower bulb waste, green waste processing and wood chippings, with tebuconazole, epoxiconazole, and prothioconazole being detected as the most frequently found fungicide residues (Schoustra et al., 2019). Furthermore, studies have shown that the storage of decaying plant waste in the bulb-

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[10] Verweij, et al., 2020. The one health problem of azole resistance in Aspergillus fumigatus: current insights and future research agenda. Fungal Biology Reviews, Volume 34, Pages 202-214, ISSN 1749-4613, https://doi.org/10.1016/j fbr.2020.10.003.

[11] Knowledge and knowledge gaps on azole resistance in a One Health perspective. Report 3-2019: Azole resistance in a One Health perspective. ISSN 1890-3290 Norwegian Veterinary Institute 2019.

[12] Snelders E, Camps SMT, Karawajczyk A, Schaftenaar G, Kema GHJ, et al. (2012) Triazole Fungicides Can Induce Cross-Resistance to Medical Triazoles in *Aspergillus fumigatus*. PLOS ONE 7(3): e31801. https://doi.org/10.1371/journal.pone.0031801

[13] Schoustra SE, Debets A, Rijs A, Zhang J, Snelders E, Leendertse PC, et al. Environmental Hotspots for Azole Resistance Selection of Aspergillus fumigatus, the Netherlands. Emerg Infect Dis. 2019;25(7):1347-1353. https://dx.doi.org/10.3201/eid2507.181625

[14] Richardson M. and Rautemaa-Richardson R., Exposure to Aspergillus in Home and Healthcare Facilities' Water Environments: Focus on Biofilms. Microorganisms 2019, 7(1), 7; doi:10.3390/microorganisms7010007

growing industry facilitates the selection of azole resistance in *A. fumigatus*, when residues of azoles are present[15]. In the Netherlands, a more detailed study on the hotspots identified in green waste processing and wood chippings is ongoing as well as an exploratory study on possible agricultural hotspots. Both studies are expected to be published in 2021. In addition, the research project ‘Circular agriculture and health risk’ will investigate *Aspergillus fumigatus* in waste streams of the flower bulb industry in more detail in the coming years. The knowledge acquired will be used to design circular agriculture in such a way that health risks can be prevented in the future.

Research in France has also shown the presence of azole-resistant *A. fumigatus* strains in sawmills.[16]

In Denmark, several studies have been carried out to obtain a better knowledge on resistance development and to provide information on fungicides that are important drivers to development of resistance and to investigate whether Denmark may have similar hotspots to those identified in the Netherlands, which can provide an optimal environment for development of azole resistant *Aspergillus*:

- Survey of potential hot-spots for development of environmental azole resistance (pilot project on composting practices)[17]
- Identification of azole fungicides and metabolites with a potential role in resistance development in *Aspergillus*[18]

A further 3 year project has been initiated (running from 1 July 2020 to 30 June 2023) to follow up on the pilot studies:

- Investigations for environmental hotspots and application practices for azole resistant *Aspergillus fumigatus*.

Recently, the project “Human exposure to antifungal resistant fungi across environments and time” was initiated (running from 1 July 2021 to 30 June 2024) on the presence of antifungal resistance across a wide range of environments, the potential occupational risk that this poses, and possible routes for transmission[19].

Several research projects are also underway in Norway:

- Pathogenic fungi in Norwegian barns - are they resistant to fungicides? (the ‘Barns’ project) - The aim of this project is to examine if animal sheds and barns could be a hot spot for azole resistant *A. fumigatus* in Norway, and thus be a potential health risk for animals and farmers.

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[15] Schoustra, S.E. *et al.*, 2019, New insights in the development of azole-resistance in *Aspergillus fumigatus*, RIVM Letter report 2018-0131

[16] Jeanvoine A, Rocchi S, Reboux G, Crini N, Crini G, Millon L. Azole-resistant Aspergillus fumigatus in sawmills of Eastern France. J Appl Microbiol. 2017 Jul;123(1):172-184. doi: 10.1111/jam.13488. Epub 2017 Jun 9. PMID: 28497646.

[17] Azeolresistens: Kortlægning af mulige hotspots for azolresistensdannelse i miljøet i Danmark. Rapport fra DCA - Nationalt Center for Fødevarer og Jordbrug Dato: 6.10.2020. Seniorforsker Lise Nistrup Jørgensen Institut for Agroøkologi, AU https://pure.au.dk/portal/files/198093640/Levering_Unders_gelse_af_forekomsten_af_Azolresistente_Aspergilus_i_Danmark.pdf (Summary in English, otherwise in Danish)

[18] Jørgensen, K.M.; Helleberg, M.; Hare, R.K.; Jørgensen, L.N.; Arendrup, M.C. Dissection of the Activity of Agricultural Fungicides against Clinical *Aspergillus* Isolates with and without Environmentally and Medically Induced Azole Resistance. *J. Fungi* 2021, *7*, 205. https://doi.org/10.3390/jof7030205 https://www mdpi.com/2309-608X/7/3/205

[19] Both projects are funded by the ‘Pesticide Research Programme’ under the Danish EPA

- Navigating the threat of azole resistance development in human, plant and animal pathogens in Norway (the 'Navazole' project). This project is a concerted action to establish methods, networks and routines for diagnostics and surveillance, It is spanning the human, animal and plant health sectors as well as the environment, i.e. One Health, and will seek to prevent further spread of azole resistance while enabling sustainable use of the azole class of fungicides in the future.

In Belgium the Sciensano Institute has scheduled a 4-year study[20] (including field trials in cereals and ornamental crops) to identify potential hot spots from the use of plant protection products containing triazoles, with the purpose to understand the possible role of such uses in the development of resistance to triazoles used in the therapy of aspergillosis.

Recent research[21] has also looked at whether the use of azoles in cereal crops might lead to the emergence of azole resistance of *Aspergillus spp* in the environment, in which it has been indicated that arable production represents a 'coldspot' for resistance development.

The development of a risk matrix for the selection of resistance in Aspergillus strains, looking at levels of exposure to azoles and exposure of *A. fumigatus* has also been put forward[22].

A project carried out at Rothamsted Research in the UK, focused on understanding the sources and spread of azole resistance in environmental *A. fumigatus* populations remains ongoing - peer-reviewed articles are under preparation and expected in the near future[23].

Other routes of exposure to azole resistant *A. fumigatus*

There is currently no indication (i.e. from the EU pharmacovigilance system or from national experts) on azole-resistant strains of *Aspergillus spp.* in animals (companion and food-producing animals) which therefore suggests that animals are not a source of the spread of resistant *Aspergillus spp.* to humans.

Environmental azole resistant *A. fumigatus* has also been found on traded products – an Irish investigation found environmental azole resistant *A. fumigatus* on Dutch flower bulbs in a plant nursery[24]. This raises the questions about whether other plants, flowers of vegetable products might be potential sources of spread via trade e.g. environmental resistant isolates have been found in connection with flower production in Colombia and Tanzania, two countries which have major exports to Europe[25].

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20 https://www.sciensano.be/en/projects/investigation-resistance-mechanisms-emerging-pathogens-onehealth-concept-missing-link

21 Fraaije B, Atkins S, Hanley S, Macdonald A, Lucas J. The Multi-Fungicide Resistance Status of *Aspergillus fumigatus* Populations in Arable Soils and the Wider European Environment. Front Microbiol. 2020 Dec 15;11:599233. doi: 10.3389/fmicb.2020.599233. PMID: 33384673; PMCID: PMC7770239.

22 Gisi, U. (2014), Assessment of selection and resistance risk for demethylation inhibitor fungicides in *Aspergillus fumigatus* in agriculture and medicine: a critical review. Pest. Manag. Sci., 70: 352-364. https://doi.org/10.1002/ps.3664

23 An abstract of the project's results can be found here: https://apsnet.confex.com/apsnet/2019/meetingapp.cgi/Paper/12610)

24 Dunne K, Hagen F, Pomeroy N, Meis JF, Rogers TR. Intercountry Transfer of Triazole-Resistant Aspergillus fumigatus on Plant Bulbs. Clin Infect Dis. 2017 Jul 1;65(1):147-149. doi: 10.1093/cid/cix257. PMID: 28369271.

25 Anuradha Chowdhary, Cheshta Sharma, Mara van den Boom, Jan Bart Yntema, Ferry Hagen, Paul E. Verweij, Jacques F. Meis, Multi-azole-resistant *Aspergillus fumigatus* in the environment in Tanzania, *Journal of Antimicrobial Chemotherapy*, Volume 69, Issue 11, November 2014, Pages 2979–2983, https://doi.org/10.1093/jac/dku259

## 2. TERMS OF REFERENCE

In view of the One Health nature of this subject, a Scientific Report is requested under Article 31 of Regulation (EC) No 178/2002 (for EFSA), Article 75 (1)(g) of Regulation (EU) No 528/2012 (for ECHA), Article 5(3) and Article 30(3) of Regulation (EU) No 726/2004 (for EMA) and Article 7(1)(a) of Regulation (EC) No 851/2004 (for ECDC). EEA is requested to contribute to the task by providing data, where available. The JRC has agreed to provide support to the task, including some experimental work.

Taking into account the background information provided in this Annex and other available sources, the Scientific Report should address the following points regarding the development of azole resistance:

- provide details about the use of azole fungicides, other than as human medicines, in the EU/EEA giving information about the types of use, the current and trend in quantities used and as much detail as possible on geographical variation.
- to what extent the current information suggests or supports the possibility of a causative link between the use of azole fungicides, other than as human medicines, and the development of azole resistant *Aspergiullus* species. Experimental work carried out by the JRC to strengthen the available data on development of resistance should be taken into account.
- In case a link is confirmed between exposure to azoles (in the environment) and development of resistance (point above):
    - a consideration of the epidemiology of azole resistant human infections caused by *Aspergillus* species acquiring resistance in the environmentshould be provided, considering patient exposure and transmission routes, in particular aerial dispersal;
    - where the possibility of a significant risk to human health is identified, the risk to health should be quantified as far as possible in terms of levels of morbidity/mortality (including from occupational exposure e.g. to workers involved in agriculture or waste processing) now and in the future, in particular assessing the current scale of the problem and its relevance and impact on human health (and animal health, if relevant) in the EU, as well as an estimation of how the situation could develop in the future;
- review the drivers/selection dynamics behind the development of environmental resistance in *Aspergillus* (in so called “hotspots”), in particular identification of specific types of uses, individual classes of substances, and the use conditions that lead to the development of environmental resistance (including conditions during storage and processing of (waste) materials with azole residues), providing, if possible, an indication of the selection risk from different uses and the contribution of sectoral uses of azoles. Experimental work should be considered in order to provide additional information on this point.

In addition, taking into account the above, the agencies and JRC should provide advice on to what extent further actions by the EU could be considered to reduce any risks to human health identified and if so what those actions may be in view of providing the agencies, Member States and/or the Commission with information that could be taken into account during the assessment and decision-making of azole substances and/or products containing them. Where relevant sectorial specific advice should be included. In particular the following points should be considered:

— identify measures that could be implemented with respect to the use of azole fungicides and storage/processing of (waste) materials with azole residues and metabolites to prevent or minimise the development of environmental resistance or minimise the spread of resistant *Aspergillus* into patients;

— identify the type of studies that could be provided by applicants when submitting applications for approval of azole substances for use other than as a human medicines which would help to determine the risk of inducing mutations conferring resistance to medicinal azoles and which methodology should be used to assess that risk;

— identify any uncertainties and further research needs that will provide information to inform decision-making for azole substances and/or products containing them at EU and national level.

The agencies and JRC should endeavour to cover all the available scientific evidence in their assessment, including search and analysis following systematic literature review methodology.