

# Preliminary SGV – Proposal by the Ecotox Centre for

Azoxystrobin



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#### Scientific Support

Due to the delayed but soon expected European review assessment dossier for azoxystrobin, the results of this SGV dossier have not been peer-reviewed externally (also see the Policy disclaimer below).

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## Policy disclaimer

According to the Action Plan for PPP (AP-PPP) (measure 6.3.3.7), pesticides in soil should be monitored in order to verify the evaluation carried out within the framework of the registration regarding the persistence of pesticides in the environment and their effect on soil organisms and soil functions. Therefore, a suitable method (indicator) for effects of PPP on soil fertility has to be developed and applied in field studies. Risk-based reference values for PPP residues should be available by 2025, and bioindicators for the effects of PPP residues on soil fertility should be developed by 2027.

In response to the AP-PPP and tasked by FOEN and FOAG, experts from the Ecotox Centre and EnviBioSoil have been working since 2018 on an integrative concept to assess the effects of PPP residues in soil. The following dossier represents the full evaluation, derivation and proposal of a Soil Guideline Value (a risk-based reference value), according to the recommended methodology developed within the AP-PPP project (Marti-Roura *et al.* 2023), and does not have a regulatory nature that goes beyond their intended use within the ongoing AP-PPP project. Further information on the ConSoil project and its framework can be found at: <a href="https://www.ecotoxcentre.ch/projects/soil-ecotoxicology/monitoring-concept-for-plant-protection-products-in-soils?ga=2.170121120.1893072167.1726132886-1891293576.1686657912.</a>

Due to the delay of the European review assessment dossier for azoxystrobin that is expected to be launched for consultation in the near future (dossiers were submitted at the end of 2021 by several applicants but neither IUCLID dossiers nor a draft Assessment Report from the Rapporteur Member State are available to date), the SGV has had to be based mostly on scientific literature data. As a result, this dossier has not been peer reviewed externally yet and the final result is considered as a preliminary SGV that is expected to change after the inclusion of the new regulatory data.



## **Executive summary**

As part of the Federal Action Plan on Plant Protection Products (Bundesrat, 2017), the Ecotox Centre develops proposals for Soil Guideline Values (SGV). These values are intended to provide an initial screening tool for assessing the potential risk for the long-term fertility of agricultural soils and for the soil ecosystem in general. Based on existing effect data for azoxystrobin and applying the methodology described in the EU Technical Guidance Document on risk assessment (EC TGD 2003), with adaptations as described in Marti-Roura *et al.* (2023), a preliminary generic SGV for azoxystrobin of 14 µg a.s./kg soil d.w. is proposed for a standard soil with 3.4 % organic matter.

## Zusammenfassung

Im Rahmen des Aktionsplans Pflanzenschutzmittel (Bundesrat, 2017) erarbeitet das Ökotoxzentrum Vorschläge für Bodenrichtwerte (SGV). Diese Werte sollen ein erstes Screening-Instrument zur Bewertung der potenziellen Risiken für die langfristige Fruchtbarkeit landwirtschaftlicher Böden und für das Ökosystem Boden im Allgemeinen darstellen. Auf der Grundlage vorhandener Wirkungsdaten für Azoxystrobin und unter Anwendung der im Technischen Leitfaden der EU zur Risikobewertung beschriebenen Methodik (EC TGD 2003) und den in Marti-Roura *et al.* (2023) beschriebenen Anpassungen wird **ein voläufiger generischer SGV** für Azoxystrobin **von 14 µg a.s. pro kg Bodentrockengewicht für einen Standardboden mit 3,4 % organischer Substanz** vorgeschlagen.

## Résumé

Dans le cadre du plan d'action Produits phytosanitaires (Conseil fédéral, 2017), le Centre Ecotox élabore des propositions de valeurs guides pour les sols (SGV). Ces valeurs sont destinées à fournir un outil de dépistage initial pour évaluer le risque potentiel pour la fertilité à long terme des sols agricoles et pour l'écosystème du sol en général. Sur la base des données existantes relatives aux effets du azoxystrobine et en appliquant la méthodologie décrite dans le document d'orientation technique de l'UE sur l'évaluation des risques (EC TGD 2003), avec les adaptations décrites dans Marti-Roura *et al.* (2023), une SGV générique préliminaire pour le azoxystrobine de 14 µg a.s./kg de sol p.s. est proposée pour un sol standard contenant 3,4 % de matière organique.

#### Sommario

Nell'ambito del Piano d'azione dei prodotti fitosanitari (Consiglio federale svizzero, 2017), il Centro Ecotox sviluppa proposte di valori guida per il suolo (SGV). Questi valori sono destinati a fornire uno strumento di screening iniziale per valutare il rischio potenziale per la fertilità a lungo termine dei suoli agricoli e per l'ecosistema del suolo in generale. Sulla base dei dati esistenti sugli effetti del azossistrobina e applicando la metodologia descritta nel documento tecnico di orientamento dell'UE sulla valutazione del rischio (EC TGD 2003), con gli adattamenti descritti in Marti-Roura *et al.* (2023), viene proposto un SGV generico preliminare per il azossistrobina di 14 µg a.s./kg di suolo (peso secco) per un suolo standard con il 3,4% di materia organica.



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## 1 General information

Information on the pesticide active substance azoxystrobin in relation to the soil environment is presented in this chapter. Registration information and risk assessments – listed chronologically – referred to are as follows:

- DE (1997): Monograph for Azoxystrobin Report and Proposed Decision. Rapporteur Member State: Germany. 29 January 1997 [Volume 1 and partial access to Volume 3 including B.1-B.5.5]
- EC (1998): Review report for the active substance azoxystrobin. Finalised in the Standing Committee on Plant Health at its meeting on 22.4.1998 in view of the inclusion of azoxystrobin in Annex I of Directive 91/414/EEC. European Commission, Directorate General for Agriculture, DG VI-B.II-1. Azoxystrobin, 7581/VI/97-Final, 22 April 1998.
- UK (2009): Report and Proposed Decision of the United Kingdom made to the European Commission under Commission Regulation 737/2007 for Azoxystrobin. Rapporteur Member State: United Kingdom. May 2009.
- EFSA (2010): Conclusion on the peer review of the pesticide risk assessment of the active substance azoxystrobin. Conclusion on Pesticide Peer Review, European Food Safety Authority (EFSA), Parma, Italy.
- UK (2014): Addendum Confirmatory Information: B.8 Environmental Fate; B.9 Ecotoxicology to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 6 of Commission Regulation (EC) No 737/2007, taking account of confirmatory data specified in Commission Implementing Regulation (EU) No 703/2011. RMS: United Kingdom. December 2014.
- EFSA (2014): Outcome of the consultation with Member States, applicant and EFSA on the pesticide risk assessment of confirmatory data for the active substance azoxystrobin. European Food Safety Authority (EFSA), Parma, Italy.
- EC (2015c): Final Review report for the active substance azoxystrobin finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 17 June 2011 in view of the approval of azoxystrobin as active substance in accordance with Regulation (EC) No 1107/2009. Commission staff working document. European Commission, Directorate-General for Health and Food Safety, Safety of the Food Chain, Pesticides and Biocides. Azoxystrobin, SANCO/11027/2011 Rev 3, 20 March 2015.

**For azoxystrobin, the first evaluation dossier in Europe** was completed by Germany as Rapporteur Member State (RMS) (DE 1997). For this evaluation, the representative products included Priori 250 SC (suspension concentrate; YF9247), Amistar 250 SC (YF9246) and Amistar 500 WG (water dispersable granule; YF8287). The following peer review and consultation with the Member States resulted in support of including azoxystrobin as a fungicide in Annex I to Directive 91/414/EEC (EC 1998).

Then azoxystrobin was included in the framework of the 1<sup>st</sup> European program for the renewal of approvals of pesticide active substances (EU 2007). This time the United Kingdom acted as RMS finalising their Assessment Report (AR; UK (2009)) and an addendum to the AR in 2009 that resulted in a supporting EFSA conclusion (EFSA 2010) and Commission decision. The approved plant protection products were Amistar and Ortiva (SC formulations with 250 g/L azoxystrobin) at the time. For the renewal evaluation, the representative product was also an SC formulation containing 250 g a.s./L ('250 SC'/YF10537). The inclusion of azoxystrobin in Annex I to Directive 91/414/EEC was renewed in 2012 deeming it to be approved under Regulation (EC) No 1107/2009, however, with special



provisions that required submitting confirmatory information regarding the risk assessment on groundwater and aquatic organisms (EFSA 2014). The addendum compiled by the UK as RMS contains the latest updated endpoints and risk assessment available so far (UK 2014, EC 2015c).

Since then, azoxystrobin got included in the framework of the 4th European program for renewing the approvals of pesticide active substances (Group 4 (2): Substances with current expiry dates between 31 July 2019 and 31 December 2021 that will be postponed three years; EC (2024)). It is noted that according to the draft working document on the AIR IV Renewal Programme (2024), a renewal dossier for azoxystrobin was submitted on 31/03/2022. Conversely, according to the Open EFSA website, the new dossier was received on 16/12/2021 (EFSA-Q-2023-00763, https://open.efsa.europa.eu/). So far, neither a draft Renewal Assessment Report nor any IUCLID dossiers (the publicly available dossiers submitted by the applicants – two Task Forces and several individual applicants in the case of azoxystrobin) have been made publicly available so far (EC 2025, EU 2020).

## 1.1 Identity and physico-chemical properties

Azoxystrobin (CAS 131860-33-8) is a broad-spectrum strobilurin fungicide. Its provisional minimum purity as manufactured is 965 g/kg (UK 2009, 2014). The technical active substance consists of one geometric isomer, the *E-isomer*, with *Z-isomer* as an impurity (a.k.a. metabolite R230310; max. 25 g/kg; UK (2009), Vol. 1, LoEP; see structural formulas in Figure 1). The technical grade material also contains *toluene* as a relevant impurity (maximum 2 g/kg; EC (2011)). The pure material is a white crystalline powder; the technical material (purity of 962 g/kg) is a pale-brown crystalline powder (see further details on physico-chemical properties in Figure 1 below).

| Name (synonyms)                         | Structural formula                    | Reference |
|---|---------------------------------------|-----------|
| Azoxystrobin                            | CN OCH <sub>3</sub>                   | UK (2014) |
| Z-isomer of azoxystrobin (R230310; M09) | CN CH <sub>3</sub> O OCH <sub>3</sub> | UK (2014) |

Figure 1: Two possible stereoisomeric forms of azoxystrobin

 $Table\ 1: Identification\ and\ physico-chemical\ properties\ of\ azoxystrobin.$ 

| Characteristics             | Values  | References               |
|-----------------------------|---|--------------------------|
| Common name                 | Azoxystrobin  | UK (2009)<br>EFSA (2010) |
|                             |   | UK (2014)                |
| Producer's development code | R215504   | DE (1997)                |
| number                      | E5504<br>ICIA5504   |                          |
| IUPAC name                  | methyl (E)-2-{2[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate | EFSA (2010)              |
| Chemical group              | Strobilurin fungicide   | Lewis et al. (2016)      |



| Characteristics  | Values   | References   |
|--|--|--|
| Structural formula   | See Error! Reference source not found. above   |  |
| Molecular formula<br>CAS   | C <sub>22</sub> H <sub>17</sub> N <sub>3</sub> O <sub>5</sub><br>131860-33-8   | EFSA (2010)  |
| EC Number<br>SMILES code (canonical SMILES)                                | 603-524-3<br>COC=C(C1=CC=CC=C1OC2=NC=NC(=C2)OC<br>3=CC=CC=C3C#N)C(=O)OC  | Lewis <i>et al.</i> (2016)<br>Lewis <i>et al.</i> (2016) |
| International Chemical Identifier key (InChIKey)                           | WFDXOXNFNRHQEC-GHRIWEEISA-N  | Lewis et al. (2016)                                      |
| Molecular weight [g/mol] Melting point [°C] Boiling point [°C]             | 403.4<br>116 (purity: 990 g/kg)<br>Above 360   | EFSA (2010)<br>EFSA (2010)<br>EFSA (2010)                |
| Solubility   |  |  |
| Water solubility [mg/L]  | pH 5.2: 6.7 at 20°C (purity: 962 g/kg)<br>pH 7.0: 6.7 at 20°C (purity: 962 g/kg)<br>pH 9.2: 5.9 at 20°C (purity: 962 g/kg)                                       | EFSA (2010)  |
| Solubility in organic solvents [g/L]                                       | (At 20°C; purity: 962 g/kg)  Hexane: 0.057  Octan-1-ol: 1.4  Methanol: 20  Toluene: 55  Acetone: 86  Ethyl acetate: 130  Acetonitrile: 340  Dichloromethane: 400 | EFSA (2010)  |
| Dissociation constant (pKa)  | Neither acidic nor basic properties  | EFSA (2010)  |
| Stability  |  |  |
| Aqueous hydrolysis [d]<br>Aqueous photolysis [d]                           | Hydrolytically stable (pH 5-9 at 25°C) DT50 = 8.7 ( <sup>14</sup> C-pyrimidinyl) 11.9 ( <sup>14</sup> C-phenylacrylate) 13.9 ( <sup>14</sup> C-cyanophenyl)      | EFSA (2010)<br>EFSA (2010)                               |
| Photochemical degradation in air   | Not studied  | EFSA (2010)  |
| Volatilisation   |  |  |
| Vapour pressure [Pa]<br>Henry's law constant [Pa·m³·mol-¹]                 | 1.1 x 10 <sup>-10</sup> (20°C; purity: 990 g/kg)<br>7.4 x 10 <sup>-9</sup>   | EFSA (2010)<br>EFSA (2010)                               |
| Partition/Adsorption   |  |  |
| Octanol-water partition coefficient (log Kow)                              | 2.5 (20°C, no pH-dependence)   | EFSA (2010)  |
| Organic carbon normalised<br>Freundlich partitioning coefficient<br>(Kfoc) | See section 1.5.3, Table 3   |  |

#### 1.2 Mode of action

Azoxystrobin is a post-emergence strobilurin fungicide with systemic and translaminar properties and is translocated in the xylem (UK (2009), Vol. 1). It inhibits spore germination and the development of fungi belonging to the groups of Ascomycetes, Basidiomycetes, Deuteromycetes, and Oomycetes (UK (2009), Vol. 1). Azoxystrobin acts by inhibiting electron transport, consequently inhibiting mitochondrial respiration in fungi by binding to the Qo site of the cytochrome bc1 complex, effectively stopping energy production and halting ATP synthesis. Due to the general mode of action, it shows high risk of cross-resistance between all members of the FRAC group Code 11 fungicides (FRAC 2024).

In the latest European evaluations, it was not highlighted if the metabolite Z-isomer would be more toxic than the parent substance, the E-isomer (oral LD50 in mice > 5000 mg/kg bw, negative in Ames test; UK 2009, EFSA 2010, UK 2014).



The endocrine disrupting (ED) properties were not considered during the previous review assessment of azoxystrobin, and the new assessment from the the AIR IV renewal programme is not available yet.

Apart from the missing evaluation and conclusion, the current evaluation of ED properties focuses on vertebrates, however the endocrine system of soil invertebrates displays substantial differences. With this in mind, extrapolation of the endocrine mode of action from vertebrates to soil invertebrates is not possible. At present, no validated tools are available for the determination of any invertebrate endocrine mode of action (OECD 2018, Crane *et al.* 2022). Additionally, a specific literature search on azoxystrobin yielded no data on endocrine-relevant endpoints for in-soil organisms (status 06.2025).

With regard to human toxicology, the potential of genotoxic, carcinogenic and reproductive effects on azoxystrobin were investigated. In a battery of *in vitro* and *in vivo* genotoxicity assays, no evidence for genotoxicity could be identified (EFSA 2010). Azoxystrobin showed no evidence of carcinogenicity in rats or mice. Fertility and reproductive performance were not impaired, and no teratogenicity was observed in either rats or rabbits. Neurotoxic effects were also not found.

#### 1.3 Use and emissions

Azoxystrobin is a fungicide that was originally evaluated at EU level as a foliar treatment against various fungal species in cereals (max. 3 x 250 g a.s./ha) and in grapevines (max. 8 x 375 g a.s./ha) for field use (DE 1997). During the first renewal assessment, the representative uses at EU level included cereals (barley and wheat) and Brassica vegetables (broccoli, cauliflower, Brussels sprouts and kale) both with maximum application rate of 2 x 250 g a.s./ha a year (UK 2009).

In Switzerland, approximately three dozen products containing azoxystrobin as a single active substance are available with several appearing under the same product name but manufactured by different companies and/or under different authorisation numbers from the same company, e.g. Amistar (5 x), Legado (2 x), Ortiva (4 x), Ortolan (3 x) or Zoxy 250 (5 x). The crops include, amongst others, various berries, stone fruits as well as leafy, fruit and root vegetables at maximum 2 x 250 g a.s./ha rate (BLV 2025).

#### 1.4 Classification and environmental limit values

During the inclusion and renewed inclusion in the Annex I to Directive 91/414/EEC (EC 1998, EFSA 2010), azoxystrobin was classified according to the previous legislation (Directive 1999/45/EC) as a substance that is

- Dangerous for the environment (N),
- *Toxic* (T),
- Toxic by inhalation (R23) and
- Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment (R50/R53).

According to the harmonised classification and labelling approved by the European Union (Regulation (EC) No 1272/2008; ECHA (2025)), azoxystrobin is bearing the hazard statements of

- Toxic if inhaled (H331, human acute toxicity category 3),
- Very toxic to aquatic life (H400, aquatic acute toxicity category 1) and
- Very toxic to aquatic life with long-lasting effect (H410, aquatic chronic toxicity category 1) with
- the signal word *Danger* and
- the pictograms of *Toxic* (GHS06) and



- Hazardous to the environment (GHS09).

In addition to the harmonised ones, the classification and labelling notified by companies and other stakeholders also include the following hazard statement (ECHA 2025):

- Harmful if inhaled (H332, human acute toxicity category 1).

Azoxystrobin is not listed as a candidate substance for substitution (EC 2011, 2015a, PSMV 2010). To date, no soil protection value could be found for azoxystrobin. Please note that the information included here may have changed since the finalisation of this dossier.

#### 1.5 Environmental fate in soil

#### Isomer-specific behaviour

Due to limited data availability, stereoselective degradation and environmental fate of the Z-isomer of azoxystrobin (metabolite R230310) in soil remains a knowledge gap. The only information available is related to photolytic degradation in water, where R230310 was the only degradate above 10 % (EFSA 2010). For the structural formula, please refer to Section 1.1.

#### Volatilisation from soil surface

No significant tendency for volatilisation was observed from soil and bean leaf surfaces up to 24 hours after the application of radiolabelled azoxystrobin (UK 2014).

## 1.5.1 Route of degradation

#### Aerobic degradation in soil

Only one major soil metabolite, R234886 was found at 28.8 % of applied radioactivity (AR) in the aerobic degradation studies (after 360 days; UK (2014)).

#### Anaerobic degradation in soil

Under anaerobic conditions, R234886 occurred at 67.7 % AR after 181 days (UK 2014).

#### Soil photolysis

Soil photolysis studies resulted in two major metabolites (here: greater than 5 % at two consecutive time points) that required further consideration for the risk assessment: R401553 occurred at 5.0 % and 5.7 % AR, while R402173 occurred at 5.4 % and 7.6 % AR on day 9.8 and day 31.3, respectively (UK 2014).

Table 2 summarises the major transformation products of azoxystrobin in soil.

Table 2: Major soil metabolites of azoxystrobin.

| Code name (synonym)                            | Chemical name  | Structural formula | Reference       |
|--|--|--------------------|-----------------|
| R234886<br>(Compound 2)                        | (2E)-2-(2-{[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3-methoxyprop-2-enoic acid              | N N N OCH3         | UK (2014)       |
| R401553<br>(Compound<br>28, M28,<br>SYN501657) | 4-(2-cyanophenoxy)-6-hydroxypyrimidine<br>or<br>2-[(6-hydroxypyrimidin-4-<br>yl)oxy]benzonitrile | O OH               | UK (2009, 2014) |



| Code name (synonym)                            | Chemical name   | Structural formula | Reference       |
|--|---|--------------------|-----------------|
| R402173<br>(Compound<br>30, M30,<br>SYN501114) | 2-[6-(2-cyanophenoxy)pyrimidin-4-<br>yloxy]benzoic acid | CN HOOO            | UK (2009, 2014) |

#### Mineralisation and non-extractable residues

Aerobic mineralisation ranged between 1.8 and 27.0 % AR, while non-extractable residues between 6.2 and 24.5 % AR after 120 d depending on the placing of the <sup>14</sup>C labelling on the parent compound (UK 2014).

Anaerobic mineralisation varied between 0.0 and 4.7 % AR, while non-extractable residues between 3.4 and 9.0 % AR after 120 d also depending on the placing of the <sup>14</sup>C labelling on the parent compound (UK 2014).

#### 1.5.2 Rate of degradation

#### Laboratory degradation studies

In regulatory studies, aerobic degradation of **azoxystrobin** was investigated in nine soils with non-normalised DT50 values of 56.4-248 d and a geometric mean of 109.4 d (all calculated with SFO – single first order – method); these indicated *moderate to high persistence* in soil. The degradation did not show pH-dependence (UK 2014). In another laboratory study from scientific literature, azoxystrobin degraded with a half-life of 66 d (15°C, darkness; Sopeña & Bending (2013)). In soil mesocosm studies the calculated degradation rate (*k*) related inversely to the applied azoxystrobin dose with increasing DT50 values of 36.5, 57.8 and 86.6 d at 2.90, 14.65 and 35.0 mg a.s./kg soil concentrations, respectively (22°C, darkness; Aleksova *et al.* (2021)).

In the available regulatory documents, the laboratory anaerobic degradation of azoxystrobin was investigated only in two soils with non-normalised DT50 values of 49.0 and 59.8 d (both at pH 7; UK (2014)).

Under aerobic conditions, the **metabolite R234886** degraded in soil somewhat faster than the parent compound with non-normalised DT50 values of 18.3-102 d, which, however, also indicated *moderate to high persistence*. No geometric mean of the DT50 values was calculated for the metabolite at EU level as according to the confirmatory data evaluation, the degradation indicated pH-dependence (UK 2014).

The non-normalised DT50 values indicated *low persistence* of **R401553 and R402173** under aerobic conditions in soil (1.36-2.01 d) and (4.24-9.80 d), respectively; (4.24-9.80 d) are respectively.

## Field dissipation studies

Dissipation of azoxystrobin was investigated in field studies under aerobic conditions following soil surface treatment with and without incorporation (UK 2014). Following incorporation, azoxystrobin showed similar rate of degradation for the normalised values (actual DisT50 of 120.9-261.9 d, normalised DisT50 of 56.1-106.7 d, normalised geometric mean DisT50 of 80.2 d, SFO method, n=3) as without incorporation (normalised slow phase DisT50 of 34.5-121.6 d, normalised slow phase geometric mean DisT50 of 75.9 d, DFOP – double first-order in parallel – kinetics).



#### Additional studies

The investigation on the degradation of azoxystrobin resulted in similar DT50 values in the cases of undisturbed versus disturbed soil structures (median DT50 of 36.0 and 34.1 d, respectively; SFO kinetics, static moisture content) and under static versus variable moisture conditions (median DT50 of 36.0 and 35.5 d, respectively; SFO kinetics, undisturbed cores; Hand *et al.* (2021)). The intact core structure with undisturbed microbiological characteristics and the variable moisture conditions were thought to be more representative of field conditions and could lead to faster degradation. However, in the case of azoxystrobin, these factors resulted in no difference in the rate of degradation (Hand *et al.* 2021).

At higher concentration azoxystrobin degraded slower (DT50 = 47 d at 25 mg a.s./kg) than at lower concentration (DT50 = 19 d at 5 mg a.s./kg), although there was no clear dose-response relationship at all tested concentrations (1, 5, 10 and 25 mg a.s./kg; Howell *et al.* (2014)).

Under solar radiation, azoxystrobin showed 2.3 times accelerated dissipation (natural sunlight in Indonesia, 26-35°C, DisT50 of 3.4 d) in a loam soil as compared to darkness (25°C, DisT50 of 7.6 d; Purnama *et al.* (2025)).

A study on three Swiss soils indicated a quicker dissipation of the bioavailable part of azoxystrobin in soil (10-32 % of initial concentration, mild extraction method) compared to the total concentration (25-49 % of initial concentration, exhaustive extraction method) after 56 d under greenhouse conditions (temperature, humidity, light irradiation etc. were not specified; Riedo *et al.* (2023)).

#### 1.5.3 Adsorption/desorption properties and bioavailability

#### **Adsorption**

Based on laboratory adsorption tests, **azoxystrobin** can be classified as *low to medium mobile* in soil  $(n = 6; EFSA\ (2010))$ , while the metabolites as medium to highly or very highly mobile (n = 15, 6 and 6 for R234886, R401553 and R402173, respectively; (EFSA 2010, UK 2014)). For R234886 it was concluded that its adsorption inversely correlated with pH (i.e. decreasing adsorption with increasing pH; UK (2014)). The adsorption properties of azoxystrobin and its soil metabolites are summarised in Table 3.

Table 3: Summary of soil adsorption of the active substance azoxystrobin and the major soil metabolites. Abbreviations: Kfoc – organic carbon-normalised Freundlich distribution coefficients; 1/n – Freundlich exponent. Source: (EFSA 2010, UK 2014).

| Substance    | Range of Kfoc<br>[mL/g] | Arithmetic<br>mean of Kfoc<br>[mL/g] | Arithmetic<br>mean of 1/n | pH dependence | Mobility category      |
|--------------|-------------------------|--------------------------------------|---------------------------|---------------|------------------------|
| Azoxystrobin | 207-594                 | 423                                  | 0.86                      | no            | low to medium          |
| R234886      | 21-490                  | [228]*                               | [0.85]*                   | yes           | medium to high         |
| R401553      | 66-500                  | 188                                  | 0.85                      | no            | medium to high         |
| R402173      | 25-200                  | [91.8]*                              | [0.95]*                   | yes           | medium to very<br>high |

Note: \* Arithmetic means were calculated previously. No arithmetic means should be calculated due to the observed pH-dependence.



A literature study reported higher Koc values for azoxystrobin than the available EU regulatory data, namely 633, 2737 and 3600 mL/g, in three Chinese soils (Wu *et al.* 2016) that would indicate *low mobility*.

## Leaching

No leaching was observed in the column leaching study; lysimeter/field leaching study was not required (UK 2014).

#### **Bioavailability**

The bioavailability of a chemical compound and in turn the actual toxicity of a substance to in-soil organisms is dependent on various factors including the soil physical and chemical properties (e.g. organic matter content, texture/clay content, pH and/or cation exchange capacity) as well as the physiology and behaviour of the organism considered (e.g. surface-volume ratio, anatomy, feeding strategy and/or preferences in habitat) (Peijnenburg 2020, Marti-Roura et al. 2023). Proper consideration of bioavailability can help with reducing the overestimation of the actual risk. In order to account only for the bioavailable portion of the tested substance, the test results need to be normalised to the above-mentioned soil properties. However, in the absence of appropriate equations that can mirror the whole complex system, in regulatory context normalisation takes place only to the organic matter content that is considered the main factor influencing bioavailability for organic compounds (Marti-Roura et al. 2023).

It should be noted that for the prospective environmental risk assessment of pesticides, no specific normalisation takes place with regard to the OM/OC content of the test soil or other soil parameters. The EU terrestrial guidance (EC 2002) – that is still in place for evaluating soil micro- and macroorganisms – requires to account for the availability of lipophilic organic contaminants to earthworms as the "toxicity of lipophilic organic contaminants to soil organisms usually depends on the organic carbon content (foc) of the substrate as this governs adsorption and thus pore water concentration." The difference should be accounted for "by dividing the LC50 and the NOEC values by 2 where log Kow is greater than 2 unless it can be demonstrated by soil sorption data or other evidence that the toxicity is independent of foc". This provision was not used consequently later on, sometimes for earthworms only, even after the compulsory data requirements were broadened to include Folsomia and Hypoaspis; also, sometimes the EPPO scheme (EPPO 2003) was followed – that was referenced in the terrestrial guidance - meaning that the correction was used only for test soils with 10 % peat content but not with 5 % peat content. The issue was further discussed in an EFSA expert meeting on general recurring issues in ecotoxicology (EFSA 2015). It was agreed upon that the correction factor of 2 should be applied in the case of artificial test soils containing both 5 and 10 % peat and for all Tier 1 soil macro-organism tests. As a refinement, the independence of toxicity from soil OM content can be shown and/or sufficiently representative natural soils can be used for testing. Instead of applying this correction, for the SGV derivation the ecotoxicological data are normalised to a standard organic matter content as explained above.

In the case of azoxystrobin, regulatory data for soil pH and texture do not seem to affect the adsorption of the compound to soil particles (EFSA 2010, UK 2014). For non-ionized organic compounds like azoxystrobin (Figure 1: Two possible stereoisomeric forms of azoxystrobin



Table 1), it is assumed that bioavailability is mainly driven by the organic matter content of the soil (EC TGD 2003); therefore the test results are normalised to organic matter content for the SGV (see Section 3).

## 1.6 Bioaccumulation and biomagnification

Substances, such as lipophilic organic compounds, can potentially accumulate along the food chain resulting in a risk for higher vertebrates, such as worm-eating birds and mammals. Especially compounds with a log Kow greater than three can pose a risk of secondary poisoning to animals at higher trophic levels. Azoxystrobin has a log Kow of 2.5 (Table 1), and thus there is likely no potential for bioaccumulation and biomagnification and there is no need for further consideration.

## 2 Chemical analysis and environmental concentrations

Comprehensive techniques are necessary for the extraction of plant protection product residues from soil and for their analysis. Through a recent development, a new multi-residue method has been developed and will be used for soil monitoring in Switzerland (Acosta-Dacal et al. 2021, Rösch et al. 2023). Pesticides are extracted using an optimised QuEChERS (quick, easy, cheap, effective, rugged and safe) approach followed by chemical analysis via liquid chromatography coupled to tandem mass spectrometry with electrospray ionisation (LC-ESI-MS/MS, triple quadrupole). In the case of azoxystrobin, the limit of quantification for the method (MLOQ) was determined as 0.5 ng a.s./g soil (corresponding to 0.0005 mg a.s./kg soil; Rösch et al. 2023).<sup>1</sup>

The soil guideline value that is derived in this dossier for azoxystrobin will be used in conjunction with the actual soil concentrations monitored in Swiss soils by using the above-described measurement method. The initial measurements on some selected, partly agricultural, Swiss soils resulted in azoxystrobin concentrations between < 0.0005 mg a.s./kg soil (< MLOQ) and 0.028 mg a.s./kg soil (Rösch et al. 2023, Table S12).

For the latest evaluated representative uses of azoxystrobin in the EU (two applications on Brassica vegetables and on cereals), the initial predicted environmental concentrations in soil (PECsoil) were 0.394 and 0.196 mg a.s./kg soil for two applications; while the predicted plateau values resulting from accumulation after 100 d, were 0.346 and 0.173 mg a.s./kg soil, respectively (EFSA 2010). However, the long-term predicted maximum accumulation value after years of uses is 0.636 mg a.s./kg (Brassica vegetables) as the maximum accumulation is reached later, after 8 years of continuous use. [It is noted that a maximum PECsoil, accumulation (called there plateau concentration) of 0.646 mg a.s./kg and a steady-state concentration (i.e. soil concentration just before the next application) of 0.246 mg a.s./kg were reported in the EFSA and UK documents (EFSA 2010, UK 2014). The PECsoil calculations were checked by the Ecotox Centre using the HSE CRD PECsoil Calculator Version 1.0 (2015) that resulted in a slightly lower maximum/plateau value as mentioned above. The reason for the difference is not clear.]

## 3 Effect data on azoxistrobin

Effect data for soil organisms were collected from studies retrieved from the European registration information (DE 1997, EC 1998, UK 2009, EFSA 2010, UK 2014). Additionally, a bibliographic search was performed for azoxystrobin and its CAS number (131860-33-8) in the ECOTOX Knowledgebase

<sup>&</sup>lt;sup>1</sup> Unless it is specified otherwise, active substance concentrations in soil are meant per kg soil <u>dry weight</u>.



(US EPA 2025) and in the database of the German Federal Environment Agency (UBA 2025). Furthermore, a literature search was performed on Scopus by using a combination of key words (Soil, EC50, LC50, NOEC, LOEC, LCx, ECx, toxicity and the English and Latin names of various soil organisms such as earthworm, Collembola or mite) and the compound's name or CAS number. The search was completed by additional manual searches based on reviewed toxicity results (e.g. Zhang *et al.* (2020)). Studies performed with formulated products were included in the dataset, unless the amount of active substance within the formulation was unknown or the formulation contained other active substances in addition to azoxystrobin.

In general, only reliable and relevant data should be used for SGV derivation. Different approaches to assessment and classification of (eco)toxicological data have been published. An established method introduced by Klimisch *et al.* (1997) uses four levels of quality: (1) reliable, (2) reliable with restrictions, (3) not reliable, (4) not assignable. The CRED approach (criteria for reporting and evaluating ecotoxicity data; Moermond *et al.* 2016) is based on a similar classification scheme but takes into account the relevance of test results in a more detailed way. This assessment method was originally developed for the aquatic environment and therefore in order to assess and classify (eco)toxicological studies performed in the soil compartment, the CRED approach needed to be adapted by incorporating soil specific aspects ((Casado-Martinez *et al.* 2024)). This modified approach is applied for the assessment of the studies in this dossier and used for evaluating the reliability and relevance of the studies (see scores for "R" and "C", respectively, in Table 4 and Table A1-Table A5).

A short summary of the main points of considerations are given below. For further details on the consideration with regard to the study evaluation and the SGV derivation, please refer to Appendix 1 as well as to the above mentioned soil CRED article (Casado-Martinez *et al.* 2024) and the methodological proposal for deriving soil guideline values (Marti-Roura *et al.* 2023).

Since the bioavailability of non-ionized organic compounds, like azoxystrobin, to soil organisms is assumed to be mainly driven by the organic matter (OM) content of soil (EC TGD 2003), effect data should be normalised to a standard organic matter content in order to make the results comparable among different soil types. The EC TGD (2003, p.116) recommends for non-ionic organic compounds, a normalisation to a standard organic matter content of 3.4 % (corresponding to 2 % organic carbon (OC)). This is in line with the findings in Swiss agricultural soils (Meuli *et al.* (2014); personal communication from NABO). The normalisation has been performed according to the following equation:

$$Effect\ concentration\ [standard] = Effect\ concentration\ [exp] imes \frac{Fom\ soil\ (standard)}{Fom\ soil\ (exp)}$$

Where:

Effect concentration [standard] – effect concentration in standard soil [mg/kg]
Effect concentration [exp] – effect concentration in experiment [mg/kg]
Fom soil (standard) – fraction of organic matter in standard soil (0.034) [kg/kg]
Fom soil (exp) – fraction of organic matter in experimental soil [kg/kg]

Studies, where the information about the organic matter (or carbon) content is missing are classified as "not assignable" (R4) in accordance with the CRED criteria. Besides the organic matter content, other soil properties such as pH and texture (clay content) need to be also considered. The pH (CaCl<sub>2</sub> method) for Swiss agricultural soils ranges between 4.5 and 7.5 (median 6.0) whereas clay content ranges between 5 % and 50 % (median 20 %; Marti-Roura et al. 2023). As there is no evidence that adsorption and in turn bioavailability of azoxystrobin is affected by soil pH or clay content (EFSA 2010, UK 2014), studies outside the recommended range (or without knowing the pH or the clay content) were not excluded from the data set.



In the course of the evaluation, reproduction endpoints are considered the most relevant endpoints as they are good indicators of the long-term sustainability of the population. Other chronic endpoints affecting survival and growth (biomass) of individuals are also accepted, since they are traditionally measured endpoints frequently extrapolated to represent the impact at population level (Marti-Roura *et al.* 2023). If multiple comparable toxicity values for the same species and the same measured effect are available, the geometric mean of the effect values is calculated.

Regulatory studies and their endpoints are either accepted without additional assessment (at face value, although without applying the additional divison of the endpoint by two in case of log Kow  $\geq 2$ ; see explanation in Section 1.5.3) or partially/fully re-considered if needed to set the endpoints in line with our criteria as summarised in Appendix 1. This is the case, for example, when organisms were not exposed through soil (e.g. plant vegetative vigour tests *via* foliar application); normalisation to a standard organic matter content was not possible due to lack of data or not the most statistically robust effect concentration was proposed/agreed upon as a final endpoint.

If more than one endpoint is available from the same study for the same effect, the statistically more robust one is preferred. This means that the statistically more robust endpoint is chosen even if it is higher than another one or it includes more than 10% effect (choosing non-significant endpoints with < 10% effects is a precautionary approach that is often used at European level). If the latter is the case, it will be highlighted and discussed further in the uncertainty analysis (see later below). If both NOEC and EC10 are available from the same study and statistically both are equally robust, due to the inherent uncertainties of the NOEC, the EC10 is preferred over the NOEC (for further explanation, please refer to Appendix 1).

Considering the limited regulatory dataset along with the old-style short study summaries, the soil OM/OC content was derived from the applied standard study guideline in the case of certain GLP studies where no deviation to the guideline occurred (i.e. Collembola reproduction test and seedling emergence test on terrestrial plants).

Complete lists of laboratory and a field studies reporting soil effect values for azoxystrobin and its transformation products are shown in Appendix 2 (for azoxystrobin, Table A1 with laboratory and Table A2 with field studies) and in Appendix 3 (for the major soil metabolites, Table A3-Table A5). If necessary, some clarifications and/or justifications of the assessment are provided in form of Notes to those tables (see Notes A1 and Notes A2) in Appendix 2 and 3, respectively, and also the same respective notes for the a.s. in Table 4. In Table 4 of the main text, all the reliable and relevant results are summarised. In Table 4 the lowest values per species per test setup per duration are shown in bold. If there are only greater-than values available, the highest one is shown in bold as they mean that up to the highest tested concentration no adverse effects were observed. The geometric mean, if it is possible to calculate from the results (i.e. there are equal-to values for the same species/effect/duration/type of effect concentration), is used for choosing the lowest value rather than the individual effect concentrations. This sifting procedure helps to choose the lowest effect concentrations per species/group for the SGV derivation (see Table 5).

## 3.1 Comparison between data for active substance and formulated products

A statistical analysis of potential differences in the toxicity of the active substance and the formulated products was not possible due to the scarcity of data. Therefore, toxicity data obtained with the active ingredient and the formulations were merged (see data for the parent in Table 4 and Table A1). When multiple comparable toxicity values for the same species and the same endpoint were available, the geometric mean of the effect values was calculated, irrespective of whether the data was obtained with the active ingredient or a formulation.

The relevant and reliable soil toxicity data on azoxystrobin is listed in Table 4.



Table 4: Azoxystrobin – All reliable (R1-R2) and relevant (C1-C2) effect data. The lowest reliable and relevant effect data per species per test setup are shown in bold. Calculated data are rounded to three significant figures. Abbreviations: OM – organic matter. The full set of studies can be found in Appendix 1 (Table A1). Data were evaluated for reliability and relevance according to the modified CRED criteria (see R/C scores) or taken at face value from regulatory dossiers (Assessment score 1-3). The explanation of notes are included after this table (Notes 1). Data in squared brackets are based on the specific guideline requirements applied in the respective GLP study (for further information, please refer to the respective notes to the study).

| Species<br>(Taxonomic<br>group        | Test<br>substance                       | Measured<br>effect                          | Duration | Type of effect concent ration | Effect<br>concentration<br>[mg a.s./kg soil] | Total<br>OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Asses<br>sment<br>score | Source  |
|---------------------------------------|---|---|----------|-------------------------------|--|--------------------|--|---|-------|-------------------------|---|
| Eisenia fetida<br>(Earthworm)         | Azoxystrobin<br>250 g/L SC<br>(YF10537) | adult<br>mortality                          | 28 d     | NOEC                          | ≥ 20   | 10                 | ≥ 6.80   | Artificial soil: 10 %<br>sphagnum peat, pH 6 ±<br>5, 50 ± 10 % moisture<br>of dry weight soil                     | E     | 1<br>(R2/C1)            | Moser & Römbke (2000)<br>cited in (UK 2009), Vol. 3<br>B.9.6.2, p.734 |
| Eisenia fetida<br>(Earthworm)         | Azoxystrobin<br>250 g/L SC<br>(YF10537) | biomass<br>(adult body<br>weight<br>change) | 28 d     | NOEC                          | ≥ 20   | 10                 | ≥ 6.80   | Artificial soil: 10 % sphagnum peat, pH $6 \pm 5$ , $50 \pm 10$ % moisture of dry weight soil                     | E     | 1<br>(R2/C1)            | Moser & Römbke (2000)<br>cited in (UK 2009), Vol. 3<br>B.9.6.2, p.734 |
| Eisenia fetida<br>(Earthworm)         | Azoxystrobin<br>250 g/L SC<br>(YF10537) | reproduction<br>(number of<br>juveniles)    | 56 d     | NOEC                          | ≥ 20   | 10                 | ≥ 6.80   | Artificial soil: 10 % sphagnum peat, pH 6 $\pm$ 5, 50 $\pm$ 10 % moisture of dry weight soil                      | E     | 1<br>(R2/C1)            | Moser & Römbke (2000)<br>cited in (UK 2009), Vol. 3<br>B.9.6.2, p.734 |
| Eisenia fetida<br>(Earthworm)         | Ortiva<br>(250 g a.s./L)                | adult<br>mortality                          | 28 d     | LC50                          | > 500  | 5.74               | > 296  | Natural soil (sandy clay loam, Portugal): 54.4 % sand, 22.1 % silt, 23.5 % silt, pH 5.9, 50 % of MWHC             | P     | R2/C2                   | Leitão <i>et al.</i> (2014)   |
| Eisenia fetida<br>(Earthworm)         | Ortiva<br>(250 g a.s./L)                | biomass<br>(adult body<br>weight<br>change) | 28 d     | NOEC                          | < 50   | 5.74               | < 29.6   | Natural soil (sandy clay<br>loam, Portugal): 54.4<br>% sand, 22.1 % silt,<br>23.5 % silt, pH 5.9, 50<br>% of MWHC | P     | R2/C1                   | Leitão <i>et al</i> . (2014)  |
| Eisenia fetida<br>(Earthworm)         | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles)    | 56 d     | NOEC                          | < 50   | 5.74               | < 29.6   | Natural soil (sandy clay<br>loam, Portugal): 54.4<br>% sand, 22.1 % silt,<br>23.5 % silt, pH 5.9, 50<br>% of MWHC | P     | R2/C1                   | Leitão <i>et al</i> . (2014)  |
| Enchytraeus<br>crypticus<br>(Potworm) | Azoxystrobin<br>(≥ 98 %<br>purity)      | adult<br>mortality                          | 21 d     | NOEC                          | 17.5   | 1.77               | 33.6   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 %<br>sand, 50 % of MWHC                      | M     | R2/C1                   | Gomes et al. (2021)   |



| Species<br>(Taxonomic<br>group        | Test<br>substance                       | Measured<br>effect                       | Duration | Type of effect concent ration | Effect<br>concentration<br>[mg a.s./kg soil] | Total<br>OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Asses<br>sment<br>score | Source   |
|---------------------------------------|---|--|----------|-------------------------------|--|--------------------|--|---|-------|-------------------------|--|
| Enchytraeus<br>crypticus<br>(Potworm) | Azoxystrobin<br>(≥ 98 % purity)         | adult<br>mortality                       | 21 d     | LC50                          | 39   | 1.77               | 74.9   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 % sand,<br>50 % of MWHC          | M     | R2/C2                   | Gomes et al. (2021)                                    |
| Enchytraeus<br>crypticus<br>(Potworm) | Azoxystrobin<br>(≥ 98 %<br>purity)      | reproduction<br>(number of<br>juveniles) | 21 d     | NOEC                          | 17.5   | 1.77               | 33.6   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 %<br>sand, 50 % of MWHC          | M     | R2/C1                   | Gomes et al. (2021)                                    |
| Enchytraeus<br>crypticus<br>(Potworm) | Azoxystrobin<br>(≥ 98 % purity)         | reproduction<br>(number of<br>juveniles) | 21 d     | EC50                          | 37   | 1.77               | 71.1   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 % sand,<br>50 % of MWHC          | M     | R2/C2                   | Gomes et al. (2021)                                    |
| Enchytraeus<br>albidus<br>(Potworm)   | Azoxystrobin<br>(≥ 98 %<br>purity)      | reproduction<br>(number of<br>juveniles) | 42 d     | NOEC                          | 4  | 10                 | 1.36   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC                  | 0     | R2/C1                   | Kovačević <i>et al.</i> (2022)                         |
| Enchytraeus<br>albidus<br>(Potworm)   | Azoxystrobin (≥ 98 % purity)            | reproduction<br>(number of<br>juveniles) | 42 d     | EC50                          | > 8  | 10                 | > 2.72   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC                  | 0     | R2/C2                   | Kovačević <i>et al.</i> (2022)                         |
| Enchytraeus<br>albidus<br>(Potworm)   | Quadris<br>(25 % a.s.)                  | reproduction<br>(number of<br>juveniles) | 42 d     | EC50                          | 2.94   | 10                 | 1.00   | Artificial soil: 70 %<br>sand, 20 % kaolin clay,<br>10 % sphagnum peat,<br>pH 6, 60 % of MWHC         | 0     | R2/C2                   | Kovačević et al. (2022)                                |
| Enchytraeus<br>crypticus<br>(Potworm) | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles) | 28 d     | EC20                          | 42.6   | 5.74               | 25.2   | Natural soil (sandy clay loam, Portugal): 54.4 % sand, 22.1 % silt, 23.5 % silt, pH 5.9, 50 % of MWHC | P     | R2/C2                   | Leitão et al. (2014)                                   |
| Enchytraeus<br>crypticus<br>(Potworm) | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 99.2   | 5.74               | 58.8   | Natural soil (sandy clay loam, Portugal): 54.4 % sand, 22.1 % silt, 23.5 % silt, pH 5.9, 50 % of MWHC | P     | R2/C2                   | Leitão et al. (2014)                                   |
| Folsomia candida<br>(Collembola)      | Azoxystrobin<br>250 g/L SC<br>(YF10537) | adult<br>mortality                       | 28 d     | NOEC                          | 200  | [10]               | [68]   | Artificial soil: pH 5.96-6.28, 35.10-37.69 % moisture content of dry weight                           | F     | (1)<br>R2/C1            | Barth (2001) cited in (UK 2009), Vol. 3 B.9.7.1, p.740 |



| Species<br>(Taxonomic<br>group   | Test<br>substance                       | Measured<br>effect                       | Duration | Type of effect concent ration | Effect<br>concentration<br>[mg a.s./kg soil] | Total<br>OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Asses<br>sment<br>score | Source   |
|----------------------------------|---|--|----------|-------------------------------|--|--------------------|--|--|-------|-------------------------|--|
| Folsomia candida<br>(Collembola) | Azoxystrobin<br>250 g/L SC<br>(YF10537) | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 167.3  | [10]               | [56.9]   | Artificial soil: pH 5.96-<br>6.28, 35.10-37.69 %<br>moisture content of dry<br>weight                                  | F     | (1)<br>R2/C2            | Barth (2001) cited in (UK 2009), Vol. 3 B.9.7.1, p.740       |
| Folsomia candida<br>(Collembola) | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles) | 28 d     | EC20                          | 54.9   | 5.74               | 32.5   | Natural soil (sandy clay<br>loam, Portugal): 54.4<br>% sand, 22.1 % silt,<br>23.5 % silt, pH 5.9, 50<br>% of MWHC      | P     | R2/C2                   | Leitão <i>et al.</i> (2014)                                  |
| Folsomia candida<br>(Collembola) | Azoxystrobin<br>250 g/L SC<br>(YF10537) | reproduction<br>(number of<br>juveniles) | 28 d     | NOEC                          | 50   | [10]               | [17.0]   | Artificial soil: pH 5.96-<br>6.28, 35.10-37.69 %<br>moisture content of dry<br>weight                                  | F     | (1)<br>R2/C1            | Barth (2001) cited in<br>(UK 2009), Vol. 3 B.9.7.1,<br>p.740 |
| Folsomia candida<br>(Collembola) | Quadris<br>(250 g a.s./L)               | reproduction<br>(number of<br>juveniles) | 28 d     | NOEC                          | 15   | 10                 | 5.10   | Artificial soil: 70 % sand, 20 % kaolinite clay, 10 % sphagnum peat, pH 6, 60 % of MWHC                                | R     | R2/C1                   | Kovačević <i>et al.</i> (2023a)                              |
| Folsomia candida<br>(Collembola) | Quadris<br>(250 g a.s./L)               | reproduction<br>(number of<br>juveniles) | 28 d     | NOEC                          | 30   | 5                  | 20.4   | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R2/C1                   | Szabó et al. (2023)  |
| F 1 · 1·1                        | 0.1:                                    | Geometric mean                           |          | NOEG                          | > 200  | 10                 | 12.1   | A ('C' : 1 - 11 70 0)  | D     | D0/C01                  | W Y '' ( 1 (2022 )   |
| Folsomia candida<br>(Collembola) | Quadris<br>(250 g a.s./L)               | adult<br>mortality                       | 28 d     | NOEC                          | ≥ 200  | 10                 | ≥ 68   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC                                   | R     | R2/C21                  | Kovačević et al. (2023a)                                     |
| Folsomia candida<br>(Collembola) | Quadris<br>(250 g a.s./L)               | adult<br>mortality                       | 28 d     | NOEC                          | 30   | 5                  | 20.4   | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R2/C1                   | Szabó <i>et al.</i> (2023)                                   |
| Folsomia candida<br>(Collembola) | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 92.0   | 5.74               | 54.5   | Natural soil (sandy clay loam, Portugal): 54.4 % sand, 22.1 % silt, 23.5 %   | P     | R2/C2                   | Leitão et al. (2014)   |



| Species<br>(Taxonomic<br>group                        | Test<br>substance             | Measured<br>effect                       | Duration | Type of effect concent ration | Effect<br>concentration<br>[mg a.s./kg soil] | Total<br>OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Asses<br>sment<br>score | Source  |
|---|-------------------------------|--|----------|-------------------------------|--|--------------------|--|--|-------|-------------------------|---|
|   |                               |  |          |                               |  |                    |  | silt, pH 5.9, 50 % of<br>MWHC  |       |                         |   |
| Folsomia candida<br>(Collembola)                      | Quadris<br>(250 g a.s./L)     | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 61.28  | 10                 | 21.2   | Artificial soil: 70 % sand, 20 % kaolinite clay, 10 % sphagnum peat, pH 6, 60 % of MWHC                                | R     | R2/C2                   | Kovačević et al. (2023a)  |
| Folsomia candida<br>(Collembola)                      | Quadris<br>(250 g a.s./L)     | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 65.6   | 5                  | 44.6   | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R2/C2                   | Szabó <i>et al.</i> (2023)  |
| Soil bacteria<br>(Microorganisms                      | Azoxystrobin<br>(99 % purity) | CFU <sup>DE</sup>                        | 28 d     | NOEC                          | ≥ <b>0.1</b> (< <b>1</b> )                   | 9.62               | ≥ 0.0353<br>(< 0.353)  | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt  | V     | R2/C2                   | Wang et al. (2018)  |
| Soil fungi<br>(Microorganisms                         | Azoxystrobin (99 % purity)    | CFU <sup>DE</sup>                        | 28 d     | NOEC                          | ≥ 10   | 9.62               | ≥ 3.53   | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt  | V     | R2/C2                   | Wang et al. (2018)  |
| Soil<br>Actinomycetes<br>(Microorganisms              | Azoxystrobin<br>(99 % purity) | CFU <sup>DE</sup>                        | 28 d     | NOEC                          | ≥ 0.1 (< 1)                                  | 9.62               | $\geq$ 0.0353 (< 0.353)  | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt  | V     | R2/C2                   | Wang et al. (2018)  |
| Microorganisms  | Azoxystrobin (99 % purity)    | urease<br>activity <sup>EE</sup>         | 28 d     | NOEC                          | ≥ 10   | 9.62               | ≥ 3.53   | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt  | V     | R2/C2                   | Wang et al. (2018)  |
| Microorganisms  | Azoxystrobin (99 % purity)    | dehydrogena<br>se activity <sup>EE</sup> | 28 d     | NOEC                          | < 0.1  | 9.62               | < 0.0353   | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt  | V     | R2/C2                   | Wang et al. (2018)  |
| Microorganisms  | Azoxystrobin (99 % purity)    | catalase<br>activity <sup>EE</sup>       | 28 d     | NOEC                          | < 0.1  | 9.62               | < 0.0353   | Natural soil (cambisol): 17.43 % clay, 44.62 % sand, 37.95 % silt  | V     | R2/C2                   | Wang et al. (2018)  |
| Microorganisms  | Azoxystrobin (99 % purity)    | protease<br>activity <sup>EE</sup>       | 28 d     | NOEC                          | ≥ 10   | 9.62               | ≥ 3.53   | Natural soil (cambisol): 17.43 % clay, 44.62 % sand, 37.95 % silt  | V     | R2/C2                   | Wang et al. (2018)  |
| Lactuca sativa <sup>D</sup><br>(Terrestrial<br>plant) | Azoxystrobin (98.6 % purity)  | seedling<br>emergence,<br>mortality,     | 18 d     | NOEC                          | ≥ 20   | [3]                | [≥ 22.7]   | n.r.   | J     | (1)<br>R2/C1            | Porch & Krüger (2002)<br>cited in (UK 2009), Vol. 3<br>B.9.9.1, p.758 |



| Species<br>(Taxonomic<br>group                             | Test<br>substance               | Measured<br>effect   | Duration | Type of effect concent ration | Effect<br>concentration<br>[mg a.s./kg soil] | Total<br>OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil | Notes | Asses<br>sment<br>score | Source  |
|--|---------------------------------|--|----------|-------------------------------|--|--------------------|--|-----------|-------|-------------------------|---|
| Raphanus<br>sativus <sup>D</sup><br>(Terrestrial<br>plant) | Azoxystrobin (98.6 % purity)    | biomass (dry<br>shoot weight)<br>seedling<br>emergence,<br>mortality,<br>biomass (dry<br>shoot weight) | 18 d     | NOEC                          | ≥ 20   | [3]                | [≥ 22.7]   | n.r.      | J     | (1)<br>R2/C1            | Porch & Krüger (2002)<br>cited in (UK 2009), Vol. 3<br>B.9.9.1, p.758 |
| Triticum aestivum <sup>M</sup> (Terrestrial plant)         | Azoxystrobin<br>(98.6 % purity) | seedling<br>emergence,<br>mortality,<br>biomass (dry<br>shoot weight)                                  | 18 d     | NOEC                          | ≥ 20   | [3]                | [≥ 22.7]   | n.r.      | J     | (1)<br>R2/C1            | Porch & Krüger (2002)<br>cited in (UK 2009), Vol. 3<br>B.9.9.1, p.758 |

Notes 1: Notes on soil studies for azoxystrobin (reliable and relevant data).

| Е | The study was conducted with an SC (suspension concentrate) product containing 251 g a.s./L (23.0 % w/w), according to the BBA VI, 2-2 guideline from 1994 (no reference provided).  |  |  |  |  |
|---|--|--|--|--|--|
|   | The test was conducted with 3 concentrations mixed into the soil.  |  |  |  |  |
|   | The study seems to have been conducted broadly in line with the OECD 222 guideline (OECD 2016a). The validity criteria were met.   |  |  |  |  |
| F | The study was conducted according to the ISO 11267:1999 guideline (ISO 1999) that is broadly in line with the current OECD 232 guideline (OECD 2016b).   |  |  |  |  |
|   | The OM/peat content of the soil was not summarised but it was noted that there was no deviation to the guideline. Thus, the guideline requirement of 10 % soil peat content is used as a surrogate of the soil OM content for normalising the results. The data derived in this way are shown in squared brackets.   |  |  |  |  |
|   | The details of the statistical evaluation was not provided but based on the results it could be confirmed.   |  |  |  |  |
| J | The seedling emergence test was conducted under GLP in accordance with the 1984 version of the OECD 208 guideline (OECD 2006).   |  |  |  |  |
|   | The three test concentrations – in exponential series – were mixed into the soil. The test duration was 14 days after 50 % of the control emerged (18 days altogether).  |  |  |  |  |
|   | There were no statistically significant effects for any of the species for any of the measured effects (seed germination, seedling survival and shoot dry weight; Dunnett's test, significance level of 0.05). At the highest test concentration (20 mg a.s./kg), 18-22 % increase occurred in lettuce, 5 % increase and 13 % decrease in radish, as well as 29 % decrease in wheat. Based on the summarised mean and SD values, the statistical results could be affirmed.  |  |  |  |  |
|   | No deviation to the guideline was noted in the study summary; however, the soil properties were not summarised. The old version of the OECD 208 guideline requires that the carbon content of the test soil not exceed 1.5 % (or 3 % organic matter). Using the maximum 3 % OM content, the normalised NOEC is $\geq$ 22.7 mg a.s./kg. Any lower OM values would result in higher effect concentrations. The test was conducted to GLP following a standard guideline; thus it is considered that the guideline requirements were met and the test soil did not contain more than 3 % OM. For the normalisation of the results the worst case scenario of 3 % OM is accepted and used. The data derived in this way are shown in squared brackets. |  |  |  |  |
| M | The study was conducted in line with the OECD 220 (OECD 2016c) guideline that was originally developed for <i>Enchytraeus albidus</i> . The test duration for both adult mortality and reproduction was 21 d, which is in line with the common practice for the tested <i>E. crypticus</i> due to its smaller size and shorter generation time (Castro-Ferreira <i>et al.</i> 2012, OECD 2010).  |  |  |  |  |



The robustness of the estimated LC/EC10 values could not be fully evaluated in the absence of the LC/EC20 values and their confidence intervals (CI). The CI of the reproduction EC10 had a "poor" normalised width (NW), of the mortality LC10 a "fair" NW (see details in EFSA (2019)). The results were shown mostly graphically, thus the nonlinear regression and the ECx estimations could not be repeated/checked. The LCx/ECx values (with 95 % CI in brackets) were as follows:

- 21-d LC10 for adult mortality: 21 (15-30) mg a.s./kg soil
- 21-d LC50 for adult mortality: 39 (34-45) mg a.s./kg soil
- 21-d EC10 for reproduction: 17 (2-33) mg a.s./kg soil
- 21-d EC50 for reproduction: 37 (25-49) mg a.s./kg soil

Although the spacing of the test concentrations were somewhat broader than recommended in the test guideline (factor of 3.1-3.2 instead of  $\leq$  1.8), the estimated EC10 values fell around the NOEC values and therefore the NOEC values are regarded as equal-to values for further consideration.

O Using Enchytraeus albidus as test organism, a 7-d non-standard range-finding test (instead of 14-d) and a standard 42-d reproduction test (OECD 2016c) were conducted.

It was reported that tested formulation, Quadris (Syngenta) contained 25 % azoxystrobin as well as the following co-formulants:

- 1,2-benzisothazol-3(2H)-one (0.025–0.05 %)
- naphthalene and alkyl naphthalene sulfonic acid formaldehyde condensate
- sodium salts  $(1 \le 10\%)$

The 21-d adult mortality results of the definitive test were not reported, only the 7-d adult mortality results from the range-finding test. The 7-d results are short-term results (in between the acute and long-term results) and as such are not considered relevant amongst the chronic data.

The reliability of the LC10/EC10 values could not be fully assessed as recommended in EFSA (2019) as the related LC20/EC20 values and their CIs were not reported; the reliability of these are scored as *not assignable* (R4). For the effect concentrations with 10 and 50 % effects, the following median values and 95 % CIs (in brackets) were reported:

- 7-d LC10 for adult mortality with the a.s.: 11.95 (11.21–12.70) mg a.s./kg
- 7-d LC50 for adult mortality with the a.s.: 16.76 (16.51–17.01) mg a.s./kg
- 42-d EC10 for reproduction with the a.s.: > 8 mg a.s./kg
- 42-d EC50 for reproduction with the a.s.: > 8 mg a.s./kg
- 7-d LC10 for adult mortality with the formulation: 10.65 (10.14–11.17) mg a.s./kg
- 7-d LC50 for adult mortality with the formulation: 15.29 (14.88–15.71) mg a.s./kg
- 42-d EC10 for reproduction with the formulation: 1.23 (0.40–2.06) mg a.s./kg
- 42-d EC50 for reproduction with the formulation: 2.94 (2.41–3.73) mg a.s./kg

The tested concentrations were 0 (control), 0.085, 0.17, 1.45, 2.7, 4 and 8 mg a.i./kg soil in the definitive test with spacing factors of 2, 8.5, 1.9, 1.5 and 2, respectively. Due to the broad spacing between the test concentrations of 0.17 and 1.45, the reproduction NOEC of 0.17 with the formulation is considered as a greater-than/equal-to value. The reason for the high spacing and the missing in-between concentrations are not clear. There are also high deviations at the two lowest test concentrations. Considering all of these uncertainties, the reliability of the reproduction NOEC tested with the formulation is *not assignable* (R4).

P Standard tests with Eisenia andrei (ISO 1998), Enchytraeus crypticus (ISO 2004) and Folsomia candida (ISO 1999) were conducted using natural soil.

12 concentrations (10, 15, 20, 35, 50, 80, 120, 200, 300, 450, 650, 1000 mg a.s./kg) with 2 replicates were applied in the potworm and the collembolan treatments and 4 replicates in the controls. In the earthworm test 5 test concentrations (50, 100, 200, 300, and 500 mg a.s./kg) and a control were used, all with 4 replicates. In addition to the natural soil controls (for comparison of the treatments in the test), artificial soil controls were also used (for comparing the performance of the animals in the control).

Adult mortality was measured in all tests but was only reported for eathworms.

Concerning reproduction, instead of NOEC/EC10 values, only EC20 and EC50 values were reported with the following CIs (in brackets):

- Earthworm 56-d EC20 for reproduction: 12.2 (1.2–23.1) mg a.s./kg
- Earthworm 56-d EC50 for reproduction: 42.0 (23.2–60.8) mg a.s./kg



- Potworm 28-d EC20 for reproduction: 42.6 (25.2–60.0) mg a.s./kg
- Potworm 28-d EC50 for reproduction: 99.2 (73.3–125.7) mg a.s./kg
- Collembola 28-d EC20 for reproduction: 54.9 (23.0–86.9) mg a.s./kg
- Collembola 28-d EC50 for reproduction: 92.0 (57.9–126.1) mg a.s./kg

The EC20 and EC50 values for earthworms are extrapolations out of the range of the tested concentrations and thus statistically not robust and as a result they are scored as *not reliable* (R3). Both the biomass and the reproduction NOEC for earthworms were reported as lower than the lowest tested concentration. It is unclear why NOEC values were not reported for potworms and collembolans.

Reproduction and avoidance tests with *Folsomia candida* were conducted on artificial soil. The food choice test was conducted on filter paper and as such not relevant here.

It was reported that the reproduction test parameters followed the standard OECD guideline (OECD 2016b). However, the test concentrations were 0, 0.003, 0.03, 3, 30, 90 and 300 mg a.s./kg soil with spacing factors of 10, 100, 10, 3 and 3.3, respectively, instead of the required factor of  $\leq$  1.8. The NOEC values are still considered *reliable with restrictions* (R2) as the spacing factor between the NOEC and LOEC values was 3. While this is still larger than the guideline recommendation, usually – especially if a standard guideline was not followed – a spacing factor up to 3 is accepted. Altogether this is considered as a minor deviation to the guideline.

The LCx values are not reliable as no CI could be calculated due to the steepness of the fitted curve.

In the absence of detailed results as well as an established relevance at population level, the avoidance test results are scored as not assignable (R4/C4).

R Reproduction test with *Folsomia candida* was conducted to the standard guidelines (ISO 2014a, OECD 2016b). The relevance of the additionally conducted biomarker test and its results have not been established at the population level and as such the results are not listed and not considered further in the SGV.

The validity criteria were reported as met.

The robustness of the estimated EC10 values could not be fully evaluated in the absence of the EC20 values and their CI. For the effect concentrations with 10 and 50 % effects, the following median values and 95 % CIs (in brackets) were reported:

- Reproduction EC10: 11.73 (5.04, 18.42) mg a.s./kg
- Reproduction EC50: 61.28 (48.05, 74.508) mg a.s./kg

V A dose-response test (0, 0.1, 1 and 10 mg a.s./kg) was conducted on soil microorganisms. No detailed history was provided for the fields where the soil was sampled; although, it was noted that no pesticides had been applied in the area.

No standard guideline was followed. All parameters were measured after 7, 14, 21 and 28 days. Considering the minimum 28-d duration of the standard nitrogen and carbon transformation tests (OECD 2000a, 2000b), results after 28 d are listed here. Also, in line with the regulatory principles for prospective risk assessment, any kind of effects (increase or decrease) are considered relevant.

It seems, for the soil respiration test no replicates were included – replication is not mentioned in the text and no standard deviation is shown for the results. Also, the method is very briefly described and it remained unclear how comparable the results are to results from other studies that followed the standard guideline. The fulfilment of the standard guideline validity criterion (i.e. if the coefficient of variation in the control was < 15 %) cannot be checked. Due to these uncertainties, the respiration results are scored as *not assignable* (R4).

While the enzymatic and diversity endpoints are not the preferred ones, they can provide a good indication of effects (or lack of them); both relevance and reliability of these endpoints are considered *acceptable with restrictions* (C2/R2). Due to the wide spacing (factor of 10), the NOEC values are recorded as unbound values and the respective interval (to the LOEC value) is indicated if possible.



## 3.2 Graphic representation of effect data

The lowest relevant and reliable data (R1-2/C1-2) per test – normalised to a standard organic matter content of 3.4 % – are plotted in Figure 2. If more values for the same endpoint from the same test are available (e.g. EC10 vs NOEC), the statistically more robust one is shown in the figure. If both EC10 and NOEC are equally robust, EC10 is preferred (for further explanation, please refer to Appendix 1 Considerations for the evaluation of the studies). If values for more measured effects for the same species from the same test are available (e.g. reproduction, biomass, mortality), the lowest one is included in the figure.

This figure aims to provide an overview of the distribution of the effect concentrations, i.e. to indicate the most sensitive species/group. Microorganisms seem to be the most sensitive group of organisms with the lowest NOEC of < 0.0353 mg a.s./kg. Less sensitivity can be seen for potworms (1.36-33.6 mg a.s./kg). Earthworms ( $\ge 6.80$  and < 29.6 mg a.s./kg) and collembolans (5.10-32.5 mg a.s./kg) show even less sensitivity, while plants do not seem to be sensitive ( $\ge 22.7$  mg a.s./kg; Figure 2). There is no data on predatory mites.

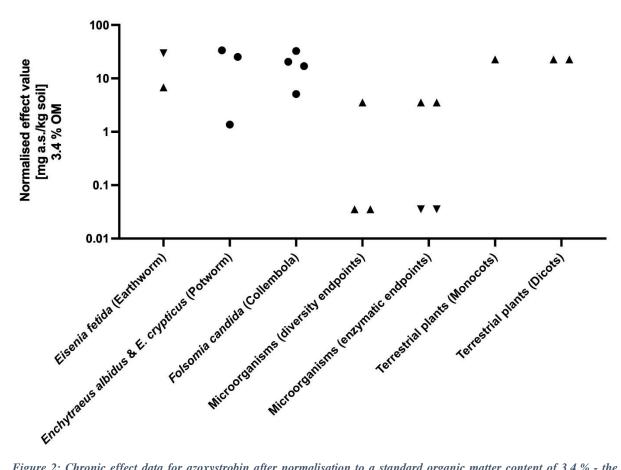


Figure 2: Chronic effect data for azoxystrobin after normalisation to a standard organic matter content of 3.4 % - the statistically most robust lowest effect values of the relevant and reliable endpoints per species/group per test setup. All are NOEC values with the exception of the EC20 of 25.2 mg a.s./kg for Enchytraeus crypticus ( $2^{nd}$  highest value) and the EC20 of 32.5 mg a.s./kg for Folsomia candida (highest value). Dots represent equal-to values; triangles represent unbound data with the triangle facing up symbolising  $\geq or >$  values and the triangle facing down symbolising  $\leq or <$  values.



## 4 Derivation of SGV

For the SGV derivation for azoxystrobin, the relevant and reliable effect concentrations of the active substance were normalised to a standard organic matter content of 3.4 %. Data on formulations were recalculated to active substance content. Then the lowest toxicity endpoints per species/group were summarised (Table 5).

Table 5: The lowest relevant and reliable chronic data for azoxystrobin per species/group, rounded to three significant figures, summarised from Table 4. Effect concentrations are expressed as concentrations normalised to 3.4 % of soil organic matter content. For multiple comparable toxicity values for the same species and the same endpoint, a geometric mean was calculated.

| Trophic level  | Species, family (Group)                           | Type of<br>effect<br>concentra<br>tion | Normalised<br>effect<br>concentration<br>[mg a.s./kg soil] | Reference  |
|--|---|--|--|--|
| Primary producers  | Lactuca sativa, Asteraceae                        | NOEC                                   | ≥ 22.7*  | Porch & Krüger (2002) cited in   |
| (terrestrial plants)                                       | (Dicots)  |  |  | (UK 2009), Vol. 3 B.9.9.1,   |
|  | Raphanus sativus, Brassiceae<br>(Dicots)          | NOEC                                   | ≥ 22.7*  | p.758  |
|  | Triticum aestivum, Poaceae (Monocots)             | NOEC                                   | ≥ 22.7*  |  |
| Decomposers  | Soil bacteria                                     | NOEC                                   | ≥ 0.0353   | Wang et al. (2018)   |
| (nutrient transformers)                                    | (Microorganisms)                                  |  | (< 0.353)**  |  |
| <ul><li>diversity</li><li>endpoints</li></ul>              | Soil fungi (Microorganisms)                       | NOEC                                   | ≥ 3.53   | •  |
| •  | Soil Actinomycetes                                | NOEC                                   | ≥ 0.0353   | -  |
|  | (Microorganisms)                                  |  | (< 0.353)**  |  |
| Decomposers<br>(nutrient                                   | Microorganisms,<br>urease activity                | NOEC                                   | ≥ 3.53   |  |
| transformers)  |   |  |  | -  |
| <ul><li>enzymatic</li><li>endpoints</li></ul>              | Microorganisms,<br>dehydrogenase activity         | NOEC                                   | < 0.0353   |  |
|  | Microorganisms, catalase activity                 | NOEC                                   | < 0.0353   |  |
|  | Microorganisms, protease activity                 | NOEC                                   | ≥ 3.53   | •  |
| Decomposers<br>(litter transformers/<br>primary consumers) | Eisenia fetida, Lumbricidae<br>(Earthworm)        | NOEC                                   | ≥ 6.80   | Moser & Römbke (2000) cited in (UK 2009), Vol. 3 B.9.6.2, p.734  |
|  | Enchytraeus albidus,<br>Enchytraeidae (Potworm)   | NOEC                                   | 1.36   | Kovačević et al. (2022)  |
|  | Enchytraeus crypticus,<br>Enchytraeidae (Potworm) | EC20                                   | 25.2   | Leitão <i>et al.</i> (2014)  |
|  | Folsomia candida,<br>Isotomidae (Collembola)      | NOEC                                   | 12.1   | Geometric mean: Barth (2001) cited in UK (2009), Vol. 3 B.9.7.1, p.740*** Kovačević et al. (2023b) Szabó et al. (2023) |

Note: \*The normalised plant data is based on the highest soil OM content acceptable in the guideline used for the seedling emergence test. Any soils with lower OM content would result in a higher normalised NOEC. \*\* There is a factor of 10 spacing



between the NOEC and the LOEC, therefore the NOEC is considered as a greater-than/equal-to value (along with being less than the LOEC in brackets). \*\*\* The regulatory data included in the geometric mean is based on 10 % peat content, as a surrogate for soil OM content, as required in the applied guideline.

#### 4.1 Derivation of SGV using the assessment factor method

The SGV<sub>AF</sub> is determined using an assessment factor (AF) applied to the lowest valid toxicity endpoint (e.g. NOEC, EC10) from long-term toxicity tests – if chronic data is available. The magnitude of the AF is selected according to the adapted methods of the European guidance document on environmental risk assessment (EC TGD 2003, Marti-Roura *et al.* 2023).

The lowest relevant and reliable toxicity endpoint available for azoxystrobin is the NOEC of < 0.0353 mg a.s./kg soil for microorganisms (Table 5).

Azoxystrobin is a fungicide that inhibits spore germination and the development of certain fungi. Thus it is expected that soil microorganisms, especially fungi – **decomposers** (**nutrient transformers**) – are the most sensitive group of organisms. Greater-than/equal-to diversity endpoints are available for soil fungi (no effect at the highest test concentration); for soil bacteria and soil Actinomycetes (Grampositive, facultatively anaerobic bacteria) the NOEC values are listed as a range between the lowest and the second lowest test concentrations due to the high spacing between the concentrations, with no statistically significant effects at 0.0353 mg a.s./kg soil (Wang *et al.* 2018). There was approx. 35 and 28 % decrease on bacterial and Actinomycetes populations, respectively, at 0.353 mg a.s./kg soil concentration after 28 d. Enzymatic endpoints are also available, of which dehydrogenase and catalase activity provided the lowest effect concentrations (NOEC < 0.0353 mg a.s./kg soil, approx. 20 % decrease and increase, respectively), while urease and protease activity did not change significantly after 28 days (NOEC  $\geq$  3.53; Wang *et al.* (2018)). These unbound values are not suitable for SGV derivation. As the overall lowest normalised effect concentrations appeared as less-than values for microorganisms, a data gap needs to be considered for soil microorganisms.

The second highest sensitivity was shown by *Enchytraeus albidus* (NOEC of 1.36 mg a.s./kg tested with azoxystrobin technical; Kovačević et al. (2022)). The implications of a possible higher toxicity of a formulation (Quadris, 25 % a.s.; Kovačević et al. (2022)) is discussed in the uncertainty analysis below (Section 7). The other **decomposers** (**litter transformers/primary consumers**) are approx. 5-8 times less sensitive: per species the lowest NOEC is  $\geq$  6.80 mg a.s./kg for *Eisenia fetida* (Moser & Römbke (2000) cited in (UK 2009), Vol. 3 B.9.6.2, p.734) and 12.1 mg a.s./kg for *Folsomia candida* (a geometric mean value based on Barth (2001) cited in UK (2009), Vol. 3 B.9.7.1, p.740, Kovačević et al. (2023b) and Szabó *et al.* (2023)).

The EC20 of 25.2 mg a.s./kg for *Enchytraeus crypticus* (Leitão *et al.* 2014) is not directly comparable to NOEC values (the comparable NOEC or EC10 was not reported) and as such it is not considered for the SGV derivation; although along with the EC50 of 58.8 mg a.s./kg (from the same test, see Table 4) the data may indicate that *E. crypticus* is not the most sensitive species and as such it is not critical for the SGV derivation.

**Terrestrial plants (primary producers)** seem to be the least sensitive to azoxystrobin (NOECs of ≥ 22.7 mg a.s./kg for three species; Porch & Krüger (2002) cited in (UK 2009), Vol. 3 B.9.9.1, p.758). The normalised greater-than/equal-to NOEC values are based on the highest soil OM content that is allowed in the followed guideline (1984 version of OECD (2006)) as soil OM content was not included in the available study summary. If soil with lower OM content was used, the OM-normalised NOEC values could be even higher.

No data on **secondary consumers** is available.



When long-term test results (NOEC or EC10 values) are available for at least two species representing two trophic levels with different living and feeding conditions, the EC TGD (2003) recommends the application of an assessment factor of 50 to the lowest valid effect datum (Table 20 in EC TGD (2003)). In the case of azoxystrobin, relevant and reliable equal-to data are available at two trophic levels with the lowest value on *E. albidus*. In order to account for the uncertainties in the available data and the data gap for the most sensitive group (microorganisms), an **AF of 100** – instead of 50 – is applied to the lowest equal-to effect concentration on *E. albidus*:

$$SGV_{AF} = \frac{lowest\ EC10\ or\ NOEC}{AF}$$

$$SGV_{AF} = \frac{1.36 \left(\frac{mg \ a.s.}{kg \ soil}\right)}{100} = 0.0136 \left(\frac{mg \ a.s.}{kg \ soil}\right)$$

The application of an AF of 100 to the lowest equal-to chronic datum results in a  $SGV_{AF} = 0.014$  mg a.s./kg soil for a standard soil with 3.4 % OM content (shown to two significant figures).

## 4.2 Derivation of SGV using the species sensitivity distribution method

The minimum data requirements recommended for the application of the species sensitivity distribution (SSD) approach for  $SGV_{SSD}$  is at least ten exact data points (NOEC/EC<sub>10</sub>) from three taxonomic groups whereas data from microbial functional processes should not be used in the distribution (Marti-Roura *et al.* 2023). In the case of azoxystrobin, exact data are available for potworms and collembolans. In total, equal-to values for three species are available from two taxonomic groups. Thus, the minimum data requirements for an SSD are not met.

## 4.3 Derivation of SGV using the equilibrium partitioning approach

If no reliable data on terrestrial organisms is available, the equilibrium partitioning (EqP) utilizing aquatic toxicity data can be used to estimate the  $SGV_{EqP}$  (EC TGD 2003). In the case of azoxystrobin, sufficient amount of data is available for soil organisms to cover a wide range of different types of physiology and behaviour at various trophic levels. Therefore, the derivation of  $SGV_{EqP}$  using the equilibrium partitioning approach is not required.

## 4.4 Determination of SGV using mesocosm/field data

A field study on straw degradation (litter bag test) via soil macro- and microorganisms was evaluated in the UK Assessment Report (UK 2009). The nominal plateau concentration (incorporated at a depth of 10 cm) and the annual rate (not incorporated) were applied to the soil separately, the latter 11 days later. The time of the year and the soil parameters were not summarised. Litterbags were buried at 5 cm depth 10 days after the application of adding the plateu concentration and one day before applying the annual application rate. The soil parameters and the time of the year of applications were not summarised, thus the results cannot be normalised to a standard organic matter content and they cannot be put in a larger context.

The applied amounts of a.s. were confirmed by analytical measurements right after the applications via sampling 10-cm soil cores. While the mean recovery values per plot were between 89 and 140 %, the individual measurement values showed a much higher deviation between 50 and 150 %.

The second measurement occurred 39 days before the first sampling for straw mass loss, and was not repeated later, thus the actual test item concentrations at the time of the straw samplings are not known.



Without analytical confirmation at the time of sampling, the results cannot be considered reliable for SGV derivation (for further explanation on the requirements for field studies, please refer to Appendix 1).

## 5 Toxicity of major transformation products

Effect data are available for the three major soil metabolites of azoxystrobin: R401553 (a.k.a. SYN501657), R401553 (a.k.a. SYN501657) and R402173 (a.k.a. SYN501114).

The full effect data tables are presented in Appendix 2 (Table A3, Table A4 and Table A5), whereas Table 6 below summarises the lowest effect concentrations for these metabolites with regard to relevant and reliable data available per organism/group of organisms.

The existing relevant and reliable data is not suitable to indicate if there is similar toxicity of the metabolites to earthworms (or especially to potworms) and microorganisms as to the active substance. As a result, it remains unclear if these metabolites would require further evaluation in a mixture risk assessment or if the risk from the metabolites is covered by the SGV and the risk assessment derived and conducted for the parent.

Table 6: Lowest relevant and reliable soil effect data for azoxystrobin transformation product R401553 (a.k.a. SYN501657). Endpoint is shown as effect concentration normalised to 3.4 % soil organic matter.

| Species                       | Type of effect<br>concentration | R401553 (a.k.a.<br>SYN501657)<br>[mg/kg soil] | References  |
|-------------------------------|---------------------------------|---|---|
| Eisenia fetida<br>(Earthworm) | LC50                            | > 340   | Friedrich (2008a) cited in (UK 2009), Vol. 3 B.9.6.1, p.731 |

## 6 Proposed SGV to protect soil organisms

Depending on the degree of uncertainty or the representativeness of the derivation method and/or the assessment factor used for the derivation of the SGV, the final SGV can be classified as *preliminary* or *definitive*. Due to the applied AF of 100, the derived SGV is preliminary.

Depending on the relevance and possible application area of the SGV, it can be *site-specific* – related to a certain field or area – or *generic*. This SGV is derived for a generic use in Switzerland.

In light of the delayed results of the ongoing European review assessment (see Policy disclaimer above) and that no external peer-review of the SGV dossier took place, the current SGV is also considered provisional.

A preliminary generic SGV of 0.014 mg a.s./kg soil for azoxystrobin is suggested.

## 7 Protection of soil organisms and uncertainty analysis

The preliminary SGV of 0.014 mg a.s./kg soil for azoxystrobin has been derived based on a dataset containing values for various microbial processes, earthworms (*Eisenia fetida*), potworms (*Enchytraeus albidus* and *E. crypticus*), collembolans (*Folsomia candida*) and terrestrial plants (*Lactuca sativa, Raphanus sativus* and *Triticum aestivum*), with microorganisms and potworms showing the highest sensitivity.



Due of to the delay the European review assessment dossier (EC (2024),https://open.efsa.europa.eu/questions/EFSA-Q-2023-00763), the SGV value had to be based mostly on scientific literature data. In the hope that the renewed EU data will be published soon, the SGV dossier has not been peer-reviewed externally yet. The dossier will be amended and reconsidered after the availability of the EU renewal assessment data.

Azoxystrobin is a fungicide, thus according to its mode of action, it is expected that fungi would be the most sensitive taxonomic group. Several literature studies dealing with the changes of microbial communities (decomposers – nutrient transformers) in structure and function as a result of exposure to azoxystrobin have been listed in this report. Most of these results were not fully conclusive as they indicated effects occurring below the lowest test concentration or higher than the highest test concentration. In addition, critical details were missing from most of these studies on microorganisms in order to accept results as reliable (see details in Table A1 and Table A2 in Appendix 2). The relevant and reliable toxicity data on diversity of fungi did not show a special sensitivity but the study indicated higher sensitivity of soil bacteria and soil Actinomycetes species (Wang et al. 2018). The data on microbial dehydrogenase and catalase activity (enzymatic effects) showed the highest sensitivity to microorganisms but could not be used directly for SGV derivation because only unbound values were available (Wang et al. 2018). This study was conducted at three test concentrations in exponential series (with a spacing factor of 10 as compared to the related guideline requirement of five; (OECD 2000a, 2000b)). Thus the NOEC values at the lowest test concentrations are considered as greater-than/equalto values (along with being less than the LOEC, shown in brackets). The lowest NOEC values from this study (Wang et al. 2018) are less than the lowest test concentration. Since these less-than values were the overall lowest relevant and reliable effect concentrations in the dataset and as such critical for the SGV derivation, a data gap for microorganisms had to be considered and this SGV dossier requires a revision when equal-to relevant and reliable data for microorganisms that are lower than the current lowest valid NOEC become available.

The *available EU regulatory data* on microorganisms (N- and C-transformation) was listed as no effect values up to 2.5 kg a.s./ha applied as a 250 SC formulation (EC 1998). No study summaries or any background information on these studies are available. These values are not directly comparable to the other effect concentrations. Considering the respective OECD guidelines that were published around that time (OECD 2000a, 2000b), 5 cm soil layer can be used to calculate the concentration in the soil from the application rate. The resulting no-effect concentrations up to 3.33 mg a.s./kg soil then can be normalised according to the recommendations of the above mentioned OECD guidelines (i.e. using test soil with 0.5-1.5 % OC content) leading to the normalised NOECs of ≥ 4.44-≥13.3 mg a.s./kg soil. These estimated values <u>may indicate that the proposed SGV is protective of microorganisms based on the currently available regulatory data.</u> Altogether, the differences between the available regulatory and literature data can highlight the different sensitivity of the measured microbial effects (see functional endpoints vs diversity and enzymatic endpoints), at least in the case of azoxystrobin.

The lowest relevant and reliable data on potworms (decomposers – litter transformers/primary producers), a NOEC of 1.36 mg a.s./kg is used as a basis for the SGV derivation (Kovačević *et al.* 2022). This equal-to value was reached via testing on potworms with azoxystrobin technical substance ( $\geq$  98 % purity) and the LOEC was the highest test concentration. The two highest test concentrations were spaced by a factor of two (just slightly above the respective guideline requirement of max. 1.8; OECD (2016c)). The test with the formulation (Quadris, 25 % a.s.; Kovačević *et al.* (2022)) might have indicated higher toxicity to *E. albidus* resulting in a NOEC at the second lowest concentration. However, at this level there was a spacing factor of 8.5 between the NOEC and the LOEC, 4.3-5.7 times higher than the spacing between the other test concentrations (i.e. spacing factors of 1.5-2 used otherwise). Also, there were much higher deviations at the two lowest test concentrations than in other concentrations in this test or in the test with the technical active substance. The surprisingly high spacing occurring only between the  $2^{nd}$  and  $3^{rd}$  lowest test concentrations along with the high standard deviations



at the two lowest concentrations raised uncertainties: if there were further test concentrations in between (at least two based on the other spacing factors used in the test; min. three based on the relevant OECD guideline, OECD (2016c)), the results could indicate a much higher NOEC value; on the other hand, if there were smaller deviations at the two lowest test concentrations, there might be statistically significant effects at a lower concentration. Due to the wide spacing between the critical test concentrations with unknown reasons for the missing in-between concentrations along with the high deviations, the resulted unbound value for *E. albidus* with the formulation (NOEC of  $\geq 0.0578$  (< 0.493) mg a.s./kg; Kovačević *et al.* (2022)) was not found reliable and could not be considered for the SGV derivation. It is noted though that **the proposed SGV is protective of the potentially lowest but** *not assignable* **unbound <b>NOEC for potworms.** 

For earthworms (decomposers – litter transformers/primary producers), a single normalised EU regulatory NOEC of  $\geq 6.80$  mg a.s./kg is available as relevant and reliable effect concentration (Moser & Römbke (2000) cited in (UK 2009), Vol. 3 B.9.6.2, p.734). The proposed SGV is protective of this value. In addition, a reproductive toxicity NOEC of 3.0 kg a.s./ha was also listed in a previous review report and DAR (Anonymous cited in EC (1998), p.14 and in UK (2009), Vol. 3 B.9.6.3, p.737). In the absence of further information on this study, it was considered as not reliable and it remained uncertain if it could have indicated higher sensitivity of earthworms than the other studies. It is unclear how the rate was calculated as compared to the tested soil concentrations. It is also unclear, how much peat the test soil contained (assuming it was an artificial soil). A worst-case calculation assuming 20 cm soil layer and 10 % peat content in the soil would result in a normalised NOEC of 0.340 mg a.s./kg that is approx. 24-times higher than the proposed SGV. There was also a literature study (Leitão et al. 2014) with a relevant and reliable non-normalised effect concentration of < 50 mg a.s./kg soil for earthworms (normalised NOEC for both biomass and reproduction was < 29.6 mg a.s./kg soil, less than the lowest test concentration). At this concentration, there was approx. 33 % decrease on adult biomass change (28 d). The effect on reproduction (number of juveniles, 56 d) at the lowest test concentration was not reported but the EC50 and EC20 values were calculated via extrapolation as 42.0 and 12.2 mg a.s./kg soil, respectively. Based on these concentrations, it is presumed that there could be less than 10 % effect on reproduction at 0.014 mg a.s./kg soil concentration. Therefore the proposed SGV is considered to be protective of earthworms based on the available regulatory and literature data.

There were several relevant and reliable chronic data on **collembolans** (**decomposers** – **litter transformers/primary producers**), all indicating lower sensitivity than microorganisms and potworms. Two literature studies (**Kovačević** *et al.* 2023b, Szabó *et al.* 2023) were reported in detail, while for another one only EC20 and EC50 – rather than EC10 or NOEC – values were provided (**Leitão** *et al.* 2014). The soil OM content for the regulatory study was not summarised (**Barth** (2001) cited in UK (2009), Vol. 3 B.9.7.1, p.740). As this study was conducted to GLP following a standard guideline without any deviation (see the study summary), the 10 % peat content as a surrogate for soil OM content, as required in the applied guideline, was used for normalising the results. None of the data indicated an increased sensitivity of the tested *F. candida*. The proposed SGV is protective of collembolans based on the available regulatory and open literature data.

Terrestrial plants (primary producers) seem to be the least sensitive to azoxystrobin. There was no statistically significant effects in the only available – regulatory – study; although it is noted that only three species were tested (2 dicots and 1 monocot; Porch & Krüger (2002) cited in (UK 2009), Vol. 3 B.9.9.1, p.758). For this study, the test substance was incorporated into the test soil (i.e. concentrations rather than rates were tested and reported); however, the soil OM content was not summarised. As it was a GLP study that followed the standard OECD guideline in force at the time and no deviation to the guideline in the study summary was noted by the RMS, it is assumed that the parameters of the test soil also followed the guideline requirements, i.e. it contained max. 3 % OM. The normalisation of the resulting NOEC values was conducted using this highest recommended soil OM content as a worst-case approach (lower soil OM content would result in even higher normalised NOEC values). Altogether,



the resulting NOEC of  $\geq$  22.7 mg a.s./kg soil for the tested three species indicated no considerable sensitivity and thus it is presumed that the proposed SGV is protective of terrestrial plant species.

The data requirements at the time of DAR and the follow-up confirmatory data evaluation (UK 2009, 2014) did not include **secondary consumers** like *Hypoaspis aculeifer*. Based on our EU regulatory data collection for prioratising pesticide active substances (based on potential ecotoxicity, measured residue frequency and measured environmental concentrations) including EU regulatory data collection on the prioratised 63 substances, *Hypoaspis aculeifer* did not prove to be the most sensitive species for any pesticides in any pesticide group (Lauber and Junghans, *Ad hoc* SGV derivation guideline, *in prep.*). Based on our data collection, **it is highly unlikely that the predatory** *H. aculeifer* (**secondary consumer**) **would be more sensitive than the other species/groups discussed above and therefore the missing data for this species/group is not considered critical**.

Out of the **major soil transformation products**, only an acute earthworm LC50 for R401553 (a.k.a. SYN501657) could be derived as relevant and reliable. This value (< 340 mg a.s./kg soil) is much higher than the proposed preliminary SGV; however, an overall conclusion on azoxystrobin soil metabolites cannot be drawn.

According to the current analytical methods described in Section 2, the concentration range around the proposed SGV is possible to be detected and quantified during the national soil monitoring (SGV of 0.014 mg a.s./kg soil vs MLOQ of 0.0005 mg a.s./kg soil). Therefore, **no analytical issues are foreseen for the use of the derived SGV.** 



## 8 References

- Acosta-Dacal, A., Rial-Berriel, C., Díaz-Díaz, R., Bernal-Suárez, M.d.M. and Luzardo, O.P. (2021) Optimization and validation of a QuEChERS-based method for the simultaneous environmental monitoring of 218 pesticide residues in clay loam soil. Science of The Total Environment 753, 142015. https://doi.org/10.1016/j.scitotenv.2020.142015
- Adetutu, E.M., Ball, A.S. and Osborn, A.M. (2008) Azoxystrobin and soil interactions: Degradation and impact on soil bacterial and fungal communities. Journal of Applied Microbiology 105(6), 1777-1790. https://doi.org/10.1111/j.1365-2672.2008.03948.x
- Aleksova, M., Kenarova, A., Boteva, S., Georgieva, S., Chanev, C. and Radeva, G. (2021) Effects of increasing concentrations of fungicide QuadrisR on bacterial functional profiling in loamy sand soil. Archives of Microbiology 203(7), 4385-4396. <a href="https://doi.org/10.1007/s00203-021-02423-2">https://doi.org/10.1007/s00203-021-02423-2</a>
- Álvarez-Martín, A., Hilton, S.L., Bending, G.D., Rodríguez-Cruz, M.S. and Sánchez-Martín, M.J. (2016) Changes in activity and structure of the soil microbial community after application of azoxystrobin or pirimicarb and an organic amendment to an agricultural soil. Applied Soil Ecology 106, 47-57. https://doi.org/10.1016/j.apsoil.2016.05.005
- Anderson, R.H., Prues, A.G. and Kravitz, M.J. (2010) Determination of the biologically relevant sampling depth for terrestrial ecological risk assessments. Geoderma 154(3), 336-339. https://doi.org/10.1016/j.geoderma.2009.11.004
- Azimonti, G., Galimberti, F., Marchetto, F., Menaballi, L., Ullucci, S., Pellicioli, F., Alessandra, C., Lidia, C., Alessio, I., Angelo, M., Boer, W.d. and Voet, H.v.d. (2015a) Comparison of NOEC values to EC10/EC20 values, including confidence intervals, in aquatic and terrestrial ecotoxicological risk assessment. EFSA Supporting Publications 12(12), EN-906. https://doi.org/10.2903/sp.efsa.2015.EN-906
- Azimonti, G., Galimberti, F., Marchetto, F., Menaballi, L., Ullucci, S., Pellicioli, F., Alessandra, C., Lidia, C., Alessio, I., Angelo, M., Boer, W.d. and Voet, H.v.d. (2015b) Comparison of NOEC values to EC10/EC20 values, including confidence intervals, in aquatic and terrestrial ecotoxicological risk assessment. EFSA Supporting Publications 12(12), EN-906. https://doi.org/10.2903/sp.efsa.2015.EN-906
- Baćmaga, M., Kucharski, J. and Wyszkowska, J. (2015) Microbial and enzymatic activity of soil contaminated with azoxystrobin. Environmental Monitoring and Assessment 187(10) https://doi.org/10.1007/s10661-015-4827-5
- Baćmaga, M., Wyszkowska, J. and Kucharski, J. (2024) Response of Soil Microbiota, Enzymes, and Plants to the Fungicide Azoxystrobin. International Journal of Molecular Sciences 25(15) https://doi.org/10.3390/ijms25158104
- Bao, X., Wang, Z., Liu, L., Wang, D., Gu, Y., Chen, L., Chen, X. and Meng, Z. (2024) The combined effects of azoxystrobin and different aged polyethylene microplastics on earthworms (*Eisenia fetida*): A systematic evaluation based on oxidative damage and intestinal function. Science of The Total Environment 923 https://doi.org/10.1016/j.scitotenv.2024.171494
- BASF (2021) Dossier for the renewal of the active substance Pendimethalin (BAS 455 48 H), BASF Agro B.V. Arnhem (NL) Freienbach Branch. <a href="https://pub-efsa.echa.europa.eu/iuclid6-web/dossier/6e23c33a-f00f-40f3-8dcb-da009b74e3fd/(main-content/iuclid6:%2F6e23c33a-f00f-40f3-8dcb-da009b74e3fd/2Fheader/)?node=6e23c33a-f00f-40f3-8dcb-da009b74e3fd</a>
- Bending, G.D., Lincoln, S.D. and Edmondson, R.N. (2006) Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. Environmental Pollution 139(2), 279-287. <a href="https://doi.org/10.1016/j.envpol.2005.05.011">https://doi.org/10.1016/j.envpol.2005.05.011</a>
- Bending, G.D., Rodríguez-Cruz, M.S. and Lincoln, S.D. (2007) Fungicide impacts on microbial communities in soils with contrasting management histories. Chemosphere 69(1), 82-88. https://doi.org/10.1016/j.chemosphere.2007.04.042
- BLV (2025) Bundesamt für Lebensmittelsicherheit und Veterinärwesen Pflanzenschutzmittelverzeichnis, <a href="https://www.psm.admin.ch/de/wirkstoffe">https://www.psm.admin.ch/de/wirkstoffe</a>.



- Casado-Martinez, M., Dell'Ambrogio, G., Campiche, S., Kroll, A., Lauber, E., Marti-Roura, M., Mendez-Fernandez, L., Renaud, M., Tierbach, A., Wildi, M., Wong, J.W.Y., Werner, I., Junghans, M. and Ferrari, B.J.D. (2024) Incorporation of sediment- and soil-specific aspects in the Criteria for Reporting and Evaluating Ecotoxicity Data (CRED). Integr Environ Assess Manag https://doi.org/10.1002/jeam.4948
- Castro-Ferreira, M.P., Roelofs, D., van Gestel, C.A.M., Verweij, R.A., Soares, A.M.V.M. and Amorim, M.J.B. (2012) Enchytraeus crypticus as model species in soil ecotoxicology. Chemosphere 87(11), 1222-1227. https://doi.org/10.1016/j.chemosphere.2012.01.021
- Crane, M., Dungey, S., Lillicrap, A., Thompson, H., Weltje, L., Wheeler, J.R. and Lagadic, L. (2022) Commentary: Assessing the endocrine disrupting effects of chemicals on invertebrates in the European Union. Environmental Sciences Europe 34, 36. <a href="https://doi.org/10.1186/s12302-022-00613-3">https://doi.org/10.1186/s12302-022-00613-3</a>
- DE (1997) Monograph for Azoxystrobin Report and Proposed Decision. Rapporteur Member State: Germany. 29 January 1997 [Volume 1 and partial access to Volume 3 including B.1-B.5.5].
- EC (1998) Review report for the active substance azoxystrobin. Finalised in the Standing Committee on Plant Health at its meeting on 22.4.1998 in view of the inclusion of azoxystrobin in Annex I of Directive 91/414/EEC. European Commission, Directorate General for Agriculture, DG VIB.II-1. Azoxystrobin, 7581/VI/97-Final, 22 April 1998. <a href="https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/backend/api/active\_substance/download/854">https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/backend/api/active\_substance/download/854</a>.
- EC (2002) Draft working Document Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev 2 final, 17 October 2002. <a href="https://food.ec.europa.eu/system/files/2016-10/pesticides">https://food.ec.europa.eu/system/files/2016-10/pesticides</a> ppp appproc guide ecotox terrestrial.pdf.
- EC (2011) Consolidated text (02.12.2024): Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. <a href="https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02011R0540-20240901">https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02011R0540-20240901</a>.
- EC (2015a) Consolidated text (24.11.2024): Commission Implementing Regulation (EU) 2015/408 of 11 March 2015 on implementing Article 80(7) of Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and establishing a list of candidates for substitution. <a href="https://eurlex.europa.eu/eli/reg\_impl/2015/408/2023-07-31">https://eurlex.europa.eu/eli/reg\_impl/2015/408/2023-07-31</a>.
- EC (2015b) Draft Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible renewal of the approval of pendimethalin under Regulation (EC) 1107/2009. Rapporteur Member State (RMS): The Netherlands; Co-RMS: Spain. https://open.efsa.europa.eu/questions/EFSA-Q-2014-00744?search=pendimethalin
- EC (2015c) Final Review report for the active substance azoxystrobin finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 17 June 2011 in view of the approval of azoxystrobin as active substance in accordance with Regulation (EC) No 1107/2009. Commission staff working document. European Commission, Directorate-General for Health and Food Safety, Safety of the Food Chain, Pesticides and Biocides. Azoxystrobin, SANCO/11027/2011 Rev 3, 20 March 2015. <a href="https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/backend/api/active\_substance/download/856">https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/backend/api/active\_substance/download/856</a>
- EC (2019) Draft Renewal Assessment Report (DRAR) prepared according to the Commission Regulation (EU) No. 1107/2009 by the rapporteur Member State Spain for the existing active substance difenoconazole. <a href="https://www.efsa.europa.eu/sites/default/files/consultation/consultation/Difenoconazole.zip">https://www.efsa.europa.eu/sites/default/files/consultation/consultation/Difenoconazole.zip</a>.
- EC (2021) Draft Assessment Report and Proposed decision of the Netherlands prepared in the context of the possible renewal of the approval of pendimethalin under Regulation (EC) 1107/2009. Rapporteur Member State (RMS): The Netherlands; Co-RMS: Spain [updated after the evaluation of confirmatory data]. <a href="https://open.efsa.europa.eu/questions/EFSA-Q-2021-00631?search=pendimethalin">https://open.efsa.europa.eu/questions/EFSA-Q-2021-00631?search=pendimethalin</a>
- EC (2022) Updated draft Renewal Assessment Report prepared according to the Commission Regulation (EU) No. 1107/2009, provided by the Rapporteur Member State Sweden for the



- existing active substance pirimicarb. https://open.efsa.europa.eu/consultations/a0c09000008TWrwAAG?search=pirimicarb.
- EC (2024) Draft working document AIR IV Renewal Programme. European Commission, Directorate-General for Health and Food Safety; Food and feed safety, innovation; Pesticides and biocides. SANTE-2016-10616-rev 14, February 2024. <a href="https://food.ec.europa.eu/document/download/77b36a75-ebe5-43b8-80e0-eff6fa1d4591\_en?filename=pesticides\_ppp\_app-proc\_air-4\_renewal-program.pdf">https://food.ec.europa.eu/document/download/77b36a75-ebe5-43b8-80e0-eff6fa1d4591\_en?filename=pesticides\_ppp\_app-proc\_air-4\_renewal-program.pdf</a>.
- EC (2025) AIR IV Renewal Programme Applications for renewal of approval submitted under Article 14 of Regulation (EU) No 1107/2009 and in accordance with Regulation (EU) No 844/2012. European Commission, Directorate-General for Health and Food Safety; Food and feed safety, innovation; Pesticides and biocides. SANTE-2016-11734-rev.32, May 2025. <a href="https://food.ec.europa.eu/document/download/d6517b04-9f3f-41ca-bad3-daf491f06c41\_en?filename=pesticides\_ppp\_app-proc\_air-4\_sante-2016-11734.pdf">https://food.ec.europa.eu/document/download/d6517b04-9f3f-41ca-bad3-daf491f06c41\_en?filename=pesticides\_ppp\_app-proc\_air-4\_sante-2016-11734.pdf</a>.
- EC TGD (2003) Technical Guidance Document on Risk Assessment (Part II). European Commission. Joint Research Centre. EUR 20418 EN/2. <a href="https://op.europa.eu/s/y4d4">https://op.europa.eu/s/y4d4</a>.
- ECHA (2017) European Chemical Agency Guidance on Biocidal Products Regulation: Volume IV Environment Assessment and Evaluation (Parts B+C). ECHA-17-G-23-EN; Cat. Number: ED-01-17-897-EN-N; ISBN: 978-92-9020-151-9. https://doi.org/10.2823/033935
- ECHA (2025) Substance Infocard, azoxystrobin (ISO); methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate (IUPAC); European Chemicals Agency. https://echa.europa.eu/substance-information/-/substanceinfo/100.127.964
- EFSA (2010) Conclusion on the peer review of the pesticide risk assessment of the active substance azoxystrobin. Conclusion on Pesticide Peer Review, European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 8(4), 1542. <a href="https://doi.org/10.2903/j.efsa.2010.1542">https://doi.org/10.2903/j.efsa.2010.1542</a>
- EFSA (2014) Outcome of the consultation with Member States, applicant and EFSA on the pesticide risk assessment of confirmatory data for the active substance azoxystrobin. European Food Safety Authority (EFSA), Parma, Italy. . EFSA supporting publication EN-718 https://doi.org/10.2903/sp.efsa.2014.EN-718
- EFSA (2015) Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. European Food Safety Authority. EFSA Supporting Publications 12(12), 924E. https://doi.org/10.2903/sp.efsa.2015.EN-924
- EFSA (2016) Conclusion on the peer review of the pesticide risk assessment of the active substance pendimethalin. EFSA Journal 2016;14(3):4420, 212 pp. <a href="https://doi.org/10.2903/j.efsa.2016.4420">https://doi.org/10.2903/j.efsa.2016.4420</a>
- EFSA (2019) Technical report on the Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 16(7), EN-1673. https://doi.org/10.2903/sp.efsa.2019.EN-1673
- EPPO (2003) Environmental risk assessment scheme for plant protection products. Chapter 8: Soil organisms and functions. European and Mediterranean Plant Protection Organization. EPPO Bulletin 33(2), 195-209. <a href="https://doi.org/10.1046/j.1365-2338.2003.00654.x">https://doi.org/10.1046/j.1365-2338.2003.00654.x</a>
- EU (2007) Commission Regulation (EC) No 737/2007 of 27 June 2007 on laying down the procedure for the renewal of the inclusion of a first group of active substances in Annex I to Council Directive 91/414/EEC and establishing the list of those substances. 29.6.2007. Official Journal of the European Union L 169/10. https://eur-lex.europa.eu/eli/reg/2007/737/oj
- EU (2020) Commission Implementing Regulation (EU) 2020/1740 of November 2020 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council, and repealing Commission Implementing Regulation (EU) No 844/2012. 23.11.2020. Official Journal of the European Union L 392/20. https://eur-lex.europa.eu/eli/reg\_impl/2020/1740/oj
- Filimon, M.N., Voia, S.O., Vladoiu, D.L., Isvoran, A. and Ostafe, A. (2015) Temperature dependent effect of difenoconazole on enzymatic activity from soil. J. Serb. Chem. Soc. 80(9), 1127-1137. http://dx.doi.org/10.2298/JSC141218030F
- FOCUS (1997) Soil persistence models and EU registration. The final report of the work of the Soil Modelling Work group of FOCUS (FOrum for the Co-ordination of pesticide fate models and



- their Use). 77 pp. <a href="https://food.ec.europa.eu/system/files/2016-10/pesticides\_ppp\_app-proc\_guide\_fate\_soil-persistance-1997.pdf">https://food.ec.europa.eu/system/files/2016-10/pesticides\_ppp\_app-proc\_guide\_fate\_soil-persistance-1997.pdf</a>.
- FRAC (2024) Code List. Fungicide Resistance Action Committee, pp. 18. <a href="https://www.frac.info/media/s1zfrjqa/frac-code-list-2024.pdf">https://www.frac.info/media/s1zfrjqa/frac-code-list-2024.pdf</a>
- Gomes, S.I.L., Ammendola, A., Casini, S. and Amorim, M.J.B. (2021) Toxicity of fungicides to terrestrial non-target fauna Formulated products versus active ingredients (azoxystrobin, cyproconazole, prothioconazole, tebuconazole) A case study with *Enchytraeus crypticus* (Oligochaeta). Science of The Total Environment 754, 142098. <a href="https://doi.org/10.1016/j.scitotenv.2020.142098">https://doi.org/10.1016/j.scitotenv.2020.142098</a>
- Guo, P., Zhu, L., Wang, J., Wang, J., Xie, H. and Lv, D. (2015) Enzymatic activities and microbial biomass in black soil as affected by azoxystrobin. Environmental Earth Sciences 74(2), 1353-1361. <a href="https://doi.org/10.1007/s12665-015-4126-z">https://doi.org/10.1007/s12665-015-4126-z</a>
- Han, L., Liu, Y., Fang, K., Zhang, X., Liu, T., Wang, F. and Wang, X. (2020) Azoxystrobin dissipation and its effect on soil microbial community structure and function in the presence of chlorothalonil, chlortetracycline and ciprofloxacin. Environmental Pollution 257 <a href="https://doi.org/10.1016/j.envpol.2019.113578">https://doi.org/10.1016/j.envpol.2019.113578</a>
- Hand, L.H., Marshall, S.J., Dougan, C., Nichols, C., Kende, A., Ritz, K. and Oliver, R.G. (2021) The Impact of Disturbed Soil Structure on the Degradation of 2 Fungicides Under Constant and Variable Moisture. Environmental Toxicology and Chemistry 40(10), 2715-2725. https://doi.org/10.1002/etc.5167
- Howell, C.C., Hilton, S., Semple, K.T. and Bending, G.D. (2014) Resistance and resilience responses of a range of soil eukaryote and bacterial taxa to fungicide application. Chemosphere 112, 194-202. <a href="https://doi.org/10.1016/j.chemosphere.2014.03.031">https://doi.org/10.1016/j.chemosphere.2014.03.031</a>
- ISO (1998) Soil quality Effects of pollutants on earthworms (*Eisenia fetida*) Part 2: Determination of effects on reproduction. International Standards Organisation, Geneva, Switzerland (ISO 11268-2:1998). https://www.iso.org/standard/20993.html
- ISO (1999) Soil quality Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Standards Organisation, Geneva, Switzerland (ISO 11267:1999). https://www.iso.org/standard/19245.html
- ISO (2004) Soil quality Effects of pollutants on *Enchytraeidae* (*Enchytraeus* sp.) Determination of effects on reproduction and survival. International Standards Organisation, Geneva, Switzerland (BS ISO 16387:2004). <a href="https://www.iso.org/standard/30946.html">https://www.iso.org/standard/30946.html</a>
- ISO (2014a) Soil quality Inhibition of reproduction of Collembola (*Folsomia candida*) by soil contaminants. International Standards Organisation, Geneva, Switzerland (ISO 11267:2014). <a href="https://www.iso.org/standard/57582.html">https://www.iso.org/standard/57582.html</a>
- ISO (2014b) Soil quality Effects of pollutants on earthworms Part 3: Guidance on the determination of effects in field situations. International Standards Organisation, Geneva, Switzerland (ISO 11268-3:2014). https://www.iso.org/standard/57583.html
- Klimisch, H.J., Andreae, M. and Tillmann, U. (1997) A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology 25, 1-5. <a href="https://doi.org/10.1006/rtph.1996.1076">https://doi.org/10.1006/rtph.1996.1076</a>
- Kovačević, M., Hackenberger, D.K. and Hackenberger, B.K. (2021) Effects of strobilurin fungicides (azoxystrobin, pyraclostrobin, and trifloxystrobin) on survival, reproduction and hatching success of *Enchytraeus crypticus*. Science of The Total Environment 790 <a href="https://doi.org/10.1016/j.scitotenv.2021.148143">https://doi.org/10.1016/j.scitotenv.2021.148143</a>
- Kovačević, M., Stjepanović, N., Hackenberger, D.K. and Hackenberger, B.K. (2022) Toxicity of fungicide azoxystrobin to *Enchytraeus albidus*: Differences between the active ingredient and formulated product. Pesticide Biochemistry and Physiology 187 <a href="https://doi.org/10.1016/j.pestbp.2022.105198">https://doi.org/10.1016/j.pestbp.2022.105198</a>
- Kovačević, M., Stjepanović, N., Zelić, L. and Lončarić, Ž. (2023a) Temporal dynamics of biomarker response in *Folsomia candida* exposed to azoxystrobin. Agriculture (Switzerland) 13(7) <a href="https://doi.org/10.3390/agriculture13071443">https://doi.org/10.3390/agriculture13071443</a>
- Kovačević, M., Stjepanović, N., Zelić, L. and Lončarić, Ž. (2023b) Multigenerational and transgenerational effects of azoxystrobin on *Folsomia candida*. Environmental Pollution 336 <a href="https://doi.org/10.1016/j.envpol.2023.122398">https://doi.org/10.1016/j.envpol.2023.122398</a>



- Lauber, E., Tierbach, A., Dell'Ambrogio, G., Renaud, M. and Junghans, M. (2024a) SGV Proposal by the Ecotox Centre for Difenoconazole. Swiss Centre for Applied Ecotoxicology, Dübendorf, Switzerland; 68 pp. https://www.ecotoxcentre.ch/media/ogyn4xav/sgv\_difenoconazole\_2025\_ext.pdf.
- Lauber, E., Tierbach, A., Renaud, M. and Junghans, M. (2024b) SGV Proposal by the Ecotox Centre for Pendimethalin. Swiss Centre for Applied Ecotoxicology, Dübendorf, Switzerland; 97 pp. <a href="https://www.oekotoxzentrum.ch/media/00yjnuh3/sgv\_pendimethalin\_final\_2024.pdf">https://www.oekotoxzentrum.ch/media/00yjnuh3/sgv\_pendimethalin\_final\_2024.pdf</a>.
- Lauber, E., Balk, F., Renaud, M. and Junghans, M. (2025) SGV Proposal by the Ecotox Centre for Fluazinam. Swiss Centre for Applied Ecotoxicology, Dübendorf, Switzerland; 82 pp. <a href="https://www.oekotoxzentrum.ch/media/xxbpxkgn/sgv\_fluazinam\_final.pdf">https://www.oekotoxzentrum.ch/media/xxbpxkgn/sgv\_fluazinam\_final.pdf</a>.
- Leitão, S., Cerejeira, M.J., Van den Brink, P.J. and Sousa, J.P. (2014) Effects of azoxystrobin, chlorothalonil, and ethoprophos on the reproduction of three terrestrial invertebrates using a natural Mediterranean soil. Applied Soil Ecology 76, 124-131. https://doi.org/10.1016/j.apsoil.2013.12.013
- Lewis, K.A., Tzilivakis, J., Warner, D. and Green, A. (2016) An international database for pesticide risk assessments and management. Human and Ecological Risk Assessment 22(4), 1050-1064. https://doi.org/10.1080/10807039.2015.1133242
- Lu, C., Hou, K., Zhou, T., Wang, X., Zhang, J., Cheng, C., Du, Z., Li, B., Wang, J., Wang, J. and Zhu, L. (2023) Characterization of the responses of soil micro-organisms to azoxystrobin and the residue dynamics of azoxystrobin in wheat—corn rotation fields over two years. Chemosphere 318 https://doi.org/10.1016/j.chemosphere.2023.137918
- Marti-Roura, M., Dell'Ambrogio, G., Campiche, S., Wong, J., Junghans, M., Renaud, M. and Ferrari, B.J.D. (2023) Methodology proposal for the derivation of Soil Guideline Values for Plant Protection Product residues. Part 2 Recommendations for the derivation of Soil Guideline Values. Swiss Centre for Applied Ecotoxicology, Dübendorf and Lausanne, Switzerland. <a href="https://www.oekotoxzentrum.ch/media/czdbwdbi/2023\_consoil\_sgv\_report\_part\_2.pdf">https://www.oekotoxzentrum.ch/media/czdbwdbi/2023\_consoil\_sgv\_report\_part\_2.pdf</a>.
- Meuli, R.G., Schwab, P., Wächter, D. and Ammann, S. (2014) Nationale Bodenbeobachtung (NABO) 1985-2004. Zustand und Veränderung der anorganischen Schadstoffe und Bodenbegleitparameter. Bundesamt für Umwelt, Bern. Umwelt-Wissen Nr. 1409: 94 S. http://www.bafu.admin.ch/uz-1507-d.
- Moermond, C.T.A., Kase, R., Korkaric, M. and Ågerstrand, M. (2016) CRED: Criteria for reporting and evaluating ecotoxicity data. Environmental Toxicology and Chemistry 35(5), 1297-1309. https://doi.org/10.1002/etc.3259
- Muñoz-Leoz, B., Garbisu, C., Charcosset, J.Y., Sánchez-Pérez, J.M., Antigüedad, I. and Ruiz-Romera, E. (2013) Non-target effects of three formulated pesticides on microbially-mediated processes in a clay-loam soil. Sci Total Environ 449, 345-354. https://doi.org/10.1016/j.scitotenv.2013.01.079
- NEPC (2011) Schedule B5b Guideline on Methodology to Derive Ecological Investigation Levels in Contaminated Soils. National Environment Protection Council, National Environment Protection (Assessment of Site Contamination) Measure. Australia, April 2011. <a href="https://www.nepc.gov.au/sites/default/files/2022-09/schedule-b5b-guideline-methodology-derive-eils-sep10.pdf">https://www.nepc.gov.au/sites/default/files/2022-09/schedule-b5b-guideline-methodology-derive-eils-sep10.pdf</a>.
- OECD (1984) Test No. 207: Earthworm, Acute Toxicity Tests. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. <a href="https://doi.org/10.1787/9789264070042-en">https://doi.org/10.1787/9789264070042-en</a>
- OECD (2000a) Test No. 216: Soil Microorganisms: Nitrogen Transformation Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. <a href="https://doi.org/10.1787/9789264070226-en">https://doi.org/10.1787/9789264070226-en</a>
- OECD (2000b) Test No. 217: Soil Microorganisms: Carbon Transformation Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. https://doi.org/10.1787/9789264070240-en.
- OECD (2006) Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. <a href="https://doi.org/10.1787/9789264070066-en">https://doi.org/10.1787/9789264070066-en</a>



- OECD (2010) Test No. 317: Bioaccumulation in Terrestrial Oligochaetes, OECD Guidelines for the Testing of Chemicals, Section 3, OECD Publishing, Paris. <a href="https://doi.org/10.1787/9789264090934-en">https://doi.org/10.1787/9789264090934-en</a>
- OECD (2016a) Test No. 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*). OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. <a href="https://doi.org/10.1787/9789264264496-en">https://doi.org/10.1787/9789264264496-en</a>
- OECD (2016b) Test No. 232: Collembolan Reproduction Test in Soil. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. <a href="https://doi.org/10.1787/9789264264601-en">https://doi.org/10.1787/9789264264601-en</a>
- OECD (2016c) Test No. 220: Enchytraeid Reproduction Test. OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. <a href="https://doi.org/10.1787/9789264264472-en">https://doi.org/10.1787/9789264264472-en</a>
- OECD (2018) Revised Guidance Document 150 on standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption, OECD Series on Testing and Assessment, OECD Publishing, Paris. https://doi.org/10.1787/9789264304741-en
- Peijnenburg, W.J.G.M. (2020) Implementation of bioavailability in prospective and retrospective risk assessment of chemicals in soils and sediments. in Ortega-Calvo, J. J. and Parsons, J. R. (eds.), Bioavailability of organic chemicals in soil and sediment, Handbook of Environmental Chemistry, 100: 391-422, Springer Nature Switzerland AG, Published online: 30 April 2020. https://doi.org/10.1007/698 2020 516
- PSMV (2010) SR 916.161, Verordnung vom 12. Mai 2010 über das Inverkehrbringen von Pflanzenschutzmitteln (Pflanzenschutzmittelverordnung, PSMV) Stand am 1. Februar 2025 <a href="https://www.fedlex.admin.ch/eli/cc/2010/340/de">https://www.fedlex.admin.ch/eli/cc/2010/340/de</a>.
- Purnama, I., Malhat, F.M., Mutamima, A., Rusdiarso, B. and Noegrohati, S. (2025) Enhanced dissipation of azoxystrobin in loam soil under direct sunlight exposure. International Journal of Environmental Science and Technology 22(6), 4521-4534. <a href="https://doi.org/10.1007/s13762-024-06152-z">https://doi.org/10.1007/s13762-024-06152-z</a>
- Revesz, A., Szabo, B. and Boros, G. (2022) Testing the effects of pesticides with *Enchytraeus albidus* avoidance test. Journal of Landscape Ecology 20, 65-75. https://doi.org/10.56617/tl.3978
- Riedo, J., Yokota, A., Walther, B., Bartolomé, N., van der Heijden, M.G.A., Bucheli, T.D. and Walder, F. (2023) Temporal dynamics of total and bioavailable fungicide concentrations in soil and their effect upon nine soil microbial markers. Science of The Total Environment 878 https://doi.org/10.1016/j.scitotenv.2023.162995
- RIVM (2007) Guidance for the derivation of environmental risk limits within the framework of 'International and national environmental quality standards for substances in the Netherlands' (INS). Authors: P.L.A. van Vlaardingen and E.M.J. Verbruggen, National Institute for Public Health and the Environment. Bilthoven, The Netherlands. RIVM report 601782001/2007. <a href="https://www.rivm.nl/bibliotheek/rapporten/601782001.pdf">https://www.rivm.nl/bibliotheek/rapporten/601782001.pdf</a>.
- Rösch, A., Wettstein, F.E., Wächter, D., Reininger, V., Meuli, R.G. and Bucheli, T.D. (2023) A multiresidue method for trace analysis of pesticides in soils with special emphasis on rigorous quality control. Analytical and Bioanalytical Chemistry 415(24), 6009-6025. <a href="https://doi.org/10.1007/s00216-023-04872-8">https://doi.org/10.1007/s00216-023-04872-8</a>
- Sim, J.X.F., Doolette, C.L., Vasileiadis, S., Drigo, B., Wyrsch, E.R., Djordjevic, S.P., Donner, E., Karpouzas, D.G. and Lombi, E. (2022) Pesticide effects on nitrogen cycle related microbial functions and community composition. Science of The Total Environment 807 https://doi.org/10.1016/j.scitotenv.2021.150734
- Sopeña, F. and Bending, G.D. (2013) Impacts of biochar on bioavailability of the fungicide azoxystrobin: A comparison of the effect on biodegradation rate and toxicity to the fungal community. Chemosphere 91(11), 1525-1533. <a href="https://doi.org/10.1016/j.chemosphere.2012.12.031">https://doi.org/10.1016/j.chemosphere.2012.12.031</a>
- Szabó, B., Révész, A. and Boros, G. (2023) Additive and dose-dependent mixture effects of Flumite 200 (flufenzin, acaricide) and Quadris (azoxystrobin, fungicide) on the reproduction and survival of *Folsomia candida* (Collembola). Ecotoxicology and Environmental Safety 263 <a href="https://doi.org/10.1016/j.ecoenv.2023.115219">https://doi.org/10.1016/j.ecoenv.2023.115219</a>
- Toschki, A., Hammers-Wirtz, M., Possberg, C., Ross-Nickoll, M., Schäffer, A., Schmidt, B., Scholz Starke, B., Römbke, J., Scheffzyk, A., Klein, M. and Hommen, D. (2020) Evaluation of the risk for soil organisms under real conditions. Development of a national position for amending



- downstream legislations of the new EU Plant Protection Products Regulation. Texte 201/2020, Report No. EF001129-ENG, German Environment Agency. <a href="https://www.umweltbundesamt.de/en/publikationen/evaluation-of-the-risk-for-soil-organisms-under">https://www.umweltbundesamt.de/en/publikationen/evaluation-of-the-risk-for-soil-organisms-under</a>.
- UBA (2025) ETOX Information System, Ecotoxicology and Environmental Quality Targets. Federal Environment Agency (Umweltbundesamt), Dessau-Roßlau, Germany. <a href="http://webetox.uba.de/webETOX/index.do">http://webetox.uba.de/webETOX/index.do</a>.
- UK (2009) Report and Proposed Decision of the United Kingdom made to the European Commission under Commission Regulation 737/2007 for Azoxystrobin. Rapporteur Member State: United Kingdom. May 2009. <a href="https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-azoxystrobin">https://www.efsa.europa.eu/en/consultations/call/public-consultation-active-substance-azoxystrobin</a>.
- UK (2014) Azoxystrobin. Addendum Confirmatory Information: B.8 Environmental Fate; B.9 Ecotoxicology to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 6 of Commission Regulation (EC) No 737/2007, taking account of confirmatory data specified in Commission Implementing Regulation (EU) No 703/2011. RMS: United Kingdom. December 2014. https://open.efsa.europa.eu/question/EFSA-Q-2014-00704.
- US EPA (2025) ECOTOXicology Knowledgebase curated toxicity data. US Environmetal Protection Agency. <a href="https://cfpub.epa.gov/ecotox/">https://cfpub.epa.gov/ecotox/</a>.
- Wang, F., Li, X., Zhu, L., Du, Z., Zhang, C., Wang, J., Wang, J. and Lv, D. (2018) Responses of soil microorganisms and enzymatic activities to azoxystrobin in cambisol. Polish Journal of Environmental Studies 27(6), 2775-2784. https://doi.org/10.15244/pjoes/81086
- Wang, F.H., Zhu, L.S., Lv, D.D., Guo, P.P., Wang, J. and Wang, J.H. (2015) Effects of azoxystrobin on soil micro-organisms and enzymatic activities. Fresenius Environmental Bulletin 24(10A), 3336-3343.
- Wang, X., Lu, Z., Miller, H., Liu, J., Hou, Z., Liang, S., Zhao, X., Zhang, H. and Borch, T. (2020) Fungicide azoxystrobin induced changes on the soil microbiome. Applied Soil Ecology 145, 103343. https://doi.org/10.1016/j.apsoil.2019.08.005
- Wang, Y., Wu, S., Chen, L., Wu, C., Yu, R., Wang, Q. and Zhao, X. (2012) Toxicity assessment of 45 pesticides to the epigeic earthworm *Eisenia fetida*. Chemosphere 88(4), 484-491. https://doi.org/10.1016/j.chemosphere.2012.02.086
- Wang, Y., Yang, X., Harkes, P., van Steenbrugge, J.J.M., Xu, M. and Geissen, V. (2024) Soil microeukaryotic communities and phosphorus-cycling microorganisms respond to chloropicrin fumigation and azoxystrobin application. Science of The Total Environment 933 <a href="https://doi.org/10.1016/j.scitotenv.2024.172871">https://doi.org/10.1016/j.scitotenv.2024.172871</a>
- Wehrli, M., Slotsbo, S., Fomsgaard, Inge S., Laursen, Bente B., Gröning, J., Liess, M. and Holmstrup, M. (2024) A Dirt(y) World in a Changing Climate: Importance of Heat Stress in the Risk Assessment of Pesticides for Soil Arthropods. Global Change Biology 30(10), e17542. https://doi.org/10.1111/gcb.17542
- Wu, P., Wu, W.Z., Han, Z.H. and Yang, H. (2016) Desorption and mobilization of three strobilurin fungicides in three types of soil. Environmental Monitoring and Assessment 188(6) <a href="https://doi.org/10.1007/s10661-016-5372-6">https://doi.org/10.1007/s10661-016-5372-6</a>
- Xu, Y., Li, B., Hou, K., Du, Z., Allen, S.C., Zhu, L., Li, W., Zhu, L., Wang, J. and Wang, J. (2021) Ecotoxicity evaluation of azoxystrobin on *Eisenia fetida* in different soils. Environmental Research 194 <a href="https://doi.org/10.1016/j.envres.2020.110705">https://doi.org/10.1016/j.envres.2020.110705</a>
- Yamaguchi, T., Mahmood, A., Ito, T. and Kataoka, R. (2021) Non-target impact of dinotefuran and azoxystrobin on soil bacterial community and nitrification. Bulletin of Environmental Contamination and Toxicology 106(6), 996-1002. <a href="https://doi.org/10.1007/s00128-021-03163-1">https://doi.org/10.1007/s00128-021-03163-1</a>
- Zhang, C., Zhou, T., Xu, Y., Du, Z., Li, B., Wang, J., Wang, J. and Zhu, L. (2020) Ecotoxicology of strobilurin fungicides. Science of The Total Environment 742 <a href="https://doi.org/10.1016/j.scitotenv.2020.140611">https://doi.org/10.1016/j.scitotenv.2020.140611</a>



### **Appendix 1 Considerations for the evaluation of the studies**

### **General considerations**

- *Effects on target species* (pests) against which the active substance can be used are not considered (they are not included in any of the data tables in the SGV dossier).
- *Efficacy studies on terrestrial plants* with the aim to evaluate the effectiveness of the chemical compound on target species (pests) are not considered for the evaluation (they are not included in any of the data tables). The potential increase of the plant health due to a reduction of the pest is unrelated to the ecotoxicological effects of the substance.
- Only the effects of the substance *via soil exposure* is considered relevant. Effects resulting from using sand or other material instead of soil, or from direct over spraying of the test organism instead of exposure through soil, are *not* considered *relevant* (C3).
- For seedling emergence tests following the standard OECD 208 guideline, the use of 15-cm containers is recommended and followed by many of the contract labs. A 15-cm pot usually has a depth of approx. 13-14 cm and – based on photos of the test in contract labs (e.g. Ibacon, Eurofins etc.) – the planted pots are usually filled up to the lower end of the brim, i.e. approx. to 10-11-12 cm. In other studies for instance it was specified that they used pots with 11-cm diameter and 10-cm depth (see Anonymous (2016) cited in (BASF 2021) or 7cm depth trays (Fleming et al. (1996a) cited in (EC 2022)). The specific container size/soil depth is used if it is reported/summarised. Otherwise the use of an average soil depth of 10 cm along with 1.5 g/cm<sup>3</sup> soil bulk density for converting the applied rate of the test item to a concentration in the soil is considered reasonable and pragmatic (also see the recommendation in Info-box 13 in (ECHA 2017), p.149). This is based on the above detailed information, i.e. the test guideline recommendation in conjunction with available information in standard regulatory study reports, information available publicly on the methods used by contract laboratories as well as personal communication with experts conducting such studies. While the soil depth can slightly vary depending on the plant species/test facility, ten centimetres soil depth is considered as a reasonable average for studies where the container size is not reported, which also allows comparability of the nontarget terrestrial plant results with other studies, where either the test item is mixed into the soil, i.e. the test item concentration in the soil is known (most laboratory studies) or the upper 10-cm layer is sampled for analytical measurements (see e.g. field earthworm studies). If specific information is available for a certain study, the concentrations are calculated accordingly.

It is noted that the behaviour of the test substances can vary and can result in different distributions in the soil in case of over-spraying. However, choosing and considering a certain soil depth is a pragmatic approach and a pragmatic solution that is already applied for the authorisation/registration of pesticides (but with different depths, i.e. 5 cm for permanent crops and 20 cm for crops where ploughing in the season takes place, even if the substance is actually not mixed into the soil after application, see e.g. (FOCUS 1997) and (EC 2002)) as well as of biocides (ECHA 2017).

- Reproductive endpoints are considered the most relevant endpoints as they are good indicators of the sustainability of the population in the long-term. Other endpoints affecting survival and growth (biomass) of individuals are also accepted, since they were traditionally measured endpoints frequently extrapolated to represent the impact at population level. If multiple comparable toxicity values for the same species and the same measured effect are available, the *geometric mean* of the effect values is calculated.
- Following a critical consideration (Azimonti *et al.* 2015b, EFSA 2019), the statistically more robust endpoint of *EC10 vs NOEC* is chosen. If both endpoints seem to be equally robust (e.g. details of statistical methods and results are reported; clear dose-response;



descriptive statistics; NOEC: also statistically significant LOEC is reported; EC10: width/lower/higher limits of confidence intervals for EC10/20/50; steepness of curve etc. are available), then EC10 is preferred due to the general inherent uncertainties a NOEC is surrounded by (Azimonti *et al.* 2015a). When no or not statistically robust EC10median is available, the statistically robust NOEC is preferred. It is noted that statistically non-robust (but "biologically significant") NOEC values are often preferred during the EU pesticide authorisation/renewal processes, to provide long-term endpoints with not higher than 10 % effects. However, such endpoint could not account for the variability of data in soil studies (where coefficient of variation in the control is accepted up to 15, 30 or 50 %). The uncertainty in a NOEC value with higher level of effects may need to be highlighted and discussed. In the absence of a statistically robust endpoint, the study results are considered *not reliable* (**R3**) or *not assignable* (**R4**) depending on the actual flaws.

- Regulatory studies and their endpoints (e.g. EFSA, US EPA) are generally accepted without additional assessment (at face value) or partially re-considered if needed to set the endpoints in line with our criteria as summarised here and detailed above (Moermond et al. 2016, Marti-Roura et al. 2023). This is the case, for example, when organisms are not exposed through soil (e.g. plant vegetative vigour tests via foliar application); normalisation to a standard organic matter content is not possible due to lack of data; not the statistically most robust effect concentration is proposed/agreed upon as an endpoint etc. A full reassessment may also be carried out for regulatory studies, where the study summary is not sufficiently detailed and we can get access to the original study report.
- Study *endpoints from authorisation reports* (e.g. EFSA, US EPA) are subjected to the same scrutiny as open literature data. These include but are not limited to careful consideration of the study design (e.g. number of replicates and test concentrations), the way the tests were conducted (e.g. environmental conditions, observations), their results (e.g. performance of control, validity criteria, dose-response, deviation) as well as the statistical analysis (e.g. methods and reported details). Authorisation reports are accepted at face value and used in the risk assessment if they meet the criteria of reliability and relevance as detailed above (Moermond *et al.* 2016, Marti-Roura *et al.* 2023). If they have flaws in terms of reliability and relevance or other requirements as detailed here and in the above cited documents (e.g. validity criteria of the study were not met; no statistically robust EC10median could be derived; endpoint could not be standardised due to lacking information on OM/OC content of the test soil etc.), the regulatory endpoints are listed at face value and not considered further but not used in deriving an SGV.
- In general, *biomarker studies* are not included in the tables since they are based on endpoints, whose relationship to effects at population level is uncertain. However, some exo-enzymes produced by soil microorganisms can be used as biomarkers of soil fertility and are important in the ecological functioning of the soil (e.g. Filimon *et al.* 2015, NEPC 2011, RIVM 2007). For this reason, microbial-mediated enzymatic activities are included in the assessment as "relevant with restrictions" (C2).
- The relationship between *microbial biodiversity and function* is quite complex. Although it cannot be denied that loss of microbial diversity can have an impact on function, the role of biodiversity in supporting microbial functions needs a better understanding (EFSA 2019). For this reason, in this report, microbial endpoints directly involved in soil functions are preferred over microbial diversity endpoints.
- **Recovery of effects** that can be seen e.g. in earthworm field studies is not considered acceptable within the scope of SGV that is used in relation to long-term pesticide residues, not immediate effects after application of pesticides.
- Long-term endpoints from *field studies* are considered as supportive information unless there is analytical verification. A robust effect concentration can only be derived when it is



confirmed by analytical verification and it should be within approximately a month of the assessment of the effect endpoint to ensure its reliability with regards to any potential loss of the test substance through degradation/dissipation and as a result to underestimate the risk. In order to derive effect concentration(s) for the whole duration of a field study, the test substance concentration should be monitored regularly until the end of the study. When the test substance concentrations are measured only at the beginning of the study, the derivation of an approx, one-month endpoint is considered reliable enough for a quantitative use (see e.g. field earthworm studies). As the actual degradation/dissipation of a pesticide can be affected by a mixture of various biotic and abiotic factors, without measured residues in the test site it is not possible to calculate a meaningful (time-weighted average) concentration in the soil and derive a robust endpoint (see e.g. concentration-dependent dissipation of pesticides in Muñoz-Leoz et al. (2013), but also the wide range of DissT50 values for pendimethalin in Section 1.5.2 above (EC 2019)). It is noted that, for instance, according to the often used field earthworm study guideline (ISO 2014b) 50 % deviation from the nominal concentration is acceptable. However, as we compare the derived effect concentrations - and in turn the derived SGV - directly to the measured environmental concentrations, it is more reasonable to base the effect values on the measured amount of test substance present in the soil during the study. Altogether it is considered a pragmatic approach to use the analytical verification results for the upper 10-cm soil layer. It is noted that the sampled upper 10-cm soil layer does not cover the whole depth where earthworms can occur. However, a) while it is not ideal, it is usually the only analytical information available (see e.g. the respective requirement in ISO (2014b)); b) depending on the ecological group (i.e. epigeic, endogeic or anecic species) the exposure of earthworms to pesticides can highly vary anyway. In a pilot study it was shown that even anecic species living usually in deep burrows can be affected by pesticide treatments due to their feeding and mating habits, i.e. gathering food and mating on the contaminated soil surface (Toschki et al. 2020). The abundance, diversity and activity of soil biota are in general the highest in the top soil layer (Toschki et al. 2020, Anderson et al. 2010).

### Soil organic matter content

- When only *total organic carbon* is reported in a study, the total organic carbon value is transformed to organic matter by using a factor of 1:1.7.
- If only a *percentage of sphagnum peat* is reported in laboratory studies with artificial soil, the soil organic matter content is estimated assuming that the only source of organic matter in the soil comes from the sphagnum peat and that the organic matter content of the sphagnum peat is approximately 100 %.
- If *no organic carbon/matter content* is reported, the study endpoint cannot be normalised and thus is not suitable for further use. As a result the study is scored as *not assignable: Information needed to assess the study is missing* (**R4**; Moermond *et al.* 2016, Casado-Martinez *et al.* 2024).

For the adapted criteria – that were mainly based on the European technical guidance document (EC TGD 2003) – and further details on the parameters and methods that are used for the SGV derivation, please refer to Marti-Roura *et al.* (2023). The criteria beyond these resources will be included in an updated methodological report.



# Appendix 2 Data on the active substance

Table A1: Soil effect data for azoxystrobin from laboratory experiments. The lowest reliable and relevant effect data per species per test setup are shown in bold. Unreliable, not relevant and not assignable data are greyed out. Calculated data are rounded to three significant figures. Abbreviations: n.r. - not reported; n.a. - not applicable; cc. - concentration; MWHC - maximum water holding capacity; OC - organic carbon; OM - organic matter; CFU - colony forming units; CD - colony development index; EP - ecophysiological index. Data were evaluated for reliability and relevance according to the modified CRED criteria (see R/C scores) or taken at face value from regulatory dossiers (Assessment score 1-3). For notes, please refer to the end of Appendix 2 (Notes A1). Data in squared brackets are based on the specific guideline requirements applied in the respective GLP study (for further information, please refer to the respective notes to the study).

| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                | Measured<br>effect <sup>3</sup> | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source  |
|--|-------------------------------|---------------------------------|----------|-------------------------------|---|-----------------|--|--|-------|-------------------------|---|
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin (a.s., ICIA5504) | adult<br>mortality              | 14 d     | LC50                          | 283   | n.r.            | n.a.   | n.r.   | A     | (1)<br>R4/C2            | Anonymous cited in (EC 1998), p.14; Fleming <i>et al.</i> (1993) cited in (UK 2009), Vol. 3 B.9.6.3, p.737    |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin (a.s., ICIA5504) | adult<br>mortality              | 14 d     | NOEC                          | 180   | n.r.            | n.a.   | n.r.   | A     | (1)<br>R4/C2            | Anonymous in (EC 1998),<br>p.14; Fleming <i>et al.</i> (1993)<br>cited in (UK 2009), Vol. 3<br>B.9.6.3, p.737 |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>250 SC        | adult<br>mortality              | acute    | LC50                          | 881   | n.r.            | n.a.   | n.r.   | A     | (1)<br>R4/C2            | Bembridge <i>et al.</i> (1994) cited in (UK 2009), Vol. 3 B.9.6.3, p.737                                      |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>250 SC        | adult<br>mortality              | acute    | NOEC                          | 10  | n.r.            | n.a.   | n.r.   | A     | (1)<br>R4/C2            | Bembridge <i>et al.</i> (1994) cited in (UK 2009), Vol. 3 B.9.6.3, p.737                                      |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>500 WG        | adult<br>mortality              | acute    | LC50                          | > 1000  | n.r.            | n.a.   | n.r.   | A     | (1)<br>R4/C2            | Bembridge <i>et al.</i> (1995) cited in (UK 2009), Vol. 3 B.9.6.3, p.737                                      |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>500 WG        | adult<br>mortality              | acute    | NOEC                          | 10  | n.r.            | n.a.   | n.r.   | A     | (1)<br>R4/C2            | Bembridge <i>et al.</i> (1995) cited in (UK 2009), Vol. 3 B.9.6.3, p.737                                      |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>(98 % purity) | adult<br>mortality              | 7 d      | LC50                          | 362   | 10              | 123  | Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay, 70 % fine sand, pH $6.0 \pm 0.5$ , water 35 % of soil dry weight | W     | R4/C2                   | Wang et al. (2012)  |

 $<sup>^{2~</sup>M}$  – monocotyledonous,  $^{D}$  – dicotyledonous plant species  $10^{3~DE}$  – diversity endpoint,  $^{ECE}$  – ecological endpoint,  $^{EE}$  – enzymatic endpoint,  $^{FE}$  – functional endpoint



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                          | Measured<br>effect <sup>3</sup>             | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source   |
|--|---|---|----------|-------------------------------|---|-----------------|--|---|-------|-------------------------|--|
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>(98 % purity)           | adult<br>mortality                          | 14 d     | LC50                          | 327   | 10              | 111  | Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay, 70 % fine sand, pH 6.0 ± 0.5, water 35 % of soil dry weight | W     | R4/C2                   | Wang et al. (2012)   |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin (purity not reported)      | adult<br>mortality                          | 14 d     | LC50                          | 32.5  | 1.33            | 83.1   | Natural soil (fluvo-<br>aquic): 20.1 % sand,<br>57.5 % silt, 22.4 % clay,<br>pH 7.95                                  | X     | R4/C2                   | Xu et al. (2021)   |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin (purity not reported)      | adult<br>mortality                          | 14 d     | LC50                          | 241   | 2.93            | 280  | Natural soil (black):<br>39.0 % sand, 29.0 % silt,<br>32 % clay, pH 6.07  | X     | R4/C2                   | Xu et al. (2021)   |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin (purity not reported)      | adult<br>mortality                          | 14 d     | LC50                          | 21.3  | 0.72            | 101  | Natural soil (red clay):<br>14.9 % sand, 13.8 % silt,<br>71.3 % clay, pH 5.47   | X     | R4/C2                   | Xu et al. (2021)   |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin (purity not reported)      | adult<br>mortality                          | 14 d     | LC50                          | 529   | 4.28            | 420  | Artificial soil: 10 %<br>sphagnum peat, 20 %<br>kaolinite clay, 70 % fine<br>sand, pH 5.87                            | X     | R4/C2                   | Xu et al. (2021)   |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>250 SC                  | reproductive<br>toxicity                    | 56 d     | NOEC                          | (3.0 kg a.s./ha)                                | n.r.            | n.a.   | n.r.  | A     | (1)<br>R4/C2            | Anonymous cited in (EC 1998), p.14 and in (UK 2009), Vol. 3 B.9.6.3, p.737 |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>250 g/L SC<br>(YF10537) | adult<br>mortality                          | 28 d     | NOEC                          | ≥ 20  | 10              | ≥ 6.80   | Artificial soil: 10 %<br>sphagnum peat, pH 6 ±<br>5, 50 ± 10 % moisture<br>of dry weight soil                         | Е     | 1<br>(R2/C1)            | Moser & Römbke (2000)<br>cited in (UK 2009), Vol. 3<br>B.9.6.2, p.734      |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>250 g/L SC<br>(YF10537) | biomass<br>(adult body<br>weight<br>change) | 28 d     | NOEC                          | ≥ 20  | 10              | ≥ 6.80   | Artificial soil: 10 % sphagnum peat, pH 6 $\pm$ 5, 50 $\pm$ 10 % moisture of dry weight soil                          | E     | 1<br>(R2/C1)            | Moser & Römbke (2000)<br>cited in (UK 2009), Vol. 3<br>B.9.6.2, p.734      |
| Eisenia fetida<br>(Earthworm)                | Azoxystrobin<br>250 g/L SC<br>(YF10537) | reproduction<br>(number of<br>juveniles)    | 56 d     | NOEC                          | ≥ 20  | 10              | ≥ 6.80   | Artificial soil: 10 % sphagnum peat, pH 6 ± 5, 50 ± 10 % moisture of dry weight soil                                  | E     | 1<br>(R2/C1)            | Moser & Römbke (2000)<br>cited in (UK 2009), Vol. 3<br>B.9.6.2, p.734      |
| Eisenia fetida<br>(Earthworm)                | Ortiva<br>(250 g a.s./L)                | adult<br>mortality                          | 28 d     | LC50                          | > 500   | 5.74            | > 296  | Natural soil (sandy clay loam, Portugal): 54.4 % sand, 22.1 % silt, 23.5 %  | P     | R2/C2                   | Leitão et al. (2014)   |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                     | Measured<br>effect <sup>3</sup>             | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source                       |
|--|------------------------------------|---|----------|-------------------------------|---|-----------------|--|--|-------|-------------------------|------------------------------|
| Eisenia fetida<br>(Earthworm)                | Ortiva<br>(250 g a.s./L)           | biomass<br>(adult body<br>weight<br>change) | 28 d     | NOEC                          | < 50  | 5.74            | < 29.6   | silt, pH 5.9, 50 % of<br>MWHC<br>Natural soil (sandy clay<br>loam, Portugal): 54.4<br>% sand, 22.1 % silt,<br>23.5 % silt, pH 5.9, 50<br>% of MWHC | P     | R2/C1                   | Leitão <i>et al</i> . (2014) |
| Eisenia fetida<br>(Earthworm)                | Ortiva<br>(250 g a.s./L)           | reproduction<br>(number of<br>juveniles)    | 56 d     | NOEC                          | < 50  | 5.74            | < 29.6   | Natural soil (sandy clay<br>loam, Portugal): 54.4<br>% sand, 22.1 % silt,<br>23.5 % silt, pH 5.9, 50<br>% of MWHC                                  | P     | R2/C1                   | Leitão <i>et al.</i> (2014)  |
| Eisenia fetida<br>(Earthworm)                | Ortiva<br>(250 g a.s./L)           | reproduction<br>(number of<br>juveniles)    | 56 d     | EC20                          | 12.2  | 5.74            | 7.23   | Natural soil (sandy clay<br>loam, Portugal): 54.4 %<br>sand, 22.1 % silt, 23.5 %<br>silt, pH 5.9, 50 % of<br>MWHC                                  | Р     | R3/C2                   | Leitão <i>et al</i> . (2014) |
| Eisenia fetida<br>(Earthworm)                | Ortiva<br>(250 g a.s./L)           | reproduction<br>(number of<br>juveniles)    | 56 d     | EC50                          | 42.0  | 5.74            | 24.9   | Natural soil (sandy clay<br>loam, Portugal): 54.4 %<br>sand, 22.1 % silt, 23.5 %<br>silt, pH 5.9, 50 % of<br>MWHC                                  | Р     | R3/C2                   | Leitão <i>et al</i> . (2014) |
| Enchytraeus<br>crypticus<br>(Potworm)        | Azoxystrobin<br>(≥ 98 %<br>purity) | adult<br>mortality                          | 21 d     | NOEC                          | 17.5  | 1.77            | 33.6   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 %<br>sand, 50 % of MWHC   | M     | R2/C1                   | Gomes et al. (2021)          |
| Enchytraeus<br>crypticus<br>(Potworm)        | Azoxystrobin<br>(≥ 98 % purity)    | adult<br>mortality                          | 21 d     | LC10                          | 21  | 1.77            | 40.3   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 % sand,<br>50 % of MWHC   | M     | R4/C1                   | Gomes et al. (2021)          |
| Enchytraeus<br>crypticus<br>(Potworm)        | Azoxystrobin<br>(≥ 98 % purity)    | adult<br>mortality                          | 21 d     | LC50                          | 39  | 1.77            | 74.9   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 % sand,<br>50 % of MWHC   | M     | R2/C2                   | Gomes et al. (2021)          |
| Enchytraeus<br>crypticus<br>(Potworm)        | Azoxystrobin<br>(≥ 98 %<br>purity) | reproduction<br>(number of<br>juveniles)    | 21 d     | NOEC                          | 17.5  | 1.77            | 33.6   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 %<br>sand, 50 % of MWHC   | M     | R2/C1                   | Gomes et al. (2021)          |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance               | Measured<br>effect <sup>3</sup>                    | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source                         |
|--|------------------------------|--|----------|-------------------------------|---|-----------------|--|--|-------|-------------------------|--------------------------------|
| Enchytraeus<br>crypticus<br>(Potworm)        | Azoxystrobin (≥ 98 % purity) | reproduction<br>(number of<br>juveniles)           | 21 d     | EC10                          | 17  | 1.77            | 32.7   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 % sand,<br>50 % of MWHC | M     | R3/C1                   | Gomes et al. (2021)            |
| Enchytraeus<br>crypticus<br>(Potworm)        | Azoxystrobin (≥ 98 % purity) | reproduction<br>(number of<br>juveniles)           | 21 d     | EC50                          | 37  | 1.77            | 71.1   | Natural soil: standard<br>LUFA 2.2, 7.3 % clay,<br>13.8 % silt, 78.9 % sand,<br>50 % of MWHC | M     | R2/C2                   | Gomes et al. (2021)            |
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)    | adult<br>mortality                                 | 21 d     | NOEC                          | 75  | 1.73            | 147  | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C1                   | Kovačević <i>et al.</i> (2021) |
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)    | adult<br>mortality                                 | 21 d     | LC10                          | 132   | 1.73            | 259  | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C1                   | Kovačević <i>et al.</i> (2021) |
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)    | adult<br>mortality                                 | 21 d     | LC50                          | ≥ 150   | 1.73            | ≥ 295  | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C2                   | Kovačević <i>et al.</i> (2021) |
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)    | reproduction<br>(number of<br>juveniles)           | 21 d     | NOEC                          | 75  | 1.73            | 147  | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C1                   | Kovačević <i>et al.</i> (2021) |
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)    | reproduction<br>(number of<br>juveniles)           | 21 d     | EC10                          | 57  | 1.73            | 112  | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C1                   | Kovačević <i>et al.</i> (2021) |
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)    | reproduction<br>(number of<br>juveniles)           | 21 d     | EC50                          | 93  | 1.73            | 183  | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C2                   | Kovačević <i>et al.</i> (2021) |
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)    | hatching rate<br>(number of<br>hatched<br>cocoons) | 19 d     | NOEC                          | 75  | 1.73            | 147  | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C4                   | Kovačević <i>et al.</i> (2021) |
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)    | hatching rate<br>(number of<br>hatched<br>cocoons) | 19 d     | EC10                          | 27  | 1.73            | 53.1   | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C4                   | Kovačević <i>et al.</i> (2021) |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                     | Measured<br>effect <sup>3</sup>                    | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source                         |
|--|------------------------------------|--|----------|-------------------------------|---|-----------------|--|--|-------|-------------------------|--------------------------------|
| Enchytraeus<br>crypticus<br>(Potworm)        | Quadris<br>(250 g a.s./L)          | hatching rate<br>(number of<br>hatched<br>cocoons) | 19 d     | EC50                          | 104   | 1.73            | 204  | Natural soil: standard<br>LUFA 2.2, 8.3 % clay,<br>14.9 % silt, 76.8 % sand,<br>60 % of MWHC | N     | R4/C4                   | Kovačević et al. (2021)        |
| Enchytraeus<br>albidus<br>(Potworm)          | Azoxystrobin<br>(≥ 98 % purity)    | adult<br>mortality                                 | 7 d      | LC10                          | 11.95   | 10              | 4.06   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | O     | R4/C3                   | Kovačević <i>et al.</i> (2022) |
| Enchytraeus<br>albidus<br>(Potworm)          | Azoxystrobin<br>(≥ 98 % purity)    | adult<br>mortality                                 | 7 d      | LC50                          | 16.76   | 10              | 5.70   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | 0     | R2/C3                   | Kovačević et al. (2022)        |
| Enchytraeus<br>albidus<br>(Potworm)          | Azoxystrobin<br>(≥ 98 %<br>purity) | reproduction<br>(number of<br>juveniles)           | 42 d     | NOEC                          | 4   | 10              | 1.36   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | 0     | R2/C1                   | Kovačević <i>et al.</i> (2022) |
| Enchytraeus<br>albidus<br>(Potworm)          | Azoxystrobin<br>(≥ 98 % purity)    | reproduction<br>(number of<br>juveniles)           | 42 d     | EC10                          | > 8   | 10              | > 2.72   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | 0     | R4/C1                   | Kovačević <i>et al.</i> (2022) |
| Enchytraeus<br>albidus<br>(Potworm)          | Azoxystrobin<br>(≥ 98 % purity)    | reproduction<br>(number of<br>juveniles)           | 42 d     | EC50                          | > 8   | 10              | > 2.72   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | 0     | R2/C2                   | Kovačević <i>et al.</i> (2022) |
| Enchytraeus<br>albidus<br>(Potworm)          | Quadris<br>(25 % a.s.)             | adult<br>mortality                                 | 7 d      | LC10                          | 10.65   | 10              | 3.62   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | 0     | R4/C3                   | Kovačević <i>et al.</i> (2022) |
| Enchytraeus<br>albidus<br>(Potworm)          | Quadris<br>(25 % a.s.)             | adult<br>mortality                                 | 7 d      | LC50                          | 15.29   | 10              | 5.20   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | 0     | R2/C3                   | Kovačević et al. (2022)        |
| Enchytraeus<br>albidus<br>(Potworm)          | Quadris<br>(25 % a.s.)             | reproduction<br>(number of<br>juveniles)           | 42 d     | NOEC                          | ≥ 0.17 (< 1.45)                                 | 10              | ≥ 0.0578 (< 0.493)   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | 0     | R4/C1                   | Kovačević <i>et al.</i> (2022) |
| Enchytraeus<br>albidus<br>(Potworm)          | Quadris<br>(25 % a.s.)             | reproduction<br>(number of<br>juveniles)           | 42 d     | EC10                          | 1.23  | 10              | 0.418  | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC         | O     | R4/C1                   | Kovačević <i>et al.</i> (2022) |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                          | Measured<br>effect <sup>3</sup>          | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source   |
|--|---|--|----------|-------------------------------|---|-----------------|--|---|-------|-------------------------|--|
| Enchytraeus<br>albidus<br>(Potworm)          | Quadris<br>(25 % a.s.)                  | reproduction<br>(number of<br>juveniles) | 42 d     | EC50                          | 2.94  | 10              | 1.00   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC                              | 0     | R2/C2                   | Kovačević et al. (2022)                                      |
| Enchytraeus<br>crypticus<br>(Potworm)        | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles) | 28 d     | EC20                          | 42.6  | 5.74            | 25.2   | Natural soil (sandy clay<br>loam, Portugal): 54.4<br>% sand, 22.1 % silt,<br>23.5 % silt, pH 5.9, 50<br>% of MWHC | P     | R2/C2                   | Leitão <i>et al</i> . (2014)                                 |
| Enchytraeus<br>crypticus<br>(Potworm)        | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 99.2  | 5.74            | 58.8   | Natural soil (sandy clay<br>loam, Portugal): 54.4 %<br>sand, 22.1 % silt, 23.5 %<br>silt, pH 5.9, 50 % of<br>MWHC | Р     | R2/C2                   | Leitão <i>et al.</i> (2014)                                  |
| Folsomia candida<br>(Collembola)             | Azoxystrobin<br>250 g/L SC<br>(YF10537) | adult<br>mortality                       | 28 d     | NOEC                          | 200   | [10]            | [68.0]   | Artificial soil: pH 5.96-<br>6.28, 35.10-37.69 %<br>moisture content of dry<br>weight                             | F     | (1)<br>R2/C1            | Barth (2001) cited in (UK 2009), Vol. 3 B.9.7.1, p.740       |
| Folsomia candida<br>(Collembola)             | Azoxystrobin<br>250 g/L SC<br>(YF10537) | reproduction<br>(number of<br>juveniles) | 28 d     | NOEC                          | 50  | [10]            | [17.0]   | Artificial soil: pH 5.96-<br>6.28, 35.10-37.69 %<br>moisture content of dry<br>weight                             | F     | (1)<br>R2/C1            | Barth (2001) cited in<br>(UK 2009), Vol. 3 B.9.7.1,<br>p.740 |
| Folsomia candida<br>(Collembola)             | Azoxystrobin<br>250 g/L SC<br>(YF10537) | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 167.3   | [10]            | [56.9]   | Artificial soil: pH 5.96-<br>6.28, 35.10-37.69 %<br>moisture content of dry<br>weight                             | F     | (1)<br>R2/C2            | Barth (2001) cited in (UK 2009), Vol. 3 B.9.7.1, p.740       |
| Folsomia candida<br>(Collembola)             | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles) | 28 d     | EC20                          | 54.9  | 5.74            | 32.5   | Natural soil (sandy clay loam, Portugal): 54.4 % sand, 22.1 % silt, 23.5 % silt, pH 5.9, 50 % of MWHC             | P     | R2/C2                   | Leitão <i>et al</i> . (2014)                                 |
| Folsomia candida<br>(Collembola)             | Ortiva<br>(250 g a.s./L)                | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 92.0  | 5.74            | 54.5   | Natural soil (sandy clay loam, Portugal): 54.4 % sand, 22.1 % silt, 23.5 % silt, pH 5.9, 50 % of MWHC             | Р     | R2/C2                   | Leitão <i>et al.</i> (2014)                                  |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L)               | adult<br>mortality                       | 28 d     | NOEC                          | ≥ 200   | 10              | ≥ 68   | Artificial soil: 70 % sand, 20 % kaolin clay, 10 % sphagnum peat, pH 6, 60 % of MWHC                              | R     | R2/C21                  | Kovačević <i>et al.</i> (2023a)                              |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance            | Measured<br>effect <sup>3</sup>                   | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source                           |
|--|---------------------------|---|----------|-------------------------------|---|-----------------|--|---|-------|-------------------------|----------------------------------|
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles)          | 28 d     | NOEC                          | 15  | 10              | 5.10   | Artificial soil: 70 % sand, 20 % kaolinite clay, 10 % sphagnum peat, pH 6, 60 % of MWHC             | R     | R2/C1                   | Kovačević et al. (2023a)         |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles)          | 28 d     | EC10                          | 11.73   | 10              | 3.99   | Artificial soil: 70 % sand, 20 % kaolinite clay, 10 % sphagnum peat, pH 6, 60 % of MWHC             | R     | R4/C1                   | Kovačević <i>et al.</i> (2023a)  |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles)          | 28 d     | EC50                          | 61.28   | 10              | 21.2   | Artificial soil: 70 % sand, 20 % kaolinite clay, 10 % sphagnum peat, pH 6, 60 % of MWHC             | R     | R2/C2                   | Kovačević <i>et al.</i> (2023a)  |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | adult<br>mortality (F0,<br>F1, F2, F3)            | 32 d     | NOEC                          | ≥ 200   | n.r.            | n.a.   | Natural soil (LUFA 2.2,<br>Speyer, Germany): no<br>specific parameters<br>provided; 60 % of<br>MWHC | S     | R4/C1                   | Kovačević <i>et al</i> . (2023b) |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles of<br>F0) | 32 d     | NOEC                          | 50  | n.r.            | n.a.   | Natural soil (LUFA 2.2,<br>Speyer, Germany): no<br>specific parameters<br>provided; 60 % of<br>MWHC | S     | R4/C1                   | Kovačević <i>et al.</i> (2023b)  |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles of<br>F1) | 32 d     | NOEC                          | <0.15   | n.r.            | n.a.   | Natural soil (LUFA 2.2,<br>Speyer, Germany): no<br>specific parameters<br>provided; 60 % of<br>MWHC | S     | R4/C4                   | Kovačević et al. (2023b)         |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles of<br>F2) | 32 d     | NOEC                          | 15  | n.r.            | n.a.   | Natural soil (LUFA 2.2,<br>Speyer, Germany): no<br>specific parameters<br>provided; 60 % of<br>MWHC | S     | R4/C4                   | Kovačević et al. (2023b)         |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles of<br>F3) | 32 d     | NOEC                          | 1.5   | n.r.            | n.a.   | Natural soil (LUFA 2.2,<br>Speyer, Germany): no<br>specific parameters<br>provided; 60 % of<br>MWHC | S     | R4/C4                   | Kovačević et al. (2023b)         |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance            | Measured<br>effect <sup>3</sup>          | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source                     |
|--|---------------------------|--|----------|-------------------------------|---|-----------------|--|--|-------|-------------------------|----------------------------|
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | adult<br>mortality                       | 28 d     | NOEC                          | 30  | 5               | 20.4   | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R2/C1                   | Szabó et al. (2023)        |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | adult<br>mortality                       | 28 d     | LC10                          | 0.514   | 5               | 0.350  | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R3/C1                   | Szabó <i>et al.</i> (2023) |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | adult<br>mortality                       | 28 d     | LC50                          | 386   | 5               | 262  | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R3/C2                   | Szabó <i>et al.</i> (2023) |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles) | 28 d     | NOEC                          | 30  | 5               | 20.4   | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R2/C1                   | Szabó et al. (2023)        |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles) | 28 d     | EC10                          | 31.8  | 5               | 21.6   | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R4/C1                   | Szabó <i>et al.</i> (2023) |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | reproduction<br>(number of<br>juveniles) | 28 d     | EC50                          | 65.6  | 5               | 44.6   | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH 6.025 ± 0.116, 40 % of MWHC | Q     | R2/C2                   | Szabó <i>et al.</i> (2023) |
| Folsomia candida<br>(Collembola)             | Quadris<br>(250 g a.s./L) | soil avoidance                           | 48 h     | NOEC                          | 90  | 5               | 61.2   | Artificial soil: 74 % sand, 20 % kaolin clay, 5 % sphagnum peat, 1 % calcium carbonate, pH                             | Q     | R4/C4                   | Szabó <i>et al.</i> (2023) |



| Species<br>(Taxonomic<br>group) <sup>2</sup>  | Test substance         | Measured<br>effect <sup>3</sup>              | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source  |
|---|------------------------|--|----------|-------------------------------|---|------------------------|--|--|-------|-------------------------|---|
|   |                        |  |          |                               |   |                        |  | $6.025 \pm 0.116, 40 \% \text{ of }$ MWHC  |       |                         |   |
| Microorganisms                                | Azoxystrobin<br>250 SC | nitrogen<br>transformatio<br>n <sup>FE</sup> | n.r.     | NOEC                          | (2.5 kg a.s./ha)                                | n.r.                   | n.a.   | n.r.   | A     | (1)<br>R4/C2            | Anonymous in (EC 1998),<br>p.14 and (UK 2009), Vol.<br>3 B.9.8.2, p.757 |
| Microorganisms                                | Azoxystrobin<br>250 SC | carbon<br>transformatio<br>n <sup>FE</sup>   | n.r.     | NOEC                          | (2.5 kg a.s./ha)                                | n.r.                   | n.a.   | n.r.   | A     | (1)<br>R4/C2            | Anonymous in (EC 1998),<br>p.14 and (UK 2009), Vol.<br>3 B.9.8.2, p.757 |
| Organotrophic<br>bacteria<br>(Microorganisms) | Amistar 250 SC         | CFU <sup>DE</sup>                            | 30 d     | NOEC                          | ≥ 22.50   | 24.3<br>(14.3 %<br>OC) | ≥ 3.15   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015)  |
| Actinomycetes<br>(Microorganisms)             | Amistar 250 SC         | CFU <sup>DE</sup>                            | 30 d     | NOEC                          | ≥ 22.50   | 24.3<br>(14.3 %<br>OC) | ≥ 3.15   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015)  |
| Fungi<br>(Microorganisms)                     | Amistar 250 SC         | CFU <sup>DE</sup>                            | 30 d     | NOEC                          | ≥ 22.50   | 24.3<br>(14.3 %<br>OC) | ≥ 3.15   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015)  |
| Organotrophic<br>bacteria<br>(Microorganisms) | Amistar 250 SC         | $CD^{DE}$                                    | 30 d     | NOEC                          | < 0.075   | 24.3<br>(14.3 %<br>OC) | < 0.0105   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015)  |
| Actinomycetes<br>(Microorganisms)             | Amistar 250 SC         | CD <sup>DE</sup>                             | 30 d     | NOEC                          | ≥ 22.50   | 24.3<br>(14.3 %<br>OC) | ≥ 3.15   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015)  |
| Fungi<br>(Microorganisms)                     | Amistar 250 SC         | $CD^{DE}$                                    | 30 d     | NOEC                          | 2.250   | 24.3<br>(14.3 %<br>OC) | 0.315  | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015)  |



| Species<br>(Taxonomic<br>group) <sup>2</sup>  | Test substance | Measured<br>effect <sup>3</sup>                   | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source                       |
|---|----------------|---|----------|-------------------------------|---|------------------------|--|--|-------|-------------------------|------------------------------|
| Organotrophic<br>bacteria<br>(Microorganisms) | Amistar 250 SC | EP <sup>DE</sup>                                  | 30 d     | NOEC                          | ≥ 22.50   | 24.3<br>(14.3 %<br>OC) | ≥ 3.15   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga et al. (2015)        |
| Actinomycetes<br>(Microorganisms)             | Amistar 250 SC | EP <sup>DE</sup>                                  | 30 d     | NOEC                          | 2.250   | 24.3<br>(14.3 %<br>OC) | 0.315  | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015) |
| Fungi<br>(Microorganisms)                     | Amistar 250 SC | EP <sup>DE</sup>                                  | 30 d     | NOEC                          | ≥ 22.50   | 24.3<br>(14.3 %<br>OC) | ≥ 3.15   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga et al. (2015)        |
| Microorganisms                                | Amistar 250 SC | dehydrogenas<br>e activity <sup>EE</sup>          | 30 d     | NOEC                          | ≥ 22.50   | 24.3<br>(14.3 %<br>OC) | ≥ 3.15   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga et al. (2015)        |
| Microorganisms                                | Amistar 250 SC | catalase<br>activity <sup>EE</sup>                | 30 d     | NOEC                          | 2.250   | 24.3<br>(14.3 %<br>OC) | 0.315  | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga et al. (2015)        |
| Microorganisms                                | Amistar 250 SC | urease<br>activity <sup>EE</sup>                  | 30 d     | NOEC                          | 0.075   | 24.3<br>(14.3 %<br>OC) | 0.0105   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015) |
| Microorganisms                                | Amistar 250 SC | acid<br>phosphatase<br>activity <sup>EE</sup>     | 30 d     | NOEC                          | 2.250   | 24.3<br>(14.3 %<br>OC) | 0.315  | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2015) |
| Microorganisms                                | Amistar 250 SC | alkaline<br>phosphatase<br>activity <sup>EE</sup> | 30 d     | NOEC                          | < 0.075   | 24.3<br>(14.3 %<br>OC) | < 0.0105   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga et al. (2015)        |



| Species<br>(Taxonomic<br>group) <sup>2</sup>  | Test substance                   | Measured<br>effect <sup>3</sup> | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source                        |
|---|----------------------------------|---------------------------------|----------|-------------------------------|---|------------------------|--|--|-------|-------------------------|-------------------------------|
| Organotrophic<br>bacteria<br>(Microorganisms) | Amistar 250 SC<br>(250 g a.s./L) | CFU <sup>DE</sup>               | 30 d     | NOEC                          | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2024)  |
| Actinomycetes<br>(Microorganisms)             | Amistar 250 SC<br>(250 g a.s./L) | CFU <sup>DE</sup>               | 30 d     | NOEC                          | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al</i> . (2024) |
| Fungi<br>(Microorganisms)                     | Amistar 250 SC<br>(250 g a.s./L) | CFU <sup>DE</sup>               | 30 d     | NOEC                          | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al</i> . (2024) |
| Organotrophic<br>bacteria<br>(Microorganisms) | Amistar 250 SC<br>(250 g a.s./L) | $CD^{DE}$                       | 30 d     | NOEC                          | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2024)  |
| Actinomycetes<br>(Microorganisms)             | Amistar 250 SC<br>(250 g a.s./L) | CD <sup>DE</sup>                | 30 d     | NOEC                          | ≥ 0.110   | 24.3<br>(14.3 %<br>OC) | ≥ 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al</i> . (2024) |
| Fungi<br>(Microorganisms)                     | Amistar 250 SC<br>(250 g a.s./L) | CDDE                            | 30 d     | NOEC                          | ≥ 0.110   | 24.3<br>(14.3 %<br>OC) | ≥ 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2024)  |
| Organotrophic<br>bacteria<br>(Microorganisms) | Amistar 250 SC<br>(250 g a.s./L) | EP <sup>DE</sup>                | 30 d     | NOEC                          | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga et al. (2024)         |
| Actinomycetes<br>(Microorganisms)             | Amistar 250 SC<br>(250 g a.s./L) | EP <sup>DE</sup>                | 30 d     | NOEC                          | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00 | K     | R4/C2                   | Baćmaga <i>et al.</i> (2024)  |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                                 | Measured<br>effect <sup>3</sup>                   | Duration | Type of effect concentration | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source                       |
|--|--|---|----------|------------------------------|---|------------------------|--|---|-------|-------------------------|------------------------------|
| Fungi<br>(Microorganisms)                    | Amistar 250 SC<br>(250 g a.s./L)               | EP <sup>DE</sup>                                  | 30 d     | NOEC                         | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00  | K     | R4/C2                   | Baćmaga et al. (2024)        |
| Microorganisms                               | Amistar 250 SC<br>(250 g a.s./L)               | dehydrogenas<br>e activity <sup>EE</sup>          | 30 d     | NOEC                         | ≥ 0.110   | 24.3<br>(14.3 %<br>OC) | ≥ 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00  | K     | R4/C2                   | Baćmaga <i>et al.</i> (2024) |
| Microorganisms                               | Amistar 250 SC<br>(250 g a.s./L)               | catalase<br>activity <sup>EE</sup>                | 30 d     | NOEC                         | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00  | K     | R4/C2                   | Baćmaga et al. (2024)        |
| Microorganisms                               | Amistar 250 SC<br>(250 g a.s./L)               | urease<br>activity <sup>EE</sup>                  | 30 d     | NOEC                         | ≥ 32.92   | 24.3<br>(14.3 %<br>OC) | ≥ 4.60   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00  | K     | R4/C2                   | Baćmaga et al. (2024)        |
| Microorganisms                               | Amistar 250 SC<br>(250 g a.s./L)               | acid<br>phosphatase<br>activity <sup>EE</sup>     | 30 d     | NOEC                         | ≥ 0.110   | 24.3<br>(14.3 %<br>OC) | ≥ 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00  | K     | R4/C2                   | Baćmaga <i>et al.</i> (2024) |
| Microorganisms                               | Amistar 250 SC<br>(250 g a.s./L)               | alkaline<br>phosphatase<br>activity <sup>EE</sup> | 30 d     | NOEC                         | < 0.110   | 24.3<br>(14.3 %<br>OC) | < 0.0154   | Natural soil (Eutric<br>Cambisol, sandy loam):<br>69.41 % sand, 27.71 %<br>silt, 2.88 % clay, pH<br>7.00  | K     | R4/C2                   | Baćmaga <i>et al.</i> (2024) |
| Microorganisms                               | Azoxystrobin<br>formulation (not<br>specified) | microbial<br>biomass-N <sup>ECE</sup>             | 30 d     | NOEC                         | ≥ 5.0   | 2.01<br>(1.18 %<br>OC) | ≥ 8.47   | Natural soil (sandy loam,<br>Long Close,<br>conventionally<br>maintained field): 73 %<br>sand, 12 % silt, 14 %<br>clay, pH 6.5, 40 % of<br>MWHC | L     | R4/C2                   | Bending <i>et al.</i> (2007) |
| Microorganisms                               | Azoxystrobin formulation (not specified)       | microbial<br>biomass-N <sup>ECE</sup>             | 30 d     | NOEC                         | ≥ 5.0   | 2.75<br>(1.62 %<br>OC) | ≥ 6.17   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field): 73 %   | L     | R4/C2                   | Bending et al. (2007)        |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                                 | Measured<br>effect <sup>3</sup>   | Duration | Type of effect concentration | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil sand, 12 % silt, 14 %  | Notes | Assess<br>ment<br>score | Source                       |
|--|--|---|----------|------------------------------|---|------------------------|--|--|-------|-------------------------|------------------------------|
| Microorganisms                               | Azoxystrobin formulation (not specified)       | dehydrogenas<br>e activity <sup>EE</sup>  | 30 d     | NOEC                         | < 5.0   | 2.01<br>(1.18 %<br>OC) | < 8.47   | clay, pH 6.5, 40 % of<br>MWHC<br>Natural soil (sandy loam,<br>Long Close):<br>conventionally<br>maintained, 73 % sand,   | L     | R4/C2                   | Bending et al. (2007)        |
| Microorganisms                               | Azoxystrobin<br>formulation (not<br>specified) | dehydrogenas<br>e activity <sup>EE</sup>  | 30 d     | NOEC                         | ≥ 5.0   | 2.75<br>(1.62 %<br>OC) | ≥ 6.17   | 12 % silt, 14 % clay, pH<br>6.5, 40 % of MWHC<br>Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field): 73 %<br>sand, 12 % silt, 14 %<br>clay, pH 6.5, 40 % of<br>MWHC | L     | R4/C2                   | Bending <i>et al.</i> (2007) |
| Microorganisms                               | Azoxystrobin (purity not reported)             | dehydrogenas<br>e activity <sup>EE</sup>  | 1 month  | NOEC                         | < 25  | n.r.                   | n.a.   | Natural soil (sandy<br>loam): 74 % sand, 12%<br>silt, 14 % clay, pH 6.07,<br>40 % of MWHC  | Т     | R4/C2                   | Sopeña & Bending (2013)      |
| Soil fungi<br>(Microorganisms)               | Azoxystrobin<br>(purity not<br>reported)       | T-RFLP<br>(terminal<br>restriction<br>fragment<br>length<br>polymorphis<br>m) <sup>DE</sup> | 1 month  | NOEC                         | < 25  | n.r.                   | n.a.   | Natural soil (sandy loam): 74 % sand, 12% silt, 14 % clay, pH 6.07, 40 % of MWHC   | Т     | R4/C2                   | Sopeña & Bending (2013)      |
| Microorganisms                               | Azoxystrobin<br>(purity not<br>reported)       | dehydrogenas<br>e activity <sup>EE</sup>  | 1 month  | NOEC                         | < 1 (25 % effect)                               | n.r.                   | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC  | U     | R4/C2                   | Howell et al. (2014)         |
| Soil fungi<br>(Microorganisms)               | Azoxystrobin<br>(purity not<br>reported)       | overall<br>number (T-<br>RFLP based<br>on DNA) <sup>DE</sup>                                | 1 month  | NOEC                         | ≥ 25  | n.r.                   | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC  | U     | R4/C2                   | Howell <i>et al.</i> (2014)  |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                           | Measured<br>effect <sup>3</sup>   | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source                      |
|--|--|---|----------|-------------------------------|---|-----------------|--|---|-------|-------------------------|-----------------------------|
| Soil fungi<br>(Microorganisms)               | Azoxystrobin<br>(purity not<br>reported) | active<br>community<br>(T-RFLP<br>based on<br>RNA) <sup>DE</sup>        | 1 month  | NOEC                          | < 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell et al. (2014)        |
| Soil fungi<br>(Microorganisms)               | Azoxystrobin<br>(purity not<br>reported) | overall<br>number<br>(Shannon<br>index based<br>on DNA) <sup>DE</sup>   | 1 month  | NOEC                          | 10  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil fungi<br>(Microorganisms)               | Azoxystrobin<br>(purity not<br>reported) | active<br>community<br>(Shannon<br>index based<br>on RNA) <sup>DE</sup> | 1 month  | NOEC                          | < 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil nematodes                               | Azoxystrobin<br>(purity not<br>reported) | overall<br>number (T-<br>RFLP based<br>on DNA) <sup>DE</sup>            | 1 month  | NOEC                          | < 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell et al. (2014)        |
| Soil nematodes                               | Azoxystrobin<br>(purity not<br>reported) | active<br>community<br>(T-RFLP<br>based on<br>RNA) <sup>DE</sup>        | 1 month  | NOEC                          | < 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil nematodes                               | Azoxystrobin<br>(purity not<br>reported) | overall<br>number<br>(Shannon<br>index based<br>on DNA) <sup>DE</sup>   | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil nematodes                               | Azoxystrobin (purity not reported)       | active<br>community<br>(Shannon   | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,   | U     | R4/C2                   | Howell <i>et al.</i> (2014) |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                           | Measured<br>effect <sup>3</sup>   | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source                      |
|--|--|---|----------|-------------------------------|---|-----------------|--|---|-------|-------------------------|-----------------------------|
|  |  | index based<br>on RNA) <sup>DE</sup>                                    |          |                               |   |                 |  | 14.3 % clay, pH 6.5,<br>40 % of MWHC  |       |                         |                             |
| Soil bacteria<br>(Microorganisms)            | Azoxystrobin<br>(purity not<br>reported) | overall<br>number (T-<br>RFLP based<br>on DNA) <sup>DE</sup>            | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil bacteria<br>(Microorganisms)            | Azoxystrobin<br>(purity not<br>reported) | active<br>community<br>(T-RFLP<br>based on<br>RNA) <sup>DE</sup>        | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell et al. (2014)        |
| Soil bacteria<br>(Microorganisms)            | Azoxystrobin<br>(purity not<br>reported) | overall<br>number<br>(Shannon<br>index based<br>on DNA) <sup>DE</sup>   | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil bacteria<br>(Microorganisms)            | Azoxystrobin<br>(purity not<br>reported) | active<br>community<br>(Shannon<br>index based<br>on RNA) <sup>DE</sup> | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell et al. (2014)        |
| Soil archaea<br>(Microorganisms)             | Azoxystrobin<br>(purity not<br>reported) | T-RFLP <sup>DE</sup>  | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil archaea<br>(Microorganisms)             | Azoxystrobin<br>(purity not<br>reported) | overall<br>number<br>(Shannon<br>index based<br>on DNA) <sup>DE</sup>   | 1 month  | NOEC                          | ≥25   | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |



| Species<br>(Taxonomic<br>group) <sup>2</sup> | Test substance                           | Measured<br>effect <sup>3</sup>   | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source                      |
|--|--|---|----------|-------------------------------|---|-----------------|--|---|-------|-------------------------|-----------------------------|
| Soil archaea<br>(Microorganisms)             | Azoxystrobin<br>(purity not<br>reported) | active<br>community<br>(Shannon<br>index based<br>on RNA) <sup>DE</sup> | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil<br>pseudomonads<br>(Microorganisms)     | Azoxystrobin<br>(purity not<br>reported) | T-RFLP <sup>DE</sup>  | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil archaea<br>(Microorganisms)             | Azoxystrobin<br>(purity not<br>reported) | overall<br>number<br>(Shannon<br>index based<br>on DNA) <sup>DE</sup>   | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil archaea<br>(Microorganisms)             | Azoxystrobin<br>(purity not<br>reported) | active<br>community<br>(Shannon<br>index based<br>on RNA) <sup>DE</sup> | 1 month  | NOEC                          | ≥ 25  | n.r.            | n.a.   | Natural soil (sandy loam,<br>Hunts Mill, organically<br>maintained field):<br>73.4 % sand, 12.3 % silt,<br>14.3 % clay, pH 6.5,<br>40 % of MWHC | U     | R4/C2                   | Howell <i>et al.</i> (2014) |
| Soil bacteria<br>(Microorganisms             | Azoxystrobin<br>(99 % purity)            | CFU <sup>DE</sup>   | 28 d     | NOEC                          | ≥ 0.1 (< 1)                                     | 9.62            | $\geq$ 0.0353 (< 0.353)  | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt   | V     | R2/C2                   | Wang et al. (2018)          |
| Soil fungi<br>(Microorganisms                | Azoxystrobin<br>(99 % purity)            | CFU <sup>DE</sup>   | 28 d     | NOEC                          | ≥ 10  | 9.62            | ≥ 3.53   | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt   | V     | R2/C2                   | Wang et al. (2018)          |
| Soil<br>Actinomycetes<br>(Microorganisms     | Azoxystrobin<br>(99 % purity)            | CFU <sup>DE</sup>   | 28 d     | NOEC                          | ≥ 0.1 (< 1)                                     | 9.62            | ≥ 0.0353<br>(< 0.353)  | Natural soil (cambisol): 17.43 % clay, 44.62 % sand, 37.95 % silt   | V     | R2/C2                   | Wang et al. (2018)          |
| Microorganisms                               | Azoxystrobin<br>(99 % purity)            | soil<br>respiration <sup>FE</sup>                                       | 28 d     | NOEC                          | 0.1   | 9.62            | 0.0353   | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt   | V     | R4/C1                   | Wang et al. (2018)          |
| Microorganisms                               | Azoxystrobin<br>(99 % purity)            | urease<br>activity <sup>EE</sup>  | 28 d     | NOEC                          | ≥ 10  | 9.62            | ≥ 3.53   | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt   | V     | R2/C2                   | Wang et al. (2018)          |



| Species<br>(Taxonomic<br>group) <sup>2</sup>                | Test substance                | Measured<br>effect <sup>3</sup>                                       | Duration | Type of effect concentr ation | Effect<br>concentration<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source  |
|---|-------------------------------|---|----------|-------------------------------|---|-----------------|--|---|-------|-------------------------|---|
| Microorganisms  | Azoxystrobin<br>(99 % purity) | dehydrogena<br>se activity <sup>EE</sup>                              | 28 d     | NOEC                          | < 0.1   | 9.62            | < 0.0353   | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt | V     | R2/C2                   | Wang et al. (2018)  |
| Microorganisms  | Azoxystrobin (99 % purity)    | catalase<br>activity <sup>EE</sup>                                    | 28 d     | NOEC                          | < 0.1   | 9.62            | < 0.0353   | Natural soil (cambisol):<br>17.43 % clay, 44.62 %<br>sand, 37.95 % silt | V     | R2/C2                   | Wang et al. (2018)  |
| Microorganisms  | Azoxystrobin (99 % purity)    | protease<br>activity <sup>EE</sup>                                    | 28 d     | NOEC                          | ≥ 10  | 9.62            | ≥ 3.53   | Natural soil (cambisol): 17.43 % clay, 44.62 % sand, 37.95 % silt       | V     | R2/C2                   | Wang et al. (2018)  |
| Lactuca sativa <sup>D</sup><br>(Terrestrial<br>plant)       | Azoxystrobin (98.6 % purity)  | seedling<br>emergence,<br>mortality,<br>biomass (dry<br>shoot weight) | 18 d     | NOEC                          | ≥ 20  | [3]             | [≥ 22.7]   | n.r.  | J     | (1)<br>R2/C1            | Porch & Krüger (2002)<br>cited in (UK 2009), Vol. 3<br>B.9.9.1, p.758 |
| Raphanus<br>sativus <sup>D</sup><br>(Terrestrial<br>plant)  | Azoxystrobin (98.6 % purity)  | seedling<br>emergence,<br>mortality,<br>biomass (dry<br>shoot weight) | 18 d     | NOEC                          | ≥ 20  | [3]             | [≥ 22.7]   | n.r.  | J     | (1)<br>R2/C1            | Porch & Krüger (2002)<br>cited in (UK 2009), Vol. 3<br>B.9.9.1, p.758 |
| Triticum<br>aestivum <sup>M</sup><br>(Terrestrial<br>plant) | Azoxystrobin (98.6 % purity)  | seedling<br>emergence,<br>mortality,<br>biomass (dry<br>shoot weight) | 18 d     | NOEC                          | ≥ 20  | [3]             | [≥ 22.7]   | n.r.  | J     | (1)<br>R2/C1            | Porch & Krüger (2002)<br>cited in (UK 2009), Vol. 3<br>B.9.9.1, p.758 |

Table A2: Soil effect data for azoxystrobin from field studies. Abbreviations: n.r. – not reported; n.a. – not applicable; WHC – water holding capacity; OC – organic carbon; OM – organic matter; CFU – colony forming units. Values resulting from calculations are rounded to three significant figures.

| Species<br>(Taxonomic<br>group) | Test substance              | Measured<br>effect   | Duration | Type of<br>effect<br>concentrati<br>on | Effect value<br>[mg a.s./kg<br>soil]             | Total<br>OM [%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil  | Notes | Assess<br>ment<br>score | Source   |
|---------------------------------|-----------------------------|----------------------|----------|--|--|-----------------|--|--|-------|-------------------------|--|
| Soil macro- and microorganisms  | A 12705 A<br>(248 g a.s./L) | straw<br>degradation | 188 d    | NOEC                                   | 0.0514<br>(nominal<br>plateau) and<br>0.5 (750 g | n.r.            | n.a.   | Field study/natural soil<br>(Arbon, Switzerland) | G     | (1)<br>R3/C2            | Kollman (2004) cited in (UK 2009), Vol. 3 B.9.7.1, p.743 |



| Species<br>(Taxonomic<br>group) | Test substance | Measured<br>effect | Duration | Type of<br>effect<br>concentrati<br>on | Effect value<br>[mg a.s./kg<br>soil] | Total<br>OM [%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil | Notes | Assess<br>ment<br>score | Source |
|---------------------------------|----------------|--------------------|----------|--|--------------------------------------|-----------------|--|-----------|-------|-------------------------|--------|
|                                 |                |                    |          |  | a.s./ha annual<br>rate)              |                 |  |           |       |                         |        |

Notes A1: Notes on soil studies for azoxystrobin.

| _ | The distribution of the DAD State of the |
|---|--|
| A | The studies were listed in the old review report and the DAR without a study summary or any further details on the results (EC 1998, UK 2009).   |
| E | The study was conducted with an SC (suspension concentrate) product containing 251 g a.s./L (23.0 % w/w), according to the BBA VI, 2-2 guideline from 1994 (no reference provided).  |
|   | The test was conducted with 3 concentrations mixed into the soil.  |
|   | The study seems to have been conducted broadly in line with the OECD 222 guideline (OECD 2016a). The validity criteria were met.   |
| F | The study was conducted according to the ISO 11267:1999 guideline (ISO 1999) that is broadly in line with the current OECD 232 guideline (OECD 2016b).   |
|   | The OM/peat content of the soil was not summarised but it was noted that there was no deviation to the guideline. Thus, the guideline requirement of 10 % soil peat content is used as a surrogate of the soil OM content for normalising the results. The data derived in this way are shown in squared brackets.   |
|   | The details of the statistical evaluation was not provided but based on the results it could be confirmed.   |
| G | A litter-bag test was conducted to some guidelines that were not properly elaborated. The test item was applied at a nominal plateau concentration (0.0514 mg a.s./kg soil) on the soil surface and then incorporated in the upper 10 cm soil layer. 11 days later azoxystrobin was applied at 750 g a.s./kg soil rate (equivalent to 0.5 mg a.s./kg soil, calculated with 10 cm soil layer) but without incorporation. Another 10 days later litter bags containing 4 g straw were buried at a depth of 5 cm. Straw mass loss was assessed after 29, 61, 124 and 188 d later.   |
|   | The soil parameters and the time of the year of applications were not summarised (or reported), thus the results cannot be normalised to a standard organic matter content and put in a bigger context.  |
|   | The applied amounts of a.s. were confirmed by analytical measurements right after the applications sampling 10-cm soil cores. While the mean recovery values per plot were between 89 and 140 %, the individual measurement values showed much higher deviation between 50 and 150 %.  |
|   | The second measurement occurred 39 days before the first sampling for straw mass loss, and was not repeated later, thus the actual test item concentrations at the time of the straw samplings are not known. Without analytical confirmation, the results cannot be considered reliable for SGV derivation (for further explanation, please refer to Appendix 1).   |
| J | The seedling emergence test was conducted under GLP in accordance with the 1984 version of the OECD 208 guideline (OECD 2006).   |
|   | The three test concentrations – in exponential series – were mixed into the soil. The test duration was 14 days after 50 % of the control emerged (18 days altogether).  |
|   | There were no statistically significant effects for any of the species for any of the measured effects (seed germination, seedling survival and shoot dry weight; Dunnett's test, significance level of 0.05). At the highest test concentration (20 mg a.s./kg), 18-22 % increase occurred in lettuce, 5 % increase and 13 % decrease in radish, as well as 29 % decrease in wheat. Based on the summarised mean and SD values, the statistical results could be affirmed.  |
|   | No deviation to the guideline was noted in the study summary; however, the soil properties were not summarised. The old version of the OECD 208 guideline requires that the carbon content of the test soil not exceed 1.5 % (or 3 % organic matter). Using the maximum 3 % OM content, the normalised NOEC is $\geq$ 22.7 mg a.s./kg. Any lower OM values would result in higher effect concentrations. The test was conducted to GLP following a standard guideline; thus it is considered that the guideline requirements were met and the test soil did not contain more than 3 % OM. For the normalisation of the results the worst case scenario of 3 % OM is accepted and used. The data derived in this way are shown in squared brackets.   |



| K | The microorganism studies (Baćmaga <i>et al.</i> 2015, Baćmaga <i>et al.</i> 2024) with the same soil and methods were conducted using a natural soil sampled in northeast Poland. The history of the soil, i.e. previous pesticide use or contamination with other pollutants, was not included in the methods.   |
|---|--|
|   | The studies were not conducted in accordance with any standard guideline. No positive control was used and standard expected values for the negative control are not available, i.e. the sensitivity of the test is not known. The statistical power of the test is also not clear.  |
|   | The tests in Baćmaga <i>et al.</i> (2015) were conducted with four test concentrations and a control. The tests in Baćmaga <i>et al.</i> (2024) were conducted with a low and a high test concentration (0.110 and 32.92 mg a.s./kg soil) and a control. Due to the almost 300 times difference between the test concentrations, NOEC of 0.110 mg a.s./kg soil are recorded as $\geq$ values.  |
|   | According to the described methods, the plant tests in Baémaga <i>et al.</i> (2024) were conducted using the Phytotoxkit test. This is a 3-d long test with all the material, including the seeds and the soil, provided by the manufacturer. The species were selected for their known rapid seed germination and root growth (see <a href="https://www.microbiotests.com/toxkit/phytotoxicity-test-with-phytotoxkit-solid-samples/">https://www.microbiotests.com/toxkit/phytotoxicity-test-with-phytotoxkit-solid-samples/</a> ). The test kit adheres to the standard ISO 18763:2016 method. It was controversially described in the study method (Baémaga <i>et al.</i> 2024) that shoot and root lengths were measured after 72-h incubation but also that the plant growth and development were assessed on day 30, 60 and 90. The amount of test soil provided in the kit (110 g) along with the thin growing plates would not be suitable continuing the test for 30/60/90 days. Also, no results for the controls were reported, only the changes in the treatments relative to the control. As a result, it remained unclear if there were statistically significant effects as compared to the control and if the validity criteria to the ISO standard were met. Due to these uncertainties, the plant results are not suitable for inclusion in Table A1 and for any further consideration in the SGV. |
|   | As a consequence of the expected degradation of azoxystrobin in soil (without analytical verification) and that recovery of effects is not accepted for SGV, only the results after 30 days can be considered here. The bare minimum of 3 replicates were used in both studies, but the deviations to the mean values were not reported/shown thus the reliability of the statistical evaluation and results cannot be considered.   |
|   | Due to the above noted missing information and uncertainties, the reliability of the study results is considered as not assignable (R4).   |
| L | The study on soil microbial communities did not follow a standard guideline. No positive control was used, the sensitivity of the test system is not known. A single test concentration was used (limit test). Only the mean values of four replicates were reported without individual values or standard deviation. The statistically non-significant data were not shown.   |
|   | Due to the above noted missing information and uncertainties, the reliability of the study results is considered as not assignable (R4).   |
| M | The study was conducted in line with the OECD 220 (OECD 2016c) guideline that was originally developed for <i>Enchytraeus albidus</i> . The test duration for both adult mortality and reproduction was 21 d, which is in line with the common practice for the tested <i>E. crypticus</i> due to its smaller size and shorter generation time (Castro-Ferreira <i>et al.</i> 2012, OECD 2010).  |
|   | The robustness of the estimated LC/EC10 values could not be fully evaluated in the absence of the LC/EC20 values and their confidence intervals (CI). The CI of the reproduction EC10 had a "poor" normalised width (NW), of the mortality LC10 a "fair" NW (see details in EFSA (2019)). The results were shown mostly graphically, thus the nonlinear regression and the ECx estimations could not be repeated/checked. The LCx/ECx values (with 95 % CI in brackets) were as follows:   |
|   | <ul> <li>21-d LC10 for adult mortality: 21 (15-30) mg a.s./kg soil</li> <li>21-d LC50 for adult mortality: 39 (34-45) mg a.s./kg soil</li> <li>21-d EC10 for reproduction: 17 (2-33) mg a.s./kg soil</li> <li>21-d EC50 for reproduction: 37 (25-49) mg a.s./kg soil</li> </ul>  |
|   | Although the spacing of the test concentrations were somewhat broader than recommended in the test guideline (factor of 3.1-3.2 instead of $\leq$ 1.8), the estimated EC10 values fell around the NOEC values and therefore the NOEC values are regarded as equal-to values for further consideration.   |
| N | Using Enchytraeus crypticus as test organism, a standard 21-d reproduction test (OECD 2010, 2016c) and a non-standard 19-d hatching test were conducted.   |
|   | The relevance of the hatching test results at the population level has not been established and the hatching success is included in the reproduction test, thus the hatching test results are scored as <i>not assignable</i> (C4) and not considered further in the SGV. It is noted that the NOEC for hatching rate is the same (75 mg a.s./kg soil) as for adult mortality and reproduction.  |
|   | The validity criteria of the reproduction test were met.   |
|   | The measured effects and the statistical evaluation were not reported in detail, so they could not be repeated. The reliability of the LC10/EC10 values could not be fully assessed as recommended in EFSA (2019) as the related LC20/EC20 values and their CIs were not reported; the reliability of these are scored as <i>not assignable</i> (R4). For the effect concentrations with 10 and 50 % effects the following median values and 95 % CIs (in brackets) were reported:   |
|   |  |



• 21-d LC10 for adult mortality: 132 (100-157) mg a.s./kg

• 21-d LC50 for adult mortality: ≥ 150 mg a.s./kg

21-d EC10 for reproduction: 57 (40-75) mg a.s./kg

• 21-d EC50 for reproduction: 93 (79-103) mg a.s./kg

• 19-d EC10 for hatching rate: 27 (14 – 55) mg a.s./kg

• 19-d EC50 for hatching rate: 104 (62–147) mg a.s./kg

The spacing of the test concentrations were somewhat broader than recommended in the test guideline (factor of 2-3 instead of  $\leq$  1.8). There was a factor of two difference between the highest and second highest test concentrations (only slightly above the recommendation), thus the NOEC values at the second highest test concentration are accepted as equal-to values.

In addition, an OM content of 1.73 % was reported for the standard LUFA 2.2 soil (Speyer, Germany) used in the test. From our historical data collection (see table A1-1 below) it seems that this value might have fitted more likely in the OC content of the LUFA 2.2 soil parameters. Due to this uncertainty, the results are scored here as *not assignable* (R4). It is noted that even if the normalised effect concentrations were accepted, they would be not critical for the SGV derivation (there are lower values for *Enchytraeus*).

Table A1-1: Our historical data for standard LUFA 2.2 soil (Speyer, Germany) – in chronological order

|   |   | * -         | Ŭ      |                 |                                  |   |
|---|---|-------------|--------|-----------------|----------------------------------|---|
| SGV Dossier                                       | Source of data  | Year        | OM [%] | OC [%]          | Test for which the soil was used | Study used for                                |
| Pendimethalin (Lauber <i>et al.</i> 2024b)        | dRAR (EC 2021), EFSA conclusion (EFSA 2016)                           | 2001        | (3.69) | 2.17            | Adsorption                       | Müller (2001a)                                |
| Pendimethalin<br>(Lauber <i>et al.</i><br>2024b)  | dRAR (EC 2015b), Vol. 3CP (AG)  | 2008        | (3.62) | 2.16            | Allium cepa                      | Fiebig (2008)                                 |
| NA  | LUFA Speyer website   | 2015        | (2.70) | $1.59 \pm 0.13$ | NA                               | NA  |
| Azoxystrobin                                      | Kovačević (2021) – referencing LUFA 2.2 soil with detailed properties | 2021 (2020) | 1.73   | (1.02)          | Enchytraeus                      | Kovačević (2021)                              |
| Difenoconazole<br>(Lauber <i>et al.</i><br>2024a) | LUFA Speyer website   | 2022        | (3.57) | 2.1             | Collembola                       | Pitombeira de Figueirêdo <i>et al.</i> (2019) |
| Azoxystrobin                                      | Kovačević (2023a) – referencing LUFA 2.2 soil in general              | 2023 (2022) | ?      | ?               | Collembola                       | Kovačević (2023a) – see Note S below          |
| Fluazinam<br>(Lauber <i>et al.</i><br>2025)       | Wehrli et al. (2024)  | 2024 (2023) | (2.82) | $1.66 \pm 0.60$ | Collembola                       | Wehrli et al. (2024)                          |
| NA  | LUFA Speyer website   | 2025 (2024) | (3.08) | $1.81 \pm 0.44$ | NA                               | NA  |

Notes:

0

OC - organic carbon content; OM - organic matter content; numbers without brackets were reported, numbers in brackets were calculated assuming a factor of 1.7 between the OM and OC content; NA - not applicable.

The parameters of the standard soils get updated each year and previous years' results are not available on the LUFA Speyer company website (<a href="https://www.lufa-speyer.de/standardboeden-bestellen">https://www.lufa-speyer.de/standardboeden-bestellen</a>). Questionable values are in red (also see Note S below).

Using *Enchytraeus albidus* as test organism, a 7-d non-standard range-finding test (instead of 14-d) and a standard 42-d reproduction test (OECD 2016c) were conducted. It was reported that tested formulation, Quadris (Syngenta) contained 25 % azoxystrobin as well as the following co-formulants:

• 1,2-benzisothazol-3(2H)-one (0.025–0.05 %)



- naphthalene and alkyl naphthalene sulfonic acid formaldehyde condensate
- sodium salts  $(1 \le 10\%)$

The 21-d adult mortality results of the definitive test were not reported, only the 7-d adult mortality results from the range-finding test. The 7-d results are short-term results (in between the acute and long-term results) and as such are not considered relevant amongst the chronic data.

The reliability of the LC10/EC10 values could not be fully assessed as recommended in EFSA (2019) as the related LC20/EC20 values and their CIs were not reported; the reliability of these are scored as *not assignable* (R4). For the effect concentrations with 10 and 50 % effects, the following median values and 95 % CIs (in brackets) were reported:

- 7-d LC10 for adult mortality with the a.s.: 11.95 (11.21–12.70) mg a.s./kg
- 7-d LC50 for adult mortality with the a.s.: 16.76 (16.51–17.01) mg a.s./kg
- 42-d EC10 for reproduction with the a.s.: > 8 mg a.s./kg
- 42-d EC50 for reproduction with the a.s.: > 8 mg a.s./kg
- 7-d LC10 for adult mortality with the formulation: 10.65 (10.14–11.17) mg a.s./kg
- 7-d LC50 for adult mortality with the formulation: 15.29 (14.88–15.71) mg a.s./kg
- 42-d EC10 for reproduction with the formulation: 1.23 (0.40–2.06) mg a.s./kg
- 42-d EC50 for reproduction with the formulation: 2.94 (2.41–3.73) mg a.s./kg

The tested concentrations were 0 (control), 0.085, 0.17, 1.45, 2.7, 4 and 8 mg a.i./kg soil in the definitive test with spacing factors of 2, 8.5, 1.9, 1.5 and 2, respectively. Due to the broad spacing between the test concentrations of 0.17 and 1.45, the reproduction NOEC of 0.17 with the formulation is considered as a greater-than/equal-to value. The reason for the high spacing and the missing in-between concentrations are not clear. There are also high deviations at the two lowest test concentrations. Considering all of these uncertainties, the reliability of the reproduction NOEC tested with the formulation is *not assignable* (R4).

P Standard tests with Eisenia andrei (ISO 1998), Enchytraeus crypticus (ISO 2004) and Folsomia candida (ISO 1999) were conducted using natural soil.

12 concentrations (10, 15, 20, 35, 50, 80, 120, 200, 300, 450, 650, 1000 mg a.s./kg) with 2 replicates were applied in the potworm and the collembolan treatments and 4 replicates in the controls. In the earthworm test 5 test concentrations (50, 100, 200, 300, and 500 mg a.s./kg) and a control were used, all with 4 replicates. In addition to the natural soil controls (for comparison of the treatments in the test), artificial soil controls were also used (for comparing the performance of the animals in the control).

Adult mortality was measured in all tests but was only reported for eathworms.

Concerning reproduction, instead of NOEC/EC10 values, only EC20 and EC50 values were reported with the following CIs (in brackets):

- Earthworm 56-d EC20 for reproduction: 12.2 (1.2–23.1) mg a.s./kg
- Earthworm 56-d EC50 for reproduction: 42.0 (23.2–60.8) mg a.s./kg
- Potworm 28-d EC20 for reproduction: 42.6 (25.2–60.0) mg a.s./kg
- Potworm 28-d EC50 for reproduction: 99.2 (73.3–125.7) mg a.s./kg
- Collembola 28-d EC20 for reproduction: 54.9 (23.0–86.9) mg a.s./kg
- Collembola 28-d EC50 for reproduction: 92.0 (57.9–126.1) mg a.s./kg

The EC20 and EC50 values for earthworms are extrapolations out of the range of the tested concentrations and thus statistically not robust and as a result they are scored as *not reliable* (R3). Both the biomass and the reproduction NOEC for earthworms were reported as lower than the lowest tested concentration. It is unclear why NOEC values were not reported for potworms and collembolans.

Q Reproduction and avoidance tests with *Folsomia candida* were conducted on artificial soil. The food choice test was conducted on filter paper and as such not relevant here.

It was reported that the reproduction test parameters followed the standard OECD guideline (OECD 2016b). However, the test concentrations were 0, 0.003, 0.03, 3.0, 90 and 300 mg a.s./kg soil with spacing factors of 10, 100, 10, 3 and 3.3, respectively, instead of the required factor of  $\le 1.8$ . The NOEC values are still considered *reliable with restrictions* (R2) as the spacing factor between the NOEC and LOEC values was 3. While this is still larger than the guideline recommendation, usually – especially if a standard guideline was not followed – a spacing factor up to 3 is accepted. Altogether this is considered as a minor deviation to the guideline.



|   | The LCx values are not reliable as no CI could be calculated due to the steepness of the fitted curve.   |
|---|--|
|   | In the absence of detailed results as well as an established relevance at population level, the avoidance test results are scored as not assignable (R4/C4).   |
| R | Reproduction test with <i>Folsomia candida</i> was conducted to the standard guidelines (ISO 2014a, OECD 2016b). The relevance of the additionally conducted biomarker test and its results have not been established at the population level and as such the results are not listed and not considered further in the SGV.  |
|   | The validity criteria were reported as met.  |
|   | The robustness of the estimated EC10 values could not be fully evaluated in the absence of the EC20 values and their CIs. For the effect concentrations with 10 and 50 % effects, the following median values and 95 % CIs (in brackets) were reported:  |
|   | <ul> <li>Reproduction EC10: 11.73 (5.04, 18.42) mg a.s./kg</li> <li>Reproduction EC50: 61.28 (48.05, 74.508) mg a.s./kg</li> </ul>   |
| S | Multigenerational reproduction test of Folsomia candida using using standard LUFA 2.2 soil (Speyer, Germany).  |
|   | Abbreviations for the generations: Parent – F0; 1 <sup>st</sup> generation – F1; 2 <sup>nd</sup> generation – F2; 3 <sup>rd</sup> generation – F3  |
|   | The specific parameters of the LUFA 2.2 soil, including the OC or OM content, were not reported. It can be seen from the historical data provided in Note N that the OC/OM content can change by years/batches, therefore a standard value cannot be assumed. As a result, no normalisation could be conducted and all endpoints/effect concentrations are scored as <i>not assignable</i> (R4).   |
|   | Also, the use of results from F1-F3 generations in the risk assessment is not established and therefore their relevance are scored as not assignable (C4).   |
|   | As recovery is not acceptable for SGV, the results of the transgenerational effects (i.e. effects on juveniles emerged from F1-F3 and placed on clean soil) are not considered here.   |
| T | A limit test with 25 mg a.s./kg was conducted on soil microorganisms. The purity of test substance was not reported; however, it is considered acceptable at ~100 % as the company who supplied azoxystrobin supplies analytical grade substances.   |
|   | After treatment, incubation occurred in darkness at 15°C. Only 1-month results with no biochar addition are considered relevant for the SGV.   |
|   | No organic carbon content of the soil was reported, only total carbon content, so the results cannot be normalised and thus their reliability is scored as not assignable (R4).  |
| U | A dose-response test (0, 1, 5, 10 and 25 mg a.s./kg) was conducted on soil microorganisms. The purity of test substance was not reported; however, it is considered acceptable at ~100 % as the company who supplied azoxystrobin supplies analytical grade substances.  |
|   | After treatment, incubation occurred in darkness at 15°C. Only 1-month results are considered relevant for the SGV.  |
|   | The organic carbon content of the soil was not measured or reported. The cited OC content measured for a previous study (Bending <i>et al.</i> 2007) was based on samples taken from the same field 5 years earlier than in this study and as such could be different. As a consequence the results cannot be normalised and the reliability is scored as <i>not assignable</i> (R4).  |
| V | A dose-response test (0, 0.1, 1 and 10 mg a.s./kg) was conducted on soil microorganisms. No detailed history was provided for the fields where the soil was sampled; although, it was noted that no pesticides had been applied in the area.   |
|   | No standard guideline was followed. All parameters were measured after 7, 14, 21 and 28 days. Considering the minimum 28-d duration of the standard nitrogen and carbon transformation tests (OECD 2000a, 2000b), results after 28 d are listed here. Also, in line with the regulatory principles for prospective risk assessment, any kind of effects (increase or decrease) are considered relevant.  |
|   | It seems, for the soil respiration test no replicates were included – replication is not mentioned in the text and no standard deviation is shown for the results. Also, the method is very briefly described and it remained unclear how comparable the results are to results from other studies that followed the standard guideline. The fulfilment of the standard guideline validity criterion (i.e. if the coefficient of variation in the control was < 15 %) cannot be checked. Due to these uncertainties, the respiration results are scored as <i>not assignable</i> (R4). |



|   | While the enzymatic and diversity endpoints are not the preferred ones, they can provide a good indication of effects (or lack of them); both relevance and reliability of these endpoints are considered <i>acceptable with restrictions</i> (C2/R2). Due to the wide spacing (factor of 10), the NOEC values are recorded as unbound values and the respective interval (to the LOEC value) is indicated if possible.  |
|---|--|
| W | The 48-h filter paper test is not relevant and not included in the table. For the 14-d acute toxicity test neither the detailed results nor the dose-response curve were reported. It cannot be checked if the validity criterion was met. Altogether the reliability of the results are considered as <i>not assignable</i> (R4).   |
| X | The purity of the test substance was not reported. The filter paper test is not relevant and not included in the table. For the 14-d acute toxicity test the tested concentrations, the detailed results or the dose-response curves were not reported. Although the degradation of residues were measured in soil, no degradation rate or DT50 was reported. For the chronic toxicity test only biomarkers (antioxidant enzyme activity, oxidative damage) were measured that are not considered relevant for earthworms in the absence of an established link between the measured effects and the effects at population level. Thus the biomarker results are not included in the table. Considering the above detailed uncertainties, the reliability of the results are considered as <i>not assignable</i> (R4). |

It is noted that the following studies were considered potentially relevant but did not report enough details of the methods and/or the results to be able to derive a reliable quantitative endpoint (reliability *not assignable*, CRED score R4; not included in the tables above, in alphabetical order):

- (Adetutu et al. 2008): Azoxystrobin and soil interactions: Degradation and impact on soil bacterial and fungal communities
- (Álvarez-Martín *et al.* 2016): Changes in activity and structure of the soil microbial community after application of azoxystrobin or pirimicarb and an organic amendment to an agricultural soil
- (Bao et al. 2024): The combined effects of azoxystrobin and different aged polyethylene microplastics on earthworms (Eisenia fetida): A systematic evaluation based on oxidative damage and intestinal function
- (Bending *et al.* 2006): Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties
- (Guo et al. 2015): Enzymatic activities and microbial biomass in black soil as affected by azoxystrobin
- (Han *et al.* 2020): Azoxystrobin dissipation and its effect on soil microbial community structure and function in the presence of chlorothalonil, chlortetracycline and ciprofloxacin
- (Lu *et al.* 2023): Characterization of the responses of soil micro-organisms to azoxystrobin and the residue dynamics of azoxystrobin in wheat–corn rotation fields over two years
- (Revesz et al. 2022): Testing the effects of pesticides with Enchytraeus albidus avoidance test
- (Sim et al. 2022): Pesticide effects on nitrogen cycle related microbial functions and community composition
- (Wang et al. 2015): Effects of azoxystrobin on soil micro-organisms and enzymatic activities
- (Wang et al. 2020): Fungicide azoxystrobin induced changes on the soil microbiome
- (Wang et al. 2024): Soil microeukaryotic communities and phosphorus-cycling microorganisms respond to chloropicrin fumigation and azoxystrobin application
- (Yamaguchi et al. 2021): Non-target impact of dinotefuran and azoxystrobin on soil bacterial community and nitrification



# Appendix 3 Data on the metabolites

Table A3: Soil effect data for R234886, a transformation product of azoxystrobin. Values resulting from calculations are shown with three significant figures. The lowest effect datum per organism is shown in bold. Unreliable, not relevant and not assignable data are greyed out. Abbreviations: MWHC – maximum water holding capacity; OM – organic matter. For notes, please refer to the end of Appendix 3 (Notes A2).

| Species<br>(Taxonomic<br>group)         | Measured effect <sup>4</sup>             | Duration | Type of effect concentr ation | Effect value<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Note<br>s | Assess<br>ment<br>score | Source   |
|---|--|----------|-------------------------------|--------------------------------------|------------------------|--|---|-----------|-------------------------|--|
| Eisenia fetida<br>andrei<br>(Earthworm) | adult mortality                          | 14 d     | NOEC                          | ≥ 1000                               | 10                     | ≥ 340  | Artificial soil: 10 % sphagnum peat, pH 5.6-5.7, approx. 66.1 % of MWHC       | В         | (1)<br>R4/C2            | Friedrich (2002) cited in (UK 2009), Vol. 3 B.9.6.1, p.730 |
| Eisenia fetida<br>andrei<br>(Earthworm) | adult mortality                          | 14 d     | LC50                          | > 1000                               | 10                     | > 340  | Artificial soil: 10 % sphagnum peat, pH 5.6-5.7, approx. 66.1 % of MWHC       | В         | (1)<br>R4/C2            | Friedrich (2002) cited in (UK 2009), Vol. 3 B.9.6.1, p.730 |
| Eisenia fetida<br>andrei<br>(Earthworm) | biomass (adult<br>weight change)         | 14 d     | NOEC                          | 320                                  | 10                     | 109  | Artificial soil: 10 % sphagnum peat, pH 5.6-5.7, approx. 66.1 % of MWHC       | В         | (1)<br>R4/C2            | Friedrich (2002) cited in (UK 2009), Vol. 3 B.9.6.1, p.730 |
| Microorganisms                          | nitrogen<br>transformation <sup>FE</sup> | 28 d     | NOEC                          | 1 and 10                             | 1.38<br>(0.81 %<br>OC) | 2.47 and 24.7  | Natural soil (Braunschweig,<br>DE): 6 % clay, 44 % silt, 50 %<br>sand, pH 6.6 | Н         | (1)<br>R4/C1            | Lemnitzer (2002) cited in (UK 2009), Vol. 3 B.9.8.1, p.749 |
| Microorganisms                          | carbon<br>transformation <sup>FE</sup>   | 28 d     | NOEC                          | 1 and 10                             | 1.38<br>(0.81 %<br>OC) | 2.47 and 24.7  | Natural soil (Braunschweig,<br>DE): 6 % clay, 44 % silt, 50 %<br>sand, pH 6.6 | Н         | (1)<br>R4/C2            | Lemnitzer (2002) cited in (UK 2009), Vol. 3 B.9.8.1, p.749 |

Table A4: Soil effect data for R401553 (a.k.a. SYN501657), a transformation product of azoxystrobin. Values resulting from calculations are shown with three significant figures. The lowest effect datum per organism is shown in bold. Unreliable, not relevant and not assignable data are greyed out. Abbreviations: MWHC – maximum water holding capacity; OC – organic carbon; OM – organic matter. For notes to the studies, please refer to the end of Appendix 3 (Notes A2).

| Species<br>(Taxonomic<br>group)         | Measured effect <sup>4</sup> | Duration | Type of effect concentr ation | Effect value<br>[mg a.s./kg<br>soil] | Total OM<br>[%] | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil                                       | Notes | Assess<br>ment<br>score | Source  |
|---|------------------------------|----------|-------------------------------|--------------------------------------|-----------------|--|---|-------|-------------------------|---|
| Eisenia fetida<br>andrei<br>(Earthworm) | adult mortality              | 14 d     | NOEC                          | ≥ 1000                               | 10              | ≥ 340  | Artificial soil: 10 % sphagnum peat, pH 5.8-5.9 | С     | 1<br>(R2/C2)            | Friedrich (2008a) cited in (UK 2009), Vol. 3 B.9.6.1, p.731 |

<sup>&</sup>lt;sup>4 DE</sup> – diversity endpoint, <sup>EE</sup> – enzymatic endpoint, <sup>FE</sup> – functional endpoint

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| Species<br>(Taxonomic<br>group)         | Measured effect <sup>4</sup>             | Duration | Type of effect concentr ation | Effect value<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source  |
|---|--|----------|-------------------------------|--------------------------------------|------------------------|--|---|-------|-------------------------|---|
| Eisenia fetida<br>andrei<br>(Earthworm) | adult mortality                          | 14 d     | LC50                          | > 1000                               | 10                     | > 340  | Artificial soil: 10 % sphagnum peat, pH 5.8-5.9   | С     | 1<br>(R2/C2)            | Friedrich (2008a) cited in (UK 2009), Vol. 3 B.9.6.1, p.731 |
| Eisenia fetida<br>andrei<br>(Earthworm) | biomass (adult<br>weight change)         | 14 d     | NOEC                          | ≥ 1000                               | 10                     | ≥ 340  | Artificial soil: 10 % sphagnum peat, pH 5.8-5.9   | С     | 1<br>(R2/C2)            | Friedrich (2008a) cited in (UK 2009), Vol. 3 B.9.6.1, p.731 |
| Microorganisms                          | nitrogen<br>transformation <sup>FE</sup> | 28 d     | NOEC                          | 0.528 and<br>2.643                   | 2.43<br>(1.43 %<br>OC) | 0.739 and 3.70   | Natural soil (Wassergut<br>Canitz): 10.1 % clay, 38.0 %<br>silt, 51.9 % sand, pH 6.5, 45 %<br>of MWHC | I     | (1)<br>R4/C1            | Schulz (2008) cited in (UK 2009), Vol. 3 B.9.8.1, p.752     |
| Microorganisms                          | carbon<br>transformation <sup>FE</sup>   | 28 d     | < 25 %<br>effect              | 0.528 and<br>2.643                   | 2.43<br>(1.43 %<br>OC) | 0.739 and 3.70   | Natural soil (Wassergut<br>Canitz): 10.1 % clay, 38.0 %<br>silt, 51.9 % sand, pH 6.5, 45 %<br>of MWHC | I     | (1)<br>R4/C2            | Schulz (2008) cited in (UK 2009), Vol. 3 B.9.8.1, p.752     |

Table A5: Soil effect data for R402173 (a.k.a. SYN501114), a transformation product of azoxystrobin. Values resulting from calculations are shown with three significant figures. The lowest effect datum per organism is shown in bold. Unreliable, not relevant and not assignable data are greyed out. Abbreviations: n.r. – not reported; n.a. – not applicable; MWHC – maximum water holding capacity; OC – organic carbon; OM – organic matter. For notes to the studies, please refer to the end of Appendix 3 (Notes A2).

| Species<br>(Taxonomic<br>group)         | Measured effect <sup>4</sup>             | Duration | Type of effect concentr ation | Effect value<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source  |
|---|--|----------|-------------------------------|--------------------------------------|------------------------|--|---|-------|-------------------------|---|
| Eisenia fetida<br>andrei<br>(Earthworm) | adult mortality                          | 14 d     | NOEC                          | ≥ 1000                               | n.r.                   | n.a.   | Artificial soil: pH 5.7-6.0   | D     | (1)<br>R4/C2            | Friedrich (2008b) cited in (UK 2009), Vol. 3 B.9.6.1, p.733 |
| Eisenia fetida<br>andrei<br>(Earthworm) | adult mortality                          | 14 d     | LC50                          | > 1000                               | n.r.                   | n.a.   | Artificial soil: pH 5.7-6.0   | D     | (1)<br>R4/C2            | Friedrich (2008b) cited in (UK 2009), Vol. 3 B.9.6.1, p.733 |
| Eisenia fetida<br>andrei<br>(Earthworm) | biomass (adult<br>weight change)         | 14 d     | NOEC                          | ≥ 1000                               | n.r.                   | n.a.   | Artificial soil: pH 5.7-6.0   | D     | (1)<br>R4/C2            | Friedrich (2008b) cited in (UK 2009), Vol. 3 B.9.6.1, p.733 |
| Microorganisms                          | nitrogen<br>transformation <sup>FE</sup> | 28 d     | < 25 %<br>effect              | 0.826 and<br>4.131                   | 2.43<br>(1.43 %<br>OC) | 1.16 and 5.78  | Natural soil (Wassergut<br>Canitz): 10.1 % clay, 38.0 %<br>silt, 51.9 % sand, pH 6.5, 45 %<br>of MWHC | I     | (1)<br>R4/C1            | Schulz (2000) cited in (UK 2009), Vol. 3 B.9.8.1, p.754     |



| Species<br>(Taxonomic<br>group) | Measured effect <sup>4</sup>           | Duration | Type of effect concentr ation | Effect value<br>[mg a.s./kg<br>soil] | Total OM<br>[%]        | Normalised<br>effect value<br>[mg a.s./kg<br>soil]<br>3.4 % OM | Test soil   | Notes | Assess<br>ment<br>score | Source  |
|---------------------------------|--|----------|-------------------------------|--------------------------------------|------------------------|--|---|-------|-------------------------|---|
| Microorganisms                  | carbon<br>transformation <sup>FE</sup> | 28 d     | < 25 %<br>effect              | 0.826 and<br>4.131                   | 2.43<br>(1.43 %<br>OC) | 1.16 and 5.78  | Natural soil (Wassergut<br>Canitz): 10.1 % clay, 38.0 %<br>silt, 51.9 % sand, pH 6.5, 45 %<br>of MWHC | I     | (1)<br>R4/C2            | Schulz (2000) cited in (UK 2009), Vol. 3 B.9.8.1, p.754 |

Notes A2: Notes on soil effect data for azoxystrobin metabolites.

| В | The study was very briefly summarised in UK (2009). The study was conducted to the OECD 207 guideline (OECD 1984) and the validity criterion was summarised as met. Also, a test with a reference substance was conducted and showed acceptable results.  |
|---|---|
|   | The derived endpoints were not well described. The LC50 was understandably > 1000 mg/kg soil as there was no mortality after 14 d. Thus, it is understood that the LOEC of 500 and NOEC of 320 mg/kg soil were agreed for the effects on biomass. In the study summary only a limit test result was summarised with statistically significant effect (16.4 % decrease) in adult weight change at 1000 mg/kg soil; although the summary said that it was a dose-response test with five concentrations (100, 180, 320, 500 and 1000 mg/kg soil). |
|   | The study was not summarised detailed enough to consider reliability (not assignable, R4).  |
| С | The study was very briefly summarised in UK (2009). The study was conducted to the OECD 207 guideline (OECD 1984) and the validity criterion was summarised as met. Also, a test with a reference substance was conducted and showed acceptable results.  |
|   | The test was conducted with five or six concentrations and there was no effect on mortality or body weight change.  |
| D | The study was very briefly summarised in UK (2009). The study was conducted to the OECD 207 guideline (OECD 1984) and the validity criterion was summarised as met. Also, a test with a reference substance was conducted and showed acceptable results. The OM/peat content of the test soil was not summarised.   |
|   | The test was conducted with six concentrations and there was no effect on mortality or body weight change.  |
|   | Due to the missing details in the study summary, the reliability of the results is not assignable (R4).   |
| Н | The study was conducted to the OECD 216 and 217 guidelines (OECD 2000a, 2000b).   |
|   | The validity of the nitrification test cannot be confirmed based on the summarised results (no deviation or coefficient of variation). For the carbon transformation test, the CV was summarised as < 15 % in the control throughout the test. According to the notifier's comment, "the coefficient of variation in the control were $\geq$ 15 %." – It is not clear for which test(s) this statement referred to.   |
|   | For the nitrogen transformation test, NH <sub>4</sub> -N and NO <sub>3</sub> -N contents were summarised after 7, 14 and 28 days in the test, but not the rates as required in the guideline. In the absence of the detailed results, the rates cannot be calculated and the test results are considered <i>not assignable</i> (R4).  |
|   | Due to the missing details on the methods and the results as well as the unclear statement in the study summary, the study results overall are considered as not assignable (R4).   |
| I | The study was conducted to the OECD 216 and 217 guidelines (OECD 2000a, 2000b).   |
|   | For the nitrogen transformation test, NO <sub>3</sub> -N content was summarised after 7, 14 and 28 days in the test, but not the rates as required in the guideline. In the absence of the detailed results, the rates cannot be calculated.  |
|   | Due to the very short study summary and missing details on the methods and results, the study results are considered as not assignable (R4).  |