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SGV – Proposal by the Ecotox Centre for:

Difenoconazole

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Authors

Eva Lauber*, Alena Tierbach*, Gilda Dell'Ambrogio, Mathieu Renaud, Marion Junghans, Swiss Centre for Applied Ecotoxicology

*Shared first authorship

Scientific Support

Stephan Jänsch and Karen Duis, ECT Oekotoxikologie GmbH, Böttgerstraße 2-14, 65439 Flörsheim/Main, Germany

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Contact

Eva Lauber: eva.lauber@oekotoxzentrum.ch
Marion Junghans: marion.junghans@oekotoxzentrum.ch

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*Shared first authorship

Oekotoxzentrum | Eawag | Überlandstrasse 133 | 8600 Dübendorf | Schweiz
T +41 (0)58 765 55 62 info@oekotoxzentrum.ch | www.oekotoxzentrum.ch

Centre Ecotox | EPFL-ENAC-IIE-GE | Station 2 | CH-1015 Lausanne | Suisse
T +41 (0)21 693 62 58 | info@centreecotox.ch | www.centreecotox.ch

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: https://www.ecotoxcentre.ch/projects/soil-ecotoxicology/monitoring-concept-for-plant-protection-products-in-soils?_ga=2.170121120.1893072167.1726132886-1891293576.1686657912.

Oekotoxzentrum | Eawag | Überlandstrasse 133 | 8600 Dübendorf | Schweiz
T +41 (0)58 765 55 62 info@oekotoxzentrum.ch | www.oekotoxzentrum.ch

Centre Ecotox | EPFL-ENAC-IIE-GE | Station 2 | CH-1015 Lausanne | Suisse
T +41 (0)21 693 62 58 | info@centreecotox.ch | www.centreecotox.ch

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 difenoconazole and applying the methodology described in the EU Technical Guidance Document on risk assessment (EC TGD 2003), with adaptations described in Marti-Roura *et al.* (2023), **a generic SGV** for difenoconazole of **42 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 Oekotoxzentrum 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 Difenoconazol 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 generischer SGV** für Difenoconazol von **42 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 difénoconazole 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** pour le difénoconazole de **42 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 difenoconazolo 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 per** il difenoconazolo di **42 µg a.s./kg di suolo (peso secco) per un suolo standard con il 3,4% di materia organica.**

Table of content

1 General information

Information on the substance difenoconazole in relation to the soil environment is presented in this chapter. Registration information and risk assessments referred to are as follows:

- *EC (2006): Draft Assessment Report (DAR) on the active substance difenoconazole prepared by the rapporteur Member State Sweden in the framework of Directive 91/414/EEC, December 2006.*
- *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.*
- *EFSA (2024): Answer to "Application for public access to documents 28 February 2024 Ref. No.: PAD 2024/028 (00011188)" Legal Affairs Services, Parma, 23 April 2024. Ref. LV/BL/mm (2024) - out-305348441 and Legal Affairs Services, Parma, 22 May 2024. Ref. LV/BL/mm (2024) – out-30729742.*
- *US EPA Environmental Fate and Effects Division (EFED) (2015): Difenoconazole: Preliminary problem formulation for environmental fate, ecological risk, endangered species, and drinking water exposure assessments in support of registration review.*[1]

Additional information, i.e. partial access to full study reports, was provided by EFSA under the EU regulation about public access to documents (PAD regulation, EC (2001)). In line with the PAD regulation, additional information accessed via EFSA (2024) that are not included in publicly available documents got redacted from the externally published version of the dossier and replaced by the abbreviation of *[CPIR]* (confidentially provided information, redacted).

1.1 Identity and physico-chemical properties

The substance difenoconazole is also commonly referred to by the producer`s development code number, CGA 169374. The active ingredient is produced at a minimum purity of ≥ 940 g/kg and contains toluene as a relevant impurity in technical material (maximum level of 0.5 %; EC 2019). The substance has two asymmetric carbon atoms, therefore four stereoisomeric forms are possible: (2R,4R), (2S,4S), (2S,4R) and (2R,4S) (Figure 1); the first two are trans, the last two are cis-isomers. The authorised standard isomer composition of difenoconazole is not available publicly in the registration dossier (EC 2019).

During the manufacturing procedure, no stereoselective processes occur (EC 2019). According to its European patent, difenoconazole is normally produced in a ratio of about 60:40 of the stereoisomers (i.e. cis:trans pairs), with a ratio of 1:1 between cis and trans racemates, that is 60:40 of [(2S,4R) and (2R,4S)] to [(2R,4R) and (2S,4S)]; and 1:1 of (2S,4R) to (2R,4S) and 1:1 of (2R,4R) to (2S,4S). As a result, in typical manufacture difenoconazole contains about 30 % (2S,4R), about 30 % (2R,4S), about 20 % (2R,4R) and about 20 % (2S,4S), see for example the product Score 250EC™ (EPO 2019). A low phytotoxicity version of difenoconazole with at least 40 % of (2R,4S) and at least 50 % (2S,4S)-isomers (by weight) as well as various other isomer combinations were patented (EPO 2019).

[1] US EPA document is included for checking the completion of the data that were submitted to the EU. Recently it has been revealed that some manufacturers did not hand in all the studies to EFSA that they handed in to EPA (Mie and Rudén, 2023).

Difenoconazole is a moderately water soluble (15 mg/L) chemical. It has a relatively low vapour pressure (3.32 x 10^{-08} Pa) and Henry's law constant (9.0 x 10^{-07} Pa·m^3/mol), which suggest that volatilisation is not expected to be a major route of dissipation from soil and water. The log Kow value is 4.36 (Table 1).

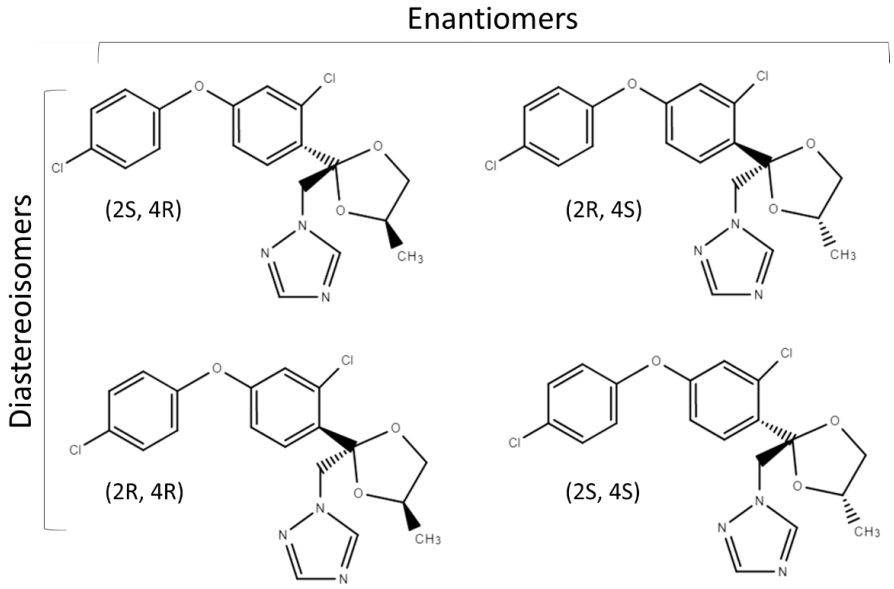
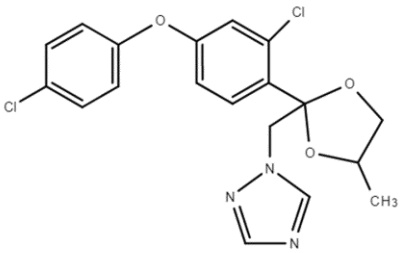


Figure 1: Four possible stereoisomeric forms of difenoconazole (EC 2019).

Table 1 summarises the identity and physico-chemical properties of difenoconazole. Where available, experimentally collected data is identified as (exp.) and calculated data as (cal.). When not identified, no indication is available in the cited literature.

Table 1: Identification and physico-chemical properties of difenoconazole. Abbreviations: exp. – experimental data; cal. – calculated value

Characteristics	Values	References
Common name	Difenoconazole	EC (2019)
Producer's development code number	CGA 169374	EC (2019)
IUPAC name	3-chloro-4-[(2RS,4RS;2RS,4SR)-4-methyl-2-(1H-1,2,4-triazol-1- ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether	EC (2019)
Chemical group	Triazole	EC (2019)
Structural formula		EC (2019)
Molecular formula	$C_{19}H_{17}Cl_2N_3O_3$	EFSA (2011)
CAS	119446-68-3	EC (2019)

EC Number	601-613-1	ECHA (2023)
SMILES code (canonical SMILES)	CC1COC(O1)(CN2C=NC=N2)C3=C(C=C(C=C3)OC4=CC=C(C=C4)Cl)Cl	Lewis (2016)
International Chemical Identifier key (InChIKey)	BQYJATMQXGBDHF-UHFFFAOYSA-N	Lewis (2016)
Molecular weight [g/mol]	406.3	EC (2019)
Melting point [°C]	82.0-83.0 (purity 99.3 %; exp., capillary method)	Das (1999a) cited in EC (2006) and EC (2019), Vol. 3CA B.2.1/01
Boiling point [°C]	Not relevant at atmospheric pressure as decomposition occurs. 100.8 at 3.7 mPa (purity 99.3 %; exp., Siwoloboff method)	Das (1997) cited in EC (2006), EFSA (2011) and EC (2019), Vol. 3CA B.2.1/02
Water solubility [mg/L]	15 ± 1.3, at pH 7.2, 25°C No pH effect is anticipated at environmentally relevant pH (purity 99.0 %; exp., shake flask method)	Stulz (1994) cited in EC (2006), EFSA (2011) and (EC 2019), Vol. 3CA B.2.5/01
Solubility in organic solvents [mg/L]	Acetone: > 500 000 Dichloromethane: > 500 000 Ethyl acetate: > 500 000 Hexane: 3000 Methanol: > 500 000 Octanol: 110 000 Toluene: > 500 000 (purity 94.6 %; exp., flask method)	Kettner (1999a) cited in EC (2006) and EC (2019), Vol. 3CA B.2.6/01
Dissociation constant (pKa)	1.07 At environmentally relevant pH, non-ionized form predominates (purity 99.3 %; exp., titration method)	Hörmann (1999) cited in EC (2006) and EC (2019), Vol. 3CA B.2.8/01
Volatilisation		
Vapour pressure [Pa]	3.32×10^{-08} at 25°C, low volatile (purity 99.0 %; exp., gas saturation method)	Rordorf (1988) cited in EC (2006) and (EC 2019), Vol. 3CA B.2.2/01
Henry's law constant [$Pa \cdot m^3 \cdot mol^{-1}$]	9.0×10^{-07} at 25°C (cal.)	Burkhard (1998) cited in EC (2006) and EC (2019), Vol. 3CA B.2.2/02
Partition/Adsorption		
Octanol-water partition coefficient (log Kow)	4.36, at pH 8, 25°C (purity 99.3 %; exp., shake flask method)	Kettner (1999b) cited in EC (2006) and (EC 2019), Vol. 3CA B.2.7/01
Organic carbon normalised Freundlich partitioning coefficient (Kfoc)	See section 1.5.3, Table 3	

1.2 Classification and environmental limit values

According to the previous legislation (Directive 1999/45/EC), the compound difenoconazole was classified as harmful (Xn, R22) and dangerous for the environment (N, R50/53; EFSA 2011). According to criteria of the CLP Regulation ((EC) No 1272/2008), the compound poses multiple health hazards (e.g. H302, H332, H318, H319) and environmental hazards (H400, H410). It is noted that no harmonised

classification is available just yet for difenoconazole, i.e. it is not listed in Annex VI to the CLP Regulation. The draft RAR proposes classification of H302, H319, H400 and H410 (EC 2019, Volume 1); while on the ECHA website for notified classification and labelling (self-classified by manufacturers, importers or downstream users), beyond the above listed most common hazard statement codes several other health hazards were notified (H226, H304, H315, H335, H336, H351, H371, H373; ECHA 2023). Due to its persistence and toxic properties, difenoconazole is included in the list of candidates for substitution (PSMV 2010, EC 2015). Up to date, no soil protection values for retrospective analysis could be found for difenoconazole. Please note that the information included here may have changed since the finalisation of this dossier.

1.3 Use and emissions

As a broad-spectrum triazole fungicide, difenoconazole is used to control fungal diseases on a wide variety of crops. In Switzerland, it is registered for use on plants or seeds of a number of fruits, vegetables, cereals, field crops and ornamental plants. This includes (but is not limited to): rye, wheat, rape, barley, oats, pome fruit, vines, fodder and sugar beet, cabbage, cucumbers, aubergines, beans, carrots, potatoes, garlic, onions, leeks, asparagus, tomatoes, celery, trees and shrubs, flower crops and ornamental plants such as roses. Furthermore, professional and non-professional users can apply difenoconazole in non-agricultural, landscaped areas. Difenoconazole can be formulated alone or in combination with other active ingredients as an emulsifiable concentrate, soluble concentrate, emulsion in water, flowable concentrate, or ready-to-use formulation. This fungicide is effective against multiple pests, such as leafspot, ring spot, early blight, barley stripe, covered smut damping-off and seed rots (BLV 2022).

1.4 Mode of action

Difenoconazole is a triazole fungicide, which is active against organisms belonging to the phyla Deuteromycota, Basidiomycota and Ascomycota. Similar to other triazoles (sterol demethylation inhibitors = DMIs), it acts by inhibiting the key enzyme, cytochrome P-450 sterol 14α-demethylase, (P-450_{14DM}), of the sterol biosynthetic pathway of fungi. The resulting effect on the C-14-demethylation of sterols leads to disruption of membrane morphology and function, and ultimately inhibits fungal growth (EC 2019).

As a chiral molecule, difenoconazole can exist in four stereoisomeric forms (2R,4R), (2S,4S), (2S,4R) and (2R,4S) (Figure 1). It was shown that the different stereoisomeric forms exhibit differences in bioactivity against target pests as well as in toxicity against non-target organisms. (2R,4S)-difenoconazole was identified as the stereoisomer with the highest bioactivity against four different target pests and as the stereoisomer with the lowest toxicity toward aquatic organisms (*Scenedesmus obliquus* (green algae), *Daphnia magna* (crustacean) and *Danio rerio* (fish)). On the other hand, (2S,4S)-difenoconazole was identified as the stereoisomer with the lowest bioactivity against pathogenic fungi and as the stereoisomer with the highest toxicity against aquatic organisms (Dong *et al.* 2013).

During the EU registration renewal, concerns were raised regarding the potential of difenoconazole to have endocrine disrupting (ED) properties in vertebrate species. In published scientific literature, there are some indications that difenoconazole is an aromatase inhibitor. Due to persisting data gaps regarding its ED potential, difenoconazole was listed as a substance "with insufficient data to conclude whether or not it is an endocrine disruptor" (EC 2007, 2004, INERIS 2013). Difenoconazole is also

included in the European Commission DG Environment study report on updating the priority list of low-tonnage endocrine disruptors (Petersen *et al.* 2007) and is classified as category 3b (substances with no or insufficient data gathered) for human health and wildlife (INERIS 2013). Studies submitted by the applicant for authorisation in the EU did not allow a clear conclusion that there were no endocrine disruptive effects on vertebrates (EFSA 2014). The ongoing EU renewal review of difenoconazole will provide an answer to that (EC 2019).

Apart from the missing conclusion, the current evaluation of ED properties is focussing 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 difenoconazole yielded no data on endocrine-relevant endpoints (status 02.2022).

The potential genotoxicity, carcinogenicity and reproductive toxicity of difenoconazole have been investigated. In a battery of *in vitro* and *in vivo* genotoxicity assays, no evidence for genotoxicity could be identified (EC 2019). Difenoconazole showed no evidence of carcinogenicity in rats, but in mice liver adenomas/carcinomas were observed in laboratory studies. However, as the carcinogenic response occurred only at high doses where toxicity was also observed, difenoconazole is considered unlikely to pose a carcinogenic risk (EC 2019).

1.5 Environmental fate in soil

Isomer-specific behaviour

The vast majority of data on the environmental fate and behaviour of difenoconazole is not resolved for each individual difenoconazole enantiomer or each diastereoisomer pair (EFSA 2011). However, one study investigated the stereoselective degradation of difenoconazole in the environment (Dong *et al.* 2013) and reported that the (2R,4S)-difenoconazole is preferentially degraded in the soil. Additionally, among the four stereoisomers no evidence of interconversion was observed (Dong *et al.* 2013). Nonetheless, due to limited data availability, stereoselective degradation and environmental fate remains a knowledge gap.

Volatilisation from soil surface

As difenoconazole has a low vapour pressure (3.32×10^{-08} Pa), the potential to volatilise from soil is expected to be low (EC 2019, US EPA 2015). This was confirmed in experiments, where volatiles were shown to be negligible (not exceeding 0.1 % of the applied radioactivity; EC 2019).

Photodegradation

The compound is stable to soil photolysis (EC 2019, US EPA 2015).

1.5.1 Route of degradation

Aerobic degradation in soil

In soil, difenoconazole degrades to a ketone (CGA 205374) which is subsequently transformed to the alcohol derivative (CGA 205375), the cleavage product 1,2,4-triazole (CGA 71019), carbon dioxide and to some minor compounds (US EPA 2015). Under laboratory conditions, the cleavage product 1,2,4-

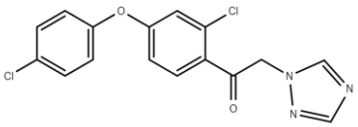
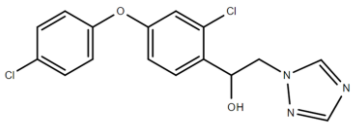
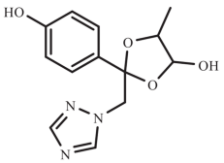
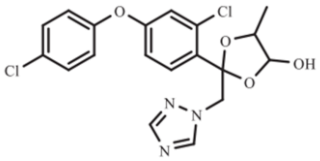
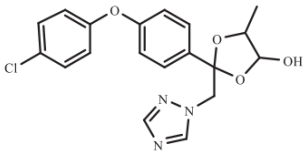
triazole (CGA 71019) was identified as the major difenoconazole transformation product, with a maximum formation of 7.9-23.4 % applied radioactivity (AR) after 190-271 days. (EFSA 2011). It needs to be highlighted that the cleavage product 1,2,4-triazole (CGA 71019) is not unique to difenoconazole, but is also a characteristic transformation product of other triazole fungicides, including propiconazole, myclobutanil, epoxiconazole and fenbuconazole.

In laboratory studies, the alcohol derivative (GCA 205375) reached a maximum of 4.4-16.0 % AR after 33-372 days, while in a radiolabelled terrestrial field study, it was detected at a maximum of 11.9 % AR, and thus was identified as another major soil metabolite (EFSA 2011).

One additional study investigated difenoconazole degradation pathways in different environmental compartments, including soil (Man *et al.* 2021). In the study three transformation products were newly identified, two of them forming directly from difenoconazole: TP295 through cleavage of the ether link between the two benzene rings, and TP421A *via* hydroxylation. The latter, with further de-chlorination, could degrade to TP387G.

Table 2 summarises the most important transformation products of difenoconazole in soil.

Table 2: Difenoconazole transformation products in soil.

Code/Trivial name	Chemical name	Structural formula	Reference
Difenoconazole-ketone CGA 205374	1-[2-chloro-4-(4-chlorophenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanone		(US EPA 2015), (EC 2019)
Difenoconazole-alcohol CGA 205375	2-chloro-4-(4-chloro-phenoxy)- benzoic acid		US EPA (2015), (EC 2019)
1,2,4-triazole CGA 71019	1H-1,2,4-triazole		US EPA (2015), (EC 2019)
TP295			Man *et al.* (2021)
TP421A			Man *et al.* (2021)
TP387G			Man *et al.* (2021)

Anaerobic degradation in soil

In soil, difenoconazole is stable under anaerobic conditions (EFSA 2011).

Mineralisation and non-extractable residues

Mineralisation (triazole and chlorophenyl ring radiolabels, respectively) to carbon dioxide was low and accounted for 0-2 % AR (after 90-100 days) and 4-19 % AR (after 90-120 days) (EFSA 2011). A major part of radioactivity was associated with the non-extractable fractions (EC 2019). The non-extractable fraction reached 8-37 % (after 90-100 days) and 7-34 % (after 90-120 days) of the total applied difenoconazole with labelled triazole and chlorophenyl rings, respectively (EFSA 2011). Those residues were strongly associated with organic matter fractions (i.e., fulvic, humic and humin; EC (2019), Vol. 3CA B.8.1.1.1.1) and their release is expected to be dependent on the turnover of organic fractions in soil.

1.5.2 **Rate of degradation**

Laboratory degradation studies

Under aerobic conditions, considering non-normalised DT50 values, laboratory degradation half-lives of difenoconazole ranged from 53 to 235 days (EFSA 2011) and, after the re-evaluation, from 51 to 191 days (EC 2019, LoEP), which classifies the substance as moderately persistent to persistent in soil.[2] The major metabolite 1,2,4-triazole exhibited low to moderate persistence (DT50 of 6-12 days) (EFSA 2011).

Field dissipation studies

The persistency of difenoconazole in soils from Germany and Switzerland was moderate to high with DissT50 values of 20-265 days (EFSA 2011) and 21-322 days after the re-evaluation (EC 2019, LoEP), which are consistent with the laboratory study results. The DT90 of difenoconazole exceeded one year with a maximum of 879 and > 1000 days (EFSA 2011, EC 2019, LoEP) and therefore further accumulation studies were conducted. These included a 5 and a 10-year long study in Switzerland, two 4-year long studies in northern Italy and a 3-year long one in the UK (EFSA 2011, EC 2019, LoEP). No accumulation was observed after up to 10 years of continuous use of difenoconazole in various crops.

Additional studies

Further studies were available from the scientific literature that were performed with different soil types under diverse conditions. The reported degradation half-life for difenoconazole ranged from nine to over 300 days (Muñoz-Leoz *et al.* 2013, Zhao *et al.* 2018, Zhang *et al.* 2021). It is assumed that specific microflora plays a critical role in the degradation of difenoconazole in soil (Man *et al.* 2021, Dong *et al.* 2013, Zhang *et al.* 2021). Additionally, a common carbon source, such as milled leaf powder, was shown to accelerate difenoconazole degradation in soil and reduce the half-life considerably (Thom *et al.* 1997). The discrepancies between the studies can result from differences in soil properties, microbial communities and pre-culture conditions (Man *et al.* 2021).

[2] Values extrapolated beyond the durations of the studies, derived from incubation times beyond 200 days or obtained from tests at 10/30ºC or under dry moisture conditions were not included.

1.5.3 **Adsorption/desorption properties and bioavailability**

Adsorption

Difenoconazole is immobile to moderately mobile in soil (EFSA 2011). The Freundlich adsorption (Kf) values for difenoconazole were found to be in the range of 2.1 to 202 (EC 2019) and 14.86 to 98.93 mL/g (Wang *et al.* 2020) for various soil types (Table 3) and are directly proportional to the soil organic carbon (OC) content, indicating a considerable influence of the OC content on difenoconazole adsorption. The Kfoc values are in the range of 400–11 202 mL/g (Table 3, EC (2019)).

Regarding the transformation products, 1,2,4-triazole exhibits high to very high mobility (Kfoc of 43-120 mL/g) and CGA 205375 has low mobility or is immobile in soil (Kfoc of 1464-6432 mL/g) (EFSA 2011). Due to the high affinity of difenoconazole for soil particles, there is a potential for particle-mediated transport (US EPA 2015).

Table 3: Soil adsorption of the active substance difenoconazole. Abbreviations: OC – organic carbon (in %); Kf – Freundlich soil-water distribution coefficients (in mL/g soil); Kfoc – organic carbon-normalised Freundlich distribution coefficients (in mL/g organic carbon); 1/n – Freundlich exponent.

Soil texture	OC [%]	Soil pH	Kf [mL/g]	Kfoc [mL/g]	1/n	Reference
sand	0.36	7.9	12.8	3870	0.74	Atkins (1991a) cited in EC (2019), Vol. 3CA B.8.1.3.1.1/02
sandy loam	1.98	7.8	63	3520	0.76	
silt loam	1.74	6.5	54.8	3470	0.85	
silty clay loam	0.67	6.9	47.2	7730	0.91	
clay	2.79	5.9	97.8	3470	0.89	Spare (1998) cited in EC (2019), Vol. 3CA B.8.1.3.1.1/03
sand	0.52	6.5	2.1	400	0.8	
silt loam	0.58	7.5	35	5660	0.88	
sandy loam	0.58	8.5	11.5	1960	0.94	
loamy sand	0.3	6.8	11.6	3870	0.80	Adam (2006a) cited in EC (2019), Vol. 3CA B.8.1.3.1.1/01
sandy loam	0.5	6.1	22.9	4587	0.84	
clay loam	3.8	7.9	182	4799	0.86	
sand	1.8	5.5	202	11 202	0.91	
silty loam	1.81	8.38	17.18		0.93	Wang *et al.* (2020)
clay loam	1.35	7.24	14.86		0.91	
silty clay loam	6.6	6.12	53.79		0.84	
silty loam	3.04	4.68	79.73		0.87	
sandy loam	3.6	4.06	98.93		0.87	
pH dependence		No				EC (2019), LoEP

Leaching

In terrestrial field dissipation studies, difenoconazole did not leach below 30 cm of soil depth (with one exception up to 60 cm in a potato production study) (MRID 46950129; US EPA 2015). One further study analysed the leaching potential of difenoconazole in sandy latosol (tropical soil) under field conditions during simulated rain events (Zhao *et al.* 2018). After the first rain event, the highest concentration of difenoconazole was detected at 10 cm and the amount of difenoconazole steadily declined up to a

depth of 30 cm. With increasing rain events, difenoconazole residues were partially transported further down to a depth of 30 cm (Zhao *et al.* 2018).

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).

In the case of difenoconazole, soil pH and texture do not seem to affect the adsorption of the compound to soil particles (EFSA 2011, EC 2019). For non-ionized organic compounds like difenoconazole (Table 1), it is assumed that bioavailability is mainly driven by the organic matter content of the soil (EC TGD 2003); therefore test results are normalised to organic matter content (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 K_{ow} greater than three can pose a risk of secondary poisoning to animals at higher trophic levels. Difenoconazole has a log Kow of 4.36 (Table 1), and thus there is a potential for bioaccumulation and biomagnification that should be considered in a separate assessment (as it is out of the scope of the current SGV derivation).

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 case of difenoconazole, the limit of quantification for the method (MLOQ) was determined as 0.2 ng a.s./g (corresponding to 0.0002 mg a.s./kg soil; Rösch *et al.* 2023).[3]

The soil guideline value that is derived in this dossier for difenoconazole 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

[3] Unless it is specified otherwise, active substance concentrations in soil are meant per soil dry weight.

resulted in difenoconazole concentrations between < 0.0002 mg a.s./kg soil (< MLOQ) and 0.043 mg a.s./kg soil (Rösch *et al.* 2023, Table S12).

For difenoconazole, the initial predicted environmental concentrations in soil (PECsoil) range from 0.091 to 0.148 mg a.s./kg soil; while the predicted plateau values resulting from accumulation after long-term use, from 0.022 to 0.082 mg a.s./kg soil for multiple applications, following the EU GAPs (Good Agricultural Practices, i.e. the proposed and evaluated representative uses of difenoconazole in the EU, max. 3 x 125 g a.s./ha/season; EC 2019). So the estimated overall PECsoil,accumulation values (PECsoil,initial + PECsoil,plateau) are 0.173 (apple/pear), 0.181 (tomato), 0.119 (carrot) and 0.024 (cereal seed treatment) mg a.s./kg soil.

3 Effect data on difenoconazole

Effect data for soil organisms were collected from studies retrieved from the European registration information (EC 2019). Additionally, a bibliographic search was performed for difenoconazole and its CAS number (CAS 119446-68-3) in the ECOTOX Knowledgebase (US EPA 2022) and in the database of the German Federal Environment Agency (UBA 2022). Furthermore, a search was performed on Scopus by using a combination of key words (Soil, EC50, LC50, NOEC, LOEC, LCx, ECx, toxicity and various soil organisms such as earthworm, Collembola or mite) and the compound's name or CAS number. 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 difenoconazole.

It is noted that the isomer composition of difenoconazole used in the studies is not known with very few exceptions (e.g. Dong *et al.* 2013). Measurement of and differentiation between the isomers are lacking throughout the draft renewal assessment report; the applied analytical methods do not allow the determination of the four isomers individually (EC 2019). In relation to that it is highlighted in the dossier that "*[d]epending on the outcome of the biological, toxicological and ecotoxicological assessment for the different isomers, methods for the determination of the isomers independently would be needed for the risk assessment methods"* (EC 2019). The potential toxicity differences of the isomers and their impact on the robustness of the derived SGV is discussed further in the uncertainty analysis (Section 7).

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 A4).

Since the bioavailability of non-ionized organic compounds, like difenoconazole, 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 Swiss Soil Monitoring Network). The normalisation has been performed according to the following equation:

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

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 ($CaCl_2$ 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 difenoconazole is affected by soil pH or clay content (EFSA 2011, EC 2019), studies outside the recommended range were not excluded from the dataset.

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.

In most cases regulatory studies and their endpoints are accepted without additional assessment (at face value) or partially 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 statistically most robust effect concentration was proposed/agreed upon as final endpoint. However, for 13 regulatory studies, where the results were inconsistent or not well summarised, full re-assessments were carried out using the original study reports that we got access to (EFSA 2024).

If more endpoints are available from the same study, the statistically more robust one is preferred. This means that the statistically more robust endpoint is being chosen even if that is higher than another one or it includes more than 10 % effect (precautionary criteria, 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 the NOEC is bearing, EC10 is preferred over the NOEC. Further details of the main criteria used for the study evaluations are included in Appendix 1.

Complete lists of laboratory and field studies reporting soil effect values for difenoconazole and its transformation products are shown in Appendix 2 (for difenoconazole, Table A1 with laboratory and Table A2 with field studies) and Appendix 3 (for the major soil metabolites, Table A3 and Table A4). 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). In Table 4 of the main text, all the reliable and relevant studies are summarised.

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 6 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 formulation.

Table 4: Difenoconazole – 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. The secondary endpoints for the same effects[4] are greyed out. Calculated data are rounded to three significant figures. Abbreviations: n.r. – not reported; n.a. – not applicable; cc. – concentration; WHC – water holding capacity; OC – organic carbon; OM – organic matter; CFU – colony forming units; [CPIR] – confidentially provided information, redacted. 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).

Species (Taxonomic group)[5]	Test substance	Measured effect[6]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida **(Earthworm)**	**Difenoconazole 250 EC (250 g/L a.s.)**	**reproduction**	**56 d**	**NOEC**	**2.10**	**10**	**0.714**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % quartz sand.**	**B**	**R2/C1**	**Servajean (2009) cited in EC (2019), Vol. 3CP, B.9.7.1.1/01, DIFCOR 250 EC, p.90**
Eisenia fetida **(Earthworm)**	**Difenoconazole (a.s.)**	**reproduction**	**56 d**	**EC10**	**0.632**	**5**	**0.430**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand**	**A**	**R1/C1**	**Taylor and Allen (2016g) re-calculating Friedrich (2011), cited in EC (2019), Vol. 3CA B.9.4.1/02, p.205**
Eisenia fetida **(Earthworm)**	**A9142L (30 g/L a.s.)**	**reproduction**	**56 d**	**EC10**	**1.16**	**5**	**0.789**	**[CPIR]**	**CC**	**R1/C1**	**Anonymous cited in EC (2019), Vol. 1, Level 2, List of endpoints, Difenoconazole, p.205; Anonymous (2016) accessed via EFSA (2024)**
		geomean					***0.623***				
Eisenia fetida (Earthworm)	Score 250 EC (250 g/L a.s.)	reproduction	56 d	NOEC	≥ 5.70	10	≥ 1.94	Artificial soil	AA, D	R2/C1	Friedrich (2006) cited in EC (2019), Vol. 3CP B.9.7.1.1/01, SCORE 250 EC (A7402T), p.102

[4] The less reliable / statistically less robust / less preferred endpoint (see e.g. EC10 vs NOEC) per test / measured effect / duration

[5] [M] – monocotyledonous, [D] – dicotyledonous plant species

[6] [DE] – diversity endpoint, [EE] – enzymatic endpoint, [FE] – functional endpoint

Proposed SGV for difenoconazole

Species (Taxonomic group)[5]	Test substance	Measured effect[6]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	reproduction	56 d	NOEC	1.00	5	0.680	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand	A	R2/C1	Friedrich (2011) cited in EC (2019), Vol. 3CA B.9.4.1/01, p.202
Eisenia fetida (Earthworm)	A9142L (30 g/L a.s.)	reproduction	56 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC	R1/C1	Anonymous (2016) accessed via EFSA (2024)
Eisenia fetida (Earthworm)	Score 250 EC (250 g/L a.s.)	adult mortality	28 d	NOEC	≥ 5.70	10	≥ 1.94	Artificial soil	AA, D	R2/C2	Friedrich (2006) cited in EC (2019), Vol. 3CP B.9.7.1.1/01, SCORE 250 EC (A7402T), p.102
Eisenia fetida (Earthworm)	Difenoconazole 250 EC (250 g/L a.s.)	adult mortality	28 d	NOEC	≥ 8.7	10	≥ 2.96	Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % quartz sand.	B, D	R2/C2	Servajean (2009) cited in EC (2019), Vol. 3CP, B.9.7.1.1/01, DIFCOR 250 EC, p.90
***Eisenia fetida* (Earthworm)**	**Difenoconazole (a.s.)**	**adult mortality**	**28 d**	**NOEC**	**≥ 8**	**5**	**≥ 5.44**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz**	**F, A, BB**	**1**	**Friedrich (2011), cited in EC (2019), Vol. 3CA B.9.4.1/02, p.205**
Eisenia fetida (Earthworm)	A9142L (30 g/L a.s.)	adult mortality	28 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC	R1/C2	Anonymous (2016) accessed via EFSA (2024)
Eisenia fetida (Earthworm)	Score 250 EC (250 g/L a.s.)	biomass change (adult growth)	28 d	NOEC	≥ 5.70	10	≥ 1.94	Artificial soil	AA, D	R2/C2	Friedrich (2006) cited in EC (2019), Vol. 3CP B.9.7.1.1/01, SCORE 250 EC (A7402T), p.102
***Eisenia fetida* (Earthworm)**	**Difenoconazole (a.s.)**	**biomass change (adult growth)**	**28 d**	**NOEC**	**2.00**	**5**	**1.36**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand**	**A**	**R1/C2**	**Friedrich (2011) cited in EC (2019), Vol. 3CA, B.9.4.1/01, p.202**
Eisenia fetida (Earthworm)	A9142L (30 g/L a.s.)	biomass change (adult growth)	28 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC	R1/C2	Anonymous (2016) accessed via EFSA (2024)

Species (Taxonomic group)[5]	Test substance	Measured effect[6]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	biomass change (adult growth)	28 d	EC10low[7]	0.24	5	0.163	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz	F, A	1	Taylor and Allen (2016g) re-calculating Friedrich (2011), cited in EC (2019), Vol. 3CA B.9.4.1/02, p.205
Earthworm	Difenoconazole 250 EC (250 g/L a.s.)	population abundance (total/species-specific number of adults/juveniles), field study	1 month	NOEC	≥ 0.317 (Appl. rate: 375 g a.s./ha)	[CPIR]	≥ 0.593	Field study/natural soil from Germany (silty loamy sand (DIN 4220) or loam (USDA)	CC, Z	R1/C1	Schulz (2015) cited in EC (2019), Vol. 3CP B.9.7.1.2/01, DIFCOR 250 EC, p.92; Anonymous (2015) accessed via EFSA (2024)
Earthworm	Difenoconazole 250 EC (250 g/L a.s.)	biomass (total/species-specific weight of adults/juveniles), field study	1 month	NOEC	≥ 0.317 (Appl. rate: 375 g a.s./ha)	[CPIR]	≥ 0.593	Field study/natural soil from Germany (silty loamy sand (DIN 4220) or loam (USDA)	CC, Z	R1/C1	Schulz (2015) cited in EC (2019), Vol. 3CP B.9.7.1.2/01, DIFCOR 250 EC, p.92; Anonymous (2015) accessed via EFSA (2024)
Enchytraeus crypticus **(Potworm)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**21 d**	**NOAEC**	**100**	**15**	**22.7**	**Natural soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively**	**BB**	**R2/C1**	**de Menezes Oliveira *et al.* (2018)**
Folsomia candida **(Collembola)**	**Difenoconazole 250 EC (250 g/L a.s.)**	**reproduction**	**28 d**	**NOEC**	**7.71**	**5**	**5.24**	**Artificial soil: 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 75 % industrial fine sand**	**E**	**R2/C1**	**Servajean (2015) cited in EC (2019), Vol. 3CP B.9.7.2.1/01, DIFCOR 250 EC, p.101**
Folsomia candida **(Collembola)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**28 d**	**EC10**	**33.5**	**5**	**22.8**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand**	**G, H**	**R1/C1**	**Friedrich (2015) cited in EC (2019), Vol. 3CA B.9.7.2.1/01, SCORE 250 EC (A7402T), p.119**

[7] EC10low is the lower limit of the 95 % confidence interval of the EC10

Species (Taxonomic group)[5]	Test substance	Measured effect[6]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
***Folsomia candida* (Collembola)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**28 d**	**EC10**	**23**	**3.57 (2.1 % TOC)**	**21.9**	**Natural soil: LUFA 2.2 (soil pHCaCl2: 5.5; water-holding capacity: 46.5%; LUFA-Speyer, Speyer, Germany)**	**H**	**R2/C1**	**Pitombeira de Figueirêdo *et al.* (2019)**
		geomean					***13.8***				
Folsomia candida (Collembola)	Score 250 EC (250 g/L a.s.)	reproduction	28 d	NOEC	10	3.57 (2.1 % TOC)	9.52	Natural soil: LUFA 2.2 (soil pHCaCl2: 5.5; water-holding capacity: 46.5 %; LUFA-Speyer, Speyer, Germany)	H	R2/C1	Pitombeira de Figueirêdo *et al.* (2019)
Folsomia candida (Collembola)	Difenoconazole (a.s.)	reproduction	28 d	NOEC	≥ 333	3.57 (2.1 % TOC)	≥ 317	Natural soil: LUFA 2.2 (soil pHCaCl2: 5.5; water-holding capacity: 46.5 %; LUFA-Speyer, Speyer, Germany)	D	R2/C1	Pitombeira de Figueirêdo *et al.* (2019)
Folsomia candida (Collembola)	Score 250 EC (250 g/L a.s.)	reproduction	28 d	NOEC	23.56	5	16.0	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand	G, H	R1/C1	Friedrich (2015) cited in EC (2019), Vol. 3CP B.9.7.2.1/01, SCORE 250 EC (A7402T), p.119
Folsomia candida (Collembola)	A9142L (30 g/L a.s.)	reproduction	28 d	NOEC	≥ 27.8	5	≥ 18.9	[CPIR]	CC, JJ	R1/C1	Anonymous cited in EC (2019), Vol. 1, Level 2, List of endpoints, Difenoconazole, p.206; Anonymous (2015) accessed via EFSA (2024)
Folsomia candida (Collembola)	A9142L (30 g/L a.s.)	adult mortality	28 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC, JJ	R1/C2	Anonymous (2015) accessed via EFSA (2024)
***Folsomia candida* (Collembola)**	**Score 250 EC (250 g/L a.s.)**	**adult mortality**	**28 d**	**NOEC**	**42.4**	**5**	**28.8**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand**	**F**	**1**	**Friedrich (2015) cited in EC (2019), Vol. 3CP B.9.7.2.1/01, SCORE 250 EC (A7402T), p.119**

Species (Taxonomic group)[5]	Test substance	Measured effect[6]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Hypoaspis aculeifer **(Mite)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**14 d**	**EC10**	**14.2**	**5**	**9.66**	**Artificial soil: 5 % sphagnum peat, 20% kaolinite clay, 74.8 % industrial quartz sand and 0.2 % calcium carbonate**	**H, L**	R1/C1	**Schulz (2015b) cited in EC (2019), Vol. 3CP B.9.7.2.1/02, SCORE 250 EC (A7402T), p.123**
Hypoaspis aculeifer **(Mite)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**14 d**	**NOAEC**	**75**	**15**	**17.0**	**Natural tropical soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively**	**BB**	**R2/C1**	**de Menezes Oliveira *et al.* (2018)**
		Geom. mean					***12.8***				
Hypoaspis aculeifer (Mite)	A9142L (30 g/L a.s.)	reproduction	14 d	NOEC	≥ 27.8	5	≥ 18.9	[CPIR]	CC, D, JJ	R1/C1	Anonymous cited in EC (2019), Vol. 1, Level 2, List of endpoints, Difenoconazole, p.207; Anonymous (2015) accessed via EFSA (2024)
Hypoaspis aculeifer (Mite)	Score 250 EC (250 g/L a.s.)	reproduction	14 d	NOEC	13.1	5	8.91	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.8 % industrial quartz sand and 0.2 % calcium carbonate	H, L	R1/C1	Schulz (2015b) cited in EC (2019), Vol. 3CP B.9.7.2.1/02, SCORE 250 EC (A7402T), p.123
Hypoaspis aculeifer (Mite)	Difenoconazole 250 EC (250 g/L a.s.)	reproduction	14 d	NOEC	≥ 70	5	≥ 47.6	Artificial soil: 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 74.7 % quartz sand	CC, D, M	R1/C1	Jansen 2016 cited in EC (2019), Vol. 3CP B.9.7.2.1/02, DIFCOR 250 EC, p.104; Anonymous (2016) accessed via (EFSA 2024)
Hypoaspis aculeifer **(Mite)**	**Score 250 EC (250 g/L a.s.)**	**adult mortality**	**14 d**	**NOEC**	**76.7**	**5**	**52.2**	**Artificial soil: 5% sphagnum peat, 20% kaolinite clay, 74.8% industrial quartz sand and 0.2% calcium carbonate**	**F**	**1**	**Schulz (2015b) cited in EC (2019), Vol. 3CP B.9.7.2.1/02, SCORE 250 EC (A7402T), p.123**

Species (Taxonomic group)[5]	Test substance	Measured effect[6]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Hypoaspis aculeifer (Mite)	A9142L (30 g/L a.s.)	adult mortality	14 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC, D, JJ	R1/C2	Anonymous (2015) accessed via EFSA (2024)
Hypoaspis aculeifer (Mite)	Difenoconazole 250 EC (250 g/L a.s.)	adult mortality	14 d	NOEC	≥ 70	5	≥ 47.6	Artificial soil: 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 74.7 % quartz sand	CC, D, M	R1/C1	Jansen 2016 cited in EC (2019), Vol. 3CP B.9.7.2.1/02, DIFCOR 250 EC, p.104; Anonymous (2016) accessed via (EFSA 2024)
Microorganisms	**Score 250 EC (250 g/L a.s.)**	**Respiratory quotient (Q_R)[FE]**	**90 d**	**NOEC**	**< 3.54**	**2.89 (1.7 % OC)**	**< 4.16**	**Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5%), pH 8.3, 2.3 g total N/kg dw, C/N ratio 7.8, conductivity 0.18 dS/m**	**R, D**	**R2/C1**	**Muñoz-Leoz *et al.* (2013)**
Microorganisms	Score 250 EC (250 g/L a.s.)	Potentially mineralizable nitrogen (N_{min}) [FE]	90 d	NOEC	≥ 3.54	2.89 (1.7 % OC)	≥ 4.16	Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5 %), pH 8.3, 2.3 g total N/kg dw, C/N ratio 7.8, conductivity 0.18 dS/m	R, D	R2/C1	Muñoz-Leoz *et al.* (2013)
Microorganisms	Score 250 EC (250 g/L a.s.)	Nitrification rate (NH_4^+ cc.)[FE]	90 d	NOEC	≥ 472	2.89 (1.7 % OC)	≥ 555	Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5 %), pH 8.3, 2.3 g total N/kg dw, C/N ratio 7.8, conductivity 0.18 dS/m	R, D	R2/C1	Muñoz-Leoz *et al.* (2013)
Microorganisms	**Score 250 EC (250 g/L a.s.)**	**Nitrification rate (NO_3^- cc.)[FE]**	**90 d**	**NOEC**	**< 3.54**	**2.89 (1.7 % OC)**	**< 4.16**	**Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5 %), pH 8.3, 2.3 g total N/kg dw, C/N ratio 7.8, conductivity 0.18 dS/m**	**R, D**	**R2/C1**	**Muñoz-Leoz *et al.* (2013)**
Microorganisms	**Score 250 EC (250 g/L a.s.)**	**Nitrogen transformation (NO_3^- cc.)[FE]**	**28 d**	**< 25 % effect (NOEC)**	**(≥) 2.04**	**[CPIR]**	**(≥) 2.85**	**Natural soil: loamy sand, pH 6.3-6.6**	**CC, HH**	**R1/C1**	**Schulz (2016) cited in EC (2019), Vol. 3CP B.9.9/01, SCORE 250 EC (A7402T),**

Species (Taxonomic group)[5]	Test substance	Measured effect[6]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
											p.131; Anonymous (2016) accessed via EFSA (2024)
Microorganisms	**Score 250 EC (250 g/L a.s.)**	**Carbon transformation (O_2 cc.)[FE]**	**28 d**	**< 25 % effect (NOEC)**	**(≥) 2.04**	**[CPIR]**	**(≥) 2.85**	**Natural soil: loamy sand, pH 6.3-6.6**	**CC, HH**	**R1/C1**	**Schulz (2016) cited in EC (2019), Vol. 3CP B.9.9/01, SCORE 250 EC (A7402T), p.131; Anonymous (2016) accessed via EFSA (2024)**
Allium cepa[M] ***Lolium perenne***[M] ***Triticum aestivum***[M] ***Zea mays***[M] ***Brassica oleracea***[D] *Glycine max*[D] ***Lycopersicon esculentum***[D] ***Raphanus sativus***[D] (Terrestrial plants)	**Score 250 EC (250 g/L a.s.)**	**seedling emergence, growth (seedling height) and biomass (shoot dry weight)**	**21 d**	**< 25 % effect**	**0.0933 (Appl. rate: 140 g a.s./ha; approx.. 10 cm soil depth)**	**1.2**	**≥ 0.264** **≥ 0.264** **≥ 0.264** **≥ 0.264** **≥ 0.264** ≥ 0.264 **≥ 0.264** **≥ 0.264**	**Loamy sand: composed of kaolinite clay, industrial quartz sand, and peat. The soil consisted of 85 % sand, 6 % silt, and 9 % clay**	**F, Y**	**1**	**Porch *et al*. (2011) cited in EC (2019), Vol. 3CP B.9.11.1/01, SCORE 250 EC (A7402T), p.135**
Avena sativa[M] *Brassica napus*[D] *Glycine max*[D] (Terrestrial plants)	Difenoconazole (a.s.)	seedling emergence	21 d	NOEC	≥ 10 ≥ 10 ≥ 10	2.41	≥ 14.1 ≥ 14.1 ≥ 14.1	Artificial soil: 65 % sand, 19 % silt, 16 % clay, pH 7.4	CC, D	R2/C1	Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via (EFSA 2024)
Avena sativa[M] **(Terrestrial plants)**	**Difenoconazole (a.s.)**	**biomass (shoot fresh weight)**	**21 d**	**NOEC**	**< 0.1**	**[CPIR]**	**< 0.141**	**[CPIR]**	**CC, D**	**R2/C1**	**Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via EFSA (2024)**

Species (Taxonomic group)[5]	Test substance	Measured effect[6]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
***Brassica napus*[D] (Terrestrial plants)**	**Difenoconazole (a.s.)**	**biomass (shoot fresh weight)**	**21 d**	**NOEC**	**0.3**	**[CPIR]**	**0.423**	**[CPIR]**	**CC**	**R2/C1**	**Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via EFSA (2024)**
***Glycine max*[D] (Terrestrial plants)**	**Difenoconazole (a.s.)**	**biomass (shoot fresh weight)**	**21 d**	**NOEC**	**3**	**[CPIR]**	**4.23**	**[CPIR]**	**CC**	**R2/C1**	**Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via EFSA (2024)**

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

A	The study from Friedrich (2011) was statistically re-evaluated by Taylor and Allen (2016g); both cited in EC (2019), Vol. 3CA B.9.4.1/01 and 02. For reproduction, the robust EC10 is preferred over the NOEC. At concentration of the statistically robust NOEC, 15-21 % reduction in the mean number of juveniles occurred. For biomass (adult growth), the re-calculated EC10 was statistically not robust enough (too wide confidence intervals; the lower end of the CI for EC20 is lower than the median EC10; the steepness of the curve is shallow) and the RMS proposed the use of the EC10low. However, for the SGV dossier the statistically robust NOEC has been chosen as the most reliable endpoint. The mortality ranged in 0-7.5 % being the highest at the highest concentration. From the summary it is unclear if there was any significant effects; in the LoEP a NOEC of ≥ 8 mg a.s./kg is reported. In the absence of the detailed results it is not possible to re-evaluate the statistics for mortality. Although surrounded by some uncertainty, a NOEC of ≥ 8 mg a.s./kg is listed (R2/C2).
AA	The Applicant claimed that no statistically significant effects on mortality/ biomass change/reproduction were observed at the highest test concentration, thus all NOEC values were greater than values (and as a result not suitable for including them in a geometric mean). It should be noted that based on the 12 % decrease in the number of juveniles at the highest test concentration, the RMS proposed to set the NOEC for reproduction at the second highest test concentration of 3.80 mg a.s./kg. The statistical results – based on the summarised data (EC 2019) – were re-evaluated, confirmed and used accordingly for the SGV dossier, i.e. NOEC ≥ the highest test concentration for all measured effects. It is noted that although mortality endpoint was not derived officially, there was no mortality in any of the treatments or control and thus NOEC for mortality is also deemed to be ≥ the highest test concentration. The peat content of the artificial soil used in the test was not included in the study summary, but only listed in the LoEP, therefore there are some uncertainties about the normalised effect concentrations.
B	The statistics has been re-checked and it could be confirmed that the statistically robust NOEC for reproduction is 2.1 mg a.s./kg soil with p of 0.1393 (LOEC = 3.4 mg a.s./kg; p = 0.0003; GraphPad Prism 10 Version 10.0.2; one-way ANOVA with Dunnett's test; α = 0.05). At the level of NOEC 15 % reduction on reproduction was observed. No mortality endpoint was officially derived. The mean mortality ranged in 0-2.5 %, thus the NOEC for mortality is deemed to be ≥ the highest test concentration.

BB	NOAEC, LOAEC, EC10 and EC50 were reported in the study. The spacing of the tested concentrations did not follow the OECD recommendations of ≤ 1.8-fold (OECD 2016); the derived EC10 values could not be considered reliable (too wide confidence intervals). The NOAECs were selected in the case of potworm and mite for potentially deriving an SGV. For springtail, neither the EC10 (too wide confidence interval), nor the NOAEC has been found reliable: there is a 100-fold gap in the test concentrations right after the LOAEC with little increase in the effects at a 100 times higher concentration; there is almost a 100-fold difference between the EC10median and the proposed NOAEC and 30-fold difference between the EC10low and the NOAEC.
CC	The study could be accessed on request for public access to documents (EFSA 2024). Please be aware that not all endpoints that could be derived from the original study report are included in the LoEP.
D	Unbound value, not suitable for including in a geometric mean. (Either no effects at the highest test concentration or significant effects already at the lowest test concentration.)
E	For Servajean (2015), an EC10 of 3.51 mg a.s./kg was calculated, however this effect concentration did not prove to be statistically robust (unacceptably wide normalised width of the confidence interval; EC20low < EC10median; shallow steepness of the curve). Based on the outcome of expert discussions on recurring issues in ecotoxicology (EFSA 2015), when EC10 < NOEC and the EC10 is not reliable it is advised that the lower limit of the EC10 confidence interval should be used (i.e. EC10low = 1.18 mg a.s./kg). However, during the commenting period the Co-RMS found the EC10 not valid and a NOEC of 2.62 mg a.s./kg was proposed for use in the risk assessment. This was not supported by statistics but based on < 10 % biological effects at that concentration. It should be noted that the renewal assessment has not been finalised yet. In addition, a NOEC of 7.71 mg a.s./kg was proposed by the applicant. The statistics has been re-evaluated for the NOEC for this SGV dossier and the applicant's proposal could be statistically confirmed (LOEC = 10.8 mg a.s./kg; p = 0.0006; GraphPad Prism 10 Version 10.0.2; one-way ANOVA with Dunnett's test; α = 0.05). This statistically robust NOEC is preferred over the unreliable EC10 (either median or lower bound of CI) or a statistically not robust NOEC that was based on biological effects alone. It is noted that there was 15 % reduction in the mean number of juveniles at the concentration of the statistically robust NOEC of 7.71 mg a.s./kg.
F	The assessment from the EC (2019) report was adopted and accepted without additional assessment (i.e. at face value).
G	Both NOEC and EC10 were reported and considered reliable by the RMS. As a precautionary approach, the lower NOEC value was selected for use in the EU risk assessment.
H	When both NOEC and EC10 are available and statistically acceptable, EC10 is preferred for SGV derivation due to the inherent uncertainties a NOEC is surrounded by (see Appendix 1; Azimonti *et al.* (2015a)).
HH	There are no statistically significant effects at end of the study for neither of the endpoints (at both concentrations), therefore the results at the higher tested concentration can be considered as greater-than/equal-to NOEC values (with overall effects in both tests at both concentrations after 28 days between -0.8 and +3.0 % as compared to the controls).
JJ	Relevant and reliable study with no effects on reproduction at the highest concentration, i.e. at 27.8 mg a.s./kg soil dw; the EFSA endpoint was based on a not significant lower concentration.
L	A statistically robust NOEC of 13.1 mg a.s./kg was reported by the applicant. According to the RMS an effect above 10 % occurs at this concentration. Additionally, an EC10 of 14.2 mg a.s./kg was calculated. Since the NOEC was lower than the EC10 but the effects at the NOEC was above 10 %, the EC10low was suggested as an endpoint for use in the risk assessment in the EU. However, due to the clear dose-response and the tight confidence intervals of the EC10/EC20/EC50 values, the median EC10 is considered more suitable for SGV derivation.
M	The study results have been statistically re-evaluated based on the individual numbers per replicates. No statistically significant difference could be shown for the treatments, therefore the highest test concentration is considered for the endpoints. (In the dRAR, the reproductive endpoint was based on a concentration with < 10 % effects.)
R	In Muñoz-Leoz *et al.* (2013), natural soil was spiked with different concentrations of difenoconazole and samples for multiple measurement endpoints were taken after 7, 30, 60 and 90 days of exposure. However, within the first 30 days, the control and the treatments showed high fluctuations as the spiked cultures needed time to acclimate to the new conditions. Due to the fluctuations during the first half of the experimental timeframe and the long-term focus of the project, the effects after 90 days of incubation were considered the most relevant. Therefore and in order to synthesize the study, only the results after 90 days were reported in the table.

	During the study, difenoconazole concentrations were monitored and the dissipation half-life (DissT50) of difenoconazole calculated with a bi-exponential model. The different difenoconazole concentrations resulted in different DissT50 values that were then used in a test-concentration-specific manner to calculate specific time-weighted average factors (f_{TWA}) and then the respective TWA concentrations over the study duration following the equations used in pesticide authorisation: $f_{TWA} = (1-e^{-kt})/kt$ *Where*: e – Euler's number; k – rate constant (ln2/DissT50); t – averaging interval $AS(t) = f_{TWA} * \text{test concentration at } t_0$ *Where*: AS(t) – the TWA active substance concentration over the averaging interval; t_0 – the test concentration at the beginning For potentially mineralizable nitrogen (N_{min}), the statistically robust, normalised NOEC of 54.0 mg a.s./kg soil potentially includes an estimated 26-29 % effect. According to the BPR GD (ECHA 2017), for terrestrial microorganisms if a statistical difference is found and the effect is > 15 %, no NOEC can be derived from tests run with control and 2 test concentrations (here there are 3 test concentrations, but also with a spacing factor of 10). If in at least one concentration no statistical difference from the control is found and the effect value is ≤ 15 %, the concentration can be considered the NOEC. At normalised concentration of 4.16 mg a.s./kg soil, approximately 17-19 % effect can be estimated based on the height of the respective columns in Figure 3 in Muñoz-Leoz *et al.* (2013). As this is a very rough estimation, this concentration is considered acceptable as a NOEC for potentially mineralizable nitrogen. Due to the high spacing factors, the no effect concentration is presented as a ≥ value. Treated-soil quality index (T-SQI) is an enzyme-based index of microbial functional diversity. Considering its integrative quality of the other parameters, it is scored as not relevant (C3). Due to the high spacing factors, the no-effect concentration is presented as a ≥ value.
Y	Soil was over-sprayed after seeds had been planted. Based on the size of the pots used in the test and the volume of soil they contained, a 10 cm soil depth could be used for calculation of the concentration as mg a.s./kg soil.
Z	Effect value is based on mean initial measurement of the applied amount of difenoconazole.

3.2 Graphic representation of effect data

The lowest most 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 etc.), the lowest one is included in the figure.

The normalised equal-to effect concentrations for potworm, collembolan and mite are in a similar range (5.24-22.8 mg a.s./kg), whereas the available normalised equal-to effect data for earthworm (between 0.430 and 0.789 mg a.s./kg) and terrestrial plants (between 0.423 and 4.23 mg a.s./kg) indicate a higher sensitivity. For microorganisms, only unbound values could be determined from the studies but they enclose a small range between 2.85 and 4.16 mg a.s./kg soil for a potential NOEC/EC10 (functional endpoints; see triangles facing down and up in Figure 2).

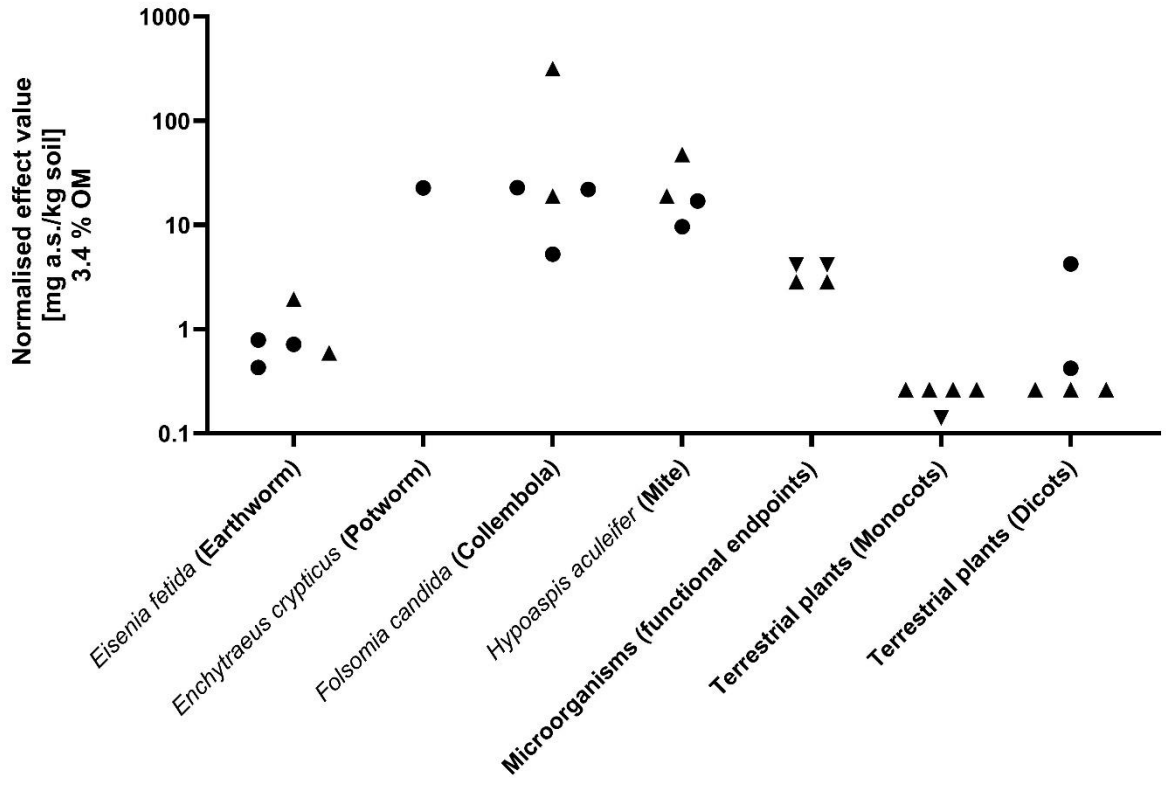


Figure 2: Effect data for difenoconazole after normalisation to a standard organic matter content of 3.4 % - the statistically most robust lowest effect values of the relevant and reliable endpoints per species per test setup. Triangles represent unbound data with the triangle facing up symbolising ≥ or > values and the triangle facing down symbolising ≤ or < values. For terrestrial plants, all the upward-facing triangles represent < 25 % effect concentrations (n = 7), while the other plant data are NOEC values (n = 3), also see Table 5 below.

4 Derivation of SGV

For the derivation of SGV for difenoconazole, the relevant and reliable data of the active ingredient and formulations were normalised to a standard organic matter content of 3.4 %.

4.1 Derivation of SGV_{AF} using the assessment factor (AF) method

The SGV_{AF} is determined using assessment factors applied to the lowest valid toxicity endpoint (e.g. NOEC, EC10) from long-term toxicity tests. 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 toxicity endpoint available for difenoconazole (Table 5) is the NOEC of < 0.141 mg a.s./kg soil for terrestrial plants (oat – monocot species).

Table 5: The statistically most robust lowest relevant and reliable chronic data for difenoconazole 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	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Reference
Primary producers (Terrestrial plants)	*Avena sativa* (Monocot)	NOEC	< 0.141	Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via (EFSA 2024)*
	Glycine max (Dicot)	NOEC	4.23	
	Brassica napus (Dicot)	NOEC	0.423	
	Allium cepa (Monocot)	< 25 % effect	≥ 0.264	Porch *et al*. (2011) cited in EC (2019), Vol. 3CP B.9.11.1/01, SCORE 250 EC (A7402T), p.135**
	Lolium perenne (Monocot)	< 25 % effect	≥ 0.264	
	Triticum aestivum (Monocot)	< 25 % effect	≥ 0.264	
	Zea mays (Monocot)	< 25 % effect	≥ 0.264	
	Brassica oleracea (Dicot)	< 25 % effect	≥ 0.264	
	Lycopersicon esculentum (Dicot)	< 25 % effect	≥ 0.264	
	Raphanus sativus (Dicot)	< 25 % effect	≥ 0.264	
Decomposers (Nutrient transformers)	Microorganisms (Functional endpoints)	NOEC	< 4.16	Muñoz-Leoz *et al.* (2013)
		NOEC***	≥ 2.85	Schulz (2016) cited in EC (2019), Vol. 3CP B.9.9/01, SCORE 250 EC (A7402T), p.131; Anonymous (2016) accessed via (EFSA 2024)
Decomposers (Litter transformers/ primary consumers)	*Eisenia fetida* (Earthworm)	NOEC/EC10	0.623	***Geomean:*** Taylor and Allen (2016g) re-calculating Friedrich (2011), cited in EC (2019), Vol. 3CA B.9.4.1/02, p.205; Servajean (2009) cited in EC (2019), Vol. 3CP, B.9.7.1.1/01, DIFCOR 250 EC, p.90; Anonymous cited in EC (2019), Vol. 1, Level 2, List

Trophic level	Species	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Reference
				of endpoints, Difenoconazole, p.205; Anonymous (2016) accessed via (EFSA 2024)
	Enchytraeus crypticus (Potworm)	NOAEC	22.7	de Menezes Oliveira *et al.* (2018)
	Folsomia candida (Collembola)	NOEC/EC10	13.8	***Geomean:*** Servajean (2015) cited in EC (2019), Vol. 3CP B.9.7.2.1/01, DIFCOR 250 EC, p.101 Friedrich (2015) cited in EC (2019), Vol. 3CA B.9.7.2.1/01, SCORE 250 EC (A7402T), p.119 Pitombeira de Figueirêdo *et al.* (2019)
Secondary consumers	*Hypoaspis aculeifer* (Mite)	NOAEC/EC10	12.8	***Geomean:*** Schulz (2015b) cited in EC (2019), Vol. 3CP B.9.7.2.1/02, SCORE 250 EC (A7402T), p.123 de Menezes Oliveira *et al.* (2018)

Notes: * Dose-response test with five test concentrations. ** Limit test with one test concentration resulting in -6 to +23 % effects. *** Originally nitrification and carbon transformation endpoints with < 25 % effects that actually cover effects between -0.8 and +3.0 % as compared to the controls. As only two concentrations were tested and there were no significant effects at the higher test concentration after 28 days, the results are considered as equal-to/greater-than NOEC values.

Difenoconazole is a fungicide, which acts by disrupting the production of ergosterols. Thus, it is plausible that soil fungi are among the most sensitive organisms. However, relevant and reliable toxicity data specifically on soil fungi are missing. The available effect data on fungi is not considered acceptable for SGV derivation, since the specific organic matter content of the soil, needed for the normalisation of the toxicity data and thus for the comparison of the toxicity between different organisms/tests, was lacking (see Annex 2, Table A1). It is noted that data on soil microorganisms – **decomposers (nutrient transformers)** – are available in general and based on that information, they do not seem to be the most sensitive group (see < 4.16 mg a.s./kg soil values for respiratory quotient and nitrification rate and >= 2.85 mg a.s./kg soil for nitrogen and carbon transformation; Muñoz-Leoz *et al.* 2013, EC 2019).

The dataset indicates that terrestrial plants – **primary producers** – could be ***the most sensitive group*** of organisms. Although several studies with plants are available, only studies where plants were exposed to difenoconazole uniquely *via* soil could be considered relevant for the assessment. One study with three species resulting in NOEC values of < 0.141, 0.423 and 4.23 mg a.s./kg soil (Balluff 2004) and another one with seven species resulting in < 25 % effects of ≥ 0.264 mg a.s./kg soil could fulfil the relevance and reliability requirements (both cited in EC 2019). Altogether there are relevant and reliable data on seedling emergence and biomass for five monocot and five dicot species. Unbound

values cannot be used for SGV derivation, even if < 0.141 mg a.s./kg soil is the overall lowest effect value of the relevant and reliable dataset on difenoconazole. The observed effect at this concentration was 16 % on biomass (shoot fresh weight) for oat. The lowest equal-to value was also measured for plants (0.423 mg a.s./kg for oilseed rape, 1.32 % effects on biomass) and this is used for SGV derivation.

The lowest most relevant data for **decomposers (litter transformers/primary consumers)** is based on a geometric mean calculated for three long-term effect values for earthworms (0.623 mg a.s./kg soil; Servajean (2009), Taylor and Allan (2016g) and Anonymous (2016) cited in EC (2019)). For earthworms a limit test in form of a field study is also available that provided similar results as the laboratory studies (NOEC ≥ 0.593 mg a.s./kg soil; see discussion below).

Secondary consumers, namely *Hypoaspis aculeifer* (predatory mite), did not show an exceptional sensitivity (geomean of 12.8 mg a.s./kg soil, Schulz (2015b) cited in (EC 2019); de Menezes Oliveira *et al.* (2018)).

When long-term test results (NOEC or EC_{10} values) are available for at least three species representing at least three trophic levels with different living and feeding conditions, the EC TGD (2003) recommends the application of an assessment factor of 10 to the lowest valid effect datum (Table 20 in (EC TGD 2003)). In the case of difenoconazole, the lowest endpoint as equal-to value is available for terrestrial plants (primary producers), decomposers (litter transformers/primary consumers) and secondary consumers. For microorganisms a less than value and a greater than/equal to value is available providing a small interval for a potential NOEC/EC10. It is noted that the lowest endpoint is a less than value for the potentially most sensitive groups of organisms, terrestrial plants. To account for the uncertainties in the available data, an AF of 10 is applied to the lowest equal-to effect value on plants:

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

$$SGV_{AF} = \frac{0.423\left(\frac{mg\ a.s.}{kg\ soil}\right)}{10} = 0.042\left(\frac{mg\ a.s.}{kg\ soil}\right)$$

The application of an AF of 10 to the lowest equal-to chronic datum results in a SGV_{AF} = **0.042 mg a.s./kg soil** for a standard soil with 3.4 % OM content (shown to two significant figures).

4.2 Derivation of SGV_{SSD} using the species sensitivity distribution (SSD) method

There is not enough data for applying the SSD method. The minimum data requirements recommended for the application of the SSD approach for SGV_{SSD} is at least ten exact data points ($NOEC/EC_{10}$) 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 difenoconazole, exact data are available for dicotyledonous plants (*Glycine max* and *Brassica napus*), Annelida (*Eisenia fetida* and *Enchytraeus crypticus*), Hexapoda (*Folsomia candida*), and Chelicerata (*Hypoaspis aculeifer*). In total, equal-to values for six species are available from four taxonomic groups. Thus, the minimum data requirements for an SSD are not met.

4.3 Derivation of SGV_{EqP} using the equilibrium partitioning (EqP) approach

If no reliable data on terrestrial organisms is available, the equilibrium partitioning utilizing aquatic toxicity data can be used to estimate the SGV_{EqP} (EC TGD 2003). In the case of difenoconazole, 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

The field studies are not considered suitable for SGV derivation. Two field studies on earthworms and a study on microorganisms could be obtained for difenoconazole (see Table A2 in Appendix 2). Only the earthworm study that was a limit test (with limited use for setting an SGV) could be considered relevant and reliable (Schulz (2015) cited in EC (2019)). This study indicated a NOEC equal to/above the single tested concentration, i.e. ≥ 0.593 mg a.s./kg soil. While this value is in line with the laboratory test results, it is not suitable for an SGV derivation as it is an unbound value, furthermore earthworms are not the most sensitive group (thus it would also not affect the choice of the assessment factor). Although the other earthworm field study included four application rates, the actual concentrations in the test could not be calculated due to the modified analytical sampling method they used (see the notes to the study in Appendix 2; Hamberger (2015) cited in EC (2019)). For the literature study (Filimon *et al.* 2015), the organic matter content of the soil was not reported, thus, normalisation of effect data to soil organic matter content and subsequently comparison of the outcome with other studies are not possible. As a result, these studies could also not be used for deriving an SGV.

5 Toxicity of major transformation products

Effect data are available for two major degradation products of difenoconazole: alcohol derivative (CGA 205375) and the cleavage product 1,2,4-triazole (CGA 71019). The full effect data tables are presented in the Appendix 2 (Table A3 and Table A4), whereas Table 6 below summarises the lowest effect concentrations for each respective organism. Due to the unbound values for earthworm, it is unclear whether *Folsomia candida* (Collembola) could be the most sensitive organism for 1,2,4 triazole (CGA 71019). For CGA 205375 *F. candida* is definitely the most sensitive organism. *F. candida* has been proven to be more sensitive to the transformation products than to the parent compound (17 and 23 times more sensitive for CGA 205375 and CGA 71019, respectively). For mixture risk assessment these metabolites also need to be considered.

Table 6: Lowest reliable and relevant soil effect data for the transformation products CGA 205375 (aka Metabolite 2 or CGA 211391) and 1,2,4-triazole (CGA 71019). Endpoints are shown as effect concentrations normalised to 3.4 % soil organic matter.

Species	Type of effect concentration	CGA 205375 (CGA 211391) concentration [mg/kg soil]	1,2,4-triazole (CGA 71019) concentration [mg/kg soil]	References
Eisenia fetida (Earthworm)	NOEC	≥ 3.26	≥ 0.340	Friedrich (2006) cited in EC (2019), Vol. 3CA B.9.4.1/06, p.213

Species	Type of effect concentration	CGA 205375 (CGA 211391) concentration [mg/kg soil]	1,2,4-triazole (CGA 71019) concentration [mg/kg soil]	References
				Moser and Scheffczyk (2004) cited in EC (2019), Vol. 3CA B.9.4.1/10, p.221
Folsomia candida (Collembola)	NOEC	0.816	0.612	Friedrich (2006a) cited in EC (2019), Vol. 3CA B.9.4.3.1/03, p.235 Moser and Scheffczyk (2002) cited in EC (2019), Vol. 3CA B.9.4.3.1/05, p.239
Hypoaspis aculeifer (Mite)	NOEC/ EC10	≥ 13.1	129	Schulz (2015a) cited in EC (2019), Vol. 3CA B.9.4.3.1/07, p.242 Schulz (2014) cited in EC (2019), Vol. 3CA B.9.8.4.2.1/07, p.245

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. With the available data for difenoconazole, only the assessment factor (AF) method could be applied for deriving an SGV. Since the dataset included enough relevant and reliable data, the AF is not exceeding 50 and, consequently, the SGV is considered definitive.

A definitive **SGV of 0.042 mg a.s./kg soil for difenoconazole** is suggested.

7 Protection of soil organisms and uncertainty analysis

The SGV of 0.042 mg a.s./kg soil for difenoconazole has been derived based on a dataset containing values for various microbial processes, earthworms (*Eisenia fetida*), potworms *(Enchytraeus crypticus*), collembolans (*Folsomia candida*), mites (*Hypoaspis aculeifer*) and terrestrial plants, with the latter showing the highest sensitivity.

Difenoconazole is a fungicide, thus according to its mode of action, it is expected that fungi would be the most sensitive taxonomic group. However, relevant and reliable toxicity data specifically on fungi are lacking. On the other hand it is worth noting that the results on soil microorganisms in general do not indicate the highest sensitivity.

Several studies dealing with the changes of microbial communities in structure and function as a result of exposure to difenoconazole have been listed in this report. These greater than/less than results indicated a narrow range for a potential NOEC/EC10.

A lower-than NOEC value for *Avena sativa* (oat) was the lowest effect concentration for primary producers (plants, monocot), which was about three times lower than the lowest bound reliable and relevant NOEC value (*Brassica napus*; plants, dicot). Since only 16 % effect on biomass (fresh shoot weight) was observed at the lowest tested concentration in the *Avena* test, an assessment factor of 10 applied to the lowest bound value still seems to appropriately cover the uncertainty with regard to the

effects on plants. The uncertainty for this group can later be investigated further in laboratory/field tests.

The transformation products CGA 205375 and 1,2,4-triazole (CGA 71019) exhibit considerable toxicity to soil organisms and thus the protectiveness of the SGV for the active substance difenoconazole over the metabolites needs further consideration – however that is beyond the scope of this dossier. In addition, 1,2,4-triazole is a common metabolite of triazole pesticides, so it is strongly recommended to perform a separate effect assessment for this transformation product and, if it is not the case already, to include it in a soil monitoring campaign.

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 0.042 mg a.s./kg soil vs MLOQ of 0.0002 mg a.s./kg soil). Therefore, no analytical issues are foreseen for the use of the derived SGV.

Statistically robust NOEC values, above 10 % average effect sizes are accepted for SGV derivation. Three NOEC values with approximately 15 % average effect sizes are accepted as relevant and reliable – one for earthworm, one for Collembola and one for microorganisms (Servajean (2009), Servajean (2015) cited in (EC 2019) and Muñoz-Leoz *et al.* (2013), respectively). For authorisation/registration purposes lower values were selected as NOEC with ≤ 10 % effects, however these were statistically not significant. Due to the natural biological variability in soil tests (accepted control coefficient of variation up to 15-30-50 %) lower average effect sizes are often statistically not significant (see also the consideration on NOEC in Appendix 1). Therefore, the statistically robust NOEC values are included in the respective geometric means for earthworm and Collembola. The earthworm NOEC of 0.714 mg a.s./kg (Servajean 2009) is within the other reliable and relevant effect data for earthworm (EC10 values of 0.430 and 0.789 mg a.s./kg; Taylor and Allen (2016g) and Anonymous (2016), respectively). The other concerned NOEC value for Collembola is actually the lowest of the dataset of reliable and relevant data on Collembola (5.24 mg a.s./kg; Servajean (2015)), lower than the other two EC10 values (21.9 and 22.8 mg a.s./kg; Pitombeira de Figueirêdo *et al.* (2019) and Friedrich (2015), respectively). The NOEC value for microorganisms (< 4.16 mg a.s./kg; Muñoz-Leoz *et al.* (2013)) has already been lowered to include supportable amount of effects (approx. 15 %, see notes to the study in Table 4 as well as the guidance on deriving endpoint for soil microorganism studies in ECHA (2017)). Altogether, these NOEC values – falling within the range or being the lowest of the other existing effect values for the same group/species and relying on a value supported by statistics – increase the robustness of the SGV.

The isomeric composition of difenoconazole, and the toxicity and behaviour of the isomers are not expected to lower the protectiveness of the SGV. As it is explained in its European patent (EPO 2019), the isomeric composition of difenoconazole that is typical for plant protection products like Score 250EC is as follows: about 30 % (2S,4R), about 30 % (2R,4S), about 20 % (2R,4R) and about 20 % (2S,4S). The faster degradation of the (2R,4S) and the (2R,4R) isomers in soil (under aerobic conditions DT50 of 173.2 and 169 days, respectively) potentially can lead to an enhanced ratio of the (2S,4R) and (2S,4S)-isomers in the residues (DT50 of 223 and 238.9 days, respectively; Dong *et al.* 2013). Along with the slower degradation, the (2S,4R) and (2S,4S)-isomers also showed higher acute aquatic toxicity (Dong *et al.* 2013), however no isomer-specific toxicity data is available on soil organisms. The comparison of the EC50 values indicated that (2S,4R)-difenoconazole is about 1.9-fold more toxic to aquatic organisms than the (2R,4S)-isomer. There is also no information about any differences in bioavailability

of the isomers in soil. The potential difference in the toxicity is 1.9-fold in aquatic organisms. Taking into account the relatively wide range of data available and the chosen AF of 10, the derived SGV can be considered protective.

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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 7-cm 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^3 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 non-target 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 re-assessment 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, including difenoconazole, in Muñoz-Leoz *et al.* (2013), but also the wide range of DissT50 values for difenoconazole in Section 1.5.2 above (EC 2019)). It is noted that, for instance, according to the often used field earthworm study guideline (ISO 2014) 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 (2014)); 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 make an assessment of the study is missing* (**R4**; Moermond *et al.* 2016).

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 difenoconazole 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; WHC – water holding capacity; OC – organic carbon; OM – organic matter; CFU – colony forming units; [CPIR] – confidentially provided information, redacted. 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).

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida **(Earthworm)**	**Difenoconazole 250 EC (250 g/L a.s.)**	**reproduction**	**56 d**	**NOEC**	**2.10**	**10**	**0.714**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % quartz sand.**	**B**	**R2/C1**	**Servajean (2009) cited in EC (2019), Vol. 3CP, B.9.7.1.1/01, DIFCOR 250 EC, p.90**
Eisenia fetida **(Earthworm)**	**Difenoconazole (a.s.)**	**reproduction**	**56 d**	**EC10**	**0.632**	**5**	**0.430**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand**	**A**	**R1/C1**	**Taylor and Allen (2016g) re-calculating Friedrich (2011), cited in EC (2019), Vol. 3CA B.9.4.1/02, p.205**
Eisenia fetida **(Earthworm)**	**A9142L (30 g/L a.s.)**	**reproduction**	**56 d**	**EC10**	**1.16**	**5**	**0.789**	**[CPIR]**	**CC**	**R1/C1**	**Anonymous cited in EC (2019), Vol. 1, Level 2, List of endpoints, Difenoconazole, p.205; Anonymous (2016) accessed via EFSA (2024)**
		geomean					***0.623***				
Eisenia fetida (Earthworm)	Score 250 EC (250 g/L a.s.)	reproduction	56 d	NOEC	≥ 5.70	10	≥ 1.94	Artificial soil	AA, D	R2/C1	Friedrich (2006) cited in EC (2019), Vol. 3CP B.9.7.1.1/01, SCORE 250 EC (A7402T), p.102

[8] M – monocotyledonous, D – dicotyledonous plant species

[9] DE – diversity endpoint, EE – enzymatic endpoint, FE – functional endpoint

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Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	A9142L (30 g/L a.s.)	reproduction	56 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC	R1/C1	Anonymous (2016) accessed via EFSA (2024)
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	reproduction	56 d	NOEC	1.00	5	0.680	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand	A	R2/C1	Friedrich (2011) cited in EC (2019), Vol. 3CA B.9.4.1/01, p.202
Eisenia fetida (Earthworm)	Score 250 EC (250 g/L a.s.)	adult mortality	28 d	NOEC	≥ 5.70	10	≥ 1.94	Artificial soil	AA, D	R2/C2	Friedrich (2006) cited in EC (2019), Vol. 3CP B.9.7.1.1/01, SCORE 250 EC (A7402T), p.102
Eisenia fetida (Earthworm)	Difenoconazole 250 EC (250 g/L a.s.)	adult mortality	28 d	NOEC	≥ 8.7	10	≥ 2.96	Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % quartz sand.	B, D	R2/C2	Servajean (2009) cited in EC (2019), Vol. 3CP, B.9.7.1.1/01, DIFCOR 250 EC, p.90
***Eisenia fetida* (Earthworm)**	**Difenoconazole (a.s.)**	**adult mortality**	**28 d**	**NOEC**	**≥ 8**	**5**	**≥ 5.44**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz**	**F, A, BB**	**1**	**Friedrich (2011), cited in EC (2019), Vol. 3CA B.9.4.1/02, p.205**
Eisenia fetida (Earthworm)	A9142L (30 g/L a.s.)	adult mortality	28 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC	R1/C2	Anonymous (2016) accessed via EFSA (2024)
Eisenia fetida (Earthworm)	Score 250 EC (250 g/L a.s.)	biomass change (adult growth)	28 d	NOEC	≥ 5.70	10	≥ 1.94	Artificial soil	AA, D	R2/C2	Friedrich (2006) cited in EC (2019), Vol. 3CP B.9.7.1.1/01, SCORE 250 EC (A7402T), p.102
***Eisenia fetida* (Earthworm)**	**Difenoconazole (a.s.)**	**biomass change (adult growth)**	**28 d**	**NOEC**	**2.00**	**5**	**1.36**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand**	**A**	**R1/C2**	**Friedrich (2011) cited in EC (2019), Vol. 3CA, B.9.4.1/01, p.202**

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	biomass change (adult growth)	28 d	EC10low [10]	0.24	5	0.163	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz	F, A	1	Taylor and Allen (2016g) re-calculating Friedrich (2011), cited in EC (2019), Vol. 3CA B.9.4.1/02, p.205
Eisenia fetida (Earthworm)	A9142L (30 g/L a.s.)	biomass change (adult growth)	28 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC	R1/C2	Anonymous (2016) accessed via EFSA (2024)
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	reproduction	56 d	NOEC	≥ 1	5	≥ 0.68	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.5 % industrial fine sand	F, V	3	Sacker (2009) cited in EC (2019), Vol. 3CA B.9.4.1/03, p.207
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	biomass change (adult growth)	28 d	NOEC	≥ 1	5	≥ 0.68	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.5 % industrial fine sand	F, V	3	Sacker (2009) cited in EC (2019), Vol. 3CA B.9.4.1/03, p.207
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	adult mortality	28 d	NOEC	≥ 1	5	≥ 0.68	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.5 % industrial fine sand	F, V	3	Sacker (2009) cited in EC (2019), Vol. 3CA B.9.4.1/03, p.207
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	reproduction	56 d	NOEC	n.a. (≥ Appl. rate: 500 g a.s./ha)	n.r.	n.a.	Artificial soil	F, V	3	Nienstedt (1999) cited in EC (2019), Vol. 3CA B.9.4.1/04, p.210
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	biomass change (adult growth)	28 d	NOEC	n.a. (≥ Appl. rate: 500 g a.s./ha)	n.r.	n.a.	Artificial soil	F, V	3	Nienstedt (1999) cited in EC (2019), Vol. 3CA B.9.4.1/04, p.210
Eisenia fetida (Earthworm)	Difenoconazole (a.s.)	adult mortality	28 d	NOEC	n.a. (≥ Appl. rate: 500 g a.s./ha)	n.r.	n.a.	Artificial soil	F, V	3	Nienstedt (1999) cited in EC (2019), Vol. 3CA B.9.4.1/04, p.210
***Enchytraeus crypticus* (Potworm)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**21 d**	**NOAEC**	**100**	**15**	**22.7**	**Natural tropical soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and**	**BB**	**R2/C1**	**de Menezes Oliveira *et al.* (2018)**

[10] EC10low is the lower limit of the 95 % confidence interval of the EC10

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Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
								10 % of fine, medium, and coarse sand, respectively			
Enchytraeus crypticus (Potworm)	Score 250 EC (250 g/L a.s.)	reproduction	21 d	EC10	59.5	15	13.5	Natural tropical soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively	BB	R3/C1	de Menezes Oliveira *et al.* (2018)
***Folsomia candida* (Collembola)**	**Difenoconazole 250 EC (250 g/L a.s.)**	**reproduction**	**28 d**	**NOEC**	**7.71**	**5**	**5.24**	**Artificial soil: 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 75 % industrial fine sand**	**E**	**R2/C1**	**Servajean (2015) cited in EC (2019), Vol. 3CP B.9.7.2.1/01, DIFCOR 250 EC, p.101**
***Folsomia candida* (Collembola)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**28 d**	**EC10**	**33.5**	**5**	**22.8**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand**	**G, H**	**R1/C1**	**Friedrich (2015) cited in EC (2019), Vol. 3CA B.9.7.2.1/01, SCORE 250 EC (A7402T), p.119**
***Folsomia candida* (Collembola)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**28 d**	**EC10**	**23**	**3.57 (2.1 % TOC)**	**21.9**	**Natural soil: LUFA 2.2 (soil pHCaCl2: 5.5; water-holding capacity: 46.5%; LUFA-Speyer, Speyer, Germany)**	**H**	**R2/C1**	**Pitombeira de Figueirêdo *et al.* (2019)**
		geomean					***13.8***				
Folsomia candida (Collembola)	Score 250 EC (250 g/L a.s.)	reproduction	28 d	NOEC	10	3.57 (2.1 % TOC)	9.52	Natural soil: LUFA 2.2 (soil pHCaCl2: 5.5; water-holding capacity: 46.5 %; LUFA-Speyer, Speyer, Germany)	H	R2/C1	Pitombeira de Figueirêdo *et al.* (2019)
Folsomia candida (Collembola)	Difenoconazole (a.s.)	reproduction	28 d	NOEC	≥ 333	3.57 (2.1 % TOC)	≥ 317	Natural soil: LUFA 2.2 (soil pHCaCl2: 5.5; water-holding capacity: 46.5 %; LUFA-Speyer, Speyer, Germany)	D	R2/C1	Pitombeira de Figueirêdo *et al.* (2019)

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Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Folsomia candida (Collembola)	Score 250 EC (250 g/L a.s.)	reproduction	28 d	NOEC	23.56	5	16.0	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand	G, H	R1/C1	Friedrich (2015) cited in EC (2019), Vol. 3CP B.9.7.2.1/01, SCORE 250 EC (A7402T), p.119
Folsomia candida (Collembola)	A9142L (30 g/L a.s.)	reproduction	28 d	NOEC	≥ 27.8	5	≥ 18.9	[CPIR]	CC	R1/C1	Anonymous cited in EC (2019), Vol. 1, Level 2, List of endpoints, Difenoconazole, p.206; Anonymous (2015) accessed via EFSA (2024)
***Folsomia candida* (Collembola)**	**Score 250 EC (250 g/L a.s.)**	**adult mortality**	**28 d**	**NOEC**	**42.4**	**5**	**28.8**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand**	**F**	**1**	**Friedrich (2015) cited in EC (2019), Vol. 3CP B.9.7.2.1/01, SCORE 250 EC (A7402T), p.119**
Folsomia candida (Collembola)	A9142L (30 g/L a.s.)	adult mortality	28 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC	R1/C2	Anonymous (2015) accessed via EFSA (2024)
Folsomia candida (Collembola)	Difenoconazole (a.s.)	adult mortality	28 d	NOEC	500	10	170	Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay, 69 % industrial quartz sand	CC, J	R3/C2	Meister (2002) cited in EC (2019), Vol. 3CA B.9.4.3/01, p.231; Anonymous (2002) accessed via (EFSA 2024)
Folsomia candida (Collembola)	Difenoconazole 250 EC (250 g/L a.s.)	reproduction	28 d	EC10	3.51	5	2.39	Artificial soil: 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 75 % industrial fine sand	E	R3/C1	Servajean (2015) cited in EC (2019), Vol. 3CP B.9.7.2.1/01, DIFCOR 250 EC, p.101
Folsomia candida (Collembola)	Difenoconazole (a.s.)	reproduction	28 d	NOEC	31.25	10	10.6	Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay, 69 % industrial quartz sand	CC, J	R3/C1	Meister (2002) cited in EC (2019), Vol. 3CA B.9.4.3/01, p.231; Anonymous (2002) accessed via (EFSA 2024)
Folsomia candida (Collembola)	Score 250 EC (250 g/L a.s.)	reproduction	28 d	NOAEC	0.12	15	0.027	Natural tropical soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and	BB	R4/C1	de Menezes Oliveira *et al.* (2018)

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
								10 % of fine, medium, and coarse sand, respectively			
Folsomia candida (Collembola)	Score 250 EC (250 g/L a.s.)	reproduction	28 d	EC10	11.35	15	2.57	Natural tropical soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively	BB	R3/C1	de Menezes Oliveira *et al.* (2018)
Folsomia candida (Collembola)	Score 250 EC (250 g/L a.s.)	reproduction	28 d (exposure 2 h after application)	NOEC	n.a. (≥ Appl. rate: 20 g a.s./ha)	11.06	n.a.	Natural soil (lattosolo; cation exchange capacity (CEC) 3.52 (cmolc/kg); 35 % clay, 21 % silt, 22 % fine sand, 20 % medium sand, 2 % coarse sand)	K, D	R4/C1	Pitombeira de Figueirêdo *et al.* (2020)
Folsomia candida (Collembola)	Score 250 EC (250 g/L a.s.)	reproduction	28 d (exposure 3 d after the 3rd application)	NOEC	n.a. (≥ Appl. rate: 3 x 20 g a.s./ha; 7 d intervals)	11.06	n.a.	Natural soil (lattosolo; cation exchange capacity (CEC) 3.52 (cmolc/kg); 35 % clay, 21 % silt, 22 % fine sand, 20 % medium sand, 2 % coarse sand)	K, D	R4/C1	Pitombeira de Figueirêdo *et al.* (2020)
***Hypoaspis aculeifer* (Mite)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**14 d**	**EC10**	**14.2**	**5**	**9.66**	**Artificial soil: 5 % sphagnum peat, 20% kaolinite clay, 74.8 % industrial quartz sand and 0.2 % calcium carbonate**	**H, L**	R1/C1	**Schulz (2015b) cited in EC (2019), Vol. 3CP B.9.7.2.1/02, SCORE 250 EC (A7402T), p.123**
***Hypoaspis aculeifer* (Mite)**	**Score 250 EC (250 g/L a.s.)**	**reproduction**	**14 d**	**NOAEC**	**75**	**15**	**17.0**	**Natural tropical soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively**	**BB**	**R2/C1**	**de Menezes Oliveira *et al.* (2018)**
		Geom. mean					***12.8***				

Proposed SGV for difenoconazole

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Hypoaspis aculeifer **(Mite)**	**Score 250 EC (250 g/L a.s.)**	**adult mortality**	**14 d**	**NOEC**	**76.7**	**5**	**52.2**	**Artificial soil: 5% sphagnum peat, 20% kaolinite clay, 74.8% industrial quartz sand and 0.2% calcium carbonate**	**F**	**1**	**Schulz (2015b) cited in EC (2019), Vol. 3CP B.9.7.2.1/02, SCORE 250 EC (A7402T), p.123**
Hypoaspis aculeifer (Mite)	Score 250 EC (250 g/L a.s.)	reproduction	14 d	NOEC	13.1	5	8.91	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.8 % industrial quartz sand and 0.2 % calcium carbonate	H, L	R1/C1	Schulz (2015b) cited in EC (2019), Vol. 3CP B.9.7.2.1/02, SCORE 250 EC (A7402T), p.123
Hypoaspis aculeifer (Mite)	Difenoconazole 250 EC (250 g/L a.s.)	reproduction	14 d	NOEC	≥ 70	5	≥ 47.6	Artificial soil: 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 74.7 % quartz sand	CC, D, M	R1/C1	Jansen 2016 cited in EC (2019), Vol. 3CP B.9.7.2.1/02, DIFCOR 250 EC, p.104; Anonymous (2016) accessed via (EFSA 2024)
Hypoaspis aculeifer (Mite)	Difenoconazole 250 EC (250 g/L a.s.)	adult mortality	14 d	NOEC	≥ 70	5	≥ 47.6	Artificial soil: 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate, 74.7 % quartz sand	CC, D, M	R1/C1	Jansen 2016 cited in EC (2019), Vol. 3CP B.9.7.2.1/02, DIFCOR 250 EC, p.104; Anonymous (2016) accessed via (EFSA 2024)
Hypoaspis aculeifer (Mite)	A9142L (30 g/L a.s.)	reproduction	14 d	NOEC	≥ 27.8	5	≥ 18.9	[CPIR]	CC	R1/C1	Anonymous cited in EC (2019), Vol. 1, Level 2, List of endpoints, Difenoconazole, p.207; Anonymous (2015) accessed via EFSA (2024)
Hypoaspis aculeifer (Mite)	A9142L (30 g/L a.s.)	adult mortality	14 d	NOEC	[CPIR]	5	[CPIR]	[CPIR]	CC	R1/C2	Anonymous (2015) accessed via EFSA (2024)
Marasmius oreades (Fungi)	Difenoconazole (a.s.)	mycelial growth	6 d	NOEC	1.64	n.a.	n.a.	Loamy sand soil	O	R1/C3	Grade (2000) cited in EC (2019), Vol. 3CA B.9.5/02, p.251

Proposed SGV for difenoconazole

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Mucor circinelloides (Fungi)	Difenoconazole (a.s.)	mycelial growth	3 d	NOEC	4.9	n.a.	n.a.	Loamy sand soil	O	R1/C3	Grade (2000) cited in EC (2019), Vol. 3CA B.9.5/02, p.251
Paecilomyces marquandii (Fungi)	Difenoconazole (a.s.)	mycelial growth	17 d	NOEC	≥ 16.4	n.a.	n.a.	Loamy sand soil	O	R1/C3	Grade (2000) cited in EC (2019), Vol. 3CA B.9.5/02, p.251
Phytophtora nicotianae (Fungi)	Difenoconazole (a.s.)	mycelial growth	17 d	NOEC	≥ 16.4	n.a.	n.a.	Loamy sand soil	O	R1/C3	Grade (2000) cited in EC (2019), Vol. 3CA B.9.5/02, p.251
Fungi spp.	Score 250 EC (250 g/L a.s.)	population (CFU)	20 d	NOEC	< 0.44	n.r.	n.a.	Natural soil	N	R4/C1	Meenakshi *et al.* (2007)
Actinomycetes spp. (Fungi)	Score 250 EC (250 g/L a.s.)	population (CFU)	20 d	NOEC	> 22	n.r.	n.a.	Natural soil	N	R4/C1	Meenakshi *et al.* (2007)
Bacteria spp.	Score 250 EC (250 g/L a.s.)	population (CFU)	20 d	NOEC	> 22	n.r.	n.a.	Natural soil	N	R4/C1	Meenakshi *et al.* (2007)
Bacteria spp. (Microorganisms)	Difenoconazole (a.s.)	Bacterial community diversity	60 d	NOEC	< 3.75	1	< 12.6	Soil "LF": Natural soil: loam, pH 8.29, Total N 0.07 %, CEC 14.60 cmol/kg	P	R3/C1	Zhang *et al.* (2021)
Bacteria spp. (Microorganisms)	Difenoconazole (a.s.)	Bacterial community diversity	60 d	NOEC	< 3.75	1.32	< 9.66	Soil "HF": Natural soil: clay, pH 5.24, Total N 0.06 %, CEC 6.02 cmol/kg	P	R3/C1	Zhang *et al.* (2021)
Bacteria spp. (Microorganisms)	Difenoconazole (a.s.)	Bacterial community diversity	60 d	NOEC	≥ 10	4.84	≥ 7.02	Soil "CJ": Natural soil: clay, pH 4.37, Total N 0.26 %, CEC 14.15 cmol/kg	P	R3/C1	Zhang *et al.* (2021)
Bacteria spp. (Microorganisms)	Difenoconazole (a.s.)	Bacterial community diversity	60 d	NOEC	< 3.75	3.10	< 4.11	Soil "HZ": Natural soil: silty clay loam, pH 6.80, Total N 0.14 %, CEC 10.6 cmol/kg	P	R3/C1	Zhang *et al.* (2021)

Proposed SGV for difenoconazole

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Bacteria spp. (Microorganisms)	Difenoconazole (a.s.)	Bacterial community diversity	60 d	NOEC	3.75	5.02	2.54	Soil "JX": Natural soil: silty clay loam, , pH 6.52, Total N 0.28 %, CEC 21.30 cmol/kg	P	R3/C1	Zhang *et al.* (2021)
Azotobacter vinelandi (Bacteria)	Score 250 EC (250 g/L a.s.)	abundance	7 d	NOEC	37	n.r.	n.a.	Natural soil	N, Q	R4/C2	Filimon *et al.* (2018)
Clostridium sp. (Bacteria)	Score 250 EC (250 g/L a.s.)	abundance	7 d	NOAEC	≥ 150	n.r.	n.a.	Natural soil	N, Q	R4/C2	Filimon *et al.* (2018)
Ammonifying bacteria	Score 250 EC (250 g/L a.s.)	abundance	7 d	NOEC	< 37	n.r.	n.a.	Natural soil	N, Q	R4/C2	Filimon *et al.* (2018)
Nitrifying bacteria	Score 250 EC (250 g/L a.s.)	abundance	7 d	NOEC	< 37	n.r.	n.a.	Natural soil	N, Q	R4/C2	Filimon *et al.* (2018)
Denitrifying bacteria	Score 250 EC (250 g/L a.s.)	abundance	7 d	NOEC	37	n.r.	n.a.	Natural soil	N, Q	R4/C2	Filimon *et al.* (2018)
Bacteria spp.	Score 250 EC (250 g/L a.s.)	Population (CFU)	7 d	NOEC	< 37	n.r.	n.a.	Natural soil	N, Q	R4/C2	Filimon *et al.* (2018)
Microorganisms	**Score 250 EC (250 g/L a.s.)**	**Respiratory quotient (Q_R)[FE]**	**90 d**	**NOEC**	**< 3.54**	**2.89 (1.7 % OC)**	**< 4.16**	**Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5%), pH 8.3, 2.3 g total N/kg dw, C/N ratio 7.8, conductivity 0.18 dS/m**	**R, D**	**R2/C1**	**Muñoz-Leoz *et al.* (2013)**
Microorganisms	Score 250 EC (250 g/L a.s.)	Potentially mineralizable nitrogen (N_{min}) [FE]	90 d	NOEC	≥ 3.54	2.89 (1.7 % OC)	≥ 4.16	Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5 %), pH 8.3, 2.3 g total N/kg dw, C/N ratio 7.8, conductivity 0.18 dS/m	R, D	R2/C1	Muñoz-Leoz *et al.* (2013)
Microorganisms	Score 250 EC (250 g/L a.s.)	Nitrification rate (NH_4^+ cc.)[FE]	90 d	NOEC	≥ 472	2.89 (1.7 % OC)	≥ 555	Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5 %), pH 8.3, 2.3 g total N/kg dw,	R, D	R2/C1	Muñoz-Leoz *et al.* (2013)

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
								C/N ratio 7.8, conductivity 0.18 dS/m			
Microorganisms	**Score 250 EC (250 g/L a.s.)**	**Nitrification rate (NO_3^- cc.)[FE]**	**90 d**	**NOEC**	**< 3.54**	**2.89 (1.7 % OC)**	**< 4.16**	**Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5 %), pH 8.3, 2.3 g total N/kg dw, C/N ratio 7.8, conductivity 0.18 dS/m**	**R, D**	**R2/C1**	**Muñoz-Leoz *et al.* (2013)**
Microorganisms	Score 250 EC (250 g/L a.s.)	Treated-soil quality index (T-SQI)[EE]	90 d	NOEC	≥ 45.9	2.89 (1.7 % OC)	≥ 54.0	Natural soil: clay-loam (sand-clay-silt : 29.8-38.7-31.5 %), pH 8.3, 2.3 g total N/kg dw, C/N ratio 7.8, conductivity 0.18 dS/m	R	R2/C3	Muñoz-Leoz *et al.* (2013)
Microorganisms	Score 250 EC (250 g/L a.s.)	Dehydrogenase, urease, phosphatase and protease activity[EE]	21 d	NOEC	< 37	n.r.	n.a.	Natural soil: pH 6.44	N, S, T	R4/C1	Filimon *et al.* (2015)
Microorganisms	Difenoconazole (a.s.)	Nitrogen transformation (nitrification; (unamended soil)[FE]	28 d	< 25 % effect	≥ 16.7	3.23 (1.9 % OC)	≥ 17.6	Natural soil: Collombey loamy sand (sand-clay-silt: 83-2.8-14.7 %), pH 7.3, microbial biomass 53.6 mgC/100 g soil, max. WHC 47 %	CC, F, U	4 (C1/R4)	Ellgehausen (1990) cited in EC (2019), Vol. 3CA B.9.5/01, p.248; Anonymous (1990) accessed via (EFSA 2024)
Microorganisms	Difenoconazole (a.s.)	Nitrogen transformation (nitrification; (amended soil)[FE]	28 d	< 25 % effect	≥ 16.7	3.23 (1.9 % OC)	≥ 17.6	Natural soil: Collombey loamy sand (sand-clay-silt : 83-2.8-14.7 %), pH 7.3, microbial biomass 53.6 mgC/100 g soil, max. WHC 47 % Amendment: Lucerne meal	CC, F, U	4 (C1/R4)	Ellgehausen (1990) cited in EC (2019), Vol. 3CA B.9.5/01, p.248; Anonymous (1990) accessed via (EFSA 2024)
Microorganisms	Difenoconazole (a.s.)	Nitrogen transformation	28 d	< 25 % effect	≥ 16.7	3.23 (1.9 % OC)	≥ 17.6	Natural soil: Collombey loamy sand (sand-clay-silt:	CC, F, U	4 (C1/R4)	Ellgehausen (1990) cited in EC (2019), Vol. 3CA

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
		(nitrification; (amended soil)[FE]						83-2.8-14.7 %), pH 7.3, microbial biomass 53.6 mgC/100 g soil, max. WHC 47 % Amendment: Ammonium sulphate			B.9.5/01 , p.248; Anonymous (1990) accessed via (EFSA 2024)
Microorganisms	Difenoconazole (a.s.)	Nitrogen transformation (nitrification; (unamended soil)[FE]	28 d	< 25 % effect	≥ 16.7	3.06 (1.8 % OC)	≥ 18.6	Natural soil: Les Evouettes silty loam (sand-clay-silt: 58-11-31 %), pH 5.4, microbial biomass 93.4 mgC/100 g soil, max. WHC 86 %	CC, F, U	4 (C1/R4)	Ellgehausen (1990) cited in EC (2019), Vol. 3CA B.9.5/01 , p.248; Anonymous (1990) accessed via (EFSA 2024)
Microorganisms	Difenoconazole (a.s.)	Nitrogen transformation (nitrification; (amended soil)[FE]	28 d	< 25 % effect	≥ 16.7	3.06 (1.8 % OC)	≥ 18.6	Natural soil: Les Evouettes silty loam (sand-clay-silt: 58-11-31 %), pH 5.4, microbial biomass 93.4 mgC/100 g soil, max. WHC 86 % Amendment: Lucerne meal	CC, F, U	4 (C1/R4)	Ellgehausen (1990) cited in EC (2019), Vol. 3CA B.9.5/01 , p.248; Anonymous (1990) accessed via (EFSA 2024)
Microorganisms	Difenoconazole (a.s.)	Nitrogen transformation (nitrification; (amended soil)[FE]	28 d	< 25 % effect	≥ 16.7	3.06 (1.8 % OC)	≥ 18.6	Natural soil: Les Evouettes silty loam (sand-clay-silt: 58-11-31 %), pH 5.4, microbial biomass 93.4 mgC/100 g soil, max. WHC 86 % Amendment: Ammonium sulphate	CC, F, U	4 (C1/R4)	Ellgehausen (1990) cited in EC (2019), Vol. 3CA B.9.5/01 , p.248; Anonymous (1990) accessed via (EFSA 2024)
Microorganisms	Difenoconazole (a.s.)	Basal and substrate-induced respiration[FE]	28d	< 25 % effect	≥ 16.7	3.23 (1.9 % OC)	≥ 17.6	Natural soil: Collombey loamy sand (sand-clay-silt: 83-2.8-14.7 %), pH 7.3, microbial biomass 53.6 mgC/100 g soil, max. WHC 47 % Amendment: Lucerne meal	CC, F, U	4 (C1/R4)	Ellgehausen (1990) cited in EC (2019), Vol. 3CA B.9.5/01 , p.248; Anonymous (1990) accessed via (EFSA 2024)
Microorganisms	Difenoconazole (a.s.)	Basal and substrate-	28d	< 25 % effect	≥ 16.7	3.06 (1.8 % OC)	≥ 18.6	Natural soil: Les Evouettes silty loam (sand-clay-silt: 58-	CC, F, U	4 (C1/R4)	Ellgehausen (1990) cited in EC (2019), Vol. 3CA

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
		induced respiration[FE]						11-31 %), pH 5.4, microbial biomass 93.4 mgC/100 g soil, max. WHC 86 % Amendment: Lucerne meal			B.9.5/01 , p.248; Anonymous (1990) accessed via (EFSA 2024)
Microorganisms	Difenoconazole 250 EC (250 g/L a.s.)	Nitrogen transformation[FE]	28 d	< 25 % effect	3.2	n.r.	n.a.	Natural soil. Amendment: 2.5 g Lucerne-grass-gree meal	N	R4/C1	Servajean (2009b) cited in EC (2019), Vol. 3CP B.9.9/01, DIFCOR 250 EC, p.108
Microorganisms	Score 250 EC (250 g/L a.s.)	Nitrogen transformation[FE]	28 d	< 25 % effect (NOEC)	2.04	[CPIR]	2.85	Natural soil: loamy sand, pH 6.3-6.6	CC, HH	R1/C1	Schulz (2016) cited in EC (2019), Vol. 3CP B.9.9/01, SCORE 250 EC (A7402T), p.131; Anonymous (2016) accessed via EFSA (2024)
Microorganisms	Score 250 EC (250 g/L a.s.)	Carbon transformation[FE]	28 d	< 25 % effect (NOEC)	2.04	[CPIR]	2.85	Natural soil: loamy sand, pH 6.3-6.6	CC, HH	R1/C1	Schulz (2016) cited in EC (2019), Vol. 3CP B.9.9/01, SCORE 250 EC (A7402T), p.131; Anonymous (2016) accessed via EFSA (2024)
Microorganisms	Score 250 EC (250 g/L a.s.)	Soil respiration[FE]	21 d	NOEC	≥ 44	n.r.	n.a.	Natural soil	N	R4/C1	Meenakshi *et al.* (2007)
Microorganisms	Score 250 EC (250 g/L a.s.)	Cellulase activity[EE]	28 d	NOEC	≥ 44	n.r.	n.a.	Natural soil	N	R4/C1	Meenakshi *et al.* (2007)
Microorganisms	Score 250 EC (250 g/L a.s.)	Xylanase and protease activity[EE]	14 d	NOEC	≥ 44	n.r.	n.a.	Natural soil	N	R4/C1	Meenakshi *et al.* (2007)
Microorganisms	Score 250 EC (250 g/L a.s.)	Urease activity[EE]	14 d	NOEC	0.44	n.r.	n.a.	Natural soil	N	R4/C1	Meenakshi *et al.* (2007)
Microorganisms	Score 250 EC (250 g/L a.s.)	Acid phosphatase activity[EE]	21 d	NOEC	≥ 44	n.r.	n.a.	Natural soil	N	R4/C1	Meenakshi *et al.* (2007)

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Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Avena sativa[M] *Lactuca sativa*[D] *Brassica napus*[D] *Triticum aestivum*[M] *Glycine max*[D] *Lepidium sativum*[D] (Terrestrial plants)	Difenoconazole (a.s.)	seedling emergence	21 d	NOEC	< 0.1 < 0.1 < 0.1 < 0.1 [CPIR] < 0.1	1.67 (0.98 % OC)	< 0.204 < 0.204 < 0.204 < 0.204 [CPIR] < 0.204	Standard loamy sand, type 2	CC, D, UU	R3/C1	Aniol (2009) cited in EC (2019), Vol. 3CA B.9.6.1/02, p.258; Anonymous (2009) accessed via EFSA (2024)
Avena sativa[M] (Terrestrial plants)	Difenoconazole (a.s.)	growth (shoot dry weight)	21 d	NOEC	< 0.1	1.67 (0.98 % OC)	< 0.204	Standard loamy sand, type 2	CC, D, UU	R3/C1	Aniol (2009) cited in EC (2019), Vol. 3CA B.9.6.1/02, p.258; Anonymous (2009) accessed via EFSA (2024)
Glycine max[D] *Lactuca sativa*[D] *Triticum aestivum*[M] (Terrestrial plants)	Difenoconazole (a.s.)	growth (shoot dry weight)	21 d	NOEC	0.1 0.1 0.1	1.67 (0.98 % OC)	0.204 0.204 0.204	Standard loamy sand, type 2	CC, UU	R3/C1	Aniol (2009) cited in EC (2019), Vol. 3CA B.9.6.1/02, p.258; Anonymous (2009) accessed via EFSA (2024)
Brassica napus[D] *Lepidium sativum*[D] (Terrestrial plants)	Difenoconazole (a.s.)	growth (shoot dry weight)	21 d	NOEC	10 10	1.67 (0.98 % OC)	20.4 20.4	Standard loamy sand, type 2	CC, UU	R3/C1	Aniol (2009) cited in EC (2019), Vol. 3CA B.9.6.1/02, p.258; Anonymous (2009) accessed via EFSA (2024)
Sinapis alba[D] (Terrestrial plants)	Difenoconazole 250 EC (250 g/L a.s.)	growth	n.r	NOER	n.a. (< Appl. rate: 250 g a.s./ha)	n.r.	n.a.	Natural soil: sandy loam	N, W	R4/C3	Servajean (2009c) cited in EC (2019), Vol. 3CP B.9.11.1/01, DIFCOR 250 EC, p.111
Triticum aestivum[M] *Allium cepa*[M] *Lactuca sativa*[D] *Daucus carota*[D]	Difenoconazole 250 EC (250 g/L a.s.)	growth	n.r	NOER	n.a. (≥ Appl. rate: 250 g a.s./ha)	n.r.	n.a.	Natural soil: sandy loam	N, W	R4/C3	Servajean (2009c) cited in EC (2019), Vol. 3CP B.9.11.1/01, DIFCOR 250 EC, p.111

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Lycopersicon esculentum[D] (Terrestrial plants)											
Beta vulgaris[D] *Zea mays*[M] *Brassica napus*[D] *Avena sativa*[M] *Allium cepa*[M] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	seedling emergence	14 d	NOER	n.a. (> Appl. rate: 100 g a.s./ha)	[CPIR]	n.a.	[CPIR]	CC, VV	R3/C1	Walder (2000) cited in EC (2019), Vol. 3CP B.9.11.2/04, SCORE 250 EC (A7402T), p.154; Anonymous (2000) accessed via EFSA (2024)
Glycine max[D] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	seedling emergence	14 d	NOER	n.a. (= Appl. rate: 3.13 g a.s./ha)	[CPIR]	n.a.	[CPIR]	CC, VV	R3/C1	Walder (2000) cited in EC (2019), Vol. 3CP B.9.11.2/04, SCORE 250 EC (A7402T), p.154; Anonymous (2000) accessed via EFSA (2024)
Avena sativa[M] *Allium cepa*[M] *Brassica napus*[D] *Beta vulgaris*[D] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	vegetative vigour	21 d	NOER	n.a. (> Appl. rate: 100 g a.s./ha)	[CPIR]	n.a.	[CPIR]	CC, VV, W	R3/C3	Walder (2000) cited in EC (2019), Vol. 3CP B.9.11.2/04, SCORE 250 EC (A7402T), p.154; Anonymous (2000) accessed via EFSA (2024)
Glycine max[D] *Zea mays*[M] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	vegetative vigour	21 d	NOER	n.a. (= Appl. rate: 3.13 g a.s./ha)	[CPIR]	n.a.	[CPIR]	CC, VV, W	R3/C3	Walder (2000) cited in EC (2019), Vol. 3CP B.9.11.2/04, SCORE 250 EC (A7402T), p.154; Anonymous (2000) accessed via EFSA (2024)
Avena sativa[M] *Brassica napus*[D] *Glycine max*[D] (Terrestrial plants)	Difenoconazole (a.s.)	seedling emergence	21 d	NOEC	≥ 10 ≥ 10 ≥ 10	[CPIR]	≥ 14.1 ≥ 14.1 ≥ 14.1	[CPIR]	CC	R2/C1	Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via EFSA (2024)

Proposed SGV for difenoconazole

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Avena sativa[M] (Terrestrial plants)	**Difenoconazole (a.s.)**	**biomass (shoot fresh weight)**	**21 d**	**NOEC**	**< 0.1**	**[CPIR]**	**< 0.141**	**[CPIR]**	**CC**	**R2/C1**	**Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via EFSA (2024)**
Brassica napus[D] (Terrestrial plants)	**Difenoconazole (a.s.)**	**biomass (shoot fresh weight)**	**21 d**	**NOEC**	**0.3**	**[CPIR]**	**0.423**	**[CPIR]**	**CC**	**R2/C1**	**Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via EFSA (2024)**
Glycine max[D] (Terrestrial plants)	**Difenoconazole (a.s.)**	**biomass (shoot fresh weight)**	**21 d**	**NOEC**	**3**	**[CPIR]**	**4.23**	**[CPIR]**	**CC**	**R2/C1**	**Balluff (2004) cited in EC (2019), Vol. 3CA B.9.6.1/01, p.256; Anonymous (2004) accessed via EFSA (2024)**
Allium cepa[M] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	growth (dry weight at 23 and 33ºC; stem length at 23ºC)	18 d	NOER	n.a. (< Appl. rates: 20 g a.s./ha; 3 applications; 7 d intervals)	11.06	n.a.	Natural soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively	X	R4/C3	Pitombeira de Figueirêdo *et al.* (2020)
Allium cepa[M] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	growth (fresh weight, 23 and 33ºC; stem length at 33ºC)	18 d	NOER	n.a. (≥ Appl. rates: 20 g a.s./ha; 3 applications; 7 d intervals)	11.06	n.a.	Natural soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively	X	R4/C3	Pitombeira de Figueirêdo *et al.* (2020)
Lycopersicon esculentum[D] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	growth (fresh and dry weight and stem length)	18 d	NOER	n.a. (≥ Appl. rates: 20 g a.s./ha; 3 applications; 7 d intervals)	11.06	n.a.	Natural soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively	X	R4/C3	Pitombeira de Figueirêdo *et al.* (2020)

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Lycopersicon esculentum[D] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	growth (major length)	18 d	NOER	n.a. (< Appl. rates: 20 g a.s./ha; 3 applications; 7 d intervals)	11.06	n.a.	Natural soil: CEC 3.33 (meq/100 g); 48 % clay; 12 % silt; and 19, 11, and 10 % of fine, medium, and coarse sand, respectively	X	R4/C3	Pitombeira de Figueirêdo *et al.* (2020)
Allium cepa[M] ***Lolium perenne***[M] ***Triticum aestivum***[M] ***Zea mays***[M] ***Brassica oleracea***[D] ***Glycine max***[D] ***Lycopersicon esculentum***[D] ***Raphanus sativus***[D] **(Terrestrial plants)**	**Score 250 EC (250 g/L a.s.)**	seedling emergence, growth (seedling height) and biomass (shoot dry weight)	**21 d**	**< 25 % effect**	**≥ 0.0933 (Appl. rate: 140 g a.s./ha; approx. 10 cm soil depth)**	**1.2**	**≥ 0.264** **≥ 0.264** **≥ 0.264** **≥ 0.264** **≥ 0.264** **≥ 0.264** **≥ 0.264** **≥ 0.264**	**Loamy sand: composed of kaolinite clay, industrial quartz sand, and peat. The soil consisted of 85 % sand, 6 % silt, and 9 % clay**	**F, Y**	**1**	**Porch et al. (2011) cited in EC (2019), Vol. 3CP B.9.11.1/01, SCORE 250 EC (A7402T), p.135**
Beta vulgaris[D] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	seedling emergence, growth (seedling height) and biomass (shoot dry weight)	21 d	< 25 % effect	≥ 0.0933 (Appl. rate: 140 g a.s./ha; approx. 10 cm soil depth)	1.2	≥ 0.264	Loamy sand: composed of kaolinite clay, industrial quartz sand, and peat. The soil consisted of 85 % sand, 6 % silt, and 9 % clay	F, V, Y	3	Porch et al. (2011) cited in EC (2019), Vol. 3CP B.9.11.1/01, SCORE 250 EC (A7402T), p.135
Lactuca sativa[D] (Terrestrial plants)	Score 250 EC (250 g/L a.s.)	seedling emergence, growth (seedling height) and biomass (shoot dry weight)	21 d	< 25 % effect	< 0.0933 (Appl. rate: 140 g a.s./ha; approx. 10 cm soil depth)	1.2	< 0.264	Loamy sand: composed of kaolinite clay, industrial quartz sand, and peat. The soil consisted of 85 % sand, 6 % silt, and 9 % clay	F, V, Y	3	Porch et al. (2011) cited in EC (2019), Vol. 3CP B.9.11.1/01, SCORE 250 EC (A7402T), p.135
Allium cepa[M] *Lolium perenne*[M]	Score 250 EC (250 g/L a.s.)	vegetative vigour	21 d	< 25 % effect	n.a. (= Appl. rate: 140 g a.s./ha)	1.8	n.a.	Sandy loam: composed of kaolin clay, industrial quartz sand and peat. The soil	W	C3	Porch et al. (2011a) cited in EC (2019), Vol. 3CP

Species (Taxonomic group)[8]	Test substance	Measured effect[9]	Duration	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Triticum aestivum[M] *Zea mays*[M] *Beta vulgaris*[D] *Brassica oleracea*[D] *Glycine max*[D] *Lactuca sativa*[D] *Lycopersicon esculentum*[D] *Raphanus sativus*[D] (Terrestrial plants)		(biomass, height)						consisted of 79 % sand, 9 % silt, and 12 % clay			B.9.11.1/02, SCORE 250 EC (A7402T), p.140

Table A2: Soil effect data for difenoconazole 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; [CPIR] – confidentially provided information, redacted. Values resulting from calculations are rounded to three significant figures.

Species (Taxonomic group)	Test substance	Measured effect[11]	Duration	Type of effect concentration	Effect value [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Earthworm	Difenoconazole 250 EC (250 g/L a.s.)	population abundance (total/species-specific number of adults/juveniles)	1 month	NOEC	≥ 0.317 (Appl. rate: 375 g a.s./ha)	[CPIR]	≥ 0.593	Field study/natural soil from Germany (silty loamy sand (DIN 4220) or loam (USDA)	CC, Z	R1/C1	Schulz (2015) cited in EC (2019), Vol. 3CP B.9.7.1.2/01, DIFCOR 250 EC, p.92; Anonymous (2015) accessed via EFSA (2024)
Earthworm	Difenoconazole 250 EC (250 g/L a.s.)	biomass (total/species-specific weight of adults/juveniles)	1 month	NOEC	≥ 0.317 (Appl. rate: 375 g a.s./ha)	[CPIR]	≥ 0.593	Field study/natural soil from Germany (silty loamy sand (DIN 4220) or loam (USDA)	CC, Z	R1/C1	Schulz (2015) cited in EC (2019), Vol. 3CP B.9.7.1.2/01, DIFCOR 250 EC, p.92; Anonymous (2015) accessed via EFSA (2024)

[11] [DE] – diversity endpoint, [EE] – enzymatic endpoint, [FE] – functional endpoint

Species (Taxonomic group)	Test substance	Measured effect[11]	Duration	Type of effect concentration	Effect value [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	Difenoconazole EC (265 g/L a.s.)	abundance (total/species-specific number of adults/juveniles)	1 month	NOEC	< (Appl. rate: 150 g a.s./ha)	[CPIR]	n.a.	Field study/natural soil from Germany: [CPIR]	CC, NN	R3/C1	Hamberger (2015) cited in EC (2019), Vol. 3CP B.9.7.1.2/01, SCORE 250 EC (A7402T), p.106; Anonymous (2015) accessed via EFSA (2024)
Eisenia fetida (Earthworm)	Difenoconazole EC (265 g/L a.s.)	biomass change (total/species-specific weight of adults/juveniles)	1 month	NOEC	< (Appl. rate: 150 g a.s./ha)	[CPIR]	n.a.	Field study/natural soil from Germany: [CPIR]	CC, NN	R3/C1	Hamberger (2015) cited in EC (2019), Vol. 3CP B.9.7.1.2/01, SCORE 250 EC (A7402T), p.106; Anonymous (2015) accessed via EFSA (2024)
Bacteria	Score 250 EC (250 g/L a.s.)	dehydrogenase activity (field study)[EE]	21 d	NOEC	< 37	n.r.	n.a.	Natural soil, pH 6.20	N	R4/C2	Filimon *et al.* (2015)
Bacteria	Score 250 EC (250 g/L a.s.)	urease activity (field study)[EE]	21 d	NOEC	75	n.r.	n.a.	Natural soil, pH 6.20	N	R4/C2	Filimon *et al.* (2015)
Bacteria	Score 250 EC (250 g/L a.s.)	phosphatase activity (field study)[EE]	21 d	NOEC	< 37	n.r.	n.a.	Natural soil, pH 6.20	N	R4/C2	Filimon *et al.* (2015)
Bacteria	Score 250 EC (250 g/L a.s.)	protease activity (field study)[EE]	21 d	NOEC	< 37	n.r.	n.a.	Natural soil, pH 6.20	N	R4/C2	Filimon *et al.* (2015)

Notes A1: Notes on soil studies for difenoconazole.

A	The study from Friedrich (2011) was statistically re-evaluated by Taylor and Allen (2016g); both cited in EC (2019), Vol. 3CA B.9.4.1/01 and 02. For reproduction, the robust EC10 is preferred over the NOEC. At concentration of the statistically robust NOEC, 15-21 % reduction in the mean number of juveniles occurred.

	For biomass (adult growth), the re-calculated EC10 was statistically not robust enough (too wide confidence intervals; the lower end of the CI for EC20 is lower than the median EC10; the steepness of the curve is shallow) and the RMS proposed the use of the EC10low. However, for the SGV dossier the statistically robust NOEC has been chosen as the most reliable endpoint. The mortality ranged in 0-7.5 % being the highest at the highest concentration. From the summary it is unclear if there was any significant effects; in the LoEP a NOEC of ≥ 8 mg a.s./kg is reported. In the absence of the detailed results it is not possible to re-evaluate the statistics for mortality. Although surrounded by some uncertainty, a NOEC of ≥ 8 mg a.s./kg is listed (R2/C2).
AA	The Applicant claimed that no statistically significant effects on mortality/ biomass change/reproduction were observed at the highest test concentration, thus all NOEC values were greater than values (and as a result not suitable for including them in a geometric mean). It should be noted that based on the 12 % decrease in the number of juveniles at the highest test concentration, the RMS proposed to set the NOEC for reproduction at the second highest test concentration of 3.80 mg a.s./kg. The statistical results – based on the summarised data (EC 2019) – were re-evaluated, confirmed and used accordingly for the SGV dossier, i.e. NOEC ≥ the highest test concentration for all measured effects. It is noted that although mortality endpoint was not derived officially, there was no mortality in any of the treatments or control and thus NOEC for mortality is also deemed to be ≥ the highest test concentration. The peat content of the artificial soil used in the test was not included in the study summary, but only listed in the LoEP, therefore there are some uncertainties about the normalised effect concentrations.
B	The statistics has been re-checked and it could be confirmed that the statistically robust NOEC for reproduction is 2.1 mg a.s./kg soil with p of 0.1393 (LOEC = 3.4 mg a.s./kg; p = 0.0003; GraphPad Prism 10 Version 10.0.2; one-way ANOVA with Dunnett's test; α = 0.05). At the level of NOEC 15 % reduction on reproduction was observed. No mortality endpoint was officially derived. The mean mortality ranged in 0-2.5 %, thus the NOEC for mortality is deemed to be ≥ the highest test concentration.
BB	NOAEC, LOAEC, EC10 and EC50 were reported in the study. The spacing of the tested concentrations did not follow the OECD recommendations of ≤ 1.8-fold (OECD 2016); the derived EC10 values could not be considered reliable (too wide confidence intervals). The NOAECs were selected in the case of potworm and mite for potentially deriving an SGV. For springtail, neither the EC10 (too wide confidence interval), nor the NOAEC has been found reliable: there is a 100-fold gap in the test concentrations right after the LOAEC with little increase in the effects at a 100 times higher concentration; there is almost a 100-fold difference between the EC10median and the proposed NOAEC and 30-fold difference between the EC10low and the NOAEC.
C	Study listed only in the "List of Endpoints" of the draft RAR (EC 2019). The summary and assessment of the study could not be found in the dossier, thus there is not enough information about the study for further consideration.
CC	The full study report was accessed on request for public access to documents (EFSA 2024). Please be aware that not all endpoints that could be derived from the original study report are included in the LoEP.
D	Unbound value, not suitable for including in a geometric mean. (Either no effects at the highest test concentration or significant effects already at the lowest test concentration.)
E	For Servajean (2015), an EC10 of 3.51 mg a.s./kg was calculated, however this effect concentration did not prove to be statistically robust (unacceptably wide normalised width of the confidence interval; EC20low < EC10median; shallow steepness of the curve). Based on the outcome of expert discussions on recurring issues in ecotoxicology (EFSA 2015), when EC10 < NOEC and the EC10 is not reliable it is advised that the lower limit of the EC10 confidence interval should be used (i.e. EC10low = 1.18 mg a.s./kg). However, during the commenting period the Co-RMS found the EC10 not valid and a NOEC of 2.62 mg a.s./kg was proposed for use in the risk assessment. This was not supported by statistics but based on < 10 % biological effects at that concentration. It should be noted that the renewal assessment has not been finalised yet. In addition, a NOEC of 7.71 mg a.s./kg was proposed by the applicant. The statistics has been re-evaluated for the NOEC for this SGV dossier and the applicant's proposal could be statistically confirmed (LOEC = 10.8 mg a.s./kg; p = 0.0006; GraphPad Prism 10 Version 10.0.2; one-way ANOVA with Dunnett's test; α = 0.05). This statistically robust NOEC is preferred over the unreliable EC10 (either median or lower bound of CI) or a statistically not robust NOEC that was based on biological effects alone. It is noted that there was 15 % reduction in the mean number of juveniles at the concentration of the statistically robust NOEC of 7.71 mg a.s./kg.

F	The assessment from the EC (2019) report was adopted and accepted without additional assessment (i.e. at face value).
G	Both NOEC and EC10 were reported and considered reliable by the RMS. As a precautionary approach, the lower NOEC value was selected for use in the EU risk assessment.
H	When both NOEC and EC10 are available and statistically acceptable, EC10 is preferred for SGV derivation due to the inherent uncertainties a NOEC is surrounded by (see Appendix 1; Azimonti *et al.* (2015a)).
HH	There are no statistically significant effects at end of the study for neither of the endpoints (at both concentrations), therefore the results at the higher tested concentration can be considered as greater-than/equal-to NOEC values (with overall effects in both tests at both concentrations after 28 days between -0.8 and +3.0 % as compared to the controls).
J	No reliable EC10/EC20/EC50 could be calculated by the applicant. There was no clear dose-response in the results. The access to the original study report and the statistical re-evaluation did not help with finding out what happened in the study, what could lead to such inconsistent results. Due to the high variation in the results and the lack of clear dose-response, the study results are not considered reliable and not used for SGV derivation.
JJ	Relevant and reliable study with no effects on reproduction at the highest concentration, i.e. at 27.8 mg a.s./kg soil dw; the EFSA endpoint was based on a not significant lower concentration.
K	Exposure 2 h after application: There are uncertainties about the actual difenoconazole concentration in the sampled soil that was used for the test (the upper 5 cm). On one hand, the way of application (spraying onto the soil surface without mixing it) and the sorption properties of difenoconazole (immobile to medium mobile) would indicate that 2 hours after the application the whole amount of the applied a.s. could be found in the sampled upper 5 cm. On the other hand, the treated soils were wetted with the amount of water that corresponds to 225 mm precipitation. Due to the uncertainties in the actual difenoconazole concentration in the sampled soil, the effect values could not be calculated per kg basis and therefore they are not suitable for SGV derivation. Exposure 3 d after the 3rd application: without analytical measurements it is difficult to reliably estimate the actual difenoconazole concentration in the sampled soil following 3 applications of 20 g a.s./ha with 7 d intervals therefore the results from this test are not suitable for SGV derivation.
L	A statistically robust NOEC of 13.1 mg a.s./kg was reported by the applicant. According to the RMS an effect above 10 % occurs at this concentration. Additionally, an EC10 of 14.2 mg a.s./kg was calculated. Since the NOEC was lower than the EC10 but the effects at the NOEC was above 10 %, the EC10low was suggested as an endpoint for use in the risk assessment in the EU. However, due to the clear dose-response and the tight confidence intervals of the EC10/EC20/EC50 values, the median EC10 is considered more suitable for SGV derivation.
M	The study results have been statistically re-evaluated based on the individual numbers per replicates. No statistically significant difference could be shown for the treatments, therefore the highest test concentration is considered for the endpoints. (In the dRAR, the reproductive endpoint was based on a concentration with < 10 % effects.)
N	Concentrations of total organic carbon or total organic matter were not reported in the study. For this reason, a normalised value cannot be calculated and the study is considered "*not assignable*" (R4).
NN	High standard deviation occurred throughout the study: e.g. effects > 40 % after a month without statistical significance (e.g. decrease in the number of L. terrestris at 1.8 L/ha, biomass of epilobous juveniles at 0.6 and 1.4 L/ha) as well as significant effect (i.e. 56 % decrease in the biomass of epilobous juveniles at 1.8 L/ha). As there were sigificant/considerable effects at all rates after a month, but recovery is not accepted for SGV derivation/retrospective RA, no endpoint could be derived and the study endpoints are not considered reliable. The soil was not sampled for the analytical verification from the upper 10 cm after the treatment as usually done, but had been sampled and dried before and spred on sheets on to the soil surface in approx. 3 cm thick layers just before the application and gathered afterwards. Therefore the recovery of the treatment might have been altered (probably improved) using this modified method. Thus the actual concentrations are not calculated from the given rates.
O	The results are considered not relevant (C3) as the tests were conducted on a mixture of loamy sand and maltextract agar. Also the concentrations were based on the amount of a.s. mixed into the soil, but after that agar was added too to the soil and topped onto the soil too.
P	In Zhang *et al.* (2021), the endpoints *bacterial community diversity*, *microbial network complexity* and *relative abundance* were reported for five soil types. Only the results related to the endpoint *bacterial community diversity (Shannon index)*, which were derived for each of the five tested soils separately, are considered suitable for further use in general and only these endpoints are shown here.

	However, no solvent control was included in the test, while difenoconazole was addid in an acetone solution. Consequently, the study results cannot be considered reliable and they are not used for an SGV derivation.
Q	Following the exposure in soil, the microorganisms were inoculated in selective culture mediums for analysis.
R	In Muñoz-Leoz *et al.* (2013), natural soil was spiked with different concentrations of difenoconazole (as well as deltamethrin and ethofumesate) and samples for multiple measurement endpoints were taken after 7, 30, 60 and 90 days of exposure. However, within the first 30 days, the control and the treatments showed high fluctuations as the spiked cultures needed time to acclimate to the new conditions. Due to the fluctuations during the first half of the experimental timeframe and the long-term focus of the project, the effects after 90 days of incubation were considered the most relevant. Therefore and in order to synthesize the study, only the results after 90 days were reported in the table. During the study, difenoconazole concentrations were monitored and the dissipation half-life (DissT50) of difenoconazole calculated with a bi-exponential model. The different difenoconazole concentrations resulted in different DissT50 values that were then used in a test-concentration-specific manner to calculate specific time-weighted average factors (f_{TWA}) and then the respective TWA concentrations over the study duration following the equations used in pesticide authorisation: $f_{TWA} = (1-e^{-kt})/kt$ *Where*: e – Euler's number; k – rate constant (ln2/DissT50); t – averaging interval $AS(t) = f_{TWA} * \text{test concentration at } t_0$ *Where*: AS(t) – the TWA active substance concentration over the averaging interval; t_0 – the test concentration at the beginning For potentially mineralizable nitrogen (N_{min}), the statistically robust, normalised NOEC of 54.0 mg a.s./kg soil potentially includes an estimated 26-29 % effect. According to the BPR GD (ECHA 2017), for terrestrial microorganisms if a statistical difference is found and the effect is > 15 %, no NOEC can be derived from tests run with control and 2 test concentrations (here there are 3 test concentrations, but also with a spacing factor of 10). If in at least one concentration no statistical difference from the control is found and the effect value is ≤ 15 %, the concentration can be considered the NOEC. At normalised concentration of 4.16 mg a.s./kg soil, approximately 17-19 % effect can be estimated based on the height of the respective columns in Figure 3 in Muñoz-Leoz *et al.* (2013). As this is a very rough estimation, this concentration is considered acceptable as a NOEC for potentially mineralizable nitrogen. Due to the high spacing factors, the no effect concentration is presented as a ≥ value. Treated-soil quality index (T-SQI) is an enzyme-based index of microbial functional diversity. Considering its integrative quality of the other parameters, it is scored as not relevant (C3). Due to the high spacing factors, the no-effect concentration is presented as a ≥ value.
S	Relevant information about the soil properties and the controls was missing.
T	In Filimon *et al.* (2015), microbial enzymatic activity was assessed either under laboratory or under field conditions. Results of the laboratory conditions are reported in Appendix 2 Data on the active substance, Table A1; and results of the field conditions in Table A2.
U	The fulfilment of the validity criteria was not fully reported. Therefore the study was considered as "*not assignable*" (R4).
UU	In this study concentrations between 0.1 and 10 mg a.s./kg soil (non-normalised values) were tested for six plant species. However, the results did not follow a dose-response. Due to the high variation and up-and-downs in the results, no reliable endpoints could be derived (R3).
V	The validity criteria as specified in the relevant guideline were not met.
VV	Non-guideline, non-GLP study with semi-quantitative results. There was not enough number of replicates. Instead of actual numbers, a scoring was invented that included phytotoxicity effects. The results are not suitable for quantitative uses.
W	The only exposure route considered relevant for this report is *via* soil, other ways of exposure (e.g. foliar application) are out of the scope of the SGV derivation. Thus this study is considered "*not relevant*" (C3). It is noted that through mixed or foliar exposure, an exposure through soil in term of mg a.s./kg soil cannot be calculated.

X	The seeds were planted on the day of the first application (i.e. exposure was through soil). However, later two other applications also took place (with 7-day intervals) in the form of spraying (i.e. foliar exposure of the emerging seedlings). For the SGV derivation, only toxicity data following soil exposure exclusively are considered.
Y	Soil was over-sprayed after seeds had been planted. Based on the size of the pots used in the test and the volume of soil they contained, a 10 cm soil depth could be used for calculation of the concentration as mg a.s./kg soil.
Z	Effect value is based on mean initial measurement of the applied amount of difenoconazole.

Appendix 3 Data on the metabolites

Table A3: Soil effect data for CGA 205375 (aka Metabolite 2 or CGA 211391), a transformation product of difenoconazole. 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; WHC – water holding capacity; OC – organic carbon; OM – organic matter; CFU – colony forming units. For notes, please refer to the end of Appendix 3 (Notes A2).

Species (Taxonomic group)	Measured effect[12]	Duration	Type of effect concentration	Effect value [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida **(Earthworm)**	**reproduction**	**8 weeks**	**NOEC**	**≥ 9.6**	**10**	**≥ 3.26**	**Artificial soil**	**F, I**	**1**	**Friedrich (2006) cited in EC (2019), Vol. 3CA B.9.4.1/06, p.213**
Eisenia fetida (Earthworm)	reproduction	8 weeks	NOEC	1	5	0.680	Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.5 % industrial fine sand (> 50 % of the particles between 0.05 mm and 0.2 mm) and 0.5 % calcium carbonate	F, GG	3	Sacker (2009b) cited in EC (2019), Vol. 3CA B.9.4.1/08, p.217
Folsomia candida **(Collembola)**	**reproduction**	**28 d**	**NOEC**	**2.4**	**10**	**0.816**	**Artificial crumbly structured soil**	**DD**	**1**	**Friedrich (2006a) cited in EC (2019), Vol. 3CA B.9.4.3.1/03, p.235**
Hypoaspis aculeifer **(Mite)**	**reproduction, mortality**	**14 d**	**NOEC**	**≥ 19.2**	**5**	**≥ 13.1**	**Artificial soil: 5 % sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand and 0.2 % calcium carbonate**	**F**	**1**	**Schulz (2015a) cited in EC (2019), Vol. 3CA B.9.4.3.1/07, p.242**

[12] DE – diversity endpoint, EE – enzymatic endpoint, FE – functional endpoint

Species (Taxonomic group)	Measured effect[12]	Duration	Type of effect concentration	Effect value [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Microorganisms	nitrogen transformation (nitrification; amended soil)^{FE}	28 d	< 25 % effect	0.22	1.12 (0.66 % OC)	0.667	Sandy loam soil, Speyer 2.3. Amendment: Lucerne meal	U, EE	4	Seyfried (2002) cited in EC (2019), Vol. 3CA B.9.5/04, p.254
Microorganisms	respiration rate (amended soil)^{FE}	28 d	< 25 % effect	0.22	1.12 (0.66 % OC)	0.667	Sandy loam soil, Speyer 2.3. Amendment: glucose/talc mixture	U, EE	4	Seyfried (2002) cited in EC (2019), Vol. 3CA B.9.5/04, p.254

Table A4: Soil effect data for 1,2,4-triazole (aka CGA 71019), a transformation product of difenoconazole. 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; WHC – water holding capacity; OC – organic carbon; OM – organic matter; CFU – colony forming units. For notes to the studies, please refer to the end of Appendix 3 (Notes A2).

Species (Taxonomic group)	Measured effect[13]	Duration	Type of effect concentration	Effect value [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	reproduction	8 weeks	NOEC	≥ 0.071	n.r.	n.a.	n.r.	N	R4/C1	Ehlers (2000) cited in EC (2019), Vol. 3CA B.9.4.1/09, p.220
***Eisenia fetida* (Earthworm)**	**reproduction**	**8 weeks**	**NOEC**	**≥ 1**	**10**	**≥ 0.340**	**Artificial soil (OECD 1984 standard) with 10 % peat**	**F**	**1**	**Moser and Scheffczyk (2004) cited in EC (2019), Vol. 3CA B.9.4.1/10, p.221**
***Folsomia candida* (Collembola)**	**reproduction**	**28 d**	**NOEC**	**1.8**	**10**	**0.612**	**Artificial soil**	**I, DD**	**R1/C1**	**Moser and Scheffczyk (2002) cited in EC (2019), Vol. 3CA B.9.4.3.1/05, p.239**
Folsomia candida (Collembola)	reproduction	28 d	EC_{20low}	0.13	10	0.044	Artificial soil	I, FF	R3/C3	Friedrich (2014a) recalculation of results from Moser and Scheffczyk (2002) cited in EC

[13] ^{DE} – diversity endpoint, ^{EE} – enzymatic endpoint, ^{FE} – functional endpoint

Species (Taxonomic group)	Measured effect[13]	Duration	Type of effect concentration	Effect value [mg a.s./kg soil]	Total OM [%]	Normalised effect value [mg a.s./kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
										(2019), Vol. 3CA B.9.4.3.1/06, p.240
Hypoaspis aculeifer (Mite)	reproduction	14 d	NOEC	171	5	116	Artificial soil: 5 % sphagnum peat, 20 % kaolin clay, 74.7 % industrial quartz sand and 0.2 % calcium carbonate	H	R1/C1	Schulz (2014) cited in EC (2019), Vol. 3CA B.9.8.4.2.1/07, p.245
***Hypoaspis aculeifer* (Mite)**	**reproduction**	**14 d**	**EC10**	**190**	**5**	**129**	**Artificial soil: comprising 5 % sphagnum peat, 20 % kaolin clay, 74.7 % industrial quartz sand and 0.2 % calcium carbonate**	**H**	**R1/C1**	**Schulz (2014) cited in EC (2019), Vol. 3CA B.9.8.4.2.1/07, p.245**
Microorganisms	Nitrogen transformation (nitrification; amended soil)[FE]	28 d	< 25 % effect	0.353	1.1 (0.66 % OC)	1.1	Sandy-loam soil, Speyer 2.3. Amendment: Lucerne meal	U, EE	4	Völkel (2000) cited in EC (2019), Vol. 3CA B.9.5/03, p.252
Microorganisms	Respiration rate (amended soil)[FE]	28 d	< 25 % effect	0.353	1.1 (0.66 % OC)	1.1	Sandy-loam soil, Speyer 2.3. Amendment : glucose/talc mixture	U, EE	4	Völkel (2000) cited in EC (2019), Vol. 3CA B.9.5/03, p.252

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

F	The assessment from the EC (2019) report was adopted and accepted without additional assessment (i.e. at face value).
H	When both NOEC and EC10 are available and statistically acceptable, EC10 is preferred for SGV derivation due to the inherent uncertainties a NOEC is surrounded by (see Appendix 1; Azimonti *et al.* (2015a).
I	In the study description (EC 2019), the organic matter content of the soil is not reported. However, the List of Endpoints contains the information. Thus, the organic matter value refers to the information available in the List of Endpoints.
N	Total organic carbon or organic matter content were not reported in the study. For this reason, a normalised value cannot be calculated and the study is considered "*not assignable*" (R4).
U	The fulfilment of the validity criteria was not fully reported. Therefore the study was considered as "*not assignable*".

DD	The statistically robust NOEC is accepted for further use in the SGV derivation.
EE	Concentrations of total organic carbon or total organic matter were not reported in the study. However, the standard artificial soil used in the experiment was reported and the information about the organic carbon content could be retrieved (https://www.lufa-speyer.de/images/stories/V5-Chemical_and_physical_of_standard_soils_according_to_GLP14.07.2022.pdf).
FF	No EC10 and EC50 could be calculated. The calculated EC20 alone is not considered sufficient to derive a reliable long-term endpoint from the study. In addition, the normalised width of the confidence interval of the EC20 (1.74) falls into the *poor* category (see Appendix E in EFSA 2019).
GG	Limit test, the control group does not fulfil the validity criterion for coefficient of variation with regard to reproduction.