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SGV – Proposal by the Ecotox Centre for

Tebufenozide

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Policy disclaimer

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

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

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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 tebufenozide 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 tebufenozide of **310 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 Tebufenozide 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 Tebufenozide von **310 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 tébufénozide 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 tébufénozide de **310 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 tebufenozide 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 tebufenozide di **310 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 pesticide active substance tebufenozide 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), Initial risk assessment provided by the rapporteur Member State Germany for the existing active substance tebufenozide of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC.*
- *EC (2009): Additional Report to the Draft Assessment Report on the active substance tebufenozide prepared by the rapporteur Member State Germany in the framework of Commission Regulation (EC) No 33/2008.*
- *EC (2010): Final Addendum to the Draft Assessment Report (DAR) and Additional Report. Risk assessment provided by the rapporteur Member State Germany for the existing active substance tebufenozide of the third stage Part A of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC and upon resubmission in the framework of the accelerated procedure as laid down in Commission Regulation (EC) No 33/2008. September 2010.*
- *EFSA (2010): Conclusion on the peer review of the pesticide risk assessment of the active substance tebufenozide. European Food Safety Authority. EFSA Journal, 8:12, 1871.*
- *US EPA (2015b): Transmittal of Preliminary Environmental Fate and Ecological Risk Assessment for Registration Review of the Insect Growth Regulator, Tebufenozide. September 17, 2015. PC Code: 129026. DP Barcode: D414537. Doc ID: EPA-HQ-OPP-2008-0824-0028.*[1]
- *US EPA (2015a): Tebufenozide: Literature Data Cited in the September 2015. Preliminary Environmental Fate and Ecological Risk Assessment. Doc ID: EPA-HQ-OPP-2008-0824-0029.*
- *EC (2018): Confirmatory Information - Tebufenozide. Addendum to the Additional Report of 18 November 2009. Rapporteur Member State: Germany, 06 March 2018.*
- *Nisso (2021): Dossier for the renewal of the active substance Tebufenozide, Nisso Chemical Europe GmbH.*

A draft assessment report (DAR; EC 2006) with several amendments (EC 2009, 2010) are available for the active substance and a representative product, on which the EFSA conclusion was based (EFSA 2010). The later summarised confirmatory information (EC 2018) did not result in a renewed EFSA conclusion. The representative formulated product for the evaluation in the EU was a 240 g/L suspension concentrate (SC) formulation registered under different names in Europe (e.g. Confirm, Confirm 2F, Mimic, Mimic 2F, Confirm 240F) (EC 2006, EFSA 2010).

At the end of the first peer review process of the DAR (EC 2006) the applicant withdrew the support for the inclusion in Annex I to Council Directive 91/414/EEC. Later the applicant made a resubmission application including additional data (EFSA 2010) that subsequently resulted in the inclusion of the substance in Annex I (EC 2011). Since then, tebufenozide got included in the framework of the 4th European program for the renewal of approvals of pesticide active substances (AIR IV, Group 4 (2) – *Substances with current expiry dates between 31 July 2019 and 31 December 2021 that will be postponed three years*) under Regulation (EC) No 1107/2009, for which a new dossier was submitted in the EU (Nisso 2021). The public version of the dossier for the representative product is available online and if necessary (e.g. newly submitted study, insufficient information in the study summary for

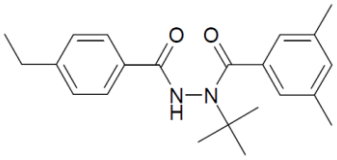
[1] US EPA documents are included for checking the completion of the data that were submitted to the EU or found through literature search. Recently it has been revealed that some manufacturers did not hand in all the studies to EFSA that they handed in to EPA (Mie & Rudén, 2023). However, data presented in US EPA documents are usually of limited use as these documents do not contain enough details to consider the relevance and reliability of a study.

a previously evaluated study) the study evaluations are reconsidered and/or amended based on the available original study reports. The active substance part of the new dossier is not available publicly.

1.1 Identity and physico-chemical properties

Tebufenozide (CAS 112410-23-8; development code number: RH-5992) is a carbohydrazide/ diacylhydrasine insecticide. Its provisional minimum purity as manufactured is ≥ 97 % (≥ 970 g/kg) (EC 2006, Lewis 2016). The technical grade material contains the relevant impurity *t*-butyl hydrazine in maximum 0.001 g/kg amount (EFSA 2010). The pure material (99.6 %) is a white powder, the technical material (97.5 %) is an off-white powder with low water solubility (see further details on physical-chemical properties in Table 1 below).

Table 1: Identification and physico-chemical properties of tebufenozide.

Characteristics	Values	References
Common name	Tebufenozide	EC (2006) and EFSA (2010)
Producer's development code number	RH-5992	EC (2006)
IUPAC name	N-tert-butyl-N′-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide	EFSA (2010)
Chemical group	Carbohydrazide compound	Lewis (2016)
Structural formula		EFSA (2010)
Molecular formula	$C_{22}H_{28}N_2O_2$	EFSA (2010)
CAS	112410-23-8	EFSA (2010)
EC Number	412-850-3	Lewis (2016)
SMILES code (canonical SMILES)	CCC1=CC=C(C=C1)C(=O)NN(C(=O)C2=CC(=CC(=C2)C)C)C(C)(C)C	Lewis (2016)
International Chemical Identifier key (InChIKey)	QYPNKSZPJQQLRK-UHFFFAOYSA-N	Lewis (2016)
Molecular weight [g/mol]	352.5	EFSA (2010)
Melting point [°C]	191-192 (purity 99.6 %)	EFSA (2010)
Boiling point [°C]	Decomposition in the range of 200 to 300°C (99.6 % purity)	EFSA (2010)
Solubility		
Water solubility [mg/L]	0.83 (purified water pH 6.5, 25°C, 99.6 % purity) No pH-dependence is expected	EFSA (2010)
Solubility in organic solvents [g/L]	Acetone: 75 Acetonitrile: 30 *n*-Butyl acetate: 16 Ethyl acetate: 24 *n*-Hexane: < 1 Methanol: 130 Methylene chloride: 460 Toluene: 3.2 (purity 97.5 %; 25°C)	EFSA (2010)
Dissociation constant (pKa)	No dissociation expected	EFSA (2010)
Stability		
Aqueous hydrolysis [d]	DT50 at pH 5: 568 (20°C) DT50 at pH 7: 1034 (20°C) DT50 at pH 9: 517 (20°C)	EFSA (2010)
Aqueous photolysis [d]	DT50: 1593	EFSA (2010)

Photochemical degradation in air	Not studied	EFSA (2010)
Volatilisation		
Vapour pressure [Pa]	≤ 1.56 x 10^{-7} (25°C, 99.9 %; extrapolated from measurement between 65 and 85°C)	EFSA (2010)
Henry's law constant [Pa·m^3·mol^{-1}]	≤ 6.6 x 10^{-5} (25°C)	EFSA (2010)
Partition/Adsorption		
Octanol-water partition coefficient (log Kow)	4.25 (buffer solution, pH 7, 25°C, 98.9 % purity)	EFSA (2010)
Organic carbon normalised Freundlich partitioning coefficient (Kfoc)	See section 1.5.3, Table 3	

1.2 Mode of action

With regard to the mode of action, tebufenozide is a non-steroidal ecdysteroid receptor agonist belonging to the insect growth regulator (IGR) group of insecticides (Oetken *et al.* 2004). Ecdysteroids are hormons that control the moulting (i.e. ecdysis) in insects. Ecdysteroid receptor agonists can bind to the ecdysone receptors mimicking the moulting hormone ecdysone (the active form of ecdysteroids, of which the most common one is 20H-hydroxyecdysone). As a result, the animal enters into a premature moulting cycle that can lead to a delayed postembryonic development – with extra larval moult cycles – and nymphal-adult intermediates (Oetken *et al.* 2004). The stimulated precocious moulting is usually incomplete and thus fatal. While tebufenozide and other ecdysteroid agonists, such as methoxyfenozide and RH 5849, are mostly effective on lepidopteran larvae, some effects of tebufenozide on certain coleopteran and aquatic crustacean larvae as well as on dipteran cell lines and larvae were also shown (Smagghe & Degheele 1998, Sundaram *et al.* 1999, Song *et al.* 1997). The selectivity of tebufenozide to caterpillars is likely based on its selective affinity to binding to the lepidopteran ecdysone receptors (Sundaram *et al.* 1999) as well as the retention of tebufenozide by the lepidopteran cells as compared to the active exlusion by the tested dipteran cells (Retnakaran *et al.* 2001).

The endocrine disrupting (ED) properties were not considered during the previous review assessment of tebufenozide (EFSA 2010), the newly submitted dossier (Nisso 2021) is only partially available without an ED assessment and the draft Renewal Assessment Report (RAR) from AIR IV is not available publicly yet.

Apart from the missing conclusion, the current evaluation of ED properties focusses on vertebrates, but 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 other than arthropods going through moulting 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). Furthermore, tebufenozide was intentionally designed to interact with the hormonal system of insects, specifically in lepidopteran species. While the number of Lepidoptera species with larvae living in the soil is lower than the number of soil-dwelling species in many other insect orders (Legal 2023), they can occur in in-field situation and could be impacted. No study was found on investigating the effects of tebufenozide on soil-dwelling caterpillars (of either target or non-target species). The possible endocrine effects of tebufenozide and other IGRs on invertebrates other than insects are also not widely studied, probably because their endocrine system is still not well understood (Oetken *et al.* 2004). Additionally, a systematic literature search on tebufenozide yielded no data on specific endocrine-related endpoints for in-soil organisms other than arthropods (status 09.2024).

With regard to human toxicology, tebufenozide showed no evidence of genotoxic, carcinogenic or neurotoxic potential, neither of developmental toxicity in the studies available during the previous peer-review (EFSA 2010).

1.3 Use and emissions

Tebufenozide is a lepidopteran-specific insecticide that is authorised at EU level against various moth species in grape (max. 4 x 172 g a.s./ha with 7-d intervals) and pome fruit crops (max. 2 x 288 g a.s./ha with a 15-d interval) for field use – see the GAP table (good agricultural practices) for the representative uses in the latest EFSA conclusion (EFSA 2010). In the dossier newly submitted for the EU renewal assessment of the active substance (Nisso 2021), the applicant also included representative uses in maize, citrus fruits, tomato, pepper and aubergine (Nisso 2021).

In Switzerland one product is available for home garden use (original authorisation) and three products for professional use only (parallel imports) (BLV 2025). All four products are authorised for uses only in permanent greenhouses in cabbage, salad and spinach crops (1 x 120 g a.s./ha).

1.4 Classification and environmental limit values

During the last finalised EU assessment (EFSA 2010), tebufenozide was classified according to the previous legistlation (Directive 1999/45/EC) as a substance that is

- *dangerous for the environment* (N) and
- *toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment* (R51/R53).

According to the harmonised classification and labelling approved by the European Union ((EC) No 1272/2008), the substance is

- *toxic to aquatic life with long lasting effects* (H411, aquatic chronic class category 2) and
- *hazardous to the environment* (GHS09 pictogram).

In addition to the harmonised one, the notified classification and labelling proposed the following (ECHA 2024):

- *very toxic to aquatic life* (H400, aquatic acute class category 1),
- *very toxic to aquatic life with long-lasting effects* (H410, aquatic chronic class category 1),
- *suspected damaging fertility or the unborn child* (H361, reproductive category 2),
- *causes damage to organs* (H372, specific target organ toxicity, repeated exposure) and
- *aspiration hazard* (GHS08 pictogram)

Tebufenozide is not listed as a candidate substance for substitution (EC 2011, 2015, PSMV 2010).

Up to date, no soil protection value was derived that could be found for tebufenozide.

Please note that the information included here may have changed since the finalisation of this dossier.

1.5 Environmental fate in soil

Volatilisation from soil surface

Considering the physico-chemical properties of tebufenozide (see vapour pressure in Table 1), volatilisation from soil was not considered relevant (EFSA 2010).

Photodegradation

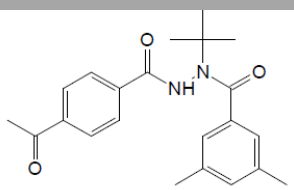
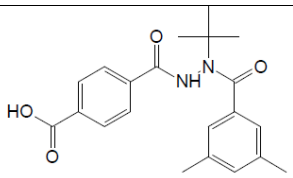
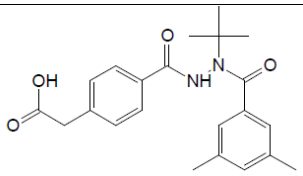
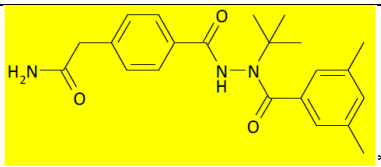
Photolysis was considered as not being a major route of degradation in soil with a first order DT50 of 98 days (Reynolds (1991) cited in Vol.3 B.8, p.367, EC (2006); EFSA (2010)).

1.5.1 Route of degradation

Aerobic degradation in soil

Four soil metabolites of tebufenozide were identified in aerobic degradation studies in soil (duration: 63-120 days) requiring further consideration (EC 2010, EFSA 2010, EC 2018): RH-2651, RH-6595, RH-2703 and the primary amide of RH-2703 (formerly M2). Table 2 summarises these transformation products of tebufenozide in soil.

Table 2: Tebufenozide soil metabolites. Abbreviation: AR – applied radioactivity [%]

Code/Trivial name	Chemical name	Structural formula	Maximum AR [%]	Reference
RH-6595	N'-[(4-acetylphenyl)carbonyl]-N-tert-butyl-3,5-dimethylbenzohydrazide		8.8	EFSA (2010)
RH-2651	4-(((2-tert-butyl-2-[(3,5-dimethylphenyl)carbonyl]hydrazinyl)carbonyl)benzoic acid		20.0	EFSA (2010)
RH-2703	[4-(((2-tert-butyl-2-[(3,5-dimethylphenyl)carbonyl]hydrazinyl)carbonyl)phenyl] acetic acid		8.0	EFSA (2010)
primary amide of RH-2703 (formerly M2)	2-[4-((2-tert-butyl-2-[(3,5-dimethylphenyl) carbonyl]hydrazinyl)carbonyl)phenyl]-acetamide	 *	9.1	Wendelburg & Balcer (2012) cited in EC (2018), B.8, p.81

Note: *The figure for the newly identified metabolite (formerly M2) has been copied over from EC (2018), where it was coloured in yellow highlighting the changes that were made later to the document as compared to the original version.

Anaerobic degradation in soil

The anaerobic degradation of tebufenozide for the Annex I evaluation was not investigated (EFSA 2010), but confirmatory information on anaerobic degradation was required later together with

degradation in soils with alkaline pH (EC 2011). To address the issue, a study investigating anaerobic aquatic metabolism of tebufenozide was submitted and evaluated (Reynolds (1992), cited in EC (2018), Vol. 3 B.8.1.1.2, p.106). While the conditions in this study were quite different from the respective standard anaerobic degradation study conditions, according to the evaluation of the rapporteur member state (RMS) the submitted study provided useful evidence about the slower degradation of tebufenozide and formation of the metabolites in lower amounts and likely without additional metabolites as compared to aerobic conditions.

Mineralisation and non-extractable residues

Mineralisation ranged from 27.2 to 38.7 %, while non-extractable residues were between 34.2 and 42.0 % in various sand, loamy sand and sandy loam soils after 92-120 days (EFSA 2010).

1.5.2 **Rate of degradation**

Laboratory degradation studies

Tebufenozide was moderately to highly persistent, the soil metabolites were moderately persistent in aerobic conditions (EFSA 2010, EC 2018). The **non-normalised aerobic degradation half-life (DT50) for tebufenozide was 27.8-277 days** at pH ($CaCl_2$) 5.52-7.39 (OC 0.98-2.5 %). The normalisation (20°C, pF2/10 kPa) resulted in similar values (20.8-277 d) with a geometric mean of 51.8 days (n = 6). Even though the two soils with higher pH values of 7.28 and 7.39 resulted in the much higher DT50s of 158 and 277 days than the other four soils (pH 5.52-6.40; DT50 of 20.8-31.2 d), the Kendall rank correlation test run by the RMS did not show statistically significant relation between the normalised DT50 and the corresponding soil pH values (EC 2018). In addition, the aerobic degradation was investigated in 15 soils (Rieder (2013), cited in EC (2018), Vol. 3 B.8.1.1.1, p.95) and showed no pH-dependence (13.9-91.1 % AR at termination on day 29, at pH 5.2-7.9).

Using only the four soils with lower pH ($CaCl_2$) of 5.52-6.4, the metabolites showed similar persistence to each other with normalised geomean DT50s of 28.9, 26.4 and 32.6 days for RH-2703, RH-2651 and RH-6595, respectively. The degradation of these metabolites did not show pH-dependence and can be considered moderately persistent in soil. M2 was investigated only in one soil with normalised DT50 of 32.4 days (EC 2018) falling in the same persistence category.

As it has been mentioned above (1.5.1 Route of degradation), there is no quantitative results for the anaerobic degradation, only an indication that it is slower with lower levels of metabolites.

Field dissipation studies

Under field conditions, **the non-normalised dissipation half-life of tebufenozide** showed higher variation than the laboratory results ranging from **14.2 to 154.3 days** (Germany, n = 4, OC 0.75-1.26 %, pH 4.35-7.0). After normalisation (20°C, not to moisture content) they ranged between 10.2 and 81.7 with a geomean of 24.2 days (EFSA 2010). The non-normalised values indicate low to high persistence of tebufenozide in field conditions with the highest DissT50 of 154.3 days relating to the lowest pH of 4.35. It can be argued that soils with pH below 5.5 (and especially below 5) are not suitable for fruit or vegetable growing, thus high persistence of tebufenozide in the place of application in such crops is not expected.

Nevertheless, at EU level the potential accumulation was calculated based on the highest field DissT50 of 154.3 days along with the highest representative use in the EU resulting in an overall predicted accumulation value of 0.1848 mg a.s./kg (for details on the predicted environmental concentrations in the EU, please refer to Section 2).

Additional studies

The persistence and metabolic fate of tebufenozide, applied as an aqueous flowable and an emulsion suspension formulation at 35, 70 and 140 g a.s./ha, were investigated in a field microcosm study in forest soil, litter and conifer matrices in Ontario, Canada (Sundaram 1995; only results for the forest soil are summarised here). Before application, the litter, moss and organic detritus were removed from the top of the soil (clay loam, organic matter content of 3.04 %; of remainder: sand 17.6 %, silt 40.4 %, clay 42.0 %; pH 5.28). The residues were analysed by HPLC. The efficiency of the analytical method regarding the recovery of the analytes were determined via including external control samples in between the test samples (mean recovery of 91 to 104 % with coefficient of variation from 6 to 11 %); the reported test results were corrected accordingly. In addition, accuracy and precision of the method was also monitored. Limits of detection (LOD) and limits of quantification (LOQ) for all matrices were 0.005 and 0.01 µg/g, respectively. The measured depositions at ground level were 77-97 % of the applied nominal rates. **The DissT50 values of tebufenozide in clay loam forest soil ranged between 32.1 and 34.0 as well as between 39.2 and 45.0 days** for the aqueous flowable and the emulsion suspension formulation, respectively, measuring the upper 2.5 cm soil layer. Some residues in soil could still be measured after 344-460 days after application. It seemed that the presence of oil as adjuvant in the emulsion suspension formulation could enhance the persistence of tebufenozide in soil. The deeper soil layers of 2.5-5.0 and 5.0-7.5 cm did not contain residues when they were sampled, 20-167 days after the application.

A forest dissipation study, similar to the previous one, was conducted in the same area in another year using a suspension concentrate formulation of tebufenozide (240 g a.s./L) at 35, 70 and 140 g a.s./ha rates (Sundaram 1997). In this case the forest was situated on sandy soil (organic matter content of 3.9 %; of remainder: sand 51 %, silt 42 %, clay 7 %; pH 5.9). Additionally, in this study the behaviour of tebufenozide was also investigated in sandy and clay soils in laboratory microcosms. The soil samples were collected in the areas of the forest dissipation studies (Sundaram 1995, 1997). The vertical and lateral movement of tebufenozide was investigated through artificial rainfall, the dissipation via photolysis through imitating light intensities and wavelengths similar to the natural sunlight measured in the sampled forest areas, and volatilisation with artificial airflow at 7 and 30°C. **The field DissT50 values of tebufenozide in the upper 2.5 cm layer of sandy forest soil ranged between 52.4 and 62.2 days at the applied rates.** In the soil cores sampled from 2.5 and 5.0 cm depth, no tebufenozide was detected at any rates up to 85 days after the application (LOD and LOQ were 0.020 and 0.050 µg/g). On day 107, traces of tebufenozide (< LOQ) could be measured at 35 g a.s./ha with no detection afterwards. At 70 and 140 g a.s./ha rates, traces of tebufenozide – reaching the level of LOQ on two occasions – could be measured from day 107 on. No residues were found in the sample cores of 5-10 and 10-15 cm depth. In the laboratory tests, downward movement of tebufenozide to lower soil layers was more pronounced after higher intensity, higher amount or continuous rainfall as compared to lower intensity, lower amount or intermittent rainfall, respectively. Lateral movement could occur with rainwater running off from the treated area. Loss of tebufenozide due to light radiation was greater from the sandy than from the clay substrates. The amount of loss also showed a positive relationship with light of longer duration or higher intensity. More volatilisation occurred at 30°C compared to 7°C (10 days), but the highest volatilisation was observed at 15°C with slightly shorter duration (8 days).

All DissT50 values reported in the additional studies are within the range of the values reported for the regulatory field dissipation studies.

1.5.3 **Adsorption/desorption properties and bioavailability**

Adsorption

Based on the results of laboratory adsorption studies, tebufenozide can be classified as low to medium mobile in soil, and the metabolites as highly or very highly mobile (EFSA 2010). The adsorption properties of tebufenozide and its metabolites are summarised in Table 3 below.

Table 3: Summary of soil adsorption of the active substance tebufenozide and the major soil metabolites. M2 was not identified and investigated for mobility at that time. Abbreviations: Kfoc – organic carbon-normalised Freundlich distribution coefficients; 1/n – Freundlich exponent. Source: EFSA (2010).

Substance	Range of K_{foc} [mL/g]	Arithmetic mean of Kfoc [mL/g]	Arithmetic mean of 1/n	pH dependence	Mobility category
Tebufenozide	351-894	572	1.005	no	low to medium
RH-2651	76-156	105	0.987	no	medium to high
RH-6595	no agreed values*	(105)*	(0.987)*	(no)*	(medium to high)*
RH-2703	27-127	79	0.753	no	high to very high

Note: *There were no agreed values for metabolite RH-6595; mean Kfoc and 1/n values of RH-2651 were used for RH-6595 as worst-case surrogates.

Leaching

Information on the mobility in soil was supplemented by a column leaching and an aged column leaching study (EFSA 2010). When the substance was freshly mixed into the soil, there was max. 5.5 % of the applied active substance in the leachate after two days. When the soil mixture was aged for 40 days, the leachate contained 7 % of the applied radioactivity as tebufenozide; 1.5 % AR as RH-2703 and 4 % AR as RH-2651.

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 tebufenozide, soil pH and texture do not seem to affect the adsorption of the compound to soil particles (EFSA 2010, EC 2018). For non-ionized organic compounds like tebufenozide (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 soil 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 Kow greater than three can pose a risk of secondary poisoning to animals at higher trophic levels. Tebufenozide has a log Kow of 4.25 (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 the case of tebufenozide, the limit of quantification for the method (MLOQ) was determined as 0.2 ng a.s./g soil (corresponding to 0.0002 mg a.s./kg soil; Rösch *et al.* 2023).[2]

The soil guideline value that is derived in this dossier for tebufenozide will be used in conjunction with the actual soil concentrations monitored in Swiss soils by using the above-described measurement method. The initial measurements on some selected, partly agricultural, Swiss soils resulted in tebufenozide concentrations between < 0.0002 mg a.s./kg soil (< MLOQ) and 0.0003 mg a.s./kg soil (Rösch *et al.* 2023, Table S12).

At EU level the potential accumulation was calculated based on the highest field DissT50 of 154.3 days in combination with the highest representative use (pome fruit, 2 x 288 g/ha, 14 d interval, 80 % crop interception, 5 cm tillage depth for permanent crop). The predicted environmental concentration in soil (PEC*soil*) for a plateau value after 10 years was calculated as 0.0359 mg a.s./kg soil. The initial PEC*soil,actual* on day 14 right after the second application was calculated as 0.1489 mg a.s./kg soil. These altogether resulted in the overall PEC*soil,accumulation* (= PEC*soil,actual* + PEC*soil,plateau*) value of 0.1848 mg a.s./kg (EFSA 2010).

3 Effect data on tebufenozide

Effect data for soil organisms were collected from studies retrieved from the European registration information (EC 2006, 2009, 2010, 2018). Additionally, a bibliographic search was performed for tebufenozide and its CAS number (CAS 112410-23-8) in the ECOTOX Knowledgebase (US EPA 2024) and in the database of the German Federal Environment Agency (UBA 2024). Furthermore, a literature search was performed on Scopus by using a combination of key words (Soil, EC50, LC50, NOEC, LOEC, LCx, ECx, toxicity and the English and Latin names of various soil organisms such as earthworm, Collembola or mite) and the compound's name or CAS number. 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 tebufenozide.

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

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 A6).

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

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

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 tebufenozide is affected by soil pH or clay content (EFSA 2010, EC 2018), studies outside the recommended range (or without knowing the pH or the clay content) were not excluded from the data set.

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

In most cases regulatory studies and their endpoints are accepted without additional assessment (at face value) or re-considered if needed to set the endpoints in line with our criteria as summarised in detail 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. Where the results are inconsistent or not well summarised, full

re-assessments may be performed using the original study reports (depending on their availability and the importance of their results).

If more endpoints are available from the same study, the statistically more robust one is preferred. This means that the statistically more robust effect concentration is chosen even if it is higher than another one or is based on more than 10 % mean effect (it is acknowledged that at European level, 10 % are often used as a threshold for biologically relevant effects as a precautionary approach). If the latter is the case, it will be highlighted and discussed further in the uncertainty analysis (see later below). If both NOEC and EC10 are available from the same study and statistically both are equally robust, due to the inherent uncertainties of the NOEC, the EC10 is preferred over the NOEC. 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 tebufenozide and its transformation products are shown in Appendix 2 (for tebufenozide, Table A1 with laboratory and Table A2 with field studies) and Appendix 3 (for the soil metabolites, Table A3-Table A6). 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 study results are summarised and the lowest values per species/group per test setup are shown in bold. If there are only greater-than values available for the same species/group from different setups, the highest one is considered decisive as they mean that up to the highest tested concentration no adverse effects could be observed. The geomean, if it is possible to calculate from the results (i.e. there are equal-to values for the same species/effect/duration/type of effect concentration), is used for choosing the lowest value rather than the individual effect concentrations. This sifting procedure helps to choose the lowest effect concentrations per species/group for the SGV derivation (see Table 5).

3.1 Comparison between data for active substance and formulated products

A statistical analysis of potential differences in the toxicity of the active substance and the formulated products was not possible due to the scarcity of data. Therefore, toxicity data obtained with the active ingredient and the formulations were merged (see data for the parent in Table 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: Tebufenozide – All reliable (R1-R2) and relevant (C1-C2) effect data. The lowest relevant and reliable effect data per species/group per test setup are shown in bold. Calculated data are rounded to three significant figures. Abbreviations: n.r. – not reported; n.a. – not applicable; cc. – concentration; MWHC – maximum water holding capacity; OC – organic carbon; OM – organic matter. 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)[3]	Test substance	Measured effect[4]	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)	Tebufenozide (a.s., 97.5 %)	adult mortality	14 d	LC50	> 1000	10	> 340	Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5	F	1	Garvey (1992) cited in EC (2006), Vol. 3 B.9.6.1, p.559
***Eisenia fetida* (Earthworm)**	**Tebufenozide (a.s., 97.5 %)**	**adult mortality**	**14 d**	**NOEC**	**≥ 1000**	**10**	**≥ 340**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5**	**F**	**1**	**Garvey (1992) cited in EC (2006), Vol. 3 B.9.6.1, p.559**
Eisenia fetida (Earthworm)	Tebufenozide (a.s., 97.5 %)	biomass (adult weight)	14 d	LC50	> 1000	10	> 340	Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5	A, F	1	Garvey (1992) cited in EC (2006), Vol. 3 B.9.6.1, p.559
***Eisenia fetida* (Earthworm)**	**Tebufenozide (a.s., 97.5 %)**	**biomass (adult weight)**	**14 d**	**NOEC**	**≥ 1000**	**10**	**≥ 340**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5**	**A, F**	**1**	**Garvey (1992) cited in EC (2006), Vol. 3 B.9.6.1, p.559**
***Eisenia fetida* (Earthworm)**	**RH-73719 (Mimic 2F, SC, nominal and measured 240 g a.s./L)**	**adult mortality**	**28 d**	**NOEC**	**≥ 7.68**	**10**	**≥ 2.61**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial sand, pH 5.61-6.21, 60.0-64.1 % MWHC**	**E, F**	**1 (R1/C1)**	**Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.2, p.563; Anonymous (2002) included in Nisso (2021), Sponsor's Project No: 021107**
***Eisenia fetida* (Earthworm)**	**RH-73719 (Mimic 2F, SC, nominal and measured 240 g a.s./L)**	**biomass (adult weight)**	**28 d**	**NOEC**	**≥ 7.68**	**10**	**≥ 2.61**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial sand, pH 5.61-**	**E, F**	**1 (R1/C1)**	**Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.2, p.563; Anonymous (2002) included in Nisso**

[3] M – monocotyledonous, D – dicotyledonous plant species
[4] DE – diversity endpoint, EE – enzymatic endpoint, FE – functional endpoint

Species (Taxonomic group)[3]	Test substance	Measured effect[4]	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
								6.21, 60.0-64.1 % MWHC			**(2021), Sponsor's Project No: 021107**
***Eisenia fetida* (Earthworm)**	**RH-73719 (Mimic 2F, SC, nominal and measured 240 g a.s./L)**	**reproduction (no. of juveniles)**	**56 d**	**NOEC**	**≥ 7.68**	**10**	**≥ 2.61**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial sand, pH 5.61-6.21, 60.0-64.1 % MWHC**	**E, F**	**1 (R1/C1)**	**Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.2, p.563; Anonymous (2002) included in Nisso (2021), Sponsor's Project No: 021107**
Folsomia candida (Collembola)	Tebufenozide (99.9 % purity)	adult mortality	28 d	LC50	> 730	10	> 248	Artificial soil: 70 % quartz, 20 % kaolinite clay, 10 % sphagnum peat, pH 5.5-6.5, 50 % of MWHC	L	R2/C2	Campiche *et al.* (2006)
***Folsomia candida* (Collembola)**	**Tebufenozide (99.9 % purity)**	**reproduction (number of juveniles)**	**28 d**	**NOEC**	**9**	**10**	**3.06**	**Artificial soil: 70 % quartz, 20 % kaolinite clay, 10 % sphagnum peat, pH 5.5-6.5, 50 % of MWHC**	**L**	**R2/C1**	**Campiche *et al.* (2006)**
Yuukianura szeptyckii (Collembola)	Tebufenozide (> 99 % purity)	adult mortality	28 d	LC50	> 700	10	> 238	Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay and 70 % sand, pH 6.0 ± 0.5	M	R2/C2	Lee *et al.* (2018)
***Yuukianura szeptyckii* (Collembola)**	**Tebufenozide (> 99 % purity)**	**adult mortality**	**28 d**	**NOEC**	**≥ 700**	**10**	**≥ 238**	**Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay and 70 % sand, pH 6.0 ± 0.5**	**M**	**R2/C1**	**Lee *et al.* (2018)**
***Folsomia candida* (Collembola)**	**Tebufenozide 240 SC (240 g/L nominal, 23.6 % w/w, measured)**	**adult mortality**	**28 d**	**NOEC**	**≥ 236**	**5**	**≥ 160**	**Artificial soil: 5 % peat, 20 % kaolinite clay, approx. 75 % sand, < 1 % calcium carbonate, pH 5.5-5.9, MWHC 45.2-50.2 %**	**P**	**R1/C1**	**Anonymous (2019a) included in Nisso (2021), Document No: RD-06740, Test facility's Project No: S18-00218**
***Folsomia candida* (Collembola)**	**Tebufenozide 240 SC (240 g/L nominal, 23.6 % w/w, measured)**	**reproduction (number of juveniles)**	**28 d**	**NOEC**	**≥ 236**	**5**	**≥ 160**	**Artificial soil: 5 % peat, 20 % kaolinite clay, approx. 75 % sand, < 1 % calcium carbonate, pH 5.5-5.9, MWHC 45.2-50.2 %**	**P**	**R1/C1**	**Anonymous (2019a) included in Nisso (2021), Document No: RD-06740, Test Facility's Project No: S18-00218**
***Hypoaspis aculeifer* (Mite)**	**Tebufenozide 240 SC (240**	**adult mortality**	**14 d**	**NOEC**	**≥ 236**	**5**	**≥ 160**	**Artificial soil: 5 % peat, 20 % kaolinite clay,**	**R**	**R1/C1**	**Anonymous (2019b) included in Nisso (2021),**

Species (Taxonomic group)[3]	Test substance	Measured effect[4]	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
	g/L nominal, 23.6 % w/w, measured)							approx. 75 % sand, < 1 % calcium carbonate, pH 5.7-5.9, MWHC 49.0-50.5 %			Document No: RD-06741, Test Facility's Project No: S18-00219
Hypoaspis aculeifer (Mite)	Tebufenozide 240 SC (240 g/L nominal, 23.6 % w/w, measured)	reproduction (number of juveniles)	14 d	NOEC	≥ 236	5	≥ 160	Artificial soil: 5 % peat, 20 % kaolinite clay, approx. 75 % sand, < 1 % calcium carbonate, pH 5.7-5.9, MWHC 49.0-50.5 %	R	R1/C1	Anonymous (2019b) included in Nisso (2021), Document No: RD-06741, Test Facility's Project No: S18-00219
Microorganisms	RH 5992 2F (Hoe 105540 00 SC23 A103; nominal 240 g a.s./L, measured 24.5 % w/w)	nitrogen transformation[FE]	28 d	< 25 % effect (< 10 % effect)	≥ 1.6	1.41 (0.83 % OC)	≥ 3.86	Natural soil: loamy sand, pH 6.15, MWHC 31.6 %; after 28 d pH 6.67-7.23	H	R2/C1	Frings & Baedelt (1993) cited in EC (2006), Vol. 3 B.9.8, p.572; Anyonymous (1993) included in Nisso (2021), document No. RD-05442, report No. 93RC-1094
Microorganisms	RH 5992 2F (Hoe 105540 00 SC23 A103; nominal 240 g a.s./L, measured 24.5 % w/w)	nitrogen transformation[FE]	28 d	< 25 % effect (< 10 % effect)	≥ 1.6	1.99 (1.17 % OC)	≥ 2.74	Natural soil: silty loam, pH 6.7, MWHC 40.4 %; after 28 d pH 7.37-7.40	H	R2/C1	Frings & Baedelt (1993) cited in EC (2006), Vol. 3 B.9.8, p.572; Anyonymous (1993) included in Nisso (2021), document No. RD-05442, report No. 93RC-1094
Allium cepa[M]	Confirm 2F TEP (22.84 % a.s.)	emergence, survival, shoot height, shoot dry weight, phytotoxicity	21 d	< 15 % effect	≥ 1.22	1.2	≥ 3.46	Artificial soil: 85 % sand, 6 % silt, 9 % clay, pH 6.2	K	R1/C1	Anonymous (2011a) included in Nisso (2021); document No. RD-06598
Lolium perenne[M]					≥ 1.22		≥ 3.46				
Triticum aestivum[M]					≥ 1.22		≥ 3.46				
Zea mays[M]					≥ 1.22		≥ 3.46				
Brassica oleracea[D]					≥ 1.22		≥ 3.46				
Glycine max[D]					≥ 1.22		≥ 3.46				
Lactuca sativa[D]					≥ 1.22		≥ 3.46				
Lycopersicon esculentum[D]					≥ 1.22		≥ 3.46				
Raphanus sativus[D] (Terrestrial plants)					≥ 1.22		≥ 3.46				

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

A	There was approx. 20 % decrease of adult weight at the highest test concentration after 14 days compared to day 0, but only approx. 13 % decrease as compared to the control on day 14. There was some decrease of adult weight at all test concentrations.
E	The study summary said that the test was conducted to the ISO (1998) guideline, but not enough details of the study were provided. Therefore the study details have been checked by OZ based on the study report submitted by the applicant recently (Anonymous (2002) included in Nisso (2021), Sponsor's Project No: 021107). Two nominal concentrations of RH-73719 were tested, 6.80 and 34.0 mg RH-73719/kg soil, corresponding to 1.54 and 7.68 mg a.s./kg soil (the density of the tested product was 1.0622 g/mL). It was reported that the artificial soil used in the test was prepared as described in the test guideline. The detailed information could confirm the composition of the soil used as well as the fulfilment of the validity criteria. 13 and 15 % decrease in the number of juveniles with 12 and 31 % coefficient of variation occurred at the lower and higher test concentration, respectively, which differences were not found to be statistically significant.
F	The evaluation from the assessment reports (EC 2006, 2018) was adopted and accepted without additional assessment (i.e. at face value). The results were re-calculated according to the actual measured active substance content of the applied formulation (if it was available) thus slight differences to the EU-listed endpoints may occur (if they used the nominal a.s. content).
H	The study was conducted to the BBA Guidelines VI 1-1 (BBA 1990). It is noted in the study summary that "*The BBA guideline is a specified guideline in the SETAC document and is the forerunner of the now adopted OECD Guidelines, Nos. 216 and 217*." It should be added that while the measured parameters are similar in this study, according to the OECD 216 guideline the results should be based on the nitrate-N formation rates (expressed in mg nitrate/kg soil dw/day) rather than the measured nitrate-N concentrations (here expressed as mg nitrate-N/100 g soil). The differences between the nitrate formation rates in the control and the treatments may result in different outcomes. Therefore, and in order for the results to be comparable with results for other substances, OZ calculated the nitrate formation rates for both soils and also checked the validity criterion according to the OECD 216 guideline (OECD 2000). The coefficient of variation in the control amended with lucerne meal was < 15 % for both soils, therefore both tests are considered valid. The deviation of the calculated nitrate-N transformation rates varied between -2 and +5 % after 28 days in both soils, but they were also ≤ 10 % after 7 and 14 days. It is noted that the results for the individual replicates were not reported, only the mean values with standard deviations – this slightly lowers the reliability of the study (R2).
K	The seedling emergence test was conducted to the US EPA Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.4100 (US EPA 2012), which is very similar to the OECD 208 test guideline (OECD 2006) with similar requirements and the same validity criteria (although the report was finalised in 2011, they used the validity criteria of the 2012 version of the US EPA guideline). It was reported that the seeds were planted in pots with 16 cm in diameter and 12 cm depth. The holes that were made for the seeds were closed by depressing the soil surface. The treatments were done with a spraying solution of 200 L/ha. The study aimed to evaluate any adverse effects on emergence (rate, biomass and survival) as percent decrease compared to the control. The adverse effects were not assessed for statistically significant differences. The ≥ 70 % emergence validity criterion was not met for the control of *Beta vulgaris*, where only a mean of 65 % of the seeds emerged. While this was considered acceptable according to the study author(s), it is definitely a breach of the validity criteria outlined in the guidelines. Therefore the results for *B. vulgaris* have not been considered reliable (R3) for this SGV dossier. One-one mortality (3 %) occurred in the control for *Allium cepa* and *Brassica oleracea* and no mortality for the other species, so the validity criteria regarding the survival of emerged seedlings were met (required: ≥ 90 % survival). Also, some phytotoxicity effects appeared on 1-2 control seedlings of *Zea mays* (score 20 and 80, i.e. slight and severe effects) and *Glycine max* (score 40, i.e. moderate effect). These were considered acceptable in the study report as it was explained that such symptoms sparingly can occur under natural conditions too. This reasoning has been accepted for the SGV dossier and these endpoints are considered valid. It is noted that phytotoxicity effects were evaluated and recorded qualitatively and as such these have not been found suitable for further consideration and not used for deriving effect concentrations.

	The treatments took place at two occasions: first for all species except *Allium cepa* and *Lycopersicon esculentum*, and second time for *A. cepa* and *L. esculentum*. The analytical verification of the application solutions resulted in mean measured concentrations of 5493 and 6489 ppm a.s. as well as 10 943 and 10 996 ppm a.s. for the lower as well as the higher concentrations of the spray mixtures, representing 103 and 121 as well as 102 and 103 % of the nominal concentrations, respectively. Based on the amount of spraying solution (200 L/ha), the analytically verified treatments were 1099 and 1298 g a.s./ha as well as 2189 and 2199 g a.s./ha at the lower as well as the higher test concentration, respectively. The concentrations in the soil were calculated based on the amount solution per hectare and the 12 cm depth of the pots (for further details on calculating the concentrations in the soil for terrestrial plants, please refer to Appendix 1).
L	28-d *Folsomia candida* reproduction test conducted according to the ISO standard 11267 (ISO 1999). The ISO guideline requires at least five concentrations to be tested "*in a geometric series at a factor not exceeding 2*". In the test, there were eight test concentrations with spacing factors varying between 2.2 and 2.5. Details of the results per treatment were shown only graphically.
M	Reproduction effects of technical tebufenozide at concentrations of 0 (control), 43.75, 87.5, 175, 350, and 700 mg a.s./kg soil were studied on *Yuukianura szeptyckii* (Collembola) according to the ISO 11267 guideline ((ISO 1999, Lee *et al.* 2018). The fulfilment of the validity criteria was not fully reported: A) Mean adult mortality was reported as equal to 15 % after 28 days (required: ≤ 20 %); B) Based on Fig. 1, the coefficient of variation in the control could be estimated as < 30 % (required: ≤ 30 %); C) It was reported that "The mean numbers of juveniles produced in the controls with acetone and distilled water were 54.0 and 42.3 per container, respectively." However, it was not reported, how many juveniles can/should be expected in the control after 28 days. In order to consider the third validity criterion (required: minimum 100 juveniles per control vessel, i.e. per 10 females, for *Folsomia candida*), the breeding and reproduction parameters of *Y. szeptyckii* – studied in Lee *et al.* (2016) – were also investigated (for getting a minimum number of juveniles that can be expected in the control). However, the information reported in Lee *et al.* (2016) regarding the number of eggs was unclear and controversial for further consideration of the validity in the test with tebufenozide (Lee *et al.* 2018), thus the reliability of the reproduction results of the test with tebufenozide was considered as not assignable (R4). It is noted that as major deviations from the guideline, the test was conducted at 25 ± 0.5°C with continuous darkness rather than at 20 ± 2°C with a light:dark cycle of between 12:12 and 16:8 hours.
P	Collembola reproduction study conducted to the OECD 232 guideline (OECD 2016a) at 17.7-1000 mg test item/kg soil, corresponding to 4.18-236 mg a.s./kg soil. Ten synchronised juveniles per replicate were tested in the control and in the test concentrations in artificial soil with 8 and 4 replicates, respectively. All validity criteria were met. There was neither dose-response, nor statistically significant effects up to the highest test concentration.
R	*H. aculeifer* reproduction study conducted to the OECD 226 guideline (OECD 2016b) at 17.7-1000 mg test item/kg soil, corresponding to 4.18-236 mg a.s./kg soil. Ten synchronised adult females per replicate were tested in the control and in the test concentrations in artificial soil with 8 and 4 replicates, respectively. All validity criteria were met. There was neither dose-response, nor statistically significant effects up to the highest test concentration.

3.2 Graphic representation of effect data

The lowest most relevant and reliable data (R1-2/C1-2) per test setup – normalised to a standard organic matter content of 3.4 % – are plotted in Figure 1. 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 setup are available (e.g. reproduction, biomass, mortality etc.), the lowest one is included in the figure. If in a test setup more measured effects resulted in the same lowest effect concentration, it is included only once.

The effect concentrations are in the same order of magnitude for the earthworm reproduction data (NOEC ≥ 2.62 mg a.s./kg), for the lower collembolan reproduction data (NOEC = 3.06 mg a.s./kg), for microorganisms (< 10 % effect at ≥ 2.74 and ≥ 3.86 mg a.s./kg soil) and for terrestrial plants (≤ 15 % effect at ≥ 3.46 mg a.s./kg). The acute earthworm, the other collembolan and the mite results (NOEC values of ≥ 340, ≥ 160, ≥ 238 and ≥ 160 mg a.s./kg, respectively) are two orders of magnitude higher unbound values. Altogether, there is only one equal-to effect concentration, the reproduction NOEC of 3.06 mg a.s./kg effect concentration for *Folsomia candida* (Collembola), the others are unbound values (see triangles facing up in Figure 1).

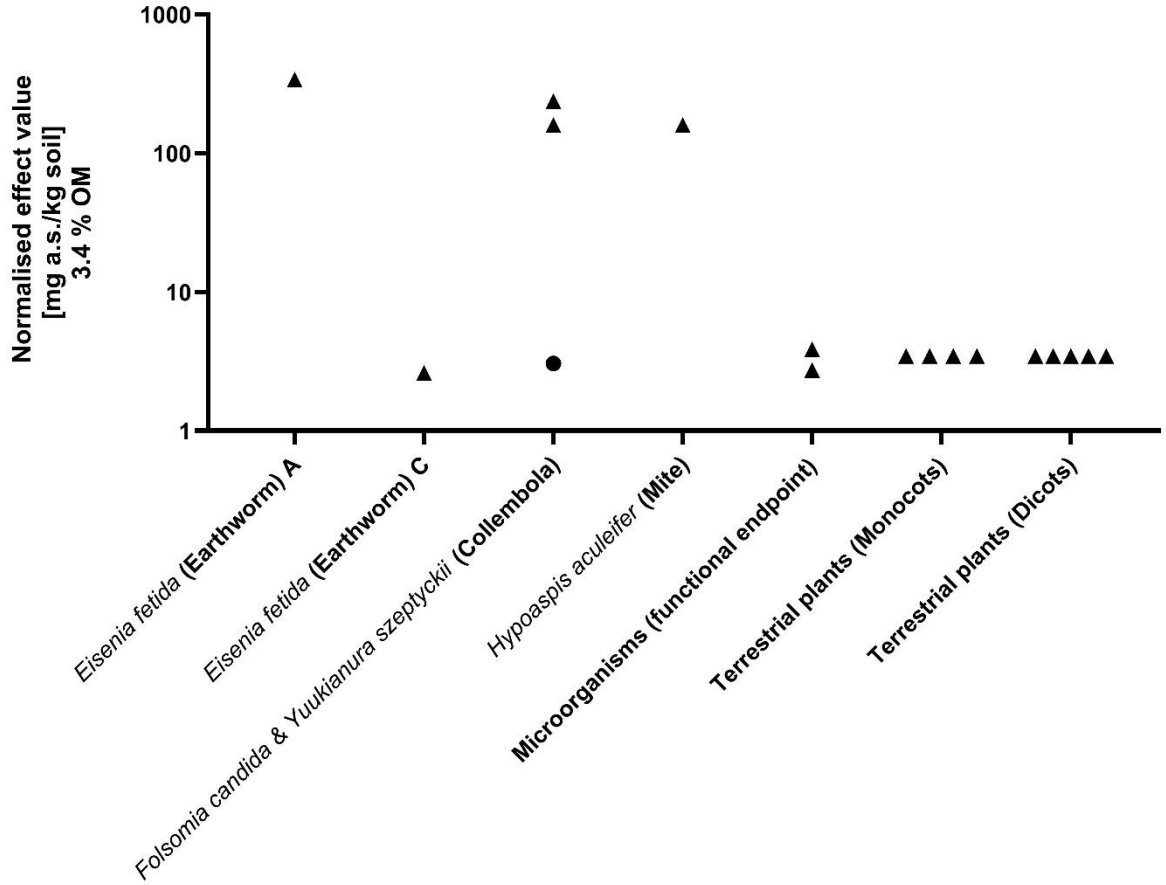


Figure 1: Effect data for tebufenozide after normalisation to a standard organic matter content of 3.4 % - the statistically most robust lowest effect values of the relevant and reliable endpoints per species/group per test setup. For earthworms the acute (A) and chronic (C) data are shown separately. For the other groups chronic data (NOEC/EC10) or equivalent to that (≤ 10 % effect) are presented with the exception of terrestrial plants, for which the results cover < 10 to < 15 % effects. Dots represent equal-to, triangles unbound data with the triangle facing up symbolising ≥ or > values and the triangle facing down symbolising ≤ or < values.

4 Derivation of SGV

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

Table 5: The lowest relevant and reliable chronic data for tebufenozide per species/group, rounded to three significant figures, summarised from Table 4. Effect concentrations are expressed as concentrations normalised to 3.4 % soil organic matter content.

Trophic level	Species	Type of effect concentration	Effect concentration [mg a.s./kg soil]	Reference
Primary producers (terrestrial plants)	*Allium cepa* (Monocots)	< 15 % effect	≥ 3.46	Anonymous (2011a) included in Nisso (2021); document No. RD-06598**
	Lolium perenne (Monocots)	< 10 % effect	≥ 3.46	
	Triticum aestivum (Monocots)	< 10 % effect	≥ 3.46	
	Zea mays (Monocots)	< 10 % effect	≥ 3.46	
	Brassica oleracea (Dicots)	< 15 % effect	≥ 3.46	
	Glycine max (Dicots)	< 10 % effect	≥ 3.46	
	Lactuca sativa (Dicots)	≤ 10 % effect	≥ 3.46	
	Lycopersicon esculentum (Dicots)	< 15 % effect	≥ 3.46	
	Raphanus sativus (Dicots)	< 15 % effect	≥ 3.46	
Decomposers (nutrient transformers)	Microorganisms (Functional endpoints)	< 10 % effect	≥ 3.86*	Frings & Baedelt (1993) cited in EC (2006), Vol. 3 B.9.8, p.572; Anyonymous (1993) included in Nisso (2021), document No. RD-05442, report No. 93RC-1094
Decomposers (litter transformers/ primary consumers)	*Eisenia fetida* (Earthworm)	NOEC	≥ 2.61	Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.2, p.563; Anonymous (2002) included in Nisso (2021), Sponsor's Project No: 021107
	Folsomia candida (Collembola)	NOEC	3.06	Campiche *et al*. (2006)
	Yuukianura szeptyckii (Collembola)	NOEC	≥ 238	Lee *et al.* (2018)
Secondary consumers	*Hypoaspis aculeifer* (Mite)	NOEC	≥ 160	Anonymous (2019) included in Nisso (2021), Document No: RD-06741, Test Facility's Project No: S18-00219

Note: *Microorganisms data were originally reported for ≤ 25 % effect. The re-evaluation of the data resulted in < 10 % effect and as such the result is considered similarly to other EC10/NOEC values. From the results with two soils, the higher greater-

than/equal-to value is shown (for explanation, please refer to section 3). **Plant data were originally evaluated for and reported as < 25 % adverse effects. The effect sizes shown here are based on the study report.

4.1 Derivation of SGV 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).

Tebufenozide is an insecticide that acts by provoking a precocious moulting of caterpillars, which is usually incomplete and thus fatal. While it is mostly effective on lepidopteran larvae, effects on other aquatic and terrestrial arthropods were observed and therefore cannot be exluded (for further details, please refer to section 1.2).

Decomposers, litter transformers/primary consumers

The lowest equal-to toxicity endpoint available for tebufenozide is the reproduction NOEC of 3.06 mg a.s./kg soil for the collembolan *Folsomia candida* (see Table 5) with the active substance used for testing. The sensitivity of collembolans to tebufenozide is not unanticipated considering their continuous moulting during their whole lifespan. The much higher reproduction NOEC of ≥ 160 mg a.s./kg soil for *F. candida* using the product might indicate a higher toxicity of the technical active substance as compared to the product tested for the new EU renewal assessment dossier (Tebufenozide 240 SC; Anonymous (2019a) included in Nisso (2021)). The reproduction NOEC of 14.9 mg a.s./kg soil for the collembolan *Yuukianura szeptyckii* obtained with the active substance (Lee *et al.* 2018) could not be validated (R4, *not assignable*; see Table A1 in Appendix 2). The difference between the two effect values for reproduction with the technical active substance might be explained by the different species tested and/or the differences in test conditions. The test with *Y. szeptyckii* was performed at 25°C as opposed to 20°C with *F. candida* that might have resulted in a faster breakdown of the test substance. The results on adult mortality – tested either with the technical active substance or the product – are two orders of magnitude higher than the lowest effect concentration for reproduction.

The chronic earthworm effect concentration is a greater-than/equal-to value (NOEC ≥ 2.61 mg a.s./kg soil; Hayward (2002) included in Nisso (2021)). Due to its unbound nature and close proximity to the lowest equal-to Collembola value, the chronic earthworm value is not considered critical.

Primary producers

Terrestrial plants showed less than 15 % adverse effects up to the maximum label use rate of the active substance in the USA (Anonymous (2011a) included in Nisso (2021)). There were no considerable differences in the effect sizes between the monocotyledonous and the dicotyledonous species (< 15 % effects at ≥ 3.46 mg a.s./kg soil).

Decomposers, nutrient transformers

Only greater-than/equal-to effect concentrations are available for microorganisms with < 10 % effects in two natural soils, of which the higher is shown here (see explanation in section 3; < 10 % effect at ≥ 3.86 mg a.s./kg soil). The detailed results are based on the study report (Anonymous (1993) included in Nisso (2021)).

Secondary consumers

In the case of *Hypoaspis aculeifer* (predatory mite), there were no statistically significant differences up to the concentration of 1000 mg product/kg soil (NOEC ≥ 160 mg a.s./kg soil; Anonymous (2019b) included in Nisso (2021)).

When long-term test results (NOEC or EC_{10} values) are available for at least three species representing 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 (see Table 20 in EC TGD (2003)). In the case of tebufenozide, effect concentrations are available for 13 species and microorganisms at four trophic levels. The lowest equal-to effect concentration suitable for SGV derivation is available for decomposers (litter transformers/primary consumers). To account for the uncertainties in the available data, an AF of 10 is applied to the lowest equal-to effect value on Collembola:

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

$$SGV_{AF} = \frac{3.06\left(\frac{mg\ a.s.}{kg\ soil}\right)}{10} = 0.31\left(\frac{mg\ a.s.}{kg\ soil}\right)$$

The application of an AF of 10 to the lowest equal-to chronic datum results in a SGV_{AF} = **0.31 mg a.s./kg soil** for a standard soil with 3.4 % OM content (shown to two significant figures).

4.2 Derivation of SGV using the species sensitivity distribution (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 tebufenozide, exact data are available for Collembola only (*Folsomia candida*). Thus, the minimum data requirements for an SSD are not met.

4.3 Derivation of SGV 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 tebufenozide, 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 field/semi-field data

One field study – a regulatory litter bag study – is available, where the soil was treated twice, one week before and five days after burying the litter bags (Mallett (2003) cited in EC (2006),Vol. 3 B.9.7, p.570). This double application – with some degradation in between – is not considered relevant for the SGV derivation. Also, the soil properties, including the organic matter or carbon (OM/OC) content, were not described in the study summary.

A study from the scientific literature was considered for the last finalised EU assessment of tebufenozide (Addison (1996), also cited in EC (2006),Vol. 3 B.9.7, p.568). The earthworm species *Dendrobaena octaedra* and four collembolan species (*Folsomia candida, F. nivalis, Onychiurus parvicornis* and

Hypogastrura pannosa) were tested in microcosms filled with collected deciduous maple leaf litter (for earthworms) or coniferous litter-fermentation-humus (for collembolans) followed by a tebufenozide application. The exposure was not provided through soil but leaf litter/litter-humus substrates that are not considered relevant for the SGV derivation. The a.s. content of the applied test item as well as the OM/OC content of the test substrates were not reported.
Due to the lacking OM/OC contents together with the other deficiencies summarised above, these study results have not been found relevant and are not considered further in the SGV.

5 Toxicity of soil metabolites

Relevant and reliable effect data are only available for RH-2651, the metabolite with the highest maximum formation out of the four soil metabolites of tebufenozide that would require further consideration (EFSA 2010).

There were no effects in the acute earthworm limit test (Boeri & Ward (2002) cited in EC (2006), Vol. 3 B.9.6.1, p.561), and there were less than 25 % effects on nitrate-formation rate and soil respiration at the end of the microrganisms study (Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.1, p.575). The results are summarised in Table 6 below.

Table 6: Lowest reliable and relevant soil effect data for the soil metabolite RH-2651. Endpoints are shown as effect concentrations normalised to 3.4 % soil organic matter.

Species	Type of effect concentration	Normalised effect value [mg metabolite/kg soil]	References
Eisenia fetida (Earthworm)	NOEC	≥ 34	Boeri & Ward (2002) cited in EC (2006), Vol. 3 B.9.6.1, p.561
Microorganisms	< 25 % effect	≥ 4	Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.1, p.575

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 tebufenozide, 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 0.31 mg a.s./kg soil for tebufenozide** is suggested.

7 Protection of soil organisms and uncertainty analysis

The SGV of 0.31 mg a.s./kg soil for tebufenozide has been derived based on a dataset containing values for earthworms (*Eisenia fetida*), collembolans (*Folsomia candida* and *Yuukianura szeptyckii*), mites (*Hypoaspis aculeifer*), microorganisms and terrestrial plants (four monocots and five dicots).

Tebufenozide is an insecticide with effects on moulting, thus, it is not unexpected that arthropods going through moulting may be sensitive. Data are available on collembolans and mites that belong to the

potentially sensitive Arthropoda phylum, and Collembola showed the highest sensitivity to tebufenozide with the lowest equal-to value. The reproduction NOEC of ≥ 2.61 mg a.s./kg soil for earthworms (vs. 3.06 mg a.s./kg soil for collembolans) is a greater-than/equal-to NOEC value. The earthworm study resulted in 13 and 15 % statistically non-significant effect on the mean number of juveniles as compared to the control at 0.524 and 2.61 mg a.s./kg soil concentration, respectively. Hence, it is considered highly unlikely that a statistically significant difference would have already been reached below or at a concentration of 3.06 mg a.s./kg soil if higher concentrations would have been tested. Therefore, based on the current dataset, the more robust equal-to NOEC of 3.06 mg a.s./kg soil for Collembola is considered protective for potential adverse effects on earthworms.

Based on the maximum formation of 20 % AR and the available toxicity data on earthworms and microorganisms, the soil metabolite RH-2651 is not expected to pose greater risk to the environment than the parent compound. However, there is no data on collembolans, the most sensitive organism group to the active substance. The toxicity of the other soil metabolites could not be assessed conclusively as the results of the new metabolite studies were not reported in sufficient detail in the new summary document for the representative product (M-CP, Section 10 included in Nisso (2021)) and the summary document for the active substance as well as the study reports related to the active substance – including the metabolite studies – were not included in the public dossier submitted for the current EU renewal assessment of tebufenozide (Nisso 2021). It is noted that according to the newly submitted dossier of the applicant (Nisso 2021), only chronic earthworm studies were conducted for the soil metabolites. Concerning the other soil organisms, ten times higher toxicity was assumed for the soil metabolites than the parent compound in the proposed risk assessment. If needed, further assessment can be conducted for the metabolites in a separate dossier as that is out of the scope of the current project.

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 0.31 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.

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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 (OECD 2006), 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 in Muñoz-Leoz *et al.* (2013), but also the wide range of DissT50 values for tebufenozide in Section 1.5.2 above). 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, Casado-Martinez *et al.* 2024).

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

Appendix 2 Data on the active substance

Table A1: Soil effect data for tebufenozide from laboratory experiments. The lowest reliable and relevant effect data per species per test setup are shown in bold. Unreliable, not relevant and not assignable data are greyed out. Calculated data are rounded to three significant figures. Abbreviations: n.r. – not reported; n.a. – not applicable; cc. – concentration; MWHC – maximum water holding capacity; OC – organic carbon; OM – organic matter. 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)[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)	Tebufenozide (a.s., 97.5 %)	adult mortality	14 d	LC50	> 1000	10	> 340	Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5	A, F	1	Garvey (1992) cited in EC (2006), Vol. 3 B.9.6.1, p.559
***Eisenia fetida* (Earthworm)**	**Tebufenozide (a.s., 97.5 %)**	**adult mortality**	**14 d**	**NOEC**	**≥ 1000**	**10**	**≥ 340**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5**	**A, F**	**1**	**Garvey (1992) cited in EC (2006), Vol. 3 B.9.6.1, p.559**
Eisenia fetida (Earthworm)	Tebufenozide (a.s., 97.5 %)	biomass (adult weight)	14 d	LC50	> 1000	10	> 340	Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5	A, F	1	Garvey (1992) cited in EC (2006), Vol. 3 B.9.6.1, p.559
***Eisenia fetida* (Earthworm)**	**Tebufenozide (a.s., 97.5 %)**	**biomass (adult weight)**	**14 d**	**NOEC**	**≥ 1000**	**10**	**≥ 340**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5**	**A, F**	**1**	**Garvey (1992) cited in EC (2006), Vol. 3 B.9.6.1, p.559**
Eisenia fetida (Earthworm)	RH-5992 2F (Mimic 2 F, 23 % a.s.)	adult mortality	14 d	LC50	> 230	(10)	(> 78.2)	Artificial soil: composition not summarised, pH 5.46-5.53	C, N	R4/C2	Candolfi (1996) cited in EC (2006), Vol. 3 B.9.6.1, p.562
Eisenia fetida (Earthworm)	RH-5992 2F (Mimic 2 F, 23 % a.s.)	adult mortality	14 d	NOEC	≥ 230	(10)	(≥ 78.2)	Artificial soil: composition not	C, N	R4/C2	Candolfi (1996) cited in EC (2006), Vol. 3 B.9.6.1, p.562

[5] M – monocotyledonous, D – dicotyledonous plant species
[6] DE – diversity endpoint, EE – enzymatic endpoint, FE – functional endpoint

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
								summarised, pH 5.46-5.53			
Eisenia fetida (Earthworm)	RH-5992 2F (Mimic 2 F, 23 % a.s.)	biomass (adult weight)	14 d	LC50	> 230	(10)	(> 78.2)	Artificial soil: composition not summarised, pH 5.46-5.53	C, N	R4/C2	Candolfi (1996) cited in EC (2006), Vol. 3 B.9.6.1, p.562
Eisenia fetida (Earthworm)	RH-5992 2F (Mimic 2 F, 23 % a.s.)	biomass (adult weight)	14 d	NOEC	≥ 230	(10)	(≥ 78.2)	Artificial soil: composition not summarised, pH 5.46-5.53	C, N	R4/C2	Candolfi (1996) cited in EC (2006), Vol. 3 B.9.6.1, p.562
Eisenia fetida (Earthworm)	Hoe 105540 SC (Mimic 2F, nominal 240 g a.s./L, measured 24.5 % a.s.)	adult mortality	14 d	LC50	> 245	(10)	(> 83.3)	Artificial soil: composition not summarised, pH 6.0-6.1	D, N	R4/C2	Heusel (1994) cited in EC (2006), Vol. 3 B.9.6.1, p.562
Eisenia fetida (Earthworm)	Hoe 105540 SC (Mimic 2F, nominal 240 g a.s./L, measured 24.5 % a.s.)	adult mortality	14 d	NOEC	≥ 245	(10)	(≥ 83.3)	Artificial soil: composition not summarised, pH 6.0-6.1	D, N	R4/C2	Heusel (1994) cited in EC (2006), Vol. 3 B.9.6.1, p.562
Eisenia fetida (Earthworm)	Hoe 105540 SC (Mimic 2F, nominal 240 g a.s./L, measured 24.5 % a.s.)	biomass (adult weight)	14 d	LC50	> 245	(10)	(> 83.3)	Artificial soil: composition not summarised, pH 6.0-6.1	D, N	R4/C2	Heusel (1994) cited in EC (2006), Vol. 3 B.9.6.1, p.562
Eisenia fetida (Earthworm)	Hoe 105540 SC (Mimic 2F, nominal 240 g a.s./L, measured 24.5 % a.s.)	biomass (adult weight)	14 d	NOEC	≥ 245	(10)	(≥ 83.3)	Artificial soil: composition not summarised, pH 6.0-6.1	D, N	R4/C2	Heusel (1994) cited in EC (2006), Vol. 3 B.9.6.1, p.562
Eisenia fetida (Earthworm)	Tebufenozide (92 % purity)	adult mortality	14 d	LC50	386.7	10	131	Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay, 70 % sand, pH 5.5-6.5	G	R4/C2	Wang *et al.* (2012)
***Eisenia fetida* (Earthworm)**	**RH-73719 (Mimic 2F, SC, nominal and measured 240 g a.s./L)**	**adult mortality**	**28 d**	**NOEC**	**≥ 7.68**	**10**	**≥ 2.61**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial sand, pH 5.61-6.21, 60.0-64.1 % MWHC**	**E, F**	**1 (R1/C1)**	**Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.2, p.563; Anonymous (2002) included in Nisso (2021), Sponsor's Project No: 021107**

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)**	**RH-73719 (Mimic 2F, SC, nominal and measured 240 g a.s./L)**	**biomass (adult weight)**	**28 d**	**NOEC**	**≥ 7.68**	**10**	**≥ 2.61**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial sand, pH 5.61-6.21, 60.0-64.1 % MWHC**	**E, F**	**1 (R1/C1)**	**Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.2, p.563; Anonymous (2002) included in Nisso (2021), Sponsor's Project No: 021107**
***Eisenia fetida* (Earthworm)**	**RH-73719 (Mimic 2F, SC, nominal and measured 240 g a.s./L)**	**reproduction (no. of juveniles)**	**56 d**	**NOEC**	**≥ 7.68**	**10**	**≥ 2.61**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial sand, pH 5.61-6.21, 60.0-64.1 % MWHC**	**E, F**	**1 (R1/C1)**	**Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.2, p.563; Anonymous (2002) included in Nisso (2021), Sponsor's Project No: 021107**
Folsomia candida (Collembola)	Tebufenozide (99.9 % purity)	adult mortality	28 d	LC50	> 730	10	> 248	Artificial soil: 70 % quartz, 20 % kaolinite clay, 10 % sphagnum peat, pH 5.5-6.5, 50 % of MWHC	L	R2/C2	Campiche *et al.* (2006)
***Folsomia candida* (Collembola)**	**Tebufenozide (99.9 % purity)**	**reproduction (number of juveniles)**	**28 d**	**NOEC**	**9**	**10**	**3.06**	**Artificial soil: 70 % quartz, 20 % kaolinite clay, 10 % sphagnum peat, pH 5.5-6.5, 50 % of MWHC**	**L**	**R2/C1**	**Campiche *et al.* (2006)**
Folsomia candida (Collembola)	Tebufenozide (99.9 % purity)	reproduction (number of juveniles)	28 d	EC10	9.2	10	3.13	Artificial soil: 70 % quartz, 20 % kaolinite clay, 10 % sphagnum peat, pH 5.5-6.5, 50 % of MWHC	L	R3/C1	Campiche *et al.* (2006)
Yuukianura szeptyckii (Collembola)	Tebufenozide (> 99 % purity)	adult mortality	28 d	LC50	> 700	10	> 238	Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay and 70 % sand, pH 6.0 ± 0.5	M	R2/C2	Lee *et al.* (2018)
***Yuukianura szeptyckii* (Collembola)**	**Tebufenozide (> 99 % purity)**	**adult mortality**	**28 d**	**NOEC**	**≥ 700**	**10**	**≥ 238**	**Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay and 70 % sand, pH 6.0 ± 0.5**	**M**	**R2/C1**	**Lee *et al.* (2018)**
Yuukianura szeptyckii (Collembola)	Tebufenozide (> 99 % purity)	reproduction (number of juveniles)	28 d	NOEC	43.75	10	14.9	Artificial soil: 10 % sphagnum peat, 20 % kaolinite clay and 70 % sand, pH 6.0 ± 0.5	M	R4/C1	Lee *et al.* (2018)

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)	Tebufenozide 240 SC (240 g/L nominal, 23.6 % w/w, measured)	adult mortality	28 d	NOEC	≥ 236	5	≥ 160	Artificial soil: 5 % peat, 20 % kaolinite clay, approx. 75 % sand, < 1 % calcium carbonate, pH 5.5-5.9, MWHC 45.2-50.2 %	P		Anonymous (2019a) included in Nisso (2021), Sponsor's Project No: S18-00218
Folsomia candida (Collembola)	Tebufenozide 240 SC (240 g/L nominal, 23.6 % w/w, measured)	reproduction (number of juveniles)	28 d	NOEC	≥ 236	5	≥ 160	Artificial soil: 5 % peat, 20 % kaolinite clay, approx. 75 % sand, < 1 % calcium carbonate, pH 5.5-5.9, MWHC 45.2-50.2 %	P		Anonymous (2019a) included in Nisso (2021), Sponsor's Project No: S18-00218
Hypoaspis aculeifer (Mite)	Tebufenozide 240 SC (240 g/L nominal, 23.6 % w/w, measured)	adult mortality	14 d	NOEC	≥ 236	5	≥ 160	Artificial soil: 5 % peat, 20 % kaolinite clay, approx. 75 % sand, < 1 % calcium carbonate, pH 5.7-5.9, MWHC 49.0-50.5 %	R	R1/C1	Anonymous (2019b) included in Nisso (2021), Document No: RD-06741, Test Facility's Project No: S18-00219
Hypoaspis aculeifer (Mite)	Tebufenozide 240 SC (240 g/L nominal, 23.6 % w/w, measured)	reproduction (number of juveniles)	14 d	NOEC	≥ 236	5	≥ 160	Artificial soil: 5 % peat, 20 % kaolinite clay, approx. 75 % sand, < 1 % calcium carbonate, pH 5.7-5.9, MWHC 49.0-50.5 %	R	R1/C1	Anonymous (2019b) included in Nisso (2021), Document No: RD-06741, Test Facility's Project No: S18-00219
Microorganisms	RH 5992 2F (Hoe 105540 00 SC23 A103; nominal 240 g a.s./L, measured 24.5 % w/w)	nitrogen transformation[FE]	28 d	< 25 % effect (< 10 % effect)	≥ 1.6	1.41 (0.83 % OC)	≥ 3.86	Natural soil: loamy sand, pH 6.15, MWHC 31.6 %; after 28 d pH 6.67-7.23	H	R2/C1	Frings & Baedelt (1993) cited in EC (2006),Vol. 3 B.9.8, p.572; Anyonymous (1993) included in Nisso (2021), document No. RD-05442, report No. 93RC-1094
Microorganisms	RH 5992 2F (Hoe 105540 00 SC23 A103; nominal 240 g a.s./L, measured 24.5 % w/w)	nitrogen transformation[FE]	28 d	< 25 % effect (< 10 % effect)	≥ 1.6	1.99 (1.17 % OC)	≥ 2.74	Natural soil: silty loam, pH 6.7, MWHC 40.4 %; after 28 d pH 7.37-7.40	H	R2/C1	Frings & Baedelt (1993) cited in EC (2006),Vol. 3 B.9.8, p.572; Anyonymous (1993) included in Nisso (2021), document No. RD-05442, report No. 93RC-1094

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
Microorganisms	Hoe 105540 (Hoe 105540 00 SC23 A103; 240 g a.s./L)	soil respiration[FE] (O_2 consumption)	28 d	< 25 % effect (< 10 % effect)	≥ 1.6	n.r.	n.a.	Natural soil: loamy sand	I, N	R4/C1	Frings & Bock (1993) cited in EC (2006),Vol. 3 B.9.8, p.574
Microorganisms	Hoe 105540 (Hoe 105540 00 SC23 A103; 240 g a.s./L)	soil respiration[FE] (O_2 consumption)	28 d	< 25 % effect (< 10 % effect)	≥ 1.6	n.r.	n.a.	Natural soil: silty loam	I, N	R4/C1	Frings & Bock (1993) cited in EC (2006),Vol. 3 B.9.8, p.574
Echinocloa crus-galli[M] *Setaria viridis*[M] *Sorghum halpense*[M] *Cyperus esculentus*[M] *Avena fatua*[M] *Xanthium pennsylvanicum*[D] *Ipomoea lacunose*[D] *Amaranthus retroflexus*[D] *Polygonum lapathifolium*[D] *Abutilon theophrastis*[D] (Terrestrial plants)	MIMIC (240 g a.s./L)	phytotoxicity (chlorosis, necrosis, inhibition of growth, tip burning)	14 d (after sowing)	NOEC	≥ 2400 g a.s./ha	n.r.	n.a.	soil / sand mixture (2:1)	J, N	R4/C4	Nunez (1997) cited in EC (2006),Vol. 3 B.9.9.2, p.580
Echinocloa crus-galli[M] *Setaria viridis*[M] *Sorghum halpense*[M] *Cyperus esculentus*[M] *Avena fatua*[M] *Xanthium pennsylvanicum*[D] *Ipomoea lacunose*[D]	MIMIC (240 g a.s./L)	phytotoxicity (chlorosis, necrosis, inhibition of growth, tip burning)	14 d (after treating seedlings)	NOEC	≥ 2400 g a.s./ha	n.r.	n.a.	soil / sand mixture (2:1)	J, N	R4/C4	Nunez (1997) cited in EC (2006),Vol. 3 B.9.9.2, p.580

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
Amaranthus retroflexus[D] *Polygonum lapathifolium*[D] *Abutilon theophrastis*[D] (Terrestrial plants)											
Allium cepa[M] ***Lolium perenne***[M] ***Triticum aestivum***[M] ***Zea mays***[M] ***Brassica oleracea***[D] ***Glycine max***[D] ***Lactuca sativa***[D] ***Lycopersicon esculentum***[D] ***Raphanus sativus***[D] **(Terrestrial plants)**	**Confirm 2F TEP (22.84 % a.s.)**	**emergence, survival, shoot height, shoot dry weight**	**21 d**	**< 25 % effect**	**≥ 1.22** **≥ 1.22** **≥ 1.22** **≥ 1.22** **≥ 1.22** **≥ 1.22** **≥ 1.22** **≥ 1.22** **≥ 1.22**	**1.2**	**≥ 3.46** **≥ 3.46** **≥ 3.46** **≥ 3.46** **≥ 3.46** **≥ 3.46** **≥ 3.46** **≥ 3.46** **≥ 3.46**	**Artificial soil: 85 % sand, 6 % silt, 9 % clay, pH 6.2**	**K**	**R1/C1**	**Anonymous (2011a) included in Nisso (2021); document No. RD-06598**
Beta vulgaris[D] (Terrestrial plants)	Confirm 2F TEP (22.84 % a.s.)	emergence, survival, shoot height, shoot dry weight	21 d	< 25 % effect	≥ 1.22	1.2	≥ 3.46	Artificial soil: 85 % sand, 6 % silt, 9 % clay, pH 6.2	K	R3/C1	Anonymous (2011a) included in Nisso (2021); document No. RD-06598

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

Species (Taxonomic group)	Test substance	Measured effect[7]	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	RH-73719 (tebufenozide, not specified)	Litter decomposition	9 m	NOEC	≥ 2.58 (295 g a.s./ha & 1195 g a.s./ha)	n.r.	n.a.	Arable site (Penclose Farm, Winterbourne, Newbury, Berkshire, UK)	N, O	R4/C3	Mallett (2003) cited in EC (2006),Vol. 3 B.9.7, p.570

Notes A1: Notes on soil studies for tebufenozide.

A	There was approx. 20 % decrease of adult weight at the highest test concentration after 14 days as compared to day 0, but only approx. 13 % decrease as compared to the control on day 14. There was some decrease of weight at all test concentrations.
C	Preliminary test was conducted with 5 concentrations ranging from 0.1 to 1000 mg product/kg soil; the definitive test was a limit test with 1000 mg product/kg soil and a control. While the summary said that the test was conducted to the OECD 207 guideline, the soil parameters were not reported and thus the results cannot be normalised. If the composition of the artificial soil given in the guideline was followed using 10 % peat content, 10 % OM content can be assumed (for further explanation, please refer to Appendix 1) – based on this assumption the normalised results are given in brackets, but their reliability is considered as not assignable (R4). As the acute earthworm study is not an EU data requirement anymore, the study report was not re-submitted by the applicant (Nisso 2021).
D	The definitive test was conducted with 5 test concentrations between 100 and 1000 mg product/kg soil. While the summary said that the test was conducted to the OECD 207 guideline, the soil parameters were not reported and thus the results cannot be normalised. If composition of the artificial soil given in the guideline was followed using 10 % peat content, 10 % OM content can be assumed (for further explanation, please refer to Appendix 1) – based on this assumption the normalised results are given in brackets, but their reliability is considered as not assignable (R4). As the acute earthworm study is not an EU data requirement anymore, the study report was not re-submitted by the applicant (Nisso 2021).
E	The study summary said that the test was conducted to the ISO (1998) guideline, but not enough details of the study were provided. Therefore the study details have been checked by OZ based on the study report submitted by the applicant recently (Anonymous (2002) included in Nisso (2021), Sponsor's Project No: 021107). Two nominal concentrations of RH-73719 were tested, 6.80 and 34.0 mg RH-73719/kg soil, corresponding to 1.54 and 7.68 mg a.s./kg soil (the density of the tested product was 1.0622 g/mL). It was reported that the artificial soil used in the test was prepared as described in the test guideline. The detailed information could confirm the composition of the soil used as well as the fulfilment of the validity criteria. 13 and 15 % decrease in the number of juveniles with 12 and 31 % coefficient of variation occurred at the lower and higher test concentration, respectively, which differences were not found to be statistically significant.
F	The assessment from the (EC 2006, 2018) reports was adopted and accepted without additional assessment (i.e. at face value). The results were re-calculated according to the actual measured active substance content of the applied formulation (if it was available) thus slight differences to the EU-listed endpoints may occur (if they used the nominal a.s. content).

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

G	Acute earthworm test more or less following the (OECD 1984) guideline. No detailed results for the control and the treatments (including the test concentrations) were reported. Also no fitted curves were included in the article, thus the results cannot be checked. Also the fulfilment of the validity criterion (≤ 10 % mortality) cannot be examined.
H	The study was conducted to the BBA Guidelines VI 1-1 (BBA 1990). It is noted in the study summary that "*The BBA guideline is a specified guideline in the SETAC document and is the forerunner of the now adopted OECD Guidelines, Nos. 216 and 217*." It should be added that while the measured parameters are similar in this study, according to the OECD 216 guideline the results should be based on the nitrate-N formation rates (expressed in mg nitrate/kg soil dw/day) rather than the measured nitrate-N concentrations (here expressed as mg nitrate-N/100 g soil). The differences between the nitrate formation rates in the control and the treatments may result in different outcomes. Therefore, and in order for the results to be comparable with results for other substances, OZ calculated the nitrate formation rates for both soils and also checked the validity criterion according to the OECD 216 guideline (OECD 2000). The coefficient of variation in the control amended with lucerne meal was < 15 % for both soils, therefore both tests are considered valid. The deviation of the calculated nitrate-N transformation rates varied between -2 and +5 % after 28 days in both soils, but they were also ≤ 10 % after 7 and 14 days. It is noted that the results for the individual replicates were not reported, only the mean values with standard deviations – this slightly lowers the reliability of the study (R2).
I	The study seems to be the "sister study" of the Frings & Baedelt (1993) study on nitrogen cycle (same year, same first author, the next report number, same soil types, same guideline etc.). However, the original study report was not included in the latest submission of the owner (Nisso 2021). In the absence of the soil properties of the natural soils used in the study, it is not possible to normalise the results. It also remained unclear, what and how was considered for accepting the validity of the study in the assessment report (EC 2006).
J	This is a screening test not following any guideline, thus it could not be validated. The study summary did not provide many details and the full study report was not included in the latest submission of the substance owner (Nisso 2021).
K	The seedling emergence test was conducted to the US EPA Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.4100 (US EPA 2012), which is very similar to the OECD 208 test guideline (OECD 2006) with similar requirements and the same validity criteria (although the report was finalised in 2011, they used the validity criteria of the 2012 version of the US EPA guideline). It was reported that the seeds were planted in pots with 16 cm in diameter and 12 cm depth. The holes that were made for the seeds were closed by depressing the soil surface. The treatments were done with a spraying solution of 200 L/ha. The study aimed to evaluate any adverse effects on emergence (rate, biomass and survival) as percent decrease compared to the control. The adverse effects were not assessed for statistically significant differences. The ≥ 70 % emergence validity criterion was not met for the control of *Beta vulgaris*, where only a mean of 65 % of the seeds emerged. While this was considered acceptable according to the study author(s), it is definitely a breach of the validity criteria outlined in the guidelines. Therefore the results for *B. vulgaris* have not been considered reliable (R3) for this SGV dossier. One-one mortality (3 %) occurred in the control for *Allium cepa* and *Brassica oleracea* and no mortality for the other species, so the validity criteria regarding the survival of emerged seedlings were met (required: ≥ 90 % survival). Also, some phytotoxicity effects appeared on 1-2 control seedlings of *Zea mays* (score 20 and 80, i.e. slight and severe effects) and *Glycine max* (score 40, i.e. moderate effect). These were considered acceptable in the study report as it was explained that such symptoms sparingly can occur under natural conditions too. This reasoning has been accepted for the SGV dossier and these endpoints are considered valid. It is noted that phytotoxicity effects were evaluated and recorded qualitatively and as such these have not been found suitable for further consideration and not used for deriving effect concentrations. The treatments took place at two occasions: first for all species except *Allium cepa* and *Lycopersicon esculentum*, and second time for *A. cepa* and *L. esculentum*. The analytical verification of the application solutions resulted in mean measured concentrations of 5493 and 6489 ppm a.s. as well as 10 943 and 10 996 ppm a.s. for the lower as well as the higher concentrations of the spray mixtures, representing 103 and 121 as well as 102 and 103 % of the nominal concentrations, respectively. Based on the amount of spraying solution (200 L/ha), the analytically verified treatments were 1099 and 1298 g a.s./ha as well as 2189 and 2199 g a.s./ha at the lower as well as the higher test concentration, respectively. The concentrations in the soil were calculated based on the amount solution per hectare and the 12 cm depth of the pots (for further details on calculating the concentrations in the soil for terrestrial plants, please refer to Appendix 1).

L	28-d *Folsomia candida* reproduction test conducted according to the ISO standard 11267 (ISO 1999). The ISO guideline requires at least five concentrations to be tested "*in a geometric series at a factor not exceeding 2*". In the test, there were eight test concentrations with spacing factors varying between 2.2 and 2.5. Details of the results per treatment were shown only graphically. The reproduction EC10 of 9.2 mg a.s./kg soil tabled in the article is not considered reliable (R3) due to the wide confidence interval including zero (0-63.3 mg a.s./kg soil).
M	Reproduction effects of technical tebufenozide at concentrations of 0 (control), 43.75, 87.5, 175, 350, and 700 mg a.s./kg soil were studied on *Yuukianura szeptyckii* (Collembola) according to the ISO 11267 guideline ((ISO 1999, Lee *et al.* 2018). The fulfilment of the validity criteria was not fully reported: A) Mean adult mortality was reported as equal to 15 % after 28 days (required: ≤ 20 %); B) Based on Fig. 1, the coefficient of variation in the control could be estimated as < 30 % (required: ≤ 30 %); C) It was reported that "The mean numbers of juveniles produced in the controls with acetone and distilled water were 54.0 and 42.3 per container, respectively." However, it was not reported, how many juveniles can/should be expected in the control after 28 days. In order to consider the third validity criterion (required: minimum 100 juveniles per control vessel, i.e. per 10 females, for *Folsomia candida*), the breeding and reproduction parameters of *Y. szeptyckii* – studied in Lee *et al.* (2016) – were also investigated (for getting a minimum number of juveniles that can be expected in the control). However, the information reported in Lee *et al.* (2016) regarding the number of eggs was unclear and controversial for further consideration of the validity in the test with tebufenozide (Lee *et al.* 2018), thus the reliability of the reproduction results of the test with tebufenozide was considered as not assignable (R4). It is noted that as major deviations from the guideline, the test was conducted at 25 ± 0.5°C with continuous darkness rather than at 20 ± 2°C with a light:dark cycle of between 12:12 and 16:8 hours.
N	Concentration of total organic carbon or total organic matter in the soil was not reported in the study. For this reason, a normalised effect concentration cannot be calculated and the study is considered "*not assignable*" (R4).
O	Seven days after the first tebufenozide treatment on bare soil (295 g a.s./ha, over-watered and raked into the upper 5 cm) the litter bags containing organic wheat straw were buried at a depth of 5 cm. Then five days later tebufenozide was sprayed onto the soil surface once more (1195 g a.s./ha). For the analytical measurement, samples were taken from the top 10-cm soil layer right after the second application. The soil properties were not described in the study summary. The double application – one before and one after burying the litter bags – is not considered relevant for the SGV dossier (C3). It is noted that while the difference between the treated and the control groups was statistically not significant, in tendency there was a slower straw litter decomposition in the tebufenozide-treated plots throughout the study as compared to the control with the biggest difference after one month (mean 10.2 % vs 3.3 % ash-free dry weight reduction in control vs treatment, relative to the start).
P	Collembola reproduction study conducted to the OECD 232 guideline (OECD 2016a) at 17.7-1000 mg test item/kg soil, corresponding to 4.18-236 mg a.s./kg soil. Ten synchronised juveniles per replicate were tested in the control and in the test concentrations in artificial soil with 8 and 4 replicates, respectively. All validity criteria were met. There were neither dose-response, nor statistically significant effects up to the highest test concentration.
R	*H. aculeifer* reproduction study conducted to the OECD 226 guideline (OECD 2016b) at 17.7-1000 mg test item/kg soil, corresponding to 4.18-236 mg a.s./kg soil. Ten synchronised adult females per replicate were tested in the control and in the test concentrations in artificial soil with 8 and 4 replicates, respectively. All validity criteria were met. There were neither dose-response, nor statistically significant effects up to the highest test concentration.

It is noted that the following studies were considered potentially relevant but did not meet the most important requirement with regard to the way of exposure through soil (and they may have other deficiencies as well), thus they have not been evaluated and listed in detail (they are considered as not relevant, i.e. C3):

- Anonymous (2011b) CONFIRM 2F TEP – A toxicity test to determine the effects on the vegetative vigour of ten plant species, cited in (Nisso 2021), document No. RD-06599.

- Addison (1996), also cited in EC (2006),Vol. 3 B.9.7, p.568 – Earthworm testing in deciduous maple leaf litter and Collembola testing in coniferous litter-fermentation-humus layer following application with tebufenozide.

Appendix 3 Data on the metabolites

Table A3: Effect data on RH-2651, a soil metabolite of tebufenozide. Values resulting from calculations are shown to three significant figures. The lowest relevant and reliable 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. For notes, please refer to the end of Appendix 3 (Notes A2).

Species (Taxonomic group)	Measured effect[8]	Duration	Type of effect concentration	Effect value [mg metabolite/ kg soil]	Total OM [%]	Normalised effect value [mg metabolite/kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	**adult mortality**	**14 d**	**NOEC**	**≥ 100**	**10**	**≥ 34**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5**	**B, F**	**1**	**Boeri & Ward (2002) cited in EC (2006), Vol. 3 B.9.6.1, p.561**
Eisenia fetida (Earthworm)	**biomass (adult weight)**	**14 d**	**NOEC**	**≥ 100**	**10**	**≥ 34**	**Artificial soil: 10 % sphagnum peat, 20 % kaolin clay, 70 % industrial quartz sand, pH 6.0 ± 0.5**	**B, F**	**1**	**Boeri & Ward (2002) cited in EC (2006), Vol. 3 B.9.6.1, p.561**
Eisenia fetida (Earthworm)	not specified	56 d	NOEC	1000	5	680	not reported	S	R4/C1	Anonymous (2019c) cited in MCP (Mimic), Section 10, p.277 included in Nisso (2021).
Microorganisms	**nitrogen transformation (nitrate-N formation rate)[FE]**	**28 d**	**< 25 % effect**	**≥ 1.6**	**1.36 (0.8 % OC)**	**≥ 4**	**Natural soil: sandy soil, 71 % sand, pH 5.79-6.12**	**F**	**1**	**Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.1, p.575**
Microorganisms	**soil respiration [FE]**	**28 d**	**< 25 % effect (< 10 % effect)**	**≥ 1.6**	**1.36 (0.8 % OC)**	**≥ 4**	**Natural soil: sandy soil, 71 % sand, pH 5.79-6.12**	**F**	**1**	**Hayward (2002) cited in EC (2006), Vol. 3 B.9.6.1, p.575**

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

Table A4: Effect data on RH-2703, a soil metabolite of tebufenozide. Values resulting from calculations are shown to 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. For notes, please refer to the end of Appendix 3 (Notes A2).

Species (Taxonomic group)	Measured effect[9]	Duration	Type of effect concentration	Effect value [mg metabolite/ kg soil]	Total OM [%]	Normalised effect value [mg metabolite/kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	not specified	56 d	NOEC	1000	5	680	not reported	S	R4/C1	Anonymous (2019d) cited in MCP (Mimic), Section 10, p.277 included in Nisso (2021).

Table A5: Effect data on RH-6595, a soil metabolite of tebufenozide. Values resulting from calculations are shown to 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. For notes, please refer to the end of Appendix 3 (Notes A2).

Species (Taxonomic group)	Measured effect[10]	Duration	Type of effect concentration	Effect value [mg metabolite/ kg soil]	Total OM [%]	Normalised effect value [mg metabolite/kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	not specified	56 d	NOEC	1000	5	680	not reported	S	R4/C1	Anonymous (2019e) cited in MCP (Mimic), Section 10, p.277 included in Nisso (2021).

9 DE – diversity endpoint, EE – enzymatic endpoint, FE – functional endpoint
10 DE – diversity endpoint, EE – enzymatic endpoint, FE – functional endpoint

Table A6: Effect data on the primary amide of RH-2703 (formerly M2), a soil metabolite of tebufenozide. Values resulting from calculations are shown to 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. For notes, please refer to the end of Appendix 3 (Notes A2).

Species (Taxonomic group)	Measured effect[11]	Duration	Type of effect concentration	Effect value [mg metabolite/ kg soil]	Total OM [%]	Normalised effect value [mg metabolite/kg soil] 3.4 % OM	Test soil	Notes	Assessment score	Source
Eisenia fetida (Earthworm)	not specified	56 d	NOEC	1000	5	680	not reported	S	R4/C1	Anonymous (2019f) cited in MCP (Mimic), Section 10, p.277 included in Nisso (2021).

Notes A2: Notes on soil effect data for tebufenozide metabolite.

B	Limit test with one test concentration (100 mg metabolite/kg soil) and a control with four replicates per treatment.
F	The assessment from the (EC 2006, 2018) reports was adopted and accepted without additional assessment (i.e. at face value). The results were re-calculated according to the actual measured active substance content of the applied formulation (if it was available) thus slight differences to the EU-listed endpoints may occur (if they used the nominal a.s. content).
S	The study results were listed in the M-CP, Section 10 document summarising ecotoxicological studies and risk assessment for the representative product Mimic (240 g/L, SC) as part of the new dossier of the applicant submitted for the EU renewal review of tebufenozide (Nisso 2021). The active substance part – including the summary documents (M-CA sections) and the study reports – are not included in the submitted IUCLID dossier.

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