CONCLUSION ON PESTICIDES PEER REVIEW

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Peer review of the pesticide risk assessment of the active substance milbemectin

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Abstract

The conclusions of the European Food Safety Authority (EFSA) following the peer review of the initial risk assessment carried out by the competent authorities of the rapporteur Member State, Germany, and co-rapporteur Member State, the Netherlands, for the pesticide active substance milbemectin are reported. The context of the peer review was that required by Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The conclusions were reached on the basis of the evaluation of the representative uses of milbemectin as an acaricide and insecticide in strawberry (field and greenhouse), berries and black and white currant (field and greenhouse), apple, pear, cherry and plum (field) and ornamentals (field and greenhouse). The reliable end points, appropriate for use in regulatory risk assessment, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are reported where identified.

Keywords: milbemectin, peer review, risk assessment, pesticide, acaricide, insecticide

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Summary

Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659, lays down the procedure for the renewal of the approval of active substances submitted under Article 14 of Regulation (EC) No 1107/2009. The list of those substances is established in Commission Implementing Regulation (EU) No 686/2012. Milbemectin is one of the active substances listed in Regulation (EU) No 686/2012.

In accordance with Article 1 of the Regulation (EU) No 844/2012, the rapporteur Member State (RMS), Germany, and the co-rapporteur Member State (co-RMS), the Netherlands, received an application from Belchim Crop Protection NV for the renewal of approval of the active substance milbemectin.

An initial evaluation of the dossier on milbemectin was provided by the RMS in the renewal assessment report (RAR) and, subsequently, a peer review of the pesticide risk assessment on the RMS evaluation was conducted by EFSA in accordance with Article 13 of Commission Implementing Regulation (EU) No 844/2012, as amended by Commission Implementing Regulation (EU) No 2018/1659. The following conclusions are derived.

The uses of milbemectin according to the representative uses proposed at the European Union (EU) level result in a sufficient acaricidal and insecticidal efficacy against the target organisms.

The assessment of the data package revealed no issues that could not be finalised or that needed to be included as critical areas of concern with respect to **identity, physical, chemical and technical properties** of the active substance and the formulation for representative uses, and **analytical methods**.

In the area of **mammalian toxicology**, the acceptability of the proposed maximum levels of the impurities in the reference specification and the representativeness of the batches used in the toxicity studies with regard to the reference specifications could not be finalised. In addition, interspecies differences in metabolism with possible identification of unique human metabolites and potential for phototoxicity of milbemectin could not be concluded. Critical areas of concern were not identified.

In the area of **residues,** the assessment of the data package revealed no issues that could not be finalised or that needed to be included as critical areas of concern.

The data available on **environmental fate and behaviour** were sufficient to carry out the required environmental exposure assessments at EU level for the representative uses, with the exception that the exposure assessment to aquatic systems could not be finalised while information on the fate and behaviour in these systems was not available for the active substance component milbemycin A_3.

In the area of **ecotoxicology,** the risk assessment for aquatic organisms could not be finalised. A high acute risk was identified to bees for the uses in field and other non-permanent protected structures. A high risk to Collembola could not be excluded for the representative uses in ornamentals (high rate, in field and other non-permanent protected structures) for the metabolite 27-keto MA_4/ MA_3. Critical areas of concern were not identified.

With regard to the **endocrine disruption** (ED) properties, based on the available information, it can be concluded that milbemectin does not meet the ED criteria for humans and non-target organisms according to points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) 2018/605.

Table of contents

Background

Commission Implementing Regulation (EU) No 844/2012[1], as amended by Commission Implementing Regulation (EU) No 2018/1659[2] (hereinafter referred to as 'the Regulation'), lays down the provisions for the procedure of the renewal of the approval of active substances, submitted under Article 14 of Regulation (EC) No 1107/2009[3]. This regulates for the European Food Safety Authority (EFSA) the procedure for organising the consultation of Member States, the applicant(s) and the public on the initial evaluation provided by the rapporteur Member State (RMS) and/or co-rapporteur Member State (co-RMS) in the renewal assessment report (RAR), and the organisation of an expert consultation where appropriate.

In accordance with Article 13 of the Regulation, unless formally informed by the European Commission that a conclusion is not necessary, EFSA is required to adopt a conclusion on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009 within 5 months from the end of the period provided for the submission of written comments, subject to an extension of an additional 3 months where additional information is required to be submitted by the applicant(s) in accordance with Article 13(3). Furthermore, in accordance with Article 13(3a), where the information available in the dossier is not sufficient to conclude the assessment on whether the approval criteria for endocrine disruption are met, additional information can be requested to be submitted in a period of minimum 3 months, not exceeding 30 months, depending on the type of information requested.

In accordance with Article 1 of the Regulation, the RMS, Germany, and co-RMS, the Netherlands, received an application from Belchim Crop Protection NV for the renewal of approval of the active substance milbemectin. Complying with Article 8 of the Regulation, the RMS checked the completeness of the dossier and informed the applicant, the co-RMS (the Netherlands), the European Commission and EFSA about the admissibility.

The RMS provided its initial evaluation of the dossier on milbemectin in the RAR, which was received by EFSA on 29 June 2017 (Germany, 2017).

In accordance with Article 12 of the Regulation, EFSA distributed the RAR to the Member States and the applicant, Belchim Crop Protection NV, for consultation and comments on 28 August 2017. EFSA also provided comments. In addition, EFSA conducted a public consultation on the RAR. EFSA collated and forwarded all comments received to the European Commission on 13 December 2017. At the same time, the collated comments were forwarded to the RMS for compilation and evaluation in the format of a reporting table. The applicant was invited to respond to the comments in column 3 of the reporting table. The comments and the applicant's response were evaluated by the RMS in column 3.

The need for experts' consultation and the necessity for additional information to be submitted by the applicant in accordance with Article 13(3) of the Regulation were considered in a telephone conference between EFSA and the RMS on 19 February 2018. On the basis of the comments received, the applicant's response to the comments and the RMS's evaluation thereof, it was concluded that additional information should be requested from the applicant and that EFSA should conduct an experts' consultation in the areas of mammalian toxicology, residues and ecotoxicology.

The outcome of the telephone conference, together with EFSA's further consideration of the comments, is reflected in the conclusions set out in column 4 of the reporting table. All points that were identified as unresolved at the end of the comment evaluation phase and which required further consideration, including those issues to be considered in an experts' consultation, were compiled by EFSA in the format of an evaluation table.

The conclusions arising from the consideration by EFSA, and as appropriate by the RMS, of the points identified in the evaluation table, together with the outcome of the expert consultation and the written consultation on the assessment of additional information, where these took place, were reported in the final column of the evaluation table.

[1] Commission Implementing Regulation (EU) No 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 252, 19.9.2012, pp. 26–32.

[2] Commission Implementing Regulation (EU) No 2018/1659 of 7 November 2018 amending Implementing Regulation (EU) No 844/2012 in view of the scientific criteria for the determination of endocrine-disrupting properties introduced by Regulation (EU) 2018/605.

[3] Regulation (EC) No 1107/2009 of 21 October 2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, pp. 1–50.

Commission Regulation (EU) 2018/605[4] introduced new scientific criteria for the determination of endocrine-disrupting (ED) properties, applicable as of 10 November 2018 to all applications for the approval/renewal of active substances, including pending applications. The peer review on the active substance milbemectin was already in an advanced stage at the time of entry into force of the new criteria, and an assessment of the ED potential in line with the EFSA/ECHA (2018) guidance document[5] for this substance was not available. Therefore, EFSA has performed an assessment of the ED properties of the active substance milbemectin in line with the EFSA/ECHA (2018) guidance for further consideration in the peer review.

Following a consultation with Member States in the Pesticides Peer Review Experts' Meeting 05 Mammalian toxicology – Ecotoxicology (joint session on endocrine disruption) (7–8 May 2019), it was considered necessary to apply an additional clock stop of max. 30 months in accordance with Commission Implementing Regulation (EU) No 2018/1659, to be able to conclude whether the approval criteria for endocrine disruption in line with the scientific criteria for the determination of endocrine-disrupting properties, as laid down in Commission Regulation (EU) 2018/605[6], are met.

Therefore, in accordance with the provisions of Commission Regulation (EU) No 2018/1659[7], in June 2019, the applicant was given the opportunity to submit, within a period of up to 30 months, additional information to address the approval criteria set out in point 3.6.5 and/or 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by Commission Regulation (EU) No 2018/605, and/or documentary evidence demonstrating that milbemectin may be used such that exposure is negligible, and/or the conditions for application of the derogation under Art.4(7) of Regulation (EC) No 1107/2009 are met. The additional information submitted by the applicant was subsequently evaluated by the RMS.

A public consultation on the revised RAR on the endocrine properties assessment made available by the RMS after the 30-month clock stop (Germany, March 2022) was conducted in May–July 2022. All comments received, including those from the applicant and Member States, were collated in the format of a reporting table and were considered during the finalisation of the peer review. As a result of the public consultation, the need for an additional experts' consultation in the area of ecotoxicology was identified.

A final consultation on the conclusions arising from the peer review of the risk assessment took place with Member States via a written procedure in May–June 2023.

This conclusion report summarises the outcome of the peer review of the risk assessment of the active substance and the representative formulation, evaluated on the basis of the representative uses of milbemectin as an acaricide and insecticide in strawberry (field and greenhouse), berries and black and white currant (field and greenhouse), apple, pear, cherry and plum (field) and ornamentals (field and greenhouse), as proposed by the applicant. In accordance with Article 12(2) of Regulation (EC) No 1107/2009, risk mitigation options identified in the RAR and considered during the peer review, if any, are presented in the conclusion. A list of the relevant end points for the active substance and the formulation for representative uses is provided in Appendix B. In addition, the considerations as regards the cut-off criteria for milbemectin according to Annex II of Regulation (EC) No 1107/2009 are summarised in Appendix A.

In addition, a key supporting document to this conclusion is the peer review report (EFSA, 2023), which is a compilation of the documentation developed to evaluate and address all issues raised in the peer review, from the initial commenting phase to the conclusion. The peer review report comprises the following documents, in which all views expressed during the course of the peer review, including minority views, where applicable, can be found:

[4] Commission Regulation (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine-disrupting properties. OJ L 101, 20.4.2018, pp. 33–36.

[5] ECHA (European Chemicals Agency) and EFSA (European Food Safety Authority) with the technical support of the Joint Research Centre (JRC), Andersson N, Arena M, Auteri D, Barmaz S, Grignard E, Kienzler A, Lepper P, Lostia AM, Munn S, Parra Morte JM, Pellizzato F, Tarazona J, Terron A and Van der Linden S, 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311, 135 pp. https://doi.org/10.2903/j.efsa.2018.5311. ECHA-18-G-01-EN.

[6] Commission Regulation (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine-disrupting properties. OJ L 101, 20.4.2018, pp. 33–36.

[7] Commission Implementing Regulation (EU) 2018/1659 of 7 November 2018 amending Implementing Regulation (EU) No 844/2012 in view of the scientific criteria for the determination of endocrine-disrupting properties introduced by Regulation (EU) 2018/605. OJ L 278, 8.11.2018, pp. 3–6.

- the comments received on the RAR;
- the comments received on the EFSA addendum on endocrine assessment (March 2019)[8];
- the reporting tables (20 February 2018 and 3 October 2022[9]);
- the evaluation table (26 June 2023);
- the reports of the scientific consultation with Member State experts (where relevant);
- the comments received on the assessment of the additional information (where relevant);
- the comments received on the draft EFSA conclusion.

Given the importance of the RAR, including its revisions (Germany, 2023), as well as the peer review report and the EFSA addendum on endocrine assessment (EFSA, 2019), both documents are considered as background documents to this conclusion and thus are made publicly available.

It is recommended that this conclusion report and its background documents would not be accepted to support any registration outside the EU for which the applicant has not demonstrated that it has regulatory access to the information on which this conclusion report is based.

The active substance and the formulation for representative uses

Milbemectin is the ISO common name for a mixture of 70% (10*E*,14*E*,16*E*)-(1*R*,4*S*,5′ *S*,6*R*,6′*R*,8*R*,13*R*,20*R*,21*R*,24*S*)-6′-ethyl-21,24-dihydroxy-5′,11,13,22-tetramethyl-(3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$]pentacosa-10,14,16,22-tetraene)-6-spiro-2′-(tetrahydropyran)-2-one (milbemycin A_4) and 30% (10*E*,14*E*,16*E*)-(1*R*,4*S*,5′ *S*,6*R*,6′*R*,8*R*,13*R*,20*R*,21*R*,24*S*)-21,24-dihydroxy-5′,6′,11,13,22-pentamethyl-(3,7,19-trioxatetracyclo[15.6.1.$1^{4,8}$.$0^{20,24}$]pentacosa-10,14,16,22-tetraene)-6-spiro-2′-(tetrahydropyran)-2-one (milbemycin A_3) (IUPAC).

The formulation for representative uses for the evaluation was 'Milbemectin 1% EC', an emulsifiable concentrate (EC) containing 9.3 g/L milbemectin.

The representative uses evaluated were foliar spray applications to control harmful mites (such as *Panonychus ulmi, Tetranychus spp., Tarsonemus spp*.) in strawberry (field and greenhouse), berries and black and white currant (field and greenhouse), apple, pear, cherry and plum (field), and mites (such as *Panonychus ulmi, Tetranychus spp., Tarsonemus spp*.) and insects (such as *Liriomyza spp*.) in ornamentals (field and greenhouse). The greenhouse uses include both permanent greenhouses and other protected structures (e.g. walk-in tunnels). Full details of the good agricultural practices (GAPs) can be found in the list of end points in Appendix B.

Data were submitted to conclude that according to the representative uses proposed at EU level the uses of milbemectin result in a sufficient acaricidal and insecticidal efficacy against the target organisms, following the guidance document SANCO/2012/11251-rev. 4 (European Commission, 2014b).

Conclusions of the evaluation

1. Identity, physical/chemical/technical properties and methods of analysis

The following guidance documents were followed in the production of this conclusion: European Commission, 2000a,b, 2010.

The proposed specification for milbemectin is based on batch data from industrial plant production and quality control (QC) data. The proposed minimum purity of the technical material is 950 g/kg with a ratio milbemycin A_3:milbemycin A_4 of approximately 30:70%. It should be mentioned that an assessment of the toxicological relevance of the impurities was not provided (see Section 2). Based on the available data, it could not be concluded that the batches used in the (eco)toxicological studies were sufficiently representative of the specifications (the newly proposed and the current reference specifications) (see Sections 2 and 5). There is no Food and Agriculture Organization (FAO) specification available for milbemectin.

The main data regarding the identity of milbemectin and its physical and chemical properties are given in Appendix B.

[8] ED assessment performed by EFSA before the timepoint of the ED additional information request (stop of the clock). The ED assessment including evaluation of the newly provided additional information on the endocrine disruption properties following the ED clock stop is available in the revised RAR (Germany, 2023) with the final outcome presented in the current EFSA Conclusion (see Section 6).

[9] Reporting table following consultation on the revised RAR on the assessment of the endocrine-disrupting properties made available after the 30-month clock stop.

Adequate methods are available for the generation of data required for the risk assessment except for short-term dog studies (**data gap,** see Sections 2 and 10). Methods of analysis are available for the determination of the active substance in the technical material and in the formulation for representative uses and for the determination of the respective impurities in the technical material.

The components of the residue definition (sum of milbemycin A_3 and milbemycin A_4, expressed as milbemectin) in food and feed of plant origin can be monitored by the quick, easy, cheap, effective and safe method (QuEChERS) using liquid chromatography with tandem mass spectrometry (LC–MS/MS) with a limit of quantification (LOQ) of 0.01 mg/kg for each analyte in all commodity groups. A validated QuEChERS method using LC–MS/MS with a LOQ of 0.01 mg/kg (per analyte) exists for monitoring of milbemycin A_3 and milbemycin A_4 in compliance with the maximum residue level (MRL) set in food of animal origin (Commission Regulation (EU) No 1317/2013[10]). It is noted that the extraction efficiency used in both monitoring methods was not verified as residues above the LOQ as a result of the representative uses are not expected.

Milbemycin A_4 can be monitored in soil and water by LC–MS/MS with LOQs of 0.01 mg/kg and 0.05 μg/L, respectively. However, the residue definition for monitoring for both compartments was concluded as sum of milbemycin A_3 and milbemycin A_4; as a consequence, **a data gap** for monitoring methods for determination of all components of the residue definition in soil and water was identified (see Section 10).

Residues of milbemycin A_3 and milbemycin A_4 in air can be determined after derivatisation (to the corresponding fluorescent anhydrides) by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) with a LOQ of 0.42 $\mu g/m^3$; however, the efficiency of the derivatisation step was not demonstrated. LC–MS method (without prior derivatisation) exists for analysis of milbemycin A_3 and milbemycin A_4 residues in air with a LOQ of 0.42 $\mu g/m^3$; however, the method is not validated for warm and humid air (35°C, 80% relative humidity). Therefore, additional validation data for the existing methods or a new method for determination of residues in air are required (**data gap**, see Section 10).

LC–MS/MS can be used for monitoring of milbemycin A_3 and milbemycin A_4 in body fluids and tissues with LOQ of 0.05 mg/L (per analyte) and 0.01 mg/kg (per analyte), respectively. However, the residue definition for monitoring in body fluids and tissues was concluded by EFSA as sum of milbemycin A_3 and milbemycin A_4 and the metabolite 13-hydroxy MA_4 (see Section 2); as a consequence, a **data gap** for a monitoring method for analysis of 13-hydroxy MA_4 in body fluids and tissues was identified (see Section 10).

2. Mammalian toxicity

The following guidance documents were followed in the production of this conclusion: European Commission, 2003, 2012; EFSA PPR Panel, 2012; EFSA, 2014, 2022; ECHA, 2017.

Milbemectin was discussed at the Pesticides Peer Review Experts' Meeting TC 183 in June 2018. The compound is a mixture of milbemycin A_3 (methyl component, approximately 30%) and milbemycin A_4 (ethyl component, approximately 70%), and they can be expected to have a similar toxicological profile.

Considering that insufficient information for the assessment of the toxicological relevance of the impurities has been provided (data gap), it could not be concluded if the proposed maximum levels of the impurities are acceptable and if the batches used in the toxicological studies were representative of the current and/or the newly proposed **reference specifications (issue not finalised, see Section 9.1)**. It is noted that only one batch could be considered representative of the newly proposed reference specification (not of the current reference specification). The analytical methods used in the toxicity studies were considered fit-for-purpose, except for the short-term dog studies for which no information was provided (**data gap**, see Section 10). This was considered as an additional source of uncertainty which should not prevent the use of these studies (in the most sensitive species) for risk assessment.

Based on experimental data with milbemycin A_4, the **oral absorption** value for milbemectin is 47% (taking into account biliary and urinary excretion). No bioaccumulation potential was identified. For the purpose of human biomonitoring, the residue definition for body fluids (plasma and urine) and

[10] Commission Regulation (EU) No 1317/2013 of 16 December 2013 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 2,4-D, beflubutamid, cyclanilide, diniconazole, florasulam, metolachlor and S-metolachlor, and milbemectin in or on certain products. OJ L 339, 17.12.2013. pp. 1–43.

tissues should include the major metabolite identified in the toxicokinetic studies with rats, i.e. the metabolite 13-hydroxy MA_4. Interspecies differences in metabolism of milbemectin with possible identification of unique human metabolites could not be finalised in the absence of an *in vitro* comparative metabolism study (**data gap and issue not finalised**, see Section 9.1).

With regard to its **acute** toxic properties, the available data support the harmonised classification[11] for acute toxicity category 4 (for oral and inhalation exposure). Neither acute dermal toxicity, skin and eye irritation, nor skin sensitising properties were shown for milbemectin. *In vitro* phototoxicity assay (and photomutagenicity if positive results) with milbemectin, testing the range of wavelengths (between 290 and 700 nm) where the absorption coefficient is $> 10\ L \times mol^{-1} \times cm^{-1}$, should be provided (**data gap and issue not finalised**, see Section 9.1).

In the **short-term** dietary toxicity studies, the most sensitive species was the dog with a no observed adverse effect level (NOAEL) of 3 mg/kg body weight (bw) per day for both the 13-week and 12-month studies.

With regard to **genotoxicity**, the available *in vitro* studies (Ames test, cytogenetic assay with mammalian cells, mouse lymphoma assay) gave negative results; however, polyploidy was not investigated (as recommended in the current OECD test guideline). For the *in vivo* mouse micronucleus test, in the absence of sufficient evidence of bone marrow exposure, the negative results were not considered reliable.[12] Consequently, the aneugenic potential of milbemectin could not be concluded (**data gap,** see Section 10). The experts agreed that, since aneuploidy is likely to be induced by a threshold-based mechanism, milbemectin can be considered as unlikely to be a non-threshold genotoxic compound.

In **long-term toxicity** studies, a carcinogenic and systemic NOAEL of 0.7 mg/kg bw per day was identified for the rats, based on increased incidences of uterine endometrial stromal polyps (considered as benign tumours) and increased kidney weights. The criteria for classification as **Carc. Cat. 2**[13] according to Regulation (EC) No 1272/2008 (ECHA, 2017) may be met for milbemectin on the basis of uterine tumours in rats.

With regard to **reproductive toxicity**, the multigeneration study with rats concluded on a parental and offspring NOAEL of 12.4 mg/kg bw per day on the basis of slightly decreased body weight in parental animals, decreased litter size, increased perinatal mortality and decreased fetal/pup weight, respectively. No adverse effect was observed on the reproductive parameters up to 53.3 mg/kg bw per day. On the basis of the developmental effects (litter size, survival and foetal weight), it was agreed that the criteria for classification as **Repr. Cat. 2**[14] ('Suspected of damaging the unborn child') according to Regulation (EC) No 1272/2008 (ECHA, 2017) may be met for milbemectin.

For all **teratogenicity** studies, the impurity profile of the batch was missing. In the rat study, the maternal NOAEL is 20 mg/kg bw per day based on decreased food consumption and body weight gain; whereas the developmental NOAEL is 60 mg/kg bw per day (highest dose). From the second rabbit study, the relevant maternal NOAEL is 50 mg/kg bw per day based on clinical signs and decreased food consumption and body weight, as well as the developmental NOAEL where prenatal mortality and decreased body weight occurred with severe maternal toxicity. No teratogenic effects were observed.

Neurotoxic effects were observed in acute (but not repeated) **neurotoxicity** studies with rats and in short-term dog studies (clinical signs including also vomiting). On the basis of neurotoxic effects in the dog studies, it was concluded that the criteria for classification as **STOT-RE Cat 2** according to Regulation (EC) No 1272/2008 (ECHA, 2017) may be met for milbemectin.[15] An assessment of the potential **immunotoxicity** of milbemectin has not been provided in the RAR, but no indication of such an effect was observed in the available studies.

The **acceptable daily intake** (ADI) for milbemectin is 0.007 mg/kg bw per day based on the 2-year rat study, applying an uncertainty factor (UF) of 100. The **acceptable operator exposure level** (AOEL) is 0.014 mg/kg bw per day based on the dog studies (13 weeks and 12 months) and applying an UF of 100 and a correction for an oral absorption value of 47%. The **acute reference dose**

[11] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, pp. 1–1355.
[12] Refer to the peer review report (EFSA, 2023), experts' consultation point 2.3, data requirement 2.4 and data gap 2.10 in the Evaluation Table.
[13] See expert consultation point 2.4 in the Report of Pesticides Peer Review Experts' TC 183 (EFSA, 2023).
[14] See expert consultation point 2.5 in the Report of Pesticides Peer Review Experts' TC 183 (EFSA, 2023).
[15] See expert consultation point 2.2 in the Report of Pesticides Peer Review Experts' TC 183 (EFSA, 2023).

(ARfD) is 0.03 mg/kg bw based on the 13-week dog study (acute effects occurring 1–6 h after treatment) and applying an UF of 100. The **acute acceptable operator exposure level** (AAOEL) is 0.014 mg/kg bw based on the 13-week dog study and applying an UF of 100 and a correction for an oral absorption value of 47%. During the first peer review (European Commission, 2005), an ADI and ARfD of 0.03 mg/kg bw (per day) were derived on the basis of the dog studies, and an AOEL of 0.014 was derived based on the 12-month dog study with a correction for an oral absorption value of 47%.

The **dermal absorption** values for the formulation for the representative uses 'Milbemectin 1% EC' are 2% for the concentrate, 18% for intermediate dilution and 19% for the highest dilution.[16]

For the outdoor uses, the estimated **operator exposure** does not exceed the systemic AOEL/ AAOEL without the use of personal protective equipment (PPE) with at least one of the models (German model (Germany, 2023), UK POEM or EFSA model (EFSA, 2014), the latter not implemented when the renewal dossier for milbemectin was submitted).[17] The exposure of **bystanders and residents** (considering the worst-case use on fruit crops) is predicted to be below the AOEL with Martin et al., 2008[18] (Germany, 2023) and with EFSA model (EFSA, 2014). **Workers** handling ornamentals treated against leaf miners should use gloves in addition to workwear (covering arms, body and legs) as shown by the EFSA model (EFSA, 2014).

For the greenhouse uses, the estimated **operator exposure** does not exceed the systemic (A) AOEL when using data from the study of Mich[19] and the German model (for mixing/loading phase) while PPE are required when using the EFSA model (EFSA, 2022, not implemented when the renewal dossier for milbemectin was submitted). Exposure of **bystanders and residents** is considered covered by the outdoor uses, therefore predicted to be below the (A)AOEL. Workers handling treated ornamentals in greenhouses should use gloves in addition to workwear as shown by the EFSA model (EFSA, 2022).

Toxicological studies have been provided for two **metabolites** (photoisomers 8,9Z-MA_4 and 8,9Z-MA_3) (not needed for the representative uses, see Appendix B).

3. Residues

The following guidance documents were followed in the production of this conclusion: OECD (2009, 2011), European Commission (2011) and JMPR (2004, 2007).

Milbemectin was discussed at the Pesticides Peer Review Experts' Meeting TC 184 in June 2018.

Metabolism studies were conducted in apples and oranges after foliar application with ^{14}C-milbemycin A_4. The conditions cover the representative GAPs on berries, apples and cherries. Although only milbemycin A_4 was investigated instead of a mixture of milbemycin A_3 and milbemycin A_4, being milbemycin A_4 representative of milbemectin the studies were considered acceptable in view of the similar structure of milbemycin A_3 and milbemycin A_4 (presence of methyl or ethyl moiety, respectively, on the lateral side chain). Total residues in fruit were low (max. 0.11 mg eq/kg in orange peel) but allowed for identification of milbemycin A_4 with up to 55% total radioactive residue (TRR) in orange peel and its photo isomer 8,9Z-milbemycin A_4 (8,9Z-MA_4) which remained below 10% TRR in all plant parts. 27-keto-MA_4 was found only in orange peel and leaves and at levels below 10% TRR. In apple and orange, unknown metabolites were reported but each accounted for less than 10% TRR in the fruit parts and they were major only in the leaves. It is noted that additional metabolism data on strawberries were considered as supportive due to shortcomings in the reporting; nevertheless, they supported the findings of the other two studies.

A sufficient number of residue trials with apples/pears, cherries, plums, strawberries, black and red currant was provided, and the trials were supported by storage stability data and conducted according to the critical GAPs. Although not required, the two photoisomers (8,9Z-MA_3 and 8,9Z-MA_4) were monitored in all commodities and found to be below their LOQs. Quantifiable residues were found only in cherries and amounted up to 0.09 mg/kg (sum of milbemycin A_3 and milbemycin A_4) requiring a study investigating the nature of residues in processed commodities (**data gap,** see Section 10).

[16] See expert consultation point 2.8 in the Report of Pesticides Peer Review Experts' TC 183 (EFSA, 2023).
[17] Further details on scenarios for which PPE is required can be found in Section 8 and Appendix B.
[18] The Martin et al.'s approach (Martin et al., 2008) is no longer scientifically supported, since limited data were included for three-dimensional exposure to spray drift and no estimates are provided for exposure to vapour from low volatility compounds. Accordingly the predictions are considered underestimated and are given for informative purpose.
[19] Mich, G., Mich, 1986, Operator exposure in Greenhouses During Practical Use of Plant Protection Products; ECON GmbH Ingelheim, conducted in Germany under the sponsorship of IVA (Industrieverband Agrar).

Following the review of MRLs according to Article 12 of Regulation (EC) No 396/2005 (EFSA, 2012), a study investigating the nature of residues in rotational crops was requested as strawberries are considered semi-permanent crops. In the newly presented study, leafy lettuce, radish and sorghum/wheat were planted after soil treatment with ^{14}C-milbemycin A_4 at a rate of 0.11 kg as/ha. Radioactive residues above 0.01 mg eq/kg were detected only at day 30 in radish (top 0.025 and root 0.014 mg eq/kg) and sorghum fodder (0.01 mg eq/kg). Due to the very low residue levels, no metabolites could be identified. The submitted rotational crop study did not provide information on the incorporation of the active substance into soil. However, the analysis of soil samples before and after application and at all plant back intervals together with the results of analysis in food and feed commodities provided evidence that no significant transfer from soil to rotational crops occurs under the conditions of the presented trial.

The **residue definition for fruit crops for enforcement and risk assessment** is set as milbemectin (sum of milbemycin A_3 and milbemycin A_4, expressed as milbemectin). For any future additional use, specific attention should be paid to the continuous formation of the photoisomers (8,9Z-MA_4 and 8,9Z-MA_3) mainly in lipophilic fractions of the edible parts of the crops.

Stability for milbemycin A_3, milbemycin A_4 and their photoisomers 8,9Z-MA_3 and 8,9Z-MA_4 was demonstrated in apple, strawberry and orange up to 12 months, and for milbemycin A_3 and milbemycin A_4 in almond and peaches up to 6 months.

Animal metabolism studies are not required as apple pomace is the only feed item and it is not leading to animal dietary burden above 0.004 mg/kg bw.

From the representative uses the highest chronic exposure amounted to 4% of the ADI, based on German children and equal to 5% of the ADI, based on NL toddlers when using PRIMo version 2.0 and version 3.1, respectively. The highest acute exposure resulted in 6.5% of the ARfD (apple) and 4% of the ARfD (cherries) when using PRIMo version 2.0 and version 3.1, respectively. Despite a fivefold decrease of the ADI, there is no consumer risk identified using PRIMo 3.1 with respect to the uses evaluated in the reasoned opinion on the review of the existing MRLs for milbemectin (EFSA, 2012).

Data on residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom were not provided (**data gap**, see Section 10). It cannot be excluded that residues will be present in plant parts including flowers from the representative outdoor uses before flowering.

The MRL request for cherries was fully supported by the available data and an MRL of 0.2 mg/kg was proposed.

4. Environmental fate and behaviour

The rates of dissipation and degradation in the environmental matrices investigated were estimated using FOCUS (2006) kinetics guidance. In soil laboratory incubations under aerobic conditions in the dark, milbemectin constituent milbemycin A_4 exhibited moderate to medium persistence, forming the major (> 10% applied radioactivity (AR)) metabolites 27-hydroxy-MA_4 (max. 10.4% AR) and 27-keto-MA_4 (max. 11.6% AR), both of which exhibited low to moderate persistence. Mineralisation of the ^{14}C radiolabels to carbon dioxide accounted for 14–35% AR after 120 days. The formation of unextractable residues (not extracted by methanol followed by 50°C methanol/water and acetonitrile/water, Soxhlet) for the radiolabels accounted for 13–38% AR after 120 days. In anaerobic soil incubations, milbemectin was more stable. In a laboratory soil photolysis study, constituent milbemycin A_4 degraded slightly faster than in the dark control but novel photolysis transformation products were not identified compared to the soil incubations in the dark. Milbemectin constituent milbemycin A_4 and 27-hydroxy-MA_4 exhibited low to slight mobility in soil. 27-keto-MA_4 is considered immobile. It was concluded that the adsorption of all these compounds was not pH dependent. In field dissipation studies carried out at three sites in the USA (in California, Florida and New York state, spray application to the soil surface), milbemectin constituents milbemycin A_4 and milbemycin A_3 dissipated at comparable rates. In addition to milbemycin A_4 and milbemycin A_3, sample analyses were also carried out for 27-hydroxy-MA_4, 27-hydroxy-MA_3, 27-keto-MA_4, 27-keto-MA_3, 8,9Z-MA_4 and 8,9Z-MA_3. These compounds were either below or around the limit of quantification of the analytical method (0.002 mg/kg for 27-hydroxy and 0.005 mg/kg for 27-keto and 8,9Z metabolites). As the weather information provided for the duration of these USA field trials was insufficient, it was not possible to use the dissipation rates from the trials to support the EU assessment nor to normalise them to reference conditions (**data gap**, see Section 10). However, the results of the USA trials did provide sufficient evidence to conclude that the transformation behaviour of the MA_3 components in soil could be read across from the laboratory information in the dossier on the MA_4 components.

In laboratory incubations in dark aerobic natural sediment water systems, milbemectin constituent milbemycin A_4 exhibited moderate to medium persistence, forming the metabolites 27-hydroxy-MA_4 (max. 7.4% AR) and 27-keto-MA_4 (max. 4.5% AR). The unextractable sediment fraction (not extracted by methanol followed by acetonitrile/water Soxhlet) was the major sink for the ^{14}C radiolabels, accounting for 29–32% AR at study end (100 days). Mineralisation of the radiolabels accounted for only 6% AR at the end of the study. The rate of decline of milbemectin constituent milbemycin A_4 in a laboratory sterile aqueous photolysis experiment was very fast relative to that occurred in the aerobic sediment water incubations. The major photolysis transformation product formed was 8,9Z-MA_4 (max. 12.6% AR). Information on the fate and behaviour in aquatic systems of the milbemectin constituent milbemycin A_3 was not available. This has been identified as a **data gap** and results in the aquatic exposure **assessment being not finalised** (see Section 9.1).

The necessary surface water and sediment exposure assessments (predicted environmental concentrations (PEC) calculations) were carried out for milbemectin and the metabolites 27-hydroxy, 27-keto and 8,9Z (with the assumption that the MA_3 constituent components would behave in the same way as the MA_4 components which were those for which experimental information was available), using the FOCUS (FOCUS, 2001) step 1 and step 2 approach (version 3.2 of the Steps 1–2 in FOCUS calculator). For the active substance milbemectin and the photolysis transformation products 8,9Z-MA_4/MA_3, step 3 (FOCUS, 2001) and step 4 calculations were available, except for the representative uses on strawberries and cane fruit which is identified as a **data gap** which can contribute to the concern of the aquatic risk **assessment being not finalised** (see Sections 5 and 9.1). The step 4 calculations appropriately followed the FOCUS (FOCUS, 2007) guidance, with no-spray drift buffer zones of up to 20 m being implemented for the drainage scenarios (representing a 67–93% spray drift reduction), and combined no-spray buffer zones with vegetative buffer strips of up to 20 m (reducing solute flux in run-off by 80% and erosion runoff of mass adsorbed to soil by 95%) being implemented for the run-off scenarios. The SWAN tool (version 4.0.1) was appropriately used to implement these mitigation measures in the simulations.

For the protected uses in permanent greenhouses, the necessary surface water and sediment exposure assessments (PEC) were carried out by EFSA for milbemectin (with the assumption that the MA_3 constituent components would behave in the same way as the MA_4 components which were those for which experimental information was available) using the FOCUS (2001) step 1 and step 2 approach (version 3.2 of the steps 1–2 in FOCUS calculator), which was then modified by post-processing the spray drift input results (option no run-off or drainage was selected) to obtain a 0.2% emission (relevant if application is by ultra-low volume spray) or 0.1% emission (relevant for hydraulic spray) of milbemectin from greenhouses being re-deposited on adjacent surface water bodies. This approach has been accepted by Member State experts as an assumption that can be used in EU level surface water exposure assessments for greenhouse uses and it is referred to in FOCUS (2008) guidance as being appropriate.

The necessary groundwater exposure assessments were appropriately carried out using FOCUS (European Commission, 2014a) scenarios and the model PELMO 5.5.3.[20] The potential for groundwater exposure from the representative uses by milbemectin (milbemycin A_4/milbemycin A_3), 27-hydroxy-MA_4/MA_3 and 27-keto-MA_4/MA_3 above the parametric drinking water limit of 0.1 μg/L was concluded to be low in geoclimatic situations that are represented by all nine FOCUS groundwater scenarios.

The applicant provided appropriate information to address the effect of water treatment processes on the nature of the residues that might be present in surface water, when surface water is abstracted for drinking water. The conclusion of this consideration was that neither milbemectin nor any of its degradation products that trigger assessment (27-hydroxy-MA_4/MA_3, 27-keto-MA_4/MA_3 and 8,9Z-MA_4/MA_3) would be expected to undergo any transformation due to oxidation at the disinfection stage of usual water treatment processes that would give rise to the formation of products such as anilines, nitrosamines or polyhalogenated biphenyls.

The PEC in soil, surface water, sediment and groundwater covering the representative uses assessed can be found in Appendix B of this conclusion. A key to the persistence and mobility class wording used, relating these words to numerical DT and Koc endpoint values can be found in Appendix C.

[20] Simulations utilised the agreed Q10 of 2.58 (following EFSA, 2008) and Walker equation coefficient of 0.7.

5. Ecotoxicology

The following guidance documents were followed in the production of this conclusion: European Commission (2002), SETAC (2001), EFSA (2009), EFSA PPR Panel (2013) and EFSA (2013).

Milbemectin was discussed at the Pesticides Peer Review Experts' Meeting TC 181 in June 2018.

The compliance of the batches used in the ecotoxicological studies with the newly proposed and current reference technical specification was not fully demonstrated, which led to a **data gap** (see Section 10).

The greenhouse uses include both permanent greenhouses and other non-permanent protected structures (e.g. walk-in tunnels). In the risk assessments presented below, when uses in 'field' are reported, uses in non-permanent protected structures are also covered. For permanent greenhouses, low risk was concluded for birds and mammals, bees, non-target arthropods, soil organisms and non-target terrestrial plants, since exposure to the environment is not anticipated.

It has to be noted that 'Milbemectin 1% EC' (BCP114I-B) is the newly proposed formulation for representative uses, while 'Milbeknock 1% EC' is the formulation for representative uses presented at the time of the first approval. Suitable evidence to support the bridging between the two formulations was available, and therefore, they were considered having comparable toxicity.

Acute and chronic studies with both the active substance and the formulation 'Milbeknock 1% EC' were available to assess the risk for **birds** and **mammals**. The risk (acute and long-term) from dietary exposure to birds and mammals was assessed as low for all the representative uses. The risk from secondary poisoning was also assessed as low for fish-eating birds and mammals. However, the tier 1 risk assessment indicated a high risk for earthworm-eating birds and mammals for the worst-case use pattern. A risk assessment refinement, based on a laboratory measured bioaccumulation factor (BAF) in earthworms, indicated a low risk. According to the agreement at the experts' meeting,[21] a screening risk assessment of bioaccumulation for the pertinent lipophilic (i.e. log Pow $\geq$ 3) soil metabolites based on the no observed effect concentration (NOEC) for the parent divided by 10, was performed. Based on this screening assessment, a high risk for the metabolites could not be excluded for the worst-case use pattern (i.e. ornamentals, field use). However, when considering the high margin of safety in the risk assessment for the parent, further refinements were not considered needed for the pertinent metabolites.

Several metabolites of milbemectin have been detected in plant metabolism studies (see Section 3). However, the risk was considered either to be covered by the risk assessment with the parent or the metabolite not relevant.

Milbemectin is composed by constituents milbemycin A_4 and milbemycin A_3, with a ratio milbemycin A_3:milbemycin A_4 30:70% (see Section 1). Since a data gap was identified for milbemycin A_3 in Section 4 (leading to the exposure assessment in natural waters not finalised, see also Section 9.1), it has to be noted that the risk assessment for aquatic organisms was performed based on the assumption that milbemycin A_3 behaves in the environment as the constituent milbemycin A_4. Hence, pending on the data gap for aquatic exposure assessment for milbemycin A_3, the risk assessment for milbemectin may present some uncertainties. It has to be noted that another data gap was identified in Section 4 (leading to the exposure assessment not finalised) for the milbemycin A_3 metabolites (27-hydroxy-MA_3, 27-keto-MA_3 and 8,9Z-MA_3); hence, their risk assessment could not be performed (see Section 9.1).

A number of studies were available to assess the effects of milbemectin, the formulations Milbeknock 1% EC' and 'Milbemectin 1% EC', and the relevant metabolites 27-keto-MA_4, 27-hydroxy-MA_4 and 8,9-Z-MA_4 to **aquatic organisms**. When comparing the available toxicity data with the active substance and the formulated products, the latter appeared to be more toxic (ca. a factor of 3). Therefore, endpoints with the formulated products were used for risk assessment, where available. For the risk assessment for aquatic invertebrates, the most sensitive acute endpoint (EC_{50} = 1.41 μg a.s./L) for *Chironomus riparius* was used as acute tier 1 RAC (regulatory acceptable concentration). The tier 1 risk assessment, based on FOCUS step 1&2 PECsw, indicated a low risk for all the representative uses only for algae. For all the other groups of aquatic organisms (i.e. aquatic invertebrates and fish), a refinement of the risk assessment was needed, since low risk could not be concluded at Tier 1. For the refinement for the acute risk assessment for fish, a geometric mean endpoint of 20.67 μg as/L was calculated based on five acute toxicity studies to derive an acute tier 2 RAC for fish (0.207 μg as/L). The

[21] See expert consultation point 5.1 in the Report of the Pesticide Peer Review Experts' Meeting PRAS 181 (June 2018) (EFSA, 2023).

refined acute risk assessment for fish showed a low risk for the representative uses on strawberries and ornamentals (lower application rate) at FOCUS step 3; for the representative uses on cane fruits, orchards and ornamentals (higher application rate), the risk was low with mitigation measures (i.e. FOCUS step 4). The chronic risk assessment for fish also showed a low risk at FOCUS step 4 for the representative uses. However, for orchards, high risk could not be excluded even with the application of maximum acceptable mitigation measures.

For the refinement of the acute risk assessment for invertebrates, several of the available screening tests were considered as not reliable at the experts' meeting, and therefore, they could not be used for risk assessment to derive an acute tier 2 RAC, i.e. based on geometric mean approach or the species sensitivity distribution (SSD). Higher tier studies were also discussed by the experts, for deriving a tier 3 RAC. In particular, the available microcosm study was considered as not suitable for covering the most sensitive species (i.e. *Chironomus riparius*, as identified by the standard tests), because sediment-dwelling organisms were not present in the study.[22] Furthermore, at the same experts' meeting, the study was considered not to present the worst case with respect to the representative uses in terms of number of applications and the interval between the applications. As a consequence, a tier 3 RAC, such as the ETO-RAC was not derived. In addition, since a standard chronic study on *Chironomus riparius* was not available, a **data gap** was identified (see Section 9.1).

The chronic risk assessment was based on the currently available endpoint on *Daphnia magna* (NOEC of 0.12 μg a.s./L) for illustrative purposes only. However, considering the:

- Uncertainties in the risk assessment due to the absence of an exposure assessment of milbemectin component milbemycin A_3, which was assumed having a similar behaviour as the constituent milbemycin A_4;
- *C. riparius* was more sensitive in the acute toxicity study;
- no reliable chronic studies with *C. riparius* or related species were available;
- the risk assessment using the endpoint for *Daphnia* resulted in a high risk for the majority of the representative uses (field and non-permanent greenhouse), except for the use in permanent greenhouses where, nonetheless, the toxicity exposure ratio (TER) value reached 12.6 against the trigger value of 10;

the aquatic risk assessment is overall considered as an **issue not finalised** (see Section 9.1).

The risk assessment to aquatic organisms for the representative uses in permanent greenhouse was performed assuming both 0.1% and 0.2% emission. In the latter case, which represents ultra-low volume spray equipment, a high risk was identified for *Chironomus* and *Daphnia*, while low risk was concluded for the former case.

No toxicity data and no exposure assessment for the constituent milbemycin A_3 for all the pertinent **metabolites** (27-hydroxy-MA_3, 27-keto-MA_3 and 8,9Z-MA_3) were available. Moreover, no risk assessment to *Chironomus* (shown to be the most sensitive organisms in the toxicity studies with the parent compound) with the relevant metabolites was available. Therefore, overall, the risk assessment for the pertinent metabolites could not be finalised (see Section 9.1). It is noted that the acute risk assessment to fish and daphnia for the relevant metabolites of milbemycin A_4 (27-hydroxy-MA_4, 27-keto-MA_4 and 8,9Z-MA_4) was assessed as low for the use in ornamentals, cane fruit and strawberries at FOCUS steps 1, 2 or 3; the risk was low for the use in orchards at FOCUS step 4 with mitigation measures up to 20 m.

Acute oral and contact toxicity studies **on honeybees** were available for the active substance milbemectin and the formulation 'Milbeknock 1 % EC'. A 10-day chronic study was also available on honeybees. No studies investigating sublethal effects were provided (**data gap**, see Section 10). No laboratory studies on honeybee larvae were available (**data gap,** see Section 10). Higher tier semi-field studies (i.e. Oomen et al., 1992 and OECD 75 tests) were provided. The risk assessment was based on the calculation of acute oral and contact hazard quotients (HQs) according to the guidance document (European Commission, 2002). Based on this first-tier risk assessment available for honeybees, low risk could be concluded for oral exposure, while high acute risk was concluded for contact exposure, for the spray application uses in berry plants, ornamentals and pome or stone fruits (except for the uses in permanent greenhouse). The available higher tier studies conducted according to OECD 75 did not show significant effects on honeybees. However, the other available higher tier study (Oomen et al., 1992), conducted to address the data requirements on potential

[22] See expert consultation point 5.3 in the Report of the Pesticides Peer Review Experts' Meeting PRAS 181 (June, 2018) (EFSA, 2023).

effects on honeybee development and brood activity, showed adverse effects of milbemectin on the brood development and the brood termination rate. Hence, the available higher tier studies were not considered sufficient to exclude adverse effects on honeybees. A chronic risk assessment, i.e. based on the EFSA bee guidance document (EFSA, 2013), was not presented for honeybees (**data gap**, see Section 10). Acute oral and contact exposure toxicity studies were also available for **bumblebees**, with the formulation ('Milbeknock 1 % EC'). A risk assessment according to EFSA (2013) (both contact and oral exposure) was provided in the RAR by the RMS for adult bumblebees, which showed low risk for acute contact and oral exposure in the field uses on ornamentals (both low and high application rate), orchards, cane fruits and strawberries.

Several (standard and extended) laboratory toxicity studies on **non-target arthropods** were available on *Aphidius rhopalosiphi* and *Typhlodromus pyri* as well as on additional species, with the formulations 'Milbeknock 1 % EC' and 'Milbemectin 1% EC'. On the basis of the tier 1 risk assessment, a high in-field and off-field risk could not be excluded for the critical use patterns, although the toxicity endpoints were unbounded values (i.e. unbounded HQs values were calculated). The higher tier in-field risk assessment indicated a low risk for all field representative uses while the higher tier off-field risk assessment indicated a low risk for the field use in strawberries without mitigation measures, and low risk for all other field representative uses with mitigation measures comparable to a no-spray buffer zone of up to 5 m, and the application of drift reduction technology up to 50%, based on the most critical endpoint for *T. pyri*.

Suitable chronic toxicity studies were available on **earthworms** with the formulations 'Milbeknock 1 % EC' and 'Milbemectin 1% EC' and with the metabolites (i.e. 27-keto-MA_4, 27-hydroxy-MA_4). A tier 1 chronic risk assessment to earthworms for the active substance milbemectin indicated a high risk for all the representative uses, except the representative uses in strawberries, while a low risk for earthworms was concluded for the relevant metabolites, for all representative uses. For the parent substance, the risk was further refined based on a relevant field study on arable land where it was concluded that no measurable adverse effects were observed on natural earthworm populations. Based on the results of this study, a low risk was concluded for the parent compound milbemectin too, for all the representative uses. No chronic toxicity studies were available with the pertinent metabolites of the milbemycin A_3 constituent (27-keto-MA_3, 27-hydroxy-MA_3); however, it can be considered that the higher tier risk assessment of the parent covers also those metabolites. Therefore, low risk may be concluded.

Based on the available data, the risk for the other **soil macro-organisms** from the active substance milbemectin was assessed as low. Regarding the relevant soil metabolites for both milbemycin A_3 and milbemycin A_4 constituents, the risk assessment was performed assuming a similar toxicity for both milbemycin A_3 and milbemycin A_4. Based on this assumption, low risk was concluded for 27-hydroxy-MA_4/MA_3. For the metabolite 27-keto-MA_4/MA_3, high risk to Collembola could not be excluded for the representative uses in field and other non-permanent protected structures in ornamentals (higher rate). For all other representative uses of milbemectin, low risk to 27-keto-MA_4/ MA_3 was concluded.

On the basis of the available data and risk assessments, a low risk to **soil microorganisms**, **non-target terrestrial plants**, **organisms involved in biological methods for sewage treatment plants** was concluded for milbemectin.

6. Endocrine disruption properties

The endocrine disruption potential of milbemectin was discussed at the Pesticide Peer Review Experts' Meeting PREV 05 (Mammalian toxicology – Ecotoxicology joint session on ED in May 2019) and at the Pesticides Peer Review Experts' TC 93 (January 2023).

With regard to the assessment of the endocrine disruption (ED) potential of milbemectin for **humans** according to the ECHA/EFSA guidance (2018), in determining whether milbemectin interacts with the oestrogen, androgen and steroidogenesis (EAS) and thyroid (T) mediated pathways, the number and type of effects induced and the magnitude and pattern of responses observed across studies were considered. Additionally, the conditions under which effects occur were considered; in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in overt toxicity. The assessment is therefore providing a weight-of-evidence analysis of the potential interaction of milbemectin with the EAS and T signalling pathways using the available evidence in the data set.

For the EAS-T modalities the data set is complete, and no adversity has been observed. Therefore, in line with ECHA/EFSA guidance (2018), scenario 1a is applicable and milbemectin is not considered

to meet the ED criteria for humans as laid down in point 3.6.5 of Annex II to Regulation (EC) No 1107/2009.

The outcome of the assessment reported above for humans also applies to **wild mammals as non-target organisms**.

For non-target organisms other than mammals, one study conducted according to OECD TG 231 (Amphibian Metamorphosis Assay – AMA) was available for the assessment of the T-modality.[23] The AMA study was discussed at the experts' meeting,[24] where it was agreed that the tested concentrations applied were appropriate and the findings observed showed no consistent pattern of T-mediated endocrine activity. For the assessment of the EAS-modalities, one study conducted according to OECD TG 229 (fish short-term reproductive assay – FSTRA) was available.[25] The FSTRA did not show any evidence of EAS-mediated endocrine activity.

Overall, based on the above-mentioned assessment, milbemectin does not meet the ED criteria for the EATS-modalities, as laid down in points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by the Commission Regulation (EU) 2018/605.

7. Overview of the risk assessment of compounds listed in residue definitions triggering assessment of effects data for the environmental compartments (Tables 1–4)

Table 1: Soil

Compound (name and/or code)	Ecotoxicology
milbemycin A_4	Low risk
milbemycin A_3	Low risk
27-hydroxy-MA_4	Low risk
27-hydroxy-MA_3	Low risk
27-keto-MA_4	Low risk for earthworms, High risk for Collembola
27-keto-MA_3	Low risk for earthworms, High risk for Collembola

Table 2: Groundwater[(a)]

Compound (name and/or code)	> 0.1 µg/L at 1 m depth for the representative uses[(b)] Step 2	Biological (pesticidal) activity/ relevance Step 3a.	Hazard identified Steps 3b. and 3c.	Consumer RA triggered Steps 4 and 5	Human health relevance
milbemycin A_4 milbemycin A_3	No	Yes	–	–	Yes
27-hydroxy-MA_4	No	Assessment not triggered	Assessment not triggered	Assessment not triggered	Assessment not triggered
27-hydroxy-MA_3	No	Assessment not triggered	Assessment not triggered	Assessment not triggered	Assessment not triggered
27-keto-MA_4	No	Assessment not triggered	Assessment not triggered	Assessment not triggered	Assessment not triggered
27-keto-MA_3	No	Assessment not triggered	Assessment not triggered	Assessment not triggered	Assessment not triggered

(a): Assessment according to European Commission guidance of the relevance of groundwater metabolites (2003).
(b): FOCUS scenarios or relevant lysimeter. Ranges indicated for FOCUS scenarios include the result from the model giving the highest concentration at each scenario, as needed to comply with European Commission (2014) guidance.

[23] See expert consultation points 4–5 in the report of the Pesticides Peer Review Experts' Meeting PREV 05 May 2019 (EFSA, 2023).
[24] See expert consultation point 5.4 in the report of the Pesticides Peer Review Experts' TC 93 January 2023 (EFSA, 2023).
[25] See expert consultation points 6–7 in the report of the Pesticides Peer Review Experts' Meeting PREV 05 May 2019 (EFSA, 2023).

Table 3: Surface water and sediment

Compound (name and/or code)	Ecotoxicology
milbemycin A_4	Data gap, issue not finalised.[26]
milbemycin A_3	Data gap, issue not finalised.
27-hydroxy-MA_4	Data gap, issue not finalised.
27-hydroxy-MA_3	Data gap. Issue not finalised.
27-keto-MA_4	Data gap. Issue not finalised.
27-keto-MA_3	Data gap. Issue not finalised.
8,9Z-MA_4	Data gap. Issue not finalised.
8,9Z-MA_3	Data gap. Issue not finalised.

Table 4: Air

Compound (name and/or code)	Toxicology
milbemycin A_4	Acute Tox 4, Harmful if inhaled (Rat LC_{50} = 1.90 mg/L air /4 h)
milbemycin A_3	

[26] Illustrative risk assessment was provided for *Daphnia magna*, showing high risk for all the uses except for the ones in permanent greenhouse. See Section 5.

8. Particular conditions proposed to be taken into account by risk managers

Risk mitigation measures (RMMs) identified following consideration of Member State (MS) and/or applicant's proposal(s) during the peer review, if any, are presented in this section (Table 5). These measures applicable for human health and/or the environment leading to a reduction of exposure levels of operators, workers, bystanders/residents, environmental compartments and/or non-target organisms for the representative uses are listed below. The list may also cover any RMMs as appropriate, leading to an acceptable level of risks for the respective non-target organisms.

It is noted that final decisions on the need of RMMs to ensure the safe use of the plant protection product containing the concerned active substance will be taken by risk managers during the decision-making phase. Consideration of the validity and appropriateness of the RMMs remains the responsibility of MSs at product authorisation, taking into account their specific agricultural, plant health and environmental conditions at national level.

Table 5: Risk mitigation measures proposed for the representative uses assessed

Representative use	**Strawberry**	**Strawberry**	**Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry**)	**Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry**)	**Stone and Pome fruit (Apple, pear, Cherry, plum**)	**Ornamentals, mites**	**Ornamentals, mites**	**Ornamentals, leaf miner**	**Ornamentals, leaf miner**
						Lower application rate		**Higher application rate**	
	Foliar spray								
	Field and other protected structures	**Permanent greenhouses**	**Field and other protected structures**	**Permanent greenhouses**	**Field**	**Field and other protected structures**	**Permanent greenhouses**	**Field and other protected structures**	**Permanent greenhouses**
Operator exposure		Use of gloves (MLA), faceshield (ML) and rainsuit (A) in case of dense scenario	Use of gloves during ML in case of handheld application	Use of gloves (MLA), faceshield (ML) and rainsuit (A) in case of dense scenario	Use of gloves during ML in case of handheld application	Use of gloves during ML in case of handheld application	Use of gloves (MLA), faceshield (ML) and rainsuit (A) in case of dense scenario	Use of gloves during ML in case of handheld application	Use of gloves (MLA), faceshield (ML) and rainsuit (A) in case of dense scenario
Worker exposure								Use of gloves in addition to workwear	Use of gloves in addition to workwear
Resident/ bystander exposure									

Representative use	Strawberry	Strawberry	Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry)	Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry)	Stone and Pome fruit (Apple, pear, Cherry, plum)	Ornamentals, mites	Ornamentals, mites	Ornamentals, leaf miner	Ornamentals, leaf miner
						Lower application rate		Higher application rate	
	Foliar spray								
	Field and other protected structures	Permanent greenhouses	Field and other protected structures	Permanent greenhouses	Field	Field and other protected structures	Permanent greenhouses	Field and other protected structures	Permanent greenhouses
Risk to aquatic organisms(*)	Fish: RMM comparable to 10 m no-spray buffer zone sufficient for 4/4 scenarios (chronic); invertebrates 20 m no-spray buffer sufficient for 2/4 scenarios (acute and chronic)[(a)]	Restriction to exclude ultra-low volume spray application	Fish: RMM comparable to 10 m no-spray buffer zone sufficient for 5/5 scenarios (acute and chronic); invertebrates: 20 m sufficient for 2/5 scenarios (acute) and not sufficient for any of the scenarios (0/5 chronic)[(b)]	Restriction to exclude ultra-ow volume spray application	Fish: RMM comparable to 20 m no-spray buffer zone sufficient for 7/7 scenarios (acute) and 0/7 (chronic); invertebrates, RMM not sufficient for any of the scenarios (0/7 acute and chronic)[(c)]	Fish: RMM comparable to 20 m no-spray buffer zone sufficient for 5/5 scenarios (chronic); invertebrates: up to 20 m sufficient for 5/5 scenarios (acute), and 1/5 (chronic)[(d)]	Restriction to exclude ultra-low volume spray application	Fish: RMM comparable to 10 m no-spray buffer zone sufficient for 5/5 (acute) and 20 m sufficient for 5/5 scenarios (chronic); invertebrates: up to 20 m not sufficient for any of the scenarios (acute and chronic)[(e)]	Restriction to exclude ultra-low volume spray application
Risk to non-target arthropods			RMM comparable to a no-spray buffer zone of up to 5 m needed		RMM comparable to a no-spray buffer zone of up to 5 m	RMM comparable to a no-spray buffer zone of up to 3 m needed		RMM comparable to a no-spray buffer zone of up to 5 m and 50% drift reduction needed	

(*): The risk assessment for aquatic organism D. magna is reported for illustrative purposes only. Please note that overall, the aquatic risk assessment could not be finalised (see Section 5).
(a): Fish: all scenarios passed (chronic); invertebrates: passing scenarios D6, R2.
(b): Fish: all scenario passed (acute and chronic); invertebrates: passing scenarios R1, R4 (acute), no scenario passed (chronic).
(c): Fish: all scenarios passed (acute), no scenarios passed (chronic); invertebrates: no scenarios passed (acute and chronic).
(d): Fish: all scenarios passed (chronic); invertebrates: passing scenario R1 (chronic).
(e): Fish: all scenario passed at 10 m (acute) and at 20 m (chronic); invertebrates: no scenario passed (acute and chronic).

9. Concerns and related data gaps

9.1. Issues that could not be finalised

An issue is listed as 'could not be finalised' if there is not enough information available to perform an assessment, even at the lowest tier level, for one or more of the representative uses in line with the uniform principles in accordance with Article 29(6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011 and if the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

An issue is also listed as 'could not be finalised' if the available information is considered insufficient to conclude on whether the active substance can be expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

The following issues or assessments that could not be finalised have been identified, together with the reasons including the associated data gaps where relevant, which are reported directly under the specific issue to which they are related.

1) The acceptability of the proposed maximum levels of the impurities and the representativeness of the toxicological batches with regard to the reference specification could not be finalised (see Section 2).

   a) Further assessment of the toxicological relevance of the impurities included in the reference specification should be provided. (Relevant for all representative uses evaluated, the applicant stated that quantitative structure–activity relationship (QSAR) analysis of the impurities has been performed after the peer review meeting; see Section 2).

2) Interspecies differences in metabolism of milbemectin with possible identification of unique human metabolites could not be finalised (see Section 2).

   a) *In vitro* comparative metabolism study with milbemectin (at least in the pivotal species used to characterise milbemectin's toxicity and in comparison with human metabolism) should be provided (relevant for all representative uses evaluated; the applicant stated that an *in vitro* comparative metabolism study has been completed at a late stage after the peer review meeting; see Section 2).

3) The assessment of the potential of milbemectin for phototoxicity (and photomutagenicity if positive results are obtained in the phototoxicity test) could not be finalised (see Section 2).

   a) *In vitro* phototoxicity study with milbemectin, testing the range of wavelengths (between 290 and 700 nm) where the absorption coefficient is > 10 L $\times$ mol^{-1} $\times$ cm^{-1}, should be provided (relevant for all representative uses evaluated; see Section 2).

4) The aquatic risk assessment could not be finalised (see Sections 4 and 5) as:

   4(1) The aquatic exposure and risk assessment could not be finalised for the active substance component milbemycin A_3 and associated transformation products while investigations of its fate and behaviour in natural sediment water systems were not available. Consequently, the aquatic risk assessment could not be finalised for milbemycin A_3, 27-hydroxy-MA_3, 27-keto-MA_3 and 8,9Z-MA_3 (see Sections 4 and 5).

   a) Satisfactory information to address the fate and behaviour of the active substance component milbemycin A_3 and associated transformation products in natural water systems was not available (relevant for all representative uses evaluated, see Section 4).

   4(2) The chronic aquatic risk assessment could not be finalised (see Section 5).

   a) A chronic study on Chironomus with the active substance was not available and it was shown to be the most sensitive species in the available acute study. Additionally, the risk assessment to Chironomus for the pertinent metabolites should be addressed (relevant for all the representative uses, noting that this risk assessment might require unavailable FOCUS surface water Step 3 and 4 PEC for photolysis metabolite 8,9Z-MA_4 for the representative uses on strawberries and cane fruits, see Sections 4 and 5).

9.2. Critical areas of concern

An issue is listed as a critical area of concern if there is enough information available to perform an assessment for the representative uses in line with the uniform principles in accordance with Article 29 (6) of Regulation (EC) No 1107/2009 and as set out in Commission Regulation (EU) No 546/2011, and if this assessment does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if the assessment at a higher tier level could not be finalised due to lack of information, and if the assessment performed at the lower tier level does not permit the conclusion that, for at least one of the representative uses, it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater, or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern if, in the light of current scientific and technical knowledge using guidance documents available at the time of application, the active substance is not expected to meet the approval criteria provided for in Article 4 of Regulation (EC) No 1107/2009.

The following critical areas of concern are identified, together with any associated data gaps, where relevant, which are reported directly under the specific critical area of concern to which they are related.

Critical areas of concern were not identified.

9.3. Overview of the concerns identified for each representative use considered (Table 6)

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in Section 8, has been evaluated as being effective, then 'risk identified' is not indicated in Table 6).

Table 6: Overview of concerns reflecting the issues not finalised, critical areas of concerns and the risks identified that may be applicable for some but not for all uses or risk assessment scenarios

Representative use		Strawberry	Strawberry	Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry)	Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry)	Stone and Pome fruit (Apple, pear, cherry, plum)	Ornamentals, mites	Ornamentals, mites	Ornamentals, leaf miner	Ornamentals, leaf miner
							Lower application rate		Higher application rate	
		Foliar spray								
		Field and other protected structures	Permanent greenhouse	Field and other protected structures	Permanent greenhouse	Field	Field and other protected structures	Permanent greenhouse	Field and other protected structures	Permanent greenhouse
Operator risk	Risk identified									
	Assessment not finalised									
Worker risk	Risk identified									
	Assessment not finalised									
Resident/ bystander risk	Risk identified									
	Assessment not finalised									
Consumer risk	Risk identified									
	Assessment not finalised									
Risk to wild non-target terrestrial vertebrates	Risk identified									
	Assessment not finalised									

Representative use		Strawberry	Strawberry	Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry)	Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry)	Stone and Pome fruit (Apple, pear, cherry, plum)	Ornamentals, mites	Ornamentals, mites	Ornamentals, leaf miner	Ornamentals, leaf miner
							Lower application rate		Higher application rate	
		Foliar spray								
		Field and other protected structures	Permanent greenhouse	Field and other protected structures	Permanent greenhouse	Field	Field and other protected structures	Permanent greenhouse	Field and other protected structures	Permanent greenhouse
Risk to wild non-target terrestrial organisms other than vertebrates	Risk identified	X[(a)]		X[(a)]		X[(a)]	X[(a)]		X[(a),(b)]	
	Assessment not finalised									
Risk to aquatic organisms[(c)]	Risk identified	2/4 FOCUS scenarios (R3, R4)		All FOCUS scenarios		All FOCUS scenarios	4/5 FOCUS scenarios (D6, R2, R3, R4)		All FOCUS scenarios	
	Assessment not finalised	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]
Groundwater exposure to active substance	Legal parametric value breached									
	Assessment not finalised									

Representative use		Strawberry	Strawberry	Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry)	Cane fruit (Gooseberry; blackberry; blueberry; red, black and white currant; raspberry)	Stone and Pome fruit (Apple, pear, cherry, plum)	Ornamentals, mites	Ornamentals, mites	Ornamentals, leaf miner	Ornamentals, leaf miner
							Lower application rate		Higher application rate	
		Foliar spray								
		Field and other protected structures	Permanent greenhouse	Field and other protected structures	Permanent greenhouse	Field	Field and other protected structures	Permanent greenhouse	Field and other protected structures	Permanent greenhouse
Groundwater exposure to metabolites	Legal parametric value breached[(d)]									
	Parametric value of 10 µg/L[(e)] breached									
	Assessment not finalised									

The superscript numbers relate to the numbered points indicated in Sections 9.1 and 9.2. Where there is no superscript number, see Sections 2–7 for further information.

(a): High acute contact risk to honeybees was concluded based on the HQs for all the representative uses, except the uses in permanent greenhouse.

(b): High risk to Collembola for the representative uses in field and other non-permanent protected structures, could not be excluded in ornamentals (higher rate) with the metabolite 27-keto MA4/MA3.

(c): The risk assessment for aquatic organism *D. magna* is reported for illustrative purposes only. Please note that overall, the aquatic risk assessment could not be finalised (see Sections 5 and 9.1).

(d): When the consideration for classification made in the context of this evaluation under Regulation (EC) No 1107/2009 is confirmed under Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008.

(e): Value for non-relevant metabolites prescribed in SANCO/221/2000-rev. 10 final, European Commission, 2003.

10. List of other outstanding issues

Remaining data gaps not leading to critical areas of concern or issues not finalised but considered necessary to comply with the data requirements, and which are relevant for some or all of the representative uses assessed at EU level, unless stated otherwise. Although not critical, these data gaps may lead to uncertainties in the assessment and are considered relevant.

These data gaps refer only to the representative uses (unless stated otherwise) assessed and are listed in the order of the sections:

- Analytical methods for monitoring of all components of the residue definition (i.e. including milbemycin A_3) in soil and water (relevant for all representative uses evaluated, see Section 1).
- Additional validation data for the existing methods or a new monitoring method for determination of the residues in air (relevant for all representative uses evaluated, see Section 1).
- Analytical method for monitoring of 13-hydroxy MA_4 in body fluids and tissues (relevant for all representative uses evaluated, see Section 1).
- The aneugenicity potential of milbemectin has not been sufficiently investigated. Evidence of bone marrow exposure in the *in vivo* micronucleus test to support the reliability of the results, or an additional genotoxicity study addressing the aneugenic potential of milbemectin, e.g. with an *in vitro* micronucleus, test should be provided (relevant for all representative uses evaluated, the applicant stated that a study to clarify the bone marrow exposure in mouse has become available after the peer review meeting, see Section 2).
- Validation of the analytical methods used in the short-term toxicity studies with dogs (relevant for all representative uses evaluated, see Section 2).
- A study investigating the nature of residues in processed commodities (relevant for the representative use in cherries, see Section 3).
- Data on residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom (relevant for all representative field uses evaluated, see Section 3).
- Field soil dissipation studies demonstrated to be comparable to EU soil and climate conditions were not available. Such studies are triggered as the DT_{90} in laboratory soil incubations with milbemycin A_4 were up to 272 days (relevant for all representative uses evaluated). The exposure assessments for the representative uses assessed at EU level have been completed with the available laboratory endpoints (see Section 4).
- The identity of unknowns 4,9,10,11,12,13, A2, A5, A8, A11 and A14 in the aerobic mineralisation study is not known. Some component characterisation was completed, i.e. the identification of 14,15-epoxy MA_4 and indications that some of the compounds are isomers of milbemycin A_4, mono-oxidised MA_4, and/or di-oxidised MA_4 (these data are not needed to complete the aquatic exposure assessment for the representative uses when following agreed EU FOCUSsw guidance, but they can have utility in the context of national/zonal assessments. See Section 4 of the evaluation table (data requirement 4.4) in the peer review report, EFSA (2023)).
- Further information on the impurity profile of the batches used in the ecotoxicity studies in order to demonstrate that they are representative of the reference technical specifications (see Section 5).
- A risk assessment to honeybees according to the EFSA guidance (2013) (relevant for all field representative uses and uses in protected structures except permanent greenhouses, see Section 5).
- Studies investigating sublethal effects on bees and effects on honeybee larvae (relevant for all field representative uses and uses in protected structures except permanent greenhouses, see Section 5).

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Abbreviations

1/*n*	slope of Freundlich isotherm
a.s.	active substance
AAOEL	acute acceptable operator exposure level
ADI	acceptable daily intake
AOEL	acceptable operator exposure level
AR	applied radioactivity
ARfD	acute reference dose
bw	body weight
DT_{50}	period required for 50% dissipation (define method of estimation)
DT_{90}	period required for 90% dissipation (define method of estimation)
ECHA	European Chemicals Agency
EEC	European Economic Community
FAO	Food and Agriculture Organization of the United Nations
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
GAP	Good Agricultural Practice
HPLC	high-pressure liquid chromatography or high-performance liquid chromatography
HQ	hazard quotient

ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
iv	intravenous
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
K_{doc}	organic carbon linear adsorption coefficient
K_{Foc}	Freundlich organic carbon adsorption coefficient
LC	liquid chromatography
LC_{50}	lethal concentration, median
LC–MS	liquid chromatography–mass spectrometry
LC–MS/MS	liquid chromatography with tandem mass spectrometry
LOQ	limit of quantification
mm	millimetre (also used for mean measured concentrations)
mN	milli-Newton
MRL	maximum residue level
MS	mass spectrometry
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NPD	nitrogen–phosphorus detector
OECD	Organisation for Economic Co-operation and Development
Pa	pascal
PD	proportion of different food types
PEC	predicted environmental concentration
PEC_{air}	predicted environmental concentration in air
PEC_{gw}	predicted environmental concentration in groundwater
PEC_{sed}	predicted environmental concentration in sediment
PEC_{soil}	predicted environmental concentration in soil
PEC_{sw}	predicted environmental concentration in surface water
P_{ow}	partition coefficient between *n*-octanol and water
PPE	personal protective equipment
ppm	parts per million (10^{-6})
QSAR	quantitative structure–activity relationship
r^2	coefficient of determination
RAC	regulatory acceptable concentration
RAR	Renewal Assessment Report
SD	standard deviation
SMILES	simplified molecular-input line-entry system
SSD	species sensitivity distribution
$t_{1/2}$	half-life (define method of estimation)
TER	toxicity exposure ratio
TRR	total radioactive residue
UF	uncertainty factor
WHO	World Health Organization

Appendix A – Consideration of cut-off criteria for milbemectin according to Annex II of Regulation (EC) No 1107/2009 of the European Parliament and of the Council

Properties		Conclusion[(a)]
CMR	**Carcinogenicity (C)**	Milbemectin is not considered to be a carcinogen according to point 3.6.3 of Annex II of Regulation (EC) No 1107/2009
	Mutagenicity (M)	Milbemectin is not considered to be a mutagen according to point 3.6.2 of Annex II of Regulation (EC) No 1107/2009
	Toxic for Reproduction (R)	Milbemectin is not considered to be toxic for reproduction according to point 3.6.4 of Annex II of Regulation (EC) No 1107/2009
Endocrine-disrupting properties		Milbemectin does not meet the ED criteria for the EATS-modalities, as laid down in points 3.6.5 and 3.8.2 of Annex II to Regulation (EC) No 1107/2009, as amended by the Commission Regulation (EU) 2018/605.
POP	**Persistence**	Milbemectin is not considered to be a persistent organic pollutant (POP) according to point 3.7.1 of Annex II of Regulation (EC) 1107/2009 based on bioaccumulation evidence for milbemycin A_4.
	Bioaccumulation	
	Long-range transport	
PBT	**Persistence**	Milbemectin is not considered to be a persistent, bioaccumulative and toxic (PBT) substance according to point 3.7.2 of Annex II of Regulation (EC) 1107/2009 based on bioaccumulation evidence for milbemycin A_4.
	Bioaccumulation	
	Toxicity	
vPvB	**Persistence**	Milbemectin is not considered to be a very persistent, very bioaccumulative substance according to point 3.7.3 of Annex II of Regulation (EC) 1107/2009 based on bioaccumulation evidence for milbemycin A_4.
	Bioaccumulation	

(a): Origin of data to be included where applicable (e.g. EFSA, ECHA RAC, Regulation).

Appendix B – List of end points for the active substance and the formulation for representative uses

Appendix B can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2023.8126

Appendix C – Wording EFSA used in Section 4 of this conclusion, in relation to DT and Koc 'classes' exhibited by each compound assessed

Wording	DT_{50} normalised to 20°C for laboratory incubations[27] or not normalised DT_{50} for field studies (SFO equivalent, when biphasic, the DT_{90} was divided by 3.32 to estimate the DT50 when deciding on the wording to use)
Very low persistence	< 1 day
Low persistence	1 to < 10 days
Moderate persistence	10 to < 60 days
Medium persistence	60 to < 100 days
High persistence	100 days to < 1 year
Very high persistence	A year or more

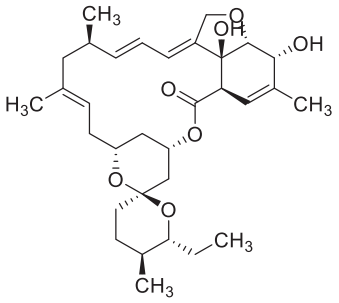
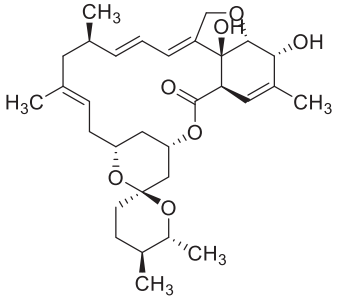
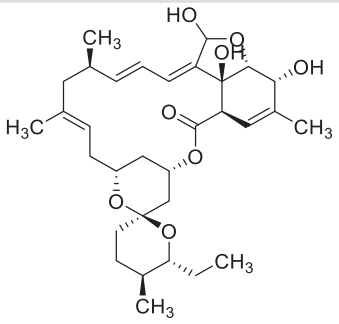
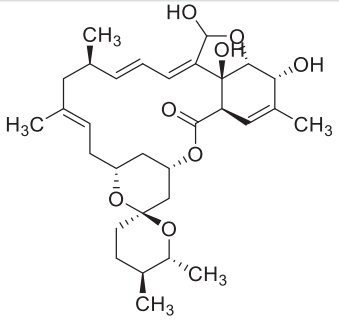
Note these classes and descriptions are unrelated to any persistence class associated with the active substance cut-off criteria in Annex II of Regulation (EC) No 1107/2009. For consideration made in relation to Annex II, see Appendix A.

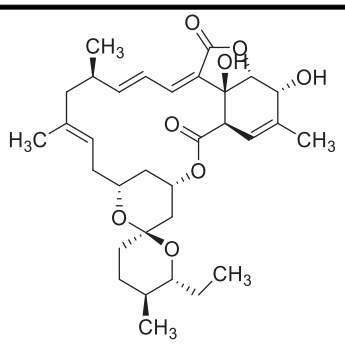
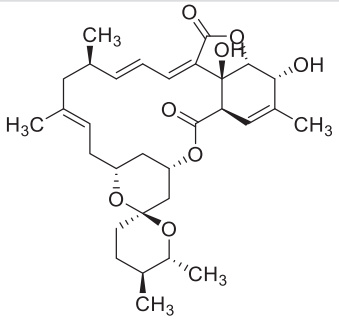
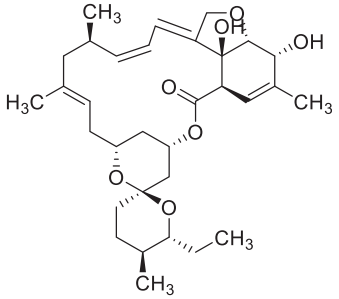
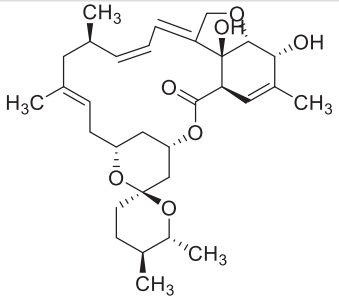
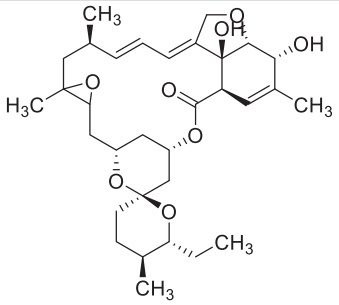
Wording	K_{oc} (either K_{Foc} or K_{doc}) mL/g
Very high mobility	0–50
High mobility	51–150
Medium mobility	151–500
Low mobility	501–2,000
Slight mobility	2,001–5,000
Immobile	> 5,000

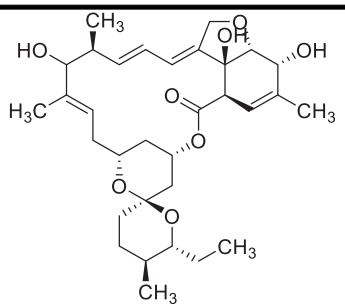
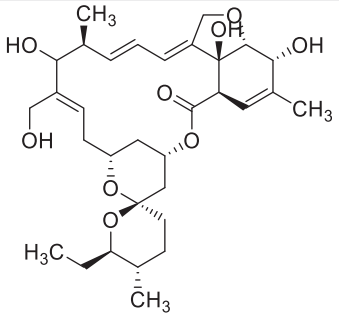
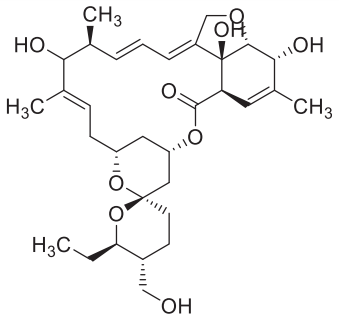
Based on McCall et al. (1980).

[27] For laboratory soil incubations normalisation was also to field capacity soil moisture (pF2/10 kPa). For laboratory sediment water system incubations, the whole system DT values were used.

Appendix D – Used compound codes

Code/trivial name[a]	IUPAC name/SMILES notation/InChiKey[b]	Structural formula[c]
Milbemectin **Milbemycin A_4** (MA4, MA_4)	(10*E*,14*E*,16*E*)-(1*R*,4*S*,5′ *S*,6*R*,6′*R*,8*R*,13*R*,20*R*,21*R*,24*S*)-6′-ethyl-21,24-dihydroxy-5′,11,13,22-tetramethyl-(3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa-10,14,16,22-tetraene)-6-spiro-2′-(tetrahydropyran)-2-one O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](CC)O4)CC=C(C)C[C@@H](C)C=CC=C3CO[C@H]1[C@]23O VOZIAWLUULBIPN-LRBNAKOISA-N and	 milbemycin A4
Milbemycin A_3 (MA3, MA_3)	(10*E*,14*E*,16*E*)-(1*R*,4*S*,5′ *S*,6*R*,6′*R*,8*R*,13*R*,20*R*,21*R*,24*S*)-21,24-dihydroxy-5′,6′,11,13,22-pentamethyl-(3,7,19-trioxatetracyclo[15.6.1.1^{4,8}.0^{20,24}]pentacosa-10,14,16,22-tetraene)-6-spiro-2′-(tetrahydropyran)-2-one O[C@@H]1C(C) = C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](C)O4)CC=C(C)C[C@@H](C)C=CC=C3CO[C@H]1[C@]23O ZLBGSRMUSVULIE-GSMJGMFJSA-N	and  milbemycin A3
27-hydroxy-MA_4	(2aZ,4E,5′S,6R,6′R,8E,11R,13R,15S,17aR,20R,20aR,20bS)-6′-ethyl-2,20,20b-trihydroxy-5′,6,8,19-tetramethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxan]-17-one O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](CC)O4)CC=C(C)C[C@@H](C)C=CC=C3C(O)O[C@H]1[C@]23O FZJPPNMBZUNEFT-ORLMHAGMSA-N	
27-hydroxy-MA_3	(2aZ,4E,5′S,6R,6′R,8E,11R,13R,15S,17aR,20R,20aR,20bS)-2,20,20b-trihydroxy-5′,6,6′,8,19-pentamethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxan]-17-one O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](C)O4)CC=C(C)C[C@@H](C)C=CC=C3C(O)O[C@H]1[C@]23O TYMMPWQKSSEJCA-KXEJHRFISA-N	

Code/trivial name[a]	IUPAC name/SMILES notation/InChiKey[b]	Structural formula[c]
27-keto-MA$_4$	(2aZ,4E,5′S,6R,6′R,8E,11R,13R,15S,17aR,20R,20aR,20bS)-6′-ethyl-20,20b-dihydroxy-5′,6,8,19-tetramethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxane]-2,17-dione O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](CC)O4)CC=C(C)C[C@@H](C)C=CC=C3C(=O)O[C@H]1[C@]23O FMBIYHICCYEPJF-WMHXARPGSA-N	
27-keto-MA$_3$	(2aZ,4E,5′S,6R,6′R,8E,11R,13R,15S,17aR,20R,20aR,20bS)-20,20b-dihydroxy-5′,6,6′,8,19-pentamethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxane]-2,17-dione O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](C)O4)CC=C(C)C[C@@H](C)C=CC=C3C(=O)O[C@H]1[C@]23O ICUKNPPPRQXUFS-JTGUENADSA-N	
8,9Z-MA$_4$ (8,9Z-milbemycin A$_4$)	(2aZ,4Z,5′S,6R,6′R,8E,11R,13R,15S,17aR,20R,20aR,20bS)-6′-ethyl-20,20b-dihydroxy-5′,6,8,19-tetramethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxan]-17-one O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](CC)O4)CC=C(C)C[C@@H](C)C=CC=C3CO[C@H]1[C@]23O VOZIAWLUULBIPN-QWYIZMBNSA-N	
8,9Z-MA$_3$ (8,9Z-milbemycin A$_3$)	(2aZ,4Z,5′S,6R,6′R,8E,11R,13R,15S,17aR,20R,20aR,20bS)-20,20b-dihydroxy-5′,6,6′,8,19-pentamethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxan]-17-one O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](C)O4)CC=C(C)C[C@@H](C)C=CC=C3CO[C@H]1[C@]23O ZLBGSRMUSVULIE-HMQIVIIHSA-N	
15,14-epoxy MA$_4$	(2aR,3R,5′S,5aR,6′R,8S,10R,12S,16R,17E,19E,19bS)-6′-ethyl-3,19b-dihydroxy-4,5′,14a,16-tetramethyl-2a,3,5a,8,9,12,13,13a,14a,15,16,19b-dodecahydro-1H,6H-spiro[8,12-methanofuro[4,3,2-pq]oxireno[i][2,6]benzodioxacyclooctadecine-10,2′-oxan]-6-one O=C1O[C@H]2C[C@@H](CC3OC3(C)C[C@@H](C)C=CC=C3CO[C@@H]4[C@H](O)C(C)=C[C@@H]1[C@@]43O)O[C@@]1(CC[C@H](C)[C@@H](CC)O1)C2 SIYMMIPRDNPBSN-RDJVMAHNSA-N	

Code/trivial name[a]	IUPAC name/SMILES notation/InChiKey[b]	Structural formula[c]
13-hydroxy MA_4	(2aE,4E,5′S,6S,6′R,8E,11R,13R,15S,17aR,20R,20aR,20bS)-6′-ethyl-7,20,20b-trihydroxy-5′,6,8,19-tetramethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxan]-17-one O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](CC)O4)CC=C(C)C(O)[C@@H](C)C=CC=C3CO[C@H]1[C@]23O OPKXKFGKQPXWIH-BMTGQWRVSA-N	
13,29-dihydroxy-MA_4	[2′R,2a(3)Z,4Z,5′S,6S,6′R,8Z,11R,15S,17aR,20R,20aR,20bS]-6′-ethyl-7,20,20b-trihydroxy-8-(hydroxymethyl)-5′,6,19-trimethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxan]-17-one O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](C)[C@@H](CC)O4)CC=C(CO)C(O)[C@@H](C)C=CC=C3CO[C@H]1[C@]23O QNRGVUGZBUINDR-OXCNGPMPSA-N	
13,30-dihydroxy-MA_4	[2′R,2a(3)E,4E,5′R,6S,6′R,8E,11R,15S,17aR,20R,20aR,20bS]-6′-ethyl-7,20,20b-trihydroxy-5′-(hydroxymethyl)-6,8,19-trimethyl-6,7,10,11,14,15,17a,20,20a,20b-decahydro-2H,17H-spiro[11,15-methanofuro[4,3,2-pq][2,6]benzodioxacyclooctadecine-13,2′-oxan]-17-one O[C@@H]1C(C)=C[C@H]2C(=O)O[C@H]3C[C@H](O[C@]4(C3)CC[C@H](CO)[C@@H](CC)O4)CC=C(C)C(O)[C@@H](C)C=CC=C3CO[C@H]1[C@]23O ZHLCZMQKIGBOMX-SGUFETDVSA-N	

(a): The name in bold is the name used in the conclusion.
(b): ACD/Name 2018.2.2 ACD/Labs 2018 Release (File version N50E41, Build 103230, 21 July 2018).
(c): ACD/ChemSketch 2018.2.2 ACD/Labs 2018 Release (File version C60H41, Build 106041, 7 December 2018).