STATEMENT

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Statement on the toxicological properties and maximum residue levels of acetamiprid and its metabolites

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Abstract

Acetamiprid is a pesticide active substance with insecticidal action whose approval was renewed by Commission Implementing Regulation (EU) 2018/113. In January 2022, the EFSA PPR Panel published a statement following a request from the European Commission to advise on human health or the environment based on new scientific evidence presented by France during the decision-making phase. In July 2022, by means of a further mandate received from the European Commission, EFSA was requested to provide advice if new information and any other scientific evidence that has become available since the assessment conducted for the renewal in 2018 warrant re-evaluation of (i) toxicological parameters used for the risk assessment of acetamiprid during the renewal process, including toxicological endpoints; (ii) the residue definition for acetamiprid in products of plant origin; and (iii) the safety of existing maximum residue levels (MRLs). Meanwhile, the applicant of acetamiprid in the EU submitted new toxicology studies regarding the toxicological profile of the metabolite IM-2-1. Furthermore, the European Commission was made aware that several recent publications in scientific literature were made available after the literature searches conducted by EFSA. As the new data could affect the advice that EFSA was expected to deliver through the 2022 mandate, EFSA was further requested to consider this information by means of a revised mandate received in September 2023. As regards re-evaluation of point (i) in this statement, this was addressed by an EFSA Working Group integrating all the available evidence. The results of the weight of evidence indicated that there are major uncertainties in the body of evidence for the developmental neurotoxicity (DNT) properties of acetamiprid and further data are therefore needed to come to a more robust mechanistic understanding to enable appropriate hazard and risk assessment. In view of these uncertainties, the EFSA WG proposed to lower the acceptable daily intake (ADI) and acute reference dose (ARfD) from 0.025 to 0.005 mg/kg body weight (per day). A revised residue definition for risk assessment was proposed for leafy and fruit crops as sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid. Regarding pulses/oilseeds, root crops and cereals, the new data received did not indicate a need to modify the existing residue definition for risk assessment, which therefore remains as parent acetamiprid. Regarding the residue definition for enforcement, the available data did not indicate a need to modify the existing definition because acetamiprid is still a sufficient marker of the residues in all crop groups. Considering the new health-based guidance values derived in the present statement, a risk for consumer has been identified for 38 MRLs currently in place in the

EU Regulation. Consequently, EFSA recommended to lower the existing MRLs for 38 commodities based on the assessment of fall-back Good Agricultural Practices received within an ad hoc data call. Some fall-back MRLs proposals require further risk management considerations.

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SUMMARY

Acetamiprid is an active substance covered by the third batch of the renewal programme for pesticides ('AIR3') in accordance with Commission Implementing Regulation (EU) No 844/2012. The active substance was first approved by Commission Directive 2004/99/EC and its approval was renewed by Commission Implementing Regulation (EU) 2018/113. A potential next renewal process needs to be initiated by 28 February 2030 at the latest. Maximum residue levels (MRLs) for this substance are set in Annex II to Regulation (EC) No 396/2005.

In January 2022, the European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues (PPR Panel) published a statement (EFSA PPR Panel, 2022) following a request from the European Commission to advise on human health based on new scientific evidence presented by France.

For human health, no conclusive evidence of higher hazards of acetamiprid compared to the previous assessment in the context of the renewal was found for genotoxicity, developmental toxicity, neurotoxicity including developmental neurotoxicity and immunotoxicity. The following recommendations were given by the PPR Panel: (i) for the assessment genotoxicity, developmental toxicity, neurotoxicity including developmental neurotoxicity and immunotoxicity, the newly submitted evidence did not change the current conclusions from EFSA and ECHA on acetamiprid and no further actions should be taken; (ii) for endocrine disruption, an assessment of endocrine-disrupting properties for acetamiprid should be conducted in line with the EFSA/ECHA guidance document for the identification of endocrine disruptors (ECHA/EFSA, 2018).

Meanwhile, further scientific publications were brought to the attention of the Commission that described methods for detection of pesticide residues in the cerebrospinal fluid (CSF) of children. Using those detection methods, low levels of N-desmethyl-acetamiprid (IM-2-1), a metabolite of acetamiprid, were detected in most samples. In addition, some Member States presented evidence to the Commission that high levels of the metabolite IM-2-1 were found in some food products, especially in spinach. While the provided data showed that the levels of this metabolite in the analysed samples were often higher than the parent compound, IM-2-1 is not included in the existing residue definition for acetamiprid in products of plant origin. Lastly, one Member State informed the Commission that a potential acute consumer risk from exposure to acetamiprid in pears and lettuces was identified if the intake calculation is performed with the most recent version of PRIMo rev. 3.1.

By means of a further mandate received in July 2022 from the European Commission, EFSA was requested to provide advice if the information referred to above and any other scientific evidence that has become available since the assessment conducted for the renewal in 2018 warrants re-evaluation of (i) toxicological parameters used for the risk assessment of acetamiprid during the renewal process, including toxicological endpoints, (ii) the residue definition for acetamiprid in products of plant origin and (iii) the safety of existing MRLs. This technical and scientific assistance addressing the terms of reference (ToRs) included in the Annex of this mandate was requested under Article 31 of Regulation (EC) No 178/2002.

Nisso Chemical Europe GmbH, as the sole applicant of acetamiprid in the EU, on 4 July 2023 submitted to EFSA new toxicology studies claimed to be relevant for the response to the mandate, in particular regarding the toxicological profile of the metabolite IM-2-1 (in vitro micronucleus test with IM-2-1; mouse lymphoma assay with IM-2-1 and a repeated dose 28-day study with IM-2-1).

Furthermore, the Commission received a letter from Pesticide Action Network (PAN) Europe that referred to several recent publications in scientific literature, which might be relevant for the response to the mandate. Following the receipt of the above new data, an extension and revision of the mandate to EFSA was received in September 2023 to consider this information in the ongoing evaluation.

The term of reference 1 (**ToR1**) concerning the toxicological properties of acetamiprid and its metabolites, including toxicological endpoints, was addressed in this statement by an EFSA working group (WG) by applying the current state of the art methodologies in a transparent and trackable evidence-based AOP-informed IATA approach (Integrated Approaches to Testing and Assessment supported by Adverse Outcome Pathway). This approach relies on the integration of different levels of evidence, i.e. systematic literature review on human epidemiological studies, in vivo studies, and in vitro studies, and on the application of weight of evidence (WoE), including the Developmental Neurotoxicity In vitro Battery (OECD, 2023). The results of the weight of evidence (WoE) indicated that acetamiprid causes activation and rapid desensitisation of the nicotinic acetylcholinesterase receptors (nAChR) at concentrations starting from 1 μM. This is considered per se as a molecular and cellular effect that could lead to an adverse outcome at organism level, and therefore representing a developmental neurotoxicity (DNT) concern. In addition, the WG noted that there are data gaps in the in vivo body of evidence (BoE), including the lack of an acceptable measurement of learning and memory, motor activity and morphometrics evaluation in the available non-guideline DNT study. This represents a regulatory data gap that should be filled to allow to come to a more robust mechanistic understanding, to identify all DNT effects of acetamiprid and obtain concordant dose–response relationships to enable hazard and risk assessment. To account for these uncertainties/limitations on the data set, the WG proposed to include an additional uncertainty factor (UF) of 5 to the current health-based guidance values (HBGVs) to cover the uncertainties in the DNT assessment. As a result of the WG proposal, the acceptable daily intake (ADI) currently set at 0.025 mg/kg body weight (bw) per day would decrease to 0.005 mg/kg bw per day; similarly, the acute reference dose (ArfD) currently set at 0.025 mg/kg bw would be set at 0.005 mg/kg bw. EFSA notes that the same additional UF would be applied for setting the (acute) acceptable operator exposure level ((A)AOEL).

Metabolite IM-2-1 is a major rat metabolite with large structural similarities with the parent compound acetamiprid, unlikely to be genotoxic based on an Ames test, an in vitro micronucleus test and a mouse lymphoma assay. The 28-day study in rats on IM-2-1 does not allow to conclude on a clearly different toxicological profile or potency compared to the parent

compound. Hence, the same HBGVs (ADI of 0.005 mg/kg bw per day and ARfD of 0.005 mg/kg bw) proposed for the parent should also apply to the metabolite.

The **ToR 2a** concerning a data call for monitoring data for plant products was addressed by collecting results of pesticide residue analysis (monitoring data) for acetamiprid and its metabolites in food of plant origin. Data were provided by national competent authorities. Samples with residue analysis for parent acetamiprid and metabolite N-desmethyl acetamiprid (IM-2-1) were reported to EFSA. The **ToR 2b** related to the consideration of the residue definitions for risk assessment and enforcement for plant products was addressed by EFSA with a detailed analysis of the data received under ToR 2a and further analysis of acetamiprid metabolism studies previously submitted and evaluated. The available studies investigating the metabolism of acetamiprid in plants gave an indication that the metabolite IM-2-1 is formed at relatively low levels in edible parts of fruit crops and leafy crops (between 2% and 8% of the TRR; up to 0.3 mg/kg in fruits; up to 1.25 mg/kg in leafy). In inedible leafy matrices, however, this metabolite occurs at higher proportions related to the parent compound, IM-2-1 representing up to 32% of the parent compound (16% of the TRR) in apple leaf at longer preharvest intervals. The monitoring data on the metabolite IM-2-1 confirmed its occurrence in several commodities belonging to the groups of leafy and fruit commodities. In these crop groups, the median proportion of metabolite IM-2-1 compared to the parent compound was found to be significant in fruit and leafy crops (median ratio IM-2-1/acetamiprid accounting for 21%–44%, respectively). It was therefore proposed to include metabolite IM-2-1 in the residue definition of risk assessment for leafy and fruit crops, which is currently limited to the parent acetamiprid. A revised residue definition for risk assessment was proposed for leafy and fruit crops as sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid. Regarding pulses/oilseeds, root crops and cereals, the new data received under ToR 2a did not indicate a need to modify the existing residue definition for risk assessment, which therefore remains as parent acetamiprid. Regarding the residue definition for enforcement, the available data do not indicate a need to modify the existing definition because acetamiprid is still a sufficient marker of the residues in all crop groups.

The **ToR 3** concerning consumer risk assessment related to the existing EU MRLs for acetamiprid in all plant and animal products was addressed by EFSA by means of three consumer risk assessment scenarios performed with the latest PRIMo rev 3.1. Considering the new health-based reference values (HBGVs)[1] derived in the present statement, a risk for consumer has been identified for 38 MRLs currently in place in the EU Regulation. Furthermore, for granate apples and aubergines, a risk for consumers is unlikely for the existing MRLs, but a risk for consumer has been identified for the higher MRLs proposed in the draft MRL Regulation SANTE/11278/2021. Consequently, EFSA recommends to lower the existing MRLs for 38 commodities and to maintain the existing MRLs for granate apples and aubergines. Data gaps were identified for some of the fall-back MRLs derived in the present statement, for which further risk management considerations are therefore required. For pears, sweet cherries, peaches, strawberries, currants and elderberries, several MRL options are given to risk managers, because of the uncertainty identified with the higher MRL proposal. For five commodities (bananas, lettuces, escaroles/ broad-leaved endives, spinaches, chards/beet leaves), it is recommended to lower the existing MRLs to the enforcement limit of quantification (LOQ) because no safe fall-back MRL options could be identified. For bovine liver and bovine (other edible offals), it is recommended to withdraw the existing Codex MRLs from the EU Regulation. Lower alternative MRL options were derived by EFSA based on an updated EU livestock dietary burden calculation.

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[1]Health-based guidance values (HBGVs) and toxicological reference values (TRVs) are often used interchangeably.

1 | INTRODUCTION

Acetamiprid is an active substance covered by the third batch of the renewal programme for pesticides ('AIR3') in accordance with Commission Implementing Regulation (EU) No 844/2012.[2] The active substance was first approved by Commission Directive 2004/99/EC[3] and its approval was renewed by Commission Implementing Regulation (EU) 2018/113.[4] A potential next renewal process needs to be initiated by 28 February 2030 at the latest. Maximum residue levels (MRLs) for this substance are set in Annex II to Regulation (EC) No 396/2005.[5]

In January 2022, the European Food Safety Authority (EFSA) Panel on Plant Protection Products and their Residues (PPR Panel) published a statement (EFSA PPR Panel, 2022) following a request from the European Commission to advise on human health or the environment based on new scientific evidence presented by France.

For human health, no conclusive evidence of higher hazards of acetamiprid compared to the previous assessment in the context of the renewal was found for genotoxicity, developmental toxicity, neurotoxicity including developmental neurotoxicity and immunotoxicity. The following recommendations were given by the PPR Panel: (i) for the assessment of the endpoint categories genotoxicity, developmental toxicity, neurotoxicity including developmental neurotoxicity and immunotoxicity, the newly submitted evidence did not change the current conclusions from EFSA and ECHA on acetamiprid and no further actions should be taken; (ii) for the endpoint category endocrine disruption, an assessment of endocrine-disrupting properties for acetamiprid should be conducted in line with the EFSA/ECHA guidance document for the identification of endocrine disruptors under Regulations (EU) No 528/2012[6] and (EC) No 1107/2009[7] (ECHA/EFSA, 2018; EFSA PPR Panel, 2022).

Recently, further scientific publications were brought to the attention of the Commission that describe methods for detection of pesticide residues in the cerebrospinal fluid (CSF) of children. Using those detection methods, low levels of N-desmethyl-acetamiprid (IM-2-1), a metabolite of acetamiprid, were detected in most samples.

In addition, some Member States presented evidence to the commission that high levels of the metabolite IM-2-1 were found in some food products, especially in spinach. While the provided data showed that the levels of this metabolite in the analysed samples were often higher than the parent compound, IM-2-1 is not included in the existing residue definition for acetamiprid in products of plant origin. Lastly, one Member State informed the commission that a potential acute consumer risk from exposure to acetamiprid in pears and lettuces was identified if the intake calculation is performed with the most recent version of PRIMo (i.e. rev. 3.1).[8]

By means of a further mandate received in July 2022 from the European Commission, EFSA was requested to provide advice if the information referred to above and any other scientific evidence that has become available since the assessment conducted for the renewal in 2018 warrant re-evaluation of (i) toxicological parameters used for the risk assessment of acetamiprid during the renewal process, including toxicological endpoints, (ii) the residue definition for acetamiprid in products of plant origin and (iii) the safety of existing MRLs. This technical and scientific assistance addressing the terms of reference (ToRs) included in the Annex of this mandate was requested under Article 31 of Regulation (EC) No 178/2002[9] (see Section 1.1).

Meanwhile, Nisso Chemical Europe GmbH, as the sole applicant of acetamiprid in the EU, submitted on 4 July 2023 new toxicology studies to EFSA that the company claimed to be relevant for the response to the mandate, in particular regarding the toxicological profile of the metabolite IM-2-1 (in vitro micronucleus test with IM-2-1; mouse lymphoma assay with IM-2-1 and repeated dose 28-day study with IM-2-1).

Furthermore, the commission received a letter from Pesticide Action Network (PAN) Europe that referred to several recent publications in scientific literature, which might be relevant for the response to the mandate. It was ascertained that these publications became available after the literature searches conducted by EFSA and, therefore, they were not included in the previous evaluation. As the new data from Nisso Chemical Europe and PAN Europe might have affected the advice that EFSA was expected to deliver in response to the mandate, EFSA was requested with an extension and revision of the mandate, to consider also this information in the ongoing evaluation. The references to the studies and publications are included in Annex I to the extension mandate.[10]

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[2]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, p. 26–32.

[3]Commission Directive 2004/99/EC of 1 October 2004 amending Council Directive 91/414/EEC to include acetamiprid and thiacloprid as active substances. OJ L 309, 6.10.2004, p. 6–8.

[4]Commission Implementing Regulation (EU) 2018/113 of 24 January 2018 renewing the approval of the active substance acetamiprid in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. OJ L 20, 25.1.2018, p. 7–10.

[5]Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC, OJ L 70, 16.3.2005, p. 1.

[6]Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. OJ L 167, 27.6.2012, p. 1–123.

[7]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, p. 1–50.

[8]The acute intake concerns for lettuce and pears were also noted in the most recent assessment of EFSA (e.g. EFSA, 2022).

[9]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

[10]https://open.efsa.europa.eu/questions/EFSA-Q-2022-00589?search=acetamiprid

The above-mentioned new data may altogether impact the evaluation of the safety of existing MRLs. Therefore, as requested in the mandate, fall-back good agricultural practices (GAPs) that could lead to safe scenarios should be investigated in relation to those uses that might lead to intake concerns. Based on a preliminary screening made by EFSA, a list of plant commodities that might lead to intake concerns was identified (full list of products included in Annex II to the extension mandate[10]). A call for data for fall-back GAPs and residue trials for the products of plant origin listed in Annex II should be launched with Member States, and the assessment should be performed taking into account the provided fall-back GAPs. In case EFSA considers that the toxicological reference values would need to be modified, the proposed revised values should be used to perform this risk assessment. In case a risk for consumers would be identified for one or more of the existing MRLs, EFSA should recommend new MRLs that ensure safety of consumers, where possible, and advise risk managers on alternative options.

The revised terms of reference with specific points to consider were provided in Annex III of the extension mandate.[10]

Considering that the additional analysis requested from EFSA would require additional efforts, and a consultation with experts from Member States in a peer-review meeting may be needed to confirm the reference values of the metabolite, EFSA was requested pursuant to Article 31 of Regulation (EC) No 178/2002, read in conjunction with Regulation (EC) No 1107/2009 and Regulation (EC) No 396/2005, to deliver a scientific output including its advice on the toxicological properties of acetamiprid and its metabolites, and the outcome of the MRL assessment by 31 March 2024.

1.1 | Background and Terms of Reference as provided by the requestor

Acetamiprid is an α-chloro-N-heteroaromatic compound that belongs to the group of neonicotinoids. The approval of acetamiprid has been renewed until 28 February 2033 by Commission Implementing Regulation (EU) 2018/113 concerning the renewal of approval of acetamiprid as active substance under Regulation (EC) No 1107/2009 that was based on the EFSA Conclusion on the peer review of the pesticide risk assessment of acetamiprid (EFSA, 2016a). This Implementing Regulation sets the conditions for the use of acetamiprid as active substance in plant protection products.

In November 2020, French authorities asked the Commission to prohibit the sale and use of acetamiprid under Article 69 of Regulation (EC) No 1107/2009, in the light of potential concerns that the substance may pose high risks to humans and the environment. The French authorities included in their notification scientific evidence to support this request, including references to published peer-reviewed studies. The Commission mandated the EFSA PPR Panel to advise on the likelihood that the body of evidence would constitute proof of serious risks to humans or the environment, in particular if the new studies indicate new or higher hazards and exposure to humans and the environment compared to previous EU assessments. In its statement published in January 2022 (EFSA PPR Panel, 2022), the EFSA PPR Panel concluded that there is no conclusive evidence of higher hazards from acetamiprid compared to the previous assessment in the context of the renewal with respect to genotoxicity, developmental toxicity, neurotoxicity including developmental neurotoxicity and immunotoxicity. However, it was recommended that an assessment of endocrine disrupting properties for acetamiprid is conducted in line with the EFSA/ECHA guidance document for the identification of endocrine disruptors under Regulations (EU) No 528/2012 and (EC) No 1107/2009 (ECHA/EFSA, 2018). Those findings were under discussion at the Standing Committee on Plants, Animals, Food and Feed, section Phytopharmaceuticals – Legislation with the view of a possible regulatory action for acetamiprid.

As regards maximum residue levels (MRLs), EFSA published a reasoned opinion on 20 July 2011 on the review of the existing MRLs for the active substance acetamiprid in compliance with Article 12(1) of Regulation (EC) No 396/2005 (EFSA, 2011). In this review, EFSA concluded that, in crops belonging to fruits and fruiting vegetables or to leafy vegetables, only the parent compound was found to be the main component, while for products of animal origin (except muscle of ruminants), the dominant compound was the metabolite N-desmethyl-acetamiprid (IM-2-1). EFSA concluded that the relevant residue definition for enforcement and risk assessment was acetamiprid only for plant products, and sum of acetamiprid and IM-2-1, expressed as acetamiprid, for products of animal origin. The MRLs resulting from this review and the confirmed residue definitions were implemented by Regulation (EU) No 87/2014,[11] and are now set in Annex II to Regulation (EC) No 396/2005.

In the beginning of 2022, an article was published (Laubscher et al., 2022) that describes methods for detection of pesticide residues in the cerebro-spinal fluid (CSF) of children. Using those detection methods, low levels of metabolite N-desmethyl-acetamiprid (IM-2-1) were detected in most examined samples. The authors concluded that despite of many uncertainties, those findings might indicate potential risks for foetal and children's health and in particular development of the nervous system, due to the mode of action of neonicotinoids. EFSA has already examined the issue of developmental neurotoxicity of acetamiprid in the EFSA PPR Panel (2022). In addition, recently, the OECD published a case study on the developmental neurotoxicity of acetamiprid as part of the OECD Integrated Approaches for Testing and Assessment (IATA) project (OECD Case Study no. 365, 2022c).

At the meeting of the Standing Committee on Plants, Animals, Food and Feed, section Phytopharmaceuticals – Pesticides residues – in April 2022, one Member State informed that it detected high levels of IM-2-1 in spinach, at times higher than

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[11]Commission Regulation (EU) No 87/2014 of 31 January 2014 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 acetamiprid, butralin, chlorotoluron, daminozide, isoproturon, picoxystrobin, pyrimethanil and trinexapac in or on certain products Text with EEA relevance. OJ L 35, 5.2.2014, p. 1–48.

those of the parent compound. So far, IM-2-1 is not part of the residue definition for acetamiprid in products of plant origin. The Commission had invited Member States to consult with their national laboratories and asked EFSA to investigate if similar findings have occurred in other countries, and to share the information with the Commission. New data were submitted to the Commission from both a Member State and EFSA, confirming that residue levels of IM-2-1 in spinach samples are often higher than the parent compound, and that similar findings occur in other crops.

In addition, another Member State brought to the attention of the Commission that the MRLs for pears and lettuces, which have been previously recommended by EFSA in the focused assessment of certain existing MRLs of concern for acetamiprid, and which have already been established by Regulation (EU) No 2019/88,[12] may pose acute risks to consumers according to intake calculations performed with the most recent version of PRIMo (rev. 3.1). The EFSA previous assessment from 2018 (EFSA, 2018c) was conducted with a previous version of PRIMo (rev. 2).

The present mandate (Ref. Ares (2023)6354306 of 20 September 2023)[10] is an extension of the mandate received in July 2022 (Ref. Ares (2022)5466053 of 29 of July 2022) by which EFSA was requested to deliver a scientific output including its advice on the toxicological properties of acetamiprid and its metabolites, and the outcome of the MRL assessment. By means of the revised mandate received in September 2023, the Commission requested EFSA to perform the following tasks as detailed in the specific revised terms of reference (**ToRs**):

1. To provide advice if new scientific evidence that has become available since the assessment conducted for the renewal in 2018 - including data/studies submitted by stakeholders and the additional studies provided by the applicant - warrant a re-evaluation of the toxicological properties of acetamiprid and its metabolites, including toxicological endpoints, in particular those related to developmental neurotoxicity.
2a. To launch a data call for monitoring data for plant products not yet submitted to EFSA.
2b. Based on monitoring data submitted under point 2a and the available metabolism studies in plants, to assess if the residue definitions for risk assessment and for enforcement derived for plant products treated with acetamiprid need to be revised.
3. To perform an assessment of the chronic and acute consumer risk related to the existing EU MRLs for acetamiprid in all plant and animal products,[13] using the newest version of the PRIMo model. This assessment should be performed considering the Good Agricultural Practices (GAPs) and supporting residue trials already available to EFSA.

In case EFSA considers that toxicological reference values would need to be modified, the proposed revised values should be used to perform this risk assessment. In case EFSA considers that a new residue definition for risk assessment in plant products should be proposed, two separate risk assessment scenarios for the existing and the proposed new residue definitions for risk assessment should be calculated. In parallel, a call for data for possible fall-back GAPs and supporting valid residue trials that could lead to safe scenarios should be launched with Member States for the products of plant origin listed in Annex II of the mandate letter. In case a risk for consumers is identified with the revised toxicological reference values and with the proposed new residue definition(s) for one of more of the existing MRLs, EFSA should recommend new MRLs that do not pose an unacceptable risk to consumers, where possible, and advise risk managers on alternative options. To identify alternative options and MRLs that do not pose an unacceptable risk to consumers, the fall-back GAPs and residue trials collected in the framework of this mandate should be considered. In case EFSA would consider that toxicological reference values and/or the residue definition for risk assessment in plants would need to be modified, the proposed revised values, and/or residue definition(s) should also be used to perform this assessment of the alternative MRL options.

EFSA was requested to deliver a scientific output including its advice on the toxicological properties of acetamiprid and its metabolites and the outcome of the MRL assessment, pursuant to Article 31 of Regulation (EC) No 178/2002, read in conjunction with Regulation (EC) No 1107/2009 and Regulation (EC) No 396/2005, by 31 March 2024.

1.2 | Interpretation of the Terms of Reference

1.2.1 | Term of Reference 1 (human health)

In the human health part, an EFSA WG was established in order to address the following ToR: To provide advice if new scientific evidence that has become available since the assessment conducted for the renewal in 2018 – including the elements referred to in Section 1 above – warrant a re-evaluation of the toxicological properties of acetamiprid and its metabolites, including toxicological endpoints, in particular those related to developmental neurotoxicity.

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[12]Commission Regulation (EU) 2019/88 of 18 January 2019 amending Annex II to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for acetamiprid in certain products). OJ L 22, 24.1.2019, p. 1–12.

[13]This assessment should also cover those MRLs that were considered safe in recent EFSA outcomes that have not been addressed yet by specific Commission Regulations (e.g. those included in the EFSA, 2021).

The EFSA WG interpreted the ToR by considering the following tasks:

1a. To conduct the assessment, including reliability and relevance for risk assessment, of the new biomonitoring data that have become available since 2016 until 2022 (in particular Laubscher et al., 2022 provided in the mandate).

1b. To use the most suitable biomonitoring data to extrapolate the level of acetamiprid and/or its metabolites in children to an external dose of acetamiprid and compare this external dose to the current HBGVs of acetamiprid.

1c. To conduct the assessment of the new evidence that has become available since 2016 until 2022, on toxicological properties that could be used for the risk assessment of acetamiprid and its metabolites.

1d. To conduct the assessment, in particular of the new evidence on DNT that has become available since 2016 until 2022, including reliability and relevance assessment for its use in DNT hazard identification and characterisation of acetamiprid. Evidence from human observational studies (HOS), in vivo studies and new approach methodologies (NAMs) (in vitro, zebrafish) will be used in an adverse outcome pathway (AOP)-informed integrated approach to testing and assessment (IATA).

The EFSA WG made use of the ToR1 to define the assessment questions. The EFSA WG considered two main aspects in the interpretation of the ToR1. The first aspect considers the exposure characterisation, and this is assessed in assessment question 1. The second aspect considers the hazard characterisation of acetamiprid and N-desmethyl-acetamiprid (IM-2-1) metabolite and this is addressed in assessment question 2.

To address tasks 1a and 1b

1. **Assessment question 1:** Based on the results of the systematic literature review for internal exposure characterisation (and in particular the presence of metabolite N-desmethyl-acetamiprid (IM-2-1) in the cerebrospinal fluid of children), does the estimated external exposure exceed the current HBGVs?

   1a. Based on the new biomonitoring evidence of acetamiprid and its metabolites, which information on internal exposure is the most appropriate (reliable, robust) to be used for the exposure pattern characterisation for acetamiprid risk assessment (i.e. derived from biomonitoring studies translated to external exposure by reverse dosimetry, using a physiologically based kinetic (PBK) model for acetamiprid)?

   1b. Based on the exposure estimation of acetamiprid from 1a and after the uncertainty analysis (including those uncertainties inherent to the modelling approaches), does the estimated external exposure exceed current HBGVs?

To address tasks 1c and 1d

2. **Assessment question 2**: Based on the results of the systematic literature review for hazard characterisation, are the current HBGVs for acetamiprid protective for the most sensitive population (fetus and children)?

   2a. Based on the newly available evidence for any toxicological endpoint for acetamiprid and its metabolites, are the current HBGVs for acetamiprid protective?

   2b. Based on the newly available evidence for DNT hazard characterisation, as outlined in the description of the task, are the current HBGVs for acetamiprid protective for the most sensitive population (fetus and children)?

1.2.2 | Term of Reference 2a (data call, monitoring data)

To address ToR 2a, EFSA launched a call for data, inviting Member States to submit to EFSA results of pesticide residue analysis for acetamiprid and its metabolites in food of plant origin derived by national competent authorities. Since the metabolites of acetamiprid are not part of the current residue definition for enforcement, information on these compounds which may be available at national level, might not have been reported in the context of the routine data collections, although such information is available at national level.

Member States were invited to send in particular data that have not been submitted previously to EFSA (i.e. in the context of the routine annual collection of pesticide monitoring results under Art. 31 of Regulation (EC) No 396/2005). The data call was restricted to the period of 2018–2022. The monitoring data should be provided in the format developed for submitting pesticide residue data under Article 31 of Regulation (EC) No 396/2005. Samples with residue analysis for parent acetamiprid and its metabolites such as N-desmethyl-acetamiprid (IM-2-1) were of major relevance for the current mandate.

In order to get a comprehensive database, the new monitoring data were combined with the previously submitted monitoring data: the combined data were assessed in detail to derive conclusions on the metabolic pattern in different crops/crop groups (see Section 3.1).

1.2.3 | Term of Reference 2b (residue definitions)

In order to address ToR 2b, EFSA applied the following approach:

1. The pesticide monitoring data (combined data from the data call under ToR 2a and monitoring data previously sent to EFSA in the context of routine data collection under Article 31 of Regulation (EC) No 396/2005) were compared with the results of the metabolism studies, to investigate whether the metabolic patterns are qualitatively and quantitatively comparable (see Section 3.1).
2. Metabolism studies relevant for plant products (i.e. metabolism studies investigating metabolism in primary crops and in rotational crops) submitted by the applicant in the framework of the renewal of the approval of acetamiprid under Regulation (EU) No 844/2012 and in other relevant applications were investigated in view of the metabolic pattern expected in different types of crops/parts of the crops (see Section 3.2).
3. EFSA identified crops/crop groups for which the metabolic pattern found in monitoring data provide evidence that metabolites are likely to be present at levels that would require a re-consideration of the residue definition (risk assessment and/or enforcement). The metabolites relevant for the different crops/crop groups were defined (see Section 3.3).
4. For those commodities where the residue definition for risk assessment was considered for being modified, and therefore became different than the residue definition for monitoring, conversion factors (CF) were derived. A non-standard methodology based on the available monitoring data was used (see Section 3.3).

1.2.4 | Term of Reference 3 (consumer risk assessment)

In order to address **ToR 3**, EFSA applied the following approach:

1. A first consumer risk assessment (CRA) was performed using PRIMo rev. 3.1. In this scenario, EFSA applied the newly derived HBGVs for acetamiprid and N-desmethyl-acetamiprid (IM-2-1) and the existing residue definitions for risk assessment, as derived in the framework of the renewal of the approval of acetamiprid under Regulation (EU) No 844/2012. EFSA assessed not only the existing MRLs but also considered those MRLs that were considered safe in the most recent EFSA outcomes (see **scenario 1** in Section 3.4.1).
2. A second consumer risk assessment (CRA) was performed using PRIMo rev. 3.1. In this scenario, EFSA applied the newly derived HBGVs for acetamiprid and N-desmethyl-acetamiprid (IM-2-1) and the newly derived residue definitions for risk assessment (including conversion factors), as proposed in the framework of the present mandate. EFSA assessed not only the existing MRLs but also considered those MRLs that were considered safe in the most recent EFSA outcomes (see **scenario 2** in Section 3.4.2).
3. EFSA launched a call for data for possible fall-back GAPs and supporting valid residue trials that could lead to safe MRL options for the plant commodities for which a risk for consumer has been identified under scenario 2. The data call was addressed to the EU Member States. Specific templates for submitting authorised good agricultural practices (GAPs) and supporting data were made available by EFSA (see Section 3.5.1).
4. EFSA screened and assessed the GAPs and supporting data received from Members States applying a stepwise approach to identify potential fall-back MRL option that would be safe for consumers (see Section 3.5.2).
5. A detailed assessment of the robustness of the identified fall-back MRL options for plant commodities was performed (see Sections 3.5.3 and 3.5.4). Further considerations were also made on the risk characterisation for those plant commodities for which no fall-back MRL could be identified (see Section 3.5.5).
6. For those commodities of animal origin for which a risk for consumer has been identified under scenario 2, further assessment was performed by EFSA to also identify fall-back MRL options.
7. A third consumer risk assessment (CRA) was performed using PRIMo rev. 3.1. EFSA applied the newly derived HBGVs for acetamiprid and N-desmethyl-acetamiprid (IM-2-1) and the newly derived residue definitions for risk assessment (including conversion factors), as proposed in the framework of the present mandate. In this scenario, EFSA used the fall-back MRLs (and risk assessment values) identified under points 4, 5 and 6 (see **scenario 3** in Section 3.5.7).
8. Based on scenario 3, EFSA recommended alternative MRLs for which risk to consumer is unlikely and provided further advice to risk managers where more than one option was identified.

2 | HUMAN HEALTH

2.1 | Hazard assessment acetamiprid

This section addresses **ToR 1a**, **ToR 1b**, **ToR 1c** and **ToR 1d**. To address **ToR 1** a WG of EFSA, including several experts of the PPR Panel was established.

2.1.1 | Data

Data related to four sources of evidence were collected and appraised to address the hazard characterisation of acetamiprid and its metabolites:

1. Data related to exposure and hazard characterisation have been retrieved conducting a systematic data collection from 2016 to 2022 and appraisal using critical appraisal tools. In addition, a protocol was developed based on EFSA (2020), which included detailed information on the search strategy, inclusion and exclusion criteria and detailed methods for the systematic review process used (see Appendix 1).
2. The OECD case study on the IATA for DNT of acetamiprid published in 2022 (https://one.oecd.org/document/env/cbc/mono(2022)27/en/pdf) and Annex I (https://one.oecd.org/document/env/cbc/mono(2022)27/ann1/en/pdf) (OECD, 2022c).
3. An in vivo developmental neurotoxicity (DNT) experimental study for regulatory purpose (OPPTS 870.6300) available for acetamiprid active substance. During the appraisal of the literature review retrieved (see source of evidence 1), the experts of the WG noted that in line with the EFSA IATA framework, all the available evidence for DNT should undergo the same reliability and relevance assessment approach (i.e. critical appraisal and uncertainty analysis) in order to conclude on the DNT potential of acetamiprid. For this reason, the DNT regulatory study was also critically appraised.
4. List of publications notified by PAN Europe and published after the period in which EFSA conducted the systematic literature review (up to end of 2022) and included in the extension of the mandate. In particular:

   - Li, He, et al. (2022). Neonicotinoid insecticides promote breast cancer progression via G protein-coupled estrogen receptor: In vivo, in vitro and in silico studies. Environment International, 170, 107568. https://doi.org/10.1016/j.envint.2022.107568
   - Mishani et al. (2022). The effect of increasing the dose of acetamiprid and dichlorvos pesticides on the reproductive performance of laboratory mice. Advanced Biomedical Research, 11, 114. https://doi.org/10.4103/abr.abr_199_22
   - Didenko et al. (2022). Dose dependence of subchronic influencing of acetamiprid on the organism of rats from data of morphological researches. Wiadomosci Lekarskie, 75, 2987–2993. https://doi.org/10.36740/WLek202212116
   - Li, Si, et al. (2022). Detection of neonicotinoid insecticides and their metabolites in human cerebrospinal fluid. Environmental Health Perspectives, 130, 127702. https://doi.org/10.1289/EHP11374
   - Yang & Liang (2023). Associations between neonicotinoids metabolites and hematologic parameters among US adults in NHANES 2015–2016. Environmental Science and Pollution Research International, 30, 26327–26337. https://doi.org/10.1007/s11356-022-23997-4
   - Mendy & Pinney (2022). Exposure to neonicotinoids and serum testosterone in men, women, and children. Environmental Toxicology. https://doi.org/10.1002/tox.23503
   - Ma et al. (2022). Long-term exposure to neonicotinoid insecticide acetamiprid at environmentally relevant concentrations impairs endocrine functions in Zebrafish: Bioaccumulation, feminization, and transgenerational effects. Environmental Science Technology, 56, 12494–12505. https://doi.org/10.1021/acs.est.2c04014

It must be noted that it was agreed to evaluate all the studies notified by PAN Europe in detail. Therefore, there was no need for consideration of inclusion/exclusion criteria for these publications as set in the protocol (Appendix 1) was performed. It is also of interest to note that the literature search by EFSA was performed in November 2022 (see Appendix 1), and that some of the studies notified by PAN Europe are from after that period.

2.1.2 | Methodologies

To address tasks 1c and 1d, the EFSA WG used the following approaches:

- An **evidence-based approach** for the data retrieval, compilation and integration in a weight of evidence (WoE). The approach to collect, appraise, synthesise/integrate evidence and analyse uncertainty followed EFSA's 'Principles and process for dealing with data and evidence' (EFSA, 2015; EFSA and EBTC, 2018; EFSA Scientific Committee, 2017) and guidance on uncertainty analysis (EFSA Scientific Committee, 2018a, 2018b). This implied planning the methods for conducting the assessment upfront, in a detailed **protocol** based on the draft protocol developed by the EFSA (2020), which included all the detailed information on the methodology of the development of the IATA, systematic literature review, critical appraisal and weight of evidence and uncertainty analysis.
- An **AOP-informed IATA** framework for assessing DNT (OECD, 2016) in line with the framework applied in the EFSA IATA Case Studies for DNT of deltamethrin and flufenacet (EFSA PPR Panel, 2021), where an evidence-based approach for the data compilation and integration in the IATA was used.

Appendix 1 contains the protocol and the details on the methodology. The protocol includes in detail the strategy and methods for the systematic review process, specifically:

- Translation of the mandate into subquestions.

- Search string to retrieve the studies for in vitro (including studies conducted in zebrafish), in vivo and human evidence and a full list of literature databases to be used.
- Studies eligibility criteria and screening for relevance.
- Critical appraisal tools (CATs) and procedures for assessing the risk of bias (RoB), including the rationale used for each line of evidence for DNT.
- Methods for evidence synthesis, integration and uncertainty analysis.

A short summary of the main methodology is reported below for easier reference.

Systematic literature review

The literature searches were conducted using three electronic bibliographic databases (PubMed, Web of Science, Toxnet). The time period considered was from 2016 until 2022. Search strings are described in the protocol (Appendix 1). A broad search was therefore conducted, and in addition, terms for the exposure were combined with relevant terms for DNT outcomes (human and in vivo studies) or methods (in vitro studies) and the studies were labelled in Distiller SR.

Critical appraisal of the evidence

The eligible in vivo studies in rodents (experimental toxicological studies) from the literature search, as well as the in vivo rodent studies notified by PAN Europe published after the systematic literature review carried out by EFSA, were appraised for the RoB by the WG using tailored versions of the OHAT-NTP RoB tool (EFSA PPR Panel, 2021; NTP, 2015). For the in vitro studies, a modified tool developed by OHAT-NTP for a specific project (NTP, 2016) was adapted. CATs were defined upfront and are described in the protocol (Appendix 1). Endpoints measured in the studies were classified as being of low (tier 1), moderate (tier 2) or high (tier 3) RoB. These tiers were derived weighing the appraisal from the individual RoB domains where some of the domains were identified as key for the overall appraisal. RoB was appraised endpoint by endpoint in each study as the diverse endpoints measured within the study may have been assessed by different methodology and thus their RoB may differ.

The study of Ma et al. (2022), which was notified by PAN Europe, was appraised in line with previous evaluations of ecotoxicological studies (see EFSA, 2023), i.e. performing a data extraction followed by the appraisal of the measured parameters. For appraising the parameters, the risk of external (relevance) and internal (reliability) bias and precision were assessed. For details about the protocol used, see EFSA (2023) and the related background documents.

Uncertainty analysis and expert knowledge elicitation

The uncertainty analysis and the expert knowledge elicitation (EKE) methodologies are reported in Appendix 1 and were performed according to EFSA's recommendations (EFSA Scientific Committee, 2018a, 2018b). An uncertainty analysis (Table 1) was therefore performed for each line of evidence and hierarchical level in order to support conclusions on the hazard identification and characterisation questions. The final purpose was to screen the evidence and identify molecular initiating events (MIEs), key events (KEs) and adverse outcomes (AOs) to be included in the putative AOP network. All data were mapped in specific endpoints and endpoint categories (obtained from the systematic review, the DNT in vitro battery (IVB) and the test guideline in vivo study) and this was used with the aim of postulating an AOP network. In the AOP, evidence was classified as MIEs, KEs or AOs based on their categorisation as molecular, cellular, organ, organism or population response. However, this was only possible for the limited available evidence that was considered reliable.

The uncertainty analysis was conducted in two steps (Steps 1 and 2; see Table 1) to identify and characterise the DNT effects in the body of evidence (BoE) by providing answers to specific assessment questions and expressing the uncertainty in a qualitative way using an EKE.

TABLE 1 Assessment questions for the uncertainty analysis for in vivo and in vitro body of evidence (BoE) for acetamiprid DNT hazard identification (see also Appendix 1).

Line of evidence	Question EKE	Possible outcome options for expression of the uncertainty qualitatively
Step 1. In vivo experimental studies	Based on the in vivo available evidence for DNT hazard characterisation and integrated following an AOP-informed IATA conceptual framework, is the WG of the opinion that the current HBGVs for acetamiprid and IM-2-1 are protective for the most sensitive population (fetus and children)? The WG must consider EFSA conclusion on acetamiprid (EFSA, 2016a) where the acceptable daily intake (ADI), the acute reference dose (ARfD), the acceptable operator exposure level (AOEL) and the acute acceptable operator exposure level (AAOEL) are set at 0.025 mg/kg bw (per day) on the basis of reduced auditory startle responses in offspring observed from 10 mg/kg bw per day (LOAEL) in the rat DNT study with an uncertainty factor (UF) of 100	**YES**, the WG considers that the current HBGVs are adequate and protective for the most sensitive population in view of the new evidence made available since the renewal of approval of acetamiprid **NO**, the WG considers that there are additional uncertainties not considered in the previous evaluation. These major limitations in the DNT BoE and data gaps would warrant a re-evaluation of the current HBGV

(Continues)

TABLE 1 (Continued)

Line of evidence	Question EKE	Possible outcome options for expression of the uncertainty qualitatively
Step 2. In vitro experimental studies	Once all the mechanistic data will be mapped in an AOP framework, the experts will be asked to answer the following question: Based on the new available evidence and on the AOP-informed IATA, is the result of 1 sufficiently protective for a DNT effect?	**YES**, the WG considers that the current HBGVs are adequate and protective for the most sensitive population in view of the new evidence made available since the renewal of approval of acetamiprid **NO**, the WG considers that there are additional uncertainties not considered in the previous evaluation. These major limitations in the DNT BoE and data gaps would warrant a re-evaluation of the current HBGV

Adverse outcome pathway (AOP)-informed IATA development and weight of evidence (WoE)

To assess the DNT evidence, an AOP-informed IATA framework was applied. Within an IATA, data from various information sources are evaluated and integrated to draw conclusions on the hazard and/or risk of chemicals (OECD, 2020). IATA is a framework developed by the OECD that allows for the integration of all available hazard and possibly exposure data, including in silico, in chemico, in vitro and in vivo, for use in chemical regulatory assessments (OECD, 2016). Important features of IATAs are the need to explicitly set out the problem formulation and context of use, since these will determine the acceptable level of uncertainty, the choice of methods (building blocks), the approach for evidence integration and the application of the WoE in an iterative process until a conclusion is reached (OECD, 2016). This approach has been previously used by EFSA for DNT hazard characterisation IATAs (EFSA PPR Panel, 2021; OECD, 2022a, 2022b) and represents a step forward in terms of the methodological approach in delivering a sound, transparent and accessible scientific advice in support to decision-making.

IATAs are pragmatic, science-based approaches for chemical evaluations in the context of hazard or risk assessments that rely on an integrated analysis of existing information, with optional use of the AOP framework, coupled with the generation of new information if necessary (OECD, 2016; Sachana & Leinala, 2017; Sakuratani et al., 2018). To fulfil these IATA needs, evidence was structured using the AOP framework. The AOP conceptual framework was applied to integrate information obtained from various lines of evidence by means of a systematic literature review, and the experimental outcome from the DNT IVB in order to provide a structured contextualisation of the molecular initiating events (MIEs) and key events (KEs) leading to the adverse outcomes (Aos) (see Figure 1). Conclusions were drafted for the AO and for the mechanistic pathway (MIE, KEs and key events relationships [KERs]) and in the subsequent step the WoE evaluation of the AOP itself was planned in line with previous EFSA AOP-informed IATAs (OECD Case Study 362 [OECD, 2022a] and OECD Case Study 363 [OECD, 2022b]).
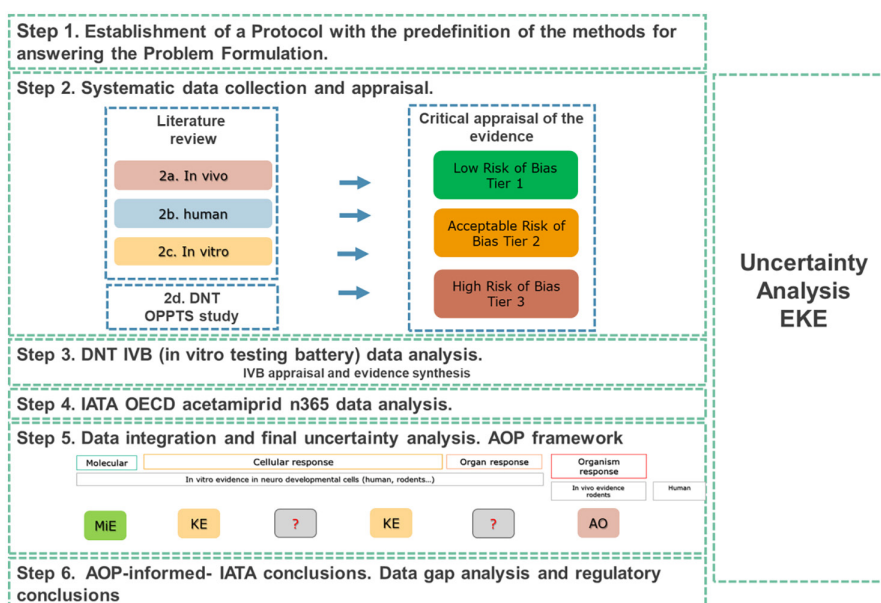

FIGURE 1 AOP-informed IATA workflow for acetamiprid assessment.

2.1.3 | Assessment for non-DNT endpoints

2.1.3.1 | *Data collection*

A total of 1702 studies were identified for acetamiprid after removing duplicates (see PRISMA flow chart, Figure 2) using an EFSA systematic literature review. Two reviewers screened in two steps the results of the broad search (step 1 title and abstract screening and step 2 full-text reading), i.e. the literature was identified through the searches in DistillerSR® (Evidence Partners, Ottawa, Canada). The evidence was clustered as (1) evidence providing data potentially relevant for any non-DNT toxicological endpoint that could be used for hazard characterisation and risk assessment of acetamiprid active substance and could warrant a re-evaluation of the current toxicological assessment (i.e. studies that could be used for setting HBGVs) and (2) evidence providing indications of a potential DNT effect in line with an AOP-informed IATA framework (i.e. where DNT is defined as any adverse effect on the normal development of nervous system structures and/or function, that is predefined in the protocol by different endpoints measured in in vivo, in vitro and human observational studies. DNT is considered as a molecular/cellular/organ/organism burden that could lead to adversity). The eligibility criteria are detailed in the protocol, Tables 4–8 (Appendix 1).

Since no publication providing evidence relevant for step 1 was retrieved, it was agreed that further assessment for non-DNT endpoints was not needed (Figure 2).
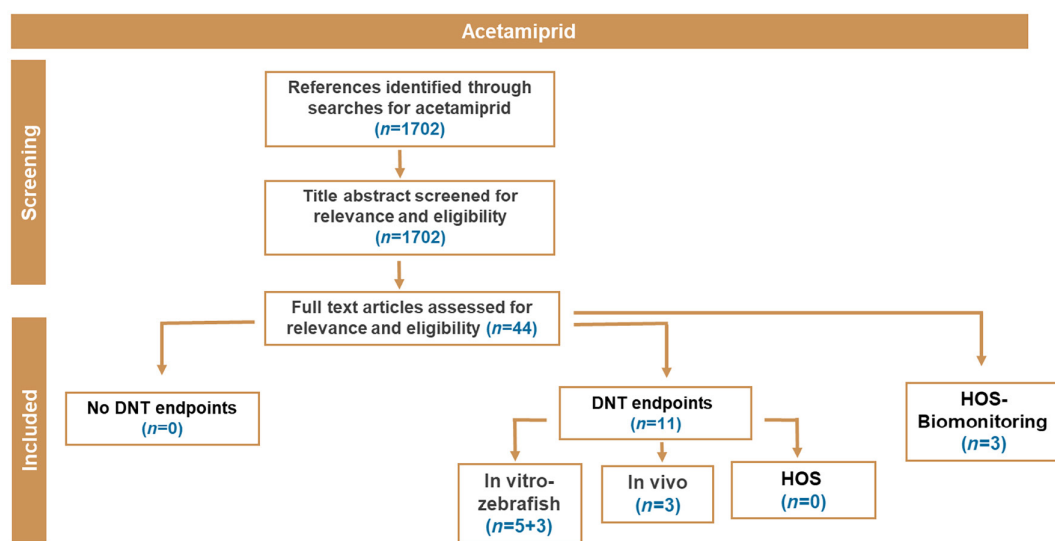
FIGURE 2 PRISMA flow chart of the literature search process for acetamiprid, including the screening for relevance of results.

However, new studies (published in 2022 and 2023) were notified by PAN Europe and included into the appraisal step. A summary of the seven studies is available in the Excel file present in Appendix 2, presenting per study information on the test systems used, the exposure conditions applied, the endpoints/readouts assessed and the results reported by the study authors.

Description of the studies

Of the seven additional publications notified by PAN Europe, three included data obtained in animal experiments (two studies using rats, one using mice), with one of these studies also including supportive mechanistic data from in vitro studies. Three studies reported on acetamiprid levels in humans, with two of them assessing a possible association between acetamiprid levels and health effects in humans. The remaining study assessed the effects of acetamiprid (and IM-2-1) on zebrafish.

In the following, a short description of the studies is provided, only focusing on acetamiprid and metabolite IM-2-1.

- In the study of Li, He, et al. (2022), the effect of acetamiprid on in situ growth of breast cancer cells in female mice and related mechanisms were studied. Ovariectomised and non-ovariectomised mice were injected with 4T1-luc cells (murine triple-negative breast cancer cell line) and exposed daily by oral gavage for 3 weeks to acetamiprid (ovariectomised mice to 0, 1, 10 or 30 mg/kg bw per day; non-ovariectomised mice to 0 or 10 mg/kg bw per day). Also, a group was included that was exposed to the G protein-coupled oestrogen receptor (GPER) antagonist G15, as well as a group that was exposed to 10 mg acetamiprid/kg bw per day together with the GPER antagonist G15. The authors reported that acetamiprid increases the growth of the in situ tumours and increased metastasis, and that these effects were inhibited by the GPER antagonist G15. In the same publication, experiments are described in which 4T1-luc cells were exposed in vitro to different concentrations of acetamiprid (in the presence or absence of the GPER antagonist G15) and effects on GPER mRNA expression,

GPER activation, as well as on cell migration and cell proliferation were assessed. The authors reported that acetamiprid induced an increase in GPER mRNA expression, GPER activation, as well as increased migration and proliferation of cells exposed to acetamiprid. The authors also reported that effects on GPER activation, cell proliferation and cell migration were inhibited by the GPER antagonist G15.

- In the study of Mishani et al. (2022), male Balb/c mice were exposed to acetamiprid via drinking water (0, 1.5 or 2.5 ppm) for 60 days. After exposure, treated males were mated with untreated females. In males, FSH, LH and testosterone levels were assessed, as well as an analysis was performed to evaluate different cell types in the testes. Pregnant females were sacrificed at gestation day (GD) 15 and the number of embryos was determined. The authors reported a decrease in number of embryos in females mated with males exposed to drinking water with 2.5 ppm acetamiprid. The authors also reported an increase in LH and FSH, a decrease in testosterone and a decrease in spermatogonial stem cells, spermatocytes and spermatogonia in testes of mice exposed to drinking water with 2.5 ppm acetamiprid.

- In the study of Didenko et al. (2022), female rats were daily exposed (5 days per week) for 90 days via oral gavage to 0, 6, 12 or 60 mg acetamiprid/kg bw. The study was performed according to OECD 408 with deviations. The rats were monitored for general effects (behaviour, feed and water consumption, skin and coat condition, mucous membranes, motor activity, tremors, gait/posture disturbances, presence of reaction to care of the animals, presence of clonic or tonic movements, stereotypic movements, chimeric behaviour). Before and during exposure (every 7 days), body weights were determined. At the end of exposure, animals were sacrificed, and absolute and relative organ weights were determined (internal organs: liver, kidneys, spleen, brain). Histological assessment was done for brain, liver, kidneys and spleen. The authors reported a decrease in body weight, absolute weight of spleen and brain and a decrease in relative spleen weight at the top dose (60 mg/kg bw per day) compared to the control group. The authors reported histological changes in various tissues at the mid and top dose levels. Effects at the mid dose were considered by the authors as adaptive changes, whereas these were considered adverse at the top dose in the liver, kidney and brain. The authors set the no observed adverse effect level (NOAEL) at 12 mg/kg bw per day, and the NOEL at 6 mg/kg bw per day.

- In the study of Li, Si, et al. (2022), neonicotinoids and their metabolites were quantified in cerebrospinal fluid (CSF) samples of 314 human donors from 4410 patients available for CSF analysis in First Affiliated Hospital of Shantou University from April 2019 to January 2021, with an age range from 1 month to 89 years. Regarding acetamiprid, the authors reported that the median concentration was below the LOD (0.002 ng/mL), the maximal concentration amounted to 2.07 ng/mL and the detection frequency was 39.5%. Regarding IM-2-1, the authors reported that the median concentration was 0.049 ng/mL, the maximal concentration amounted to 2.38 ng/mL and the detection frequency was 85.4%.

- In the study of Yang and Liang (2023), the relationship between neonicotinoid metabolites (levels measured in urine) and hematologic parameters was assessed using a cross-sectional study design in 1397 adults of the National Health and Nutrition Examination Survey (NHANES) 2015–2016, applying multivariate linear regression models. Regarding acetamiprid/IM-2-1, the authors reported that IM-2-1 levels in urine were associated with reduced white blood cells (WBC) and neutrophils counts (statistically significant for males and females together and for males as separate group). Furthermore, the authors reported that IM-2-1 levels in urine were linked to increased eosinophil counts and percentages (statistically significant only for females as separate group) and to decreased haemoglobin and haematocrit, and reduced platelet count (statistically significant only for males as separate group). The authors concluded that the results from the study provided evidence that exposure to neonicotinoid metabolites can disturb haematologic homeostasis in the general populations and that effects may be sex specific.

- The study of Mendy and Pinney (2022) assessed the association between concentrations of neonicotinoids and their metabolites determined in urine of 2014 participants to the NHANES 2015–2016 (6 years and older) and total serum testosterone concentration and free androgen index (FAI: serum concentration ratio of total testosterone to sex hormone binding globulin [SHBG]). IM-2-1 in urine was associated with reduced testosterone in serum in males (detection rate and log10 concentration). For females, the same association was observed only when adjusted for age, that is, 40 years and older (detection and log10 concentration). Both in males and females, IM-2-1 in urine was reported to be associated with reduced FAI (males only for log 10 concentration, females both for detection and log10 concentration). The authors concluded that exposure to neonicotinoids is associated with decreased serum testosterone levels in humans, but that future prospective studies are warranted to confirm these findings.

- The study from Ma et al. (2022) investigated the long-term exposure to acetamiprid on the aquatic test species zebrafish (*Danio rerio*). The study included three different experiments, (i) a fish full life cycle-type experiment (from larvae to embryos of the second generation [F1]); (ii) an experiment where F1 embryos were monitored but not exposed and (iii) a fish embryo test:

  1. Fish full life cycle type test (1_1):
     a. Zebrafish were exposed from larval stage (20 days post fertilisation – dpf) up to adulthood (174 dpf) to five nominal concentrations of acetamiprid (0.15, 1.5, 15, 150 and 1500 µg/L). Adult zebrafish were used to examine survival, sex ratio and growth responses. Negative controls and solvent controls containing 0.01% (v/v) DMSO were tested concurrently.
     b. From the adult fish, two females and two males (3 replicates per treatment) of the F0 generation were incubated, and the fish embryos (F1) were collected for 3 days. Number of eggs and percentage of fertilisation were recorded. Successfully fertilised and normally developed F1 embryos at 6 h post-fertilisation (hpf) were continuously exposed up to 168 hpf to the same levels of acetamiprid as the F0 in 96-well plates. Hatchability at 72 hpf, malformations and survival at 168 hpf were determined.

2. F1 generation raised in unexposed conditions (1_2): to monitor potential transgenerational effects of parental exposure, successfully fertilised and normally developed F1 embryos at 6 h post-fertilisation (hpf) from the F0 generation exposed to acetamiprid were raised in clean medium in 96-well plates. Hatchability at 72 hpf, malformations and survival at 168 hpf were determined.

3. Fish embryo toxicity experiment (1_3): Studies with acetamiprid and its metabolite IM-2-1 were conducted separately to evaluate their bioaccumulation potential and acute toxicity (LC50/EC50 values) to zebrafish embryos. No specific guideline study was followed/mentioned in the text. Analytically verified treatment levels were 0, 57, 81, 210, 337, 461, 477 and 734 mg/L for acetamiprid, and 0, 69, 104, 287, 366, 434, 520, 628, and 722 mg/L for IM-2-1. After exposure, hatchability at 72 hpf, malformation and survival at 168 hpf were determined.

2.1.3.2 | *Critical appraisal results*

The results of the RoB analysis conducted during the extension of the mandate are presented in **Tables 2–6** by lines of evidence (i.e. in vivo rodent studies [**Table 2**], in vitro studies [**Table 3**], human studies [**Tables 4 and 5**] and zebrafish study [Table 6]). Regarding the human studies, one study reported only internal concentrations (no health effects were measured; **Table 4**) and two studies evaluated possible associations between internal exposure data and (health) effects (**Table 5**). These studies were subdivided, because they followed different RoB assessment methods.

All in vivo rodent endpoints were rated as Tier 3 (high RoB). For the Li, He, et al. (2022) and Mishani et al. (2022) studies, risk of bias was considered regarding blinding of research personnel to study group (Q4), possible attrition or exclusion from analysis (Q5), the exposure characterisation (Q6, key questions) and the outcome assessment (Q7, key question). For some of the endpoints of the Li, He, et al. (2022) study, also a risk of bias was considered regarding randomisation of the animals (Q1, key question).

All in vitro endpoints were rated as Tier 3, except for GPER mRNA expression (Tier 2). For all in vitro endpoints, risk of bias was considered regarding the comparability of experimental conditions in control and treatment groups (Q2), outcome reporting (Q7) and internal validity: number of replicates (Q8b). For one endpoint (cell migration: wound healing assay), lack of randomisation of exposure levels was considered to provide a risk of bias (Q1), for two endpoints (cell migration: wound healing assay and cell migration: Boyden chamber assay), lack of blinding of research personnel to study group was considered to provide a risk of bias (Q3), and for two endpoints (GPER activation and cell proliferation), the outcome assessment was considered to provide a risk of bias (Q6, key question).

The human biomonitoring study on acetamiprid/IM-2-1 levels in CSF was rated as Tier 3 (high RoB). There was limited information about the method's validation (Q6) and no reporting about quality assessment/quality control (QA/QC; Q7, key question). Haematologic parameters and testosterone and free androgen index from the human observational studies were rated as Tier 1 (low RoB). More details on the assessments of the rodent, in vitro and human studies can be found in Appendix 3.

TABLE 2 Heatmap results of the RoB for the endpoints measured in the new in vivo studies notified by PAN Europe in the extension of the mandate for acetamiprid.

In vivo endpoints	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Tier
Li, He, et al., (2022): tumour growth ovariectomised mice (bioluminescence)										3
Li, He, et al., (2022): tumour growth ovariectomised mice (tumour volume)										3
Li, He, et al., (2022): tumour metastasis lung ovariectomised mice (bioluminescence)										3
Li, He, et al., (2022): tumour growth non-ovariectomised (bioluminescence)										3
Li, He, et al., (2022): tumour growth non-ovariectomised mice (tumour volume)										3
Li, He, et al., (2022): tumour metastasis non-ovariectomised mice (bioluminescence)										3
Li, He, et al., (2022): tumour metastasis non-ovariectomised mice (tumour nodules lung)										3
Mishani et al., (2022): number of embryos at GD15										3
Mishani et al., (2022): FSH										3
Mishani et al., (2022): LH										3
Mishani et al., (2022): testosterone										3
Mishani et al., (2022): stem cells in testes										3
Mishani et al., (2022): spermatocytes in testes										3
Mishani et al., (2022): spermatogonia in testes										3

(Continues)

TABLE 2　(Continued)

In vivo endpoints	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Tier
Didenko et al. (2022): organ weight										3
Didenko et al. (2022): organ histopathology										3

Note: The heat map includes the endpoint-specific ratings for the RoB for each of the nine questions in the CAT (i.e. domains of randomisation, allocation concealment, experiment conditions, research personnel blinded, attrition, exposure characterisation, outcome assessment, outcome reporting and other aspects, i.e. systemic or maternal toxicity). Each question is scored as either definitely low RoB (dark green), probably low RoB (light green), probably high RoB (light red) or definitely high RoB (dark red).The RoB questions highlighted in yellow correspond to the key RoB criteria considered in the final Tier (1 [low RoB], 2 [medium RoB] or 3 [high RoB]) of the endpoint (Q1 randomisation domain; Q6 exposure characterisation; Q7 outcome assessment method). For detailed rationale for each rating, see Appendix 3.

TABLE 3　Heatmap results of the RoB for the endpoints measured in the new in vitro studies notified by PAN Europe in the extension of the mandate for acetamiprid.

In vitro endpoints	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8a	Q8b	Tier
Li, Si, et al. (2022): GPER activation: intracellular calcium										3
Li, Si, et al. (2022): Cell migration: wound healing assay										3
Li, Si, et al. (2022): Cell migration: Boyden chamber assay										3
Li, Si, et al. (2022): Cell proliferation: MTT assay										3
Li, Si, et al. (2022): GPER mRNA expression: qPCR										2

Notes: The heatmap includes the endpoint-specific ratings for the RoB for each of the nine questions in the CAT (i.e. domains of randomisation, experiment conditions, research personnel blinded, attrition, exposure characterisation, outcome assessment, outcome reporting, other aspects, i.e. cytotoxicity and replicates). Each question is scored as either definitely low RoB (dark green), probably low RoB (light green), probably high RoB (light red) or definitely high RoB (dark red). The RoB questions highlighted in yellow correspond to the key RoB criteria considered in the final Tier (1 [low RoB], 2 [medium RoB] or 3 [high RoB]) of the endpoint (Q5 exposure characterisation; Q6 outcome assessment method; Q8a cytotoxicity). For detailed rationale for each rating, see Appendix 3.

TABLE 4　Heatmap results of the RoB for the new human biomonitoring study on reported internal acetamiprid/IM-2-1 concentrations notified by PAN Europe in the extension of the mandate for acetamiprid.

Human biomonitoring study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Tier
Li, Si, et al. (2022): acetamiprid/IM-2-1 levels CSF									3

Note: The heatmap includes the endpoint-specific ratings for the RoB for each of the eight questions in the CAT (i.e. number of participants, analyte measured and biological sample used, potential contamination or non-specific binding to the collection tube, sample storage, method's validation, QA/QC, analytical instrumentation, limited of detection/quantification reported). Each question is scored as either low RoB (green), medium RoB (orange) or high RoB (red). The RoB questions highlighted in yellow correspond to the key RoB criteria considered in the final Tier (1 [low RoB], 2 [medium RoB] or 3 [high RoB]) of the endpoint (Q2 analyte measured; Q5 method's validation; Q6 QA/QC; Q7 analytical instrumentation). For detailed rationale for each rating, see Appendix 3.

TABLE 5　Heatmap results of the RoB for the new human observational studies on acetamiprid notified by PAN Europe in the extension of the mandate for acetamiprid.

Human observational studies	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Tier
Yang and Liang (2023): hematologic parameters								1
Mendy and Pinney (2022): testosterone and free androgen index								1

Note: The heatmap includes the endpoint-specific ratings for the RoB for each of the seven questions in the CAT (i.e. selection study participants, study design confounding, attrition, exposure characterisation, outcome assessment, reporting, other: statistics). Each question is scored as either definitely low RoB (dark green), probably low RoB (light green), probably high RoB (light red) or definitely high RoB (dark red). The RoB questions highlighted in yellow correspond to the key RoB criteria considered in the final Tier (1 (low RoB), 2 (medium RoB) or 3 (high RoB)) of the endpoint (Q2 study design confounding; Q4 exposure characterisation; Q5 outcome assessment). For detailed rationale for each rating, see Appendix 3.

Table 6 presents the assessed parameters and the related RoB for external validity, internal validity and precision of the zebrafish study. The data extracted from the zebrafish study and the appraisal of the measured parameters are presented in Appendix 4.

Although the study is overall well reported, all parameters assessed have high risk of bias for internal validity (Table 6). Two main drawbacks were identified in the full life cycle type test (experiment no. 1_1): the high mortality in control animals (25%) and the high mortality across all treatment levels. Regarding the control mortality, although the study was not performed following an available test guideline, control survival in adult zebrafish should normally be 90% (OECD, 229). Based on this, the validity of the study is overall questionable. For all tested concentrations, clear signs of systemic toxicity (mortality > 10%) were observed, and thus, the tested concentrations were likely inappropriate. Moreover, although some effects were observed, those effects are likely due to excessive toxicity rather than triggered by an ED MoA.

In addition to the parameters reported in Table 6, the bioaccumulation potential of acetamiprid was assessed in the different experiments conducted on both adults of the F0 generation and on the F1 generation. The study authors reported that acetamiprid and its metabolites were observed in a dose–response manner in adult fish tissues, with a bioaccumulation factor of 0.65 ± 0.1 L/kg wet weight. For the F1, acetamiprid and metabolite N-desmethyl-acetamiprid

(IM-2-1) were found in the eggs supporting the initial hypothesis of maternal transfer. Additionally, in the Fish Embryo Acute Toxicity test (FET) the metabolism of acetamiprid was reported to be low based on the ratio of IM-2-1 to acetamiprid. Overall, when observed against the criteria and recommendations of related testing guideline on bioaccumulation in fish (i.e. OECD TG 305), some drawbacks limiting its reliability were identified. For instance, the mortality observed in the F0 generation exceeds 10% (as also mentioned in the previous paragraph). While it could be assumed that a steady state was reached after 154 days of exposure in adult fish, some uncertainties remain since the uptake curve was not reported, together with intermediate analysis of acetamiprid in fish and water over the entirety of the exposure period. Intermediate sampling of fish weight measurement should also have been carried out, to allow correction on growth dilution. In addition, the fish lipid content should have been measured so that the bioconcentration factor (BCF) can be expressed based on a 5% lipid content. It was also noted that a depuration phase was not carried out. It should also be considered that the log k_{ow} of acetamiprid is below 3; therefore, the substance is considered having low bioaccumulation potential, which was anyway confirmed by the low BCF obtained by the study authors.

TABLE 6 Heatmap of the parameters assessed in Ma et al. (2022) with zebrafish.

Experiment no.	Assessed endpoint	External validity	Internal validity	Precision
1_1	Survival F0			
1_1	Sex ratio female biased F0			
1_1	Females_Body length F0			
1_1	Females_Body weight F0			
1_1	Females_Condition factor K F0			
1_1	Females_Brain somatic Index F0			
1_1	Females_Hepatosomatic Index F0			
1_1	Females_Gonadosomatic Index F0			
1_1	Males_Body length F0			
1_1	Males_Body weight F0			
1_1	Males_condition factor K F0			
1_1	Males_Brain somatic Index F0			
1_1	Males_Hepatosomatic Index F0			
1_1	Males_Gonadosomatic Index F0			
1_1	Females_progesterone_F0			
1_1	Females_17-hydroxyprogesterone_F0			
1_1	Females_17B-estradiol_F0			
1_1	Females_estriol_F0			
1_1	Females_androstenedione_F0			
1_1	Females_testosterone_F0			
1_1	Males_progesterone_F0			
1_1	Males_17-hydroxyprogesterone_F0			
1_1	Males_estrone_F0			
1_1	Males_17B-estradiol_F0			
1_1	Males_estriol_F0			
1_1	Males_androstenedione_F0			
1_1	Males_testosterone_F0			
1_1	Females_gene expression_Brain_gnrh2 F0			
1_1	Females_gene expression_Brain_gnrh3_F0			
1_1	Females_gene expression_Brain_fshB_F0			
1_1	Females_gene expression_Brain_lhB_F0			
1_1	Females_gene expression_Brain_cyp19b_F0			
1_1	Females_gene expression_Brain_arB_F0			
1_1	Females_gene expression_Gonad_fshr_F0			
1_1	Females_gene expression_Gonad_lhr			
1_1	Females_gene expression_Gonad_cyp11a_F0			
1_1	Females_gene expression_Gonad_3Bhsd_F0			
1_1	Females_gene expression_Gonad_cyp17_F0			
1_1	Females_gene expression_Gonad_cyp19a_F0			

(Continues)

TABLE 6 (Continued)

Experiment no.	Assessed endpoint	External validity	Internal validity	Precision
1_1	Females_gene expression_Liver_vtg1_F0	green	red	red
1_1	Males_gene expression_Brain_gnrh2	green	red	red
1_1	Males_gene expression_Brain_gnrh3_F0	green	red	red
1_1	Males_gene expression_Brain_fshB	green	red	red
1_1	Males_gene expression_Brain_lhB_F0	green	red	red
1_1	Males_gene expression_Brain_cyp19b_F0	green	red	red
1_1	Males_gene expression_Brain_arB_F0	green	red	red
1_1	Males_gene expression_Gonad_fshr_F0	green	red	red
1_1	Males_gene expression_Gonad_lhr_F0	green	red	red
1_1	Males_gene expression_Gonad_cyp11a_F0	green	red	red
1_1	Males_gene expression_Gonad_3Bhsd_F0	green	red	red
1_1	Males_gene expression_Gonad_cyp17_F0	green	red	red
1_1	Males_gene expression_Gonad_cyp19a_F0	green	red	red
1_1	Males_gene expression_Liver_vtg1_F0	green	red	red
1_1	F1_hatchability_continously exposed	yellow	red	green
1_2	F1_hatchability_unexposed	red	red	green
1_1	F1_survival_continously exposed	red	red	green
1_2	F1_survival_unexposed	red	red	green
1_1	F1_malformations_continously exposed	red	red	green
1_2	F1_malformations_unexposed	red	red	green
1_3	FET survival	red	red	green
1_3	FET hatchability	red	red	green
1_3	FET abnormalities	red	red	green

Note: Each question is scored as either definitely low RoB (dark green), probably low RoB (light yellow), or definitely high RoB (dark red).

Relevance assessment

The WG discussed the papers for their possible relevance to the human hazard assessment of acetamiprid. The main points are summarised below per study.

- Li, He, et al. (2022): The WG noted that most lines of evidence were scored as Tier 3 (high RoB), except from the in vitro experiments assessing the effects of acetamiprid on mRNA expression of the GPER (Tier 2). The in vivo investigated endpoints (increase in tumour volume and metastasis of in situ-injected cancer cells) are non-standard endpoints for hazard assessment of active substances and therefore were not considered relevant for the hazard assessment of acetamiprid. The observed in vitro and in vivo effects may be of relevance in the assessment of possible estrogenic activity of acetamiprid mediated via G protein-coupled receptors. However, the lack of an ED assessment for acetamiprid prevents from drawing any conclusion from a single study.
- Mishani et al. (2022): The WG noted that all endpoints were scored as Tier 3 (high RoB); the study cannot be used for hazard characterisation, as exposure levels have not been determined (only concentration in drinking water was reported, but information on consumption of drinking water and related acetamiprid exposure are lacking). Also, in the two acetamiprid exposure groups, reported food consumption was half of the control group, and weight loss was reported within the 60-day exposure period, pointing to general toxicity that biases the study results.
- Didenko et al. (2022): The WG noted that the lines of evidence were scored as Tier 3 (high RoB). Although it was indicated that the study was conducted according to OECD 408 with deviations, providing possible relevant data for the hazard assessment, serious shortcomings in the outcome assessment were identified (scored as high risk of bias), and the study was therefore not further considered. The histopathology procedures are not sufficiently described or not fully adequate, and the histopathological findings (descriptions and figures) are of poor quality and overall indicate artefactual/autolysis findings. No obvious pathological features can be confirmed. The WG also noted that, based on the evaluation of the reported histological findings, the adequateness of organ weight assessment is questioned (e.g. although the brain congestion and oedema reported would contribute to increased brain weight, decreased brain weight was observed). Based on the available pictures of liver histology, it was noted that blood was present in portal and centrilobular veins, as well as in some capillaries, indicating incomplete exsanguination, which may have affected the organ weight findings, especially regarding liver and spleen.
- Li, Si, et al. (2022): The WG noted that the lines of evidence were scored as Tier 3 (high RoB). The relevance of this study is limited as it is a descriptive case series study using a convenience sample. This consisted of patients who experienced neurological symptoms and who underwent a lumbar puncture to obtain CSF. Therefore, results are not generalisable

to the general population. No determinants of exposure other than age and sex are reported. The study only reports on concentrations of acetamiprid and IM-2-1 in CSF, but it did not assess possible associations with health effects. The most important information provided by this study is that neonicotinoids (including acetamiprid and IM-2-1) can be detected in CSF of diseased patients in a hospital setting, but this does not necessarily entail an impairment of the nervous system. As such, the data cannot be used for hazard assessment. As concluded in Section 2.3, an estimation of related external exposure levels based on human biomonitoring data (internal concentrations of acetamiprid and/or IM-2-1) was not deemed feasible, given the lack of sufficient kinetic data to develop a sufficiently robust and reliable PBK model to allow translation of internal exposure data to related estimated external exposure levels.

- Yang and Liang (2023): The WG noted that the lines of evidence were scored as Tier 1 (low RoB). The significant associations found with changes in certain haematological parameters cannot be considered as adverse (i.e. clinically relevant) because there is no indication that these parameters are above or below the normal reference interval. Due to the cross-sectional design, no conclusion about temporality and causality can be drawn between exposure (IM-2-1) and outcome. Furthermore, potential co-exposure to other chemicals affecting haematological parameters was not studied. It is worth noting that only a single spot urine sample was measured, which is not representative of true daily exposure. Furthermore, the detection rate of acetamiprid was < 5.0%, while that for IM-2-1 was 32.6%, which may point to direct exposure to this metabolite, which can be pre-formed in the environment.
- Mendy and Pinney (2022): The WG noted that the lines of evidence were scored as Tier 1 (low RoB). Due to the cross-sectional design of this study, temporality and causality between exposure (IM-2-1) and outcome (reduced testosterone) cannot be established. Only a single spot urine sample was measured, which is not representative of true daily exposure. The accuracy of FAI as a proxy for free testosterone is matter of debate (Vermeulen et al., 1999). The potential contribution of co-exposure to other chemicals affecting serum testosterone levels was not considered. Furthermore, the detection rate of acetamiprid was considered as low, but not reported (between 0.7% and 7.9% for several neonicotinoids), in contrast to the detection rate of IM-2-1 (33.4%), which may suggest possible direct exposure to this metabolite, as IM-2-1 can be pre-formed in the environment. More studies are needed to evaluate the reproducibility of these findings in different populations and using epidemiological designs that allow inferring causality.
- Ma et al. (2022): All parameters assessed have a high risk of bias for internal validity (**Table** 6). Clear signs of systemic toxicity (mortality > 10%) were observed, and thus, the tested concentrations were likely inappropriate. Moreover, although some effects were observed, those effects are likely due to excessive toxicity rather than triggered by an ED MoA.

2.1.3.3 | *Uncertainty analysis and conclusions by the EFSA WG*

Based on the RoB analysis and relevance assessment, the WG performed an uncertainty analysis to conclude whether the new studies notified by PAN Europe in the mandate provide information that would warrant a re-evaluation of the conclusion achieved for acetamiprid for the in vivo evidence.

Regarding the new evidence from the in vitro and in vivo studies, the WG considered that, based on the RoB analysis and relevance assessment, the studies of Li, He, et al. (2022) and Mishani et al. (2022) cannot be used for the hazard assessment. The lines of evidence in Didenko et al. (2022) were considered of relevance for the hazard assessment, but were scored as high risk of bias (Tier 3), so these were not further considered.

Regarding the evidence from the human studies, the WG considered that the study of Li, Si, et al. (2022) cannot be used for the hazard assessment of acetamiprid, as it solely contains information on internal acetamiprid/IM-2-1 concentrations, but not about possible related (health) effects. Regarding the other human studies (Mendy & Pinney, 2022; Yang & Liang, 2023), the WG noted that given the cross-sectional designs, no conclusion about temporality and causality can be drawn between exposure and outcome, and that potential co-exposure to other chemicals was not controlled for. Therefore, the WG considered that these studies cannot be used for the hazard assessment of acetamiprid.

Regarding the fish study by Ma et al. (2022), overall, all the parameters measured in the study have a high RoB for internal validity (see **Table** 6). It was noted that, even if data would be considered reliable, a conclusion related to the study by Ma et al. (2022) should not be generalised to draw a conclusion on the ED properties of acetamiprid for non-target organisms, since an ED assessment in line with the ECHA/EFSA (2018) Guidance has not been performed.

In conclusion and answering problem formulation 2a, based on the EFSA systematic data collection and on the studies notified by PAN Europe for any toxicological endpoint but not DNT for acetamiprid and its metabolites, the current HBGVs for acetamiprid are considered protective. It is, however, noted that in line with the PPR Panel recommendation, an assessment of endocrine-disrupting properties for acetamiprid in line with EFSA/ECHA guidance document for the identification of endocrine disruptors is recommended and was outside the scope of this mandate (EFSA PPR Panel, 2022).

2.1.4 | Assessment for DNT: Integrated approaches to testing and assessment (IATA) for DNT hazard characterisation of acetamiprid active substance

Step 1. Establishment of a protocol with the predefinition of the methods for answering the problem formulation

In line with the summary provided in the methodologies section (see Section 2.1.2), Appendix 1 contains the protocol and the details on the methodology. This was established upfront to reduce data-driven decisions and increase transparency.

Step 2. Data collection and appraisal results

For step 2, during the title and abstract screening, publications providing potentially relevant evidence for DNT were clustered in in vivo (containing in vivo experimental studies in rodents), in vitro (containing in vitro mechanistic studies and behavioural studies conducted in zebrafish embryos up to 120-h post-fertilisation) or human (containing HOS-biomonitoring) studies. After the title and abstract screening, 44 relevant publications remained that underwent a full-text review (Figure 2). Finally, 14 publications were included and classified in three categories: (a) in vitro (8 studies; 5 in vitro and 3 zebrafish studies), (b) human evidence containing biomonitoring data (3 studies) and (c) in vivo (3 studies). Regarding the human studies, it is noted that these do not describe a link between exposure and (health) effects and were only used for the exposure question (refer to Section 2.3). The full list of relevant references from the systematic literature review is presented in Table 7. DNT was thus the only outcome to be considered in further steps in the AOP-informed IATA in order to assess whether the new available evidence for DNT hazard characterisation, including HOS, experimental data from in vivo animal studies and NAMs (in vitro, zebrafish), integrated following an AOP-informed IATA conceptual framework, could warrant a re-evaluation of the current HBGVs for acetamiprid and/or its metabolites in line with the ToR1 and to answer problem formulation 2b.

Appendix 5 shows a summary of the material and methods and data published in all the selected studies.

TABLE 7   Relevant selected studies from the systematic literature review containing evidence of acetamiprid and measuring DNT effects in in vivo and in vitro studies.

RefID*	Authors	Year	Title
219	Loser, D., Hinojosa, M. G., Blum, J., Schaefer, J., Brüll, M., Johansson, Y., Suciu, I., Grillberger, K., Danker, T., Möller, C., Gardner, I., Ecker, G. F., Bennekou, S. H., Forsby, A., Kraushaar, U., Leist, M.	2021	Functional alterations by a subgroup of neonicotinoid pesticides in human dopaminergic neurons
66	Christen, V., Rusconi, M., Crettaz, P., Fent, K.	2017	Developmental neurotoxicity of different pesticides in PC-12 cells in vitro
1603	Lee, J., Escher, B. I., Scholz, S., Schlichting, R.	2022	Inhibition of neurite outgrowth and enhanced effects compared to baseline toxicity in SH-SY5Y cells
173	Kagawa, N., Nagao, T.	2018	Neurodevelopmental toxicity in the mouse neocortex following prenatal exposure to acetamiprid
261	Nakayama, A., Yoshida, M., Kagawa, N., Nagao, T.	2019	The neonicotinoids acetamiprid and imidacloprid impair neurogenesis and alter the microglial profile in the hippocampal dentate gyrus of mouse neonates
317	Sano, K., Isobe, T., Yang, J., Win-Shwe, T. T., Yoshikane, M., Nakayama, S. F., Kawashima, T., Suzuki, G., Hashimoto, S., Nohara, K., Tohyama, C., Maekawa, F.	2016	In utero and lactational exposure to acetamiprid induces abnormalities in socio-sexual and anxiety-related behaviors of male mice
153	Hussain, A., Audira, G., Malhotra, N., Uapipatanakul, B., Chen, J. R., Lai, Y. H., Huang, J. C., Chen, K. H. C., Lai, H. T., Hsiao, C. D.	2020	Multiple screening of pesticides toxicity in zebrafish and daphnia based on locomotor activity alterations
364	Von Hellfeld, R., Ovcharova, V., Bevan, S., Lazaridi, M. A., Bauch, C., Walker, P., Hougaard Bennekou, S., Forsby, A., Braunbeck, T.	2022	Zebrafish embryo neonicotinoid developmental neurotoxicity in the FET test and behavioral assays
225	Ma, X., Li, H., Xiong, J., Mehler, W. T., You, J.	2019	Developmental Toxicity of a Neonicotinoid Insecticide, Acetamiprid to Zebrafish Embryos
277	Öztaş, E., Kara, M., Boran, T., Bişirir, E., Karaman, E. F., Kaptan, E., Özhan, G.	2021	Cellular Stress Pathways Are Linked to Acetamiprid-Induced Apoptosis in SH-SY5Y Neural Cells
58	Cheng, L., Lu, Y., Zhao, Z., Hoogenboom, R. L. A. P., Zhang, Q., Liu, X., Song, W., Guan, S., Song, W., Rao, Q.	2020	Assessing the combined toxicity effects of three neonicotinoid pesticide mixtures on human neuroblastoma SK-N-SH and lepidopteran Sf-9 cells

*RefID is the identification number in Distiller (see Appendix 1).

All the selected papers underwent the RoB appraisal step. The outcome of the RoB is presented in Appendix 6 for in vivo (including in vivo mechanistic endpoints), in vitro and zebrafish lines of evidence. For the selected HOS for biomonitoring, see Section 2.3.

Figure 3 presents an overview of the results of the appraisal by specific endpoint for each Tier (Tier 1 (low RoB), Tier 2 (moderate RoB), Tier 3 (high RoB)) and for each line of evidence.
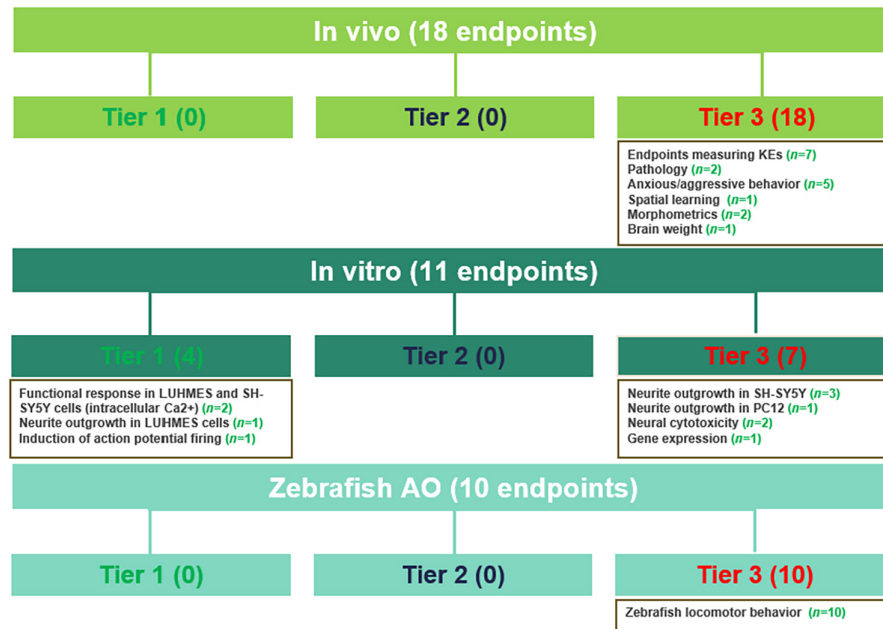
FIGURE 3 Summary of the appraisal of the endpoint categories of the in vivo, in vitro and zebrafish studies selected from the systematic literature review for acetamiprid measuring DNT endpoints.

The body of evidence of in vivo studies

Appendix 5 shows the summary of the materials and methods and results of the selected studies. During the data extraction of the studies selected from the systematic literature review, it was noted that three in vivo studies showed potential DNT effects in rodents. These studies (Kagawa & Nagao, 2018; Nakayama et al., 2019; Sano et al., 2016) underwent a detailed RoB appraisal. Sano et al. (2016) concluded that in utero and lactational exposure to acetamiprid impaired socio-sexual and anxiety-related behaviour in male mice starting from the dose of 1 mg/kg bw per day. Nakayama et al. (2019) concluded that acetamiprid changed the neurogenesis in the hippocampal dentate gyrus and the microglia profile in the hippocampal dentate gyrus at 5 mg/kg bw per day after postnatal exposure; Kagawa and Nagao (2018) concluded that neonatal exposure to 5 mg/kg bw per day resulted in a reduction of hippocampal neurogenesis and increased neuronal apoptosis after prenatal and postnatal exposure. Table 8 shows a summary of the materials and methods and measured endpoints of these studies considered relevant for DNT assessment of acetamiprid.

TABLE 8 Overview of endpoints and study and exposure characteristics of the studies selected providing evidence for key events (KE) and adverse outcomes (AO) of the postulated AOP and considered in the uncertainty analysis (UA) for in vivo evidence.

Publication	Adverse outcome/key event endpoints measured	Study characteristics	Exposure characteristics
Sano et al. (2016)	**KE: Number of AVP neurons in hypothalamus** Number of arginine vasopressin (AVP) immunoreactive neurons in paraventricular nucleus of the hypothalamus **AO: Gross pathology** Brain weight (absolute, relative) **AO: Behaviour** Male sexual behaviour Female sexual behaviour (number of lordosis) Male aggressive behaviour (chasing, boxing, wrestling, biting, tail rattling, lateral attacks) Male anxiety behaviour (light compartment: (a) time spent there, (b) total distance travelled and (c) latency to enter) Female anxiety behaviour Behavioural flexibility test	C57BL/6J mice Developmental neurotoxicity There were no effects on body weight of male and female mice at birth, PND21 or 23–26 weeks of age	0, 1, 10 mg/kg bw per day Daily exposure of dams from GD6-LD21 Acetamiprid 98% purity
Kagawa and Nagao (2018)	**KE: Neurogenesis** NSC (Ki67 + Cidu + IdU) neocortex E14 NSC (Ki67 + Cidu + IdU) neocortex PND14 Microglia profile neocortex **AO: Gross pathology** Brain weight (relative and absolute)	ICR mice Developmental neurotoxicity study There were no effects on body weight	0, 5 mg acetamiprid/kg bw per day with oral gavage to the dams from GD6 to GD13 or GD6 to GD18 Acetamiprid 99.9% purity

(Continues)

TABLE 8   (Continued)

Publication	Adverse outcome/key event endpoints measured	Study characteristics	Exposure characteristics
Nakayama et al. (2019)	**KE: Neurogenesis** NSC Proliferation (CIdU and Ki67) Neuronal count in DG Cell cycle exit of neural stem cells, stained with anti-Ki67 and anti-IdU 24 h after IdU administration at PND27 **KE: Change in microglia profile in the hippocampal Dentate Gyrus** Microglia profile **AO: Gross pathology** Brain weight (absolute, relative)	ICR mice Developmental neurotoxicity study with daily exposure (oral gavage) from PND12-PND26 males and females (12 newborns used from 3 dams)	0, 5 mg acetamiprid/kg bw per day Acetamiprid 99.9% purity

The results of the systematic literature review indicated that DNT AO and/or KE could occur at doses potentially below the current NOAEL for DNT. It was however noted that all new public literature studies measuring DNT endpoints in vivo that have been made available since the renewal of approval of acetamiprid and considered relevant by the EFSA WG (Kagawa & Nagao, 2018; Nakayama et al., 2019; Sano et al., 2016), were appraised as high RoB and considered not reliable due to major limitations in several key questions of the CAT (see **Table 9** for a summary of the appraisal and Appendix 6 for the detailed appraisal of each endpoint measured in the papers).

TABLE 9   Heatmap results of the RoB for in vivo studies from public literature.

DNT endpoints measured and appraised in Sano et al. (2016)	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Tier
Brain weight (absolute, relative)	red	green	green	red	green	red	green	green	green	3
ACE concentration in brain	red	green	green	red	green	red	green	green	green	3
Male sexual behaviour	red	green	green	red	green	red	red	green	green	3
Female sexual behaviour (number of lordosis)	red	green	green	red	green	red	red	green	green	3
Male aggressive behaviour (chasing, boxing, wrestling, biting, tail rattling, lateral attacks)	red	green	green	red	green	red	red	green	green	3
Male anxiety behaviour (light compartment: (a) time spent there, (b) total distance travelled and (c) latency to enter)	red	green	green	red	green	red	red	green	green	3
Female anxiety behaviour	red	green	green	red	green	red	red	green	green	3
Number of arginin vasopressin (AVP) immunoreactive neurons in paraventricular nucleus of the hypothalamus	red	green	green	red	green	red	red	green	green	3
Behavioural flexibility test	red	green	green	red	green	red	green	green	green	3

DNT endpoints measured and appraised in Kagawa and Nagao (2018)	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Tier
NSC (Ki67+Cidu+IdU) neocortex E14	red	green	green	red	green	red	green	green	green	3
NSC (Ki67+Cidu+IdU) neocortex PND14	red	green	green	red	green	red	green	green	green	3
Microglia profile neocortex	red	green	green	red	green	red	green	green	green	3
Brain weight (relative and absolute)	red	green	green	red	green	red	green	green	green	3

DNT endpoints measured and appraised in Nakayama et al. (2019)	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Tier
NSC Proliferation (CidU and Ki67)	red	green	green	green	green	red	green	green	green	3
Neuronal count in DG	red	green	green	red	green	red	red	green	green	3
Microglia profile	red	green	green	green	green	red	green	green	green	3
Brain weight (absolute, relative)	red	green	green	green	green	red	green	green	green	3
Cell cycle exit of neural stem cells, stained with anti-Ki67 and anti-IdU 24 h after IdU administration at PND27	red	green	green	green	green	red	red	green	green	3

Note: The heatmap includes the endpoint-specific ratings for the RoB for each of the nine questions in the CAT (i.e. domains of randomisation, allocation concealment, experiment conditions, research personnel blinded, attrition, exposure characterisation, outcome assessment, outcome reporting and other aspects, i.e. systemic or maternal toxicity). Each question is scored as either definitely low RoB (dark green), probably low RoB (light green), probably high RoB (light red) or definitely high RoB (dark red). The RoB questions highlighted in yellow correspond to the key RoB criteria considered in the final Tier (1 [low RoB], 2 [medium RoB] or 3 [high RoB]) of the endpoint (Q1 randomisation domain; Q6 exposure characterisation; Q7 outcome assessment method). For detailed rationale for each rating, see Appendicies 1 and 6.

Following the IATA framework, which requires all evidence to undergo the same appraisal and uncertainty analysis process for the WoE, an expert uncertainty analysis was conducted for all endpoints measured in the OPPTS 870.6300 regulatory study, as well as in the three in vivo studies selected from public literature. The OPPTS 870.6300 regulatory study was conducted during 1999–2000 and thus not following the current OECD 426 Test Guideline. The study was previously classified as acceptable/non-guideline (Netherlands, 2016; Table 10). It was also noted that the original report lacked methodological details and further amendments were made available providing more methodological details (Netherlands, 2016). Several reporting issues were also noted while evaluating the different reports submitted as part of the revised renewal assessment report (RAR) on acetamiprid (see this section 'Step 5. Data integration in the AOP framework and final uncertainty analysis').

Results of the appraisal are presented in Appendix 6 and a summary by endpoint in **Table 11**.

In addition, the WG conducted a thorough uncertainty analysis in line with the analysis done by EFSA and ECHA for the ECHA report of the Extended One-Generation Reproductive Toxicity Study (EOGRTS) review project (ECHA, 2023). This evaluation included the analysis of the proficiency of the laboratory of the study (e.g. HCD, PCD, dynamic range for each endpoint, statistical analysis), the reporting, including information on the equipment used, the control of extraneous experimental factors, the time of day when testing is performed, as well as recurring methodological inadequacies of these studies. This was considered necessary after the appraisal exercise because of the consolidated experience gained by EFSA on assessing these studies. This detailed assessment is provided in Appendix 7 and thoroughly discussed in the uncertainty analysis in this section ('Step 5. Data integration in the AOP framework and final uncertainty analysis').

TABLE 10 Overview of the endpoints and study and exposure characteristics of the OPPTS 870.6300 regulatory study included in the revised RAR on acetamiprid (Netherlands, 2016).

Adverse outcome/endpoints measured	Study characteristics	Exposure characteristics
AO: Functional observational battery: Detailed clinical observations in pups (i.e. ease of removal from cage and easy to handle animals; but the measurement of FOB (functional observational battery) manipulative endpoints is lacking) **AO: Motor activity** Motor activity in males and females **AO: Startle reflex** Auditory startle response in males and females **AO: Learning and memory** Learning and memory in males and females **AO: Gross pathology** Brain weight measured at PND11 and PND72 **AO: Morphometrics** Morphometrics in males and females and brain size Histopathology in males and females	Sprague–Dawley Rat Crl:CD®(SD)IGS BR Developmental neurotoxicity study with oral gavage to dams from GD6-LD21 Maternal toxicity was observed only at 45 mg/kg bw per day Mean body weight on the day of vaginal patency was statistically significantly reduced in the 45 mg/kg bw per day group	0, 2.5, 10, 45 mg/kg bw per day by oral gavage to dams from GD6-LD21 Acetamiprid > 99% purity

TABLE 11 Heatmap results of the RoB for the endpoints measured in the regulatory OPPTS 870.6300 study.

DNT endpoints measured:	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Tier
Detailed clinical observations in pups (i.e. ease of removal from cage and easy to handle animals; but the measurement of FOB manipulative endpoints is lacking)	dark red	light green	dark green	dark green	dark green	dark green	light green	dark green	dark green	3
Motor activity in males and females (PND 13, PND 17, PND 21, PND 61)	dark red	light green	dark green	dark green	dark green	dark green	dark red	dark green	dark green	3
Auditory startle response in males and females (PND 20, PND 60)	dark red	light green	dark green	dark green	dark green	dark green	light green	dark green	dark green	3
Learning and memory in males and females (PND 22 and PND 62)	dark red	light green	dark green	dark red	dark green	dark green	dark red	dark green	dark green	3
Brain weight measured at PND11 and PND72	dark red	light green	dark green	light green	dark green	dark green	light green	light green	dark green	3
Morphometrics in males and females and brain size (PND 11 and PND 72)	dark red	light green	dark green	dark red	dark green	dark green	dark red	dark green	dark green	3
Histopathology in males and females (PND 11 and PND 72)	dark red	light green	dark green	dark green	dark green	dark green	dark red	light green	dark green	3
Gross pathology in males and females (PND 11 and PND 72)	dark red	light green	dark green	dark green	dark green	dark green	dark green	dark green	dark green	3

Note: The heatmap includes the endpoint-specific ratings for the risk of bias for each of the nine questions in the CAT (i.e. domains of randomisation, allocation concealment, experiment conditions, research personnel blinded, attrition, exposure characterisation, outcome assessment, outcome reporting and other aspects – i.e. systemic or maternal toxicity). Each question is scored as either definitely low RoB (dark green), probably low RoB (light green), probably high RoB (light red) or definitely high RoB (dark red). The RoB questions highlighted in yellow (Q1 randomisation domain; Q6 exposure characterisation; Q7 outcome assessment method) corresponds to the key RoB criteria considered in the final Tier (1 [low RoB], 2 [medium RoB] or 3 [high RoB]) of the endpoint. For detailed rationale for each rating, see Appendicies 1 and 7.

In the study, a decreased maximum auditory startle response was observed in males from the dose of 10 mg/kg bw per day onwards at PND 20 and PND 60 (see **Table 12** for further details), attaining statistical significance in the 45 mg/kg bw per day group on both days ($p < 0.05$). The average response amplitudes (V_{ave}) in these males were also statistically significantly reduced compared to the control group values at PND 20 and 60, while latencies to maximum response amplitude (T_{max}) were similar to those in the control group at PND 20 and 60. On PND 20, V_{max} and V_{ave} were significantly reduced in females in the 45 mg/kg bw per day group in comparison to the control group values, while Tmax was significantly increased in comparison to the control group at this time point. The decreased maximum auditory startle response in males has been the basis for the NOAEL/LOAEL setting of the study by the peer review meeting (EFSA, 2016a; Netherlands, 2016).

TABLE 12 V_{max} effect as percentage of control in the regulatory OPPTS 870.6300 study.

V_{max} as percent control					
Sex	**Age**	**0**	**2.5**	**10**	**45**
Male	**20**	100	85	73	58
	60	100	90	60	47
Female	**20**	100	101	89	71
	60	100	100	102	102

Note: An LOAEL has been set at 10 mg/kg bw per day based on decrease in maximum auditory startle response in males from the dose of 10 mg/kg bw per day onwards at PND20 and PND60.

The body of evidence of in vitro studies

From the systematic literature review, eight studies were selected as relevant: five were conducted in vitro and three in zebrafish embryos. Two in vitro studies were not evaluated in detail (Cheng et al., 2020; Öztaş et al., 2021), due to the lack of relevance of the test systems as identified during the study appraisal. For more details on the studies, see Appendix 5.

Table 13 shows information on the methods regarding measured endpoint, test system, exposure and RoB of the studies providing evidence for the MIE, KEs and AO.

TABLE 13 Summary of endpoints measured, test systems used and exposure characteristics applied in the selected in vitro studies with the related RoB scores.

Publication	Endpoint measured/KE	Test system	Exposure characteristics (& significant effect concentrations indicated by*)	RoB
Loser et al. (2021)	**KE: Activation of calcium influx** Measurements of intracellular Ca^{2+} (preincubation with Cal-520 AM) as the mean of fluorescence signal of each well in 384 well plate (high throughput) or the single cell level	LUHMES (human DA mesencephalic neurons): 48 h pre-differentiated for Ca^{2+}–imaging	0.1, 1.0, 10* and 100* µM acetamiprid purity > 99% up to 8 min	1
Loser et al. (2021)	**KE: Activation of calcium influx** Electrophysiology measured using manual patch clamp	LUHMES (human DA mesencephalic neurons): 48 h pre-differentiated and another 7–8 days for patch clamp recording	100 µM* acetamiprid purity > 99% 5 s	1
Loser et al. (2021)	**KE: Activation of calcium influx** Measurements of intracellular Ca^{2+} using Fura-2AM after 72 h of differentiation	SH-SY5Y cell line (human neuroblastoma, differentiated during 3DIV in the presence of RA, passage 50–70)	0.1, 1.0, 10* and 100* µM acetamiprid purity > 99% 150 s	1
Christen et al. (2017)	**KE: Neurite outgrowth** Neurite outgrowth	PC12 (rat adrenal cancer) cell line differentiated towards neuronal phenotype with nerve growth factor (NGF) for 5 days	1, 10 and 100 µM of acetamiprid purity > 99%	3
Christen et al. (2017)	**KE: Neurite outgrowth** Gene expression linked to altered neurite outgrowth	PC12 (rat adrenal cancer) cell line differentiated towards neuronal phenotype with NGF for 5 days	10* and 100* µM of acetamiprid purity > 99%	3

TABLE 13 (Continued)

Publication	Endpoint measured/KE	Test system	Exposure characteristics (& significant effect concentrations indicated by*	RoB
Lee et al. (2022)	**KE: Neurite outgrowth** Neurite outgrowth Cytotoxicity	Human neuroblastoma SH-SY5Y differentiation with RA for 72 h	620, 1500 and 2600 µM of acetamiprid purity unknown 24 h	3
Hussain et al. (2020)	**AO: Locomotor behaviour** Locomotor activity in zebrafish (travelled distance during the light cycle, total travelled distance during the dark cycle, pattern of locomotor activity, burst activity)	Zebrafish AB strain	1 ppb acetamiprid in water purity ≥ 98% 24 h	3
Ma et al. (2019)	**AO: Locomotor behaviour** Locomotor activity in zebrafish (spontaneous movement, head and tail touch response)	Zebrafish AB strain	107, 537, 760 and 974 mg/L acetamiprid purity 98.1% 120 h	3
Von Hellfeld et al. (2022)	**AO: Locomotor behaviour** Locomotor activity in zebrafish (coiling assay (21–47 hpf), swimming assay (83–120 hpf))	Zebrafish West aquarium strain	6.25; 12.5; 25; 50 and 100 µM acetamiprid purity unknown 120 h	3

From the in vitro BoE, only the study from Loser et al. (2021) was appraised as low RoB. This study tested whether acetamiprid and other neonicotinoids induced changes of the free intracellular Ca^{2+} concentration ([Ca^{2+}]i) in two different test systems, SH-SY5Y human neuroblastoma cells and human dopaminergic post-mitotic neurons generated from LUHMES neuronal precursor cells. Both test systems were characterised for the presence and function of nAChR subunits including a7, a4 and b2 at different days in vitro (DIV). Nicotine, applied as a positive control, triggered typical neuronal signalling responses that were blocked by nAChR antagonists, such as tubocurarine and mecamylamine. Acetamiprid induced Ca^{2+} influx in neuronal cells mediated by the activation of nAChRs subunits (including a7 and a4b2) at 10–100 µM. This effect was strongly potentiated by PTU (an allosteric modulator of a7 nAChR subunit). The presence of functional nAChR subunits was confirmed by using a voltage clamp technique. Similar effects were observed with other neonicotinoids (i.e. imidacloprid, clothianidin and thiacloprid) but not with thiamethoxam and dinotefuran. It was also observed that pretreatment of LUHMES and SH-SY5Y cells with active neonicotinoids (at 1–10 µM) blunted the signalling response of nicotine causing cross-desensitisation starting from 1 µM. These data show that human neuronal cells are functionally affected by low micromolar concentrations of several neonicotinoids resulting in potential toxicity to human brain during development. These results are in line with earlier findings obtained with other mammalian cells, i.e. primary rat brain cultures (Kimura-Kuroda et al., 2012), which provided evidence for potential DNT effects caused by acetamiprid and imidacloprid. However, follow-up studies are required to judge the full toxicological implication for human brain development (i.e. downstream neurofunctional adverse effects).

Step 3. Data analysis of the developmental neurotoxicity in vitro battery (DNT IVB) results for acetamiprid

Recently, the OECD published the Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In Vitro Testing Battery (DNT IVB; OECD, 2023). The document contains the descriptive standardisation of a battery of 17 assays and recommendations on the use of data in the context of hazard assessment or weight of evidence determination. The assays included are not based on molecular targets, as no comprehensive list of targets is currently known, but instead on fundamental neurodevelopmental processes known to be necessary for proper nervous system development, as reported in Figure 4. The OECD document describes the assays that comprise the battery in terms of neurodevelopmental processes and provides criteria for the evaluation of the relevance of the data to DNT and assists in the determination of the degree of certainty in any positive or negative findings to better inform the use of DNT in vitro data in regulatory hazard assessments (OECD, 2023). It is noted that at the time of publishing this Statement, the OECD project on DNT is still ongoing with further activities to test more chemicals, develop more assays, validate assays already available (e.g. inter-laboratory testing of all assays using defined lists of positive and negative compounds), develop tiered testing strategies as follow-up of the DNT IVB outcomes (e.g. orthogonal assays to confirm positives or negatives or mechanistic testing in rodent assays) and propose more case studies (OECD, 2023).
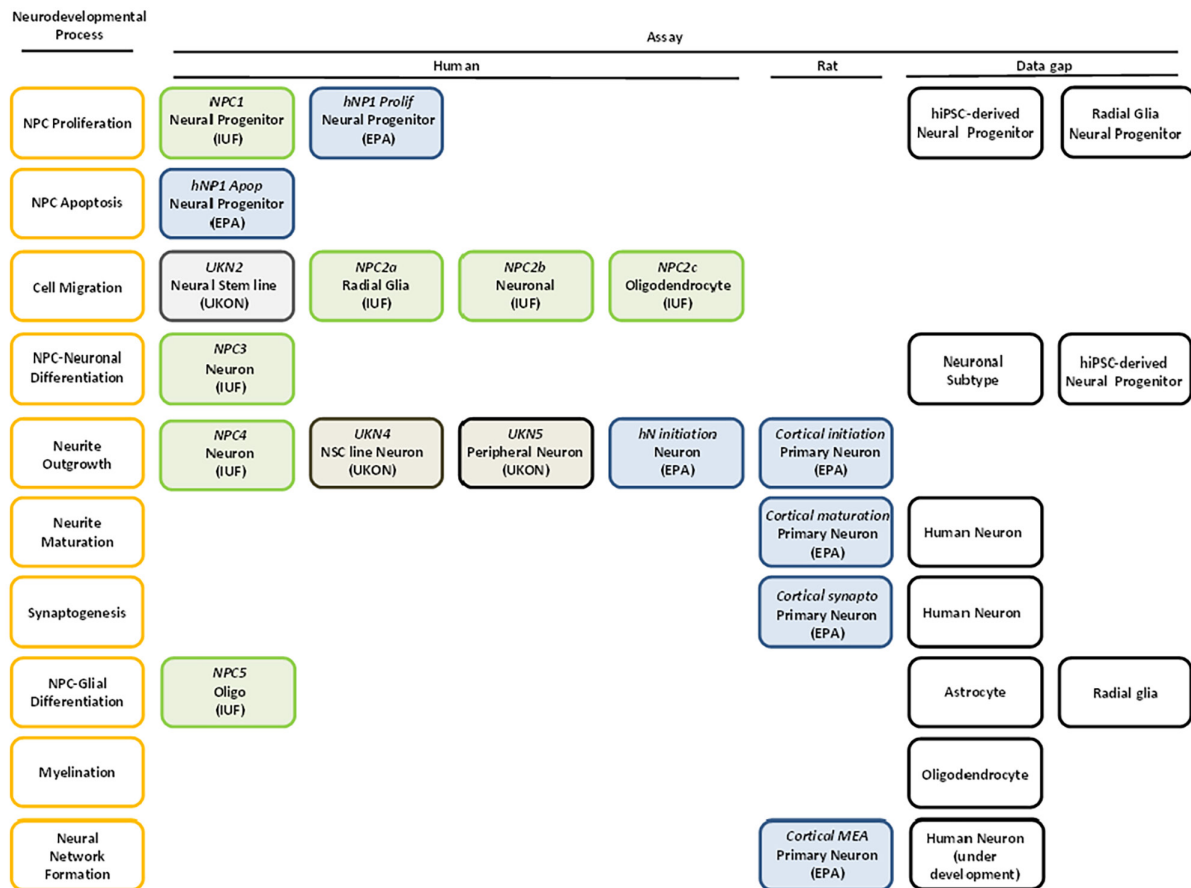
FIGURE 4   Assays in the current DNT IVB and assays identified as high priority for development (from OECD, 2023). Assays are grouped according to the neurodevelopmental process evaluated (rows) and test system used (columns). Each assay box lists the abbreviated assay name, cell type and assay development laboratory. Assays that need further development, and not included in the current DNT IVB, are identified as data gaps. Each assay is represented as a box that lists the test method name (italics), the test system (cell type used) and the home institution of the developer (IUF, Leibniz Research Institute for Environmental medicine – green; UKON, University of Konstanz - grey; EPA, US Environmental Protection Agency – blue). Other abbreviations can be found in the abbreviations list.

As indicated in the OECD Initial recommendations document Appendix E, acetamiprid has been tested in all assays included in the DNT IVB (i.e. 17 assays as described in Figure 3 of that document) and data are reported in the EFSA report (Masjosthusmann et al., 2020) and can be retrieved from the ToXCast dashboard (https://comptox.epa.gov/dashboard/).

Figures 5, 6 and 7 show the heatmap results for the DNT IVB assays of acetamiprid. The WG extracted the data of the DNT IVB for acetamiprid, nicotine and other neonicotinoids. Results for other neonicotinoids and nicotine are also shown in the figures. A detailed uncertainty analysis was performed for acetamiprid and in part for nicotine, while data for the other neonicotinoids were mainly used for comparison purposes in the uncertainty analysis and the results are presented in this section. A detailed description of the assays is reported in the OECD initial recommendation document (OECD, 2023). Figure 5 shows the heatmap of acetamiprid, nicotine and other neonicotinoids in the US EPA DNT IVB Neural Network Formation (NNF) assay (extracted from invitrodb v3.5 in June, 2023) (Shafer et al., 2019; Harrill et al., 2010; OECD, 2023). Figure 6 shows the heatmap of acetamiprid, nicotine and other neonicotinoids in the US EPA DNT High Content Image (HCI) cellular event assays for Assessing Chemical Effects on Neurodevelopment Processes (i.e. proliferation in neural progenitor cells hNP1; apoptosis in neural progenitor cells hNP1; neurite initiation in human neurons; neurite initiation cortical rat primary neurons; neurite maturation in rat primary neural culture; synaptogenesis in rat primary neural culture) (extracted from invitrodb v3.5 in June, 2023; OECD, 2023). Figure 7 shows the heatmap of acetamiprid, nicotine and other neonicotinoids in the EU DNT IVB (extracted from Blum et al., 2023). The EU DNT IVB detailed methodological details are fully reported in OECD (2023) and EFSA PPR Panel (2021).
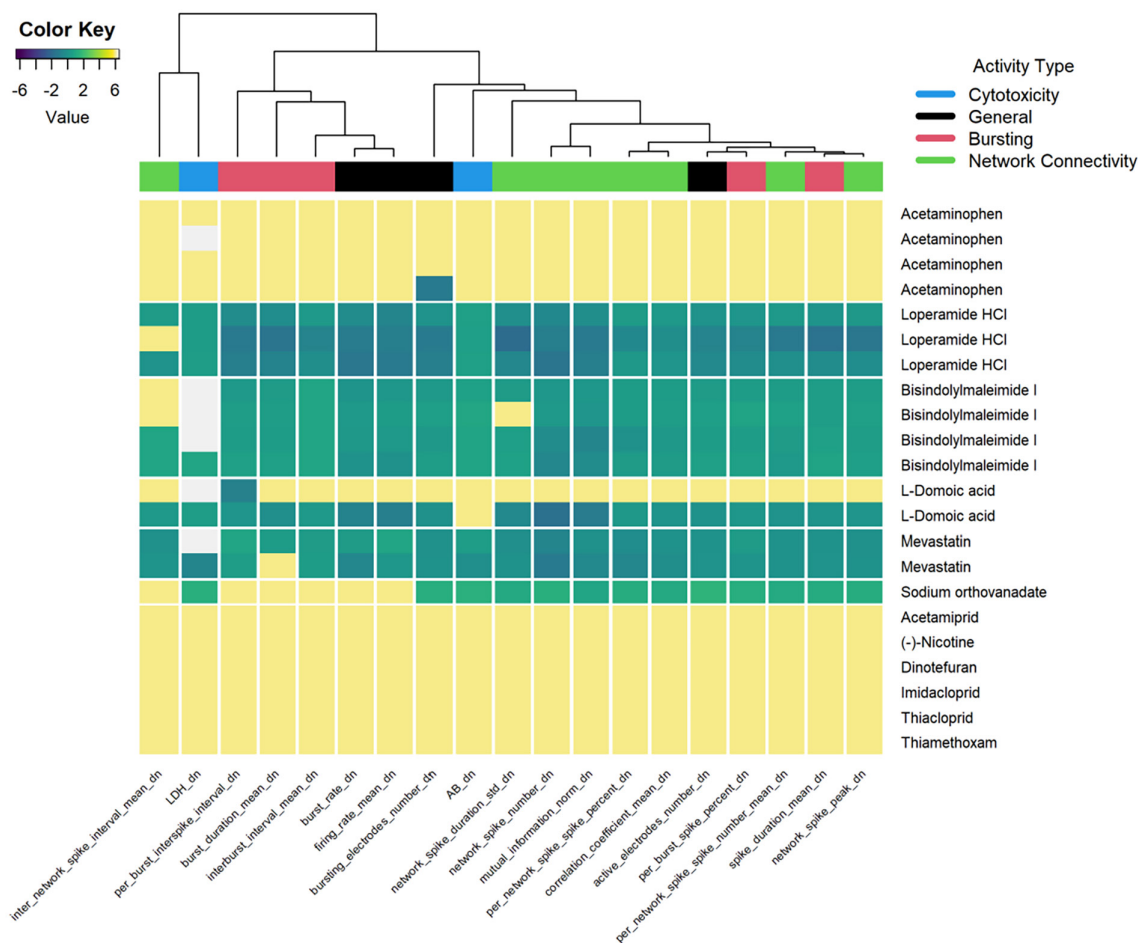
FIGURE 5 Heatmap of acetamiprid, nicotine and other neonicotinoids in the US EPA DNT IVB MEA (NNF) assay, including positive and negative performance controls. Data extracted from ToxCast (invitrodb v3.5 in June, 2023). The following filters applied: ≥ 3 ToxCast flags capturing curve-fitting behaviour such as noisy data; curve-fits with a model top less ≤ 1.2 times the cut-off for a positive and a resultant AC50 lower than the concentration range screened; concentrations series with fewer than four concentrations; excluded MEA NFA '_up' endpoints as not validated by positive performance controls.
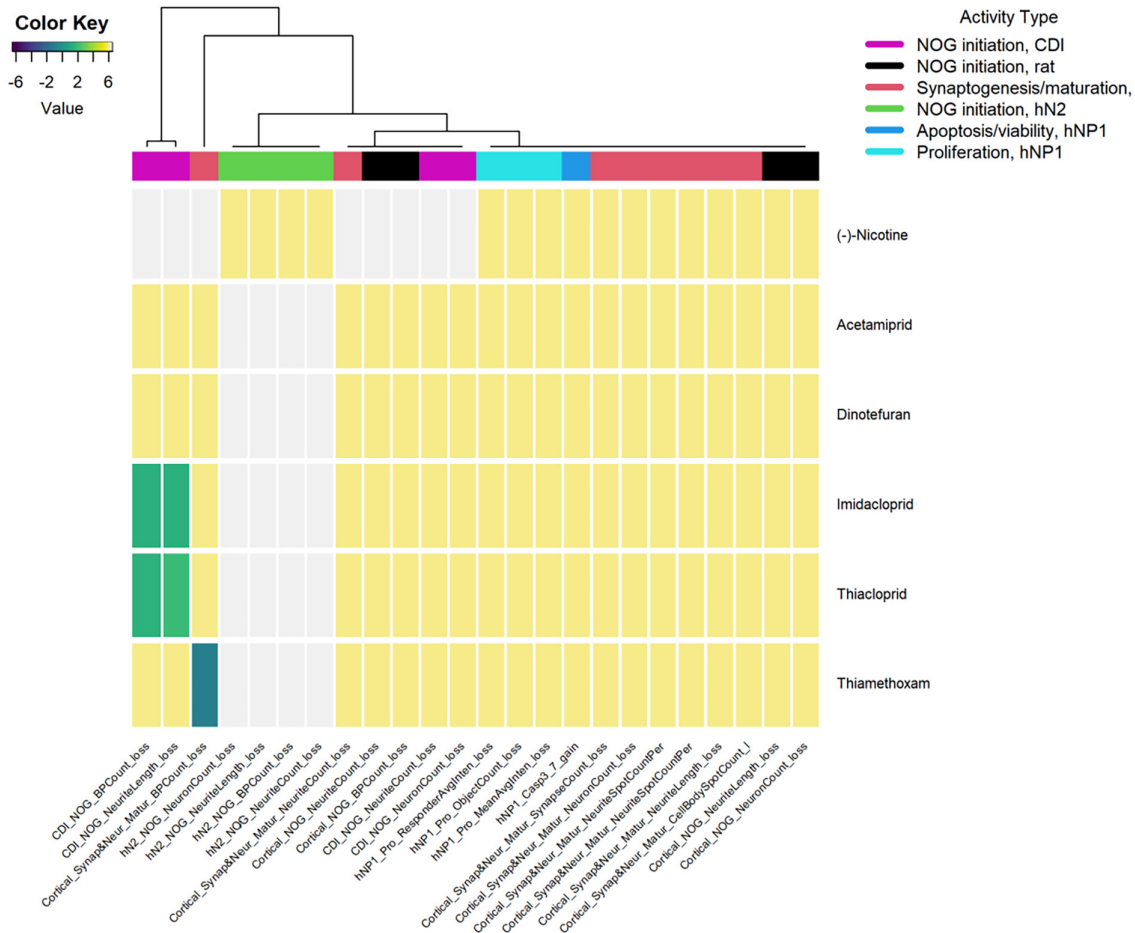
FIGURE 6 Heatmap of acetamiprid, nicotine and other neonicotinoids in the US EPA DNT high content image cellular event assays for assessing chemical effects on neurodevelopment processes. Data extracted from ToXCast (invitrodb v3.5 in June, 2023). Assays included proliferation in neural progenitor cells hNP1; apoptosis in neural progenitor cells hNP1; neurite initiation in human neurons; neurite initiation cortical rat primary neurons; neurite maturation in rat primary neural culture; synaptogenesis in rat primary neural culture.

FIGURE 7 Heatmap of acetamiprid, nicotine and other neonicotinoids in the EU DNT IVB. Data extracted from Blum et al. (2023) and EFSA PPR Panel (2021).

As indicated in Figures 5, 6 and 7, acetamiprid is inactive across the DNT IVB.

The WG, in the context of this IATA case, carefully considered the negative outcome, recognising the limitations of the DNT IVB listed in the OECD initial recommendation document, e.g. lack of coverage for some neurodevelopment processes, and the limited characterisation of some of the test systems for the expression and ontogeny of the nACh receptor subunits (OECD, 2023) (see also Figure 4).

Therefore, the WG retrieved gene expression data for the following nAChR subunits, which were analysed in some of the in vitro models (not all) included in the DNT IVB: nAChRa2, nAChRa3, nAChRa4, nAChRa6, nAChRa7, nAChRa8, nAChRa9, nAChRa10, nAChRb2, nAChRb3 and nAChRb4 and classified as present, borderline and absent according to the criteria described in Masjosthusmann et al. (2020). This exercise was part of the uncertainty analysis for the relevance of the DNT IVB for assessing DNT effects of acetamiprid. The WG concluded that:

- Human fetal neural progenitor cells (hNPC) showed borderline expression of nAChRb2 and the remaining subunits was absent. The NPC transcriptomic profile during neural differentiation was analysed using PrimeView Arrays (Affymetrix). In the neural crest cells differentiated from hiPSCs (IMR90), the absence of all nAChR subunits was confirmed, except for

nAChRa8, which was not measured.

- LUHMES cells (post-mitotic mesencephalic DA neurons, CNS) showed the presence of nAChRa3, nAChRb2, nAChRb4, borderline expression of nAChRa4 and nAChRa9. The presence of nAChRa8 subunit has not been measured.
- hiPSC-derived immature dorsal root ganglia (DRG) expressed nAChRa3, nAChRa4, nAChRb2, nAChRb4 and presence of nAChRa8 subunit has not been measured.
- Human iPSC-derived neurons (SynFire, used in the neuronal network formation (hNNF) assay) expressed nAChRa3, nAChRa4, nAChRb2, nAChRb4, borderline presence of nAChRa6 and nAChRa7. nAChRa8 subunit expression has not been measured.

The gene expression of the nAChR subunits has not been analysed yet in the following in vitro models of the DNT IVB:

- hNP1 Human Progenitors fully differentiated, derived as adherent cells from hESC WA09 line to evaluate cell viability, apoptosis and high-content imaging assay to screen for changes in neuron proliferation (DNT IVB US EPA).
- hiPSC-derived iCell GlutaNeurons (FujiFilm Cellular Dynamics, USA) for high-content imaging assay to screen for changes in neurite outgrowth.
- Rat primary cortical neurons differentiated for 12–14 days in vitro applied for evaluation of neuronal network formation (rNNF), neurite maturation, synaptogenesis, viability and high-content imaging assay to screen for changes in neurite outgrowth (US EPA). However, published data suggest that primary rat cells from brain cortex, in vitro express α4β2-nAChRs and α7-nAChRs as measured for instance by the spontaneous $[Ca^{2+}]i$ oscillations and nicotine-induced $[Ca^{2+}]i$ oscillations (Wang et al., 2016). Earlier studies also suggest that nAChR-binding sites are present in rat primary neuronal cells in culture (Dávila-García et al., 1999).

It is noted that in vitro models (preferably based on human cells) with an advanced stage of neuronal differentiation and functional nAChR subunits are necessary to produce relevant in vitro data for the assessment of the neurodevelopmental toxicity of acetamiprid. The negative results of the testing of acetamiprid in the DNT IVB are considered uncertain due to the lack of relevant models with advanced stage of neuronal differentiation and functional nAChR subunits. Before chemical testing, each model should be characterised for the presence of different neuronal and glial cell types, expression of critical receptors, neurotransmitters, enzymes, pathways/mechanisms, etc., that are relevant for the specific class of tested chemicals. Such a model characterisation will reduce uncertainty and will facilitate the interpretation of the in vitro data relevant for hazard assessment.

In view of the identified limitations of the DNT IVB, the negative results obtained with acetamiprid are associated with high uncertainty and it cannot be excluded that acetamiprid is a false-negative substance when tested in the DNT IVB.

Step 4. Data analysis of the EUTOXRisk acetamiprid IATA case study number 365

The EUToxRisk IATA case study has been published in the OECD Case Study program as Case Study no. 365 (OECD, 2022c) with the following problem formulation: Can new approach methods (NAM) data in an IATA context (integrating existing information) on acetamiprid sufficiently characterise DNT hazard? The EUTOXRisk case study postulated an AOP and concluded as follows:

'The available in vivo *data does not corroborate with each other, and no consistent effects have been established. This is based on the Scientific Opinion on the developmental neurotoxicity potential of acetamiprid and imidacloprid* (EFSA PPR Panel, 2013) *conclusion that there were uncertainties in the OPPTS 870.6300 regulatory study on acetamiprid that prevented a firm conclusion regarding motor activity and learning and memory, whereas decreased auditory startle response was found at 10 and 45 mg/ kg bw per day. The mechanistic data reported by* Kimura-Kuroda et al. *(2012) did provide some mechanistic understanding, but the study had limitations. Subsequent* in vivo *DNT studies in mice found that acetamiprid might affect neurogenesis* (Kagawa & Nagao, 2018; Nakayama et al., 2019) *and reduced anxiety-related behavior in males. Regarding the* in vitro *evidence, the OECD IATA Cs 355 concluded that acetamiprid induces Ca^{2+} influx via the nAChR as well as attenuated voltage-operated calcium channel (VOCC) function after exposure at concentrations between 1 and 100 μM. Functional endpoints in terms of neurite outgrowth have been investigated but no statically significant effects were seen, yet a reduced dendritic area was observed in rat Purkinje cells. Two of the studies reviewed performed transcriptomics after exposure and observed differentially expressed genes (DEGs) relevant for neuronal development. However, some of the studies were scored as of high RoB because only one concentration was studied, less relevant mammalian models were used, or they did not assess cytotoxicity.*

Further testing in the DNT IVB and additional systems/endpoint firmly established that neither acetamiprid nor nicotine affects these endpoints, nor do they exert agonism or antagonism on various nuclear receptors, PPARa and AhR, or activated stress pathways in the CALUX reporter gene assay. However, it is established that acetamiprid has similar, but less potent, activity than nicotine on nAChR-mediated Ca^{2+} influx in neuronal systems. There remain significant uncertainties regarding downstream KEs, including a specific adverse outcome. No effects were observed in the proposed KE3 and KE4 with the assays used after direct exposure with acetamiprid or nicotine, but the possibility cannot be ignored that if the nAChR remains bound by an agonist a conformational change of the receptor leading to a nonfunctional state (termed desensitization) may alter evoked responses, e.g., voltage operated ion channel function, transmitter release or neural network function. This major uncertainty is currently undergoing further investigations. As a first attempt, analysis of DEGs in test systems where acetamiprid and nicotine are active

showed no major effects for any of the systems. Thus, KE3 cannot be confirmed using transcriptomics. Another possibility would be investigating activation/inactivation of signaling pathways as exemplified above.'

Despite some of the data considered are also in the EFSA IATA BoE, a different approach was used by the WG to retrieve, appraise and weight the evidence. More importantly, a different problem formulation and regulatory framework was used. The WG therefore recommends EFSA to update the OECD case study no. 365 or to adapt this statement to an AOP-informed IATA and to submit it to the OECD case study project.

EFSA has evaluated the data generated and reported in the EUTOX Risk IATA as part of the BoE but notes that an independent analysis of the evidence, including a critical appraisal, would have been necessary for using the data in the regulatory decision-making process of acetamiprid.

Step 5. Data integration in the AOP framework and final uncertainty analysis

The evidence was clustered hierarchically by evidence streams first (in vivo, in vitro, zebrafish), then by publication, then by endpoint categories and finally integrated in an AOP framework as potential AOs, KEs and MIEs. Two experts of the WG were independently asked to identify the relevant DNT endpoints in the BoE and classify them as potential MIEs/KEs or AOs, taking into account the previous work in the postulated AOP of the OECD IATA Case Study no. 365. Then, the results of this exercise were discussed in a WG meeting. The postulated AOP from EUTOXRisk was used as a framework to organise the evidence retrieved and appraised independently by EFSA.

The study results are displayed together with the main experiment characteristics and AO/KEs identification in Appendix 5.

Data systematically collected and appraised, as reported in the previous sections, were integrated using the AOP conceptual framework and uncertainty analysis, in two steps. First, synthesis, integration and uncertainty analysis of the evidence on AO were performed from experimental in vivo and zebrafish studies. All endpoints measured in each study were included in the data synthesis, regardless of the Tier allocation in the appraisal. Second, synthesis, integration and uncertainty analysis of mechanistic evidence on MIE and KEs were performed from in vitro and in vivo mechanistic studies. All endpoints measured in the studies were included in the data synthesis, regardless of the Tier of the appraisal. To conduct fit for purpose hazard identification and characterisation, only endpoints falling in Tiers 1 or 2 of internal validity were used in the final uncertainty analysis for drawing conclusions on a DNT effect of acetamiprid and included in the postulated AOP. Tier 3 endpoints were not used in the uncertainty analysis due to the high risk of bias (see Appendix 5 with the data extraction summary data). The uncertainty analysis and the expert knowledge elicitation (EKE) methodologies are reported in Appendix 1 and were performed in line with EFSA Guidance on uncertainty analysis (EFSA and EBTC, 2018; EFSA Scientific Committee, 2018a, 2018b). It is noted that a similar approach and workflow was applied in the AOP-informed IATA for deltamethrin DNT hazard characterisation and this stepwise approach culminated in the development of an evidence-based stressor-based AOP (EFSA PPR Panel, 2021; OECD, case study 362 [OECD, 2022a]).

Outcome of the EKE for the BoE of in vivo studies

In order to conclude on the AO, in line with the protocol part 2 (see Appendix 1), the evidence retrieved for the AO was assessed for reliability and relevance. An uncertainty analysis was conducted using an EKE exercise in a WG meeting. For AO, seven experts participated in answering the questions (see Table 1) and conducted the exercise in an independent manner. Unanimously, all experts concluded that there are major uncertainties in the in vivo BoE available to identify and characterise the DNT potential of acetamiprid. The following main uncertainties were noted during the EKE discussions.

- Since the renewal of approval of acetamiprid active substance, as indicated in the results of the systematic literature review, new in vivo *DNT* studies became available (in particular Sano et al., 2016; Kagawa & Nagao, 2018; and Nakayama et al., 2019; see Table 7). The experts appraised these studies as of high RoB (Tier 3), reflecting major limitations in several key questions of the CAT (see Appendix 5 and Section 2.1.4.5). In accordance with the protocol, these endpoints were not included in the postulated AOP due to the high RoB.
- Regarding the DNT regulatory study (███████████), evaluated in Netherlands, 2016, all measured endpoints were also appraised using the CAT and were all assigned to Tier 3 (high RoB). This was because at least one critical question was categorised as of high RoB or probably high RoB. Table 14 shows a summary of the uncertainties in the regulatory study, identified by the WG. However, the WG considered the study as acceptable for the auditory startle response and histopathology evaluation, as the only issue identified in the RoB appraisal for these endpoints was the lack of reporting for one aspect of randomisation and the study was conducted following good laboratory practices (GLP). The experts considered the lack of reporting of randomisation as not sufficient to dismiss the positive findings in the acute startle response from 10 mg/kg bw per day onwards, which showed a dose–response pattern and reproducibility over time (decrease was observed in the measurements at PND 20 and PND60 in males, see 'Step 2. Data collection and appraisal results' of this section). In addition, the WG noted that control values are in line with the historical control data (HCD) provided for this endpoint. The WG also noted that the variability in the HCD was limited, which was considered acceptable, indicating a negligible impact of the uncontrolled variables along the different studies. In addition, the positive control data (PCD) submitted for the startle response prove the proficiency of the laboratory to conduct the assay: Propylthiouracil (PTU) showed a clear dose response with up to 50% decrease in auditory startle response in both males

and females and methimazole showed good responses in males and females at PND20 and PND60. In contrast, the RoB assessment for learning and memory, motor activity and morphometric measurements, indicated that the study has major limitations in several key questions in the RoB analysis, particularly for the outcome assessment method. Therefore, no conclusion can be drawn on these measurements. Regarding the outcome assessment for motor activity, and learning and memory, the main constraints were related to HCD (high variability in responses, indicating no confidence in the applied methodology over time and/or lack of control of external variables) and PCD (limited response and high variability). For the morphometric analysis, the methodology and results were poorly reported, making their interpretation difficult. Moreover, the statistically significant effects observed in two measurements in females at high dose should have triggered the measurement of the endpoint in the low and medium doses, which was not done. The experts also noted that only detailed clinical observations were conducted but not a complete FOB is available in the study, which is a shortcoming compared to the current standard.

TABLE 14 Selected uncertainties identified in the in vivo guideline study available.

Uncertainty	Uncertainty number	Description
HCD and PCD for motor activity not acceptable	U1	The HCD provided are not acceptable since there is a lack of control of the test methods over time (e.g. PND17 controls range from about 400 to 1800 number of movements in 60 min of session (400–1200 if three inhalation studies are also included)). This was considered as being related to uncontrolled variables in the conduction of the assay by the laboratory. In addition, there are also reporting issues and the studies included were judged inadequate (e.g. use of inhalation studies). Regarding PCD (PTU and methimazole), the selected doses were considered correct (Crofton et al., 2008); however, data were considered as not acceptable because for PTU: (1) the reporting is insufficient (only a figure indicating % of control is provided); (2) the results are not clearly positive (the only statistically significant effect was observed at mid-dose on PND21; (3) habituation was not measured. PCD with methimazole showed an appropriate dynamic range in ontogenetic pattern of habituation, in line with other studies with the test item; however, the variability observed in the control groups is not acceptable
Learning and Memory assay not acceptable	U2	The Biel Water Maze was used for measuring learning and memory. However, several major limitations were noted that makes the data not reliable: (1) In the path A for learning (with 4 trials during 2 days), there is no learning apparent in controls at PND22; learning is not demonstrated in control animals; high-dose adult males made more errors than control but standard deviation (SD) > means; the SD was as large as the means for latencies and for errors at PND60; (2) in path B, the observed variability in the control animals would obscure any treatment related effect; (3) in the path A 2 testing for memory, an inconsistent pattern was observed across sex/age/treatment and high variability was especially observed in the treated groups Overall, the assay was considered not acceptable and of not sufficient quality to determine effects on learning and memory
HCD and PCD Learning and Memory not acceptable	U3	Overall, the WG considered the provided HCD as not acceptable because there is no sufficient evidence in the report that the testing laboratory has control over time of the applied methodology. The data assessment is compromised due to inadequate reporting, as the tables only display mean values and not SD. In addition, means do not seem to be consistent across studies. Therefore, the WG could not properly assess and interpret the data The use of PTU as PCD is considered unacceptable because of inadequate reporting; there is no learning curve and there was no effect of PTU on errors at any dose. There was an increase in latencies, but it was the same at all dose levels despite the doses are considered high. The PCD for methimazole are also considered unacceptable since the observed effects are not in line with the expected positive effects (see Appendix 7 section 5)
Morphometrics evaluation	U4	The WG noted the following limitations: (1) no inclusion of brain size measurements; (2) no samples images of the measured areas are provided in the methodology; (3) the WG considered the statistical analysis inadequate (e.g. sex was not included as a factor); (4) blinding was not reported. More importantly, only control and high-dose animals were investigated. It is noted that a statistically significant effect was observed in the ventral limb of the dentate hilus in the hippocampus in female at PND72. In addition, in females, a statistically significant effect was observed on the height between hippocampal pyramidal neuron layers at PND11. The magnitude of the effect on morphometry was greater than 10% of the controls. The WG noted that these changes should have been further addressed by peer review for the correctness of the sampling, and by conducting further statistical analysis. These changes should have also triggered an investigation of the endpoint in the low and mid dose
FOB not complete	U5	Only detailed clinical observations were measured in the study and the WG noted that the FOB was not available in line with the current standard (i.e. OECD TG 426 and OECD TG 443 Annex 1). See also Sections 1 and 2 of Appendix 7
Number of animals	U6	Only 10 pups per group were included for all DNT endpoints (at least 20/sex per litter/dose are required in the OECD TG 426 for several DNT endpoints)

Additionally, the WG decided to evaluate the ontogeny of the MIE, i.e. the developmental timeline of nAChR expression, as an important step to assess the relevance of the AO. This assessment was not part of the EKE but reported here because

of the relevance of the dynamic changes in the expression and localisation of the nAChR isoforms to better understand the relevance of the in vivo models used in the assessment. The WG also considered the importance of the nAChR ontogeny for the assessment of the relevance of the in vitro models; though, the following discussion will mainly cover ontogeny-related uncertainties for in vivo studies.

nAChRs are pentameric ligand-gated cation channels consisting of various combinations of α and β subunits (e.g. α4β2 heterodimeric receptors) or only α subunits (e.g. α7 homomeric pentamers) (Broide et al., 2019). nAChRs are widely distributed throughout human and rodent brain over all phases of development (Alzu'bi et al., 2020). During ontogeny, nAChRs undergo changes in their distribution, subunit composition and function in the nervous system (Dvorakova et al., 2005). Nine out of 16 genes for human nAChR subunits are selectively expressed in human fetal cerebral cortex between 7.5 and 12 post-conceptional weeks (Alzu'bi et al., 2020). nAChRs are therefore present as early as the first trimester, gradually increasing up to mid-gestation and then declining in the third trimester (Mao et al., 2007).

The mRNA of the α7 subunit is strongly expressed as early as gestational day (GD) 13 in the cortical and hippocampal anlage of mice, and a similar temporal and spatial expression pattern has been observed in rat hippocampus (Broide et al., 2019). At GD 16, epibatidine and α-bungarotoxin binding sites (corresponding to heteromeric and α7 nAChRs, respectively) are widely distributed in the embryonic brain, although with different patterns. They are also abundant in neonate animals, with noticeable changes in some areas during the two to three postnatal weeks. The redistribution of nAChRs in the cerebral cortex, or their transient expression in the brainstem or hippocampus, may be related to the establishment of functional neuronal connections (Tribollet et al., 2004).

In rats, high transcript levels of neuronal nAChRs α4 and β2 subunits are observed at birth in cerebral cortex, medial habenula, CA1/CA3 regions of the hippocampus and several thalamic nuclei. In the latter, the expression of α4 and β2 subunit transcripts shows a biphasic pattern: the lowest levels occurring during the first and second postnatal weeks, respectively, and the highest levels during the second and fourth postnatal weeks, respectively. These findings suggest that the two nAChRs subunits are independently regulated in most of the brain areas (Cimino et al., 1995).

The α7 nAChR subtype is believed to be involved in the regulation of neuronal growth, differentiation and synapse formation during the development of the human brain (Falk et al., 2002). This receptor subtype has been implicated in hippocampal excitatory synapse formation and in the developmental GABAergic switch from excitation to inhibition, suggesting a functional role for these receptors in the developing hippocampus (Broide et al., 2019).

Transiently elevated expression of nAChRs in various brain regions during development contributes to maturation. The nAChR regulation of development occurs during 'critical periods' that are specific to each brain structure (O'Leary & Leslie, 2003).

The WG notes that the DNT OECD TG 426, with dosing during gestation and direct dosing of pups postnatally ensuring adequate exposure during lactation, would have been the appropriate study for acetamiprid to minimise the uncertainties due to the species differences in ontogeny (see Figure 8). However, several uncertainties on the proficiency of the laboratories to conduct the study as well as considerable uncertainties intrinsic to any TG 443 or TG 426 data regarding their reliability and relevance were also noted by the WG (Paparella et al., 2020).
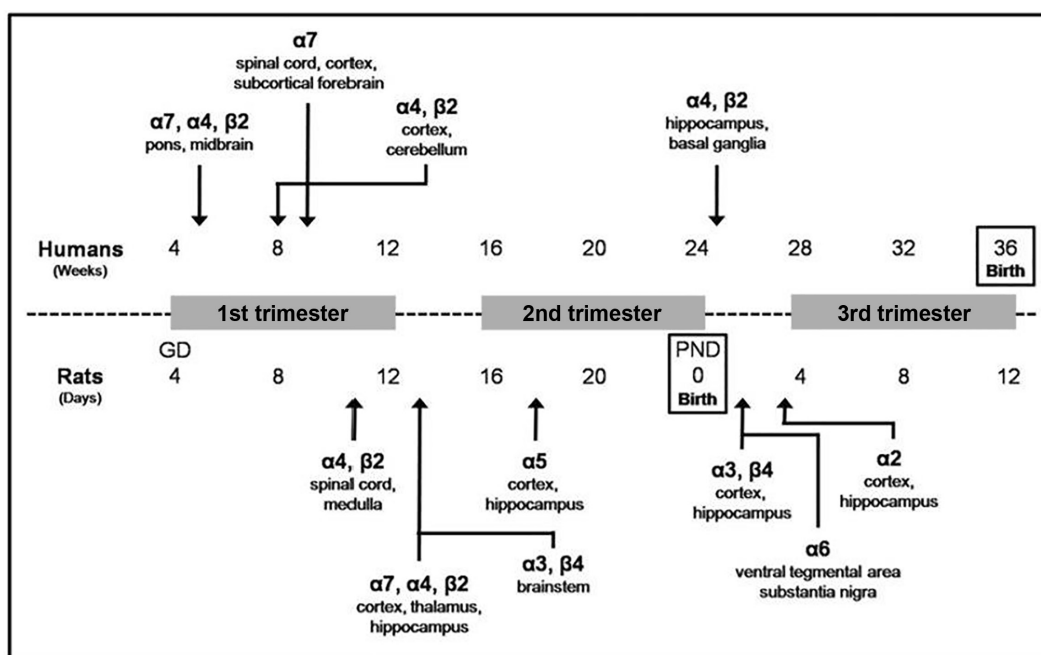

FIGURE 8 Nicotinic acetyl choline receptor (nAChR) ontogeny in rats and humans in the different brain areas. Figure extracted and adapted from OECD IATA Case Study no. 365.

In conclusion, the WG noted that the available evidence is insufficient to robustly characterise a DNT potential and to provide a conclusive pattern of dose–response for acetamiprid. This is mainly due to the lack of adequate measurement of learning and memory, motor activity and brain morphometry during the critical windows of brain development.

Outcome of the EKE for the BoE of vitro studies

In order to conclude on the mechanistic understanding, i.e. KE and KER, and in line with the pre-established protocol, the evidence retrieved for the identification of KE and their relationship (KER) was assessed for reliability and relevance. All data were extracted, and an uncertainty analysis conducted using an EKE methodology. Seven experts participated in answering the questions used to conduct the exercise (see Table 1) in an independent manner.

During the EKE, unanimously all experts concluded that there is no sufficient data to finalise an AOP-informed IATA that will allow to fully characterise the DNT mechanistic pathway of acetamiprid. The main uncertainties noted during the EKE discussions are presented in Table 15.

TABLE 15 Selected uncertainties identified in the available in vitro BoE.

Uncertainty	Uncertainty number	Description
Limited number of reliable in vitro studies from the literature	U1	New public literature studies measuring DNT endpoints in vitro have been made available since the renewal of approval of the active substance. Three have been considered relevant by the WG (Christen et al., 2017; Lee et al., 2022; Loser et al., 2021). According to the studies' authors they show evidence of DNT effects. The WG considered Lee et al. (2022) and Christen et al. (2017) as of high RoB due to limitations in one key question each (purity and cytotoxicity) Regarding Loser et al. (2021), the study was appraised as Tier 1 and a functional effect was observed from the concentration of 1 μM. This effect was considered as an immediate consequence of the nAChR activation and shows that human neuronal cells are functionally affected by low micromolar concentrations of acetamiprid and is therefore relevant
Expression of (functional) nAChR in DNT IVB test systems and relevant downstream KEs	U2	Acetamiprid has been tested in the DNT IVB (OECD, 2023) and no hit was observed. The WG noted that the expression of the nAChR in the DNT IVB test systems was not fully characterised. It is also noted that nicotine and other neonicotinoids showed negative results in the test battery. In addition, the nAChR desensitisation in the experimental conditions of the DNT IVB was considered a remaining uncertainty The WG concluded that there are important knowledge gaps that need to be addressed to identify and/or characterise downstream KEs. This may be done, for example, by the assessment of the neuronal network function (NNF) in the micro electrode array (MEA) using DA neurons derived from LUHMENS cells (Lund human mesencephalic cells representing human embryonic neuronal precursor cells)
Coverage of neurobiological processes during brain development by in vitro test systems, including those of DNT IVB	U3	The relevance of the available in vitro models, in particular the ones included in the DNT IVB, was evaluated by assessing the expression of the relevant targets and their functional activity upon activation. This assessment was not part of the EKE but reported in 'Step 3. Data analysis of the developmental neurotoxicity in vitro battery (DNT IVB) results for acetamiprid' in this section. This was considered as an important exercise to understand the relevance of the available in vitro models and correct in vitro data interpretation The WG noted that the DNT IVB test systems lack some features that are known to be critical in the development of the nervous system. Given the complex nature of brain development and the gaps in our current knowledge on these processes, the DNT IVB assays are not expected to cover the entire array of neurobiological processes during brain development. The applied in vitro models should be well characterised to know what they can or cannot detect

It was noted that limitations and uncertainties in the state of the art must be assessed for in vitro and in vivo data with the same scrutiny, and uncertainties are conceptually similar for in vitro and in vivo methods (Paparella et al., 2020). In vitro methods represent a powerful tool in examining specific mechanisms and have been proved to be fit for purpose when integrated in an AOP-informed IATA. Thus, the incorporation of the DNT IVB in the evaluation of DNT, along with the appropriate uncertainty analysis, would aid the data interpretation, by assembling multiple lines of evidence. Mechanistic in vitro data may represent DNT alerts as such and they may also contextualise negative, uncertain or positive apical endpoints in the in vivo results from guideline studies or public literature, and support a conclusion based on integrated evidence.

It was further noted that additional assays, possibly based on in vitro complex human models, should be developed as part of the DNT IVB. These additional models should be representative of key neuronal and glial subtypes at more advanced stages of morphological and functional differentiation and maturation. Before chemical testing, each model should be characterised for the presence of different neuronal and glial cell types and for the expression of critical molecular targets such as receptors, neurotransmitters, enzymes, pathways/mechanisms, etc.

Step 6. AOP-informed IATA conclusions for DNT of acetamiprid by the EFSA WG

A critical step in the IATA iterative process is the postulation of an AOP (if not already available) that will inform which KEs are essential to move from the MIE to the AO and how they can be measured using in vitro assays. The WG used the AOP

conceptual framework to organise the evidence and conclude on the mechanistic pathway of acetamiprid. However, the lack of positive outcome in the DNT IVB for acetamiprid and the several uncertainties identified in the test systems and on the DNT IVB outcomes with nicotine and other neonicotinoids, were considered as a critical uncertainty by the WG, such that an acetamiprid-specific postulation of an AOP was considered too uncertain (see also Step 5 EKE results).

Figure 9 shows the results of integrating the BoE from in vivo and in vitro studies using the AOP conceptual framework. The WG acknowledges the biological relevance of the effect of acetamiprid on the nAChR and the uncertainties within the AOP and the related methods, but nevertheless concluded that more empirical data should be made available to postulate an AOP-informed IATA for acetamiprid.
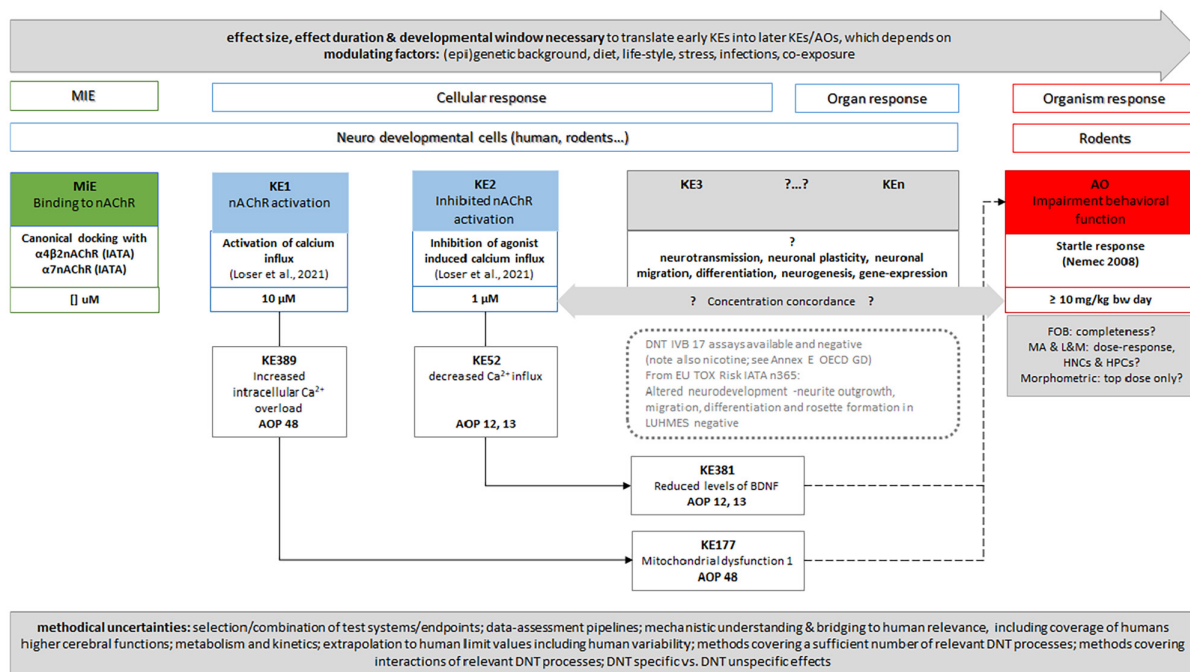
FIGURE 9 Integration of the acetamiprid DNT BoE into an AOP framework including the related uncertainties. Uncertainties are shaded in grey.

In establishing the AOP postulation, the WG considered the pesticide MoA, i.e. activation of nAChR, as a MIE.

Both neonicotinoid insecticides, as well as nicotine, bind to nAChRs and mimic the action of acetylcholine by opening the ion channels which allow the entry of Na^+ and Ca^{2+} into cells. These compounds vary in their affinity for different nAChR subtypes, with nicotine showing selective toxicity for vertebrates, whereas neonicotinoids are highly selective for insect nAChRs. The binding of neonicotinoids to insect nAChRs is virtually irreversible (EFSA PPR Panel, 2013).

Recent molecular docking studies using receptor models for the human α7 nAChR and the α4ß2 nAChR isoforms showed relevant differences on predominant orientation of the compounds in the binding site using different neonicotinoids and nicotine. This could give a molecular rationale for the observed functional differences (Loser et al., 2021; OECD IATA Case Study no. 365, OECD, 2022c).

Despite the common structural features, neonicotinoids and nicotine have different protonation states at physiological pH. Nicotine tends to be protonated in neutral aqueous solutions. This positive charge gives to nicotine a strong affinity for mammalian nAChRs as it occurs with the ammonium head of ACh; this is a requirement to interact with the mammalian nAChRs (Tomizawa & Casida, 2003). Instead of an easily protonated nitrogen, neonicotinoids have an electronegative pharmacophore, such as a nitro, or a cyano moiety, which is crucial for their insecticide activity. The electronegative pharmacophore is believed to be associated with a cationic subsite (possibly lysine, arginine or histidine) in the insect nAChR (Tomizawa & Casida, 2003, 2005; OECD IATA Case Study no. 365, OECD, 2022c). Acetamiprid shows weak affinity for mammalian nAChRs but strong affinity for insect nAChRs. Indeed, the binding characterisation is considered adequate for neonicotinoids and partially characterised in the IATA Case Study no. 365. Therefore, neonicotinoids are considered adequate stressors to measure the MIE activation.

The immediate downstream KE, following the MIE activation was identified in the activation of calcium influx. This KE was measured in two different neuron types (SH-SY5Y human neuroblastoma cells, an established model of nAChR signalling and dopaminergic neurons generated from LUHMES neuronal precursor cells) at concentrations starting from 10 μM.

Activation of nAChRs (MIE) often results in increased intracellular free Ca^{2+} levels through the direct passage of extracellular Ca^{2+} across the receptor channels in neurons, in addition to Na^+ and K^+. Agonist activation of the α7 nAChR can trigger an intracellular transient calcium increase that vary in duration, amplitude and distribution, depending on the subcellular localisation of the nAChR and its proximity to the endoplasmic reticulum (Govind et al., 2009). The raised cytoplasmic calcium levels trigger a series of calcium-dependent intracellular processes. Among the different nAChR subtypes in the brain, the homomeric α7 subtype exhibits higher permeability to Ca^{2+} than the other ligand-gated ion channels permeable to

this divalent cation, such as the NMDA (N-methyl-D-aspartate) receptor (Takarada et al., 2012). This receptor subtype is also known to desensitise rapidly (Wu et al., 2011).

During brain development, the consequences of MIE activation depend on the window of the exposure due to the different stage of cell differentiation/maturation and functional nAChR expression (see Figure 8). Regarding the α7 nAChR subtype, two features of this homomeric receptor must be considered from a toxicological perspective. First, the high levels of expression during brain development as compared to adults in both humans (Falk et al., 2002) and rodents (Tribollet et al., 2004; Zhang et al., 1998) support the important role of nAChRs for the development of the central nervous system (CNS) and are indicative of a potential vulnerability of developing mammals to early neonicotinoid exposures.

Indeed, with agonist binding, receptors become rapidly activated by opening the ion channel through the receptor. This is a metastable event and if the receptor remains bound by agonist, activation is followed by a second conformational change leading to a non-functional state termed desensitisation in which the channel is closed. Normally, when nicotine is removed, receptors rapidly recover from the desensitised state and enter in the resting state. Several lines of evidence indicate that chronic exposure to nicotine causes some of the nAChRs in the brain to undergo long-lasting state changes. These conformational changes are distinguished from activation and desensitisation by much slower kinetics, in the order of hours to days (Govind et al., 2009).

Therefore, the WG considered as a downstream consequence of the KE1, the affected functioning of the nAChR (desensitisation) as KE2. The inhibitory effect of acetamiprid on nAChR activity (i.e. inhibition of agonist-induced Ca^{2+} influx) was tested by pre-exposing LUHMES and SH-SY5Y cells with acetamiprid and acute addition of the receptor agonists nicotine or acetylcholine. Acetamiprid concentrations between 0.01 and 100 μM were tested and effects were observed at concentrations of 1 μM and higher (Loser et al., 2021; OECD IATA Case Study no. 365). The WG noted that the IATA Case Study no. 356 used the same evidence to characterise the MIE, the KE1 and the KE2, but there was no empirical support to the downstream KEs. Overall, the AOP-informed IATA for acetamiprid was considered inconclusive, due to the existing empirical data gaps between KE2 (desensitisation and loss of functionality of nAChRs) and the AO (i.e. impairment of behavioural function).

However, it is worth noting that the biological plausibility of DNT is high when taking into consideration the abundant expression of nAChRs which are functionally important in the developing brain for neurotransmission, neuronal plasticity, neuronal migration and differentiation, neurogenesis and gene expression (e.g. Lauder & Schambra, 1999). Moreover, the AOP Wiki contains three relevant OECD endorsed AOPs (see also Pitzer et al., 2023), i.e. AOPs 12 and 13, which link 'decreased Ca^{2+} influx' (KE52) to reduced levels of brain derived neurotrophic factor (BDNF) (KE381) as well as AOP 48 which links 'increased intracellular Ca^{2+} overload' (KE389) to mitochondrial dysfunction 1 (KE177). Both of these later key events may ultimately lead to 'impairment of learning and memory' (AO341). Thus, the main uncertainty is the critical effect size, exposure duration and exposure window during brain development which are necessary to translate the earlier KEs, for which acetamiprid data are available, into later KEs and finally to an organism level-based AO. Another, relevant uncertainty is that in the rodent in vivo BoE the auditory startle response is affected from 10 mg/kg/bw per day onwards, but it is unknown if other DNT AOs may be affected by acetamiprid at lower doses, since no reliable data are available (i.e. FOB, motor activity, learning and memory, morphometric changes). All these uncertainties need to be contextualised considering the baseline uncertainties resulting from the extrapolation from any experimental system to real-world scenarios. Such uncertainties include variable (epi)genetics, multiple stressors and environmental modulators, such that any early molecular or cellular KE triggered may be considered as a burden for an organism reducing its capacity to compensate for additional stress. In addition, there are methodological uncertainties for the assessment of toxicological effects which are conceptually similar between in vivo and in vitro assays (Figure 9 and Paparella et al., 2020). The utility of new data generation can be considered in relation to these baseline uncertainties. Consequently, the amount of evidence needed to regulate pesticides needs to be recognised as a science-policy task.

The WG noted that additional empirical evidence should be provided in terms of experimental data for acetamiprid from assays covering mechanisms between KE2 and the AO. This may be done by, e.g. using LUHMES cell-derived DA neurons in the MEA (neural network formation) assay for the assessment of the neuronal network formation and function. In addition, data and knowledge for other neonicotinoids should be included to minimise the uncertainties. Indeed, integration of mechanistic and AO evidence for the chemical class, by inclusion of several neonicotinoids and nicotine would increase the empirical support for the KERs in terms of critical effect size, effect duration and dose and temporal concordance within the AOP for acetamiprid.

2.2 | Hazard assessment of acetamiprid metabolites

2.2.1 | Data

Data related to two sources of evidence were obtained and appraised to address hazard characterisation of acetamiprid and its metabolites.

1. Data related to hazard characterisation of acetamiprid metabolites have been retrieved conducting a systematic literature search and data collection from 2016 to 2022 and appraisal using Critical Appraisal Tools (in the context of the same search as for the a.s. [Section 2.1.1]). In addition, a protocol was developed based on EFSA (2020),

which included detailed information on the search strategy, inclusion and exclusion criteria and detailed methods for the systematic review process used (see Appendix 1).

2. New toxicological studies submitted by the sole applicant of acetamiprid on 4 July 2023. These are studies on the toxicological profile of the metabolite IM-2-1 (in vitro micronucleus test with IM-2-1; mouse lymphoma assay with IM-2-1 and repeated dose 28-day toxicity study in rats with IM-2-1)

2.2.2 | Methodologies

2.2.2.1 | *EFSA systematic literature review for potential residue metabolites*

The methodologies applied for assessing the data retrieved from the systematic literature search and data collection for potential residue metabolites are the same as the methodologies used for acetamiprid and are described in Section 2.1.2 and in Appendix 1.

2.2.2.2 | *IM-2-1 toxicity studies submitted by sole applicant*

The IM-2-1 toxicity studies submitted by the sole applicant were evaluated by Member State experts in the context of a Peer Review Experts' Meeting. For this purpose, EFSA prepared a background document providing an overview of all the available toxicity data for IM-2-1 for the Member States participating in the Peer Review Meeting in order to facilitate drawing a conclusion on the toxicological properties of the metabolite and assessing its impact on the safety of existing MRLs.

The document takes into account all the available information (regulatory studies submitted by the applicant, public literature studies and in silico predictions) on the metabolite and contains a WoE of all the retrieved available evidence, including studies available in the context of the renewal of the active substance (i.e. included in Netherlands, 2016) and the newly submitted studies under Art 31 of Regulation (EC) No 178/2002, ref. Ref. Ares (2023)6354306 of 20 September 2023, and provides EFSA's considerations for setting toxicological reference values for the consumer risk assessment. This background document can be found in Appendix 8.

In the Peer Review Meeting TC 119 (19 November 2023), the Member State experts discussed the studies and concluded on the toxicological profile and reference values of metabolite IM-2-1 (see the experts' meeting report in Appendix 8a).

2.2.3 | Assessment

2.2.3.1 | *EFSA systematic literature review for potential residue metabolites*

A systematic literature review was performed for the list of metabolites included in Table 16. Two reviewers screened the literature identified through the searches in two steps. For potential crops/livestock metabolites, 92 unique references were identified (see PRISMA flow chart, Figure 10). No publication was considered relevant for assessing any toxicological endpoint of any metabolite. Most of the published studies were not conducted with any of the metabolites as listed in Table 16 or were not toxicological studies.

TABLE 16 List of potential crop metabolites for which a systematic literature review was done for toxicological parameters (2016–2022).

Name and SMILES code
(1*E*)-*N*-[(6-chloropyridin-3-yl)methyl]-*N*′-cyanoethanimidamide (**IM-2-1**): Clc1ccc(CNC(\C)=N\C#N)cn1
1-(6-chloropyridin-3-yl)-*N*-methylmethanamine (**IM-1-4**): Clc1ccc(CNC)cn1
(1*E*)-*N*′-cyano-*N*-methylethanimidamide (IS-1-1): C/C(=N\C#N)NC
(1*E*)-*N*′-cyanoethanimidamide (IS-2-1): C/C(N)=N\C#N
(1*E*)-*N*′-carbamoyl-*N*-[(6-chloropyridin-3-yl)methyl]-*N*-methylethanimidamide (IM-1-2): Clc1ccc(CN(C)C(\C)=N\C(N)=O)cn1
N-[(6-chloropyridin-3-yl)methyl]-*N*-methylacetamide (IM-1-3): Clc1ccc(CN(C)C(C)=O)cn1
N-[(6-chloropyridin-3-yl) methyl]acetamide (IM-2-3): Clc1ccc(CNC(C)=O)cn1
6-chloropyridine-3-carboxylic acid (IC-0): OC(=O)c1cnc(Cl)cc1
(6-chloropyridin-3-yl)methanol (IM-0): OCc1cnc(Cl)cc1

Metabolites*

References identified through
searches for metabolites
(*n*=92)

Title abstract screened for
relevance and eligibility
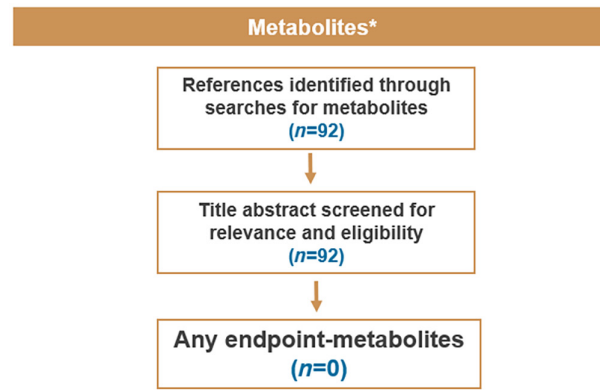(*n*=92)

Any endpoint-metabolites
(*n*=0)

FIGURE 10 PRISMA flow chart of the literature search process for acetamiprid and its metabolites, including the screening for relevance. *See list of metabolites in Table 16.

2.2.3.2 | *EFSA assessment and conclusion on the toxicological properties of IM-2-1 residue metabolite*

As IM-2-1 metabolite was considered relevant to include in the consumer risk assessment, the information on IM-2-1 metabolite in the revised RAR on acetamiprid (Netherlands, 2016), (Section B.6.8.1.2 of revised RAR and EFSA conclusion (2016)) was reviewed.

The following data on IM-2-1 were made available for the renewal of the active substance acetamiprid: (1) An acute oral toxicity study with LD50 value for IM-2-1 above 5000 mg/kg bw in both sexes (revised RAR on acetamiprid, Netherlands, 2016); (2) a negative Ames test with IM-2-1 (Netherlands, 2016). In addition, it is noted that, in rats, acetamiprid is metabolised to IM-2-1 by simple demethylation on the chain (Netherlands, 2016). In addition, IM-2-1 metabolite has been quantified as a major rat metabolite in urine (> 10% of absorbed dose).

In rats, acetamiprid is rapidly and extensively absorbed (Cmax was reached at 0.5–7 h) after single (1 or 50 mg/kg bw) (~ 96%) and repeated (1 mg/kg bw per day) (> 60%) oral administration. It is then distributed reaching the highest concentrations in liver, kidneys, adrenals and thyroid. Excretion is rapid, mainly via urine during the first 24 h after treatment, with more than 90% of the compound being excreted at 96 h. After single oral administration in rats, acetamiprid is extensively metabolised, with 50%–70% of the dose being excreted as metabolites in both urine and faeces. The main metabolic pathway in rats is the demethylation to **IM-2-1** (N-desmethyl-acetamiprid). This metabolite is further transformed to **IC-O** (6-chloronicotinic acid), with the release of **IS-2-1** (N-cyanoacetamidine derivative) after cleavage from the side-chains. In a mouse study from the literature (Ford & Casida, 2006), both acetamiprid and IM-2-1 have been detected in the brain upon intraperitoneal injection of acetamiprid (see detailed RoB assessment for the study in section 2.3). The toxicokinetic data included in the revised RAR on acetamiprid (Netherlands, 2016) also point to exposure of the brain (parent and/or metabolite(s)) upon oral acetamiprid exposure, but since only total radioactivity has been quantified, it cannot be concluded whether the brain exposure includes acetamiprid and/or IM-2-1. In Laubscher et al. (2022), IM-2-1 (but not acetamiprid) was detected in cerebrospinal fluid of 14 children (age range 3–18 years) treated for leukaemia and lymphomas and undergoing therapeutic lumbar punctions. The WG noted that biomonitoring studies may be confounded by preformed pesticide metabolites in the diet due to environmental degradation of parent compounds, as occurs with organophosphate insecticides (Hernández et al., 2019). Since IM-2-1 is an environmental degradation product of acetamiprid (Ospina et al., 2019), it is possible that the quantification of IM-2-1 in plasma, urine and cerebrospinal fluid in all children in Laubscher et al. (2022), with a concurrent lack of quantification of the parent compound acetamiprid in the same specimens may be due to direct exposure to IM-2-1 produced in the environment and not in the body (see Section 4.1.2). In addition, these findings are not generalisable to the general children population, given that the study population consisted of children with lymphohematopoietic tumours (selection bias), and tumours may affect the blood–brain barrier permeability and choroidal plexus barrier function (see Section 2.3.3), thus favouring a greater entry of chemicals into the brain.

Although no quantitative structure–activity relationship analysis is available in the revised RAR on acetamiprid (Netherlands, 2016), the WG noted that IM-2-1 represents a simple demethylated metabolite of acetamiprid, which still contains the N-cyanoamidine group as acetamiprid and thiacloprid. As described in Section 2.1.4.3, the nitro- or cyano- or equivalent electronegative pharmacophore is considered crucial for optimum nAChR binding. It is therefore plausible that IM-2-1 would also bind to mammalian nAChRs, but it is difficult to predict its affinity and potency compared to those of acetamiprid. Further data would be needed to understand the potential concern for DNT of IM-2-1.

Regarding the new toxicity studies submitted by sole applicant on IM-2-1 (see Section 2.2.2.2), details on the assessment of the studies can be found in the background document prepared by EFSA (Appendix 8). The conclusion of the Peer Review Meeting TC 119 (19 November 2023) on the toxicological profile of metabolite IM-2-1 is provided in Appendix 8a and below.

The new in vitro genotoxicity studies (micronucleus (MN) test, mouse lymphoma assays) submitted by the applicant in the context of the present mandate were considered acceptable. In addition, in the revised RAR on acetamiprid (Netherlands, 2016), an acceptable Ames test for IM-2-1 has been included. Based on these data, it was concluded that metabolite IM-2-1 did not induce clastogenicity or aneugenicity in the micronucleus assay in vitro, and it does not induce gene mutations in the mouse lymphoma assay, nor in the Ames test, and was therefore considered unlikely to be genotoxic.

The new 28-day repeated dose toxicity study of IM-2-1 in rats submitted by the applicant in the context of the present mandate was also considered acceptable. The NOAEL of the study was set at 1000 ppm (equivalent to 88.4 mg/kg body weight (bw) per day) based on decreased body weight gain in males (up to 38.0%) and supported by a decrease in body weight in males (10.4%, non-statistically significant), accompanied with a slight decrease in food consumption in male rats at 1600 ppm (138 mg/kg bw per day). The top dose of 1600 ppm (141 mg/kg bw per day) was the NOAEL for females based on the absence of treatment-related adverse effects at all dose levels. An increase in relative liver weight in females at the top dose (of 1600 ppm) was not considered adverse, as no concomitant histopathological changes were observed, and the increase in relative weight was below 15%. It was noted that at the top dose, triglyceride levels increased, and α1-globulin decreased in female rats, pointing to an effect on the liver, although not adverse. It was noted that according to the revised RAR on acetamiprid (Netherlands, 2016), acetamiprid causes liver toxicity in the available short-term study in rats (NOAEL of 90-day rat study amounts to 200 ppm, equivalent to 12.4 and 14.6 mg/kg bw per day for males and females, respectively, based mainly on bw reduction and liver effects from 800 ppm) and that the liver was considered a target organ in the available long-term toxicity study in rats. It was further noted that possible liver toxicity cannot be excluded for the metabolite IM-2-1 based only on this short-term toxicity study. However, the evidence for liver toxicity in females in the current 28-day study is limited and not robust enough to be considered as adverse for the NOAEL setting.

IM-2-1 is found as residue metabolite and the Peer Review Meeting TC 119 (19 November 2023) proposed to set the same toxicological reference values (ADI and ARfD) for IM-2-1 as those for acetamiprid, based on the following considerations:

- Structural similarities: IM-2-1 represents a simple demethylated metabolite of acetamiprid and still contains the active group (i.e. N-cyanoamidine group) as acetamiprid and thiacloprid.
- ADME: IM-2-1 metabolite is a major rat metabolite (18%–24% of dose in urine).
- Genotoxicity: IM-2-1 metabolite does not induce clastogenicity or aneugenicity in the micronucleus assay in vitro, and it does not induce gene mutations in the mouse lymphoma assay, nor in the Ames test. Overall, IM-2-1 metabolite is unlikely to be genotoxic.
- Systemic toxicity: Available 28-day rat study indicated decreased body weight gain, accompanied with bw decrease and decreased food consumption, as the critical toxicological effects in male rats (liver effects observed in females were considered treatment related, but not adverse). In the 90-day rat study with acetamiprid, liver effects were identified as the critical toxicological effects. The critical endpoint used for setting the reference values for acetamiprid, i.e. DNT, is not captured in a short-term repeated dose toxicity study.
- Relative toxicity compared to parent: The available 28-day rat study on IM-2-1 does not allow to conclude on a different qualitative or quantitative toxicological profile of the metabolite compared to the parent. Based on structural similarities to parent and being IM-2-1 a major rat metabolite, the toxicological profile of IM-2-1 is considered as covered by the parent (i.e. IM-2-1 is expected to be at least of equal toxicity as the parent). Therefore, the same toxicological reference values of the parent can apply to the metabolite.

2.3 | Human exposure estimation based on human biomonitoring data

To address **ToR1a** and **ToR1b** (assessment question 1) (see Section 1.2.1), the EFSA WG evaluated new human biomonitoring data on acetamiprid and its IM-2-1 metabolite, including the study of Laubscher et al. (2022) as included in the mandate. It was also requested to use these human biomonitoring data to estimate related external (oral) exposure levels using a PBK modelling reverse dosimetry approach (Clewell et al., 2008). These estimated oral exposure levels were then compared with the HBGVs of acetamiprid to evaluate whether exposure, based on the human biomonitoring data, exceeds the HBGVs. It must be noted that it was not intended to perform a full exposure characterisation based on the available human biomonitoring datasets. It was rather aimed to assess whether these datasets would point to possible exceedance of HBGVs of acetamiprid.

Besides application of a PBK model to estimate external oral exposure levels from human biomonitoring data (using a so-called reverse dosimetry approach), a PBK model can be applied to estimate internal exposure levels upon exposure to a given external (e.g. oral) dose (also called forward dosimetry). As such, the PBK model is also intended to estimate internal acetamiprid concentrations upon exposure to levels corresponding to the current HBGVs and to compare these predicted internal concentrations with reported in vitro effect concentrations from relevant and reliable in vitro effect studies identified (Section 2.1).

2.3.1 | Data

2.3.1.1 | *Human biomonitoring data*

The assessment primarily relied on the Laubscher et al. (2022) study, which was included in the mandate. Additionally, two other human biomonitoring studies were identified and considered relevant to estimate acetamiprid (oral) exposure levels for sensitive life stages regarding DNT effects.

2.3.1.2 | *PBK modelling*

A PBK model has been developed for acetamiprid and IM-2-1 for the OECD IATA Case Study no. 365 on acetamiprid (published in https://one.oecd.org/document/env/cbc/mono(2022)27/en/pdf and Annex I https://one.oecd.org/document/env/cbc/mono(2022)27/ann1/en/pdf) (OECD, 2022c). Furthermore, kinetic data were collected by the WG for possible rebuilding/adjustment of the PBK model.

2.3.2 | Methods

2.3.2.1 | *Human biomonitoring data*

Relevant studies were selected as described in the protocol (Appendix 1), which underwent a reliability assessment. The WG noted that the currently available appraisal tool for HOS was developed for studies addressing the relationship between compound exposure and a given (health) effect, whereas the studies to be evaluated for the exposure question should be assessed for reliability of the reported levels in biological fluids. Therefore, the WG developed a CAT tailored to assess the reliability of the reported levels of acetamiprid and/or IM-2-1 in the human biomonitoring studies. The CAT developed and applied can be found in Appendix 9. The studies were appraised by an expert of the WG and then reviewed by EFSA staff. The outcomes of the appraisals were discussed and agreed upon in a WG meeting.

2.3.2.2 | *PBK modelling*

A stepwise approach, as described in the protocol (Appendix 1) was followed to assess whether the available PBK model was adequate for the reverse dosimetry analysis (estimation of external (oral) acetamiprid dose related to reported concentrations of acetamiprid and IM-2-1 in biomonitoring studies) and forward dosimetry analysis (estimation of internal concentration of acetamiprid upon oral exposure to the HBGVs). Given that the question in step 3 of the stepwise approach *('Is the available PBK model adequate to answer the assessment question?')* was answered as 'no' (see Section 2.3.3.2), it was evaluated whether the available PBK model can be rebuilt/adjusted. Details on the approach to be used for this assessment had not been predefined in the protocol. The WG considered it relevant to perform an 'ad hoc' data collection to assess the possibility of PBK model development. To that end, the steps as described below were followed.

a. Collection of kinetic data from studies reported in the revised RAR on acetamiprid (Netherlands, 2016)
b. Collection of kinetic data from the scientific peer-reviewed literature

- Set inclusion and exclusion criteria
- Literature search
- Data extraction
- Appraisal

c. Collection of kinetic data reported in the OECD IATA Case Study no. 365 on acetamiprid
d. Evaluation of whether the available kinetic data allows for PBK model development before use in reverse and forward dosimetry analyses

More details on the approach for kinetic data collection and appraisal can be found in Appendix 10.

2.3.3 | Assessment

2.3.3.1 | *Results appraisal of human studies*

Three HOS containing biomonitoring data were selected for appraisal (Figure 2). These include the study of Laubscher et al. (2022), as requested in the mandate and two other studies that were identified (Mahai et al., 2022; Oya et al., 2021). A short description of the three studies is provided in Table 17.

TABLE 17 Identified human biomonitoring studies for exposure estimation.

Study	Subjects	Biological samples	Reported data on acetamiprid and/or metabolites
Laubscher et al. (2022)	14 children (age range 3–18 years) treated for leukaemia and lymphomas and undergoing therapeutic lumbar punctions in the Lausanne University Hospital in Switzerland	Cerebrospinal fluid, plasma, urine	Acetamiprid was not detected (< LOD) in any of the biological fluids analysed Acetamiprid's metabolite IM-2-1 was detected above the LOQ in most of the analysed samples (13 cerebrospinal fluid samples [93%], 12 plasma samples [86%] and 13 urine samples [93%])

(Continues)

TABLE 17   (Continued)

Study	Subjects	Biological samples	Reported data on acetamiprid and/or metabolites
Mahai et al. (2022)	408 pregnant women (average age of 29.5 years), residents of Wuhan undergoing prenatal cares and giving birth at Wuhan Women and Children Medical and Healthcare Center in Wuhan, Hubei Province, China	Urine samples collected in the three trimesters of pregnancy	For acetamiprid, detection frequencies amounted to 16.9%, 25.2% and 37.0% in the first, second and third trimester, respectively. For acetamiprid's metabolite IM-2-1, detection frequencies amounted to 99.8%, 99.3% and 100% in the first, second and third trimester, respectively
Oya et al. (2021)	1036 children (age range 16–23 months) living in urban and suburban locations in the Aichi prefecture, the central region of Japan	Extraction of disposable diapers, as surrogate for urine	The detection frequency for acetamiprid amounted to 37.3%. The detection frequency for acetamiprid's metabolite IM-2-1 amounted to 14.1%

The results of the reliability assessment performed for the selected human biomonitoring data sets is presented in **Table 18**. The studies of Laubscher et al. (2022) and Mahai et al. (2022) were scored as Tier 3, whereas the study of Oya et al. (2021) as Tier 2. A major limitation of the first two studies was the lack of quality assurance (QA)/quality control (QC), or lack of reporting on this. More details on the results of the appraisal can be found in Appendix 11.

TABLE 18   Heatmap results of the RoB for human biomonitoring studies retrieved from public literature.

Human biomonitoring study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Tier
Laubscher et al. (2022)									3
Mahai et al. (2022)									3
Oya et al. (2021)									2

Notes: The heatmap includes the endpoint-specific ratings for the RoB for each of the 8 questions in the CAT (i.e. number of participants, analyte measured and biological sample used, potential contamination or non-specific binding to the collection tube, sample storage, method's validation, QA/QC, analytical instrumentation, limited of detection/quantification reported). Each question is scored as either low RoB (green), medium RoB (orange), or high RoB (red). The RoB questions highlighted in yellow correspond to the key RoB criteria considered in the final Tier (1 [low RoB], 2 [medium RoB] or 3 [high RoB]) of the endpoint (Q2 analyte measured; Q5 method's validation; Q6 QA/QC; Q7 analytical instrumentation). For detailed rationale for each rating, see Appendix 11.

The WG considered the data reported by Laubscher et al. (2022) as not representative for the general children population, given that the subjects included were children with lymphohematopoietic tumours (selection bias), and tumours may affect the blood–brain barrier and choroidal plexus barrier, thus allowing chemicals to gain access to the brain. The results of the Mahai et al. (2022) and Oya et al. (2021) studies could be extrapolated to their respective contexts (geographical areas, customs, diet, ethnic peculiarities, etc.). The WG also noted that the Laubscher et al. (2022) study is the only one assessing subjects residing in Europe while the other studies with subjects residing in China and Japan (Table 17) may be of less relevance to estimate exposure in the European situation as they rather reflect exposure in these countries. In that respect, it is reiterated that it was not intended to perform a full exposure characterisation based on these biomonitoring studies, but it was rather aimed to assess whether the available human biomonitoring datasets would point to possible exceedance of HBGVs of acetamiprid for those specific studies.

2.3.3.2 | *Results stepwise approach PBK modelling*

The steps as described in the protocol (Appendix 1) were followed. The results of the assessment performed related to the predefined steps in the protocol are described below.

Step 1) Define required characteristics of PBK model to answer the assessment questions

The minimal requirements for the PBK model were defined by the WG.

Regarding the reverse dosimetry question, the WG noted that the human biomonitoring data referred to in the mandate (from the Laubscher et al. (2022) study) include only data on the metabolite IM-2-1, as acetamiprid was not detected. Therefore, the WG acknowledged the importance of adequate description of the kinetics of IM-2-1 in the PBK model. Robust modelling (informed mechanisms and compound-specific data) of the relation between oral exposure to acetamiprid and related internal concentrations (plasma and brain) and urinary levels of IM-2-1 was considered essential. For the other identified studies (Mahai et al., 2022; Oya et al., 2021), only data are included on reported levels of acetamiprid and IM-2-1 in urine. For exposure estimation of these data sets, the WG noted that a PBK model is required that provides a robust modelling of the relation between oral exposure to acetamiprid and related urinary levels of acetamiprid and IM-2-1. Regarding relevant life stages to be included in the PBK model, data from the Laubscher et al. (2022) study were obtained from children hospitalised in Europe with an age range between 3 and 18 years. The data of the Mahai et al. (2022) study

were obtained from pregnant Chinese women with an average age of 29.5 years, and the data of the Oya et al. (2021) study were obtained from Japanese toddlers with ages ranging from 16 to 23 months. For a low tier assessment of the reverse dosimetry question, the WG considered application of a PBK model of a human, adjusted with life-stage-specific physiological parameters (for children, pregnant women) as a minimum, acceptable.

Regarding the PBK modelling application for interpretation of the in vitro studies, it was noted that in vitro (DNT-related) toxicity studies were only available for the parent compound (acetamiprid) and not for the metabolite (IM-2-1). Therefore, for this application, the WG considered the minimal requirement of the PBK model to provide a robust modelling of internal acetamiprid dosimetry upon oral exposure. For a low tier assessment, the WG considered a relatively simple PBK model, e.g. describing internal (plasma) concentrations, sufficient. For a low tier assessment of the forward dosimetry question, the WG considered application of a PBK model of a human, adjusted with life stage-specific physiological parameters (for children, pregnant women), acceptable. For a higher tier assessment, a robust description of brain and/or fetal dosimetry was considered relevant.

Step 2) Provide description of available PBK model(s)

One PBK model of acetamiprid is currently available. This PBK model has been developed for the OECD IATA Case Study no. 365 on acetamiprid within the framework of the EUTOXRisk project. Whole-body rat and human PBK models were developed using the rat and human Simcyp Simulator (V19) (https://www.simcyp.com). The aims of the PBK modelling work described in the OECD IATA Case study no. 365 on acetamiprid were to develop PBK models to predict comparative human exposure of acetamiprid using quantitative in vitro to in vivo extrapolation (qIVIVE) approaches, and to examine the inter-individual variability in the simulated exposure of acetamiprid in humans. The PBK models describe the internal dosimetry (plasma and brain concentrations) and urinary levels of acetamiprid and IM-2-1 in rats and humans. Although the OECD IATA Case Study no. 365 does not report PBK model simulations for children or pregnant women (only for human adults), physiological parameters of these specific groups are available in the literature and may be selected in the Simcyp software. As such, the human PBK model was considered to be relevant for both the reverse dosimetry question (external exposure estimation based on human biomonitoring data) and the forward dosimetry question (internal exposure estimation upon exposure to HBGV).

Step 3) Determine whether available PBK model(s) is/are adequate to answer assessment questions: Yes or No

Although the Simcyp human PBK model was considered to be relevant, it could not be applied by the WG to answer assessment question 1b, since Simcyp Simulator software is a proprietary software that can only be used following a dedicated training. Therefore, given the timeframe of the mandate, the WG considered application of the available PBK model not feasible.

Given that the answer for step 3 is therefore 'no', the WG proceeded to step 5, according to the step-wise approach described in the protocol (Appendix 1).

Step 5) Assess whether PBK model(s) can be rebuilt/adjusted to answer assessment questions: Yes or No

Given that the answer for step 3 is 'no', it was concluded that assessment question 1b cannot be answered with the available PBK model. Therefore, given the timeframe of the mandate, the WG considered as best approach to attempt to rebuild the PBK model in PK-Sim software (https://www.open-systems-pharmacology.org/), which is a freely available software, providing similar possibilities as the Simcyp Simulator as relevant for the PBK modelling questions in the mandate.

First, it was assessed whether the parameters applied in the Simcyp model (as described in the OECD IATA Case Study no. 365) could be applied in the PK-Sim model. To that end, the described approach in that case study was critically assessed. As also indicated in the Case Study no. 365, the available kinetic data were limited, hampering the development of the PBK model. The WG concluded that uncertainty related to the description of the absorption and distribution processes were limited. It should be noted that the distribution parameters (partition coefficients) were predicted solely using in silico methods (on the basis of the physico-chemical properties of acetamiprid and IM-2-1, and the composition of the tissues), and were not evaluated against in vivo data. Although evaluation with in vivo data would improve the accuracy of the applied partition coefficients in the PBK model, the WG considered the associated uncertainty as acceptable.

The uncertainties in model parameterisation of metabolism and excretion processes were considered to be high and various selected chemical-specific parameter values were not supported by data. The major uncertainties identified by the WG related to metabolism and excretion processes are summarised in Table 19 and Figure 11.

TABLE 19 Overview of identified major uncertainties related to metabolism and excretion processes of the available PBK model for acetamiprid.

Uncertainty	Uncertainty number	Description
Parameter value for hepatic clearance acetamiprid	U1	There is uncertainty in the parameter values for hepatic clearance in the rat and human PBK models. In the rat PBK model, the value for hepatic clearance was estimated based on fitting it in such a way that the rat PBK model simulations describe the reported data on total radioactivity in rat serum (using kinetic data of kinetic study reported in the revised RAR on acetamiprid [Netherlands, 2016]). This was considered the best approach in the absence of information on internal exposure to acetamiprid (parent compound) in the in vivo kinetic studies available and in the absence of in vitro data on rat hepatic clearance. The limitation of the approach was acknowledged by the PBK model developers in the OECD IATA Case Study no. 365, and they assessed the impact of different values for hepatic clearance on PBK model predicted internal acetamiprid concentrations (plasma), called a 'sensitivity analysis' in the Case Study. Starting with this uncertainty in the model parameter value for hepatic clearance in rat, the approach of scaling the obtained value to a human value for the human PBK model was considered to bring an extra uncertainty in the hepatic clearance value in the human PBK model according to the WG. Based on the in vitro clearance study performed with human hepatocytes referred to in the IATA Case Study no. 365, no decrease of acetamiprid was detected, suggesting no or limited hepatic clearance based on the in vitro study. That in vitro result is not in line with the hepatic clearance value applied in the human PBK model. Altogether, the WG considered the parameter value related to the hepatic clearance in the PBK model as uncertain
Parameter value for IM-2-1 formation	U2	The formation of IM-2-1 was set as amounting to 80% of the hepatic clearance of acetamiprid in rats and humans. As far as could be evaluated by the WG, the selection of this value was arbitrary and not supported by data. Therefore, the WG considered the parameter values related to the formation of IM-2-1 as uncertain
Parameter values for metabolic (hepatic) clearance of IM-2-1	U3	For the human PBK model, a further metabolic (hepatic) clearance of IM-2-1 is described. Further biotransformation may be expected when considering the in vivo rat toxicokinetic studies as reported in the revised RAR on acetamiprid (Netherlands, 2016), showing that downstream metabolites of IM-2-1 have been detected in excreta. However, no qualitative nor quantitative information on this reaction in humans is available (a comparative in vitro metabolism study is lacking in the revised RAR on acetamiprid (Netherlands, 2016)). As far as could be evaluated by the WG, the parameter value for this reaction chosen was arbitrary and not directly supported by data. Therefore, the WG considered the parameter value related to the biotransformation of IM-2-1 as uncertain
Parameter values for renal clearance of acetamiprid	U4	Renal clearance of acetamiprid was described in the PBK model. Renal clearance for acetamiprid was reported to be determined based on in vivo kinetic data as indicated in the OECD IATA Case Study no. 365. For rats, 5%–7% of acetamiprid excreted unchanged in urine are mentioned in the Case Study, but it is not clear to the WG which data from the revised RAR on acetamiprid (Netherlands, 2016) were used to come to these values, and to which time point after dosing they apply, making the estimation of kinetic parameter values related to renal clearance uncertain. For humans, a value of 2.6% excreted unchanged in urine is mentioned in the Case Study, which is in line with the value reported by Harada et al. (2016) showing cumulative acetamiprid levels in urine 96 h after exposure ($2.6 \pm 3.4\%$). Given the high variation reported and the relatively late time point (96 h), the WG considered the estimation of kinetic parameter values related to renal clearance in humans uncertain for acetamiprid
Parameter values for renal clearance of IM-2-1	U5	The PBK model for humans also describes renal clearance of IM-2-1. The value for IM-2-1 renal clearance (0.95 L/h) is more than 10 times higher than the renal clearance value for acetamiprid (0.082 L/h) applied in the PBK model developed in the OECD IATA Case Study no. 365. The kinetic dataset used for calculating this clearance value was based on data from Harada et al. (2016), reporting urinary levels of IM-2-1 at 4 time points after dosing (24, 48, 72, 96 h). A renal clearance value was obtained by applying an arbitrary split (not supported by data) between an (arbitrary) value of hepatic IM-2-1 clearance (see U3) and renal clearance. Therefore, the WG considered the estimation of the PBK model parameter values related to renal clearance also as uncertain
Description of internal concentrations of acetamiprid	U6	Given the uncertainty in the parameter values as described for U1, U2, and U4, the related resulting description of internal concentrations of acetamiprid were considered uncertain by the WG. Practically an unlimited number of combinations of parameter values related to the 3 kinetic processes described for U1, U2, U4 could result in an adequate description of the in vivo reported concentrations of acetamiprid in urine (the only in vivo kinetic data available for acetamiprid in rats and humans). However, different choices of these parameters are expected to result in a large range of predicted internal concentrations of acetamiprid. Given that no in vivo kinetic data on internal concentrations of acetamiprid were used for model development/evaluation (because not available), the WG considered the prediction of internal concentrations of acetamiprid as uncertain
Description of internal concentrations of IM-2-1	U7	Given the uncertainty in the parameter values as described for U1-U5, the related resulting description of internal concentrations of IM-2-1 were considered uncertain by the WG. Practically an unlimited number of combinations of parameter values related to the 5 kinetic processes described for U1-U5 could result in an adequate description of the in vivo reported concentrations of IM-2-1 in urine (the only in vivo kinetic data available for IM-2-1 in rats and humans). However, different choices of these parameters are expected to result in a large range of predicted internal concentrations of IM-2-1. Given that no in vivo kinetic data on internal concentrations of IM-2-1 were used for model development/evaluation, the WG considered the prediction of internal concentrations of IM-2-1 as uncertain

FIGURE 11 Schematic overview of interrelation of identified uncertainties related to metabolism and excretion processes of acetamiprid and IM-2-1 in the available PBK model for acetamiprid. The PBK model parameter values chosen for the kinetic processes related to U1, U2 and U4 impact on predicted internal and urinary acetamiprid concentrations (U6). The PBK model parameter values chosen for the kinetic processes related to U1–U5 impact on predicted internal and urinary IM-2-1 concentrations (U7).

The WG considered that uncertainty in certain model parameters can be accepted. However, as the combination of these different uncertain model parameters will affect the PBK model predictions for the internal dosimetry and urinary levels of both acetamiprid and IM-2-1, i.e. the critical PBK model outcomes that are required to answer the assessment question 1b, the WG considered that a translation of the available PBK model in Simcyp to a similar PBK model in PK-Sim would not result in a PBK model that is sufficiently robust to answer the assessment question 1b of the mandate. Also, given the differences in the two software packages, some model parameters may not be directly transferable from the Simcyp model to a PK-Sim model.

Therefore, the WG attempted to develop a new PBK model in PK-Sim software, taking also into account possible new available kinetic data that have become available after the OECD IATA Case Study no. 365 on acetamiprid. To that end, the WG first performed a kinetic data search, to include all possible relevant kinetic data for PBK model development/evaluation and subsequently appraised the obtained studies from the scientific literature. An overview of the resulting kinetic data collection and appraisal results are presented in Appendix 12. The extracted kinetic data can be found in Appendix 13 and the detailed results of the appraisals are presented in Appendix 14.

Some new kinetic studies were identified that were not used for the PBK model development as described in the OECD IATA Case Study no. 365. After the kinetic data collection and appraisal, the WG evaluated whether the uncertainties identified for the available PBK model (see Table 19) could be resolved by the newly available kinetic data. The WG noted that only new data were identified that could be of use to parameterise PBK models for IM-2-1 formation in the rat liver (Khidkhan et al., 2021; Kolanczyck et al., 2020) and human liver (Khidkhan et al., 2021), referring to U2 in Table 19. However, given the high RoB of the studies (Tier 3) and the large difference in reported values for IM-2-1 formation kinetics for rat liver microsomes in the Kolanczyck et al. (2020) and Khidkhan et al. (2021) studies, the WG was of the opinion that uncertainty in definition of parameter values for hepatic IM-2-1 formation in the PBK model would remain. The WG concluded that none of the newly identified studies provided data that could be used to decrease the uncertainty in the parameter values as described in Table 19. The WG considered that practically an infinite number of combinations of parameter values can be fitted for the metabolism and renal clearance processes of acetamiprid and IM-2-1 to obtain an adequate fit of the limited available in vivo kinetic data of acetamiprid and IM-2-1 in rat and human urine. The different choices in parameter values for the hepatic and renal clearance processes would, however, result in different internal dosimetry of acetamiprid and IM-2-1, and therefore the WG concluded that the available kinetic data set should be considered as being too limited to develop a sufficiently robust and reliable PBK model to answer the questions of the mandate.

Altogether, the WG concluded that with the limitedly available amount of kinetic data, it was not possible to develop a PBK model that is sufficiently robust and reliable to answer the assessment question 1b of the mandate. The WG discussed what kinetic data would be required to develop a sufficiently robust PBK model. The outcome of these discussions is presented in Appendix 15.

3 | RESIDUES

3.1 | Metabolic pattern of acetamiprid and related metabolites identified in pesticide residue monitoring data for unprocessed and processed food of plant origin

3.1.1 | Data collection

In the framework of Article 31 of Regulation (EC) No 396/2005 on the yearly collection of monitoring data to draw the annual report on pesticide residues, EFSA has regularly received monitoring data on acetamiprid as the relevant marker of the residue definition for enforcement in commodities of plant origin. Data on acetamiprid main metabolite (N-desmethyl-acetamiprid (IM-2-1)) were also provided by some Member States on a voluntary basis, but since metabolites are not part of the residue definition established in Regulation (EC) No 396/2005, only limited information is available.

In line with the ToR (2a) of the present mandate, EFSA consulted EU Member States on the availability of additional monitoring data not yet submitted to EFSA, in particular monitoring data in plant commodities for the metabolite N-desmethyl-acetamiprid (IM-2-1) and other metabolites identified in the available metabolism studies performed with acetamiprid. The screening for the availability of such data was restricted to the period of 2018–2022. For IM-2-1, some Member States reported that such data are available; however, no information could be provided by Member States for other metabolites.

Following the consultation of Member States, EFSA launched an ad hoc data collection for monitoring data relevant for this mandate. Data could be submitted between 1 November and 30 November 2022 offering the possibility for Member States to provide monitoring data for N-desmethyl-acetamiprid (IM-2-1).

The LOQ reported by the national authorities for the analysis of acetamiprid in all reported samples ranged between 0.001 and 0.1 mg/kg. Most of the samples (99%) were analysed with an LOQ equal or less than 0.01 mg/kg. A small proportion of samples (0.4%) were analysed with LOQ of 0.05–0.1 mg/kg and mainly correspond to high oil content commodities (e.g. olives) or matrices difficult to analyse (e.g. tea, herbal infusion…). Therefore, these samples are all deemed relevant from the data analysis.

In 17,634 samples, both acetamiprid and metabolite IM-2-1 were analysed. These data have been reported only by Austria, Germany and Sweden (Table 20).

Most of the samples (94%) correspond to unprocessed commodities. The remaining 6% are from processed food. Considering that acetamiprid was found to be stable under conditions representative for food processing (EFSA, 2016a), EFSA decided to include the results for processed products in the data analysis.

The LOQ reported by the national authorities for the analysis of metabolite IM-2-1 in the reported samples ranged between 0.001 and 0.05 mg/kg. Most of the samples (99%) were analysed with an LOQ equal or less than 0.01 mg/kg. The samples analysed with LOQ of 0.05 mg/kg (0.2%) mainly correspond to matrices difficult to analyse (e.g. coffee, tea…). Therefore, these samples are all deemed relevant from the data analysis.

No data were excluded from the data analysis.

All the monitoring data received are available in the overview file (xls), which is published in Appendix 16 of the present statement. An overview is presented below.

TABLE 20 Number of samples analysed for both acetamiprid and metabolite IM-2-1 per reporting country and per year.

Country	2018	2019	2020	2021	2022	TOTAL
Austria	1053	975	899	943	97	3967
Germany	2701	2555	2264	2624	–	10,144
Sweden	–	1374	1408	741	–	3523
Total	3754	4904	4571	4308	97	17,634

3.1.2 | Overview of the results in samples analysed for parent and metabolite IM-2-1

Within the **17,634** samples of plant origin analysed for both acetamiprid and metabolite IM-2-1, there are commodities belonging to the groups of leafy crops, fruit crops, pulses/oilseeds, cereals, root crops, stem vegetables, fungi and algae.

Of these samples, acetamiprid was quantified above the LOQ in **1700** samples and the metabolite IM-2-1 was quantified above the LOQ in only **805** samples. The metabolite IM-2-1 was quantified above the LOQ mainly in fruit crops (645 quantifications) and leafy crops (148 quantifications) but a few positive findings were also retrieved in pulses/oilseeds and in stem vegetables (**Tables 21 and 22**).

TABLE 21 Overview of the available monitoring data.

Crop category	N samples	Number of results above LOQ (VAL) for acetamiprid	Number of results above LOQ (VAL) for metabolite IM-2-1
Leafy	2966	246	148
Fruit	10,779	1433	645
Pulses/oilseeds	914	15	11
Cereals	773	2	0
Root	1513	2	0
Stem	387	1	1
Fungi	294	1	0
Algae	4	0	0
Total	17,634	1700	805

A short list of raw commodities in which the metabolite IM-2-1 was quantified in more than 10 samples is reported in Table 22. These commodities all belong to the leafy and fruit crop groups. The max concentrations of IM-2-1 are above 0.01 mg/kg in all commodities of the short list. However, the overall average amounts of metabolite IM-2-1 is above 0.01 mg/kg in only 3 commodities: spinaches (0.05 mg/kg), Roman/rocket (0.013 mg/kg) and chilli peppers (0.011 mg/kg). These commodities are also those where the highest maximum levels of IM-2-1 were found: 4.8 mg/kg, 0.52 mg/kg and 0.23 mg/kg, respectively.

TABLE 22   Short-list of unprocessed commodities where metabolite IM-2-1 was quantified in more than 10 samples.

Commodity	Number of results above LOQ for metabolite IM-2-1	Number of raw samples analysed for metabolite IM-2-1	Average value for metabolite IM-2-1[a] (mg/kg)	Max value for metabolite IM-2-1 (mg/kg)
Sweet peppers/bell peppers	64	648	0.006	0.160
Courgettes	60	262	0.005	0.070
Cherries (sweet)	59	216	0.007	0.017
Apples	54	872	0.008	0.049
Spinaches	51	378	0.050	4.800
Table grapes	40	651	0.007	0.110
Grapefruits	37	174	0.007	0.100
Peaches	32	461	0.007	0.022
Tomatoes	31	727	0.007	0.110
Pears	30	504	0.007	0.017
Aubergines/eggplants	29	197	0.005	0.029
Cucumbers	26	550	0.007	0.013
Roman rocket/rucola	26	112	0.013	0.516
Lettuces	25	653	0.004	0.047
Blueberries	22	179	0.004	0.077
Granate apples/ pomegranates	22	232	0.005	0.030
Mandarins	22	338	0.007	0.023
Currants (black, red and white)	18	103	0.004	0.019
Plums	17	234	0.002	0.019
Chilli peppers	14	181	0.011	0.230
Melons	11	276	0.007	0.011
Oranges	10	384	0.007	0.015

[a]Average value was calculated using the upper bound approach; therefore, sample results below LOQs were considered at the value of the LOQ for the calculations.

In addition to the above results, it is noted that in commodities belonging to the pulses/oilseeds group, the metabolite IM-2-1 was found above the value of 0.01 mg/kg in only one sample of beans with pods (0.012 mg/kg), its average in each commodity not exceeding the value of 0.005 mg/kg.

Processed commodities:

The occurrence of metabolite IM-2-1 in processed commodities is slightly lower compared to raw commodities and remain overall very small (Table 23). The metabolite IM-2-1 was found in a few processed items of leafy (3) and fruit (15) commodities, mainly corresponding to drying processes (e.g. dried apricots, dried herbs, dried peppers, goji dried berries, curry leaves, paprika powder, raisins, jasmine flowers…). Goji dried berry is the commodity where the max quantified values were found (0.12 mg/kg; 3 samples > LOQ), followed by curry leaves (0.058 mg/kg; 1 sample > LOQ) and Jasmine flowers (0.033 mg/kg; 1 sample > LOQ).

TABLE 23 Occurrence of metabolite IM-2-1 in unprocessed vs processed commodities.

	N samples		Number of results above LOQ (VAL) for metabolite IM-2-1		% of occurrence above LOQ for metabolite IM-2-1	
	Unprocessed	**Processed**	**Unprocessed**	**Processed**	**Unprocessed**	**Processed**
Leafy crops	2863	103	145	3	5.1	2.9
Fruit crops	10,156	623	630	15	6.2	2.4
Pulses/oilseeds	788	126	11	0	1.4	0
Cereals	553	220	0	0	0	0
Root crops	1501	12	0	0	0	0
Stem vegetable	382	5	1	0	0.3	0
Fungi	268	26	0	0	0	0
Algae	0	4	0	0	n.r.	0
Total	16,511	1119	787	18	4.8	1.6

Abbreviation: n.r., not relevant.

Conclusion:

The available monitoring data indicate that metabolite IM-2-1 may be a relevant component of the residues in some commodities belonging to the fruit and leafy crop groups and, very unlikely in pulses and oilseeds crops. Therefore, further investigations on the relative amount of metabolite IM-2-1 compared to the parent compound was done in commodities belonging to these crop categories. No major differences were found between unprocessed and processed commodities.

3.1.3 | Relative amount of metabolite IM-2-1 compared to parent compound

For the crop groups of concern based on the above screening (fruit crops, leafy crops and pulses and oilseeds crops), the relative amount of metabolite IM-2-1 compared to acetamiprid was further investigated using the available monitoring data.

In the samples of leafy crops, fruit crops and pulses/oilseeds analysed for acetamiprid and metabolite IM-2-1, most of the results ($n = 12,921$) simply indicate both acetamiprid and IM-2-1 to remain below the LOQ; these samples are called '[LOQ-LOQ]' samples in the text and tables below. These results are not deemed useful to investigate the ratio between the two compounds.

However, the subset of results where acetamiprid and/or IM-2-1 were quantified are of interest to investigate further the ratio between the two compounds. A total of 1738 samples were deemed relevant. The following abbreviations were applied to define the different cases:

- '[VAL-LOQ]': Samples where acetamiprid was quantified (VAL) but metabolite IM-2-1 was below the LOQ.
- '[LOQ-VAL]': Samples where acetamiprid was below the LOQ but metabolite IM-2-1 was quantified (VAL).
- '[VAL-VAL]': Samples where acetamiprid and metabolite IM-2-1 were quantified (VAL).

Taking the [LOQ-LOQ] samples apart and only focusing on the [VAL-LOQ], [LOQ-VAL] and [VAL-VAL] samples, it appears that the parent compound was quantified in most of the samples (97%); the metabolite IM-2-1 was quantified in approximately half of them (46%). There are only 44 samples (22 in fruit crops and 22 in leafy crops) where only the metabolite IM-2-1 was quantified, while acetamiprid was below the LOQ (Table 24).

TABLE 24 Acetamiprid and IM-2-1 quantification in different crop groups.

	[LOQ-LOQ] acetamiprid LOQ-IM-2-1 LOQ	[VAL-LOQ] acetamiprid VAL- IM-2-1 LOQ	[LOQ-VAL] acetamiprid LOQ- IM-2-1 VAL	[VAL-VAL] acetamiprid VAL- IM-2-1 VAL
Leafy crops	2698	120	22	126
Fruit crops	9324	810	22	623
Pulses and Oilseeds	899	4	0	11
Total	**12,921**	**934**	**44**	**760**

To quantify the relative amount of metabolite IM-2-1 compared to acetamiprid, EFSA defined the ratio 'IM-2-1/acetamiprid' (concentration of IM-2-1 as reported in the monitoring data divided by concentration of acetamiprid as reported in the monitoring data) as a relevant indicator. This ratio could be calculated for each sample where data on acetamiprid and

metabolite IM-2-1 were available. However, the samples considered relevant to calculate this ratio were only those where acetamiprid and/or its metabolite IM-2-1 were quantified above the LOQ. The following considerations were made for each case:

- [VAL-LOQ]: These samples are relevant because they reflect cases of crops probably treated with acetamiprid (because of the demonstrated presence of acetamiprid) for which the parent compound was still the main component of the residues. For each sample, a ratio was calculated considering the LOQ for IM-2-1 divided by the parent concentration reported in the same sample.
- [LOQ-VAL]: These samples are relevant because they reflect cases of crops probably treated with acetamiprid (because of the demonstrated presence of IM-2-1) for which the metabolite was the main component of the residues. For each sample, a ratio was calculated considering the IM-2-1 concentration reported in the same sample divided by the LOQ for acetamiprid.
- [VAL-VAL]: These samples are relevant because they reflect cases of crops probably treated with acetamiprid (because of the presence of acetamiprid and its desmethyl metabolite). For each sample, a ratio 'metabolite IM-2-1/acetamiprid' could directly be calculated without further assumption.
- [LOQ-LOQ]: These samples were not considered to calculate ratios.
- As no major differences were found between unprocessed and processed commodities, all commodities were used together to derive ratios.

The summary statistics of the ratio calculations for each crop group are reported in Table 25. For the calculation of the average concentration for acetamiprid and metabolite IM-2-1, the upper bound approach was used. Therefore, sample results below LOQs were considered at the value of the LOQ.

TABLE 25 Summary statistics and ratios 'concentration of IM-2-1/ concentration of acetamiprid' in different crop groups.

	Average parent (± SD) [Max Val] (mg/kg)[a]	Average metabolite IM-2-1[a] (± SD) [Max VAL] (mg/kg)	Number of relevant ratio[b]	Average ratio[b] (± SD) [Min-Max]	N ratio > 1
Leafy crops	0.020 (±0.169) [4.6]	0.011 (±0.100) [4.8]	268	1.61 (±4.33) [0.01–35]	55
Fruit crops	0.011 (±0.051) [3.7]	0.006 (±0.006) [0.23]	1455	0.43 (±0.50) [0.01–10]	53
Pulses and Oilseeds	0.007 (±0.005) [0.072]	0.007 (±0.004) [0.03*][c]	15	0.56 (±0.32) [0.08–1]	0

[a]The calculated average concentrations for acetamiprid and metabolite IM-2-1 are derived from the samples where acetamiprid and/or IM-2-1 were analysed. The upper bound approach was used (sample results below LOQs were considered at the value of the LOQ).

[b]Average and median ratio derived from the individual ratios calculated for each sample, using the upper bound approach (sample results below LOQs were considered at the value of the LOQ).

[c]It is noted that a higher value of 0.03* mg/kg reported for P/O corresponded to the enforcement LOQ used to analyse the sample. The highest quantified value is 0.012 mg/kg.

Leafy crops:

Metabolite IM-2-1 is below the LOQ in 2818 samples out of 2966 samples available on leafy crops. Using the upper bound approach, the overall average concentration for the metabolite IM-2-1 in leafy crops is 0.011 mg/kg. It is two times lower than the average concentration for the parent compound in leafy crops (0.02 mg/kg) (the calculated average for acetamiprid is derived from the samples where acetamiprid and IM-2-1 were analysed).

Looking at the 268 relevant ratios 'IM-2-1/acetamiprid' calculated at sample level, the average is 1.61 with a wide distribution. This means that metabolite IM-2-1 does represent a significant part of the residues in some samples of leafy crops.

In 55 out of the 262 samples where a ratio could be calculated (21% of the cases), the concentration of IM-2-1 was higher than the concentration of acetamiprid (ratio above 1). The corresponding commodities are reported in Table 26, which also provides an overview of the occurrence of IM-2-1 and its relative amount compared to acetamiprid.

TABLE 26 IM-2-1 occurrence and relative amount compared to acetamiprid in leafy commodities where at least one ratio > 1 was observed.

Commodity	Average IM-2-1 (mg/kg)	Max IM-2-1 (mg/kg)	Average ratio (IM-2-1/acetamiprid)	N ratio > 1/N relevant ratio
Brussels sprouts	< 0.01	0.01	1.02	1/4
Chives	< 0.01	0.15	**3.62**	**3/3**
Kales	< 0.01	0.18	**1.86**	**3/7**
Lettuces	< 0.01	0.05	0.31	1/108
Parsley	< 0.01	0.04	0.77	1/13
Spinaches	0.05	4.8	**6.57**	**46/51**

The main commodities where ratios are above 1 are spinach (90% of the cases), and in minor proportions in chives and kale. The highest mean ratios calculated at commodities level were also found in spinaches, chives and kale (in bold in the table). In spinaches, the overall average for IM-2-1 is 0.05 mg/kg, which is also the highest average calculated at commodity level.

The average ratios 'IM-2-1/acetamiprid' calculated in leafy commodities other than spinach, chives and kales are significantly lower, ranging between 0.02 (curry leaves) and 1 (Brussels sprouts).

Overall, it is concluded that the metabolite IM-2-1 is a relevant component of the residues in leafy crops. However, this conclusion is mainly driven by observations made in the monitoring of a few specific commodities. In particular, very high proportions of IM-2-1 compared to acetamiprid were found in spinaches, kales and chives. Spinach was also present in the short-listed commodities where metabolite IM-2-1 was quantified in more than 10 samples (see Section 3.1.2).

Fruit crops:

Metabolite IM-2-1 is below the LOQ in 10,134 samples out of 10,779 samples available on fruit crops. Furthermore, the overall average for the metabolite IM-2-1 in fruit crops is below 0.01 mg/kg and thus remains in lower proportions compared to the parent compound. At commodity level, the average concentrations of IM-2-1 also remain low, not exceeding 0.01 mg/kg in any commodity. However, as identified in Table 22, the metabolite IM-2-1 was found in high concentrations in single samples of several commodities, with max values up to 0.23 mg/kg (chilli peppers).

Looking at the 1358 ratios calculated at sample level, the average is 0.43, with a standard deviation of 0.52. This means that metabolite IM-2-1 levels are generally lower than those of parent in fruit crops samples, however not negligeable.

In 53 out of the 1455 samples where a ratio could be calculated (4% of the cases), the concentration of IM-2-1 was higher than the concentration of acetamiprid (ratio above 1). The corresponding commodities are reported in Table 27, which also provides an overview of the occurrence of IM-2-1 and its relative amount compared to acetamiprid.

TABLE 27   IM-2-1 occurrence and relative amount compared to acetamiprid in fruit commodities where at least one ratio >1 was observed.

Commodity	Average IM-2-1 (mg/kg)	Max IM-2-1 (mg/kg)	Average ratio (IM-2-1 /acetamiprid)	N ratio > 1/N relevant ratio
Apples	< 0.01	0.05	0.45	**7/170**
Avocados	< 0.01	0.02	**1.25**	1/2
Courgettes	< 0.01	0.07	**1.01**	**21/64**
Grapefruits	< 0.01	0.10	0.49	1/54
Peaches	< 0.01	0.02	0.51	**3/89**
Plums	< 0.01	0.02	0.64	**4/45**
Prickly pears/cactus fruits	< 0.01	0.01	**5.0**	1/1
Sweet peppers/bell peppers	< 0.01	0.16	0.52	**8/105**
Tomatoes	< 0.01	0.11	0.61	**7/47**

The main commodities where ratios are above 1 are courgettes (33% of the cases), tomatoes (15%), plums (9%), sweet peppers (8%), apples (4%) and peaches (3%). The highest mean ratios calculated at commodities level were found in prickly pears/cactus fruits, avocadoes and courgettes.

In prickly pears/cactus fruits, the ratio of 5 was based on one sample only where the concentration of IM-2-1 was quantified at the level of 0.005 mg/kg while acetamiprid was not quantified (LOQ = 0.001 mg/kg). A similar situation is identified in avocadoes with the value for IM-2-1 of 0.003 mg/kg. The max values for IM-2-1 observed in these commodities are 0.01 mg/kg and 0.02 mg/kg, respectively. Therefore, the calculated ratios are not reflecting a significant occurrence of metabolite IM-2-1 in these two commodities.

In courgettes, the average concentration of IM-2-1 is low (< 0.01 mg/kg) and ranges between 0.001 and 0.07 mg/kg. The highest ratio values (above 1) are observed in samples where quantified concentrations of parent and IM-2-1 are between 0.014 and 0.017 mg/kg, respectively.

In the other commodities listed in Table 27, the average ratios are between 0.49 and 0.64. This means that in those commodities (apples, grapefruits, peaches, plums, sweet peppers/bell peppers and tomatoes), the metabolite IM-2-1 account for 50% of the parent compound concentration, on average.

Overall, it can be concluded that the metabolite IM-2-1 is a relevant component of the residues in fruit crops. This conclusion is driven by observations made in the monitoring of major fruit crops (courgettes, apples, grapefruits, peaches, plums, sweet peppers/bell peppers and tomatoes). In these commodities, the proportions of IM-2-1 are generally not higher than those of acetamiprid, but significant. It should also be noted that these commodities were all present in the short-listed commodities where metabolite IM-2-1 was quantified in more than 10 samples (see Table 22).

<u>Pulses/oilseeds:</u>

The overall average for the metabolite IM-2-1 in pulses and oilseeds is below 0.01 mg/kg. Metabolite IM-2-1 is below the LOQ in 903 out of the 914 available samples available on pulses and oilseeds. The 11 samples in which metabolite IM-2-1 was quantified correspond to beans with pods (7), peas with pods (2) and peas without pods (2). Therefore, there are no pulses and oilseeds commodities in the short-listed commodities where metabolite IM-2-1 was quantified in more than 10 samples.

The quantified values ranged between 0.001 and 0.012 mg/kg.

In all samples, the calculated ratio was below the value of 1, meaning that the concentration of IM-2-1 was always lower than the one of acetamiprid.

Overall, it is concluded that the metabolite IM-2-1 is not a relevant component of the residues in pulses and oilseeds, based to the available monitoring samples.

3.2 | Metabolic pattern identified in metabolism studies relevant for food products of plant origin

3.2.1 | Primary crop metabolism overview

In the framework of the renewal of the approval for the active substance acetamiprid under Commission Implementing Regulation 844/2012, the applicant submitted metabolism studies in primary crops representative for fruit and fruiting vegetables, root crops, leafy vegetables and pulses/oilseeds. These studies were performed to elucidate the metabolic pattern expected in food derived from crops treated with acetamiprid. The key parameters on the design of these metabolism studies are summarised in Table 28.

These studies were previously assessed at EU level and therefore the assessment of the validity of these metabolism studies was not reopened by EFSA under the present mandate. Furthermore, no new metabolism studies have been collected under the present mandate.

TABLE 28 Available primary crop plant metabolism studies performed with radiolabelled acetamiprid.

Crop group	Crops	Application	Sampling (day, DAT)	Comments
Fruit	Aubergine	Foliar (dotting on leave and fruit surface) (1× 9.5 g/100 L)	7, 14 DAT	[pyridine-2,6-^{14}C]-labelled acetamiprid
	Apple	Foliar (dotting on leave: 1× 208 g/ha)	0, 7, 14, 28, 62, 90 DAT	[pyridine-2,6-^{14}C]-labelled acetamiprid
		Foliar (dotting on fruit: 1× 104 g/ha)	0, 14, 28, 62 DAT	
Root	Carrot	Foliar (2× 100 g/ha)	14 DAT	[pyridine-2,6-^{14}C]-labelled acetamiprid
Leafy	Cabbage	Foliar (1× 301.5 g/ha) (study 1)	0, 7, 14, 21, 28 DAT	[pyridine-2,6-^{14}C]-labelled acetamiprid
		Soil treatment (1× 5940 g/ha)	7, 14, 28 DAT	
		Foliar (298.5 g/ha) (study 2)	0, 7, 14, 28, 63 DAT	Cyano-^{14}C labelled acetamiprid
Pulses and Oilseeds	Cotton	Foliar (4× 123 g/ha)	14, 28 DAT	[pyridine-2,6-^{14}C]-labelled acetamiprid

Abbreviation: DAT, days after treatment.

Acetamiprid was identified as a major component of the total radioactive residue (TRR) in all crop parts except cabbage head and cotton seed. Acetamiprid accounted for 79%–97% TRR in aubergine and apple fruits, for 61%–99% TRR in cabbage aerial parts, and for 27%–34% TRR in carrot mature samples (root and tops).

In cabbage head and cotton seeds, acetamiprid accounted for no more than 0.3% TRR and 5% TRR, respectively. The main components identified in these crop parts was the **6-chloronicotinic acid metabolite (IC-0)** (up to 46% TRR cabbage head and cotton seeds) and its methyl-ester (up to 13% TRR in cotton seed). IC-0 was also detected in carrot roots up to 26% TRR (0.02 mg/kg).

The **metabolite IM-2-1 (N-desmethyl-acetamiprid)** was below 10% of the TRR in all crop parts (≤ 4% TRR in aubergine and apple fruits, ≤ 7% TRR in cabbage samples, ≤ 6% TRR in carrot mature samples, ≤ 8% TRR in cotton seeds) except in apple leaf samples. In the latter, the metabolite IM-2-1 was found up to 16% TRR (3.64 mg eq./kg) at longer pre-harvest intervals (PHI).

The metabolite **IM-1-4** was also found in significant proportions, but only in carrot leaves: 15% TRR in mature samples (43% TRR in immature samples). In carrot tops samples taken at maturity, another compound (conjugate form of **IM-0**) was found up to 33% TRR. The IM-0 was also found in immature carrot flesh samples (14% TRR) (Table 29).

TABLE 29 Compounds identified above 10% TRR in different plant matrices.

Main compounds	Apple, aubergines (fruits) [%TRR]	Apple leaf [%TRR]	Cabbage (aerial parts) [%TRR]	Cabbage (head) [%TRR]	Carrot root/flesh (mature samples) [%TRR]	Carrot tops (mature samples) [%TRR]	Cotton seed [%TRR]
Acetamiprid	**79–97**	**49–96**	**61–99**	≤ 0.3	**33/34**	**27**	≤ 5
IM-2-1	2–4	1–**16**	≤ 7	–	4/4	6	6–8
IM-1-4	≤ 1	≤ 2	≤ 1	–	5/6	**15**	–
IC-0	≤ 1	≤ 2	≤ 1	**46**	**26/31**	1	**24–46**
IC-0-methyl ester	–	–	–	–	–	–	7–**13**
IM-0 (and conjugate)	–	≤ 1	≤ 1	–	7/7	**33**[a]	0

[a]IM-0 glc conjugate.

3.2.2 | Metabolite IM-2-1 in fruit and leafy crops according to metabolism studies

Further detailed results of metabolism studies are presented below for the metabolite IM-2-1 in fruit and leafy commodities since, based on the monitoring results this compound was significantly found in samples of crops belonging to these crop groups (see Section 3.1). Additionally, data on the leafy parts of other crops (e.g. carrots), where available, were considered regarding the occurrence of metabolite IM-2-1.

In fruit crops (apples and aubergines, see Table 30), the metabolite IM-2-1 did not exceed the absolute level of 0.03 mg/kg (maximum value identified in apples at PHI 14 days). At longer PHI, the parent compound seems to be degraded (to 80.8% TRR at PHI 62 days), and therefore, a slight increase of IM-2-1 is observed (from 0.6 mg eq./kg at PHI 0 days to 3.7 mg eq./kg at PHI 62 days). However, metabolite IM-2-1 always remains in negligeable proportions (maximum 2%) compared to the parent compound. It should be noted that the method of applications used in the metabolism assay to generate data on fruits (dotting on fruit surface only) is not fully reflecting a broadcast foliar application on fruits and leafy parts of the crops at the same time.

TABLE 30 Detailed results for metabolite IM-2-1 in fruit commodities.

Commodity/study	PHI Days	Acetamiprid [%TRR]	mg/kg (as parent)	IM-2-1 [%TRR]	mg/kg (as parent)	Ratio (IM-2-1/acetamiprid) [based on mg/kg]
Aubergines/fruit treatment	7	95.9	0.38	0.4	0.0	n.r.
	14	93.9	1.10	n.d.	n.d.	n.r.
Apples/fruit treatment	0	97.1	0.47	0.6	0	n.r.
	14	89.9	1.3	2.3	0.03	**0.02**
	28	79.2	0.42	2.1	0.01	< 0.01
	62	80.8	0.24	3.7	0.01	< 0.01

Abbreviations: n.d., not detected; n.r., not relevant.

In leafy crop (cabbage), the metabolite IM-2-1 was found at levels up to 1.25 mg eq./kg at PHI 14 days after soil treatment, and at significant levels after foliar treatments (0.13; 0.20; 0.23 mg eq./kg at PHIs of 28–63 days), generally occurring at higher levels after longer PHI. The proportion of IM-2-1 compared to the parent compound remains low but reach percentage around 6% and 11% after foliar treatments at PHI of 28 days and 63 days (study 1), respectively. It should be noted that cabbage was the only leafy crop used in the metabolism studies and may not be representative of other leafy crops (and crops cycles) on which different findings have been observed in the monitoring data. However, according to applicable guidelines, the study was considered sufficient to depict the metabolism of acetamiprid in leafy crops following foliar treatment.

Furthermore, data on leafy parts of apples and aubergines were also collected after target applications on leaves (dotting). In apples leaves, very high levels of IM-2-1 were observed at long PHI (16% TRR; 3.64 mg eq./kg at PHI 90 days), also representing high proportions compared to the parent compound (32%). In aubergine leaves, metabolite IM-2-1 was present at lower proportions compared to the parent compound (maximum 2%).

In the metabolism study performed with carrots, data of residue composition in leafy parts of the crop (tops) were provided. Following foliar treatment, at the PHI of 14 days, metabolite IM-2-1 in carrot tops was identified at 5.92% TRR, accounting for up to 22% of the parent compound.

TABLE 31 Detailed results for metabolite IM-2-1 in leafy commodities and leafy parts.

Commodity/study	PHI Days	Acetamiprid [%TRR]	mg/kg (as parent)	IM-2-1 [%TRR]	mg/kg (as parent)	Ratio (IM-2-1/acetamiprid) [based on mg/kg]
Aubergine leaves/foliar treatment	7	89.2	20.02	1.0	0.22	0.01
	14	85.2	17.02	1.8	0.35	0.02
Apples leaves/foliar treatment	0	97.4	34.85	0.6	0.21	0.01
	7	94.4	35.71	1	0.39	0.01
	14	89.7	28.03	2.1	0.67	0.02
	28	80.2	19.98	4.8	1.19	0.06
	62	61.1	15.69	10.5	2.7	0.17
	90	49	11.5	15.6	3.64	**0.32**
Cabbage/Study 1 – foliar treatment	0	84.6	6.69	0.1	0.01	0.00
	7	90.8	4.54	1.6	0.08	0.02
	14	83.9	2.97	2	0.07	0.02
	21	76.5	2.32	3.4	0.1	0.04
	28	78.8	1.89	4.7	0.11	0.06
	63	66.7	1.84	7.2	0.2	0.11
Cabbage/Study 1 – soil treatment	7	90.2	93.11	0.8	0.84	0.01
	14	86.5	58.01	1.9	1.25	0.02
	28	60.5	17.2	2	0.56	0.03
Cabbage/Study 2 – foliar treatment	0	99.3	5.12	n.d.	n.d.	n.r.
	7	95.58	4.8	0.47	0.02	< 0.01
	14	88.65	3.56	2.05	0.08	0.02
	28	78.43	4.04	4.39	0.23	0.06
	63	63.84	2.09	4.09	0.13	0.06
Carrot tops/leaves – foliar treatment	14	26.85	0.12	5.92	0.03	0.22

Abbreviations: n.d., not detected; n.r., not relevant.

3.2.3 | Other available metabolism studies (rotational crops and hydrolysis studies)

Studies investigating the uptake of residues from soil resulting from the use of acetamiprid in primary crops (rotational crop field studies) and rotational crop metabolism studies in root crops (turnips), leafy crops (spinaches), cereals and oilseed crops (wheat) have been provided in the dossier submitted in the framework of the renewal of the approval of acetamiprid. In the framework of an application to modify the existing MRLs for acetamiprid in honey and various oilseed crops, an additional rotational crops study was assessed by Austria (EMS) (EFSA, 2022).

Since acetamiprid has a low persistence in soil (highest field DT_{90} 43 days), the metabolism studies in rotational crops were not conducted with acetamiprid but using the more persistent soil metabolite IM-1-5 (DT_{50} ranging from 319 to 663 days). The metabolism of metabolite IM-1-5 has been investigated after bare soil treatment at application rates of 266 g/ha and 160 g/ha (EFSA, 2016a, 2022). In both studies, crops were planted/sown on the day of the treatment (0 day plant back interval [PBI]).

In the different rotational crops investigated (wheat, turnip, spinaches), the metabolite IM-1-5, major soil metabolite of acetamiprid (which was also the test material of the studies) was the main component of the radioactive residues accounting in mature plant at harvest for 77%–94% TRR. No other metabolites or unidentified residues were observed in significant proportions in any crop commodity. A limited metabolism of IM-1-5 was observed in the rotational crops and no metabolic pathway was proposed for IM-1-5.

Field rotational crop studies evaluated under the EU pesticides peer review with acetamiprid applied onto the bare soil at ca. 300 g/ha, confirmed that acetamiprid, IM-1-4 and IM-1-5 residues are not expected to be present in rotational crops (EFSA, 2016a).

The effect of processing on the nature of acetamiprid was also investigated in the framework of the EU pesticides peer review (EFSA, 2016a). Studies were performed with the standard processing conditions representative of pasteurisation (20 min, 90°C, pH 4), baking/brewing/boiling (60 min, 100°C, pH 5) and sterilisation (20 min, 120°C, pH 6).

The standard hydrolysis studies showed that acetamiprid is hydrolytically stable under standard processing conditions representative of pasteurisation, baking/brewing/boiling and sterilisation.

3.3 | Residue definitions in plant commodities and conversions factors

3.3.1 | Residue definitions

The existing risk assessment and enforcement residue definition in plant commodities has been set by the EU pesticides peer review and is limited to parent acetamiprid (EFSA, 2016a).

The re-assessment of the residue definition for risk assessment in plant commodities under the ToR 2b of present mandate was performed following the principles defined in OECD guidance (OECD, 2009). Available studies investigating the metabolism acetamiprid in plant matrices as well as the monitoring data collected in this mandate, including occurrence data for the metabolite IM-2-1 were considered for this assessment.

With respect to the metabolic pathway of acetamiprid in plants, particular consideration was given to the following acetamiprid metabolites, which have been identified in the available metabolism studies: metabolite IC-0 and its conjugate, metabolite IM-1-4 and metabolite IM-2-1.

Metabolite **6-chloronicotinic acid (IC-0)** and its methyl-ester were the main metabolites identified in cabbage head and cotton seeds (> 10% TRR). Metabolite IC-0 was also found in carrot roots. The metabolite 6-chloronicotinic acid (IC-0) was observed in the rat metabolism, and therefore, its toxicity was concluded to be covered by the toxicity of the parent acetamiprid (EFSA, 2016a). Furthermore, this metabolite was not deemed relevant for the risk assessment residue definition in the previous EFSA assessments due to its lower acute toxicity compared to the parent compound and its absence of genotoxic potential (EFSA, 2011). For this compound, no monitoring data were reported to EFSA and, in the framework of the present mandate, there are no new data concerning its toxicity. Therefore, there are no new elements to rediscuss the inclusion of metabolite 6-chloronicotinic acid (IC-0) in the risk assessment residue definition.

Regarding metabolite **IM-0** (and its conjugate) and metabolite **IM-1-4** which were not found in edible parts of crops, but only in immature samples of carrots, there are also no new element (monitoring data or toxicological data) to rediscuss their inclusion in the risk assessment residue definition.

Consequently, metabolite 6-chloronicotinic acid (IC-0) (and its methyl-ester), metabolite IM-0 (and its conjugate) and metabolite IM-1-4 are not proposed to be included in the updated risk assessment residue definitions for plant commodities.

The desmethyl-metabolite of acetamiprid (**IM-2-1**) was observed in the rat metabolism and its toxicity is covered by the parent compound. In the framework of the present mandate, this conclusion was confirmed.

According to the available metabolism studies in primary crops, metabolite IM-2-1 was below 10% of the TRR (between 2% and 8%) in all edible crop parts (aubergine, apple, cabbage samples, carrot mature samples, cotton seeds) and therefore not considered relevant for the risk assessment by the EU pesticides peer review. However, samples of apple leaf gave an indication that levels of metabolite IM-2-1 could reach higher proportions at longer pre-harvest intervals of 62 and 90 days (PHI), up to 17% and 32% of the parent compound (ca. 11% and 16% TRR, respectively). Also, in carrot leaves metabolite IM-2-1 was present at 5.92% TRR, accounting for up to 22% of the parent acetamiprid (see Section 3.1). Since this metabolite was not considered relevant for the risk assessment by the EU pesticides peer review, residue trial data on the magnitude of IM-2-1 are not available and have not been required to authorise acetamiprid uses.

In the framework of the present mandate, monitoring data on the metabolite IM-2-1 were collected which confirm the occurrence of this metabolite in several commodities belonging to the groups of leafy and fruit commodities (see Section 3.1). Furthermore, in 6 commodities belonging to the leafy crops group and in 9 commodities belonging to the fruit crops group, the concentration of metabolite IM-2-1 was found to be above the concentration of acetamiprid in at least one sample (see Section 3.1.3). In each of these commodities, the average proportion of metabolite IM-2-1 compared to parent compound (mean ratio 'IM-2-1/acetamiprid') has been found to be above 30% (see Section 3.1.3). This information was considered sufficiently relevant to reconsider the conclusions of the EU pesticide peer review from the renewal on the relevance of metabolite IM-2-1 residues in plant commodities.

Based on these findings, it is therefore proposed to include metabolite IM-2-1 in the residue definition for risk assessment in leafy and fruit crops (Table 32), which is currently limited to acetamiprid.

Regarding pulses/oilseeds, root crops and cereals, the collected monitoring data do not reveal a significant occurrence of IM-2-1 in commodities belonging to these categories. Therefore, it is not proposed to modify the residue definition for risk assessment in pulses/oilseeds, root crops and cereals, which therefore remains acetamiprid.

Regarding the residue definition for enforcement, the available data do not indicate a need to modify the existing definition because acetamiprid is still a sufficient marker of the residues in all crop groups.

The conclusions of EFSA derived in the present assessment on the risk assessment and enforcement residue definitions for acetamiprid residues in plant commodities are compiled in a table below:

TABLE 32   Updated residue definitions in plant commodities.

Plant residue definition for monitoring (RD-Mo)	– Acetamiprid (all metabolism groups)
Plant residue definition for risk assessment (RD-RA)	– Fruit crops: sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid – Leafy crops: sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid – Pulses/oilseeds: acetamiprid – Root crops: acetamiprid – Cereals: acetamiprid

3.3.2 | Conversion factors

For leafy and fruit crops, conversion factors (CFs) from monitoring to risk assessment should be derived to consider the newly derived residue definition in the consumer risk assessment. Conversions factors are intended to recalculate the residue concentration expressed according to the residue definition for monitoring (acetamiprid) to the residue concentration expressed according to the residue definition for risk assessment (sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid). Therefore, CFs are generally calculated according to the following formula:

$$CF = [RD - RA]\text{mg} / \text{kg} / [RD - Mo]\text{mg} / \text{kg}.$$

In the absence of supervised residues trials analysing for acetamiprid and metabolite IM-2-1, tentative CFs can be derived on the basis of monitoring data and metabolism studies.

An estimate of the CFs can be derived at crop group level, using the **median ratio** of residues in each crop group as calculated from the submitted monitoring data. To minimise the potential overestimation of the derived conversion factor due to the LOQ values, EFSA did not consider for the calculation those samples where the metabolite N-desmethyl-acetamiprid (IM-2-1) was not quantified above the LOQ. Indeed, when these samples are considered using the upper bound approach, it artificially results in high ratio 'IM-2-1/parent' in the samples where the parent compound is quantified in low amounts. The median ratios 'IM-2-1/acetamiprid' were calculated for leafy crops and fruit crops separately. The median ratios 'IM-2-1/acetamiprid' were then recalculated expressing the metabolite IM-2-1 as parent compound,[14] resulting in ratios of 0.44 and 0.21 for leafy crops and fruits crops, respectively. Consequently, the corresponding CFs from enforcement to risk assessment could be derived for leafy crops and fruit crops, as follows (Table 33):

TABLE 33 Proposed conversion factors for leafy and fruit crops based on median ratios [IM-2-1]/[acetamiprid].

	Median ratio[a]: [IM-2-1]$_{as\ reported}$/ [acetamiprid]	Median ratio recalculated[b]: [IM-2-1]$_{as\ parent}$/ [acetamiprid]	Median CF: [RD-RA]/ [RD-Mo]
Leafy crops	0.42	0.44	1.44
Fruit crops	0.20	0.21	1.21

[a]Median ratio derived from the individual ratios calculated for each sample. Only samples where metabolite IM-2-1 was quantified were considered. The ratios are derived considering metabolite IM-2-1 expressed as IM-2-1.

[b]Median ratios recalculated considering metabolite IM-2-1 expressed as parent compound.

Furthermore, the proposed conversion factors can be discussed in the light of the metabolism studies available on leafy and fruit crops.

For leafy crops, the highest ratio 'IM-2-1/acetamiprid' observed in the samples taken in the metabolism studies is 0.32 and was found in apple leaf samples taken at PHI 90 days (see Table 31). This is in the same order of magnitude as the median derived from the monitoring data (0.4). Therefore, the CF of 1.4 (derived from median ratio of 0.4 in monitoring data) is considered appropriate to perform a conservative risk assessment for leafy crops.

Regarding fruit crops, the levels of metabolite IM-2-1 found in the metabolism studies are clearly lower than those found in leafy crops. The highest concentration of IM-2-1 was found in apple samples taken at PHI 14 days: 0.03 mg eq./kg (see Table 30). Considering the concentration of parent compound (2.3 mg/kg) found in the same sample, a ratio 'IM-2-1/acetamiprid' of 0.02 is calculated. This is 10 times lower than the median ratio derived from the monitoring data (0.2). The CF of 1.2 (derived from median ratio of 0.2 in monitoring data) is considered appropriate to perform a conservative risk assessment for fruit crops.

It should be noted that the proposed CFs for leafy and fruit crops derived from monitoring data should be considered tentative only. However, in the absence of supervised field trials analysing for parent and metabolite IM-2-1 simultaneously, these are the best estimates possible based on the available evidence.

3.4 | Consumer risk assessment and identification of MRLs posing risk to consumers

As defined in the ToR 3 of the mandate, EFSA performed the assessment of the chronic and acute consumer risk related to the existing EU MRLs for acetamiprid in all plant and animal products, using revision 3.1 of the EFSA PRIMo (EFSA, 2018a, 2019). The assessment was performed considering the Good Agricultural Practices (GAPs) and supporting residue trials already available to EFSA before this mandate. It includes GAPs and residue trials assessed in the framework of the initial MRL review (EFSA, 2011) and in the framework of the focussed assessment of certain MRLs of concern for acetamiprid (EFSA, 2018c) as well as in the framework of several MRL applications submitted in accordance with Article 6 of Regulation

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[14]Considering the molecular mass of 222.67 for acetamiprid and of 208.67 for metabolite IM-2-1.

(EC) 396/2005 (EFSA, 2012, 2013, 2014, 2015, 2016c, 2021, 2022). For certain plant commodities and animal commodities the EU MRL has been set on the basis of the Codex MRLs and therefore the JMPR evaluations were also considered.

The existing toxicological reference values (ADI = 0.025 mg/kg bw per day; ARfD = 0.025 mg/kg bw) for acetamiprid were derived in the framework of the EU pesticides peer review (European Commission, 2018). However, in the framework of the present mandate, EFSA derived new toxicological reference values for acetamiprid. The newly derived toxicological reference values are lower than the existing ones: ADI = 0.005 mg/kg bw per day; ARfD = 0.005 mg/kg bw. Consequently, in line with ToR 3, the newly proposed revised values were used to perform the risk assessment.

In the framework of the present mandate, EFSA also derived a new residue definition for risk assessment in leafy and fruit crops. Consequently, in line with ToR 3, EFSA performed two separate risk assessment scenarios: for the existing residue definitions for risk assessment (**scenario 1**) and for the newly proposed residue definitions for risk assessment (**scenario 2**).

3.4.1 | Consumer risk-assessment using the newly derived HBGVs and existing residue definition for risk assessment (scenario 1)

EFSA updated the assessment performed in the framework of the most recent MRL application, modifying the existing MRLs for acetamiprid in honey and various oilseed crops (EFSA, 2022) with the newly derived toxicological reference values. It is noted that the MRL proposals derived under the most recent MRL assessment (EFSA, 2022) have not been implemented yet but are reported in the draft Regulation SANTE/11278/2021. Therefore, these MRLs and risk assessment values were considered under the present scenario. In addition, the CXL for pistachios, assessed in 2021 (FAO, 2021) and recommended by EFSA for the implementation in the EU legislation and its corresponding risk assessment values were also considered for a complete updated picture of the potential consumer exposure.

The input values were therefore the risk assessment values as derived in previous EFSA assessments or in the evaluations by the JMPR, or, in few cases, the existing EU MRL. Where available, the peeling factors were applied to refine the exposure assessment. The reliable processing factors assessed in previous EFSA opinions were also considered to assess the exposure calculated for processed commodities. Therefore, the PF of 0.35 previously derived for gherkins pickles was considered. For orange juice, it was acknowledged that the PF of 0.13 previously derived was not fully supported by data. However, considering the significant effect of peeling in orange (peeling factor of 0.03), it was considered appropriate to refine the exposure calculation for orange juice with the available PF.

The input values used in the exposure calculations are reported in Appendix 17 and the PRIMo file is published in Appendix 20.

Based on this calculation, EFSA identified an exceedance of the ARfD for **36 commodities**, for which acute exposure ranged between **102%** (Roman rocket/rucola) and **582%** (pears) of the ARfD. An overview of the commodities for which exceedance of the ARfD was identified is presented in Table 34. Exceedances of the ARfD were also identified for several processed commodities. For most of them, exceedance of the ARfD was also identified for the corresponding raw agricultural commodities (RAC) listed in Table 34. However, exceedances of ARfD were also identified for elderberries juice (204% of the ARfD) and pumpkins boiled (195% of the ARfD) while for the RAC of these commodities, no exceedances of the ARfD were identified. For the remaining commodities the short-term exposure did not exceed the ARfD.

The highest estimated long-term dietary intake was **81%** of the **ADI** (NL toddler).

Overall, considering the updated hazard assessment for acetamiprid and its metabolites and considering the existing residue definition for risk assessment, the existing MRLs (or the MRL proposed in SANTE/11278/2021) for 38 commodities (36 plant commodities, 2 commodities of animal origin) were found to lead to acute intake concerns when assessed with the newest revision 3.1 of PRIMo. Regarding the long-term dietary intake, no exceedance of the ADI was identified.

3.4.2 | Consumer risk-assessment using the new endpoints derived in the present statement (scenario 2)

The new proposed residue definition for risk assessment in leafy and fruit crops is the 'sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid'. The metabolite IM-2-1 included in the risk assessment residue definition is still concluded to be of similar toxicity as the parent compound. Tentative conversion factors from monitoring to risk assessment were also derived for leafy and fruit commodities based on monitoring data (see Section 3.3.2).

The second scenario for the consumer risk assessment was performed using the new HBGVs, and the new residue definitions and conversion factors derived in the present statement. For fruit crops and leafy crops, the tentative conversion factors (1.21 and 1.44, respectively) were applied. The input values used in the exposure calculations are reported in Appendix 17 and the PRIMo file is published in Appendix 21.

Based on this calculation, EFSA identified an exceedance of the ARfD for **38 commodities**, for which acute exposure ranged between **106%** (table olives) and **822%** (lettuces) of the ARfD. An overview of the commodities for which exceedance of the ARfD was identified is presented in Table 34. For strawberries, it is noted that the margin of safety is very low (99% of the ARfD).

Exceedances of the ARfD were also identified for several processed commodities. For most of them, exceedance of the ARfD was also identified for the corresponding raw agricultural commodities (RAC) listed Table 34. However, exceedances

of ARfD were also identified for elderberries juice (247% of the ARfD) and pumpkins boiled (236% of the ARfD) while for the RAC of these commodities, no exceedances of the ARfD were identified.

Regarding **elderberries juice**, it is noted that the acute exposure result is obtained for a large portion of 257.59 g reported for the DE children. It is likely that this large portion does not correspond to pure juice (i.e. 100% fruits). Therefore, the calculated exposure may not consider the dilution factor possibly applicable for these consumption data. In the absence of processing trials for this commodity, EFSA was not able to perform further refinement. The available results are therefore likely overestimating the exposure resulting from the existing critical authorisations identified for elderberries.

Regarding **pumpkins boiled**, in the absence of processing trials for this commodity, EFSA was not able to proceed with further refinement.

The existing authorised GAPs of acetamiprid for which the acute consumer intake concerns were identified have been assessed and reported in the previous EFSA assessments. These data are therefore not reiterated in the present assessment.

The highest estimated long-term dietary intake was **93%** of the **ADI** (NL toddler).

Overall, considering the updated hazard assessment for acetamiprid and the updated residue definition for risk assessment, the existing MRLs (or the MRL proposed in SANTE/11278/2021) for 40 commodities (38 plant commodities and 2 commodities of animal origin) were found to lead to acute intake concerns when assessed with the newest revision 3.1 of PRIMo. Regarding the long-term dietary intake, no exceedance of the ADI was identified but the margin of safety is very small.

It is noted that compared to scenario 1, where the newly proposed residue definition for risk assessment was not considered, exceedances of the ARfD are identified for the same list of commodities, with the only addition of cranberries and table olives to the list (see Table 34).

TABLE 34 Raw Agricultural Commodities for which exceedance of the ARfD is identified in scenario 1 and/or in scenario 2 for the existing MRLs and associated risk assessment values.

Commodity	Existing MRL[a]	Scenario 1		Scenario 2	
		IESTI in % ARfD	Input value for RA (mg/kg)	IESTI in % ArfD	Input value for RA (mg/kg)
Lettuces	1.5	571% (children)	0.75	822% (children)	1.08
Pears	0.4	582% (children)	0.21	704% (children)	0.25
Apples	0.4	453% (children)	0.21	548% (children)	0.25
Apricots	0.8	399% (children)	0.57	482% (children)	0.69
Table grapes	0.5	365% (children)	0.25	441% (children)	0.3
Melons	0.2	334% (children)	0.11	404% (children)	0.13
Tomatoes	0.5	326% (children)	0.28	394% (children)	0.34
Quinces	0.8	315% (children)	0.64	381% (children)	0.77
Cauliflowers	0.4	255% (children)	0.22	367% (children)	0.32
Sweet peppers/bell peppers	0.4	274% (children)	0.23	331% (children)	0.28
Watermelons	0.2	269% (children)	0.11	325% (children)	0.13
Cucumbers	0.4	262% (children)	0.2	317% (children)	0.36
Head cabbages	0.4	221% (children)	0.25	319% (children)	0.24
Broccoli	0.4	208% (children)	0.25	300% (children)	0.36
Red mustards	3	202% (adults)	1.9	209% (adults)	2.74
Escaroles/broad-leaved endives	0.4	201% (children)	0.25	289% (children)	0.36
Cherries (sweet)	1.5	215% (children)	0.88	260% (children)	1.06
Blackberries	2	214% (children)	1	259% (children)	1.21
Bananas	0.4	209% (children)	0.11	253% (children)	0.13
Peaches	0.2	190% (children)	0.1	230% (children)	0.12
Courgettes	0.4	186% (children)	0.2	225% (children)	0.24
Raspberries (red and yellow)	2	185% (children)	1	224% (children)	1.21
Blueberries	2	182% (adults)	1	221% (adults)	1.21
Medlar	0.8	177% (children)	0.64	214% (children)	0.77
Granate apples/pomegranates	0.3	176% (children)	0.16	213% (children)	0.19
Spinaches	0.6	140% (children)	0.31	202% (children)	0.45
Currants (red, black and white)	2	158% (children)	1	191% (children)	1.21
Asparagus	0.8	166% (children)	0.43	166% (children)	0.43
Chards/beet leaves	0.6	117% (adults)	0.31	169% (adults)	0.45

(Continues)

TABLE 34   (Continued)

Commodity	Existing MRL[a]	Scenario 1		Scenario 2	
		IESTI in % ARfD	Input value for RA (mg/kg)	IESTI in % ArfD	Input value for RA (mg/kg)
Lamb's lettuce/corn salads	3	107% (children)	1.9	154% (children)	2.74
Roman rocket/rucola	3	102% (children)	1.9	147% (children)	2.74
Bovine: Liver	1	144% (children)	0.89	144% (children)	0.89
Wine grapes	0.5	119% (adults)	0.25	143% (adults)	0.3
Gooseberries (green, red and yellow)	2	118% (children)	1	142% (children)	1.21
Bovine: Edible offals (other than liver and kidney)	1	130% (children)	0.89	130% (children)	0.89
Aubergines/egg plants	0.4	103% (adults)	0.19	125% (adults)	0.23
Cranberries	2	90% (children)	1	109% (children)	1.21
Table olives	3	88% (children)	1.3	106% (children)	1.57

[a]Existing MRL or MRL proposed in SANTE/11278/2021.

3.5 | Identification of MRLs that do not pose risk to consumers

In line with Terms of Reference 3, EFSA performed further assessments to identify lower MRLs for acetamiprid that do not pose unacceptable risk to consumers for all the commodities listed in Table 34. In addition, as the margin of safety was found to be very low for strawberries, further attempts to identify lower MRL options were also performed for this commodity. In addition, further attention was also given to elderberries and pumpkins for which exceedance of the ARfD was only identified for processed commodities (juice and boiled, respectively).

3.5.1 | Data call

In the framework of the extended mandate, EFSA organised a call for data to identify fall-back GAPs and supporting residue trials that would lead to safe scenarios for all plant commodities identified in Table 34 and for strawberries.

The data call was issued for EU Member States. Consequently, data directly sent by applicants were not in the scope of this mandate. In addition, only authorised GAPs for acetamiprid and supporting valid residue trials previously assessed by Member State Authorities were in the scope of this data call.

EFSA launched the data call on **3 October 2023**. Member States were invited to submit their authorised Good Agricultural Practices (GAPs) and supporting residue data, which might support the setting of fall-back safe MRLs for acetamiprid. Member States were invited to upload their GAP forms (Excel format) and Evaluation Reports (word document) within the deadline of **30 November 2023**. All documents received are available as background documents to the present statement.

A total of 13 Member States replied to the call for data. Regarding the plant commodities of concern, a total of 509 GAPs were reported to EFSA. It is noted that 179 GAPs for crops not listed in Table 34 were also reported, partly due to possible overreporting when using crop grouping. These GAPs were therefore not considered by EFSA in the framework of the present mandate.

Based on the validation criteria available in the GAP form, a GAP was considered clear if the following key parameters were available: Formulation type; Application methods; Number of application(s) and interval between applications; Application rate; PHI (or growth stage during application). All the GAPs received within this data call are available in the GAP overview file (xls), which is published in Appendix 18 of the present statement. An overview of the collected data which are relevant in the scope of the present mandate is reported in Table 35 below.

TABLE 35   Overview of the data received during the call for data October–November 2023 for fall-back GAPs and supporting residue trials.

Member State	Number of relevant GAPs	Evaluation report
Austria	27	Austria (2023) (30 pages)
Belgium	36	Belgium (2023) (55 pages)
Czech Republic	13	Czech Republic (2023) (16 pages)
Finland	10	Finland (2023) (22 pages)
France	0	*No report*
Germany	58	Germany (2023) (34 pages)

TABLE 35   (Continued)

Member State	Number of relevant GAPs	Evaluation report
Greece	123	Greece (2023) (450 pages)
Hungary	19	*No report*
Italy	79	Italy (2023) (412 pages)
Portugal	84	Portugal (2023) (195 pages)
Spain	35	Spain (2023) (183 pages)
Sweden	16	Sweden (2023) (47 pages)
The Netherlands	9	The Netherlands (2023) (76 pages)

3.5.2 | Methodology

EFSA screened the 509 relevant GAPs received during the data call using the following stepwise approach for each GAP:

– Check if GAP compliant trials are available in the evaluation report of the Member State which submitted the GAP or in the evaluation reports of other Member States.
– If no GAP compliant trials are available, the GAP was discarded.
– If more than one GAP compliant trials are available, check the highest residue value (HR) reported by the Member States.
– If the HR does not pose acute intake concerns for raw agricultural commodities using PRIMo rev. 3.1, the revised HBGVs and the revised residue definition for risk assessment (RD-RA) and CFs, the GAP was retained as a candidate to derive fall-back MRL; further assessment was then performed.
– If the HR shows acute concerns for raw agricultural commodities using PRIMo rev. 3.1, the revised HBGVs and revised RD-RA and CFs, the GAP was discarded.
– If a safe fall-back GAP (and MRL) was identified for a given commodity (i.e. no acute consumer intake concerns), no further detailed assessment was performed for the less critical GAPs leading to lower MRLs and RA values.

The result of this screening is available in the GAP overview file (xls), which is published in Appendix 18 the present statement.

For those GAPs retained for further attention, EFSA performed validity assessment of residue trials in accordance with the Technical guidelines on data requirements for setting maximum residue levels, comparability of residue trials and extrapolation on residue data on products from plant and animal origin (European Commission, 2020). According to the cited Guidance, one parameter of the residue trials (e.g. application rate) may deviate by ±25% compared to the GAP under assessment, if other parameters remain unchanged. In some cases, Member State Authorities proposed to use residue data from trials conducted with different application rates to support the reported GAPs, using the proportionality concept and applying a scaling of residue data to the nominal application rate. EFSA assessed the Member State proposals in accordance with the EFSA Technical Report on the recommendations on the use of the proportionality approach in the framework of risk assessment for pesticide residues (EFSA, 2018b). This concept was applied to data from field trials conducted with application rates falling within a range of 0.3x and 4x the GAP rate, all other parameters being the same.

It is noted that detailed assessment of the validity of the residue trial studies (e.g. validation of analytical methods) was not undertaken by EFSA, relying on the Member States that only valid residue trials data were reported.

3.5.3 | Plant commodities with identified fall-back options

Following the approach defined in Section 3.5.2, fall-back MRL options could be identified for 30 plant commodities (including strawberries). The assessment of the fall-back GAPs, the individual results of the supporting valid residue trials and the derived fall-back MRLs and associated risk assessment values are reported in this section. The full description of the fall-back GAPs identified in this section is reported in Appendix 19.

Samples from all residue trials have been analysed for parent acetamiprid and no data on the magnitude of metabolite IM-2-1 were available. Residue trials data submitted for the fall-back GAPs are summarised in Table 36.

Apples and pears:

Identified fall-back GAP: 2 × 51 g a.s./ha; PHI: 14 days (BE, NEU, Outdoor, SG formulation)

The GAP reported by Belgium is supported by 7 GAP compliant residue trials performed on apples (Belgium, 2023). Residue data can be extrapolated to pears. One additional residue trial supporting this GAP should be required to derive a robust MRL proposal. In the meantime, tentative MRL and risk assessment values can be proposed for these commodities.

Quinces

Identified fall-back GAP: 2 × 75 g a.s./ha; PHI: 14 days (AT, NEU, Outdoor, SL formulation)

The GAP reported by Austria is supported by seven residue trials performed on apples (Austria, 2023). The available trials were performed with SP formulation and deemed acceptable to support the GAP defined for SL formulation. Residue data can be extrapolated to quinces. It is noted that three trials are GAP compliant (with 25% tolerance on the application rate) and that four trials are slightly overdosed (up to 109 g a.s./ha per application). Considering that four overdosed trials are not compliant with the GAP, five additional GAP compliant residue trials supporting this GAP should be required to derive a robust MRL proposal. In the meantime, tentative MRL and risk assessment values can be proposed for this commodity.

Medlar

Identified fall-back GAP: 2 × 100 g a.s./ha; PHI: 14 days (EL/PT, SEU, Outdoor, SL formulation)

The GAP reported by Greece and Portugal is supported by eight GAP compliant residue trials performed on apples (Portugal, 2023). Residue data can be extrapolated to medlar. It is noted that the available trials were performed with SP formulation and deemed acceptable to support the GAP defined for SL formulation. MRL and risk assessment values can be proposed for this commodity.

Apricots

Identified fall-back GAP: 2 × 51 g a.s./ha; PHI: 14 days (BE, NEU, Outdoor, SG formulation)

The GAP reported by Belgium is supported by four GAP compliant trials performed on peaches (Belgium, 2023). It is noted that the available trials were performed with SP formulation and deemed acceptable to support the GAP defined for SG formulation. Furthermore, apricots being a minor crop in northern EU, four residue trials are sufficient. However, according to the current extrapolation rules, a minimum of 50% of trials performed on apricots is required to support the use and to derive an MRL on apricots. Therefore, two additional GAP compliant residue trials performed on apricots should be required to derive a robust MRL proposal. In the meantime, tentative MRL and risk assessment values can be proposed for this commodity.

Peaches:

Identified fall-back GAP: 2 × 51 g a.s./ha; PHI: 14 days (BE, NEU, Outdoor, SG formulation)

The GAP reported by Belgium is supported by four GAP compliant residue trials performed on peaches (Belgium, 2023). It is noted that the available trials were performed with SP formulation and deemed acceptable to support the GAP defined for SG formulation. Peaches being a minor crop in northern EU, 4 residue trials are sufficient. MRL and risk assessment values can be proposed for this commodity.

Cherries

Identified fall-back GAP 1: 2 × 70 g a.s./ha; PHI: 3 days (EL, SEU, Outdoor, SL formulation)

The GAP reported by Greece is supported by four GAP compliant residue trials on cherries (Greece, 2023), all performed with SL formulation. Residues in the whole fruit were calculated as (weight of flesh × residue level)/weight of fruit. Cherries being a minor crop in southern EU, four residue trials are sufficient. MRL and risk assessment values can be proposed for this commodity.

Identified fall-back GAP 2: 2 × 100 g a.s./ha; PHI: 14 days (EL, SEU, Outdoor, SL formulation)

The GAP reported by Greece is supported by six GAP compliant residue trials, all performed with SL formulation (Greece, 2023). Cherries being minor crop in southern EU, the available residue trials are sufficient. MRL and risk assessment values can be proposed for this commodity.

Table and wine grapes:

Identified fall-back GAP: 1 × 75 g a.s./ha; PHI: 14 days (PT, SEU, Outdoor, SG/SP formulations)

The GAP reported by Portugal is supported by eight GAP compliant residue trials on grapes, all performed with SG formulation (Portugal, 2023). MRL and risk assessment values can be proposed for these commodities.

Strawberries:

Identified fall-back GAP: 1 × 50 g a.s./ha; PHI: 3 days (SE, EU, Indoor, SG formulation)

EFSA notes that acute consumer intake concerns have not been identified for strawberries for the existing use. However, as the margin of safety for consumer exposure was small, a fall-back GAP was assessed to provide supporting information should risk managers decide to revise the existing EU MRL in strawberries. The GAP reported by Sweden is supported by nine residue trials on strawberries, all performed with two applications instead of one, all other trial parameters being compliant with GAP (Sweden, 2023). The nine trials were performed with SG formulation. Considering the over-estimation of residues in the crop due to the higher number of applications, tentative MRL and risk assessment values can be proposed for this commodity. Eight GAP compliant residue trials should still be required to derive a robust MRL proposal.

Blackberries, Raspberries

Identified fall-back GAP: 2 × 50 g a.s./ha; PHI: 7 days (AT/BE/DE, NEU, Outdoor, SG formulation)

The GAP reported by Austria, Belgium and Germany is fully supported by four GAP compliant residue trials performed on raspberries, all performed with SG formulation (Austria, 2023; Belgium, 2023; Germany, 2023). In three of the trials, the sample storage period exceeded the demonstrated storage stability period of 12 months (i.e. 15 months); however, this was not considered a major deviation. Blackberries and raspberries being minor crops in EU, the available residue trials are sufficient. MRL and risk assessment values can be proposed for these commodities.

Blueberries, Cranberries, Currants, Gooseberries

Identified fall-back GAP: 2 × 60 g a.s./ha; PHI: 7 days (BE, NEU, Outdoor, SG formulation)

The GAP reported by Belgium is fully supported by 9 residue trials performed on currants, all performed with SG formulation (Belgium, 2023). Residue data can be extrapolated from currants to blueberries, cranberries and gooseberries. The trials were performed with an application rate of 50 g a.s/ha and are therefore underdosed but within the acceptable deviation of 25%. Nevertheless, Belgium (2023) proposed to apply a scaling factor of 1.2 to the residue data set. This approach is acceptable in accordance with EFSA technical report (EFSA, 2018b). MRL and risk assessment values can be proposed for these commodities.

Elderberries

Identified fall-back GAP: 2 × 50 g a.s./ha; PHI: 7 days (AT, NEU, Outdoor, SG formulation)

EFSA notes that acute consumer intake concerns have not been identified for the raw agricultural commodity elderberry for the existing use. However, as an exceedance of the ARfD for elderberries juice was identified, a fall-back GAP was assessed to provide supporting information should risk managers decide to revise the existing EU MRL in elderberries. The GAP authorised in Austria is fully supported by nine residue trials performed on currants, all performed with SG formulation (Belgium, 2023). Residue data can be extrapolated from currants to elderberries. The trials were performed with an application rate of 50 g a.s/ha and are therefore compliant with GAP. MRL and risk assessment values can be proposed for this commodity.

Table olives

Identified fall-back GAP: 2 × 70 g a.s./ha; PHI: 28 days (PT, SEU, Outdoor, SG formulation)

The GAP reported by Portugal is fully supported by 8 GAP compliant residue trials performed on table olives, all performed with SG formulation (Portugal, 2023). MRL and risk assessment values can be proposed for this commodity.

Tomatoes

Identified fall-back GAP: 2 × 100 g a.s./ha; PHI: 7 days (EL, SEU, Outdoor, SL formulation)

The GAP reported by Greece is supported by eight residue trials on tomatoes. The trials were performed with an application rate ranging between 90 and 94 g a.s./ha and are therefore deemed compliant with the GAP considering that the difference compared to the authorised GAP is less than 25% (Portugal, 2023). It is noted that the available trials were performed with SP formulation and deemed acceptable to support the GAP defined for SL formulation. MRL and risk assessment values can be proposed for this commodity.

Sweet peppers

Identified fall-back GAP: 2 × 80 g a.s./ha; PHI: 7 days (ES, SEU, Outdoor, SG formulation)

The GAP reported by Spain is supported by eight residue trials on peppers (Spain, 2023). It is noted that five trials are GAP compliant (within 25% tolerance on the application rate) and three trials are slightly overdosed (up to 112 g a.s./ha) and thus not fully GAP compliant. It is also noted that four trials were performed with SG formulation while the four others were performed with SP formulation, all deemed acceptable to support the GAP defined for SG formulation. However, three additional GAP compliant residue trials supporting this GAP should be required to derive a robust MRL proposal. In the meantime, tentative MRL and risk assessment values can be proposed for this commodity.

Aubergines

Identified fall-back GAP: 2 × 50 g a.s./ha; PHI: 3 days (EL/ES, EU, Indoor, SG formulation)

The GAP reported by Greece and Spain is not supported by GAP compliant trials. However, eight overdosed trials on tomatoes performed with an application rate ranging between 94 and 109 g a.s./ha are available. Spain proposed to apply the proportionality concept and scaled the residue results accordingly. The proportionality approach is applicable. All trials were performed with SG formulation in line with the reported GAP. Consequently, MRL and risk assessment can be proposed for this commodity.

Cucumber, Courgettes

Identified fall-back GAP: 2 × 50 g a.s./ha; PHI: 3 days (ES, SEU, Outdoor, SP formulation)

The GAP reported by Spain is supported by eight residue trials performed on courgettes (Spain, 2023). Residue data can be extrapolated from courgettes to cucumbers. It is noted that only one trial is GAP compliant and that seven trials are slightly overdosed (up to 69 g a.s./ha) and thus not fully GAP compliant. It is also noted that the available trials were performed with SG formulation and deemed acceptable to support the GAP defined for SP formulation. However, seven additional GAP compliant residue trials supporting this GAP should be required to derive a robust MRL proposal. In the meantime, tentative MRL and risk assessment values can be proposed for these commodities.

Melons, Pumpkins, Watermelons

Identified fall-back GAP: 2 × 100 g a.s./ha; PHI: 14 days (EL, SEU, Outdoor, SL formulation)

The GAP reported by Greece is supported by 10 GAP compliant residue trials performed on melons (Greece, 2023). Residue data can be extrapolated from melons to other cucurbits with inedible peel. Residues in pulp samples were also collected for a refined exposure assessment. All trials were performed with SL formulation, therefore fully supporting MRL and risk assessment values for melons, pumpkins and watermelons.

Broccoli, Cauliflower

Identified fall-back GAP: 1 × 70 g a.s./ha; PHI: 14 days (EL/ES/IT, SEU, Outdoor, SL formulation)

The GAP reported by Greece, Italy and Spain is fully supported by eight GAP compliant residue trials performed on broccoli (4) and cauliflowers (4), all performed with SL formulation (Greece, 2023; Italy, 2023; Spain, 2023). An additional GAP-compliant trial on broccoli was available but was considered a statistical outlier explained by agronomical conditions by all Member States who assessed this trial (Greece, Italy, Portugal, Spain). Therefore, EFSA considered appropriate to disregard this trial. MRL and risk assessment values can be proposed for these commodities.

Head cabbages

Identified fall-back GAP: 1 × 70 g a.s./ha; PHI: 14 days (EL/ES, SEU, Outdoor, SL formulation)

The GAP reported by Greece and Spain is supported by eight GAP-compliant residue trials (within 25% tolerance on the application rate) performed on head cabbages, all performed with SL formulation (Greece, 2023). MRL and risk assessment values can be proposed for this commodity.

<u>Lamb's lettuce, Roman rocket</u>

Identified fall-back GAP: 2 × 50 g a.s./ha; PHI: 3 days (DE, NEU, Outdoor, SG formulation)

The GAP reported by Germany is supported by eight GAP compliant trials performed on lettuces already assessed in the framework of previous MRL review (EFSA, 2011) and focussed MRL assessment of acetamiprid (EFSA, 2018c). Residue data can be extrapolated from lettuce to lamb's lettuce and rocket. MRL and risk assessment values can be proposed for these commodities.

<u>Red mustards</u>

Identified fall-back GAP: 1 × 70 g a.s./ha; PHI: 7 days (IT, SEU, Outdoor, SL formulation)

The GAP reported by Italy is supported by eight GAP compliant residue trials performed on lettuces (Italy, 2023). Residue data can be extrapolated from lettuce to red mustard. All trials were performed on open leaf varieties with SL formulation, therefore fully supporting MRL and risk assessment values proposed for red mustards.

TABLE 36 Summary of residues data from the supervised residue trials supporting fall back GAPs received in the framework of the present mandate.

Commodity	Region (MS)[a]	Residue levels observed in the supervised residue trials[b] (mg/kg)	Comments/source	Calculated MRL (mg/kg)	HR[c] (mg/kg)	STMR[d] (mg/kg)	CF[b]
Enforcement residue definition: acetamiprid **New proposed risk assessment residue definition**: sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid							
Apples Pears	**NEU - Outdoor (BE)**	0.016; 0.017; 0.019; 0.022; 0.026; 0.026; 0.029	Tentative MRL can derived based on 7 GAP compliant trials performed on apples (Belgium, 2023)	0.07 (tentative)	0.029	0.022	1.21
Quinces	**NEU – Outdoor (AT)**	GAP compliant trials: 2×0.03; 0.071 Overdosed trials (96–109 g a.s./ha): 2×0.03; 0.05; 0.07	Tentative MRL can derived based on a combined data set of trials performed on apples: 3 GAP compliant (with 25% tolerance on the application rate) and 4 overdosed (Austria, 2023)	0.15 (tentative)	0.071	0.03	1.21
Medlar	**SEU – Outdoor (EL/PT)**	2×0.02; 0.05; 0.06; 0.07; 0.08; 0.09; 0.20	MRL fully supported by GAP compliant trials performed on apples (Portugal, 2023)	0.3	0.20	0.065	1.21
Apricots	**NEU – Outdoor (BE)**	< 0.01; 0.02; 0.029; 0.042	Tentative MRL can derived based on 4 GAP compliant trials performed on peaches (Belgium, 2023)	0.08 (tentative)	0.042	0.025	1.21
Cherries	**SEU – outdoor (EL GAP 1)**	0.20; 0.21; 0.22; 0.33	MRL fully supported by GAP compliant trials performed on cherries (Greece, 2023)	0.8	0.33	0.215	1.21
Cherries	**SEU – Outdoor (EL GAP 2)**	0.10; 0.11; 0.17; 0.18; 0.21; 0.23	MRL fully supported by GAP compliant trials performed on cherries (Greece, 2023)	0.5	0.23	0.18	1.21
Peaches	**NEU – Outdoor (BE)**	< 0.01; 0.02; 0.029; 0.042	MRL fully supported by GAP compliant trials performed on peaches (Belgium, 2023)	0.08	0.042	0.025	1.21
Table grapes Wine grapes	**SEU – Outdoor (PT)**	2×0.01; 3×0.02; 0.03; 2×0.04	MRL fully supported by GAP compliant trials on grapes (Portugal, 2023)	0.08	0.04	0.02	1.21
Strawberries	**NEU – Outdoor (BE)**	0.03; 0.04; 0.05; 2×0.10; 0.11; 2×0.13; 0.18	Tentative MRL can derived based on 9 trials performed with 2 applications instead of 1 (Sweden, 2023). Residue trial on strawberries	0.3 (tentative)	0.18	0.10	1.21
Blackberries Raspberries	**NEU – Outdoor (AT/BE/DE)**	0.11; 0.14; 0.21; 0.29	MRL fully supported by GAP compliant trials performed on raspberries (Austria, 2023; Belgium, 2023; Germany, 2023)	0.6	0.29	0.175	1.21
Blueberries Cranberries Currants Gooseberries	**NEU – Outdoor (BE)**	Scaled results: 0.072; 3×0.144; 2×0.204; 0.28; 0.30; 0.34 [Results from underdosed trials (50 g a.s./ha): 0.06; 3×0.12; 2×0.17; 0.23; 0.25; 0.28]	MRL fully supported by underdosed trials (within 25% tolerance on the application rate) performed on currants (Belgium, 2023). The scaling-up of residue data by a factor of 1.2 was proposed by Belgium (2023). This approach is acceptable in accordance with EFSA (2018b)	0.7	0.34	0.204	1.21
Elderberries	**NEU outdoor (AT)**	0.06; 3×0.12; 2×0.17; 0.23; 0.25; 0.28	MRL fully supported by GAP compliant trials performed on currants (Belgium, 2023)	0.5	0.28	0.17	1.21
Table olives	**SEU – Outdoor (PT)**	0.012; 0.17; 0.22; 0.23; 0.25; 0.37; 0.42; 0.48	MRL fully supported by GAP compliant trials performed on table olives (Portugal, 2023)	0.9	0.48	0.24	1.21

TABLE 36   (Continued)

Commodity	Region (MS)[a]	Residue levels observed in the supervised residue trials[b] (mg/kg)	Comments/source	Calculated MRL (mg/kg)	HR[c] (mg/kg)	STMR[d] (mg/kg)	CF[b]
Tomatoes	**SEU – Outdoor (EL)**	4×<0.01; 0.012; 0.015; 0.026; 0.04	MRL fully supported by GAP compliant trials (within 25% tolerance on the application rate) on tomatoes (Portugal, 2023)	0.06	0.04	0.011	1.21
Sweet peppers	**SEU – Outdoor (ES)**	GAP compliant trials: 0.032; 0.036; 0.038; 0.04; 0.051 Overdosed trials (103–112 g a.s./ha): <0.01; 2×0.02	Tentative MRL can derived based on a combined data set of trials performed on sweet peppers: 5 GAP compliant (within 25% tolerance on the application rate) and 3 overdosed (Spain, 2023)	0.09 (tentative)	0.051	0.034	1.21
Aubergines	**EU – Indoor (EL/ES)**	Scaled results: 2×0.03; 0.04; 0.06; 2×0.07; 0.08; 0.09 [Results from overdosed trials (94–109 g a.s./ha): 2×0.06; 0.07; 0.11; 2×0.13; 0.15; 0.19]	MRL fully supported by overdosed residue trials performed on tomatoes (down-scaling factor of 0.5). Spain applied the proportionality principle accordingly (Spain, 2023)	0.2	0.09	0.065	1.21
Cucumbers Courgettes	**SEU – Outdoor (ES)**	GAP compliant trials: 0.03 Overdosed trials (63–69 g a.s./ha): 2×<0.01; 0.01; 4×0.02	Tentative MRL can be derived based on trials performed on courgettes: 1 GAP compliant and 7 overdosed (Spain, 2023)	0.05 (tentative)	0.03	0.02	1.21
Melons Pumpkins Watermelons	**SEU – Outdoor (EL)**	2×0.011; 3×0.012; 0.013; 0.016; 0.017; 0.024; 0.06 (Pulp: 9×<0.01; 0.011)	MRL fully supported by GAP compliant trials performed on melons (Greece, 2023). One value (0.06 mg/kg) was obtained at a PHI longer than 14 days	0.08	0.06 (pulp: 0.011)	0.013 (pulp: 0.01)	1.21
Broccoli Cauliflowers	**SEU – Outdoor (EL/ES/IT)**	Broccoli: <0.01, 3×0.02 Cauliflower: 3×<0.01; 0.04	MRL fully supported by GAP compliant trials performed on broccoli and cauliflowers (Greece, 2023; Italy, 2023; Spain, 2023)	0.06	0.04	0.015	1.44
Head cabbages	**SEU – Outdoor (EL/ES)**	5×<0.01; 0.01; 0.015; 0.021	MRL fully supported by GAP compliant trials (within 25% tolerance on the application rate) performed on head cabbages (Greece, 2023)	0.03	0.021	0.010	1.44
Lamb's lettuce Roman rocket	**NEU – Outdoor (DE)**	0.15; 0.19; 0.32; 0.39; 0.58; 0.63; 0.66; 0.75	MRL fully supported by GAP compliant trials performed on lettuce (EFSA, 2018c)	1.5	0.75	0.49	1.44
Red mustards	**SEU – Outdoor (IT)**	0.02; 0.03; 0.06; 0.06; 0.24; 0.29; 0.35; 0.49	MRL fully supported by GAP compliant trials performed on lettuce (Italy, 2023)	0.9	0.49	0.15	1.44

[a]NEU: northern EU; SEU: southern EU. The Member State who reported the GAP is reported between brackets.

[b]Samples from all residue trials have been analysed for parent acetamiprid only. No data on the magnitude of metabolite IM-2-1 are available. Tentative conversion factor from enforcement to risk assessment derived in the present statement are based on monitoring data (see Section 3.3.2).

[c]Highest residue level from the available dataset derived according to the residue definition for monitoring.

[d]Median residue level from the available dataset derived according to the residue definition for monitoring.

3.5.4 | Plant commodities with LOQ as the only fall-back MRL option

For two commodities (granate apples and asparagus), the less critical GAPs reported by Member states (see Appendix 19), although supported by data or by acceptable waiver, did not allow to derive a fall-back MRL above the enforcement LOQ.

Granate apples/pomegranates

Identified fall-back GAP: 1 × 50 g a.s./ha at BBCH 59; no PHI (PT, SEU, Outdoor, SG formulation)

The GAP reported by Portugal is supported by 4 GAP compliant residue trials (Spain, 2023). All trials were performed with SG formulation, in line with the reported GAP. All samples taken at maturity show residues below LOQ. Therefore, although MRL and risk assessment can be derived for this crop, the MRL proposal is at the LOQ.

Asparagus

Identified fall-back GAP: 2 × 65 g a.s./ha; post-harvest application on the field (DE, NEU, Outdoor, SG formulation)

The GAP reported by Germany consists of applications performed on the field after harvest of asparagus. No residues are expected in the crops harvested in the succeeding year. Therefore, based on the assessment of Germany, no residue trials are necessary. Although this rationale might be acceptable, it would only allow to derive MRL proposal at the LOQ.

3.5.5 | Plant commodities with no fall-back option

For the remaining commodities (bananas, lettuces, escaroles, spinaches, chards/beet leaves), several GAPs supported by GAP compliant residue trials were reported by Member States. However, none of these GAPs allowed to derive safe fall-back MRLs. It was found that even the least critical GAPs collected in the framework of this mandate (i.e. leading to the lowest HR value and MRLs) would still result in an exceedance of the newly derived ARfD, when using the available HR for the consumer risk assessment.

A summary of the acute exposure estimates resulting from the least critical GAPs identified for bananas, lettuces, escaroles, spinaches, chards/beet leaves is reported in Table 37. A complete of overview of all the GAPs received in the framework of this mandate is available in the GAP overview file (xls), which is published in Appendix 18 of the present statement.

It should be noted that for bananas, the GAP was not reported by Portugal in the GAP overview file. However, Portugal reported the GAP in its evaluation report (Portugal, 2023). Furthermore, this GAP and its supporting residue trials were assessed in a previous EFSA opinion (EFSA, 2014).

TABLE 37 Summary assessment for the plant commodities where no safe MRL proposal could be derived in the framework of the present mandate.

Commodity	Least critical GAP identified in the data call[a]	HR[b] (mg/kg)	CF[c]	%ARfD (with CF)[d]	%ARfD (w/o CF)[e]
Bananas	1 × 100 g a.s./ha; PHI: 21 days (PT – outdoor)[f]	0.049 (pulp)[f]	1.21	115% (children)	95% (children)
Lettuces	2 × 50 g a.s./ha; PHI of 7 days (EL/IT/PT - outdoor)	0.17	1.44	186% (children)	129% (children)
Escaroles/broad-leaved endives	2 × 30 g a.s./ha; PHI: 14 days (BE - outdoor)	0.16	1.44	185% (children)	129% (children)
Spinach	1 × 60 g a.s./ha; PHI of 10 days (PT - outdoor)	0.29	1.44	189% (children)	131% (children)
Chards/beet leaves	2 × 50 a.s./ha; PHI of 7 days (BE - outdoor)	0.31	1.44	169% (adults)	117% (adults)

[a]See also GAP overview file (xls), which is published as Appendix 18 of the present statement.

[b]HRs reported in this table are based on MS assessment according to the Evaluation Reports of the respective Member States. The HR is reported for acetamiprid only because no data are available for the metabolite IM-2-1.

[c]Tentative conversion factor from enforcement to risk assessment derived from monitoring data submitted in the present statement (see Section 3.3.2).

[d]Using the newly proposed ARfD of 0.005 mg/kg bw and the newly derived residue definition for risk assessment, therefore using the conversion factor derived in the present statement.

[e]Using the newly proposed ARfD of 0.005 mg/kg bw derived in the present statement but without considering the conversion factor (i.e. considering the existing residue definition for risk assessment).

[f]The GAP on bananas was not reported in the GAP overview file. However, Portugal reported the GAP in its evaluation report (Portugal, 2023). The residue trials supporting the outdoor GAP on bananas were assessed in a previous EFSA opinion (EFSA, 2014). The value of the HR in pulp (0.49 mg/kg) is retrieved from this previous EFSA opinion.

For bananas, an exceedance of the ARfD is identified when using the conversion factor of 1.21 tentatively derived for fruit crops. This approach takes into consideration the possible occurrence of metabolite IM-2-1 (for up to 21%) in fruit

crops, in accordance with the residue definition for risk assessment proposed in the present statement. The application of the tentative CF of 1.21, based on monitoring data, is adding non-standard uncertainty to the exposure assessment. Considering the order of magnitude of the calculated acute exposure (of 115% ARfD), it is expected that additional GAP compliant residue trials on banana analysing acetamiprid and metabolite IM-2-1 may allow further refinements for this crop, however, only in case a lower CF than 1.21 would be derived.

For lettuce, escaroles/broad-leaved endive, spinaches, chards/beet leaves, an exceedance of the ARfD is identified when using the conversion factor of 1.44. This approach takes into consideration the possible occurrence of metabolite IM-2-1 (for up to 44%) in leafy crops, in accordance with the residue definition for risk assessment proposed in the present statement. The application of the tentative CF of 1.44, based on monitoring data, is adding non-standard uncertainty to the exposure assessment. However, it should be noted that even without using these CFs, exceedance of the ARfD would still be identified (117%–131% ARfD). Although additional GAP compliant residue trials analysing acetamiprid and metabolite IM-2-1 would allow further refinement, it is not expected that the reported GAPs can support safe fall-back MRLs for these crops even in case where metabolite IM-2-1 would not be quantified at levels above LOQ.

Conclusion:

For bananas, lettuces, escaroles, spinaches, chards/beet leaves, despite the data call issued in the framework of the present mandate, it was not possible to demonstrate that the alternative lower MRL options would not pose acute intake risk to consumers.

3.5.6 | Fall-back options for commodities of animal origin

The existing MRLs for commodities of bovine and swine origin correspond to Codex MRLs derived by the JMPR (FAO, 2015), which were implemented into Regulation (EU) 2017/626. These Codex MRLs were considered safe for consumers based on a risk assessment performed with the ARfD of 0.025 mg/kg bw (EFSA, 2016b).

In the framework of the present mandate, using the newly derived ARfD of 0.005 mg/kg bw (see scenario 2), a risk for consumers has been identified for the existing MRLs on bovine liver (1 mg/kg) and bovine other edible offals (1 mg/kg). The consumer risk assessment was performed with the HR of 0.89 mg/kg derived in the Codex assessment (FAO, 2015). It is noted that this HR was based on the highest residues identified in kidney for the Australian beef cattle dietary burden (maximum dietary burden of 18 mg/kg dry matter (DM)). For liver, further refinement of this assessment is in principle possible, using the HR directly derived for liver (0.67 mg/kg; FAO, 2015). Nevertheless, the calculated acute exposure would still exceed the ARfD (108% ARfD).

Consequently, risk managers may consider withdrawing the existing Codex MRLs for bovine liver and bovine other edible offals from the EU Regulation.

In order to assess the residue concentrations expected in bovine liver and bovine other edible offals based on an EU livestock diet, EFSA updated the existing EU livestock dietary burden calculations. The last assessment of the EU dietary burden was performed in the framework of the focussed MRL assessment (EFSA, 2018c). EFSA now updated these previous calculations considering that fall-back MRLs and risk assessment values were derived in the present statement for plant commodities that can be fed to livestock (apples and head cabbage) and considering the possible impact of the new risk assessment residue definition derived for fruit and leafy crops (using indicative CFs).

The livestock dietary burden was therefore recalculated, for cattle only, according to OECD guidance (OECD, 2013) considering livestock intake of all feed products containing acetamiprid residues resulting from all authorised EU and authorised import tolerance uses, except for apples and head cabbages where fall-back GAPs were considered. The input values for all relevant commodities are summarised in Appendix 17 while the livestock dietary burden calculator (xls) file is published in Appendix 23.

The calculated dietary burden for cattle and the updated EU MRLs for bovine tissues are reported in Tables 38 and 39 below.

It is noted that the MRLs for bovine tissues calculated in this section are expressed for the residue definition currently implemented in the EU legislation (sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid). During the peer review for the renewal of the active substance acetamiprid (EFSA, 2016a), it was proposed to limit the residue definition for enforcement in animal commodities to metabolite IM-2-1only, but this proposal was not further implemented in the MRL legislation. However, it is not the scope of the present mandate to recalculate the MRLs for commodities of animal origin for the residue definition proposed during the renewal of the active substance acetamiprid.

Regarding the analytical method for enforcement, it should be noted that the QuEChERS multiresidue method with HPLC–MS/MS was considered sufficiently validated to enforce both acetamiprid and metabolite IM-2-1 at the LOQ of 0.01 mg/kg for each compound (EFSA, 2016a). Therefore, it is concluded that the LOQ for enforcement for the existing residue definition (sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid) is 0.02 mg/kg.

TABLE 38 Updated dietary burden calculations for EU cattle.

	Dietary burden expressed in					
	mg/kg bw per day		mg/kg DM			Most critical
Relevant groups	Median	Maximum	Median	Maximum	Most critical diet	commodity
Cattle (all diets)	0.026	0.046	0.68	1.32	Dairy cattle	Citrus dried pulp

TABLE 39 MRL and risk assessment values calculations estimated for the updated dietary burden for EU cattle.

	Residues at the closet feeding level (mg/kg)		Estimated value at 1N level			
Animal commodity	Mean	Highest	STMR (mg/kg)	HR (mg/kg)	MRL proposal (mg/kg)	CF
Cattle (all diets)						
Closest feeding level:	0.21	mg/kg bw	4.5	N Dairy cattle (highest diet)		
Muscle	0.04	0.05	< 0.01	0.01	0.02	1
Fat	0.03	0.06	< 0.01	0.01	0.02	1
Liver	0.15	0.15	0.02	0.03	**0.03**	1
Kidney	0.24	0.25	0.02	0.04	**0.05**	1

Considering the above calculations, a fall-back MRL of 0.03 mg/kg is proposed for bovine liver and a fall-back MRL of 0.05 mg/kg is proposed for bovine other edible offals; this latter being extrapolated from the MRL derived on kidney.

3.5.7 | Consumer risk assessment using the new endpoints derived in the present statement and considering the identified fall-back MRL options (scenario 3)

A third scenario for the consumer risk assessment was performed, using the new HBGVs (ADI = 0.005 mg/kg bw per day; ARfD = 0.005 mg/kg bw), the residue definitions for plant commodities and conversion factors derived in the present statement, and considering risk mitigations measures (i.e. fall-back MRLs) for those commodities where a risk was identified in scenario 2 (see section 3.4.2).

For the plant commodities for which an exceedance of the new ARfD was identified in scenario 2 and for which safe fallback MRLs and risk assessment values could be identified in the present mandate (i.e. apples, pears, quinces, medlars, apricots, peaches, cherries, table and wine grapes, blackberries, raspberries, blueberries, cranberries, currants, gooseberries, elderberries, table olives, tomatoes, sweet peppers, aubergines, cucumbers, courgettes, melons, pumpkins, watermelons, broccoli, cauliflowers, head cabbages, lamb's lettuce, roman rocket and red mustards), the risk assessment values derived from the residue trials submitted in support of the identified fall-back GAPs were included in the consumer risk assessment calculations (see Section 3.5.3).

For granate apples and asparagus, MRLs and risk assessment values at the LOQ were included in the consumer risk assessment calculations (see Section 3.5.4).

For commodities of animal origin, the input values as derived in EFSA (2018a, 2018b, 2018c) were used for all commodities, except for bovine liver and bovine edible offals (other than liver and kidney) for which fall-back MRLs and risk assessment values derived in the present mandate were considered (see Section 3.5.6). It is noted that MRL values are expressed according to the residue definition for animal commodities currently implemented in the EU legislation (sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid). Under scenario 3, it is therefore assumed that the existing Codex MRLs for bovine liver and bovine other edible offals (other than liver and kidney) are withdrawn from the EU Regulation.

The plant commodities for which no safe fall-back MRL options could be identified (i.e. bananas, lettuces, escaroles, spinaches, chards/beet leaves) were excluded from the consumer risk assessment. For these crops, even the least critical GAPs collected in the framework of this mandate would still result in an exceedance of the newly derived ARfD (see Section 3.5.5). Consequently, EFSA recommends that MRLs for bananas, lettuces, escaroles, spinaches, chards/beet leaves are lowered to the enforcement LOQ. In this scenario 3, it is therefore assumed that the existing uses of acetamiprid on bananas, lettuces, escaroles, spinaches and chards/beet leaves are withdrawn.

The input values used in the exposure calculations are reported in Appendix 17 and the PRIMo file is published in Appendix 22.

The highest estimated long-term dietary intake is 48% of the ADI (NL toddler).

Regarding the short-term dietary intake, no exceedances of the ARfD were identified for raw agricultural commodity and for commodities of animal origin.

However, an exceedance of the ARfD was still identified for the processed commodity currants juice (141% of the ARfD, children). It is noted that this result is obtained for a large portion of 525.8 g reported for the NL children. It is likely that this

large portion does not correspond to pure juice (i.e. 100% fruits). Therefore, the calculated exposure may not consider the dilution factor possibly applicable for these consumption data. In the absence of processing trials for this commodity, EFSA was not able to perform further refinement. The available results are therefore likely overestimating the exposure resulting from the fall-back GAP identified for currants.

Furthermore, it is noted that the margin of safety is very low for strawberries (99% ARfD), sweet cherries (98% ARfD), pears (97% ARfD) and peaches (97% ARfD). Furthermore, these results are affected by an additional non-standard uncertainty related to the use of a tentative conversion factor, derived from monitoring data, for all fruit crops. Therefore, EFSA made further attempts to identify lower MRL options for these four commodities. For strawberries and sweet cherries, the less critical GAPs assessed in this mandate would allow to derive lower MRL values of 0.3 mg/kg (tentative) and 0.5 mg/kg, respectively. These MRLs would allow to decrease the acute exposure to 71% ARfD for strawberries and to 68% ARfD for sweet cherries. For pears and peaches, no other alternative than the enforcement LOQ could be identified in the framework of the present mandate.

4 | CONCLUSIONS

4.1 | Human health

4.1.1 | Hazard assessment acetamiprid

The **ToR1** concerning the toxicological properties of acetamiprid and its metabolites, including toxicological endpoints, as requested in the mandate, was addressed in the present statement by an EFSA Working Group by applying the current methodologies, including experts of the PPR Panel a transparent and trackable evidence-based AOP-informed IATA approach.

The ADI, the ARfD, the AOEL and the AAOEL of acetamiprid were set in 2016 at 0.025 mg/kg bw (per day) on the basis of the rat DNT study (uncertainty factor (UF) 100; EFSA, 2016a). In the peer review meeting conducted in the framework of the renewal process, the experts agreed that there was a treatment related reduction of auditory startle responses in offspring from 10 mg/kg bw per day onward, resulting in a NOAEL of 2.5 mg/kg bw per day for this endpoint. In addition, the experts noted that the data do not allow for any firm conclusion, since important endpoints such as motor activity, learning and memory evaluation could not be properly assessed (EFSA, 2016a).

In the present assessment, an AOP-informed IATA approach was used, relying on a combination of multiple layers of evidence (i.e. systematic literature review of human epidemiological studies, existing in vivo, in vitro, zebrafish data; and integration of the DNT IVB data). The evidence was organised according to the AOP framework, as in case of previous OECD case study projects from EFSA.

In an AOP-informed IATA framework, integration of mechanistic understanding derived from in vitro methods is crucial throughout the iterative process of implementing the postulated AOP and its use in the weight of evidence (WoE). With the currently available relevant and reliable evidence on acetamiprid, it was concluded that acetamiprid causes nAChR activation and rapid desensitisation of the receptors at concentrations starting from 1 μM. This is considered per se as a molecular and cellular effect that could lead to an adverse outcome at organism level, and therefore representing a DNT concern.

However, the results of the WoE in the AOP-informed IATA for acetamiprid indicated that there are major uncertainties in the body of evidence (BoE) for the DNT properties of acetamiprid. Further data are therefore needed to clarify the current uncertainties, to come to a more robust mechanistic understanding, to identify all DNT effects of acetamiprid and obtain concordant dose–response relationships for them to enable hazard and risk assessment.

In addition, the WG noted that the data gaps of the in vivo BoE (including the lack of an acceptable measurement of learning and memory, motor activity and morphometrics evaluation in the available non-guideline DNT study) would warrant a re-evaluation of the current HBGVs of acetamiprid and this lack of knowledge and regulatory data gap should be filled.

The WG considered that the conduction of a study following the OECD TG 426, with adequate measurement of exposure, would currently represent the most appropriate solution to minimise the identified uncertainties. Despite this, the WG acknowledged that the current in vivo DNT studies may not be sensitive enough to detect subtle effects, such as changes in cognition, or brain morphometry, which could result in false negatives, and for which lack of mechanistic understanding is an aggravating uncertainty.

It was concluded, by answering the problem formulation, that based on the EFSA systematic literature review and data collection for DNT effects of acetamiprid and its assessment, and using an AOP-informed IATA framework, the current HBGVs for acetamiprid may not be sufficiently protective.

It is also noted that the PPR Panel recommended an assessment of endocrine-disrupting properties for acetamiprid in line with the EFSA/ECHA (2018) guidance document for the identification of endocrine disruptors (EFSA PPR Panel, 2022); however, this is outside of the scope of this mandate.

To immediately address the impact of the identified uncertainties/limitations on the consumer risk assessment, the WG proposed to apply an additional UF of 5 to the current HBGVs to cover the uncertainties in the DNT assessment, as follows:

- The ADI is currently set at 0.025 mg/kg bw per day and the WG proposed to lower it to 0.005 mg/kg bw per day
- The ARfD is currently set at 0.025 mg/kg bw and the WG proposed to lower it to 0.005 mg/kg bw

The WG noted that this is in line with Regulation (EU) No 283/2013,[15] which lays down: '*When indicated by observations in other studies or the mode of action of the test substance, supplementary studies or information may be required to provide information on the postnatal manifestation of effects such as developmental neurotoxicity*' and with Annex II of the Regulation No 1107/2009, that stipulates '*When the critical effect is judged of particular significance, such as developmental neurotoxic or immunotoxic effects, an increased margin of safety shall be considered, and applied if necessary*'.

The WG recognised that although the additional UF of 5 may appear as an arbitrary decision, it is based on the standard approach of the regulatory peer review process to apply an UF of 10 when there is no regulatory DNT study, but a DNT concern exists. In the case of acetamiprid, there is a NOAEL and a LOAEL for a decreased auditory startle response, but given the uncertainties around this study, the WG considered that an additional UF of 5 would be sufficiently conservative considering that a DNT concern still remains.

The WG also noted that the current assessment of the available regulatory in vivo DNT study is more conservative than the one conducted by the PPR Panel in 2013. This is because (a) the approach used herein follows the OECD Principles and Key Elements for Establishing a Weight of Evidence for Chemical Assessment (use of AOP-informed IATA methodology); and (b) current regulatory toxicology gives more weight to mechanistic considerations in support of biological plausibility. In the case of acetamiprid, the nAChR activation and further desensitisation along with the current conceptual understanding of neuronal network/brain development raise a DNT concern. Moreover, the available in vivo data were independently re-analysed with high scrutiny leveraging on the experience gained in the last years in the assessment of DNT studies.

The WG considered that the new studies notified by PAN Europe as part of the extension of the mandate did not provide relevant and reliable new data for the assessment of DNT potential of acetamiprid or its metabolites, nor on non-DNT endpoints.

Finally, EFSA notes that the same additional UF would be applied for setting the (acute) acceptable operator exposure level ((A)AOEL).

4.1.2 | Hazard assessment of acetamiprid metabolites

New data were available only for metabolite IM-2-1, no data was available for any other metabolite, as provided by the sole applicant. Based on the available genotoxicity studies (Ames test, in vitro micronucleus test, mouse lymphoma assay), it was concluded that metabolite IM-2-1 is unlikely to be genotoxic. The available evidence on IM-2-1 (i.e. it is a major rat metabolite; with large structural similarities with the parent; and the available 28-day rat study) does not allow to conclude on a different qualitative or quantitative toxicological profile of this metabolite compared to the parent. Therefore, it was agreed that the toxicological profile of IM-2-1 is considered as covered by that of acetamiprid and the same HBGVs (ADI of 0.005 mg/kg bw per day and ARfD of 0.005 mg/kg bw) proposed for the parent should also apply to the metabolite.

4.1.3 | Human exposure estimation based on human biomonitoring data

The study of Laubscher et al. (2022), as mentioned in the mandate was appraised as High RoB (tier 3). Two other identified human studies containing biomonitoring data for exposure estimation were appraised as high RoB (Mahai et al., 2022; tier 3) or medium RoB (Oya et al., 2021; tier 2). As the WG considered the available kinetic data as being too limited to develop a sufficiently robust physiologically based kinetic (PBK) model, no external exposure estimation could be made related to the human biomonitoring data reported by Laubscher et al. (2022), nor for the other human biomonitoring data sets identified. Correspondingly, no estimation of internal acetamiprid concentrations upon exposure to acetamiprid's HBGVs was feasible.

The WG noted that, even if a robust PBK model for acetamiprid would be available, exposure estimation of acetamiprid based on reported IM-2-1 concentrations in human biomonitoring studies is cumbersome, given that human exposure to both acetamiprid and IM-2-1 can be expected (e.g. reported by Ospina et al. (2019) and based on residue assessment as presented in Section 3), which may both result in internal IM-2-1 exposure and detection of IM-2-1 concentrations in different body fluids, as reported by Laubscher et al. (2022). Hence, it is possible that a large fraction of the IM-2-1 found in the cerebrospinal fluid of 13 out of 14 children assessed in that study, in which the parent compound (acetamiprid) was not detected, may be due to direct exposure to IM-2-1 as this metabolite can be formed in the environment as a result of environmental degradation of acetamiprid (Ospina et al., 2019). This represents an additional uncertainty that hampers to carry out an exposure estimation for acetamiprid based on the biomonitoring data reported by Laubscher et al. (2022).

4.2 | Residues

The **ToR 2a** concerning a data call for monitoring data for plant products was addressed by EFSA with a call for data, inviting Member States to submit to EFSA results of pesticide residue analysis (monitoring data) for acetamiprid and its

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[15]Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with 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 93, 3.4.2013, p. 1–84.

metabolites in food of plant origin derived by national competent authorities. Data were provided in the format developed for submitting pesticide residue data under Article 31 of Regulation (EC) No 396/2005. Samples with residue analysis for parent acetamiprid and metabolite N-desmethyl-acetamiprid (IM-2-1) were reported to EFSA. The new monitoring data were combined with the monitoring data previously submitted to EFSA.

The **ToR 2b** related to the consideration of the residue definitions for risk assessment and enforcement for plant products was addressed by EFSA with a detailed analysis of the data received under ToR 2a and further analysis of acetamiprid metabolism studies previously submitted and evaluated in the framework of the renewal of the approval of acetamiprid under Regulation (EU) No 844/2012, or in other relevant applications. The available studies investigating the metabolism of acetamiprid in plants gave an indication that the metabolite IM-2-1 is formed at relatively low levels in edible parts of fruit crops and leafy crops (between 2% and 8% of the TRR; up to 0.3 mg/kg in fruits; up to 1.25 mg/kg in leafy). In inedible leafy matrices, however, this metabolite occurs at higher proportions related to the parent compound, IM-2-1 representing up to 32% of the parent compound (16% of the TRR) in apple leaf at longer preharvest intervals. The monitoring data on the metabolite IM-2-1 confirmed its occurrence in several commodities belonging to the groups of leafy and fruit commodities. In these crop groups, the median proportion of metabolite IM-2-1 compared to the parent compound was found to be significant in fruit and leafy crops (median ratio IM-2-1/acetamiprid accounting for 21%–44%, respectively). It was therefore proposed to include metabolite IM-2-1 in the residue definition of risk assessment for leafy and fruit crops, which is currently limited to the parent acetamiprid. It was confirmed that the toxicological profile of IM-2-1 is considered as covered by that of acetamiprid. A revised residue definition for risk assessment (RD-RA) was proposed for leafy and fruit crops as follows:

- **Revised RD-RA (leafy and fruit crops)**: sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid.

Regarding pulses/oilseeds, root crops and cereals, the new data received under ToR 2a did not indicate a need to modify the existing residue definition for risk assessment, which therefore remains as parent acetamiprid.

Regarding the residue definition for enforcement, the available data did not indicate a need to modify the existing definition because acetamiprid is still a sufficient marker of the residues in all crop groups.

The **ToR 3** concerning consumer risk assessment related to the existing EU MRLs for acetamiprid in all plant and animal products was addressed by EFSA by means of three consumer risk assessment scenarios performed with the latest PRIMo rev 3.1:

- **Scenario 1** (using existing HBGVs for acetamiprid and IM-2-1 and the existing residue definitions for risk assessment). These calculations indicated exceedances of the ARfD for 38 commodities. Regarding the long-term dietary intake, the exposure remained below the ADI (81% ADI).
- **Scenario 2** (using the newly derived HBGVs for acetamiprid and IM-2-1 and the newly derived residue definitions for risk assessment proposed in ToR 2b). These calculations indicated exceedances of the ARfD for 40 commodities. Regarding the long-term dietary intake, the margin of safety was small, but the exposure remained below the ADI (93% ADI).
- **In order to identify safe fall-back MRL options** for the crops for which acute intake concerns were identified in scenario 2, EFSA launched a call for data to Member States. From the information provided, EFSA was able to derive fall-back MRLs based on fall-back GAPs for several plant commodities and for 2 commodities of animal origin. For the 5 remaining commodities (bananas, lettuces, escarole/broad-leaved endives, spinach, chard/beet leaves), it was not possible to identify GAPs leading to safe MRL options.
- **Scenario 3** (using the newly derived HBGVs for acetamiprid and IM-2-1, the newly derived residue definitions for risk assessment proposed in ToR 2b and the fall-back MRL options, where possible). These calculations did not indicate exceedances of the ARfD, except for currant juice (141% ARfD). Regarding the long-term dietary intake, the exposure was below the ADI (max. 48% ADI).

Consequently, as reply to ToR3, EFSA was able to make recommendation for safe MRL options and to provide risk managers with advice on the different options (see Recommendations).

5 | RECOMMENDATIONS

5.1 | Human health

For the assessment of DNT in vivo studies

- The reliability of the DNT studies submitted for chemical safety assessment is a relevant uncertainty. The availability of the appropriate historical control data (HCD) and positive control data (PCD) from the performing laboratory should be always considered as part of the DNT data package. The assessing authority should be sufficiently confident that the reliability of the HCD and PCD is adequate (e.g. by discussing it during the pre-submission advice). It is highly recommended that all available evidence should be submitted, to allow regulators to conduct a thorough assessment and to reduce uncertainties in general.

For the AOP-informed IATA methodology

- The EFSA AOP-informed IATA framework is fit for purpose and recommended for DNT hazard identification and characterisation and is the current recommended approach for integrating the results of the DNT IVB.
- The IATA case study developed for DNT hazard characterisation of acetamiprid should be submitted to the OECD IATA Case Study Project.
- It is recommended to discuss with the OECD the IATA framework for DNT hazard characterisation used by EFSA and the lessons learnt from the 3 IATA case studies on pesticides published by EFSA (deltamethrin, flufenacet, acetamiprid; OECD, 2022a, 2022b, 2022c) in order to develop a tiered approach for DNT hazard characterisation.
- Further development of the AOP with nAChR activation (as MIE) leading to behavioural and/or other DNT AOs and its submission to the OECD AOP development programme for endorsement is recommended. This is considered of high benefit for developing an AOP-informed IATA methodology to identify and characterise the DNT potential of neonicotinoids and its metabolites. It is acknowledged that, within AOPs, the relevance of early/upstream functional KEs, such as receptor desensitisation, could be further substantiated as a basis for in vitro assay implementation within the DNT IVB and derivation of reference points (aka points of departure).

For the implementation of the DNT IVB in the risk assessment

- The test systems of the DNT IVB should be further improved as regards molecular and functional characterisation according to the purpose of the assay. Molecular and functional characterisation of the cell system remains a critical step in understanding the relevance of the test system application (e.g. receptor expression and functionality). This exercise represents a priority for pesticides with a neurotoxic mode of action (OECD, 2023). In addition, it is recommended to further characterise different cell systems, applied in the neural network formation (NNF) assay using positive controls.
- To resolve the uncertainties in the AOP-informed IATA, nicotine and other neonicotinoids should be tested in an appropriate (i.e. human cell-based system) and properly characterised test system.
- To expand the DNT IVB, further relevant in vitro test methods should be included, filling the current gaps, using test systems characterised accordingly (preferably based on human cells). Disruption of the NNF represents a downstream KE of high impact in the overall WoE for the DNT assessment. In particular, loss of neuronal electrical functionality, as measured in the NNF assay (MEA), could result from disruption of several early KEs or MIEs. The relevance and reliability of the assay is supported by reproducibility of the results using different test systems. It is therefore possible to apply the same NNF assay (MEA) to test systems representing different stages of human brain development (i.e. representative of receptor ontogeny).

For the human exposure estimation based on human biomonitoring data

To contribute to the harmonisation of the development and use of PBK models in pesticide risk assessment (and in other regulatory areas), it is recommended:

- To define a set of minimal data requirements (from in vitro and in vivo studies) for PBK model development regarding parameterisation of chemical-specific model parameters for:

  ✓ Exposure estimation based on human biomonitoring data
  ✓ Quantitative interpretation (qIVIVE) of in vitro DNT data

- To develop guidance on the performance of reliable in vitro kinetic studies for providing chemical-specific input parameter values for PBK models (such as for parameterisation of intestinal uptake, (hepatic) clearance, (hepatic) metabolite formation and fraction unbound to plasma).
- To develop guidance on the application of in silico tools (e.g. determination of applicability domain) for derivation of PBK model parameter values (such as fraction unbound to plasma proteins and tissue:plasma partition coefficients).
- To update the OECD TG 417 for in vivo toxicokinetic studies, by including the measurement of internal concentrations (at least in blood or plasma) of the parent chemical and the major metabolites in the time course studies, in addition to total radioactivity.

For the non-dietary risk assessment

It is recommended to re-evaluate the non-dietary risk assessment of acetamiprid by applying the new reference values.

5.2 | Residues

For the consumer risk assessment

It is recommended:

- To modify the residue definition for risk assessment in leafy and fruit crops as follows: 'sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid'.
- To require additional supervised residue trials analysing simultaneously for acetamiprid and N-desmethyl-acetamiprid (IM-2-1), supporting existing and any new intended uses on leafy and fruit crops.
- To derive robust conversion factors (CF) from enforcement to risk assessment. Robust CFs for each plant commodity should be derived based on supervised residue trials compliant with authorised or intended GAPs and analysing simultaneously for acetamiprid and N-desmethyl-acetamiprid (IM-2-1).

For the MRLs of acetamiprid in plant and animal commodities

A risk for consumer has been identified for 38 MRLs currently in place in the EU Regulation. Furthermore, for granate apples and aubergines, a risk for consumers is unlikely for the existing MRLs but a risk for consumer has been identified for the higher MRLs proposed in the draft MRL Regulation SANTE/11278/2021. Consequently, it is recommended to lower the existing MRLs for 38 commodities. For granate apples and aubergines, it is recommended to maintain the existing MRLs of 0.01* mg/kg (granate apples) and 0.2 mg/kg (aubergines) and not to implement the MRLs proposed in the draft MRL Regulation SANTE/11278/2021.

The lower MRL proposals derived by EFSA are reported in Table 40. Further considerations and recommendations are reported below:

- For apples, pears, quinces, apricots, sweet peppers, cucumbers and courgettes, the fall-back MRLs proposed in Table 40 are tentative, because not fully supported by residue data. Therefore, should these MRLs be implemented in the regulation, additional residue trials data should be requested.
- For pears, sweet cherries, peaches and strawberries, the MRL reported in the table is proposed for further risk management considerations, because of the low margin of safety and the non-standard uncertainty identified. For sweet cherries and strawberries, a lower MRL option is proposed for risk managers to increase the margin of safety. For pears and peaches, the only lower alternative is the LOQ.
- For elderberries, the existing MRL led to an exceedance of the ARfD for the processed commodity elderberries juice. Therefore, a lower MRL option, for which no exceedance of the ARfD is identified, was reported by EFSA. Noting the uncertainty resulting in a possible overestimation of the acute exposure calculated for elderberries juice, further risk management considerations are required. In the case where the higher MRL option would be implemented in the regulation, it is recommended to request additional trials investigating the effect of processing on the residue concentration in elderberry juice.
- For currants, the lowest fall-back MRL identified in this statement led to an exceedance of the ARfD for the processed commodity currant juice. The only lower MRL option for which no exceedance of the ARfD would be identified is the enforcement LOQ. Noting the uncertainty resulting in a possible overestimation of the acute exposure calculated for currants juice, further risk management considerations are required. In the case where the higher MRL option would be implemented in the Regulation, it is recommended to request additional trials investigating the effect of processing on the residue concentration in currant juice.
- For bananas, lettuces, escaroles/broad-leaved endives, spinaches, chards/beet leaves, it is recommended to lower the existing MRLs to the enforcement LOQ because no safe fall-back MRL options could be identified. Should the MRLs for these crops be lowered to the enforcement LOQ, the authorised uses on bananas, lettuces, escaroles/broad-leaved endives, spinaches, chards/beet leaves leading to acetamiprid residues above the LOQ may need to be reconsidered by national authorities.
- For bovine liver and bovine (other edible offals), it is recommended to withdraw the existing Codex MRLs from the EU Regulation. Lower alternative MRL options were derived by EFSA based on an updated EU livestock dietary burden calculation.

Furthermore, for plums (0.04 mg/kg), poppy seeds (0.3 mg/kg), mustard seed (0.15 mg/kg) and honey (0.3 mg/kg), it was concluded that risk for consumers was still unlikely for the new MRLs proposed in SANTE/11278/2021. For these crops, risk managers can therefore implement the MRLs proposed in SANTE/11278/2021.

TABLE 40 Recommended MRLs.

Code[a]	Commodity	Existing EU MRL (mg/kg)	SANTE/11278/2021 (not yet applicable) (mg/kg)	Proposed EU MRL (mg/kg)	Comment/justification
Enforcement residue definition: acetamiprid					
130010	Apples	0.4	0.4	0.07 (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A tentative fall-back MRL can be proposed based on a less critical GAP (1 GAP compliant trial missing) Risk for consumers unlikely with the fall-back MRL Further risk management decision required
130020	Pears	0.4	0.4	0.07 (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A tentative fall-back MRL can be proposed based on a less critical GAP (1 GAP compliant trial missing) Risk for consumers unlikely with the fall-back MRL, but the margin of safety is low (96% ARfD). The only lower MRL option is the LOQ Further risk management decision required
130030	Quinces	0.8	0.8	0.15 (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A tentative fall-back MRL can be proposed based on a less critical GAP (5 GAP compliant trials missing) Risk for consumers unlikely with the fall-back MRL Further risk management decision required
130040	Medlars	0.8	0.8	0.3	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
140010	Apricots	0.8	0.8	0.08 (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A tentative fall-back MRL can be proposed based on a less critical GAP (2 GAP compliant trials missing) Risk for consumers unlikely with the fall-back MRL Further risk management decision required
140020	Cherries (sweet)	1.5	1.5	0.8 (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL but the margin of safety is low (98% ARfD) This can be mitigated by a lower MRL option of 0.5 mg/kg, fully supported by data and resulting in 68% ARfD Further risk management decision required
140030	Peaches	0.2	0.2	0.08 (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL but the margin of safety is low (96% ARfD). The only lower MRL option is the LOQ Further risk management decision required
151010	Table grapes	0.5	0.5	0.08	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
151020	Wine grapes	0.5	0.5	0.08	
152000	Strawberries	0.5	0.5	0.5 (Further risk management considerations required)	Risk for consumers unlikely with the existing MRL but the margin of safety is low (99% ARfD) This can be mitigated by a lower MRL option of 0.3 mg/kg, tentative because based on overdosed trials, resulting in 71% ARfD Further risk management decision required
153010	Blackberries	2	2	0.6	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
153030	Raspberries (red and yellow)	2	2	0.6	
154010	Blueberries	2	2	0.7	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
154020	Cranberries	2	2	0.7	

TABLE 40 (Continued)

Code[a]	Commodity	Existing EU MRL (mg/kg)	SANTE/11278/2021 (not yet applicable) (mg/kg)	Proposed EU MRL (mg/kg)	Comment/justification
154030	Currants (black, red and white)	2	2	0.7 or 0.01* (Further risk management considerations required)	A risk for consumer is identified for the existing MRL A fall-back MRL can be derived based on a less critical GAP fully supported by data but an exceedance of the ARfD is still identified for the processed commodity currant juice (141% ARfD). No other fall-back MRLs were identified Noting the uncertainty associated to acute exposure calculated for currants juice, further risk management considerations are required
154040	Gooseberries (green, red and yellow)	2	2	0.7	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
154080	Elderberries	2	2	2 or 0.5 (Further risk management considerations required)	A risk for consumer is identified for the existing MRL because of exceedance of ARfD for elderberries juice (247%) A fall-back MRL can be proposed based on a less critical GAP fully supported by data and for which risk for consumers is unlikely Noting the uncertainty associated to acute exposure calculated for elderberries juice, further risk management considerations are required
161030	Table olives	3	3	0.9	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
163020	Bananas	0.4	0.4	0.01* (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A risk for consumer has been identified for the lowest MRL options assessed in the framework of this mandate No fall-back MRLs were identified. It is therefore proposed to lower the existing MRL at the LOQ Further risk management decision required
163050	Granate apples/ pomegranates	0.01*	0.3	0.01*	Risk for consumers unlikely with the existing MRL at the LOQ. However, a risk for consumer is identified for the MRL proposed in SANTE/11278/2021 The existing MRL of 0.01* mg/kg, which covers the less critical GAPs assessed in the framework of this mandate, should be maintained
231010	Tomatoes	0.5	0.5	0.06	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
231020	Sweet peppers/ bell peppers	0.3	0.4	0.09 (Further risk management considerations required)	Risk for consumer is identified for the existing MRL and for the MRL proposed in SANTE/11278/2021 A tentative fall-back MRL can be proposed based on a less critical GAP (3 GAP compliant trials missing) Risk for consumers unlikely with the fall-back MRL Further risk management decision required
231030	Aubergines/ eggplants	0.2	0.4	0.2	Risk for consumers unlikely with the existing MRL. However, a risk for consumer is identified for the MRL proposed in SANTE/11278/2021 The existing MRL of 0.2 mg/kg, which covers the less critical GAPs assessed in the framework of this mandate, should be maintained
232010	Cucumbers	0.3	0.4	0.05 (Further risk management considerations required)	Risk for consumer is identified for the existing MRL and for the MRL proposed in SANTE/11278/2021 A tentative fall-back MRL (mainly based on overdosed trials) can be proposed based on a less critical GAP Risk for consumers unlikely with the fall-back MRL Further risk management decision required
232030	Courgettes	0.3	0.4	0.05 (Further risk management considerations required)	

TABLE 40 (Continued)

Code[a]	Commodity	Existing EU MRL (mg/kg)	SANTE/11278/ 2021 (not yet applicable) (mg/kg)	Proposed EU MRL (mg/kg)	Comment/justification
233010	Melons	0.2	0.2	0.08	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data
233020	Pumpkins	0.2	0.2	0.08	
233030	Watermelons	0.2	0.2	0.08	Risk for consumers unlikely with the fall-back MRL
241010	Broccoli	0.4	0.4	0.06	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data
241020	Cauliflowers	0.4	0.4	0.06	Risk for consumers unlikely with the fall-back MRL
242020	Head cabbages	0.4	0.4	0.03	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
251010	Lamb's lettuces/ corn salads	3	3	1.5	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
251020	Lettuces	1.5	1.5	0.01* (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A risk for consumer has been identified for the lowest MRL options assessed in the framework of this mandate
251030	Escaroles/broad-leaved endives	0.4	0.4	0.01* (Further risk management considerations required)	No fall-back MRLs were identified. It is therefore proposed to lower the existing MRL at the LOQ Further risk management decision required
251060	Roman rocket/ rucola	3	3	1.5	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
251070	Red mustards	3	3	0.9	A risk for consumer is identified for the existing MRL. A fall-back MRL can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
252010	Spinaches	0.6	0.6	0.01* (Further risk management considerations required)	A risk for consumer is identified for the existing MRL. A risk for consumer has been identified for the lowest MRL options assessed in the framework of this mandate
252030	Chards/beet leaves	0.6	0.6	0.01* (Further risk management considerations required)	No fall-back MRLs were identified. It is therefore proposed to lower the existing MRL at the LOQ Further risk management decision required
270010	Asparagus	0.8	0.8	0.01*	A risk for consumer is identified for the existing MRL. A fall-back MRL at the LOQ can be proposed based on a less critical GAP fully supported by data Risk for consumers unlikely with the fall-back MRL
Enforcement residue definition: Sum of acetamiprid and N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid					
1012030	Bovine liver	1	1	0.03	A risk for consumer is identified for the existing MRL, which was derived from Codex MRL (AUT dietary burden)
1012050	Bovine other edible offals	1	1	0.05	Fall-back MRLs can be proposed for bovine liver and bovine other edible offals, based on an updated EU livestock dietary burden calculated in the framework of this mandate Risk for consumers unlikely with the fall-back MRLs Risk managers may consider withdrawing the existing Codex MRLs from the EU Regulation

Abbreviations: GAP, good agricultural practice; MRL, maximum residue level; NEU, northern Europe; SEU, southern Europe.

*Indicates that the MRL is set at the limit of analytical quantification (LOQ).

[a]Commodity code number according to Annex I of Regulation (EC) No 396/2005.

ABBREVIATIONS

a.s.	active substance
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AIR	Annex I Renewal
AOEL	acceptable operator exposure level
ARfD	acute reference dose
BCF	bioconcentration factor
CAT	Critical Appraisal Tool
CF	conversion factors
CRA	consumer risk assessment
CSF	cerebro-spinal fluid
CTA	Critical Appraisal Tools
DNT	developmental neurotoxicity
ECHA	European Chemicals Agency
ED	endocrine disruption
EKE	Expert Knowledge Elicitation
EOGRTS	Extended One-Generation Reproductive Toxicity Study
EU	European Union
FOB	Functional Observational Battery
GAP	Good Agricultural Practice
GD	gestation day
GPER	G protein-coupled oestrogen receptor
HBGV	Health-based guidance value
HCD	historical control data
IVB	in vitro battery
KE	Key Event
KER	Key Events Relationships
LD	lethal dose
LOQ	limit of quantification
MEA	Micro Electrode Array
MIE	Molecular Initiating Event
MoA	mode of action
MRL	maximum residues level
NAM	new approach methodologies
nAChRs	Nicotinic acetylcholine receptors
NCE	Normo- chromatic erythrocytes
NNF	Neural Network Formation
OHAT/NTP	The Office of Health Assessment and Translation/National Toxicology Programme
PCE	Polychromatic erythrocyte
PFAS	Perfluoroalkyl substances
PFOS	Perfluorooctane sulfonate, perfluorooctane sulfonic acid
PHI	pre-harvest intervals
PKB	physiologically based kinetic
PRIMo	Pesticide Residue Intake Model
QA	quality assessment
QC	quality control
RAR	Renewal Assessment Report
RoB	Risk of Bias
SCoPAFF	Standing Committee on Plants, Animals, Food and Feed
SL	Soluble (liquid) concentrate
SG	water-soluble granule
SP	water-soluble powder
ToR	term of reference
TRR	total radioactive residue
UA	uncertainty analysis
US EPA	United States Environmental Protection Agency

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CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

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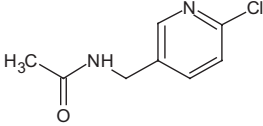
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APPENDIX A

Used compound codes

Code/trivial name[a]	IUPAC name/SMILES notation/InChiKey[b]	Structural formula[c]
Acetamiprid	(1*E*)-*N*-[(6-chloro-3-pyridyl)methyl]-*N*′-cyano-*N*-methylacetimidamide Clc1ccc(CN(C)C(\C)=N\C#N)cn1 WCXDHFDTOYPNIE-RIYZIHGNSA-N	
N-desmethyl-acetamiprid (IM-2-1)	(1*E*)-*N*-[(6-chloropyridin-3-yl)methyl]-*N*′-cyanoethanimidamide Clc1ccc(CNC(\C)=N\C#N)cn1 AYEAUPRZTZWBBF-UHFFFAOYSA-N	
IM-1-4	1-(6-chloropyridin-3-yl)-*N*-methylmethanamine Clc1ccc(CNC)cn1 XALCOJXGWJXWBL-UHFFFAOYSA-N	
IM-1-5	*N*-[(6-chloropyridin-3-yl)methyl]-*N*-methylethanimidamide Clc1ccc(CN(C)C(C)=N)cn1 JHZWQGRBAHJYIZ-UHFFFAOYSA-N	
6-chloronicotinic acid (IC-0)	6-chloropyridine-3-carboxylic acid OC(=O)c1cnc(Cl)cc1 UAWMVMPAYRWUFX-UHFFFAOYSA-N	
IC-0-methyl ester	methyl 6-chloropyridine-3-carboxylate Clc1ccc(cn1)C(=O)OC RMEDXVIWDFLGES-UHFFFAOYSA-N	
IM-0	(6-chloropyridin-3-yl)methanol OCc1cnc(Cl)cc1 GOXYBEXWMJZLJB-UHFFFAOYSA-N	
IM-0 (gly conjugate)	(2*R*,3*R*,4*S*,5*S*,6*R*)-2-[(6-chloropyridin-3-yl)methoxy]-6-(hydroxymethyl)oxane-3,4,5-triol Clc1ccc(CO[C@@H]2O[C@H](CO)[C@@H](O)[C@H](O)[C@H]2O)cn1 ZRRXFGLNJBNGQI-DVYMNCLGSA-N	
IS-2-1 (N-cyanoacetamidine derivative)	(1*E*)-*N*′-cyanoethanimidamide C/C(N)=N\C#N KKZFHAKALPLYLL-UHFFFAOYSA-N	
IS 1-1	(1*E*)-*N*′-cyano-*N*-methylethanimidamide C/C(=N\C#N)NC HGUZMABFJZHYJY-UHFFFAOYSA-N	

Code/trivial name[a]	IUPAC name/SMILES notation/InChiKey[b]	Structural formula[c]
IM 1-2	(1*E*)-*N*′-carbamoyl-*N*-[(6-chloropyridin-3-yl)methyl]-*N*-methylethanimidamide Clc1ccc(CN(C)C(\C)=N\C(N)=O)cn1 ISBUGOZWWNMPPC-VGOFMYFVSA-N	
IM- 1-3	*N*-[(6-chloropyridin-3-yl)methyl]-*N*-methylacetamide Clc1ccc(CN(C)C(C)=O)cn1 FFKDBDOYQUWIEU-UHFFFAOYSA-N	
IM-2-3	*N*-[(6-chloropyridin-3-yl)methyl]acetamide Clc1ccc(CNC(C)=O)cn1 PKLYKZAYVXYVQX-UHFFFAOYSA-N	

[a]The name in bold is the name used in the statement.

[b]ACD/Name 2021.1.3 ACD/Labs 2021.1.3 (File Version N15E41, Build 123232, 7 July 2021).

[c]ACD/ChemSketch 2021.1.3 ACD/Labs 2021.1.3 (File Version C25H41, Build 123835, 28 August 2021).

APPENDICES 1–23

Appendices 1–23 can be found in the online version of this output ('Supporting information' section): https://doi.org/10.2903/j.efsa.2024.8759

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