SCREENING OF TOXICOLOGICAL INFORMATION FOR NON-APPROVED ACTIVE SUBSTANCES AND DATA GAPS IDENTIFICATION

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BACKGROUND

In 2026, the European Commission (EC) submitted a request to EFSA, in accordance with Article 43 of Regulation (EC) No 396/2005[1], to provide a targeted review of maximum residue levels (MRLs) covering the following non-approved active substances: **carbaryl, methoprene, phorate, pyrasulfotole, quinclorac, saflufenacil and terbufos** (mandate number M-2025-00158[2]).

In particular, the European Commission mandated EFSA to examine the available toxicological information in order to screen the quality of the toxicological reference values (TRVs) set at European (EU) level and of those established by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). This screening should also consider the completeness of the set of toxicological studies used to derive the TRVs, to assess whether it would be acceptable according to the current standards and the most recent data requirements. In case data gaps are identified, these should be highlighted along with the resulting uncertainties (see mandate, Terms of Reference (ToR) No 1).

EFSA identified toxicological data gaps for all active substances under assessment, and as required, EFSA is launching a stakeholders' consultation to investigate whether additional toxicological data, complying with the most recent data requirements[3], are available at the time of the launch of the consultation (see mandate, ToR No 2).

First step of this consultation consists in a call for expression of interest in providing existing data covering the toxicological data gaps identified by EFSA. In this context, the paragraphs below present the toxicological assessment and data gaps identified for each specific active substance.

1. CARBARYL

The TRVs for carbaryl reported in Table 1 were derived by EFSA in 2006 (EFSA, 2006); the TRVs were adopted by the European Commission on this basis. A decision for non-inclusion of carbaryl in Annex I of Directive 91/414/EEC[4] was formalised by the Commission Decision 2007/355/EC[5] based on the Review Report for the active substance carbaryl from September 2006 (European Commission, 2006). In 2001, the JMPR revised the previously derived acceptable daily intake (ADI) and established an acute reference dose (ARfD) which can be found in Table 2.

The different ADI values derived by EFSA and the JMPR are due to differences in rounding. The different ARfD values can be explained by a different interpretation of the short-term toxicity studies in dogs (1-year dog study) resulting in different relevant point of departure. According to the JMPR, both the 5-week and 1-year toxicity studies in dogs present a no observed adverse effect level (NOAEL) around 3.8/3.1 mg/kg bw per day, while in the EU assessment, this dose level is considered a lowest observed adverse effect level (LOAEL) for inhibition of brain cholinesterase activity in the 1-year dog study, thus using the 90-day rat neurotoxicity study as point of departure. Also, a different uncertainty factor (UF) was used (see Tables 1 and 2).

1 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, pp. 1–16.

2 https://open.efsa.europa.eu/questions/EFSA-Q-2026-00138

3 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.

4 Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. OJ L 230, 19.8.1991, p. 1–32. Repealed by Regulation (EC) No 1107/2009.

5 Commission Decision of 21 May 2007 concerning the non-inclusion of carbaryl in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing that substance. OJ L 133, 25.5.2007, pp. 40–41.

Table 1: Toxicological reference values proposed at EU level for carbaryl.

TRV	Value	Reference	Comments
ADI	0.0075 mg/kg bw per day	EFSA (2006)	Based on the LOAEL of 15 mg/kg bw per day for the presence of vascular tumours (haemangiomas and haemangiosarcomas) observed at all doses tested in males in a carcinogenicity study in mice and applying an increased UF of 2000 to account for the use of a LOAEL as point of departure for a carcinogenic effect.
ARfD	0.01 mg/kg bw	EFSA (2006)	Based on the NOAEL of 1 mg/kg bw per day for inhibition of AChE activity in a 90-day neurotoxicity study in rats and applying a standard UF of 100.

Abbreviations: AChE, acetylcholinesterase; ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; TRV: toxicological reference value; UF, uncertainty factor.

Table 2: Toxicological reference values set by the JMPR for carbaryl.

TRV	Value	Reference	Comments
ADI	0.008 mg/kg bw per day	FAO and WHO (2001)	Based on the LOAEL of 15 mg/kg bw per day for the presence of vascular tumours (haemangiomas and haemangiosarcomas) observed at all doses tested in males in the carcinogenicity study in mice and applying an UF of 2000, an additional UF of 20 in view of the occurrence of this rare and malignant type of tumour, for which a NOAEL could not be identified.
ARfD	0.2 mg/kg bw	FAO and WHO (2001)	Based on the NOAEL of 3.8 mg/kg bw per day for inhibition of AChE activity observed in a 5-week toxicity study in dogs and applying an UF of 25 because the effects were rapidly reversible and were driven by the peak concentration in plasma (C_{max}) rather than the AUC and data indicated that the sensitivity between laboratory animals (rats and dogs) to inhibition of AChE activity by carbaryl are similar.

Abbreviations: AChE, acetylcholinesterase; ADI, acceptable daily intake; ARfD, acute reference dose; AUC, area under the blood concentration/time curve; bw, body weight; C_{max}, concentration achieved at peak blood level; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; TRV: toxicological reference value; UF, uncertainty factor.

EFSA screened the completeness and the quality of the toxicological studies that were used to derive the EU and the JMPR TRVs, focussing on the question whether the studies meet current scientific standards. EFSA did not undertake a full review of the original studies, the basis of the TRV derivation was scrutinised based on the available data reported mainly in the original draft assessment report (DAR) and addenda (Spain, 2004, 2006).

During this scrutiny, EFSA identified critical issues related to the available toxicological database and focussed on the following two main points:

- the genotoxicity data set;
- the robustness of the available data to derive toxicological reference values, i.e. the ADI, the ARfD and respective UF.

The genotoxicity data package for carbaryl contains studies assessing the three endpoints, i.e., gene mutation in bacterial and mammalian cells (in vitro), clastogenicity (in vitro and in vivo) and aneugenicity (in vivo); additional endpoints were investigated such as DNA damage, and DNA and protein binding in vitro.

The studies for gene mutation showed negative and inconclusive results. An in vitro assay for clastogenicity was positive for structural chromosome aberrations with metabolic activation and negative results were seen when tested in vivo. Aneugenicity showed negative results in vivo. Most of the studies were conducted in the 80s and 90s and therefore, conducted according to the OECD test guidelines (TG) in place at the time but updated later on, following scientific and

technical knowledge developments. One of the test guidelines (in vitro unscheduled DNA synthesis (UDS) assay; OECD TG 482 (OECD, 1986)) was considered relevant and reliable at the time of the assessment to clarify the gene mutation potential of the test substance, but this method is not considered sufficiently robust anymore and the related OECD TG has been cancelled in 2014.

The reliability of the studies is questioned due to significant deviations with regards to current test guidelines. In particular, for the gene mutation assays, one of the five bacterial strains recommended by TG 471 (OECD, 2020) has not been investigated in the available Ames test, thereby missing a strain sensitive to detect cross-linking mutagens; insufficient number of usable tested concentrations were obtained for some *S. typhimurium* strains, and the equivocal results observed in mammalian cells with metabolic activation were not repeated in an independent experiment. In regarding to in vivo studies (mammalian bone marrow cytogenetic test in rats and erythrocyte micronucleus test in mouse), deviations were also noted with regards to current standards. For the chromosome aberration test, at least 200 metaphases per animal should be scored while 100 were analysed in the study. For the micronucleus test, two samples of bone marrow were taken from all dose levels instead of the three recommended, 2000 immature erythrocytes per animal were scored, while currently a minimum of 4000 should be scored for the incidence of micronucleated immature erythrocytes. Proof of bone marrow exposure was not clearly shown in either study as plasma analysis was not performed and except for death that was the outcome of higher dose levels used in preliminary toxicity range finding tests, signs of toxicity are not clearly reported in the chromosome aberration test and only lethargy is mentioned in the micronucleus test at the highest dose tested. Accordingly, their negative results cannot be fully confirmed (EFSA Scientific Committee, 2017). This is particularly relevant to address the clastogenicity potential that was positive in vitro and aneugenicity that was not tested in vitro. In addition, as the results of all genotoxicity studies are not reported in a tabular format, it is not possible to perform an independent assessment of the overall conclusion reached for each study.

Overall, the data package available is not considered reliable. It is not possible to conclude on the genotoxicity potential of carbaryl, regarding gene mutation, chromosome aberration and aneugenicity.

With regards to the toxicological data package needed to derive an ADI and ARfD for carbaryl, limitations and missing data to comply fully with the current data requirements are listed below:

- An assessment of the validity of analytical methods used in feed, body fluids and tissues, air and any additional matrices used in support of the toxicological studies is not available.
- The presence of toxicologically relevant impurities in the technical specification and consequently in carbaryl-treated commodities is unknown. The assessment of the toxicological relevance of impurities was listed as a data gap in the EFSA conclusion (EFSA, 2006).
- An interspecies in vitro comparative metabolism study performed on animal species used in pivotal studies and on human material is not available to determine the relevance of the toxicological animal data to humans and whether additional testing of potentially unique human metabolites would be required.
- Testing of the phototoxicity/photomutagenicity potential of carbaryl is not available while the active substance meets the light absorption criteria to perform such investigations.
- The assessment of the endocrine disruptive (ED) potential of carbaryl was not conducted since insufficient investigations of the ED parameters are available according to the current ECHA (European Chemicals Agency)/EFSA Guidance (ECHA and EFSA, 2018).
- An up-to-date search for published literature is missing.
- Concerning the assessment of the individual studies, the summary of the toxicological studies reported in the DAR (Spain, 2004, 2006) is not detailed and reported as would

be expected in current standards and an independent review of the findings could not be fully undertaken.

An updated assessment of the toxicological properties of carbaryl is also needed, taking into consideration the main toxicological issues identified for carbaryl:

(1) Belonging to the chemical class of carbamate pesticides, carbaryl presents a neurotoxic mode of action and inhibition of AChE activity was identified as the most sensitive endpoint in all species tested, after single or repeated administrations; reduced motor activity and alterations in the functional observational battery (FOB) were observed in rats. Developmental neurotoxicity in vitro battery (DNT IVB) data is available through Toxcast Dashboard with DNT specific hits in network formation, synaptogenesis and migration assays starting from AC50 (concentration producing 50% of the maximum activity) 4.8 µM. A DNT study from 1997 (and re-evaluation of morphological sections in the brain from 2001) was available to the peer review and previously assessed by several regulatory bodies (Crofton and Mundy, 2024). In the study decrease in cerebrum and cerebellum morphometrics, evaluation and inhibition of brain AChE was observed in offspring animals starting from 10 mg/kg bw per day (NOAEL of 1 mg/kg bw per day) and increase tremors and occurrence of ataxia and statistically significant decrease in plasma ChE in dams starting from 10 mg/kg bw per day (NOAEL of 1 mg/kg bw per day). EFSA notes that the reliability of the old DNT studies is a relevant uncertainty, the availability of the appropriate historical control data and positive control data from the performing laboratory should be always considered as part of the DNT data package (which is not available in the carbaryl study) (Crofton and Mundy, 2024). EFSA recommends the implementation of an Adverse Outcome Pathway (AOP)-informed Integrated Approach to Testing and Assessment (IATA) framework for the DNT hazard identification and characterisation, integrating the results of the DNT IVB in the risk assessment for carbaryl (EFSA PPR Panel, 2021).

(2) With regards to the carcinogenicity potential of carbaryl, neoplastic findings were observed in several organs in rats (thyroid, liver, urinary bladder and kidney) above the maximum tolerated dose (MTD); in mice, liver and kidney tumours were also seen in the highest dose tested and vascular tumours (located predominantly in the liver and spleen) were observed at all dose level tested, for which no threshold could be established. A benchmark dose (BMD) analysis of the data may help reduce the related uncertainty and identify a more robust point of departure to the ADI.

(3) Concerns were identified in the EFSA conclusion (EFSA, 2006) with regards to two metabolites, 4-hydroxycarbaryl and 5-hydroxycarbaryl, identified in the rat metabolism studies, but also in plants and animal commodities. Both metabolites are structurally similar to carbaryl and are comparable to carbaryl with regards to inhibition of cholinesterase activity, one of them (5-hydroxycarbaryl) presenting a lower oral median lethal dose (LD50) than carbaryl. They were considered of particular concern as it is not known whether the parent or the metabolites are responsible for the carcinogenic effects observed in the long term and carcinogenicity studies.

The use of an additional uncertainty factor to derive toxicological reference values is not considered appropriate in view of the deficiencies and uncertainties identified. This is further supported by the high UF previously set at 2000, an additional UF of 20 to account for the point of departure being a LOAEL for severe effects (carcinogenic effects).

It is concluded that the existing TRVS derived at EU level in the past cannot be confirmed for carbaryl since its genotoxicity potential is inconclusive regarding gene mutation in bacterial and mammalian cells, clastogenicity and aneugenicity, the data available are considered insufficient when compared to current standards.

The JMPR values suffer from the same limitations as they appear to be based on the same toxicological studies.

2. METHOPRENE

The TRVs for methoprene (racemic mixture of *R*- and *S*-enantiomers in a 1:1 ratio) and *S*-methoprene reported in Table 3 and 4 respectively were derived by the JMPR in 2001; the active substance was never peer reviewed at the EU level as a pesticide. *S*-methoprene is approved for use in biocidal products (type 18) whose renewal of approval is ongoing at ECHA; no ADI or ARfD were derived for the biocide use of the active substance. For completeness, table 5 reports the systemic TRVs (acceptable exposure level (AEL) long-term and acute) established for *S*-methoprene as a biocide active substance that were derived by ECHA in 2016 (ECHA, 2016), noting that a re-evaluation is on-going.

Table 3: Toxicological reference values set by the JMPR for methoprene (racemic mixture of *R*- and *S*-enantiomers in a 1:1 ratio).

TRV	Value	Reference	Comments
ADI	0.09 mg/kg bw per day	(FAO and WHO, 2001)	Based on the NOAEL of 8.6 mg/kg bw per day for increased liver weight and the increase in alkaline phosphatase activity in a 90-day study in dogs performed with methoprene and applying an UF of 100.
ARfD	-	(FAO and WHO, 2001)	Unnecessary

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; NOAEL, no observed adverse effect level; TRV, toxicological reference value; UF, uncertainty factor.

Table 4: Toxicological reference values set by the JMPR for *S*-methoprene.

TRV	Value	Reference	Comments
ADI	0.05 mg/kg bw per day	(FAO and WHO, 2001)	0.5 x ADI of racemic methoprene; no studies with repeated doses were available for *S*-methoprene, the JMPR experts made the conservative assumption that, in the absence of any information to the contrary, all the toxicity of the racemate was due to the *S*-enantiomer.
ARfD	-	(FAO and WHO, 2001)	Unnecessary

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; JMPR, Joint Meeting on Pesticide Residues; TRV, toxicological reference value.

Table 5: Systemic toxicological reference values set for *S*-methoprene as a biocide active substance (currently under re-evaluation).

TRV	Value	Reference	Comments
AEL long-term	0.076 mg/kg bw per day	ECHA (2016)	Based on a NOAEL of 21.7 mg/kg bw per day for evidence of liver toxicity such as increased incidence of hepatic lesions (bile-duct proliferation and portal lymphocyte infiltration) in males and increased absolute and relative weights of the liver in females in a 2-year study in rats, applying a standard UF of 100 and correction for oral absorption of 35%.
AEL acute	0.35 mg/kg bw	ECHA (2016)	Based on the NOAEL of 100 mg/kg bw per day for intrauterine foetal growth retardation, maternal death, increase in abortions, reduced activity and vaginal bleeding observed in a developmental toxicity study in rabbits conducted with *S*-methoprene and applying an UF of 100 and correction for oral absorption of 35%

Abbreviations: AEL, acceptable exposure level (systemic value); bw, body weight; NOAEL, no observed adverse effect level; TRV, toxicological reference value; UF, uncertainty factor.

The activity of the compound as a juvenile hormone is restricted to the *S*-enantiomer.

EFSA screened the completeness and the appropriateness of the toxicological data reported in the JMPR monograph (FAO and WHO, 2001) used to derive the TRVs, focussing on the question whether the information is sufficient to assess whether they meet current quality (i.e. reliability

and reporting) standards and the EU data requirements. The original studies are not available to EFSA.

With regards to the toxicological data package needed to derive an ADI and ARfD, the following data gaps were identified for methoprene according to the current data requirements:

- An assessment of the validity of the analytical methods used in feed, body fluids and tissues, air and any additional matrices used in support of the toxicological studies is not reported.
- The presence of toxicologically relevant impurities in the technical specification and consequently in methoprene-treated commodities is unknown. This is of particular relevance since the JMPR monograph reports toxicological studies performed with varying levels of purity of the test substance used in toxicological studies between 69% to 96%.
- No study of pharmacokinetics and metabolism was performed after repeated dosing. In the studies performed with a single oral dose, a slow decline of the radioactivity from fat indicates that methoprene may build up in fat after repeated dosing.
- An interspecies comparative in vitro metabolism study performed on animal species used in pivotal studies and on human material is not available to determine the relevance of the toxicological animal data to humans and whether additional testing of potential unique human metabolites would be required.
- The available data on skin sensitisation are considered unreliable and additional data on *S*-methoprene and racemic methoprene were identified as useful to continue the evaluation of both compounds by the JMPR experts.
- No data are available on phototoxicity or photomutagenicity, and no rationale for waiving such investigations is provided.
- The design and reporting of repeated dose studies is reported as not meeting current guidelines.
- The genotoxicity data package is incomplete for either the racemate or the *S*-enantiomer; gene mutation was not examined in mammalian cells in vitro, clastogenicity was investigated only in vitro and aneugenicity was not investigated either in vitro or in vivo. The JMPR experts could not reach a definitive conclusion on the genotoxic potential of the racemate.
- Developmental toxicity studies are reported to present shortcomings such as mice and rabbits treated for 2 and 1 day less that required in the relevant test guideline; a developmental toxicity study has not been performed in rats.
- An assessment of the endocrine disruptive potential of methoprene cannot be performed since insufficient investigations of the ED parameters are available according to the current ECHA/EFSA Guidance (ECHA and EFSA, 2018).
- The neurotoxicity and immunotoxicity potential of the active substance were not assessed.
- Medical surveillance on manufacturing plant personnel and monitoring studies are missing.
- A search for the scientific peer-reviewed open literature on the active substance (and its relevant metabolites), dealing with side effects on health according to the EU guidance document (EFSA, 2011) has not been provided.

With regards to the JMPR monograph, it is not considered a source of information that can be independently reviewed due to the lack of details reported on the methods and results of the toxicological studies, such as the presentation of the tabulated results. An assessment of the relevance and reliability of each study when compared to the current OECD test guidelines would also be needed. No information is reported on the tested material, deviations from OECD test

guidelines and overall conduct and results of the studies, and therefore a reliability/relevance assessment cannot be undertaken for the individual studies.

Considering the data gaps and uncertainties identified, it is concluded that the data available are insufficient to assess the strength of the toxicological reference values compared to current standards, and uncertainty factors could not be established.

3. PHORATE

The TRVs for phorate reported in Table 6 were derived by the JMPR in 2004; the active substance was never peer reviewed at the EU level.

Table 6: Toxicological reference values set by the JMPR for phorate.

TRV	Value	Reference	Comments
Group ADI	0.0007 mg/kg bw per day	FAO and WHO (2004b)	Based on the overall NOAEL of 0.07 mg/kg bw per day for inhibition of brain AChE activity in rats and dogs and an UF of 100. This ADI includes the phorate metabolites, phorate sulfone and phorate sulfoxide.
Group ARfD	0.003 mg/kg bw	FAO and WHO (2004b)	Based on the NOAEL of 0.25 mg/kg bw for miosis in the study with single doses in rats. Although inhibition of AChE activity is a C_{max}-dependent phenomenon, an UF of 100 was used in view of the steep dose–response curve and the slow recovery of brain AChE activity because of irreversibility of its inhibition. This ARfD includes the metabolites of phorate, phorate sulfone and phorate sulfoxide.

Abbreviations: AChE, acetylcholinesterase; ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; C_{max}, concentration achieved at peak blood level; NOAEL, no observed adverse effect level; TRV, toxicological reference value; UF: uncertainty factor.

Phorate is an organophosphate insecticide and acaricide.

EFSA screened the completeness and the appropriateness of the toxicological data reported in the JMPR monograph (FAO and WHO, 2004b) used to derive the TRVs, focussing on the question whether the information is sufficient to assess whether they meet current quality (i.e. reliability and reporting) standards and the EU data requirements. The original studies are not available to EFSA.

The studies reported in the JMPR Monograph on phorate are all old studies. For instance, most genotoxicity studies predate the OECD test guidelines, being from the 70s. Pivotal toxicological endpoints such as long-term and carcinogenicity in rats and mice, 1-year dog, developmental toxicity in rats and rabbits, as well as two-generation reproductive toxicity are based on studies performed between 1981 and 1991, the newest available study, a 90-day neurotoxicity study in rats, being from 1999. No statement on the reliability of the studies is given in the monograph, whether they complied with the OECD test guidelines (if) in place at the time or with good laboratory practice.

With regards to the toxicological data package needed to derive an ADI and ARfD, the following data gaps were identified according to the current data requirements:

- An assessment of the validity of the analytical methods used in feed, body fluids and tissues, air and any additional matrices used in support of the toxicological studies is not available.
- The presence of toxicologically relevant impurities in the technical specification and in phorate-treated commodities is unknown.
- An interspecies comparative in vitro metabolism study performed on animal species used in pivotal studies and on human material is not available to determine the relevance of

the toxicological animal data to humans and whether additional testing of potential unique human metabolites would be required.

- An assessment of the phototoxicity and photomutagenicity potential of phorate is not available.
- Belonging to the chemical class of organophosphate pesticides, phorate presents a neurotoxic mode of action and inhibition of brain acetylcholinesterase activity was identified as the most sensitive endpoint in all species tested. DNT in vitro battery (IVB) data is available through Toxcast Dashboard with DNT specific hits in network formation, synaptogenesis and neurite outgrowth assays starting from AC50 2.6 µM. An in vivo DNT study from 2004 previously assessed by US Environmental Protection Agency (EPA) exists but is not available for EFSA assessment (Crofton and Mundy, 2024). The study does not satisfy the guideline requirement for a DNT study due to the inadequacies in the assessment of learning and memory in the offspring. In addition, EFSA notes that the reliability of the old DNT studies is a relevant uncertainty, the availability of the appropriate historical control data and positive control data from the performing laboratory should be always considered as part of the DNT data package (which is not available in the phorate study) (Crofton and Mundy, 2024). In the study dose concordant increase of motor activity, effect on auditory startle in males only and inhibition of brain and erythrocyte ChE was observed in offspring starting from 0.1 mg/kg bw per day (NOAEL 0.03 mg/kg bw per day) and increase salivation, tremors and gait posture in dams starting from 0.3 mg/kg bw per day (NOAEL 0.2 mg/kg bw per day). EFSA recommends the implementation of an Adverse Outcome Pathway (AOP)-informed Integrated Approach to Testing and Assessment (IATA) framework for the DNT hazard identification and characterisation, integrating the results of the DNT IVB (EFSA PPR Panel, 2021).
- An assessment of the endocrine disruptive potential of phorate cannot be performed since insufficient investigations of the ED parameters are available according to the current ECHA/EFSA Guidance (ECHA and EFSA, 2018).
- An up-to-date search for published literature would currently be required.

With regards to the JMPR monograph (FAO and WHO, 2004b), it is not considered a source of information that can be independently reviewed due to the lack of details reported on the methods and results of the toxicological studies, such as the presentation of the results in a tabulated format. An assessment of the relevance and reliability of each study when compared to the current OECD test guidelines would also be needed. For instance, in the JMPR assessment, a summary table presents the results of the genotoxicity studies with phorate. No information is reported on the tested material (except for its purity), deviations from OECD test guidelines and overall conduct and results of the studies in a tabular format, and therefore a reliability/relevance assessment cannot be undertaken for the individual studies. For the other toxicokinetic and toxicity studies, it is not possible to review the basis for the conclusion reached by the JMPR experts and the NOAEL/LOAEL established for each study.

Considering the data gaps and uncertainties identified, it is concluded that the data available are insufficient to assess the strength of the toxicological reference values compared to current standards.

4. PYRASULFOTOLE

The TRVs for pyrasulfotole reported in Table 7 were derived by the JMPR in 2021 (FAO and WHO, 2022); the active substance was never peer reviewed at the EU level.

Table 7: Toxicological reference values set by the JMPR for pyrasulfotole.

TRV	Value	Reference	Comments

ADI	0.01 mg/kg bw per day	FAO and WHO (2022)	Two-year toxicity and carcinogenicity study (rat), UF 100; based on the NOAEL of 1 mg/kg bw per day for effects on the eyes and increased plasma cholesterol observed at 10 mg/kg bw per day. The JMPR noted a margin of 56,000-fold between the ADI and the LOAEL for urinary tract carcinoma and papilloma in mice. The JMPR also noted that a parental LOAEL of 2.5 mg/kg bw per day was identified in the two-generation reproductive toxicity study in rats (lowest dose tested); however, the effects seen at this LOAEL (increased thyroid weight and histopathological changes – pigment deposition and colloid alteration) were considered of equivocal toxicological significance, therefore decided to base the ADI on the NOAEL from the 2-year rat study.
ARfD	unnecessary	FAO and WHO (2022)	-

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; NOAEL, no observed adverse effect level; LOAEL, lowest observable adverse effect level; TRV, toxicological reference value; UF: uncertainty factor.

Pyrasulfotole is an herbicide acting by inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) in susceptible plants. EFSA notes that a mode of action (MoA) shared with other active substances assessed by EFSA and approved in the EU, such as isoxaflutole, mesotrione, tembotrione or clomazone.

In 2024, EFSA reviewed the toxicological data assessed and described in the JMPR monograph and derived the following conclusions (EFSA, 2024):

- The JMPR monograph presents details on the results from the pivotal studies on the relevant endpoints, such as toxicokinetic and metabolism studies, short-term toxicity in rats and dogs, long-term toxicity and carcinogenicity, reproductive and developmental toxicity studies, as well as neurotoxicity and mechanistical research carried out by the applicant. The critical studies are reported to comply with good laboratory practice (GLP) and the more recent versions of the OECD test guidelines.
- It is noted that an assessment of the validity of the analytical methods used in feed, body fluids and tissues and any additional matrices used in support of the toxicity studies is not reported.
- A critical issue is identified for genotoxicity assessment of pyrasulfotole. Only an overview summary table of genotoxicity studies is provided for pyrasulfotole. Given the critical nature of the genotoxicity endpoint, a detailed, independent review of the data is needed to reach a conclusion on the genotoxicity profile of the active substance.
- Another critical issue is identified as regards metabolites assessment, that is based solely on read across with no quantitative structure–activity relationship (QSAR) analysis, or toxicological studies (genotoxicity or general toxicity) provided (see also below).

Furthermore, as regards the completeness of the data submitted to the JMPR, a number of data required by the EU Regulation on data requirements was not available:

- The toxicokinetic data (absorption, distribution and excretion) available to the JMPR do not fully comply with the EU data requirements as investigations were not conducted upon repeated dosing.
- An interspecies comparative in vitro metabolism study is not available and needs to be performed on animal species used in pivotal studies and human material to assess the relevance of the toxicological animal data, including the toxicological reference values to humans.

- Acute toxicity studies upon dermal or inhalation exposures, including skin and eyes irritation and skin sensitisation endpoints were not investigated, which represents data gaps when compared to the EU data requirements; however, these data are not considered relevant to the current assessment.
- No data are available on phototoxicity or photomutagenicity, and no rationale for waiving such investigations is provided.
- Specific immunotoxicity tests were not performed and the information on the immune system is too concise in the monograph to assess whether such a study would be required.
- The assessment of the endocrine-disrupting properties of pyrasulfotole was not conducted in line with the EU requirements and overall, no conclusion can be drawn on the ED potential of the active substance (ECHA and EFSA, 2018). Of note, the data set includes in vivo studies relevant to address potential adverse effects linked to endocrine-mediated MoAs. Some effects were noted in these studies, such as a delay in balanopreputial separation in a two-generation reproductive toxicity study conducted according to most recent protocol (OECD, 2001), and thyroid effects (i.e. increased thyroid weight and/or histopathological changes (pigment deposition and colloid alteration with or without diffuse follicular hypertrophy/hyperplasia) in one species (rat) in studies of different durations (i.e. 90-day, 2-year, rat and 2-generation reproductive toxicity studies). These findings would need to be further integrated into lines of evidence and MoA for the thyroid modality (T-modality) to conclude on the ED potential of the active substance. Overall, no conclusion can be drawn on the ED potential of pyrasulfotole.
- Toxicological studies are available on the metabolite pyrasulfotole-benzoic acid (MTFM-BA) that is a common metabolite to isoxaflutole (also referred as RPA 203328). The monograph reports the same studies on this metabolite as those assessed by the peer review in 2016 under the isoxaflutole review (EFSA, 2016).
- With regard to the metabolites desmethyl-pyrasulfotole and its conjugates, and pyrasulfotole-hydroxymethyl, identified as minor rat metabolites, additional data would be needed to enable a conclusion whether they are covered by the toxicity profile of the parent pyrasulfotole, such as QSAR analysis, or comparison of the physico-chemical properties of these compounds as a first step (EFSA, 2016).
- No details on the search of the scientific peer-reviewed open literature on the active substance (and its relevant metabolites), dealing with side effects on health according to the EU guidance document (EFSA, 2011) have been provided.

With regard to the interpretation of the studies reported in the monograph, although tabulated results are available, additional tables would be helpful to confirm the outcome concluded by the JMPR experts. In addition, details on historical control data are not available.

Based on the available information on pyrasulfotole and the relevant metabolites expected to occur in food and feed, EFSA concludes that:

- It is not possible to conclude on the genotoxicity potential of pyrasulfotole due to the conciseness of the provided summary (no comprehensive tabulated summaries of the studies are available).
- The interpretation and conclusion of some general toxicity studies (treatment relationship and adversity of the findings) would need further details.
- The metabolites desmethyl-pyrasulfotole (and its conjugates) and pyrasulfotole-hydroxymethyl assessments are solely based on systemic availability in toxicological studies and read-across, with no studies (genotoxicity or general toxicity), this needing further elaboration.

As regards the compliance with the EU standards, the provided toxicological data set is not fully aligned with regard to:

- toxicokinetics;
- immunotoxicity;
- ED assessment.

In addition, no information is available on the validation of the analytical methods used in feed, body fluids and tissues and any additional matrices used in support of the toxicity studies, a comparative interspecies in vitro metabolism study, a phototoxicity assessment and details on the search for published literature on the active substance and its relevant metabolites.

Based on the above, in particular due to the concise reporting of the genotoxicity studies on pyrasulfotole, EFSA is not in the position to conclude on the ADI derived by the JMPR for this substance or confirm that an ARfD is not required.

5. QUINCLORAC

The TRVs for quinclorac reported in Table 8 were derived by the JMPR in 2015; the active substance was never peer reviewed at the EU level.

Table 8: Toxicological reference values set by the JMPR for quinclorac.

TRV	Value	Reference	Comments
ADI	0.4 mg/kg bw per day	FAO and WHO (2015)	Based on the NOAEL of 35 mg/kg bw per day for increased relative kidney weights from the 1-year dog study and applying an UF of 100.
ARfD	2 mg/kg bw	FAO and WHO (2015)	Based on the NOAEL of 150 mg/kg bw per day for reductions in motor activity in the acute neurotoxicity study in rats and applying an UF of 100.

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; NOAEL, no observed adverse effect level; TRV, toxicological reference value; UF, uncertainty factor.

Quinclorac is an herbicide acting as a mimic of the plant hormone auxin.

EFSA screened the completeness and the quality of the toxicological studies that were used to derive the JMPR TRVs, focussing on the question whether the studies meet current scientific standards. EFSA did not undertake a full review of the original studies, the basis of the TRVs derivation was scrutinised based on the available data reported in the JMPR monograph (FAO and WHO, 2015).

During this scrutiny, EFSA identified critical issues related to the available toxicological database and focussed on the following two main points:

- the genotoxicity data set;
- the robustness of the available data to derive toxicological reference values, i.e. the ADI, the ARfD and respective UF.

The genotoxicity data package for quinclorac contains studies assessing the three endpoints, i.e., gene mutation in bacterial and mammalian cells (in vitro), clastogenicity (in vitro and in vivo) and aneugenicity (in vivo). Additional endpoints were investigated such as unscheduled DNA synthesis in vivo. An overview summary table of genotoxicity studies is provided for quinclorac, as well as some tabulated results (in Appendix 1 of the JMPR monograph), but not covering all genotoxicity studies.

The studies for gene mutation in bacteria and in mammalian cells showed negative, equivocal and positive results. An in vitro assay for clastogenicity was positive for structural chromosome aberrations with and without metabolic activation and negative results were seen when tested in vivo. Aneugenicity showed negative results in vivo. The studies were conducted between the 80s and early 90s and therefore, expected to be conducted according to the OECD test guidelines in place at the time but updated later on, following scientific and technical knowledge

developments. One of the test guidelines, in vivo unscheduled DNA synthesis assay (TG 486; OECD, 1997) was considered relevant and reliable at the time of the assessment to clarify the gene mutation potential of the test substance but is not considered sufficiently robust anymore (EFSA Scientific Committee, 2017). The reliability of the studies and their deviations with regards to current test guidelines cannot be assessed due to the lack of details reported in the JMPR monograph. In addition, as the results of most genotoxicity studies are not reported in a tabular format, it is not possible to perform an independent assessment of the overall conclusion reached for each study (EFSA Scientific Committee, 2011). Given the critical nature of the genotoxicity endpoint, a number of equivocal/positive results obtained in vitro for chromosome aberration, and gene mutation in bacteria and mammalian cells, a detailed independent review of the data is needed to reach a conclusion on the genotoxicity profile of the active substance. Moreover, additional studies in vivo may need to be performed to clarify the positive results seen in vitro for gene mutation.

Overall, the data package available is not considered reliable. It is not possible to conclude on the genotoxicity potential of quinclorac, regarding gene mutation, chromosome aberration and aneugenicity.

With regards to the toxicological data package needed to derive an ADI and ARfD for quinclorac, the JMPR monograph presents detailed results from most pivotal studies on the relevant endpoints, such as toxicokinetic and metabolism studies, short-term toxicity in rats and dogs, long-term toxicity and carcinogenicity, reproductive and developmental toxicity studies, as well as neurotoxicity and immunotoxicity studies performed with the active substance. Toxicokinetic and metabolism studies, as well as acute and short-term toxicity studies, are presented on the plant metabolite quinclorac methyl ester. The critical studies are reported to comply with GLP.

As regards the completeness of the data submitted to the JMPR, a number of data required by the EU Regulation to comply fully with the current data requirements is missing as listed below:

- An assessment of the validity of the analytical methods used in feed, body fluids and tissues and any additional matrices used in support of the toxicity studies is not reported.
- The presence of toxicologically relevant impurities in the technical specification and consequently in quinclorac-treated commodities is unknown. The JMPR monograph reports that initial production batches of quinclorac contained cinnoline impurities that were associated with positive results in genotoxicity studies and that improved production methods have reduced the levels of these impurities. Current batches are reported to present a purity greater than 99%, containing cinnolines at concentrations below 1 part per million (ppm). This is of particular relevance taking into consideration the genotoxicity assessment of quinclorac itself.
- An interspecies comparative in vitro metabolism study is not available and needs to be performed on animal species used in pivotal studies and human material to assess the relevance of the toxicological animal data, including the toxicological reference values to humans.
- No data are available on phototoxicity or photomutagenicity, and no rationale for waiving such investigations is provided.
- The assessment of the endocrine disruptive properties of quinclorac was not conducted in line with the EU requirements and overall, no conclusion can be drawn on the ED potential of the active substance (ECHA and EFSA, 2018). Of note, no adverse effects were reported on reproduction, fertility, offspring's development (except for reduced pup weight during lactation in rats and increase in skeletal variations in rabbits) and carcinogenicity, and the main target organs of quinclorac are the kidneys. However, the in vivo data set appears to be insufficient to address potential adverse effects linked to endocrine-mediated MoAs, since the available two-generation reproductive toxicity study performed in 1988 would not comply with the most recent protocol (e.g. OECD TG 416; OECD, 2001) and sensitive parameters to oestrogen, androgen, thyroid and

steroidogenesis (EATS) modalities were probably not fully investigated (the study protocol is not specified in the JMPR monograph). No specific in vitro or in vivo mechanistic data are available to explore the endocrine activity of the active substance. Overall, no conclusion can be drawn on the ED potential of quinclorac.

- A search for the scientific peer-reviewed open literature on the active substance (and its relevant metabolites), dealing with side effects on health according to the EU guidance document (EFSA, 2011) has not been provided and the results from epidemiological, occupational health and other such observational studies of human exposure were identified by the JMPR experts as useful information to be added to continue the evaluation of the active substance.

With regard to the interpretation of the studies reported in the monograph, although tabulated results are available, additional tables would be helpful to confirm the outcome concluded by the JMPR experts.

Based on the available information on quinclorac and the relevant metabolites expected to occur in food and feed, EFSA concludes that:

- It is not possible to conclude on the genotoxicity potential of quinclorac due to the conciseness of the provided summary and some positive results reported for bacterial and mammalian cells gene mutation and clastogenicity.
- The interpretation and conclusion of some general toxicity studies (treatment relationship and adversity of the findings) would need further details.

As regards the compliance with the EU standards, the provided toxicological data set is not fully aligned with regard to the ED assessment.

In addition, no information is available on the validation of the analytical methods used in feed, body fluids and tissues and any additional matrices used in support of the toxicity studies, the presence of toxicologically relevant impurities in the technical specification and consequently in quinclorac-treated commodities, an interspecies comparative in vitro metabolism study, a phototoxicity assessment and a search for published literature on the active substance and its relevant metabolites.

Based on the above, in particular due to the critical issues identified on the genotoxicity assessment on quinclorac, EFSA is not in the position to conclude on the ADI and ARfD derived by the JMPR for this substance.

6. SAFLUFENACIL

The TRVs for saflufenacil reported in Table 9 were derived in 2012 by the Evaluation Member State (EMS), UK, based on an import tolerance procedure under the Regulation (EC) No 396/2005 (EFSA, 2012). Saflufenacil did not go through an approval procedure according to Regulation (EC) No 1107/2009[6] and the TRVs were not formally adopted by the European Commission. The TRVs for the metabolite trifluoroacetic acid (TFA), common metabolite to several active substances were revised under the flurtamone peer review (EFSA, 2017), they are currently being re-examined following a request for a review of the TRVs for TFA (under EFSA-Q-2024-00502[7]). In 2011, the JMPR derived an ADI and an ARfD which can be found in Table 10.

The difference in ADI values derived by the UK and the JMPR is due to rounding. The difference in the ARfD setting can be explained by a different interpretation of the relevance to a single exposure of the results observed in the developmental toxicity study in rats by the two authorities.

6 Regulation (EC) No 1107/2009 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.

7 https://open.efsa.europa.eu/question/EFSA-Q-2024-00502

Table 9: Toxicological reference values proposed at EU level for saflufenacil and its metabolite TFA.

TRV saflufenacil	Value	Reference	Comments
ADI	0.046 mg/kg bw per day	EFSA (2012)	Based on the NOAEL of 4.6 mg/kg bw per day for anaemia and increased porphyrin in liver and faeces observed in an 18-month study in mice and applying an UF of 100.
ARfD	0.05 mg/kg bw	EFSA (2012)	Based on the NOAEL of 5.0 mg/kg bw per day for malformed limb bones: thickened humerus, bent radius, ulna, scapula in a developmental toxicity study in rats and applying an UF of 100.
TRV TFA (trifluoroacetic acid)			
ADI	0.05 mg/kg bw per day	EFSA (2017)	Based on the NOAEL of 10 mg/kg bw per day for increased liver weight, hepatocellular hypertrophy based on peroxisome proliferation observed in a 90-day study in rats and applying an increased UF of 200; an additional UF of 2 to extrapolate from short-term to long-term exposure.
ARfD	unnecessary	EFSA (2017)	-

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; NOAEL, no observed adverse effect level; TFA, trifluoroacetic acid; TRV, toxicological reference value; UF, uncertainty factor.

Table 10: Toxicological reference values set by the JMPR for saflufenacil.

TRV	Value	Reference	Comments
ADI	0.05 mg/kg bw per day	FAO and WHO (2011)	Based on the NOAEL of 4.6 mg/kg bw per day for MHA observed at 13.8 mg/kg bw per day in an 18-month carcinogenicity study in mice and applying an UF of 100. The ADI was supported by the NOAEL of 6.2 mg/kg bw per day for anogenital region smeared with urine in females and MHA observed in the 2-year study in rats at 24.2 mg/kg bw per day. It is further supported by the NOAEL of 5 mg/kg bw per day for increased skeletal anomalies observed in the developmental toxicity study in rats at 20 mg/kg bw per day.
ARfD	unnecessary	FAO and WHO (2011)	-

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; MHA: microcytic hypochromic anaemia; NOAEL, no observed adverse effect level; TRV, toxicological reference value; UF, uncertainty factor.

Saflufenacil is an herbicide from the uracil family, acting as a protoporphyrinogen IX oxidase (PPO) inhibitor.

EFSA screened the completeness and the quality of the toxicological studies that were used to derive the EU and the JMPR TRVs, focussing on the question whether the studies meet current scientific standards. EFSA did not undertake a full review of the original studies, the basis of the TRVs derivation was scrutinised based on the available data reported mainly in the JMPR monograph (FAO and WHO, 2011), since the evaluation report supporting the import tolerances setting (UK, 2011) does not provide a detailed assessment of each individual study.

During this scrutiny, EFSA reached the following conclusions:

- The JMPR monograph presents details on the material and methods, as well as results observed in the pivotal studies on the relevant endpoints, such as toxicokinetic and metabolism studies, short-term toxicity in rats and dogs, long-term toxicity and carcinogenicity, reproductive and developmental toxicity studies, as well as neurotoxicity,

immunotoxicity and mechanistical research carried out by the applicant. The critical studies are reported to comply with good laboratory practice.

- A critical issue is identified for genotoxicity assessment of saflufenacil. Only an overview summary table of genotoxicity studies is provided for saflufenacil. Given the critical nature of the genotoxicity endpoint, a detailed independent review of the data is needed to reach a conclusion on the genotoxicity profile of the active substance, such as reporting the compliance of each test with the more recent OECD TG, presentation of the results in a tabular format and include an assessment of the aneugenic potential of the active substance. In addition, since positive results are reported in the in vitro chromosome aberration test with metabolic activation, further evidence of bone marrow exposure should be demonstrated and reported with respect to the negative in vivo micronucleus test to overrule the in vitro results.
- With regards to metabolite TFA assessment, a common metabolite to saflufenacil and flurtamone among others, reference values for consumer risk assessment have been previously peer reviewed by EFSA. An ADI of 0.05 mg/kg bw per day (expressed as sodium trifluoroacetate) was derived and an ARfD was deemed unnecessary (EFSA, 2017). According to the latest scientific state of the art (EFSA Scientific Committee, 2021) the derivation of reference values could be affected by the conclusion on aneugenicity. However, this was not discussed during the peer review. In addition, a classification and labelling harmonised (CLH) proposal for reproductive toxicity 1B has been sent to ECHA[8], based on a new developmental toxicity study[9], having potential on the risk assessment of TFA as a residue (since the effects observed in the developmental toxicity may trigger the need to set an ARfD). As already mentioned, a re-assessment of the ADI and ARfD for TFA is currently ongoing under a specific mandate.

As regards the completeness of the data submitted to the JMPR and the EMS, the following data required according to the EU Regulation on data requirements were not available:

- An assessment of the validity of the analytical methods used in feed, body fluids and tissues and any additional matrices used in support of the toxicity studies is not reported.
- The presence of toxicologically relevant impurities in the technical specification and consequently in saflufenacil-treated commodities is unknown.
- An interspecies comparative in vitro metabolism study is not available and needs to be performed on animal species used in pivotal studies and human material to assess the relevance of the toxicological animal data, including the toxicological reference values to humans.
- No data are available on phototoxicity or photomutagenicity, and no rationale for waiving such investigations is provided.
- The assessment of the endocrine disruptive properties of saflufenacil was not conducted in line with the EU requirements (ECHA and EFSA, 2018). Of note, the data set includes in vivo studies relevant to address potential adverse effects linked to endocrine-mediated MoAs (such as a 2-generation reproductive toxicity study conducted according to the OECD TG 416; OECD, 2001), that includes the required investigations of EAS-sensitive parameters), but no in vitro or in vivo mechanistical studies are available to examine any EATS-mediated MoA. The data would need to be further integrated into lines of evidence to conclude on the ED potential of the active substance. Overall, no conclusion can be drawn on the ED potential of saflufenacil.

8 EFSA noted that TFA is currently under the registry of classification and labelling (CLH) intentions under ECHA remit and further data might become available. https://echa.europa.eu/it/registry-of-clh-intentions-until-outcome/-/dislist/details/0b0236e188e6e587

9 Available under REACH registration dossier of TFA at this link.

- No search of the scientific peer-reviewed open literature on the active substance (and its relevant metabolites), dealing with side effects on health according to the EU guidance document (EFSA, 2011) has been provided.

With regard to the interpretation of the studies reported in the monograph, although tabulated results are available, additional tables would be helpful to confirm the outcome concluded by the JMPR experts.

Based on the available information on saflufenacil and the relevant metabolites (M800H11 and M800H35)[10] expected to occur in food and feed, EFSA concludes that:

- It is not possible to conclude on the genotoxicity potential of saflufenacil due to the conciseness of the provided summary.
- The interpretation and conclusion of some general toxicity studies (treatment relationship and adversity of the findings) would need further details.
- The provided toxicological data set is not fully aligned regarding the ED assessment.

In addition, further toxicological information is needed on the validation of the analytical methods used in feed, body fluids and tissues and any additional matrices used in support of the toxicity studies, the presence of toxicologically relevant impurities, a comparative interspecies in vitro metabolism study, a phototoxicity/photomutagenicity assessment and a search for published literature on the active substance and its relevant metabolites.

Based on the above, in particular due to the concise reporting of the genotoxicity studies on saflufenacil, EFSA is not in the position to conclude on the ADI derived by the JMPR for this substance or confirm whether an ARfD would be required.

7. TERBUFOS

The TRVs for terbufos reported in Table 11 were derived by the JMPR in 2003 (FAO and WHO, 2004a); the active substance was never peer reviewed at the EU level.

Table 11: Toxicological reference values set by the JMPR for terbufos.

TRV	Value	Reference	Comments
ADI	0.0006 mg/kg bw per day	FAO and WHO (2004a)	Based on the NOAEL of 0.06 mg/kg bw per day for inhibition of brain cholinesterase activity observed in the 1-year toxicity, 13-week neurotoxicity and two-generation reproductive toxicity studies in rats, and in the 1-year toxicity study in dogs; an UF of 100 applied.
ARfD	0.002 mg/kg bw	FAO and WHO (2004a)	Based on the NOAEL of 0.15 mg/kg bw per day for miosis in the acute neurotoxicity study in rats and applying an UF of 100.

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight; NOAEL, no observed adverse effect level; TRV, toxicological reference value; UF: uncertainty factor.

Terbufos is an organophosphorus insecticide and nematicide.

EFSA screened the completeness and the quality of the toxicological studies that were used to derive the JMPR TRVs, focussing on the question whether the studies meet current scientific standards and derived the following conclusions:

- The JMPR monograph presents details on the material and methods, as well as compliance with GLP for each study. It is noted that the data package was mostly conducted between the 70s and 80s and deficiencies and limitations are reported in many studies.

10 Both metabolites have been identified in the rat metabolism but not quantified. Based on the limited information available on their toxicological properties, it is not possible to derive specific reference values, nor assessing whether the saflufenacil ADI and ARfD would apply to these metabolites as well (EFSA, 2012).

Accordingly, additional information on their adherence with OECD test guidelines would be needed to assess their reliability and conclude whether the data package is sufficient to characterise the toxicological profile of the active substance according to current standards. The presentation of the findings in a tabular format would also be needed to conduct an independent review of the conclusions reached by the JMPR experts.

- A critical issue is identified for genotoxicity assessment of terbufos. Only an overview summary table of genotoxicity studies is provided for terbufos. Given the critical nature of the genotoxicity endpoint, a detailed independent review of the data is needed to reach a conclusion on the genotoxicity profile of the active substance such as reporting the compliance of each test with the more recent OECD TG and presentation of the results in a tabular format. It is noted that aneugenicity has not been investigated in the available genotoxicity data package.
- Terbufos may produce metabolites potentially more toxic than the parent (terbufos oxon and other oxons, terbufoxon sulfoxide, terbufos sulfoxide, terbufoxon sulfone, and others) that may need further consideration.

With regards to the completeness of the data submitted to the JMPR and the toxicological data package needed to derive an ADI and ARfD for terbufos according to the current EU data requirements, the following data would be needed:

- An assessment of the validity of the analytical methods used in feed, body fluids and tissues and any additional matrices used in support of the toxicity studies.
- An assessment of the presence of toxicologically relevant impurities in the technical specification and consequently in terbufos-treated commodities.
- Toxicokinetic and metabolism studies should include intravenous administration (preferentially), or a single oral dose with assessment of biliary excretion, or a justification for waiving this requirement. An evaluation of the rate and extent of oral absorption including maximum plasma concentration (C_{max}), area under the curve (AUC), time to maximum concentration (T_{max}) and other appropriate toxicokinetic parameters, such as bioavailability needs to be provided.
- An interspecies comparative in vitro metabolism study is not available and needs to be performed on animal species used in pivotal studies and human material to assess the relevance of the toxicological animal data, including the toxicological reference values to humans.
- No data are available on phototoxicity or photomutagenicity, and no rationale for waiving such investigations is provided.
- The assessment of the endocrine disruptive properties of terbufos was not conducted in line with the EU requirements (ECHA and EFSA, 2018). Of note, the data package is insufficient to investigate all sensitive parameters relevant to EATS-mediated adversity and no in vitro or in vivo mechanistical studies are available to examine any EATS-mediated MoA. It is also noted that in the 2-generation reproductive toxicity study, reduction in pregnancy rate, male fertility and mean number of viable pups during lactation are observed at dose levels producing inhibition of brain cholinesterase activity. The data would need to be further integrated into lines of evidence to conclude on the ED potential of the active substance. Overall, no conclusion can be drawn on the ED potential of terbufos.
- Belonging to the chemical class of organophosphate pesticides, terbufos presents a neurotoxic mode of action and inhibition of brain AChE activity was identified as the most sensitive endpoint in all species tested. DNT in vitro battery (IVB) data is available through Toxcast Dashboard with DNT specific hits in network formation, synaptogenesis and neurite outgrowth assays starting from AC50 9.9 µM. A DNT study from 2004 previously assessed by US EPA exists but is not available for EFSA assessment (Crofton

and Mundy, 2024). In the study dose concordant increase of motor activity and inhibition of brain and blood ChE was observed in offspring from 0.08 mg/kg bw per day (NOAEL 0.01 mg/kg bw per day) and statistically significant decrease brain and erythrocyte ChE in dams from 0.15 mg/kg bw per day (NOAEL 0.08 mg/kg bw per day). EFSA recommends the implementation of an Adverse Outcome Pathway (AOP)-informed Integrated Approach to Testing and Assessment (IATA) framework for the DNT hazard identification and characterisation, integrating the results of the DNT IVB (EFSA PPR Panel, 2021).

- The immunotoxicity potential of terbufos has not been addressed.
- No search of the scientific peer-reviewed open literature on the active substance (and its relevant metabolites), dealing with side effects on health according to the EU guidance document (EFSA, 2011) have been provided.

Based on the available information on terbufos and the relevant metabolites expected to occur in food and feed, EFSA concludes that:

- It is not possible to conclude on the genotoxicity potential of terbufos due to the conciseness of the provided summary and aneugenicity has not been examined.
- The interpretation and conclusion of general toxicity studies (treatment relationship and adversity of the findings) need a full detailed assessment with regards to their reliability and reporting of the findings in tabular format.
- The provided toxicological data set is not aligned regarding:
    - ED assessment;
    - neurotoxicity assessment.

Moreover, additional toxicological information is needed on the validation of the analytical methods used in feed, body fluids and tissues and any additional matrices used in support of the toxicity studies, the presence of toxicologically relevant impurities in terbufos-treated commodities, additional information on toxicokinetic studies, a comparative interspecies in vitro metabolism study, phototoxicity/photomutagenicity and immunotoxicity assessments and a search for published literature on the active substance and its relevant metabolites.

Based on the above, in particular due to the concise reporting of the toxicity and genotoxicity studies on terbufos, EFSA is not in the position to conclude on the ADI and ARfD derived by the JMPR for this substance.

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ABBREVIATIONS

4-HPPD	4-hydroxyphenylpyruvate dioxygenase
AC50	concentration producing 50% of the maximum activity in the assay
AChE	acetylcholinesterase
ADI	acceptable daily intake
AEL	acceptable exposure level
AOP	adverse outcome pathway
ARfD	acute reference dose
AUC	area under the blood concentration/time curve
a.s.	active substance
BMD	benchmark dose
bw	body weight
ChE	cholinesterase
CLH	classification and labelling harmonised
C_{max}	maximum plasma concentration
CXL	Codex maximum residue limit
DAR	draft assessment report
DNA	deoxyribonucleic acid
DNT	developmental neurotoxicity
EATS	oestrogen, androgen, thyroid and steroidogenesis
EAS	oestrogen, androgen and steroidogenesis
EC	European Commission
ECHA	European Chemicals Agency
ED	endocrine disruptor
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FOB	functional observational battery
GLP	good laboratory practice
IATA	integrated approach to testing and assessment
IVB	in vitro battery
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
LD50	median lethal dose

LOAEL	lowest observable adverse effect level
MoA	mode of action
MRL	maximum residue level
MTD	maximum tolerated dose
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OJ	official Journal of the European Union
ppm	part per million
PPO	protoporphyrinogen IX oxidase
PPR Panel	EFSA panel on Plant Protection Products and their Residues
QSAR	quantitative structure–activity relationship
TFA	trifluoroacetic acid
TG	test guideline
T_{max}	time to maximum concentration
ToR	terms of reference
TRV	toxicological reference values
UDS	unscheduled DNA synthesis
UF	uncertainty factor
US	United States
WHO	World Health Organization