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REASONED OPINION

# Setting of residue definitions and toxicological reference values for ethiprole

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

## Abstract

In accordance with Article 6 of Regulation (EC) No 396/2005, the original evaluating Member State (EMS), the United Kingdom, received an application from Bayer CropScience to set an import tolerance for the non-approved active substance ethiprole in rice. In the framework of the assessment process, the application was re-allocated to the Netherlands and the purpose of the application was changed to only set European residue definitions and toxicological reference values for the active substance ethiprole. Based on the assessment of the available toxicological data, an acceptable daily intake of 0.002 mg/kg bw per day and an acute reference dose of 0.005 mg/kg bw were derived. The data submitted in support of the request were found to be sufficient to derive residue definitions in primary crops and in processed commodities. The residue definition for enforcement was derived as 'ethiprole'. Adequate analytical methods for enforcement are available to control the residues of ethiprole in dry commodities at the validated LOQ of 0.002 mg/kg. A residue definition for risk assessment was derived as the 'sum of ethiprole, ethiprole-sulfone (RPA097973) and ethiprole-amide (RPA112916), expressed as ethiprole'. This residue definition is applicable for foliar treatment in all primary crops, for both foliar and soil treatments in cereals crops, and is also valid for processed commodities.

**KEYWORDS**

consumer risk assessment, ethirpole, MRL, pesticide, residue defintion, TRV

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## SUMMARY

In accordance with Article 6 of Regulation (EC) No 396/2005, Bayer CropScience submitted an application to the original competent national authority in the United Kingdom (evaluating Member State, EMS; UK) to initially set an import tolerance for the active substance ethiprole in rice. The active substance has never been notified and authorised in the EU and therefore no residue definitions and/or toxicological reference values are set for ethiprole. The EMS UK drafted an evaluation report in accordance with Article 8 of Regulation (EC) No 396/2005, which was submitted to the European Commission and forwarded to the European Food Safety Authority EFSA on 2 September 2015. The EMS also proposed to establish maximum residue levels (MRLs) for ethiprole in rice imported from Indonesia at the proposed level of 2 mg/kg.

EFSA assessed the application and the evaluation report as required by Article 10 of the MRL regulation. In 2015 EFSA identified data gaps which needed further clarifications and requested them to the EMS. In 2016 the EMS UK submitted the requested information in a first revised evaluation report. A second request for additional data was set by EFSA in 2017. The additional data were submitted in October 2018 and the assessment was resumed. Subsequently, EFSA launched a Member State Consultation (MSC) from 24 October 2018 to 9 November 2018 on the toxicological assessment provided in the updated ER and then organised an expert meeting on Mammalian Toxicology, on 21–22 November 2018 (Pesticides peer review meeting 186). The experts' discussion raised the need for further data, including an assessment of the endocrine disrupting properties (ED) of ethiprole, in line with the newly published ECHA/EFSA guidance.

In view of that, a third request for additional data was set in November 2018. The assessment was resumed in January 2020 and EFSA run a second MSC from 12 February 2020 to 11 March 2020 on the updated information received. The consultation aimed to collect Member States feedback on the toxicological assessment of ethiprole and the proposal for new residue definitions. After the MSC, a final request for additional data was made in May 2020. It is noted that after the withdrawal of the United Kingdom from the Union on 1 February 2020, the application was re-allocated to the EMS Denmark first, and then to the Netherlands (NL) in 2021.

Furthermore, in the framework of the process the applicant decided to withdraw its intention to set an import tolerance for rice and to keep the application for setting EU residue definitions and TRVs for ethiprole. Therefore, alongside the process the purpose of the application was changed, being restricted to the setting of EU residue definitions and toxicological reference values for ethiprole, based on the new data made available within the application.

The EMS NL submitted an updated version of the ER in September 2023. The updated assessment was discussed with the MS peer review experts in two specific meetings on Mammalian toxicology (22 November 2023, TC 120) and on Residues (24 November 2023, TC 122). On 6 March 2024 the EMS submitted a final revised evaluation report, considering the outcome of the meetings, which replaced all the previously submitted evaluation reports, and EFSA resumed the assessment. The discussion on Mammalian Toxicology needed a follow up, first via written consultation and then in a second meeting round (30 September 2024, TC 148), in order to conclude on the final toxicological reference values in view of missing developmental neurotoxicity information.

It is noted that ethiprole is not approved and was never assessed in the EU. Therefore, based on the data provided by the EMS in the framework of this application, the following conclusions are derived (see also **summary table**).

In the area of mammalian toxicology, the available data were sufficient to derive an acceptable daily intake of 0.002 mg/kg bw per day and an acute reference dose of 0.005 mg/kg bw. Ethiprole's developmental neurotoxicity potential and its potential for phototoxicity and photomutagenicity could not be concluded. The metabolites included in the residue definition were considered to be of similar toxicity as the parent active substance.

The metabolism of ethiprole was investigated for foliar applications in cereals (rice), fruit crops (sweet peppers) and in pulses and oilseeds (cotton). In rice, the metabolism was also investigated for soil application (paddy rice).

Ethiprole was found as a relevant marker in all investigated crops. Therefore, the residue definition for enforcement in all plant commodities was derived as 'ethiprole'. A validated analytical method based on liquid chromatography– tandem mass spectrometry detector (LC–MS/MS) is available for the enforcement of ethiprole residues in dry matrices at or above 0.002 mg/kg (LOQ).

The main compounds found in primary crop metabolism studies were metabolites ethiprole-sulfone and ethiprole-amide. The outcome of the mammalian toxicological assessment concluded that the toxicological reference values (TRVs) of ethiprole are applicable to metabolites ethiprole-sulfone and ethiprole-amide. Therefore, the experts of the meeting concluded that a comprehensive residue definition for risk assessment (RD-RA) should contain ethiprole, ethiprole-sulfone and ethiprole-amide. The following residue definition for risk assessment was derived for all primary crops: 'sum of ethiprole, ethiprole-sulfone (RPA097973) and ethiprole-amide (RPA112916), expressed as ethiprole'. This residue definition was considered applicable for foliar treatment in all primary crops and for both foliar and soil treatments in cereals crops.

Based on the available hydrolysis studies performed with ethiprole and ethiprole-sulfone, it was concluded that degradation compounds other than ethiprole, ethiprole-sulfone and ethiprole-amide are not to be expected in processed commodities. Therefore, the same residue definitions apply to processed commodities.

As no studies investigating the nature of residues in rotational crops were submitted in the present application, it was not possible to conclude on the nature of residues in rotational crops.

The stability of ethiprole and ethiprole-sulfone in dry/high starch content matrices was sufficiently demonstrated, for each compound independently, for at least 12 months when stored at –23°C. The data on the storage stability of ethiprole-amide have not been provided.

The outcome of the assessment as regards the setting of residue definitions and toxicological reference values for ethiprole, is presented in the summary table below:

## Summary table:

| | |
|---|---|
| **ADI*** | 0.002 mg/kg bw per day |
| **ARfD*** | 0.005 mg/kg bw |
| **RD-Mo in plants and processed commodities** | Ethiprole |
| **RD-RA in primary crops and processed commodities** | Sum of ethiprole, ethiprole-sulfone (RPA097973) and ethiprole-amide (RPA112916), expressed as ethiprole<br>[For cereals: both foliar and soil applications]<br>[For other crop groups: only foliar applications] |

Abbreviations: ADI, acceptable daily intake; ARfD, acute reference dose; bw, body weight.

*ADI and ARfD apply also to metabolites RPA097973 (M01; ethiprole-sulfone) and RPA112916 (M05; ethiprole-amide).

## ASSESSMENT

The European Food Safety Authority (EFSA) received an application to initially set an import tolerance for the active substance ethiprole in rice. The EMS proposed to establish maximum residue levels (MRLs) for ethiprole in rice imported from Indonesia at the proposed level of 2 mg/kg. However, during the assessment process the purpose of the MRL application was changed, and the applicant decided to withdraw its intention to set an import tolerance for rice but to keep the application for setting EU residue definitions and TRVs for ethiprole.

Ethiprole is the ISO common name for 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1H-pyrazole-3-carbonitrile (IUPAC). The chemical structures of the active substance and its main metabolites are reported in Appendix E.

Ethirpole[1] is an active substance which was never assessed in EU (never notified or authorised) for the uses in plant protection products.

No EU MRLs are established for ethiprole and a default MRL of 0.01 mg/kg according to Art 18(1)(b) of Regulation (EC) No 396/2005[2] applies. It is noted that EFSA had assessed certain Codex MRL proposals to provide support for preparing an EU position for the 51St and 53rd Session of the codex committee on pesticide residues (CCPR) (EFSA, 2019; EFSA, 2022). However, since no specific residue definitions nor toxicological reference values (TRVs) were yet established in EU (pending the current MRL assessment to be finalised), the EC introduced reservations on the related CXL MRLs proposals (coffee beans, coffee beans roasted, edible offal, mammalian fats, meat, milk fats, milks, poultry meat, edible offal and fats, rice, rice husked and polished, eggs, soyabeans) and did not take them over in the EU legislation. The residue definitions set by JMPR for plant commodities were ethiprole (for enforcement) and sum of ethiprole, ethiprole-amide[3] and ethiprole-sulfone,[4] expressed as parent equivalents (for risk assessment).

In accordance with Article 6 of Regulation (EC) No 396/2005, Bayer CropScience submitted an application to the original competent national authority in the United Kingdom (evaluating Member State, EMS; UK) to initially set an import tolerance for the active substance ethiprole in rice. The EMS UK drafted an evaluation report in accordance with Article 8 of Regulation (EC) No 396/2005, which was submitted to the European Commission and forwarded to the European Food Safety Authority EFSA on 2 September 2015. The EMS proposed to establish maximum residue levels (MRLs) for ethiprole in rice imported from Indonesia at the proposed level of 2 mg/kg.

EFSA assessed the application and the evaluation report as required by Article 10 of the MRL regulation. In 2015 EFSA identified data gaps which needed further clarifications and requested them to the EMS. In 2016 the EMS UK submitted the requested information in a first revised evaluation report. A second request for additional data was sent by EFSA in 2017. The additional data were submitted in October 2018 and the assessment was resumed again. Subsequently, EFSA launched a Member State Consultation from 24 October 2018 to 9 November 2018 on the toxicological assessment provided in the updated ER and then organised an expert meeting on Mammalian Toxicology on 21–22 November 2018 (Pesticides peer review meeting 186). The experts' discussion raised the need for further data, including an assessment of the endocrine disrupting properties (ED) of ethiprole, in line with the newly published ECHA/EFSA guidance (ECHA-EFSA, 2018).

In view of that, a third request for additional data was set in November 2018. The assessment was resumed in January 2020 and EFSA run a second Member States Consultation from 12 February 2020 to 11 March 2020 on the updated information received. The consultation aimed to collect MSs feedback on the toxicological assessment of ethiprole and the proposal for new residue definitions. After the MSC, a final request for additional data was made in May 2020. It is noted that after the withdrawal of the United Kingdom from the Union on 1 February 2020, the application was reallocated to the EMS Denmark first, and then to the Netherlands (NL) in 2021. Furthermore, alongside the process the purpose of the application was changed, as the applicant decided to withdraw its intention to set an import tolerance for rice but to keep the application for setting EU residue definitions and TRVs for ethiprole, based on the new data made available within the application. The EMS NL submitted an updated version of the ER in September 2023. The updated assessment was discussed with the MS peer review experts in two specific meetings on Mammalian toxicology (22 November 2023, TC 120) and on Residues (24 November 2023, TC 122). On 6 March 2024 the EMS submitted a final revised evaluation report, considering the outcome of the meetings, which replaced all the previously submitted evaluation reports, and EFSA resumed the assessment. However, the discussion on Mammalian Toxicology needed a follow up, first via written and then in a second meeting round (30 September 2024, TC 148) in order to re-discuss and conclude on the final toxicological reference values in view of missing developmental neurotoxicity information.

EFSA based its assessment mainly on the evaluation report originally submitted by the EMS the United Kingdom and subsequently revised by the new appointed EMS the Netherlands (Netherlands, 2021).

[1]It should be noted that ethiprole and its three metabolites (as reported in Appendix B) are identified as a pesticide active substance/metabolites that meet the definition of per- and polyfluoroalkyl substances (PFAS) based on its chemical structure (https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas).

[2]Regulation (EC) No 396/2005 of the 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. 1–16.

[3]5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1H-pyrazole-3-carboxamide; see also Appendix B.

[4]5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethanesulfonyl)-1H-pyrazole-3-carbonitrile; see also Appendix B.

For this application, the data requirements established in Regulation (EU) No 544/2011[5] and the guidance documents applicable at the date of submission of the application to the EMS are applicable (European Commission, 1997a, 1997b, 1997c, 2000, 2010, 2023). The assessment is performed in accordance with the legal provisions of the Uniform Principles for the Evaluation and the Authorisation of Plant Protection Products adopted by Commission Regulation (EU) No 546/2011.[6]

A selected list of end points of the studies assessed by EFSA in the framework of this MRL application is presented in Appendix A.

The final revision of the evaluation report as submitted by the EMS the Netherlands (Netherlands, 2021; as revised in 2024), the reports of the two Member State Consultations (EFSA, 2018a; EFSA, 2020) and of the four experts meetings held on ethiprole (EFSA, 2018b, 2023a, 2023b; EFSA, 2024) are considered as supporting documents to this reasoned opinion and, thus, are made publicly available as background documents to this reasoned opinion.[7]

## 1 | MAMMALIAN TOXICOLOGY

The toxicological profile of ethiprole was discussed at the Pesticides Peer Review Experts' Meeting 186 in November 2018, at the Pesticides Peer Review Experts' Teleconference 120 in November 2023, and at the Pesticides Peer Review Experts' Teleconference 148 in September 2024.

Ethiprole is a non-systemic phenyl pyrazole insecticide and acts by interfering with the passage of chloride ions through the insect GABA (γ-aminobutyric acid) regulated chloride channel, thereby disrupting central nervous system activity and causing death in insects.

Ethiprole is not authorised, and it was never assessed in the EU. There is no information on the methods of analysis used in feed, body fluids and tissues, and any additional matrices in support of the toxicity studies. Ethiprole has no harmonised classification and labelling.

In the toxicokinetics studies in rats, ethiprole was extensively and rapidly absorbed. Oral absorption was greater than 83%. There was no evidence for accumulation. Excretion of substance was predominantly through the faecal route (with evidence of bile excretion) but with appreciable amounts excreted in urine. Ethiprole was extensively metabolised. In vitro interspecies comparative metabolism studies did not reveal evidence of the occurrence of unique human metabolites.[8]

The substance has low acute toxicity when administered orally to rats. No phototoxicity was observed in an in vitro BALB/c 3T3 cell NRU-PT test. However, as the test item only absorbs at wavelengths lower than the minimal wavelength used in the assay (320 nm), the assay is considered not appropriate to assess the phototoxic potential of ethiprole. Therefore, the phototoxicity (and photomutagenicity) potential of ethiprole cannot be concluded.

In short-term oral toxicity studies with rats, mice and dogs, the target organs of toxicity included the liver (all species), thyroid (rats) and thymus (dogs). Dogs also showed non-specific critical effects as reduced body weight gain. The relevant short-term oral NOAEL is 0.22 mg/kg bw per day based on increased liver weight (relative to body weight) in females in the 1-year dog study.[9]

Ethiprole was negative in gene mutation studies with bacterial and mammalian cells, in a chromosomal aberration test with mammalian cells and an in vivo micronucleus study in mice (with sufficient evidence of bone barrow exposure[10]). Based on available genotoxicity studies, the substance is unlikely to be genotoxic.

In the long-term toxicity and carcinogenicity study in rats, the NOAEL for systemic toxicity is 0.85 mg/kg bw per day based on increased body weight gain, changes in prothrombin times, clinical chemistry and urinalysis parameters, effects on thyroid hormone levels (increased TSH, decreased T4), increase in thyroid and liver weights, follicular cell hypertrophy and colloid mineralisation of the thyroid. The NOAEL for carcinogenicity in rats is 4.4 mg/kg bw per day, based on follicular cell adenoma in thyroid (males and females), liver adenomas in males, ovarian sex cord stromal tumours in females and benign subcutaneous lipomas in males. In the long-term toxicity and carcinogenicity study in mouse, the NOAEL for systemic toxicity is 26 mg/kg bw per day, based on liver weight increase and associated pathology (steatosis). The NOAEL for carcinogenicity in mice is 36 mg/kg bw per day, based on an increase of liver adenomas in females. The available information on the mode of action points to PXR/CAR-mediated liver effects, for which rats are considered more sensitive than humans. Altogether, the Peer Review Meeting considered that the tumour findings are not expected to meet the criteria for classification according to CLP regulation.[11,12]

[5]Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances. OJ L 155, 11.6.2011, p. 1–66.

[6]Commission Regulation (EU) No 546/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards uniform principles for evaluation and authorisation of plant protection products. OJ L 155, 11.6.2011, p. 127–175.

[7]Background documents to this reasoned opinion are published on OpenEFSA portal and are available at the following link: https://open.efsa.europa.eu/study-inventory/EFSA-Q-2015-00497.

[8]See experts' consultation point 2.1 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).

[9]See experts' consultation point 2.2 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).

[10]See experts' consultation point 2.1 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).

[11]See experts' consultation point 2.3 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).

[12]Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, 1–1355.

In the multigeneration study in rats, fertility and overall reproductive performance were not impaired.[13] The overall parental NOAEL is 0.7 mg/kg bw per day based on increased liver weight and reduced pituitary weight. The overall offspring NOAEL is 4.8 mg/kg bw per day based on reduced body weights during lactation and organ weights changes in liver, brain, spleen, kidney and thymus. The overall reproductive NOAEL is 32 mg/kg bw per day, the top dose level tested. In the developmental toxicity study in rats the overall maternal NOAEL is 3 mg/kg bw per day based on increased liver weight. The developmental NOAEL in rats is 10 mg/kg bw per day based on higher incidence of foetuses with enlarged thymus, ossification retardation and short 13th rib(s).[14] In the developmental toxicity study in rabbits, the overall maternal NOAEL is 0.5 mg/kg bw per day based on body weight loss and reduced food intake. The overall developmental NOAEL in rabbits is 0.5 mg/kg bw per day based on incomplete ossification (of pubis, metacarpal and/or middle phalanges).[15]

Regarding neurotoxicity assessment, the acute NOAEL is 25 mg/kg bw based on transient behavioural and motor activity effects seen in the acute neurotoxicity study in rats. In the subchronic study, no behavioural, motor activity or neuropathology findings were observed up to the top dose tested of 28.7 mg/kg bw per day. Given that ethiprole has a pesticidal mode of action, an assessment of developmental neurotoxicity is required, but a reliable developmental neurotoxicity study is not available for this compound.[16]

From the analysis of the haematological and histopathological findings in standard regulatory repeat-dose toxicity studies and a 28-day immunotoxicity study in female rats, there is no evidence of a specific or primary effect of ethiprole on the immune system.

The **acceptable daily intake (ADI)** is established at 0.002 mg/kg bw per day based on the NOAEL of the 1-year dog study, related to increased liver weight (relative to body weight) and applying a standard uncertainty factor (UF) of 100. The **acute reference dose (ARfD)** is established at 0.005 mg/kg bw based on the maternal NOAEL in the rabbit developmental toxicity study, related to decreased body weight gain and applying a standard UF of 100.[17] To cover for the missing DNT study, the experts discussed whether an extra UF should be applied to derive the TRVs. As neurotoxicity effects do not form the basis to set the TRVs and given the high margin between the TRVs and the lowest NOAEL for neurotoxicity effects (12,500 for the ADI and 5000 for the ARfD) an ADI of 0.002 mg/kg bw per day and ARfD of 0.005 mg/kg bw per day are considered sufficiently protective and the application of an extra UF is not warranted.[18]

Metabolite RPA112916 (M05) is the amide metabolite of ethiprole. It is not a major rat metabolite. As regards genotoxicity, RPA112916 (M05) is considered unlikely to be genotoxic based on a negative mutagenicity study in bacteria and a negative in vitro micronucleus study in human lymphocytes. As regards general toxicity, it is of low acute toxicity (LD50 > 5000 mg/kg bw) by the oral route. In a 28-day study in rats, adverse effects were observed in liver, thyroid and adrenals, with a relevant NOAEL of 5 mg/kg bw per day. These findings indicate that the toxicological profile of RPA112916 (M05) is quantitatively and qualitatively similar to that of ethiprole (target organs of short-term toxicity include liver, thyroid and adrenals, with a relevant NOAEL in the 28-day study of 2 mg/kg bw per day). RPA112916 (M05) is therefore covered by the toxicological profile of the parent compound, and the reference values of ethiprole are also applicable to RPA112916 (M05).[19]

RPA097973 (M01) is the sulfone metabolite of ethiprole. It is of low acute toxicity ($LD_{50}$ > 2000 mg/kg bw) by the oral route and is not mutagenic in bacteria. RPA097973 (M01) is a major rat metabolite. It is therefore covered by the genotoxicity and general toxicity profile of ethiprole, and the same toxicological reference values are applicable.[20]

The endocrine disrupting (ED) properties of ethiprole for humans were discussed at the Pesticides Peer Review Experts' Teleconference 120 (November 2023).[21] The assessment of the endocrine disruption potential of ethiprole for humans was performed according to the ECHA/EFSA guidance (ECHA-EFSA, 2018). The thyroid (T) modality is considered sufficiently investigated and a pattern of T adversity was concluded, based on increased thyroid weight and follicular cell hypertrophy observed in several rat studies with different dose regimes and exposure durations, and T endocrine activity, i.e. decrease in serum T4 and increase in TSH observed in short-term toxicity studies in rat. The proposed mode of action is CAR/PXR activation as molecular initiating events (MIEs) and induction of phase I and II liver enzymes and increased hepatic clearance of thyroid hormones due to upregulation of UDP-GT as Key Events (KEs). It is concluded that Scenario 1b of the EFSA/ECHA ED guidance (ECHA-EFSA, 2018) is applicable and that the ED criteria for the T-modality are met for ethiprole. The proposed NOAEL for thyroid effects in the dataset is 1 mg/kg bw per day based on the thyroid follicular cell hypertrophy observed from 3 to 5 mg/kg bw per day in the chronic study in rats (by diet).

Regarding the oestrogen, androgen and steroidogenesis (EAS) modalities, EAS adversity was considered sufficiently investigated and no pattern of EAS adversity was observed. Therefore, Scenario 1a is applicable and ED criteria are not met for EAS modalities.

[13]See experts' consultation point 2.4 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).
[14]See experts' consultation point 2.5 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).
[15]See experts' consultation point 2.6 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).
[16]See experts' consultation point 2.7 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).
[17]See experts' consultation point 2.10 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).
[18]See experts' consultation point 1 in the Report of the Pesticides Peer Review Experts' Teleconference 148 in September 2024 (EFSA, 2024).
[19]See experts' consultation point 2.9 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).
[20]See experts' consultation point 2.9 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).
[21]See experts' consultation point 2.8 in the Report of the Pesticides Peer Review Experts' Teleconference 120 in November 2023 (EFSA, 2023a).

# 2 | RESIDUES IN PLANTS

## 2.1 | Nature of residues and methods of analysis in plants

### 2.1.1 | Nature of residues in primary crops

The metabolism of ethiprole was investigated in cereals (rice), fruit crops (sweet peppers) and in pulses and oilseeds (cotton). Four radiolabelled metabolism studies were reported by the EMS (Netherlands, 2021). These studies were discussed during the expert meeting (TC 122, November 2023; EFSA, 2023b) in view of the setting of residue definitions in primary crops.

In **rice**, two valid metabolism studies are available, one performed with spray foliar application, the other corresponding to soil application on paddy rice. In both cases, ethiprole was found as a relevant marker in rice grain (62%–72% TRR; > 0.1 mg/kg) and straw. Ethiprole-sulfone (RPA097973; M01) was the main metabolite found in rice grain (18%–25% TRR; up to 0.54 mg eq/kg) and rice straw (23%–35% TRR; up to 5.6 mg eq/kg). Another metabolite, ethiprole-amide (RPA 112916), was also found in rice straw (11% TRR; 2.68 mg eq/kg) and in rice grain (8.3% TRR; 0.023 mg eq/kg), but only relevant after soil application. This compound was not significant after spray application (< 1% TRR in grain and straw; ≤ 0.05 mg eq/kg).[22]

The metabolism study performed on **sweet peppers** was considered valid to depict metabolism of ethiprole in fruit crops. The parent compound is the main component of the residues in immature and mature fruits (43%–60% TRR). Ethiprole-sulfone was the main metabolite found in mature red fruits (16.4% TRR; 0.07 mg/kg), followed by ethiprole-amide (5.3% TRR; 0.02 mg/kg). The experts agreed that the total rate of metabolite identification was sufficient in fruits and that the study was acceptable.[23]

Regarding the metabolism study performed on **cotton**, a good extraction (90% TRR) of the TRR was achieved. The metabolism of ethiprole in cotton foliage and gin trash, in which sufficient identification was achieved, indicate the same metabolic pattern as noted in rice and sweet peppers. However, a low identification rate of the TRR was reported in cotton seeds: total identified or characterised TRR accounted for 57.9% (1N study) and 76.2% TRR (10N study). Several polar unknown compounds were not further identified. The sum of unknowns, corresponding to 16–36 individual peaks, corresponds to 12.9%–18.4% of the TRR. The validity of the study was therefore extensively discussed by the meeting of residue experts. Despite the relatively low identification rate of the TRR in seeds, the study was considered valid by majority of experts. In cotton seeds, parent compound represented up to 7% of the TRR (0.04 mg/kg) and ethiprole-sulfone was found up to 2.9% of the TRR (0.012 mg/kg). No other compounds were found in significant amounts in cotton seeds.[24]

Overall, the metabolism studies are considered sufficient to depict metabolic pathway of ethiprole following foliar application in three crop groups and following soil application in cereals.

### 2.1.2 | Nature of residues in rotational crops

Since ethiprole is not intended to be used for applications onto crops in the EU, the nature of residues in rotational crops was not assessed by the EMS in the framework of the present application (Netherlands, 2021). No studies have been provided and therefore the nature of ethiprole residues in rotational crops could not be assessed under the present assessment.

It is noted however that a rotational crop study has been performed for the JMPR evaluation (FAO, 2018) to assess the behaviour of ethiprole residues in rotational crops represented by lettuce, radish, wheat and sorghum. In this study, radiolabelled ethiprole was sprayed on soil at a total rate of 740 g ai/ha and residues in rotational crops at plant back intervals (PBI) of 30, 90, 150 and 365 days after application were tested. The JMPR (FAO, 2018) concluded that ethiprole is extensively metabolised in rotational crops and that the metabolite ethiprole-sulfone (RPA097973; M01) is the main residue component in almost all crop commodities and all PBIs.

### 2.1.3 | Nature of residues in processed commodities

Studies investigating the effect of processing on the nature of ethiprole and its main primary crop metabolite ethiprole-sulfone (RPA097973; M01) were submitted with the current application (Netherlands, 2021). These studies were performed according to EC guidelines SANCO 7035/VI/95 rev 5 (European Commission, 1997b) and were discussed during the expert meeting (TC 122, November 2023; EFSA, 2023b).

The hydrolysis study performed with ethiprole indicate that slight degradation of ethiprole to ethiprole-amide may occur during sterilisation conditions (5.5% AR). Similarly, the hydrolysis study performed with ethiprole-sulfone indicate a slight degradation to ethiprole-sulfone-amide under sterilisation conditions (3.9% AR). These degradations were considered insignificant and therefore both compounds (ethiprole and ethiprole-sulfone) are concluded to be stable under standard hydrolysis studies.

[22]See experts' consultation point 3.1 in the Report of the Pesticides Peer Review Experts' Teleconference 122 in November 2023 (EFSA, 2023b).
[23]See experts' consultation point 3.2 in the Report of the Pesticides Peer Review Experts' Teleconference 122 in November 2023 (EFSA, 2023b).
[24]See experts' consultation point 3.2 in the Report of the Pesticides Peer Review Experts' Teleconference 122 in November 2023 (EFSA, 2023b).

The meeting also discussed the absence of hydrolysis studies performed with ethiprole-amide, which is also relevant metabolite for the risk assessment in primary crops. Experts concluded that such studies were not deemed necessary because in the studies conducted with both ethiprole and ethiprole-sulfone no significant degradation was observed and because ethiprole-amide is already formed under the hydrolysis of ethiprole.[25]

EFSA concludes that the nature of ethiprole residues in processed commodities is sufficiently addressed and the relevant residue for risk assessment in processed commodities is expected to be the same as for primary crops.

### 2.1.4 | Analytical methods for enforcement purposes in plant commodities

An analytical method using LC–MS/MS for the determination of ethiprole residues in plant matrices was submitted with the current MRL application and proposed for the enforcement purposes (Netherlands, 2021).

The EMS reported satisfactory validation data for wheat grain, which allows concluding on the validity of this method to quantify ethiprole residues at or above the LOQ of 0.002 mg/kg in crops belonging to the group of dry commodities. The validation was performed on two different mass transitions for quantification (m/z) thus a confirmatory method is not necessary. An interlaboratory validation (ILV) was also performed and satisfactory on rice grain.

It is noted that this proposed enforcement method was also validated on metabolites ethiprole-sulfone (RPA097973; M01) and ethiprole-amide (RPA 112916) with the same LOQ of 0.002 mg/kg in wheat grain. However, the ILV was only provided for ethiprole-sulfone (RPA097973; M01).

This method was also tested on other matrices (orange/high acid, tomato/high water, dry bean (seeds)/dry-high starch and avocado/high oil). However, for the other matrices, no validation data were reported by the EMS (Netherlands, 2021). Therefore, an assessment of the validity of these methods for matrices other than dry matrices was not performed.

Regarding the extraction efficiency of the analytical enforcement method, the EMS performed a comparison of the extraction techniques applied for the preparation of the samples in the enforcement method and in the metabolism studies on rice grain, where 100% of the TRR was extracted. In the enforcement methods, the extraction is performed with a solvent acetronitrile/water (9/1, v/v). In the metabolism study performed with foliar application on rice, samples were first extracted with acetonitrile/water (80:20) and then further extracted by detergent (Triton X-100) sonification followed by hot acid digestion with hydrochloric acid (1.5N) before final extraction with acetonitrile. Therefore, the extraction methods are not fully comparable. EFSA concludes that the evidence to support the extraction efficiency is not considered sufficient according to the requirements of the extraction efficiency Guidance, SANTE 2017/10632 (European Commission, 2023). To satisfy the current criteria of the guidance further investigation on this matter would be required in case import tolerance requests for ethiprole are submitted in the future. According to the data requirements applicable for the assessment of the present MRL application, the investigation of extraction efficiency of enforcement method is not required.

### 2.1.5 | Storage stability of residues in plants

Studies investigating the stability of ethiprole and ethiprole-sulfone (RPA097973; M01) in plants stored under frozen conditions were submitted in the framework of the present MRL application. The reported data provide information on the stability of residues in rice grain, tea leaves, cotton seed and oranges (Netherlands, 2021).

In the stability test performed with rice grain (samples frozen at –23°C), satisfying recoveries (≥ 87%) were observed for ethiprole and ethiprole-sulfone at month 1, 3, 6, 9 and 12. It is noted that recoveries higher than 110% were found for ethiprole at month 6 (134%–156%) and 12 (121%–117%), which could be explained by high-concurrent recoveries in freshly spiked samples at these time points.

In the stability test performed with tea leaf (samples frozen at –23°C), satisfying recoveries (≥ 75%) were observed for ethiprole and ethiprole-sulfone at month 1, 3, 6, 9 and 12. It is noted that recoveries higher than 110% were found for ethiprole and ethiprole-sulfone at month 9 and 12 (122%–139%) and 12 (124%–139%), which could be explained by high-concurrent recoveries in freshly spiked samples at these time points. There is however no information available as to whether the tea leaves corresponded to fresh green tea leaves and whether this assay can cover commodities with high-water content.

In the stability tests performed with cotton seeds and orange samples, the exact temperature of storage was not reported by the EMS; samples were simply reported as frozen. The detailed results for procedural recoveries were also not reported but ranges indicated by the EMS. In cotton seeds exceedance of 110% were reported for ethiprole (75%–151%) and ethiprole-sulfone (72%–135%). The same was observed in orange for ethiprole (81%–129%) and ethiprole-sulfone (91%–158%). Furthermore, in the study performed with orange samples, the analytical method was not strictly validated in accordance with SANCO/3029/99/rev. 4 (European Commission, 2000), as insufficient precision data was reported. Further validation of the storage stability studies performed with cotton and oranges would therefore be needed. The available results can be considered as indicative. In cotton seed, satisfying recoveries were observed for ethiprole (77%–114%) and

[25]See experts' consultation point 3.3 in the Report of the Pesticides Peer Review Experts' Teleconference 122 in November 2023 (EFSA, 2023b).

ethiprole-sulfone (80%–96%) at month 1, 3, 6, 9 and 12. In oranges, satisfying recoveries were observed for ethiprole (72%–112%) and ethiprole-sulfone (76%–103%) at month 1, 3, 6, 9, 12 and 16.

It is concluded that the stability of ethiprole and ethiprole-sulfone was sufficiently demonstrated in dry/high-starch content matrices only, for each compound independently, for at least 12 months when stored at –23°C. On cotton seeds and oranges, only indicative results are available from the study summaries reported in the present application. Regarding high-water content matrices, no conclusion could be derived in the absence of further information on the tea leaves samples used in the available studies. It is also noted that no data on the storage stability of ethiprole-amide were reported in the framework of the present application.

### 2.1.6 | Proposed residue definitions

Based on the metabolism studies performed on rice, initially submitted in support of the import tolerance MRL application, ethiprole is always present in major proportions in rice grain and was therefore concluded to be a sufficient marker for enforcement in cereal crops. In addition to ethiprole, metabolites ethiprole-sulfone and ethiprole-amide were the main compounds found in rice matrices, ethiprole-amide being only retrieved after soil treatment on paddy rice.

The outcome of mammalian toxicological assessment concluded that the toxicological reference values (TRVs) of ethiprole are applicable to metabolites ethiprole-sulfone and ethiprole-amide. Therefore, the experts of meeting concluded that a comprehensive residue definition for risk assessment (RD-RA), applicable to uses with foliar or soil application on cereal crops, should contain ethiprole, ethiprole-sulfone and ethiprole-amide.

The experts of the meeting also discussed the validity of the other metabolism studies performed in fruit crops and in pulses and oilseeds in a view to derive a general residue definition in plant commodities. It was concluded that the study performed on fruit crops was sufficient to depict the nature of residues. Regarding the study performed on oilseeds, despite shortcomings in the identification of the TRR in seeds, it was considered valid by majority of experts and thus sufficient to address the nature of residue in a third crop group. In fruit and oilseeds, no additional degradation compounds than those observed in rice were found. Consequently, majority of meeting experts was in favour to derive general residue definitions in all plant commodities as follows:

- residue definition for enforcement: ethiprole
- residue definition for risk assessment: sum of ethiprole, ethiprole-sulfone (RPA097973) and ethiprole-amide (RPA112916), expressed as ethiprole

For cereal crops, these residue definitions cover both foliar and soil treatments. For the other crops, only foliar uses are covered.

Based on the hydrolysis studies performed with ethiprole and ethiprole-sulfone, it was concluded that degradation compounds other than ethiprole, ethiprole-sulfone and ethiprole-amide are not expected in processed commodities. However, no studies investigating the nature of residues in rotational crops was assessed in the present application. Consequently, the above residue definitions are concluded for primary crops and processed commodities.

A validated analytical method for the enforcement of ethiprole in dry matrices was reported by the EMS (Netherlands, 2021).

## 2.2 | Magnitude of residues in plants

Not relevant as the purpose of the present application was only to address the nature of residues in plant commodities.

# 3 | RESIDUES IN LIVESTOCK

Not relevant as the purpose of the present application was only to address the nature of residues in plant commodities.

# 4 | CONSUMER RISK ASSESSMENT

Not relevant as the purpose of the present application was only to address the nature of residues in plant commodities.

# 5 | CONCLUSION AND RECOMMENDATIONS

The data submitted in the present application were sufficient to conclude on the mammalian toxicity of ethiprole (and its -sulfone and -amide metabolites) and to derive residue definitions and toxicological reference values for the non-approved active substance in EU ethiprole.

Specifically, the data relevant for the following endpoints were derived:

- Toxicological reference values for ethiprole and its main metabolites in plants (ethiprole-sulfone and ethiprole-amide).
- Residue definitions for enforcement and risk assessment in primary crops and processed commodities.
- Validated analytical method for enforcement of ethiprole residues in dry commodities.
- Storage stability of ethiprole and ethiprole-sulfone in dry/high-starch commodities.

The endpoints derived in the present assessment are summarised in Appendix A.

It must be noted that the current application identified some information as non-available or not sufficient, such as the methods of analysis used in feed, body fluids and tissues, and any additional matrices in support of the toxicity studies, the phototoxicity (and photomutagenicity) potential of ethiprole, the assessment of the developmental neurotoxicity and the information related to the extraction efficiency of the analytical enforcement method. Even if these data gaps did not prevent EFSA to reach the conclusions presented in this opinion, they still might need further consideration in the framework of future MRL applications on ethiprole. Nevertheless, the list of missing information mentioned in this opinion cannot be considered exhaustive, given the focussed purpose of the current application, on the exclusive setting of residue definitions and toxicological reference values for the non-approved active substance in EU ethiprole.

## ABBREVIATIONS

| | |
|---|---|
| a.s. | active substance |
| ADI | acceptable daily intake |
| AR | applied radioactivity |
| ARfD | acute reference dose |
| BBCH | growth stages of mono- and dicotyledonous plants |
| bw | body weight |
| CXL | Codex maximum residue limit |
| EMS | evaluating Member State |
| EURL | EU Reference Laboratory (former Community Reference Laboratory (CRL)) |
| FAO | Food and Agriculture Organization of the United Nations |
| GAP | Good Agricultural Practice |
| GC–MS | gas chromatography with mass spectrometry |
| GLP | Good Laboratory Practice |
| HPLC–MS/MS | high performance liquid chromatography with tandem mass spectrometry |
| HR | highest residue |
| ILV | independent laboratory validation |
| ISO | International Organisation for Standardisation |
| IUPAC | International Union of Pure and Applied Chemistry |
| JMPR | Joint FAO/WHO Meeting on Pesticide Residues |
| LC | liquid chromatography |
| LOAEL | lowest observed adverse effect level |
| LOD | limit of detection |
| LOQ | limit of quantification |
| MRL | maximum residue level |
| MS | Member States |
| MS/MS | tandem mass spectrometry detector |
| MW | molecular weight |
| NOAEL | no observed adverse effect level |
| OECD | Organisation for Economic Co-operation and Development |
| PBI | plant back interval |
| PROFile | (EFSA) Pesticide Residues Overview File |
| QuEChERS | Quick, Easy, Cheap, Effective, Rugged, and Safe (analytical method) |
| RA | risk assessment |
| RD | residue definition |
| RMS | rapporteur Member State |
| TRR | total radioactive residue |
| WHO | World Health Organization |

## REQUESTOR

European Commission

## QUESTION NUMBER

EFSA-Q-2015-00497

## REFERENCES

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

EFSA (European Food Safety Authority). (2018a). Member state consultation report on the evaluation report (mammalian toxicology section) prepared by the EMS the United Kingdom on the active substance ethiprole. October–November, 19 pp. www.efsa.europa.eu

EFSA (European Food Safety Authority). (2018b). Pesticides peer review meeting report on the active substance ethiprole held on 21–22 November 2018. Peer review meeting on mammalian toxicology 186, 22 November 2018, 5 pp. www.efsa.europa.eu

EFSA (European Food Safety Authority). (2019). Scientific Report on scientific support for preparing an EU position in the 51st session of the codex committee on pesticide residues (CCPR). *EFSA Journal*, *17*(7), 5797. https://doi.org/10.2903/j.efsa.2019.5797

EFSA (European Food Safety Authority). (2020). Member state consultation report on the evaluation report (mammalian toxicology section and residue definitions) prepared by the EMS the United Kingdom on the active substance ethiprole. February–March 2020, 39 pp. www.efsa.europa.eu

EFSA (European Food Safety Authority). (2022). Scientific support for preparing an EU position in the 53rd session of the codex committee on pesticide residues (CCPR). *EFSA Journal*, *20*(9), 7521. https://doi.org/10.2903/j.efsa.2022.7521

EFSA (European Food Safety Authority). (2023a). Pesticides Peer Review Meeting report on the active substance ethiprole held on 22 November 2018. Peer review meeting on Mammalian toxicology TC 120, 68 pp. www.efsa.europa.eu

EFSA (European Food Safety Authority). (2023b). Pesticides Peer Review Meeting report on the active substance ethiprole held on 24 November 2023. Peer review meeting on Residues TC 122, 17 pp. www.efsa.europa.eu

EFSA (European Food Safety Authority). (2024). Pesticides Peer Review Meeting report on the active substance ethiprole held on 24 November 2023. Peer Review Meeting on Mammalian Toxicology TC 148, 7 pp. www.efsa.europa.eu

European Commission. (1997a). Appendix A. Metabolism and distribution in plants. 7028/VI/95-rev.3, 22 July 1997.

European Commission. (1997b). Appendix E. Processing studies. 7035/VI/95-rev. 5, 22 July 1997.

European Commission. (1997c). Appendix H. Storage stability of residue samples. 7032/VI/95-rev. 5, 22 July 1997.

European Commission. (2000). Residue analytical methods. For pre-registration data requirements for Annex II (part A, section 4) and Annex III (part A, section 5) of Directive 91/414. SANCO/3029/99-rev. 4. 11 July 2000.

European Commission. (2010). Residue analytical methods. For post-registration control. SANCO/825/00-rev. 8.1, 16 November 2010.

European Commission. (2023). Technical Guideline on the Evaluation of Extraction Efficiency of Residue Analytical Methods. SANTE 2017/10632, Rev. 5, 11 May 2023.

FAO (Food and Agriculture Organization of the United Nations). (2018). Ethiprole. In: Pesticide residues in food – 2018. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 234.

Netherlands. (2021). Evaluation report on the setting of residue definitions for the active substance ethirprole. 27 January 2021 (superseding previous versions prepared by former EMS the United Kingdom for an import tolerance in rice), revised in March 2024, 516 pp. www.efsa.europa.eu

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# APPENDIX A

## List of end points

### A.1 | MAMMALIAN TOXICOLOGY

**Absorption, distribution, metabolism and excretion (toxicokinetics) (Regulation (EU) N° 283/2013, Annex Part A, point 5.1)**

| | |
|---|---|
| Rate and extent of oral absorption/systemic bioavailability | 83.5% (males) - 88.6% (females) upon single exposure to 5 mg/kg bw, and 10.9% (males) - 15.3% (females) upon single exposure to 1000 mg/kg bw based on total radioactivity data from urinary and biliary excretion, levels in carcass, and cage wash (by 96 h). |
| Toxicokinetics | Parameters based on total radioactivity measurements |

| Dose (mg/kg bw) | Blood $C_{max}$ (µg equiv/g) | Blood $T_{max}$ (h) | Blood $AUC_{168}$ (µg equiv.h/mL) | Blood $T_{1/2}$ (h) |
|---|---|---|---|---|
| 5 (male) | 2.1 | 8 | 95 | 48 |
| 5 (female) | 1.6 | 8 | 79 | 114 |
| 1000 (male) | 42 | 34 | 2620 | 49 |
| 1000 (female) | 30 | 48 | 2240 | 44 |

| | |
|---|---|
| Distribution | Widely distributed, with highest levels in kidney and liver. |
| Potential for bioaccumulation | No evidence for accumulation. The similarity of residue levels in tissues following single and multiple administration indicated that there was no tissue accumulation or changes in the pattern of tissue distribution and elimination following repeat administration of the test material. |
| Rate and extent of excretion | Rapid and extensive (86% within 48 h upon single dosing, 84% within 48 h upon repeated dosing). Excretion mainly via bile (males: 67%; females: 52% in 96 h) and urine (males: 11%; females: 30% in 96 h). |
| Metabolism in animals | Extensively metabolized in rat. Major metabolites (detected in urine and/or bile) include RPA104615 (M03) and RPA097973 (M01) when also considering its downstream metabolites. |
| *In vitro* metabolism | No unique nor disproportionate human metabolite identified. In vitro rate of conversion highest for mouse, and rats, intermediate for rabbits, and lowest for dogs and humans. |
| Toxicologically relevant compounds (animals and plants) | Ethiprole |
| Toxicologically relevant compounds (environment) | No data, data not relevant for import tolerances. |

**Acute toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.2)**

| | | |
|---|---|---|
| Rat $LD_{50}$ oral | > 5000 mg/kg bw | |
| Rat $LD_{50}$ dermal | No data, data not relevant for import tolerances. | |
| Rat $LC_{50}$ inhalation | No data, data not relevant for import tolerances. | |
| Skin irritation | No data, data not relevant for import tolerances. | |
| Eye irritation | No data, data not relevant for import tolerances. | |
| Skin sensitisation | No data, data not relevant for import tolerances. | |
| Phototoxicity | Negative in BALB/c 3T3 Cell NTU assay. As the test item only absorbs at wavelengths lower than the minimal wavelength used in the assay (320 nm), the assay is considered not appropriate to assess the phototoxic potential of ethiprole.<br>Phototoxicity potential of ethiprole cannot be concluded. | |

**Short-term toxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.3)**

| | | |
|---|---|---|
| Target organ / critical effect | 28-day rat: ↑ total protein, ↑ liver weight, ↑ adrenal weight<br>90-day rat: ↑ liver and thyroid weight, hepatocellular hypertrophy, thyroid follicular hypertrophy<br>28-day mouse: ↑ liver weight<br>90-day dog: ↓ body weight gain, ↓ thymus weight<br>1-year dog: ↑ liver weight | STOT-RE Cat. 2 |
| Relevant oral NOAEL | 28-day rat: 2.0 mg/kg bw per day<br>90-day rat: 1.2 mg/kg bw per day<br>28-day mouse: 9.3 mg/kg bw per day<br>90-day dog: 1.0 mg/kg bw per day<br>1-year dog: 0.22 mg/kg bw per day | |
| Relevant dermal NOAEL | No data, data not relevant for import tolerances | |
| Relevant inhalation NOAEL | No data - not required | |

**Genotoxicity (Regulation (EU) N° 283/2013, Annex Part A, point 5.4)**

| | | |
|---|---|---|
| *In vitro* studies | Negative Ames test<br>Negative chromosome aberration test in human lymphocytes | |
| | Negative gene mutation (mouse lymphoma) assay | |
| *In vivo* studies | Negative mouse bone marrow micronucleus test (sufficient evidence of bone marrow exposure)<br>Negative mammalian cell DNA repair assay (UDS) | |
| Photomutagenicity | No data. Photomutagenicity potential cannot be concluded. | |
| Potential for genotoxicity | Ethiprole is unlikely to be genotoxic. | |

**Long-term toxicity and carcinogenicity (Regulation (EU) N°283/2013, Annex Part A, point 5.5)**

| | | |
|---|---|---|
| Long-term effects (target organ/critical effect) | Rat: ↑ body weight gain, changes in prothrombin times, clinical chemistry and urinalysis parameters, ↑ TSH, ↓ T4, ↑ thyroid weight, ↑ liver weight, ↑ incidence thyroid follicular cell hypertrophy and colloid mineralisation<br>Mouse: ↑ liver weight and associated pathology (liver steatosis) | |
| Relevant long-term NOAEL | 2-year, rat: 0.85 mg/kg bw per day<br>18-month, mouse: 26 mg/kg bw per day | |
| Carcinogenicity (target organ, tumour type) | Rat: ↑ follicular cell adenoma in thyroid (males and females), ↑ liver adenomas in males, ↑ ovarian sex cord stromal tumours in females, ↑ benign subcutaneous lipomas in males<br>Mouse: ↑ liver adenomas in females<br>Ethiprole is unlikely to be carcinogenic to humans | |
| Relevant NOAEL for carcinogenicity | 2-year, rat: 4.4 mg/kg bw per day<br>18-month, mouse: 36 mg/kg bw per day | |

**Reproductive toxicity (Regulation (EU) No 283/2013, Annex Part A, point 5.6)**

**Reproduction toxicity**

| | | |
|---|---|---|
| Reproduction target / critical effect | Parental toxicity: ↑ liver weight (F0 and F1 females) ↓ pituitary weight (F1).<br>Reproductive toxicity: no adverse effect observed in rat 2-generation study<br>Offspring toxicity: ↓ pup body weight during lactation and organ weight changes in the liver, brain, spleen, kidney and thymus | |
| Relevant parental NOAEL | 0.7 mg/kg bw per day | |
| Relevant reproductive NOAEL | 32 mg/kg bw per day (highest dose tested) | |
| Relevant offspring NOAEL | 4.8 mg/kg bw per day | |

## Developmental toxicity

| | | |
|---|---|---|
| Developmental target / critical effect | Rat:<br>Maternal toxicity: ↑ liver weight<br>Developmental toxicity: ↑ incidence of foetuses with enlarged thymus, ossification retardation and short 13th rib(s)<br>Rabbit:<br>Maternal toxicity: ↓ body weight gain<br>Developmental toxicity: ↑ incidence of incomplete ossification (of pubis, metacarpal and/or middle phalanges) | |
| Relevant maternal NOAEL | Rat: 3 mg/kg bw per day<br>Rabbit: 0.5 mg/kg bw per day | |
| Relevant developmental NOAEL | Rat: 10 mg/kg bw per day<br>Rabbit: 0.5 mg/kg bw per day | |

## Neurotoxicity (Regulation (EU) No 283/2013, Annex Part A, point 5.7)

| | | |
|---|---|---|
| Acute neurotoxicity | NOAEL: 25 mg/kg bw; reversible behavioural changes on the day of treatment at LOAEL (35 mg/kg bw) and top dose (250 mg/kg bw). Not clear whether the observed behavioural and motor activity changes were specific to the nervous system or a generalized toxic/stress reaction to the treatment. | |
| Repeated neurotoxicity | NOAEL neurotoxicity: 28.7 mg/kg bw per day (highest dose tested); no sign of a specific neurotoxic potential.<br>NOAEL general toxicity: 1.4 mg/kg bw per day (↑ thyroid weight, ↑ liver weight). | |
| Additional studies (e.g. delayed neurotoxicity, developmental neurotoxicity) | No data | |

**Other toxicological studies (Regulation (EU) No 283/2013, Annex Part A, point 5.8)**

| | |
|---|---|
| Supplementary studies on the active substance | Mechanistic studies:<br>Experimental in vitro and in vivo evidence suggests that ethiprole is an activator of nuclear hormone receptors in rats, mice and humans. Rats (in vitro and in vivo): mainly PXR and CAR; mice (in vivo): PXR, CAR and AhR; humans (in vitro): PXR and CAR (weak).<br>Ethiprole stimulates cell proliferation in cultures of cryopreserved male Han Wistar rat primary hepatocytes. No effect of ethiprole was noticed on cell proliferation in primary cultures of human hepatocytes.<br>Immunotoxicity:<br>NOAEL 28-day immunotoxicity study in female rats: 41.2 mg/kg bw per day (highest dose tested).<br>Based on haematological and histopathological findings in standard regulatory repeat dose toxicity studies and the 28-day immunotoxicity study, there is no evidence of a specific or primary effect of ethiprole on the immune system. |
| Endocrine disrupting properties | The ED criteria for T-modality are met. The ED criteria for the EAS-modality are not met, based on a complete dataset on adversity.<br>The NOAEL for thyroid effects is 1 mg/kg bw per day based on the thyroid follicular cell hypertrophy observed at 3-5 mg/kg bw per day in the 2-year study in rats. |
| Studies performed on metabolites or impurities | Metabolite RPA097973 (M01; ethiprole-sulfone):<br>Negative in Ames test<br>Acute oral $LD_{50}$ in rats > 2000 mg/kg bw<br>As major metabolite observed in the rat metabolism studies (when also considering downstream metabolites), toxicological reference values of the parent apply to this metabolite.<br>Metabolite RPA112916 (M05; ethiprole-amide):<br>Negative in Ames test and in in vitro micronucleus test<br>Acute oral $LD_{50}$ in rats > 5000 mg/kg bw<br>NOAEL 28-day study in rats: 5 mg/kg bw per day. Effects at LOAEL: ↑ TSH, ↑ liver weight, ↑ incidence diffuse microvacuolation of the zona fasciculata of adrenals.<br>As this metabolite is unlikely to be genotoxic and of similar potency or lower potency than ethiprole (based on data from 28-day rat studies), toxicological reference values of the parent apply to this metabolite. |

**Medical data (Regulation (EU) No 283/2013, Annex Part A, point 5.9)**

| |
|---|
| Occupational medical surveillance of workers exposed to ethiprole in production and formulation, performed annually or every 18 months (not in each case relating to exposures) has not revealed any unwanted effects. No accidents with ethiprole have occurred and no consultations with the medical departments due to work or contact with ethiprole have been required.<br>No reports are available on any symptoms for researchers handling the product in field trials and no human poisoning cases have been published. |

| **Summary (Regulation (EU) No 1107/2009, Annex II, point 3.1 and 3.6)** | Value (mg/kg bw (per day)) | Study | Uncertainty factor |
|---|---|---|---|
| Acceptable Daily Intake (ADI)* | 0.002 | dog, 1-year | 100 |
| Acute Reference Dose (ARfD)* | 0.005 | rabbit, maternal effects in developmental toxicity study | 100 |
| Acceptable Operator Exposure Level (AOEL) | Not established, not relevant to import tolerances | - | - |
| Acute Acceptable Operator Exposure Level (AAOEL) | Not established, not relevant to import tolerances | - | - |

* ADI and ARfD apply also to metabolites RPA097973 (M01; ethiprole-sulfone) and RPA112916 (M05; ethiprole-amide)

**Classification with regard to toxicological data (Regulation (EU) N° 283/2013, Annex Part A, Section 10)**

| Substance: | ethiprole |
|---|---|
| Harmonised classification according to Regulation (EC) No 1272/2008 and its Adaptations to Technical Process [Table 3.1 of Annex VI of Regulation (EC) No 1272/2008 as amended][26] : | No harmonised classification |
| According to the Peer review, the criteria for classification may be met for: | Specific target organ toxicity–repeated exposure: (STOT-RE) Category 2:<br>H373: "May cause damage to liver and thyroid through prolonged or repeated exposure". |

[26]Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, 1-1355.

## A.2 | RESIDUES IN PLANTS

### A.2.1 | Nature of residues and analytical methods for enforcement purposes in plant commodities

#### A.2.1.1 | Metabolism studies, analytical methods and residue definitions in plants

| Primary crops (available studies) | Crop groups | Crop(s) | Application(s) | Sampling (DAT) | Comment/source |
|---|---|---|---|---|---|
| | Fruit crops | Sweet peppers | Foliar spray (indoor):<br>1st: 450 g a.s./ha<br>2nd 220 g a.s./ha | 14 | Radiolabelled active substance: phenyl-UL-$^{14}$C-ethiprole (Netherlands, 2021). |
| | Cereals/grass | Rice | Foliar spray (indoor):<br>1st: 450 g a.s./ha<br>2nd 220 g a.s./ha | 14 | Radiolabelled active substance: phenyl-UL-$^{14}$C-ethiprole (Netherlands, 2021). |
| | | Rice (paddy) | Soil (indoor):<br>2×600 g a.s./ha | 30 | |
| | Pulses/oilseeds | Cotton | Foliar spray (outdoor):<br>1st: 450 g a.s./ha<br>2nd 220 g a.s./ha | 48 | Radiolabelled active substance: phenyl-UL-$^{14}$C-ethiprole (Netherlands, 2021). |
| | | | Foliar spray (outdoor):<br>1st: 4500 g a.s./ha<br>2nd: 2200 g a.s./ha | 48 | |
| **Rotational crops (available studies)** | **Crop groups** | **Crop(s)** | **Application(s)** | **PBI (DAT)** | **Comment/Source** |
| | – | | | | No study assessed in the framework of the present application. |
| **Processed commodities (hydrolysis study)** | **Conditions** | | **Stable?** | | **Comment/Source** |
| | **Ethiprole** | | | | |
| | Pasteurisation (20 min, 90°C, pH 4) | | Yes | | Ethiprole was shown to be stable to hydrolysis under all standard simulated conditions (Netherlands, 2021).<br>Slight degradation under sterilisation conditions (5.5% of AR) to ethiprole-amide observed. |
| | Baking, brewing and boiling (60 min, 100°C, pH 5) | | Yes | | |
| | Sterilisation (20 min, 120°C, pH 6) | | Yes | | |
| | **Ethiprole-sulfone** | | | | |
| | Pasteurisation (20 min, 90°C, pH 4) | | Yes | | Ethiprole-sulfone was shown to be stable to hydrolysis under all standard simulated conditions (Netherlands, 2021). Slight degradation to ethiprole-sulfone-amide under sterilisation conditions (3.9% AR) observed. |
| | Baking, brewing and boiling (60 min, 100°C, pH 5) | | Yes | | |
| | Sterilisation (20 min, 120°C, pH 6) | | Yes | | |

| | | |
|---|---|---|
| Can a general residue definition be proposed for primary crops? | Yes | For cereals, both foliar and soil treatments are covered.<br>For other crops, only foliar treatments are covered. |
| Rotational crop and primary crop metabolism similar? | Inconclusive | No study assessed in the framework of EU assessments. |
| Residue pattern in processed commodities similar to residue pattern in raw commodities? | Yes | Based on standards hydrolysis studies performed with ethiprole and ethiprole-sulfone. |
| Plant residue definition for monitoring (RD-Mo) | Ethiprole | |
| Plant residue definition for risk assessment (RD-RA) | Primary crops (raw and processed): Sum of ethiprole,ethiprole-sulfone (RPA097973) and ethiprole-amide (RPA112916), expressed as ethiprole<br>[For cereals: both foliar and soil applications]<br>[For other crop groups: only foliar applications] | |
| Methods of analysis for monitoring of residues (analytical technique, crop groups, LOQs) | Dry matrices:<br>LC-MS/MS, LOQ 0.002 mg/kg (validated in wheat grain)<br>No confirmatory method required (two mass transitions)<br>ILV available for rice grain.<br>(The Netherlands, 2021) | |

DAT: days after treatment; PBI: plant-back interval; BBCH: growth stages of mono- and dicotyledonous plants; a.s.: active substance; MRL: maximum residue level; GC-MS: gas chromatography with mass spectrometry; LC-MS/MS: liquid chromatography with tandem mass spectrometry; HPLC-MS/MS: high performance liquid chromatography with tandem mass spectrometry; LOQ: limit of quantification; QuEChERS: Quick, Easy, Cheap, Effective, Rugged, and Safe; ILV: independent laboratory validation.

A.2.1.2 | Stability of residues in plants

| Plant products (available studies) | Category | Commodity | T (°C) | Stability period | | Compounds covered | Comment/source |
|---|---|---|---|---|---|---|---|
| | | | | Value | Unit | | |
| | Dry/High starch | Rice grain | –23 | 12 | M | Ethiprole | No data available for ethiprole-amide (The Netherlands, 2021) |
| | Dry/High starch | Rice grain | –23 | 12 | M | Ethiprole-sulfone | |
| | Dry/High starch | – | – | – | – | Ethiprole-amide | |

### A.2.2 | Magnitude of residues in plants

Not relevant.

## A.3 | Residues in livestock

Not relevant.

## A.4 | Consumer risk assessment

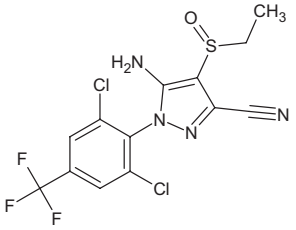
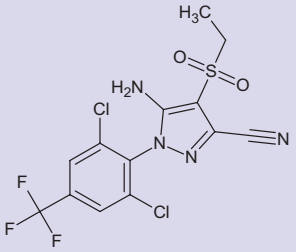
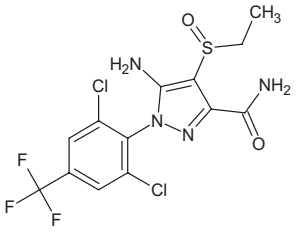
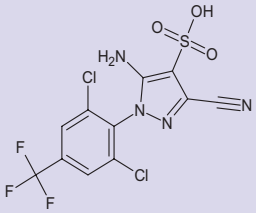
Not relevant.

## A.5 | Recommended MRLs

Not relevant.

# APPENDIX B

## Used compound codes

| Code/trivial name[a] | IUPAC name/SMILES notation/InChiKey[b] | Structural formula[c] |
|---|---|---|
| Ethiprole[d] | 5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-(ethylsulfinyl)-1*H*-pyrazole-3-carbonitrile<br>O=S(CC)c1c(C#N)nn(c1N)c1c(Cl)cc(cc1Cl)C(F)(F)F<br>FNELVJVBIYMIMC-UHFFFAOYSA-N |  |
| Ethiprole-sulfone<br>RPA097973<br>(M01) | 5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-(ethanesulfonyl)-1H-pyrazole-3-carbonitrile<br>SGTQRPFDKIRFIQ-UHFFFAOYSA-N<br>O=S(=O)(CC)c1c(C#N)nn(c1N)c1c(Cl)cc(cc1Cl)C(F)(F)F |  |
| Ethiprole-amide<br>RPA112916<br>(M05) | 5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-(ethanesulfinyl)-1H-pyrazole-3-carboxamide<br>QQVQUIRUQOHHEP-UHFFFAOYSA-N<br>O=S(CC)c1c(nn(c2c(Cl)cc(cc2Cl)C(F)(F)F)c1N)C(=O)N |  |
| RPA104615 (M03) | 5-amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-4-sulfonic acid<br>ABOHYNBXDJHVHB-UHFFFAOYSA-N<br>O=S(O)(=O)c1c(C#N)nn(c2c(Cl)cc(cc2Cl)C(F)(F)F)c1N |  |

Abbreviations: InChiKey, International Chemical Identifier Key; IUPAC, International Union of Pure and Applied Chemistry; SMILES, simplified molecular-input line-entry system.

[a]The metabolite name in bold is the name used in the conclusion.

[b]ACD/Name 2021.1.3 ACD/Labs 2021.1.3 (File Version N15E41, Build 123,232, 07 July 2021).

[c]ACD/ChemSketch 2021.1.3 ACD/Labs 2021.1.3 (File Version C25H41, Build 123,835, 28 August 2021).

[d]It should be noted that name of ethiprole and its three metabolites presented above are identified as a pesticide active substance/metabolites that meet the definition of per- and polyfluoroalkyl substances (PFAS) based on its chemical structure (https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas).