5.25 ISOXAFLUTOLE (268)

TOXICOLOGY

Isoxaflutole is the ISO-approved name for 5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole (IUPAC), with CAS No. 141112-29-0. Isoxaflutole is an isoxazole herbicide that is used as a pre-emergent or early post-emergence broadcast treatment for the control of broadleaf and grass weeds. Its primary target in plants is the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD); inhibition of the enzyme results in the bleaching of weeds due to the blockage of phenylquinone biosynthesis.

Isoxaflutole has not previously been evaluated by JMPR and was reviewed by the present Meeting at the request of CCPR.

All critical studies contained statements of compliance with GLP.

Biochemical aspects

Following oral gavage dosing of rats, isoxaflutole was rapidly absorbed: about 70% after low dose (1 mg/kg bw) administration and about 40% after high dose (100 mg/kg bw) administration. The maximum concentrations in blood (C_{max}) were achieved between 0.5 and 1 hour post-dosing. Only about 1.5–4.4% of the dose was recovered in the tissues (e.g. kidney, liver, blood and plasma) 7 days after dosing. The elimination of the radioactivity associated with [^{14}C]isoxaflutole following oral administration was rapid, with the majority (80%) of the radioactivity being eliminated within 48 hours at the high dose level and within 24 hours at the low dose level. The urine was the major route of elimination for the low-dose groups (about 69–74% of the dose), whereas faeces was the major route of elimination for the high-dose group (about 55–63% of the dose). Isoxaflutole and/or its metabolites have a mean β-phase elimination half-life of about 60 hours, irrespective of the dose level. Up to nine radiolabelled components were found in the urine, and up to 11 in the faeces. The major component identified in urine, faeces and liver was a diketonitrile (RPA 202248, or 3-cyclopropyl-2-[2-mesyf-4-trifluoromethylbenzoy]-3-oxopropane nitrile), followed by RPA 203328 (2-mesyf-4-trifluoromethylbenzoic acid). Unchanged isoxaflutole was detected primarily in faeces in the high-dose animals. There were no sex differences in absorption, distribution or metabolism.

Toxicological data

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw, and the acute dermal LD_{50} in both rats and rabbits was greater than 2000 mg/kg bw. The acute inhalation LC_{50} in rats was greater than the maximum achievable concentration of 5.23 mg/L air. Isoxaflutole was non-irritating to rabbit skin and minimally irritating to rabbit eyes. It was not a skin sensitizer in guinea-pigs, as determined by the Buehler method and the Magnusson and Kligman test.

The liver was the primary target organ in mice, rats and dogs in repeated-dose toxicity studies. Thyroid, kidney and the haematopoietic system were also target organs in dogs and rats. Corneal opacity was observed in repeated-dose toxicity studies in rats, but not in mice or dogs.

In a 28-day toxicity study in mice using dietary concentrations of 0, 175, 700, 2800 and 7000 ppm (equal to 0, 29.4, 120.7, 474.6 and 1140.1 mg/kg bw per day for males and 0, 34.7, 142.9, 534.4 and 1347.4 mg/kg bw per day for females, respectively), the NOAEL was 175 ppm (equal to 29 mg/kg bw per day), based on increases in liver enzymes (e.g. ALT, aspartate aminotransferase [AST] and AP), clinical chemistry changes (decreased bilirubin and creatinine levels) and increased liver weight at 700 ppm (equal to 120.7 mg/kg bw per day). In the absence of any other significant findings at 175 ppm, the increased liver weights were considered a minor adaptive change.

In a 90-day toxicity study in mice using dietary concentrations of 0, 50, 1000 and 2000 ppm (equal to 0, 7.6, 170.0 and 324.1 mg/kg bw per day for males and 0, 8.7, 181.2 and 376.2 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 0.87 mg/kg bw per day), based on increases in liver enzymes (e.g. ALT, aspartate aminotransferase [AST] and AP), clinical chemistry changes (decreased bilirubin and creatinine levels) and increased liver weight at 1000 ppm (equal to 17.0 mg/kg bw per day). In the absence of any other significant findings at 50 ppm, the increased liver weights were considered a minor adaptive change.
per day for females, respectively), the NOAEL of 50 ppm (equal to 7.6 mg/kg bw per day) was based on increased ALT and AST activities, increased absolute and relative liver weights and increased incidence of periacinar hepatocytic hypertrophy at 1000 ppm (equal to 170.0 mg/kg bw per day).

In a 6-week toxicity study in rats given diets providing doses of 0, 25, 100, 400 and 1000 mg/kg bw per day, the LOAEL was 25 mg/kg bw per day, based on corneal opacities and effects on the liver observed at all doses. Most of the corneal opacities were resolved by the 2nd week of the reversibility period. In a 90-day dietary toxicity study in rats at doses of 0, 1, 3, 10 and 100 mg/kg bw per day, the NOAEL was 3 mg/kg bw per day, based on haematological changes, corneal opacity and liver toxicity observed at 10 mg/kg bw per day.

In a 1-year toxicity study in dogs using dietary concentrations of 0, 240, 1200, 12 000 and 30 000 ppm (equal to 0, 8.56, 44.81, 453 and 1265 mg/kg bw per day for males and 0, 8.41, 45.33, 498 and 1254 mg/kg bw per day for females, respectively), the NOAEL was 1200 ppm (equal to 44.81 mg/kg bw per day), based on reduced weight gains, increased liver weight, histopathological findings in the liver and changes in haemological and clinical chemistry parameters at 12 000 ppm (equal to 453 mg/kg bw per day).

In a 90-day dietary study in dogs providing doses of 0, 0.5, 2, 20 and 500 mg/kg bw per day, the NOAEL was 2 mg/kg bw per day, based on liver, thyroid, ocular and nervous system toxicity in males and liver toxicity in females seen at 20 mg/kg bw per day. An increased incidence of adenomas and carcinomas of the liver was found in male and female rats at 500 mg/kg bw per day. In male rats, an increase of thyroid follicular cell adenomas was also observed at 500 mg/kg bw per day.

In a 78-week study of toxicity and carcinogenicity in mice using dietary concentrations of 0, 25, 500 and 7000 ppm (equal to 0, 3.2, 64.4 and 977.3 mg/kg bw per day for males and 0, 4.0, 77.9 and 1161.1 mg/kg bw per day for females, respectively), the NOAEL was 25 ppm (equal to 3.2 mg/kg bw per day), based on liver effects seen at 500 ppm (equal to 64.4 mg/kg bw per day). The NOAEL for carcinogenicity was 500 ppm (equal to 64.4 mg/kg bw per day), based on an increased incidence of hepatocellular adenomas and carcinomas in both sexes at 7000 ppm (equal to 977.3 mg/kg bw per day).

In a 14-day dietary study in mice and rats indicated a marked increase in microsomal enzyme induction (increased pentoxyresorufin O-depentylase [PROD] and benzoxyresorufin O-debenzylase [BROD] activities) and increased liver weights. There was no peroxisome proliferation. The data were inadequate to elucidate the precursor events leading to tumour formation and dose concordance for hepatocellular adenomas and carcinomas in mice and rats. In a 14-day oral gavage study in rats, isoxaflutole was found to decrease T\(_4\) levels, with little or no change in T\(_3\) levels, and an increased systemic clearance of \(^{125}\text{I}\)-labelled T\(_4\) was observed. The results of these mechanistic studies were suggestive of the induction of microsomal enzymes and tumour formation, but failed to establish the mode of action.

The Meeting concluded that isoxaflutole is carcinogenic in mice and rats.

Special studies conducted to evaluate the corneal opacity seen in rats suggest that the lesion may be linked to the inhibition of the enzyme HPPD in the catabolic pathway of tyrosine. The studies have shown that if HPPD is inhibited, alternative pathways may be utilized to remove excess tyrosine, and species specificity may be linked to the differences in activity of these alternative pathways. The results of the comparative metabolism study in mice and rats suggest that the elimination of tyrosine as 4-hydroxyphenyl lactate and 4-hydroxyphenyl acetate is more efficient in the mouse than in the rat, with twice as much of the administered dose of \([^{14}\text{C}]\text{tyrosine}\) observed in mouse urine as in rat urine. The results of special studies indicate that rats are more sensitive than mice, dogs and humans to tyrosinaemia.

Isoxaflutole was tested for genotoxicity in vitro and in vivo in an adequate range of assays. No genotoxicity was observed.
The Meeting concluded that isoxaflutole is unlikely to be genotoxic.

On the basis of the absence of genotoxicity and other available toxicological information, the Meeting concluded that the mode of action for the increased incidences of hepatocellular adenomas and carcinomas in both male and female mice and rats and the increased incidence of thyroid follicular cell adenomas in male rats, while not completely understood, is likely to involve a threshold. Therefore, the Meeting concluded that isoxaflutole is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study in rats given diets providing doses of 0, 0.5, 2, 20 and 500 mg/kg bw per day, the NOAEL for parental systemic toxicity and offspring toxicity was 2 mg/kg bw per day. The NOAEL for reproductive toxicity was 500 mg/kg bw per day, the highest dose tested. The parental systemic toxicity LOAEL of 20 mg/kg bw per day was based on increased liver weights, liver hypertrophy and vacuolation. The offspring toxicity LOAEL of 20 mg/kg bw per day was based on decreased pup weights and reduced pup viability.

In a developmental toxicity study in rats that tested doses of 0, 10, 100 and 500 mg/kg bw per day, the maternal NOAEL was 100 mg/kg bw per day, based on decreased body weight gain observed at 500 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, based on decreased fetal weight and delayed ossification observed at 100 mg/kg bw per day.

In a developmental toxicity study in rats given diets providing doses of 0, 0.5, 2, 20 and 500 mg/kg bw per day, the NOAEL for parental systemic toxicity and offspring toxicity was 2 mg/kg bw per day, based on decreased body weight gain observed at 500 mg/kg bw per day. The NOAEL for reproductive toxicity was 500 mg/kg bw per day, the highest dose tested. The parental systemic toxicity LOAEL of 20 mg/kg bw per day was based on increased liver weights, liver hypertrophy and vacuolation. The offspring toxicity LOAEL of 20 mg/kg bw per day was based on decreased pup weights and reduced pup viability.

The Meeting concluded that isoxaflutole is not teratogenic in rats or rabbits.

In an oral acute neurotoxicity study in rats that tested doses of 0, 125, 500 and 2000 mg/kg bw, no evidence of neurotoxicity or systemic toxicity was observed at doses up to 2000 mg/kg bw. In a 90-day neurotoxicity study in rats given diets providing doses of 0, 25, 250 and 750 mg/kg bw per day, no neurotoxicity was observed at doses up to 750 mg/kg bw per day. A NOAEL for systemic toxicity was not identified, as only limited parameters were evaluated in this study.

In a developmental neurotoxicity study in rats that tested gavage doses of 0, 5, 25 and 250 mg/kg bw per day, the maternal NOAEL was 25 mg/kg bw per day, based on decreased maternal body weight, body weight gain and feed consumption at 250 mg/kg bw per day. The NOAEL for fetal toxicity NOAEL was 25 mg/kg bw per day, based on slightly delayed development of the fetuses, decreased fetal weights and delayed ossification at 100 mg/kg bw per day.

The Meeting concluded that isoxaflutole is not neurotoxic.

Toxicological data on metabolites and/or degradates

The acute oral LD₅₀ of metabolite RPA 202248, a major metabolite of urine, faeces and liver, was greater than 5000 mg/kg bw. The metabolite was not genotoxic in the Ames test.

Metabolite RPA 203328, detected in urine and faeces, was extensively studied. The acute oral LD₅₀ in rats was greater than 5000 mg/kg bw. RPA 203328 was not genotoxic in a range of in vivo and in vitro genotoxicity assays. In a 14-day gavage toxicity study in rats, the NOAEL for RPA 203328 was 30 mg/kg bw per day, based on increased salivation, slightly decreased body weight gains and changes in the haematology and clinical chemistry parameters seen at 300 mg/kg bw per day. Dietary 28-day and 90-day toxicity studies in rats were conducted for RPA 203328 at doses up to 15 000 ppm (equal to 1178 mg/kg bw per day) and 12 000 ppm (equal to 769 mg/kg bw per day), respectively. No evidence of systemic toxicity was observed in these studies. No evidence of
teratogenicity or developmental toxicity in rats was observed in a developmental toxicity study for RPA 203328 at doses up to 750 mg/kg bw per day.

**Human data**

In reports on employees working in isoxaflutole manufacturing plants, no adverse health effects were reported.

The Meeting concluded that the existing database on isoxaflutole was adequate to characterize the potential hazards to fetuses, infants and children.

**Toxicological evaluation**

The Meeting established an ADI of 0–0.02 mg/kg bw on the basis of a NOAEL of 2 mg/kg bw per day in a 2-year dietary study of toxicity and carcinogenicity in rats, on the basis of liver, thyroid and nervous system toxicity in males and liver toxicity in females at 20 mg/kg bw per day. A safety factor of 100 was applied. This ADI is supported by a NOAEL of 2 mg/kg bw per day in a dietary two-generation reproductive toxicity study in rats, based on increased liver weights, liver hypertrophy, vacuolation, decreased pup weights and pup viability observed at 20 mg/kg bw per day. The ADI provides a margin of exposure of at least 25 000 relative to the LOAEL for liver and thyroid tumours in rats and at least 48 000 relative to the LOAEL for the liver tumour response in mice. Thus, the Meeting considered that isoxaflutole is not likely to pose a carcinogenic risk to humans from the diet.

The Meeting concluded that it was not necessary to establish an ARfD for isoxaflutole in view of its low acute toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

A toxicological monograph was prepared.

**Levels relevant to risk assessment of isoxaflutole**

<table>
<thead>
<tr>
<th>Species</th>
<th>Study</th>
<th>Effect</th>
<th>NOAEL</th>
<th>LOAEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td>Toxicity</td>
<td>50 ppm, equal to 7.6 mg/kg bw per day</td>
<td>1000 ppm, equal to 170 mg/kg bw per day</td>
</tr>
<tr>
<td></td>
<td>Six-month study of toxicity</td>
<td>Toxicity</td>
<td>25 ppm, equal to 3.2 mg/kg bw per day</td>
<td>500 ppm, equal to 64.4 mg/kg bw per day</td>
</tr>
<tr>
<td></td>
<td>Toxicity and carcinogenicity</td>
<td>Carcinogenicity</td>
<td>500 ppm, equal to 64.4 mg/kg bw per day</td>
<td>7000 ppm, equal to 977 mg/kg bw per day</td>
</tr>
<tr>
<td>Rat</td>
<td>Acute neurotoxicity study</td>
<td>Toxicity</td>
<td>2000 mg/kg bw</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Ninety-day study of toxicity</td>
<td>Toxicity</td>
<td>3 mg/kg bw per day</td>
<td>10 mg/kg bw per day</td>
</tr>
<tr>
<td></td>
<td>Two-year study of toxicity and carcinogenicity</td>
<td>Toxicity</td>
<td>2 mg/kg bw per day</td>
<td>20 mg/kg bw per day</td>
</tr>
<tr>
<td></td>
<td>Two-generation study of reproductive toxicity</td>
<td>Reproductive toxicity</td>
<td>500 mg/kg bw per day</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parental toxicity</td>
<td>2 mg/kg bw per day</td>
<td>20 mg/kg bw per day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Offspring toxicity</td>
<td>2 mg/kg bw per day</td>
<td>20 mg/kg bw per day</td>
</tr>
<tr>
<td>Species</td>
<td>Study</td>
<td>Effect</td>
<td>NOAEL</td>
<td>LOAEL</td>
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<td>---------</td>
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</tr>
<tr>
<td>Rabbit</td>
<td>Developmental toxicity study&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Maternal toxicity</td>
<td>100 mg/kg bw per day</td>
<td>500 mg/kg bw per day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryo and fetal toxicity</td>
<td>10 mg/kg bw per day</td>
<td>100 mg/kg bw per day</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Developmental toxicity study&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Maternal toxicity</td>
<td>20 mg/kg bw per day</td>
<td>100 mg/kg bw per day</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Embryo and fetal toxicity</td>
<td>20 mg/kg bw per day</td>
<td>100 mg/kg bw per day</td>
</tr>
<tr>
<td>Dog</td>
<td>One-year study of toxicity&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Toxicity</td>
<td>1200 ppm, equal to 44.8 mg/kg bw per day</td>
<td>12 000 ppm, equal to 453 mg/kg bw per day</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dietary administration.
<sup>b</sup> Gavage administration.
<sup>c</sup> Highest dose tested.

**Estimate of acceptable daily intake**

0–0.02 mg/kg bw

**Estimate of acute reference dose**

Unnecessary

**Information that would be useful for the continued evaluation of the compound**

Results from epidemiological, occupational health and other such observational studies of human exposure

**Critical end-points for setting guidance values for exposure to isoxaflutole**

*Absorption, distribution, excretion and metabolism in mammals*

- Rate and extent of oral absorption: Rapid, at least 70%
- Dermal absorption: Low, < 4.5%
- Distribution: Widely distributed (highest levels in kidney and liver)
- Potential for accumulation: None
- Rate and extent of excretion: Rapid and complete, about 80% in urine and faeces in 24 h in rats
- Metabolism in animals: Extensive; saturated at high doses
- Toxicologically significant compounds in animals, plants and the environment: Isoxaflutole, RPA 202248<sup>a</sup>, RPA 205834<sup>a</sup>, RPA 207048<sup>a</sup>

*Acute toxicity*

- Rat, LD<sub>50</sub> oral: > 5000 mg/kg bw
- Rat, LD<sub>50</sub> dermal: > 2000 mg/kg bw
- Rat, LC<sub>50</sub> inhalation: > 5.23 mg/L (whole-body exposure)
- Rabbit, dermal irritation: Non-irritating
- Rabbit, ocular irritation: Minimally irritating
Isoxaflutole

Dermal sensitization  
Non-sensitizing (Buehler method and Magnusson-Kligman test)

**Short-term studies of toxicity**

<table>
<thead>
<tr>
<th>Target/critical effect</th>
<th>Eye, liver and red blood cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest relevant oral NOAEL</td>
<td>3 mg/kg bw per day (rat)</td>
</tr>
<tr>
<td>Lowest relevant dermal NOAEL</td>
<td>1000 mg/kg bw per day (rat)</td>
</tr>
<tr>
<td>Lowest relevant inhalation NOAEC</td>
<td>No data</td>
</tr>
</tbody>
</table>

**Long-term studies of toxicity and carcinogenicity**

<table>
<thead>
<tr>
<th>Target/critical effect</th>
<th>Liver and thyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest relevant oral NOAEL</td>
<td>2 mg/kg bw per day (rat)</td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>Unlikely to pose a carcinogenic risk to humans from the diet</td>
</tr>
</tbody>
</table>

**Genotoxicity**

Not genotoxic

**Reproductive toxicity**

<table>
<thead>
<tr>
<th>Target/critical effect</th>
<th>Pup viability and pup weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest relevant parental NOAEL</td>
<td>2 mg/kg bw per day</td>
</tr>
<tr>
<td>Lowest relevant offspring NOAEL</td>
<td>2 mg/kg bw per day</td>
</tr>
<tr>
<td>Lowest relevant reproductive NOAEL</td>
<td>500 mg/kg bw per day, the highest dose tested</td>
</tr>
</tbody>
</table>

**Developmental toxicity**

<table>
<thead>
<tr>
<th>Developmental target/critical effect</th>
<th>Delayed ossification, decreased fetal weights</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest maternal NOAEL</td>
<td>20 mg/kg bw per day (rabbit)</td>
</tr>
<tr>
<td>Lowest embryo/fetal NOAEL</td>
<td>10 mg/kg bw per day (rat)</td>
</tr>
</tbody>
</table>

**Neurotoxicity**

Acute and subchronic neurotoxicity  Not neurotoxic

**Other toxicological studies**

Studies on metabolites  Rat, LD₅₀, oral: > 5000 mg/kg bw (RPA 203348 and RPA 203328)

Lowest relevant short-term NOAEL: 769 mg/kg bw per day (RPA 203328)

Not genotoxic (RPA 202248 and RPA 203328)

**Medical data**

No adverse effects

*Based on structural similarity to the parent compound.

**Summary**

<table>
<thead>
<tr>
<th>Value</th>
<th>Study</th>
<th>Safety factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADI</td>
<td>0–0.02 mg/kg bw</td>
<td>100</td>
</tr>
<tr>
<td>ARfD</td>
<td>Unnecessary</td>
<td>—</td>
</tr>
</tbody>
</table>

Two-year study of toxicity and carcinogenicity in rats
RESIDUE AND ANALYTICAL ASPECTS

Isoxaflutole was scheduled for the evaluation as a new compound by 2013 JMPR at the Forty-fourth Session of the CCPR (2012). Isoxaflutole is a synthetic compound of the isoxazole group of chemicals used as a herbicide. The mode of action of isoxaflutole is the inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), which inhibits pigment formation, causing bleaching of the developing tissues of the target plants. Isoxaflutole controls a wide spectrum of grasses and broadleaf weeds by bleaching emerging or emerged weeds following herbicide uptake via the root system.

The Meeting received information from the manufacturer on identity, metabolism, storage stability, residue analysis, use patterns, residues resulting from supervised trials on sweet corn, chickpeas, glyphosate/HPPD tolerant soya beans, maize, sugar cane and poppy seed, fates of residue during processing, and livestock feeding studies.

**Chemical name:**

Isoxaflutole

IUPAC: 5-cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl)-isoxazole

Structural formula:

![Structural formula of Isoxaflutole](image)

Metabolites referred to in the appraisal by codes:

- **IFT-DKN**
  - Isoxaflutole diketonitrile; IUPAC: 3-cyclopropyl-2-(2-mesyl-4-(trifluoromethyl)benzoyl)-3-oxopropenitrile;

- **IFT-BA**
  - Isoxaflutole benzoic acid; IUPAC: 2-mesyl-4-trifluoromethylbenzoic acid;

- **IFT-amide** (no code)
  - Isoxaflutole benzamide; IUPAC: 2-mesy1-4-trifluoromethyl benzamide

- **RPA 205834**
  - 2-aminomethylene-l-cyclopropyl-3-(2-mesy1-4-trifluoromethylphenyl)-propane-1,3-dione
Animal metabolism

The Meeting received results of animal metabolism studies in lactating goats and laying hens. Experiments were carried out with \([U-^{14}C-phenyl]-isoxaflutole\).

Metabolism in laboratory animals was summarized and evaluated by the WHO panel of the JMPR in 2013. Following oral administration in rats, \([U-^{14}C-phenyl]-isoxaflutole\) was rapidly and extensively metabolized yielding nine radioactive fractions in the urine and up to eleven in the faeces. There were no indications of any metabolites resulting from phase II (conjugation) reaction. Parent isoxaflutole was only found in the faeces of the single 100 mg/kg bw high dose group and to a lesser extent also in the urine of this group (together 5.7–8.2% of the administered dose). The major radioactive component (70–85% of the administered dose) in both urine and faeces, as well as in solvent fractions of liver was IFT-DKN for all three dose groups (single 1 mg/kg bw low dose, single 100 mg/kg bw high dose, repeated 1 mg/kg bw low dose).

The parent compound metabolized to IFT-DKN (major pathway) or RPA 205834 and subsequently further oxidized, respectively to IFT-BA (most polar, 0.6–3.5% of the administered dose) or RPA 207048. A third minor metabolic pathway includes the cleavage of the sulfonic acid group to metabolite RPA 205568 (only 1.3–2.0% of the administered dose).

Four lactating goats, orally treated twice daily for 7 consecutive days with \([U-^{14}C-phenyl]-isoxaflutole\), were sacrificed 23 hours after the last dose. The four goats received low, medium (2) and high actual doses equivalent to 1.1 (goat 1), 10 (goat 2), 13 (goat 3) and 64 (goat 4) ppm dry feed (2.0, 20, 20 and 100 mg ai/kg bw, respectively). Total recovered radioactivity amounted to 97, 88, 78, and 73% of the administered dose in goat 1 to 4, respectively. Radioactivity recovered from urine and faeces ranged from 56% of the administered dose in the high dosed goat (27% in urine, 29% in faeces) to 85% in the low dosed goat (54% in urine, 31% in faeces). Radioactivity in edible tissues and organs ranged from 5.3% of the administered dose in the high dose goat to 11% in the low dose goat. Radioactivity in milk ranged from not detectable in the low dose goat to 0.54–0.60% of the administered dose in the medium to high dosed goats. Radioactivity levels in milk peaked at 0.093–0.095 mg/kg eq in goat 3 at day 4–5, 0.059–0.060 mg/kg eq in goat 2 at day 6–7 and 0.33–0.35 mg/kg eq in goat 4 at day 5–7.
Tissues (goat 3) and milk (goat 2) were subjected to further analysis. The total radioactive residues (TRR) were 2.1 mg/kg eq (liver), 0.90 mg/kg eq (kidney), 0.26 mg/kg eq (muscle), 0.069 mg/kg eq (renal fat), 0.062 mg/kg eq (omentum fat) and 0.060 mg/kg eq (milk). Radioactive residues could be extracted with methanol (milk), phosphate buffer pH 7.5 (liver, kidney and fat) or phosphate buffer pH 7.5 in combination with protease treatment (muscle). The extracted radioactive residues in the medium dosed goats amounted to 95% TRR for milk, 98% TRR for liver, 94% TRR for kidney, 76% TRR for muscle, 98% TRR for omental fat and 93% TRR for renal fat.

Parent compound was not found in any of the goat commodities. In goat milk, and all tissues the metabolite IFT-DKN was the most abundant component of the residues in the primary extracts (18–86% TRR or 0.015–1.8 mg/kg eq), followed by the metabolite RPA 207048 (9.1–26% TRR or 0.10–0.016 mg/kg eq, respectively). Muscle contained 18.3% TRR free and 23% conjugated IFT-DKN as well as 9.1% free and 3.4% conjugated RPA 207048; (released as free metabolites by protease treatment). Metabolite RPA 205834 (8.1–18% TRR or 0.005–0.011 mg/kg eq) was only found in milk and fat.

Ten laying hens, orally treated once daily for 14 consecutive days with [U-14C-phenyl]-isoxaflutole, were sacrificed 23 hours after the last dose. Hens were treated at an actual dose rate of 1.1 (group A) or 11 (group B) ppm dry feed. Total recovered radioactivity amounted to 117% and 92% of the administered dose in group A and B, respectively. Radioactivity from the excreta amounted to 112% and 88% of the administered dose for group A and B, respectively. Low levels of radioactivity were recovered in the eggs (0.12–0.15% of the administered dose) or tissues (1.7% in group A and 0.20% in group B).

In the low dose group (A) the concentrations of radioactivity in egg whites were below 0.002 mg/kg eq at all-time points. The levels of radioactivity in egg yolk reached a steady state within 7 days after the first dose (0.022–0.028 mg/kg eq). In the high dose group (B) the concentration of radioactivity in egg whites reached a steady state (0.010–0.015 mg/kg eq) within 4 days. The levels of radioactivity in egg yolk were up to 16 times higher, with a steady state concentration of 0.14–0.15 mg/kg eq within 7 days of exposure.

The highest radioactivity concentrations in edible tissues were found in the liver (0.84 and 0.95 mg/kg eq for dose group A and B, respectively) and kidney (0.055 and 0.16 mg/kg eq, respectively for A and B). Radioactivity in fat and muscle was only observed in the high (B) dose group, being 0.028 and 0.035 mg/kg eq, respectively. Some radioactivity was found in skin (0.008 and 0.068 mg/kg eq in dose group A and B, respectively).

Radioactivity was characterized in tissues and eggs from the high dose group B. Radioactive residues were extracted sequentially by exhaustive extractions using hexane, methanol, acetonitrile, ethyl acetate, acidified methanol and/or water. The primary extractable residues amounted to 97% TRR for liver, 74% TRR for kidney, 54% TRR for muscle, 93% TRR for fat, 53% TRR for skin, 66% TRR for egg yolk and 50% TRR for egg white. After treatment with protease, another 16%, 29%, 21%, 46% and 20% TRR could be released from kidney, muscle, skin, egg yolk and egg white, respectively. After extensive acid hydrolysis (6 M HCl at 95 ºC for 7 days) another 1.9%, 43%, 7.4% and 20% TRR could be released from kidney, muscle, skin and egg white, respectively.

In egg yolk (0.137 mg/kg eq), the major compounds in the primary extracts represented IFT-DKN (26% TRR) and RPA 205834 (28% TRR). Parent, IFT-BA and RPA 207048 were not found. Exhaustive acid hydrolysis released additional IFT-DKN (17% TRR) from egg yolk. Residues in egg white (0.010 mg/kg eq) and the remaining fractions from egg yolk could not be identified, although two fractions in egg yolk contained considerable radioactivity (10% TRR or 0.014 mg/kg eq in the primary extract and 18% TRR or 0.025 mg/kg eq in the acid hydrolysate).

In hen liver (0.953 mg/kg eq), kidney (0.155 mg/kg eq) and skin (0.068 mg/kg eq), the major compound in the primary extracts represented IFT-DKN (93%, 74% and 37% TRR, respectively). Parent, RPA 207048 and RPA 205834 were not found. IFT-BA was found as minor metabolite in
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liver and kidney (3.5% TRR and 0.65% TRR, respectively). Protease digestion and exhaustive acid hydrolysis released additional IFT-DKN (18% and 7.4% TRR, respectively) from skin.

In hen muscle (0.035 mg/kg eq) the only compound identified in the primary extracts was RPA 207048 (31%TRR). Parent and RPA 205834 were not found. Exhaustive acid hydrolysis released additional RPA 207048 (17% TRR) as well low levels of IFT-DKN and IFT-BA (5.7% and 5.7% TRR, respectively) from muscle.

In hen fat (0.028 mg/kg eq) the major compounds in the primary extracts were IFT-DKN (29% TRR) and RPA 207048 (21% TRR).

The metabolic pathway of isoxaflutole in livestock involves the opening of the oxazole ring and the formation of the diketo-nitrile derivative (IFT-DKN) or the diketo-amine derivative (RPA 205834). Further degradation occurs through deamination to form the diketo-hydroxy derivative (RPA 207048) or through further cleavage to form the benzoic acid derivative (IFT-BA). Further metabolism involves conjugation of IFT-DKN, RPA 207048 and/or IFT-BA with proteins or acid cleavable compounds.

Parent compound was not found in milk, eggs, goat tissues or hen tissues. This may represent rapid metabolism within the animal or it may represent degradation during frozen storage of the samples or degradation during extraction.

The major compounds identified in goat, hen tissues, milk or eggs are: IFT-DKN, RPA 207048, RPA 205834, conjugated IFT-DKN and conjugated RPA 207048. In goats, metabolites IFT-DKN (18–86% TRR) and RPA 207048 (9.1–26% TRR) were found in all tissues and milk. Metabolite RPA 205834 (8.1–18% TRR) was only found in milk and fat. Conjugated forms of IFT-DKN (23% TRR) and RPA 207048 (3.4% TRR) were only found in goat muscle and free metabolites could be released by protease digestion. In hens, metabolite IFT-DKN (26–93% TRR) was found in eggs and all tissues, except muscle. Metabolite RPA 207048 (21–31% TRR) was only found in muscle and fat. Metabolite RPA 205834 was only found in eggs (28% TRR in egg yolk). Conjugated forms of IFT-DKN (5.7–25% TRR) were found in hen muscle, skin and eggs and free metabolites could be released by protease (skin only) or exhaustive acid hydrolysis. Conjugated forms of RPA 207048 were found in hen muscle only and free metabolites could be released by exhaustive acid hydrolysis. IFT-BA was only found as a minor metabolite in hen liver and kidney (3.5% TRR and 0.65% TRR, respectively) and as conjugate form in hen muscle (5.7% TRR).

The metabolic pathway in ruminants and poultry is identical to the metabolic pathway in rats, although in rats an additional minor pathway to RPA 205568 is found, which is not found in ruminant or poultry. All metabolites identified in livestock are also found in rats.

Plant metabolism

The Meeting received plant metabolism studies for isoxaflutole on cereals (maize and wheat), pulses/oilseeds (soya beans and poppy seeds) and sugar cane after a pre-plant, crop pre-emergence, crop post-emergence or crop foliar application.

The metabolism of [U-14C-phenyl]-isoxaflutole in outdoor grown maize was studied following pre-plant soil incorporated and pre-emergent applications. Maize forage, grains and fodder were harvested at DAT = 41, 122 and 122/138. Residue levels in maize forage, grains and fodder were 0.20–0.044–0.15 mg/kg eq, respectively, for pre-plant incorporated treatment at 0.21 kg ai/ha and 0.23–0.039–0.12 mg/kg eq, respectively, for pre-emergence treatment after 0.23 kg ai/ha. Residue levels were 0.80–0.15–0.66 mg/kg eq for pre-plant incorporated treatment at 0.66 kg ai/ha and 0.49–0.12–0.53 mg/kg eq for pre-emergence treatment at 1.1 kg ai/ha. Residue levels in maize forage, grains and fodder after pre-plant incorporated treatment were higher than those after pre-emergence treatment at the high dose rate; they were similar at the low dose rates. Exaggerated dose rates exhibited greater phytotoxicity. Radioactivity was characterized in maize from the low dose treatments. The major part of the residues could be extracted with an exhaustive range of solvents:
hexane/ethyl acetate, acetonitrile, water pH 5.5, and acidified acetonitrile: 91–99% TRR in forage, 83–87% TRR in grains and 75–79% TRR in fodder. Additional residues could be released by cellulase digestion (3.0–6.5% TRR). Parent isoxaflutole was not found in any of the maize commodities. The major compound identified was free IFT-BA (61–89% TRR) in all maize commodities. Minor metabolites represented conjugated IFT-DKN (0.49–0.53% TRR in forage only) and conjugated IFT-BA (1.1–2.7% TRR in forage and fodder).

In a second study, the metabolism of [U-\textsuperscript{14}C-phenyl]-isoxaflutole was studied in outdoor grown maize following post-emergent application at 0.21 kg ai/ha in the presence of the safener cyprosulfamide. Maize forage and sweet corn were harvested at DAT 75; maize grains and fodder were harvested at DAT = 106. Residue levels in maize forage, sweet corn, maize grains and fodder were 0.13–0.010–0.015–0.10 mg/kg eq, respectively after pre-plant incorporated treatment. These residue levels were similar to those from the pre-plant incorporated and pre-emergence treatments in the previous study. The major part of the residues could be extracted with acetonitrile and water: 93% TRR in forage, 97% TRR in sweet corn, 77% TRR in grains and 88% TRR in fodder. Aqueous fractions underwent base hydrolysis. Parent isoxaflutole was not found in any of the maize commodities. The major compound identified was free IFT-BA (52–63% TRR) in all maize commodities. Other identified metabolites represented free IFT-DKN (4.0–9.8% TRR in sweet corn, fodder and grain) and conjugated IFT-BA (4.1–15% TRR in forage and fodder).

The metabolism of [U-\textsuperscript{14}C-phenyl]-isoxaflutole in outdoor grown wheat was studied following a post-emergent application at 0.055 kg ai/ha onto immature plants (Zadoks 30). Residue levels in wheat hay, straw and grains harvested at DAT 41, 93 and 93/99 were 0.172–0.107–0.058 mg/kg eq, respectively. The major part of the residues (86–96% TRR) could be extracted with acetonitrile/water. Parent isoxaflutole was only found in wheat hay (6.5% TRR). Major metabolites identified were free IFT-BA (65–96% TRR) in all wheat commodities and free IFT-DKN (9.9–21% TRR in forage and straw only). A small fraction of the metabolites in straw might be attributed to conjugates because 3% TRR was released through acid reflux.

The metabolism of [U-\textsuperscript{14}C-phenyl]-isoxaflutole in indoor grown glyphosate/HPPD-tolerant soya was studied following a pre-plant application or a foliar application to plants in full bloom (BBCH 65, 57 days after planting) at 0.331 kg ai/ha. Soya bean forage, hay and seeds were harvested at DAT 74/17, 189/132 and 189/132, respectively, for pre-plant/foliar treatment. Residue levels in soya bean forage, hay and seeds were 0.27–0.49–0.15 mg/kg eq after pre-plant treatment and 13–1.8–0.26 mg/kg eq after foliar treatment. The major part of the residues could be extracted with acetonitrile/water: 91–100% TRR in all soya commodities. The foliar application of isoxaflutole had a significant effect on the metabolic profile. Major compounds identified in the pre-plant application were free IFT-amide (53%, 13% and 8% TRR in forage, hay and seeds, respectively), free IFT-BA (66%, 56% and 27% TRR in seeds, hay and forage, respectively) and free IFT-DKN (13–17% TRR in all soya commodities). Major compounds identified in the foliar application were parent (72% and 25% TRR in forage and hay, respectively), free IFT-BA (62%, 38% and 6% TRR in seeds, hay and forage, respectively) and free IFT-DKN (18–24% TRR in all soya commodities). Minor metabolites identified in the foliar application were free IFT-amide (3–8% TRR in hay and seeds).

FG 72 soya beans express the hppdPFW336 gene from Pseudomonas fluorescens and the 2mepps gene derived from maize. These genes confer tolerance to the herbicide isoxaflutole and glyphosate-containing herbicides, respectively, via a modification of the target enzyme which makes the modified enzymes insensitive against the herbicide. Since in the FG72 soya bean variety the tolerance against glyphosate and isoxaflutole is not based on detoxification of the pesticides, the tolerance to glyphosate is not expected to modify the nature and levels of isoxaflutole-derived residues in treated soya beans. Similarly the tolerance to isoxaflutole is not expected to modify the nature and levels of glyphosate residues.

The metabolism of [U-\textsuperscript{14}C-phenyl]-isoxaflutole in outdoor grown poppies was studied following a pre-emergent application (3 days after planting) at 0.11 kg ai/ha in the presence of the safener cyprosulfamide. Residue levels in poppy seeds, seed bolls and straw harvested at DAT 110...
were 0.056, 0.78 and 0.72 mg/kg eq, respectively. Most of the radioactivity (92–98% TRR) could be extracted with acetonitrile/water. Parent isoxaflutole was not found in any of the samples. The major compound identified was free IFT-BA (66–94% TRR) in all commodities. Free IFT-DKN was found in low levels (2.1–3.5% TRR) in seed bolls and straw, but not in poppy seeds.

The metabolism of [U-14C-phenyl]-isoxaflutole in outdoor grown sugarcane was studied following a pre-emergent application at 0.210 kg ai/ha or a foliar application (47 days after planting) at 0.133 kg ai/ha. Residue levels in sugarcane plants harvested at DAT 81, 95 and 365 were 0.12–0.15–<0.01 mg/kg eq, respectively, for pre-emergence treatment and 0.18, <0.01 and <0.01 mg/kg eq at DAT 40, 95 and 365, respectively, for foliar treatment. Radioactivity in samples with residues >0.01 mg/kg eq was characterized. Most of the radioactivity could be extracted with acetonitrile: 66%, 79% and 84% TRR in DAT 40, 81 and 95 samples, respectively. Additional residues (9.5–24% TRR) could be released by exhaustive extraction procedures including reflux with acetonitrile, reflux with 0.1 M HCl and reflux with 0.1 M ammonia. The major compound identified in immature sugarcane (DAT 40, 81 and 95) was IFT-BA (66–93% TRR). Parent isoxaflutole (11% TRR) and IFT-DKN (2.2% TRR) were only found in the foliar treated crop harvested at DAT 40. Since initial acetonitrile extracts were combined with more exhaustive extracts, it is not clear whether the identified compounds are free or conjugated.

The effect of the safener cyprosulfamide on the metabolism of isoxaflutole was investigated in 3 day old maize seedlings grown in nutrient solution. Roots were exposed for 24 h to [U-14C-phenyl]-isoxaflutole alone or in combination with cyprosulfamide. After this period the plants were grown in blank nutrient solution for 3 days. Shoots, seeds and roots were collected and extracted with acetonitrile/water. The effect of the safener cyprosulfamide is a clear reduction of leaf damage (bleaching) and a lower ratio of IFT-DKN to IFT-BA in the shoots.

From these data it is concluded that in cereal grains, seeds of pulses/oilseeds, sugarcanes, forage and fodder of cereals or pulses/oilseeds, metabolite IFT-BA is the only residue identified at significant quantities (52–99% TRR). Parent isoxaflutole (6.5–11% TRR) is only found after post-emergent or foliar treatments in wheat hay or immature sugarcane. Minor metabolites identified were IFT-DKN (2.1–21% TRR in sweet corn, maize grain, immature sugarcane, cereal forage, cereal fodder and poppy straw), conjugated IFT-DKN (0.49–0.53% TRR in maize forage) and conjugated IFT-BA (1.1–15% TRR in maize forage and maize fodder).

Glyphosate/HPPD-tolerant soya has a somewhat different metabolic profile. Major compounds identified in the pre-plant application were free IFT-amide (53%, 13% and 8% TRR in forage, hay and seeds, respectively), free IFT-BA (66%, 56% and 27% TRR in seeds, hay and forage, respectively) and free IFT-DKN (13–17% TRR in all soya commodities). Major compounds identified in the foliar application were parent (72% and 25% TRR in forage and hay, respectively), free IFT-BA (62%, 38% and 6% TRR in seeds, hay and forage, respectively) and free IFT-DKN (18–24% TRR in all soya commodities).

The first hydrolytic step in the degradation in plants is the opening of the isoxazole ring to form IFT-DKN. Further hydrolytical cleavage of the carbonyl bridge and loss of the complete isoxazole moiety leads to the corresponding benzoic acid derivative (IFT-BA). In glyphosate/HPPD tolerant soya beans loss of the complete isoxazole moiety may also lead to the benzamide derivative (IFT-amide).

Metabolites IFT-DKN and IFT-BA are also found in rat. Metabolite IFT-amide (8–53% TRR) seems to be formed in glyphosate/HPPD tolerant soya beans only. This metabolite was not found in rat.

**Environmental fate in soil**

The Meeting received information on aerobic degradation in soil, soil photolysis and fate in rotational crops.
Aerobic degradation of [U-\(^{14}\)C-phenyl]-isoxaflutole under laboratory conditions was studied at 20 °C in various soil types treated at 0.2 mg ai/kg dry soil (0.20 kg ai/ha). The half-life for isoxaflutole was estimated at 7.6–11 h in clay loam and loamy sand soils, 1.3–2.5 days for sandy loam and clay soils and 4 days for loamy soils. The major metabolites identified were IFT-DKN (max. 52–96% TAR during a period 3–10 days after treatment) and IFT-BA (max. 30–90% TAR during a period of 1–12 months after treatment). IFT-BA levels in clay loam soil were very low (max 7.1% TRR at 7 days). Carbon dioxide was formed from day 1 onwards and these levels increased with time (up to 1.8–37% TRR after 1 year). These study results show that parent isoxaflutole is unlikely to be taken up by crops (and weeds) when applied onto bare soil as pre-plant or pre-emergent application.

Using the data from these soil degradation studies, the half-life for the metabolite IFT-DKN were estimated at 20 days in a sandy loam soil, 25 days in a loam soil, 37 days in a clay soil, 41 days in a clay loam soil and 56 days in a loamy sand soil. The half-life for IFT-BA was estimated at 290 days in clay soil and 980 days in a sandy loam soil. These studies show that metabolites IFT-DKN and IFT-BA are available for take-up by the plants for a considerable period after pre-plant or pre-emergence application depending on the soil type.

Soil photolysis of [U-\(^{14}\)C-phenyl]-isoxaflutole was studied in a sandy loam soil, surface treated at 0.645 kg ai/ha and exposed for 31 days to artificial sunlight. The half-life for isoxaflutole (\(DT_{50}\) 23 hours) was similar to the one for the dark control (\(DT_{50}\) 20 hours). The formation of transformation products specific for photolytic processes was insignificant. The study shows that light has no effect on the degradation of isoxaflutole on soil.

Metabolism of [U-\(^{14}\)C-phenyl]-isoxaflutole was investigated in confined rotational crops following pre-plant incorporated soil treatment or pre-emergence treatment. A sandy loam soil was treated at a rate of 0.213 kg ai/ha under outdoor conditions. Rotational crops (radish, lettuce, mustard greens, sorghum and wheat) were sown 34, 123 and 365–375 days after application, representing first, second and third rotation. Total radioactivity for pre-plant incorporated soil treatment ranged from 0.003–0.24 mg/kg eq after first rotation, 0.001–0.030 mg/kg eq after second rotation and 0.001–0.051 mg/kg eq after third rotation. Total radioactivity for pre-emergence application ranged from 0.010–0.126 mg/kg eq after first rotation, < 0.001–0.042 mg/kg eq after second rotation and 0.001–0.030 mg/kg eq after third rotation. Total radioactivity levels above 0.05 mg/kg eq were only found in immature lettuce and sorghum commodities after the first rotation (0.126–0.24 mg/kg eq in sorghum forage, 0.13 mg/kg eq in sorghum fodder, 0.12 mg/kg in sorghum grain, 0.056 mg/kg eq in immature lettuce) and after the third rotation (0.051 mg/kg eq in sorghum forage). Parent isoxaflutole was not found in any of the rotational crops. IFT-BA represented the major compound and was present in the commodities of the first, second and third rotation at levels between < 0.001–0.11 mg/kg eq (6.9–100% TRR). IFT-DKN was only found in radish leaves and sorghum grain at levels up 0.005 mg/kg eq (0.8–27.3% TRR) in the first rotation. RPA 205834 was detected by HPLC-MS-MS in trace amounts (< 0.001 mg/kg eq) in mature lettuce of the first rotation. A fourth metabolite (U1) was found in the commodities of the first, second and third rotation at levels between < 0.001–0.022 mg/kg eq (10–100% TRR). The polarity of this compound and the molecular weight of 192, as determined by HPLC-MS-MS, suggests it is a carboxylic acid degradation product of IFT-BA with the structural formula CF\(_3\)-C\(_6\)H\(_4\)-COOH (4-trifluoromethyl benzoic acid).

In a field rotational crop study at two different locations in the USA isoxaflutole was applied as pre-plant or pre-emergent application to maize at 0.154–0.161 kg ai/ha. Rotational crops (soya beans, sugar beets, radishes, turnips, mustard greens, wheat and sorghum) were sown 29/30, 104, 119/120, 151, 166, 180, 365 days after application, representing various rotations of maize. No residues (< 0.01 mg/kg) of parent isoxaflutole, metabolites IFT-DKN or metabolite IFT-BA were found in any of the crops at any of the rotations. Metabolite 4-trifluoromethyl benzoic acid was not analysed, but since its levels are expected to be in the same order of magnitude as IFT-BA, based on the confined rotational crop study, it is not expected to be found at levels > 0.01 mg/kg.
From these data it is concluded that the first hydrolytic step in the aerobic degradation in soil is the opening of the isoxazole ring to form IFT-DKN, which is responsible for the mode of action. Further hydrolytical cleavage of the carbonyl bridge and loss of the complete isoxazole moiety leads to the corresponding benzoic acid derivative (IFT-BA). Light has no effect on the degradation of isoxaflutole on soil. Isoxaflutole has a very short half-life of 8 h to 4 days in soil, and consequently is not found in rotational crops after pre-plant or crop pre-emergence applications. Metabolites IFT-DKN and IFT-BA have long half-lives of 20–56 and 290–980 days, respectively, in soil and consequently are the main metabolites found in plants after pre-plant, crop pre-emergence or crop post-emergence applications. Since metabolites IFT-DKN and IFT-BA were also found after foliar treatments of sugarcane and glyphosate/HPPD tolerant soya beans, it is likely that metabolites IFT-DKN and IFT-BA are also formed in plants. In confined rotational crop studies, a third metabolite (4-trifluoromethyl benzoic acid (CF$_3$-C$_6$H$_4$-COOH)) was found (10–100% TRR), formed by the loss of the methylsulfonyl moiety of IFT-BA. It is not clear whether this metabolite is formed exclusively in the plants or is formed in the soil and taken up by the plants. Metabolite 4-trifluoromethyl benzoic acid was not found in rat.

Methods of Analysis

The Meeting received description and validation data for analytical methods of isoxaflutole related residues in plant and animal commodities.

For plants, a HPLC-MS-MS method was submitted as enforcement/monitoring method for the individual determination of parent and its metabolite IFT-DKN. Plant material was extracted with acidified methanol/water followed by filtration. The Meeting considers validation sufficient for commodities with high acid content, high water content, high starch content and high oil content. The LOQ was 0.01 mg/kg for each analyte.

Several other HPLC-MS-MS methods were submitted for the determination of parent and its metabolites IFT-DKN and IFT-BA in plant material. In some trials a GC-MS method was used, where residues were extracted with methanol and converted into a common moiety IFT-methylbenzoate by hydrolysis and methylation. Most analytical methods were considered fit for purpose with LOQs ranging from 0.01 mg/kg eq for total residues or 0.01–0.03 mg/kg for individual analytes.

For animal commodities, the existing multi-residue method QuEChERS was submitted as enforcement/monitoring method. The Meeting considers this method valid for the individual determination of parent and its metabolite IFT-DKN in all animal commodities. The LOQ was 0.01 mg/kg for milk, eggs, meat, fat, liver and kidney for each analyte. Three other analytical methods were submitted for the determination of isoxaflutole related residues in milk, eggs or animal tissues. HPLC-UV methods were used for milk and eggs. A HPLC-MS-MS method was used for tissues. Conjugates of IFT-DKN and RPA 207048 were not analysed by these methods. The reported LOQ of 0.05 mg/kg for each analyte in meat, fat, liver and kidney needs to be substantiated by additional data, since only 1–2 recoveries per matrix were provided at this level. Parent is degraded during extraction and is measured as increased IFT-DKN in these methods. Based on the validation data available, the methods are considered suitable for determination of parent, IFT-DKN, RPA 205834 and RPA 207048 in milk at 0.02–2.0 mg/kg and 0.05–0.25 mg/kg in eggs and tissues.

Solvents used in the analytical methods were different from the extraction methods used in the metabolism studies. Extraction efficiency, using radiolabelled samples from the metabolism studies, was not verified for any of the analytical methods.
Stability of pesticide residues in stored analytical samples

The Meeting received information on the stability of isoxaflutole, IFT-DKN and IFT-BA in plant commodities or isoxaflutole, IFT-DKN, IFT-BA, RPA 205834 and RPA 207048 in animal commodities in animal commodities stored frozen.

Storage stability studies at -10 °C and -20 °C showed that total isoxaflutole residues (sum of parent, IFT-DKN and IFT-BA, measured as common moiety) were stable for at least 11 months in commodities with high protein content (chickpea seeds), 15 months in commodities with high starch content (maize grains) and straw (maize fodder) and at least 20 months in commodities with high water content (sugar canes).

Storage stability studies showed that isoxaflutole converts to IFT-DKN after 3–6 months of storage at -10 °C in commodities with high acid content (oranges), high water content (sugar canes), high protein content (dry pinto beans) and high oil content (dry soya beans). The metabolite IFT-DKN remained stable for at least 12 months at -10 °C in commodities with high acid content (oranges), high water content (sugar canes), high protein content (dry pinto beans) and at least 16 months in commodities with high oil content (poppy seeds and dry soya bean seeds). Metabolite IFT-DKN converts to IFT-BA within a period of 23 months at -20 °C in commodities with high water content (sorghum forage, lettuce and radish leaves), high starch content (radish roots), high protein content (sorghum grain) and straw (sorghum fodder).

Based on storage stability studies at -20 °C in fortified samples of animal commodities the Meeting noted that isoxaflutole was not stable. Isoxaflutole degraded rapidly to IFT-DKN in eggs and degraded after a period of 85 days in milk and muscle. Parent isoxaflutole also degrades to IFT-DKN by the extraction method used for tissue analysis in the feeding studies. Metabolite IFT-DKN is stable for a period of at least 113–130 days in liver, kidney, muscle, fat, milk and eggs. If metabolite IFT-DKN is included in the residue definition, any degradation from isoxaflutole to IFT-DKN is covered by the total residues measured.

Metabolite RPA 205834 is stable for a period of 113–131 days in kidney, muscle, fat and milk. Metabolite RPA 205834 is stable for a maximum storage period of 94 days in liver and degrades significantly thereafter (36% remaining after 130 days). Storage stability of RPA 205834 was not investigated in eggs.

Metabolite RPA 207048 is not stable in any animal commodity. RPA 207048 degrades rapidly in kidney to a level of about 45% which is maintained from 13–115 days. RPA 207048 is stable for a maximum storage period of 28 days in muscle, 40 days in liver and 84 days in fat and thereafter remains stable at a level of about 50% of the original residue level for a period up to 85–113 days. Storage stability of RPA 207048 has not been investigated in milk and eggs. Precautions need to be taken when analysing this metabolite. Besides storage conditions, also extraction conditions are critical.

In case quantitative levels of total residues based on parent, IFT-DKN, RPA 205834 and RPA 207048 are needed, samples of animal origin need to be analysed within 30 days. And even then, metabolite RPA 207048 will be underestimated in kidney.

Definition of the residue

Parent compound isoxaflutole was not found in milk, eggs or livestock tissues. Metabolites found at significant levels in livestock commodities were: IFT-DKN, conjugated IFT-DKN, RPA 205834, RPA 207048, and conjugated RPA 207048. Metabolite IFT-DKN (18–93% TRR) was found in milk, eggs, all goat and hen tissues, except hen muscle. Conjugated forms of IFT-DKN (5.7–25% TRR) were only found in goat muscle, hen muscle, hen skin and eggs and the free metabolite could be released by protease digestion (goat muscle and hen skin) and/or exhaustive acid hydrolysis (hen muscle and eggs). Metabolite RPA 205834 (8.1–28% TRR) was found in milk, eggs (yolk) and goat fat in the metabolism studies on goat and hen, but it was also found in milk, bovine liver and bovine
kidney in cow feeding study. Metabolite RPA 207048 (9.1–31% TRR) was found in milk, all goat tissues, hen muscle and hen fat. Conjugated forms of RPA 207048 (3.4% TRR) were only found in goat and hen muscle and could be released by protease digestion (goat muscle) or exhaustive acid hydrolysis (hen muscle). Further there seems to be a metabolic shift between RPA 205834 and RPA 207048 between goat and cow. In goat, RPA 207048 seems to be present in all goat tissues and at higher levels than RPA 205834, while in cow RPA 207048 is not found. This metabolic shift might be related to the shorter period between last application and slaughter time for cows (7.5 h) compared to goats (23 h).

Isoxaflutole is easily converted to IFT-DKN. Since no discrimination can be made whether isoxaflutole is metabolized within the animal, or whether it is degraded because of its sensitivity to physical chemical conditions, isoxaflutole needs to be included in the residue definition for enforcement. Any isoxaflutole degraded because of storage or extraction conditions will be measured as IFT-DKN. The sum of IFT and IFT-DKN can therefore serve as the marker residue for enforcement.

The log $K_{ow}$ for isoxaflutole is 2.34. Isoxaflutole and its metabolite IFT-DKN are extracted with acidified aqueous solvents and highest levels of these metabolites are found in the organs kidney and liver. The sum of parent and IFT-DKN is considered not fat soluble.

Apart from isoxaflutole and IFT-DKN other metabolites found at significant levels in livestock commodities were conjugated IFT-DKN, RPA 205834, RPA 207048, and conjugated RPA 207048.

Toxicity of the free IFT-DKN, RPA 205834 and RPA 207048 is considered to be covered by toxicity studies on isoxaflutole since each of the free metabolites was found in the rat high dose group. Free IFT-DKN is the major compound found in rat urine, faeces and solvent fractions of liver, and its toxicity is considered to be similar to that of the parent compound. Since metabolites RPA 205834 and RPA 207048 have similar structures as IFT-DKN and no data are available to conclude that they are of less toxicological significance, each metabolite is considered relevant for the residue definition for dietary risk assessment. Conjugated forms of IFT-DKN and RPA 207048 are considered relevant for dietary exposure, since the free metabolites can be released during the metabolic process in humans. The Meeting proposed to include parent, IFT-DKN, conjugated IFT-DKN, RPA 205834, RPA 207048, and conjugated RPA 207048 in the residue definition for dietary risk assessment of animal commodities.

The analytical method used in the feeding study cannot determine the conjugated forms of IFT-DKN and RPA 207048. For goat muscle, conjugates represent 26.62% TRR and free compounds (IFT-DKN + RPA 205834 + RPA 207048) represent 27.43% TRR. For hen muscle, conjugates represent 22.81% TRR and free compounds represent 31.4% TRR. For hen eggs, conjugates represent 16.8% TRR and free compounds represent 54% TRR. A multiplication factor 2 for goat and hen muscle and 1.3 for hen eggs could be used on the total residues to compensate for this underestimation of dietary exposure.

In primary crops, metabolite IFT-BA is the only residue identified at significant quantities (52–99% TRR). Parent isoxaflutole is only found after post-emergent or foliar treatments in livestock feed commodities: major amounts in glyphosate/HPPD-tolerant soya bean forage and hay (25–72% TRR) and minor amounts (6.5–11% TRR) in wheat hay or immature sugarcane. Minor metabolites identified were IFT-DKN (2.1–21% TRR), conjugated IFT-DKN (0.49–0.53% TRR) and conjugated IFT-BA (1.1–15% TRR). Metabolite IFT-amide (8–53% TRR) seems only to be formed in glyphosate/HPPD tolerant soya bean (seeds, forage, hay).

Metabolite IFT-BA is the major residue and is relevant for consideration in the residue definition for enforcement. However, IFT-BA can also arise in plant commodities as a result of treatment with pyrasulfotole. For this reason IFT-BA cannot be used as a marker for isoxaflutole.
The only other compounds relevant for the residue definition for enforcement are the parent and IFT-DKN. The sum of IFT and IFT-DKN can therefore serve as the marker residue for enforcement.

Apart from isoxaflutole and IFT-DKN, other metabolites found at significant levels in plant commodities are IFT-BA (feed commodities and tolerant soya beans) and IFT-amide (tolerant soya beans only). Assessment of additional toxicological data on IFT-BA demonstrated that metabolite IFT-BA is considerably less toxic than the parent compound. Thus, from a toxicological point of view it is not necessary to include IFT-BA in the residue definition. Metabolite IFT-amide is not found in rat. But since IFT-amide is present at lower levels than IFT-BA in soya bean seeds and because of its structural similarity with IFT-BA, IFT-amide is considered not relevant for the residue definition.

The Meeting recommended the following residue definition for isoxaflutole:

Definition of the residue for compliance with the MRL and for dietary risk assessment for plant commodities: sum of isoxaflutole and isoxaflutole diketonitrile, expressed as isoxaflutole.

Definition of the residue for compliance with the MRL for animal commodities: sum of isoxaflutole and isoxaflutole diketonitrile, expressed as isoxaflutole.

The Meeting considers the residue not fat soluble.

Definition of the residue for dietary risk assessment for animal commodities: sum of isoxaflutole, isoxaflutole diketonitrile, RPA 205834 (2-aminomethylene-1-cyclopropyl-3-(2-mesyl-4-trifluoromethylphenyl)-propane-1,3-dione) and RPA 207048 (1-cyclopropyl-2-hydroxymethylene-3-(2-mesyl-4-trifluoromethylphenyl)-propane-1,3-dione), including their conjugates, expressed as isoxaflutole.

**Results of supervised residue trials on crops**

The total residue values selected for maximum residue level recommendations and dietary intake are based on the sum of isoxaflutole and IFT-DKN. In case a common moiety method is used for analysis also the IFT-BA metabolite is included. Since the relative molecular weight of IFT-DKN is identical to that of isoxaflutole, no molecular weight conversion is needed. Soil type and the addition of the safener cyprosulfamide affect the residue levels in plant commodities. Residue trials were conducted in a range of soil types, which included those with the longest half-lives.

Since IFT-DKN is included in the residue definition, any degradation from isoxaflutole to IFT-DKN is covered by the total residues measured. Since IFT-BA is not part of the residue definition, any degradation from IFT-DKN to IFT-BA means an underestimation of the original residue present in the sample. Therefore, the Meeting takes only those trials into account, where samples have been stored for a maximum of 16 months (commodities with high oil content) or 12 months (all other commodities). Also trials where IFT-BA is below LOQ can be taken into account, because it indicates that no degradation of IFT or IFT-DKN to IFT-BA occurred.

**Sweet corn (corn-on-the-cob)**

Field trials involving sweet corn were performed in Spain, Italy, Greece, Portugal, Germany, France, Netherlands and the United Kingdom.

Critical GAP for sweet corn in France is for a single post sowing pre-emergence treatment (BBCH 00–08) at 0.099 kg ai/ha, where the safener cyprosulfamide is added. Trials from Spain, Italy, Germany, France, Netherlands and the UK (0.099–0.10 kg ai/ha, growth stage BBCH 0–06, cyprosulfamide added) matched this GAP. For sweet corn harvested at BBCH 79, total residues were: < 0.02 (10) mg/kg (n=10).
Several trials, where isoxaflutole was applied at the same dose rate at a later growth stage (BBCH 13–14), as well as a metabolism study in sweet corn following a post-emergence treatment at 0.21 kg ai/ha, confirmed the non-residue situation.

The Meeting estimated a maximum residue level of 0.02* mg/kg on sweet corn (corn-on-the-cob). The Meeting estimated an STMR of 0 mg/kg.

**Pulses**

Field trials involving chick-peas (dry) were performed in Australia.

Critical GAP for chick-peas in Australia is a single post plant crop, pre-emergence application at 0.075 kg ai/ha. In trials from Australia (1× 0.075 kg ai/ha post planting pre-emergent) matching this GAP, total residues were: < 0.01 (3) and < 0.01* mg/kg (n=4) with a common moiety method (including IFT-BA). The superscript (a) indicates the addition of an adjuvant. The addition of which did not result in a difference in total residue levels.

An additional four trials at the same locations using a single post-plant crop emergence application at a higher application rate of 0.15 kg ai/ha, as well as a metabolism study in poppy seeds after post-plant crop pre-emergence treatment at 0.11 kg ai/ha, confirmed the no residue situation.

The Meeting estimated a maximum residue level of 0.01* mg/kg on chickpea, dry and an STMR of 0 mg/kg.

Field trials involving glyphosate/HPPD tolerant soya beans (FG72) (dry) were performed in the USA and Canada.

No authorised uses were available for glyphosate/HPPD tolerant soya beans (FG72). The Meeting agreed that no recommendations could be set for soya beans.

**Maize**

Field trials involving maize were performed in Spain, Italy, Greece, Portugal, Germany, France, Netherlands, the United Kingdom, the USA and Canada.

Critical GAP for maize in France is a single early post emergence treatment up to 3 leaf stage of the crop (BBCH 13) at 0.099 kg ai/ha with the addition of the safener cyprosulfamide. In field trials from Spain, Italy, Greece, Portugal, Germany, France, the Netherlands and the United Kingdom (1× 0.10 kg ai/ha, BBCH 13, with cyprosulfamide) matching this GAP, total residues for maize grain harvested at BBCH 89 were: < 0.02 (14) mg/kg.

The GAP for maize in the USA is a single pre-emergence treatment at 0.16 kg ai/ha without the addition of the safener cyprosulfamide or a single early post-emergence treatment at 0.105 kg ai/ha up to 2 leaf-collar growth stage of the crop (GS 12) with the addition of the safener cyprosulfamide. In field trials from the USA (1× 0.15–0.17 kg ai/ha, pre-emergent, no adjuvants) matching the first GAP, total residues for maize grains harvested at maturity were < 0.02 (17) mg/kg. In field trials from the USA (1× 0.13 kg ai/ha, post-emergent GS 12, with safener cyprosulfamide, no adjuvants) matching the second GAP, total residues for maize grains harvested at maturity were < 0.03 (17) mg/kg.

A metabolism study with mature maize grains at 0.21 kg ai/ha after pre plant or post-emergence application did not confirm the non-residue situation.

The Meeting estimated a maximum residue level of 0.02* mg/kg and an STMR of 0.02 mg/kg.

**Sugar cane**

Supervised residue trials on sugar cane were conducted in Australia, Mexico and Brazil.
Isoxaflutole

There were no authorised uses available for Mexico and Brazil.

Critical GAP for sugar cane in Australia is a single soil directed application at 0.15 kg ai/ha when the sugar cane was at least 0.75 m high and with a PHI of 19 weeks without adjuvant. In field trials from Australia (1 × 0.150 kg ai/ha, PHI 133–166 days) matching this GAP, total residues were < 0.01 (2) mg/kg with a common moiety method (including IFT-BA).

Two additional trials, at the same locations, at 1 × 0.225 kg ai/ha and a PHI of 133–166 days, two trials in Brazil using two applications at 0.150 kg ai/ha and a PHI of 92–95 days and a metabolism study in mature sugar cane after soil directed application to emerged plants at 0.133 kg ai/ha, confirmed the no residue situation.

The Meeting estimated a maximum residue level of 0.01* mg/kg on sugar cane and an STMR of 0 mg/kg.

Poppy seed

Field trials involving poppy seed were performed in France, Spain, Germany, Netherlands and Hungary.

Critical GAP for poppy seed in Spain is for a single pre-emergence application at 0.060 kg ai/ha without the safener cyprosulfamide. There were no trials matching this GAP.

Critical GAP for poppy seed in Hungary is for a single pre-emergence application at 0.11 kg ai/ha with the safener cyprosulfamide. In field trials from Germany, the Netherlands and Northern France (1 × 0.10 kg ai/ha, pre-emergence, with cyprosulfamide) matching this GAP, total residues poppy seeds were < 0.02 (3) mg/kg.

A metabolism study in poppy following post-plant crop pre-emergence treatment at 0.11 kg ai/ha confirmed the no residue situation.

The Meeting estimated a maximum residue level of 0.02* mg/kg in poppy seeds and an STMR of 0 mg/kg.

Legume animal feeds

Field trials involving chick-pea forage were performed in Australia.

Critical GAP for chickpeas in Australia is a single post plant crop pre-emergence application at 0.075 kg ai/ha. In trials from Australia (1 × 0.075 kg ai/ha, post planting pre-emergent) matching this GAP, total residues were: < 0.01 (3) and < 0.01*mg/kg (n=4) with a common moiety method (including IFT-BA). The superscript (a) indicates the addition of adjuvant. The addition of which did not result in a difference in total residue levels.

An additional trial at the same location using a single post-plant pre-emergence application at a higher application rate of 0.20 kg ai/ha did not confirm the non-residue situation.

The Meeting estimated a maximum residue level of 0.01* mg/kg on chickpea fodder, a median residue of 0.01 mg/kg and a highest residue of 0.01 mg/kg for livestock dietary burden calculations.

Forage and fodder of cereal grains and grasses

Field trials involving maize forage were performed in Spain, Italy, Greece, Portugal, Germany, France, the Netherlands, the United Kingdom, the USA and Canada.
Critical GAP for maize in France is a single early post-emergence treatment up to 3 leaf stage of the crop (BBCH 13) at 0.099 kg ai/ha with the addition of the safener cyprosulfamide. No PHI is mentioned. High total residues ranging from 1.3–16 mg/kg eq were found immediately after treatment (DAT=0). However, the Meeting considers DAT=0 irrelevant for maize forage harvest and considers growth stage BBCH 16 or 33 (3 nodes with 6 leaves) the earliest grazing time for maize forage. In field trials from Spain, Italy, Greece, Portugal, Germany, France, Netherlands and the United Kingdom (1× 0.099–0.10 kg ai/ha, BBCH 13, with cyprosulfamide) matching the French GAP, total residues for maize forage harvested at BBCH 19 or 33–35 (DAT 40–41) were < 0.02 (11) and 0.34 mg/kg (n=12).

Critical GAP for maize in the USA is a single early post-emergence treatment at 0.105 kg ai/ha up to 2 leaf-collar growth stage of the crop (BBCH 14 or 32) with the addition of the safener cyprosulfamide, with or without the addition of an adjuvant. The pre-harvest interval is 45 days. In field trials from the USA (1× 0.13 kg ai/ha, PHI 43–45 days, with safener cyprosulfamide, no adjuvants) matching this GAP, total residues for early maize forage were < 0.03 (15) mg/kg.

The Meeting agreed that the dataset matching French GAP could be used to estimate a median residue of 0.02 mg/kg and a highest residue of 0.34 mg/kg for livestock dietary burden calculations.

Field trials involving maize fodder were performed in Spain, Italy, Greece, Portugal, Germany, France, Netherlands, the United Kingdom, the USA and Canada.

Critical GAP for maize in France is a single early post-emergence treatment up to 3 leaf stage of the crop (BBCH 13) at 0.099 kg ai/ha with the addition of the safener cyprosulfamide. In field trials from Spain, Italy, Greece, Portugal, Germany, France, Netherlands and the United Kingdom (1× 0.099–0.10 kg ai/ha, BBCH 13, with cyprosulfamide) matching this GAP total residues for maize fodder harvested at BBCH 79 (sweet corn fodder) were < 0.02 (13) mg/kg.

Critical GAP for maize in the USA is a single pre-emergence treatment at 0.16 kg ai/ha without the addition of the safener cyprosulfamide or a single early post-emergence treatment at 0.105 kg ai/ha up to 2 leaf-collar growth stage of the crop (i.e. GS 12) with the addition of the safener cyprosulfamide, each with or without the addition of an adjuvant. In field trials from the USA (1× 0.15–0.17 kg ai/ha, pre-emergent, no adjuvants) matching the first GAP, total residues for maize fodder harvested at dent stage to maturity were < 0.02 (17) mg/kg. In field trials from the USA (1× 0.13 kg ai/ha, post-emergent GS 12, with safener cyprosulfamide, no adjuvants) matching the second GAP, total residues for maize fodder harvested at maturity were < 0.03 (14) mg/kg.

A metabolism study with mature maize fodder at 0.21 kg ai/ha after post-emergence application did not confirm the non-residue situation.

The Meeting estimated a maximum residue level of 0.02* mg/kg, a median residue of 0.02 mg/kg and a highest residue of 0.02 mg/kg for livestock dietary burden calculations.

Miscellaneous forage and fodder crops

Supervised residue trials on sugar cane tops/fodder were conducted in Australia.

The Meeting estimated a maximum residue level of 0.01* mg/kg on sugar cane fodder, a median residue of 0 mg/kg and a highest residue of 0.01 mg/kg for livestock dietary burden calculations.

Residues from rotational crops

Total residues above 0.01 mg/kg eq are not expected in rotational crops.
Fate of residues during processing

Processing studies with isoxaflutole were undertaken for soya beans. However, since no MRLs could be set for soya beans, no processing factors are needed.

Residues in animal commodities

The Meeting estimated the dietary burden of isoxaflutole residues on the basis of the livestock diets listed in the FAO manual appendix IX (OECD feedstuff table). Calculation from highest residue and STMR (some bulk commodities) provides the levels in feed suitable for estimating maximum residue levels, while calculation from STMR values from feed is suitable for estimating STMR values for animal commodities. Commodities used in the dietary burden calculation are maize grains, maize forage, chickpea fodder, maize forage, maize fodder and sugar cane tops.

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are provided in Annex 6. A mean and maximum dietary burden for livestock, based on isoxaflutole use, is shown in the table below.

Animal dietary burden for isoxaflutole total residues, expressed as ppm of dry matter diet

<table>
<thead>
<tr>
<th></th>
<th>US</th>
<th>EU</th>
<th>AU</th>
<th>JP</th>
<th>overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>max</td>
<td>max</td>
<td>max</td>
<td>max</td>
<td>max</td>
</tr>
<tr>
<td>beef cattle</td>
<td>0.146</td>
<td>0.685</td>
<td>0.688</td>
<td>0.017</td>
<td>0.688</td>
</tr>
<tr>
<td>dairy cattle</td>
<td>0.394</td>
<td>0.518</td>
<td>0.688</td>
<td>0.436</td>
<td>0.688</td>
</tr>
<tr>
<td></td>
<td>0.017</td>
<td>0.016</td>
<td>–</td>
<td>0.016</td>
<td>0.017</td>
</tr>
<tr>
<td>poultry layer</td>
<td>0.017</td>
<td>0.102</td>
<td>–</td>
<td>0.018</td>
<td>0.102</td>
</tr>
<tr>
<td>mean</td>
<td>mean</td>
<td>mean</td>
<td>mean</td>
<td>mean</td>
<td>mean</td>
</tr>
<tr>
<td>beef cattle</td>
<td>0.026</td>
<td>0.045</td>
<td>0.048</td>
<td>0.017</td>
<td>0.048</td>
</tr>
<tr>
<td>dairy cattle</td>
<td>0.034</td>
<td>0.038</td>
<td>0.048</td>
<td>0.036</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>0.017</td>
<td>0.016</td>
<td>–</td>
<td>0.016</td>
<td>0.017</td>
</tr>
<tr>
<td>poultry layer</td>
<td>0.017</td>
<td>0.022</td>
<td>–</td>
<td>0.018</td>
<td>0.022</td>
</tr>
</tbody>
</table>

*Highest mean and maximum dietary burden suitable for maximum residue level and STMR estimates for mammalian meat

*Highest mean and maximum dietary burden suitable for maximum residue level and STMR estimates for milk

*Highest mean and maximum dietary burden suitable for maximum residue level and STMR estimates for poultry meat

*Highest mean and maximum dietary burden suitable for maximum residue level and STMR estimates for eggs

Livestock feeding studies

The Meeting received a feeding study on lactating cows and laying hens. Total residues in animal commodities for enforcement are defined as the sum of isoxaflutole and IFT-DKN, expressed as isoxaflutole equivalents. Total residues for dietary risk assessment are defined as the sum of isoxaflutole, IFT-DKN, RPA 205834 and RPA 207048, and their conjugates, expressed as isoxaflutole equivalents.

Four groups of four lactating Holstein cows were dosed once daily via capsules at levels of 0.0, 4.7, 14.4 and 45.5 ppm parent compound in dry weight feed for 42 consecutive days. Milk was collected throughout the study and tissues were collected on day 42 within 7.5 h after the last dose. Milk was not analysed for RPA 207048, but the level of RPA 207048 has been estimated based on relative levels to the other metabolites in the metabolism study. Residues found at the 4.7 ppm dose level are summarized in the residues section below.

Four groups of 15 laying hens were dosed once daily via capsules at levels of 0.0, 0.18, 0.54 and 1.8 ppm parent compound in dry weight feed for 42 consecutive days. Eggs were collected throughout the study and tissues were collected on day 42 within 3 h after the last dose. Eggs and hen
tissues were not analysed for RPA 205834 and RPA 207048, but the level of these metabolites has been estimated based on relative levels to the other metabolites in the metabolism study. Residues found at the 0.18 ppm dose level are summarized in the residue section below.

**Residues in animal commodities**

**Cattle**

For maximum residue level estimation, the highest residue in the tissues and milk were calculated by interpolating the maximum dietary burden (0.688 ppm) between the relevant feeding levels (0–4.7 ppm) from the dairy cow feeding study and using the highest tissue concentrations based on the residue definition for enforcement from individual animals within those feeding groups and using the mean milk concentration from those feeding groups (see table below).

The STMR values for the tissues and milk were calculated by interpolating the mean dietary burden (0.048 ppm) between the relevant feeding levels (0–4.7 ppm) from the dairy cow feeding study and using the mean tissue and milk concentrations based on the residue definition for dietary risk assessment from those feeding groups (see table below).

<table>
<thead>
<tr>
<th>Feed level (ppm) for milk residues</th>
<th>Total residues (mg/kg eq) in milk</th>
<th>Feed level (ppm) for tissue residues</th>
<th>Total residues (mg/kg eq) in milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum residue level—beef or dairy cattle (residue definition for enforcement)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding study a</td>
<td>4.7</td>
<td>NA</td>
<td>4.7</td>
</tr>
<tr>
<td>Dietary burden and residue estimate</td>
<td>0.688</td>
<td>0</td>
<td>0.688</td>
</tr>
<tr>
<td>STMR—beef or dairy cattle (residue definition for dietary risk assessment)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding study b</td>
<td>4.7</td>
<td>NA</td>
<td>4.7</td>
</tr>
<tr>
<td>Dietary burden and residue estimate</td>
<td>0.048</td>
<td>0</td>
<td>0.048</td>
</tr>
</tbody>
</table>

NA not analysed, because highest dose level of 45.5 ppm showed residues below or just above the LOQ

- a highest residues for tissues and mean residues for milk
- b mean residues for tissues and mean residues for milk

The Meeting estimated a maximum residue level for total isoxaflutole residues of 0.01* mg/kg in meat (from mammals other than marine mammals), mammalian fats (except milk fats), milks, and 0.1 mg/kg in mammalian edible offal. The residue in animal commodities is considered not fat soluble.

The Meeting estimated an STMR for total isoxaflutole residues of 0 mg/kg in meat (from mammals other than marine mammals), mammalian fats (except milk fats) and milks, and 0.2 mg/kg in mammalian edible offal.

**Poultry**

For maximum residue level estimation, the high residue in the tissues and eggs were calculated by interpolating the maximum dietary burden (0.102 ppm) between the relevant feeding levels (0–0.18 ppm) from the laying hen feeding study and using the highest tissue and egg concentrations based on the residue definition for enforcement from individual animals within those feeding groups (see table below).
The STMR values for the tissues and eggs were calculated by interpolating the mean dietary burden (0.022 ppm) between the relevant feeding levels (0–0.18 ppm) from the laying hen feeding study and using the mean tissue and egg concentrations based on the residue definition for dietary risk assessment from those feeding groups (see table below).

<table>
<thead>
<tr>
<th>Feed level (ppm) for egg residues</th>
<th>Feed level (ppm) for tissue residues</th>
<th>Total residues (mg/kg eq) in egg</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding study a</td>
<td>0.18</td>
<td>NA</td>
<td>0.102</td>
<td>0</td>
<td>0.102</td>
<td>NA</td>
</tr>
<tr>
<td>Dietary burden and residue estimate</td>
<td>0.022</td>
<td>NA</td>
<td>0</td>
<td>0.022</td>
<td>&lt; 0.1</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA not analysed, because highest (1.8 ppm) and medium (0.54 ppm) dose level did not show residues

a highest residues for tissues and eggs

b mean residues for tissues and eggs

The Meeting estimated a maximum residue level for isoxaflutole total residues of 0.2 mg/kg in poultry edible offal, 0.01* mg/kg in poultry meat, poultry fats and poultry eggs. The residue in animal commodities is considered not fat soluble.

The Meeting estimated an STMR for isoxaflutole total residues of 0.1 mg/kg in poultry edible offal, 0 mg/kg in poultry meat and poultry fats, and eggs.

**RECOMMENDATIONS**

On the basis of the data from supervised trials the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits.

Definition of the residue for compliance with the MRL and for dietary risk assessment for plant commodities: *sum of isoxaflutole and isoxaflutole diketonitrile, expressed as isoxaflutole*.

Definition of the residue for compliance with the MRL for animal commodities: *sum of isoxaflutole and isoxaflutole diketonitrile, expressed as isoxaflutole*.

The Meeting considers the residue not fat soluble.

Definition of the residue for dietary risk assessment for animal commodities: *sum of isoxaflutole, isoxaflutole diketonitrile, RPA 205834 (2-aminomethylene-1-cyclopropyl-3-(2-mesy1-4-trifluoromethylphenyl)-propane-1,3-dione) and RPA 207048 (1-cyclopropyl-2-hydroxymethylene-3-(2-mesy1-4-trifluoromethylphenyl)-propane-1,3-dione), including their conjugates, expressed as isoxaflutole.*
DIETARY RISK ASSESSMENT

Long-term intake
The International Estimated Daily Intakes (IEDI) of for isoxaflutole was calculated from recommendations for STMRs for raw and processed commodities in combination with consumption data for corresponding food commodities. The results are shown in Annex 3.

The IEDI of in the 13 GEMS/Food cluster diets, based on the estimated STMRs were in the range 0–1% of the maximum ADI of 0.02 mg/kg bw. The Meeting concluded that the long-term intake of residues of isoxaflutole from uses considered by the Meeting is unlikely to present a public health concern.

Short-term intake
Since no ARfD is considered necessary, no short-term intake assessment is considered necessary. The Meeting concluded that the short-term intake of residues of isoxaflutole from uses considered by the Meeting is unlikely to present a public health concern.