5.20 METRAFENONE (278)

TOXICOLOGY

Metrafenone is the ISO-approved common name for (3-bromo-6-methoxy-2-methylphenyl) (2,3,4-trimethoxy-6-methylphenyl)-methanone (IUPAC), for which the CAS number is 220899-03-6.

Metrafenone is a fungicide for the control of *Erysiphe graminis* and *Pseudocercosporella herpotrichoides* (eyespot and powdery mildew) and for the control of *Uncinaria necator* (powdery mildew). It inhibits the growth of the mycelium on the leaf surface, leaf penetration, the formation of haustoria and sporulation.

Metrafenone has not been evaluated previously 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 gavage dosing in rats, absorption of metrafenone was rapid and complete (> 88%) at the low dose of 10 mg/kg bw but was limited to 15–20% at the high dose of 1000 mg/kg bw, suggesting saturation of the absorption processes. Metrafenone was widely distributed in the body, with highest residue levels found mainly in the gastrointestinal tract, liver and fat. There was no evidence of accumulation. The labelled material was relatively rapidly excreted into the gastrointestinal tract via the bile (85–90%), resulting in extensive excretion via faeces. Excretion via urine was relatively low at 10 mg/kg bw (5–6%), and even lower at the high dose of 1000 mg/kg bw (~1%). Metrafenone was extensively metabolized, with most of the labelled products excreted as glucuronic acid conjugates in bile and urine. Five possible sites of conjugation with glucuronic acid were identified, following *O*-demethylation of the molecule. Residues in faeces consisted primarily of parent compound and the aglycones of bile and urine conjugates. The transformation steps included:

- *O*-demethylation of the aromatic methoxy group(s) followed by mono-*O*-glycosidation;
- hydroxylation of the bromophenyl ring; and
- hydroxylation of the methyl substituent to hydroxymethyl followed by *O*-glycosidation or further oxidation to aldehyde or lactone.

The bond between the bromophenyl ring and trimethoxyphenyl ring remained intact.

Toxicological data

Metrafenone has low acute toxicity when administered orally and dermally (LD_{50}s > 5000 mg/kg bw) and via inhalation (LC_{50} > 5 mg/L) to rats. No studies on skin and eye irritation or skin sensitization were available.

The major target organ for toxicity was the liver in short-term and long-term studies in mice and rats. Increased liver weight was the most common finding. Hepatocyte vacuolation was observed in rats, and hepatocyte hypertrophy was observed in mice. In the long-term studies, the kidneys were also a target organ in rodents, as chronic nephropathy was observed (with or without increased kidney weights). Chronic nephropathy observed only in male rats would not normally be considered relevant for a human risk assessment; however, this finding was observed in mice and both sexes of rats and therefore was considered relevant for the risk assessment.

In two 90-day studies of toxicity, mice were treated with metrafenone either at a dietary concentration of 0, 1000, 3500 or 7000 ppm (equal to 0, 163, 622 and 1206 mg/kg bw per day for males and 0, 216, 788 and 1663 mg/kg bw per day for females, respectively) or at a dietary concentration of 0, 250 or 500 ppm (equal to 0, 42 and 84 mg/kg bw per day for males and 0, 55 and 113 mg/kg bw per day for females, respectively). The overall NOAEL was 1000 ppm (equal to

163 mg/kg bw per day), on the basis of liver effects (increased total bilirubin, increased liver weight and centrilobular hepatocellular hypertrophy) observed at 3500 ppm (equal to 622 mg/kg bw per day).

In two 90-day studies of toxicity, rats were treated with metrafenone either at a dietary concentration of 0, 1000, 5000, 10 000 or 20 000 ppm (equal to 0, 79, 404, 800 and 1663 mg/kg bw per day for males and 0, 94, 486, 967 and 1938 mg/kg bw per day for females, respectively) or at a dietary concentration of 0, 250 or 500 ppm (equal to 0, 21 and 43 mg/kg bw per day for males and 0, 24 and 48 mg/kg bw per day for females, respectively). The overall NOAEL was 1000 ppm (equal to 79 mg/kg bw per day), on the basis of effects on liver (increased cholesterol and total protein, increased liver weight and periportal hepatocellular vacuolation) noted at 5000 ppm (equal to 404 mg/kg bw per day). This overall NOAEL was supported by findings in a 28-day dose range–finding study.

In 90-day and 1-year studies, dogs received metrafenone via oral capsule at 0, 50, 100 or 500 mg/kg bw per day. The overall NOAEL was 500 mg/kg bw per day, the highest dose tested, because minor changes in liver weight and/or clinical chemistry parameters were not accompanied by any microscopic abnormality and were therefore not considered adverse. This overall NOAEL was supported by findings in a 28-day dose range–finding study.

In an 18-month dietary toxicity and carcinogenicity study, mice were treated with metrafenone at 0, 250, 1000 or 7000 ppm (equal to 0, 39, 156 and 1109 mg/kg bw per day for males and 0, 53, 223 and 1492 mg/kg bw per day for females, respectively). Significant treatment-related effects were observed in the liver, kidneys and spleen at 1000 ppm (equal to 156 mg/kg bw per day) and above. Increased incidence and severity of extramedullary haematopoiesis in the spleen of female mice were recorded. Increased incidence and severity of chronic nephropathy in the kidneys (more severe in males) were recorded. Liver effects included increased weights (more severe in females) and increased incidence and severity of centrilobular and diffuse hepatocellular hypertrophy (more severe in males). The NOAEL for chronic toxicity was 250 ppm (equal to 39 mg/kg bw per day). The NOAEL for carcinogenicity was 1000 ppm (equal to 156 mg/kg bw per day), based on a treatment-related increase in hepatocellular adenomas and carcinomas in high-dose male mice at 7000 ppm (equal to 1109 mg/kg bw per day).

In a 2-year dietary study of toxicity and carcinogenicity, rats were treated with metrafenone at 0, 500, 5000 or 20 000 ppm; the dietary concentration for the high-dose females was reduced from 20 000 to 10 000 ppm beginning with study week 69 (doses equal to 0, 25, 260 and 1069 mg/kg bw per day for males and 0, 30, 320 and 1419 mg/kg bw per day [up to the end of week 68] or 593 mg/kg bw per day [weeks 72–104] for females, respectively). Body weight gains were reduced, and the liver and kidney were the target organs. Kidney effects included increased weights and increased incidence and severity of chronic nephropathy in both sexes. Effects on the liver were generally more marked in females and included increased weights, histopathological effects consistent with liver enlargement, such as centrilobular hypertrophy, and polyploidy and necrosis. The NOAEL for chronic toxicity was 500 ppm (equal to 25 mg/kg bw per day), based on effects on body weight, liver and kidney at 5000 ppm (equal to 260 mg/kg bw per day). The NOAEL for carcinogenicity was 500 ppm (equal to 30 mg/kg bw per day), based on an increased incidence of hepatocellular adenoma at the intermediate and high dose levels in females, with an equivocal response in high-dose males (LOAEL of 5000 ppm, equal to 320 mg/kg bw per day).

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

Mechanistic studies in rats did not reveal tumour initiating potential in rat liver and showed that the treatment-related liver tumours identified in mice and rats were induced by a mechanism that is non-genotoxic and is expected to have a threshold. The postulated mechanism is that metrafenone induces an increased rate of hepatocyte proliferation (cytochrome P450 enzyme induction as an associated marker); continuing exposure to metrafenone leads to chronic stimulation of proliferation, which is a known mechanism that can give rise to tumours. Levels of exposure that are insufficient to

give rise to induction of liver enzymes and liver cell proliferation would not be expected to cause liver tumours following chronic exposure.

Metrafenone was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Meeting concluded that metrafenone is unlikely to be genotoxic.

In view of the lack of genotoxicity and the fact that the observed carcinogenicity in mice and rats is expected to have a threshold, the Meeting concluded that metrafenone is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation study of reproductive toxicity, rats were given a diet containing metrafenone at a concentration of 0, 500, 1000 or 10 000 ppm (equal to 0, 39, 79 and 811 mg/kg bw per day based on the average combined premating values for P and F_1 males and females). The NOAEL for effects on reproductive parameters was 1000 ppm (equal to 79 mg/kg bw per day), based on an increased proportion of abnormal sperm in F_1 males at 10 000 ppm (equal to 811 mg/kg bw per day), although there were no clear effects on reproductive performance at any dose. The NOAEL for parental toxicity was 500 ppm (equal to 39 mg/kg bw per day), based on effects on body weight gain in F_1 parental males at 1000 ppm (equal to 79 mg/kg bw per day). The NOAEL for effects on pups was 1000 ppm (equal to 79 mg/kg bw per day), based on adverse effects on pup weights and increased liver weights at 10 000 ppm (equal to 811 mg/kg bw per day).

In a study of prenatal developmental toxicity, rats received metrafenone via gavage at 0, 50, 500 or 1000 mg/kg bw per day. There was no evidence of either maternal or embryo/fetal toxicity. The NOAEL for both maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a study of prenatal developmental toxicity, rabbits received metrafenone via gavage at 0, 50, 350 or 700 mg/kg bw per day. The NOAEL for maternal toxicity was 50 mg/kg bw per day, based on lower body weight gains and feed consumption, increased liver weights and histopathological effects in the liver at 350 and 700 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 350 mg/kg bw per day, based on decreased fetal weights at 700 mg/kg bw per day.

The Meeting concluded that metrafenone is not teratogenic.

In an acute neurotoxicity study, rats received metrafenone via gavage at 0, 125, 500 or 2000 mg/kg bw per day. The NOAEL was 2000 mg/kg bw, the highest dose tested.

In a 28-day neurotoxicity study, rats were given a diet containing metrafenone at a concentration of 0, 1500, 5000 or 15 000 ppm (equal to 0, 143, 459 and 1371 mg/kg bw per day for males and 0, 152, 493 and 1371 mg/kg bw per day for females, respectively). The NOAEL for systemic toxicity was 1500 ppm (equal to 143 mg/kg bw per day), based on clinical signs (piloerection and red discoloured urine) at 5000 ppm (equal to 459 mg/kg bw per day). The NOAEL for neurotoxicity was 15 000 ppm (equal to 1371 mg/kg bw per day), the highest dose tested.

The Meeting concluded that metrafenone is not neurotoxic.

In a 28-day immunotoxicity study, female rats were given a diet containing metrafenone at a concentration of 0, 1000, 4000 or 12 000 ppm (equal to 0, 80, 315 and 1086 mg/kg bw per day, respectively). The NOAEL for immunotoxicity was 12 000 ppm (equal to 1086 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 1000 ppm (equal to 80 mg/kg bw per day), based on significantly increased absolute and relative liver weights observed at 4000 ppm (equal to 315 mg/kg bw per day).

The Meeting concluded that metrafenone is not immunotoxic.

Toxicological data on metabolites and/or degradates

No metabolites of concern were identified.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted.

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

Toxicological evaluation

An ADI of 0–0.3 mg/kg bw was established, on the basis of the NOAEL of 25 mg/kg bw per day for liver and kidney effects in the 2-year dietary study in rats, using a safety factor of 100. The upper bound of the ADI provides a margin of exposure of at least 1000 relative to the LOAEL for the induction of liver tumours in rats and mice.

The Meeting concluded that it was not necessary to establish an ARfD for metrafenone in view of its low acute oral 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 metrafenone

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	250 ppm, equal to 39 mg/kg bw per day	1 000 ppm, equal to 156 mg/kg bw per day
		Carcinogenicity	1 000 ppm, equal to 156 mg/kg bw per day	7 000 ppm, equal to 1 109 mg/kg bw per day
Rat	Ninety-day studies of toxicity[a,b]	Toxicity	1 000 ppm, equal to 79 mg/kg bw per day	5 000 ppm, equal to 404 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 25 mg/kg bw per day	5 000 ppm, equal to 260 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 30 mg/kg bw per day	5 000 ppm, equal to 320 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	1 000 ppm, equal to 79 mg/kg bw per day	10 000 ppm, equal to 811 mg/kg bw per day
		Parental toxicity	500 ppm, equal to 39 mg/kg bw per day	1 000 ppm, equal to 79 mg/kg bw per day
		Offspring toxicity	1 000 ppm, equal to 79 mg/kg bw per day	10 000 ppm, equal to 811 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	1 000 mg/kg bw per day[d]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[d]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	50 mg/kg bw per day	350 mg/kg bw per day
		Embryo and fetal toxicity	350 mg/kg bw per day	700 mg/kg bw per day
Dog	Ninety-day and 1-year studies of toxicity[b,c]	Toxicity	500 mg/kg bw per day[d]	–

[a] Dietary administration.
[b] Two or more studies combined.
[c] Gavage or capsule administration.

[d] Highest dose tested.

Estimate of acceptable daily intake (ADI)

0–0.3 mg/kg bw

Estimate of acute reference dose (ARfD)

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 exposures.

Critical end-points for setting guidance values for exposure to metrafenone

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	> 88% within 72 hours at 10 mg/kg bw; 15–20% within 72 hours at 1 000 mg/kg bw
Dermal absorption	1.8% for the concentrate and 19% for a 1/2 000 aqueous dilution
Distribution	Widely distributed; highest concentrations in gastrointestinal tract and liver
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	67–79% (mainly in faeces via bile) within 24 hours at 10 mg/kg bw
Metabolism in animals	Extensive; mostly O-demethylation and subsequent conjugation with glucuronic acid
Toxicologically significant compounds in animals and plants	Parent compound
Acute toxicity	
Rat, LD_{50}, oral	> 5 000 mg/kg bw
Rat, LD_{50}, dermal	> 5 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5 mg/L
Rabbit, dermal irritation	No data
Rabbit, ocular irritation	No data
Dermal sensitization	No data
Short-term studies of toxicity	
Target/critical effect	Liver / increased weights and associated histopathological findings
Lowest relevant oral NOAEL	79 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver / hepatocyte hypertrophy and hepatocellular adenomas
Lowest relevant NOAEL	25 mg/kg bw per day (2-year study in rats)

Carcinogenicity	Hepatocellular adenomas and carcinomas in male mice (at 1 109 mg/kg bw per day) and hepatocellular adenomas in female rats (at 320 mg/kg bw per day)
	Non-genotoxic mechanism proposed (chronic induction of cell proliferation/enzyme induction)
	Unlikely to pose a carcinogenic risk to humans from the diet
Genotoxicity	
	Unlikely to be genotoxic
Reproductive toxicity	
Target/critical effect	Reduced pup weights (F_1 and F_2) and increased proportion of abnormal sperm (F_1 only) with no adverse effects on reproductive performance in the presence of parental toxicity
Lowest relevant parental NOAEL	39 mg/kg bw per day
Lowest relevant offspring NOAEL	79 mg/kg bw per day
Lowest relevant reproductive NOAEL	79 mg/kg bw per day
Developmental toxicity	
Target/critical effect	Lower fetal body weight at maternally toxic doses (rabbit)
Lowest relevant maternal NOAEL	50 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	350 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw (highest dose tested)
Subchronic neurotoxicity NOAEL	1 371 mg/kg bw per day (highest dose tested)
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity NOAEL	1 086 mg/kg bw per day, highest dose tested
Studies on toxicologically relevant metabolites	No metabolites of concern were identified
Mechanistic studies	Reversible induction of P450 enzymes and cell proliferation in rat liver; no tumour initiating potential in rat liver
Medical data	
	No evidence of adverse effects in personnel exposed to metrafenone; no incident reports

Summary

	Value	Study	Safety factor
ADI	0–0.3 mg/kg bw	Two-year toxicity and carcinogenicity study (rat)	100
ARfD	Unnecessary	–	–

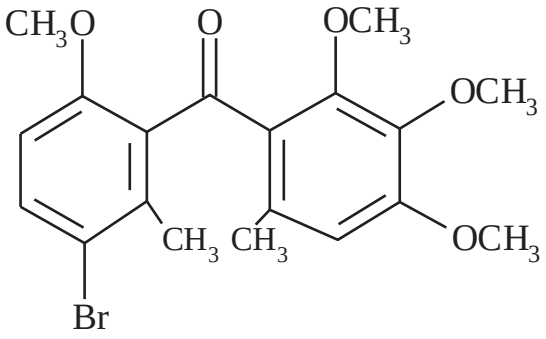
RESIDUE AND ANALYTICAL ASPECTS

Metrafenone is a benzophenone fungicide, active mainly against powdery mildews and eyespot, inhibiting mycelium growth, leaf penetration, haustoria formation and sporulation.

It was scheduled by the Forty-fifth Session of the CCPR as a new compound for consideration by the 2014 JMPR. The manufacturer submitted studies on metabolism, analytical

methods, supervised field trials, processing, freezer storage stability, environmental fate in soil and rotational crop residues.

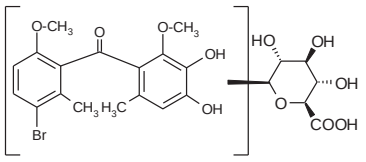
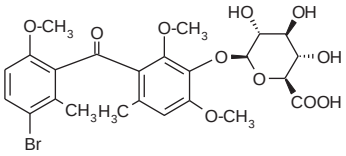
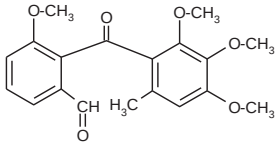
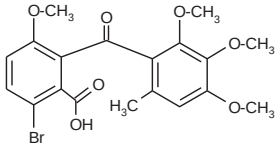
Authorisations exist for the use of metrafenone on cereals, grapes, strawberries and fruiting vegetables in over 50 countries in Europe, the Americas, Asia and the Pacific.

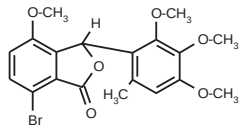
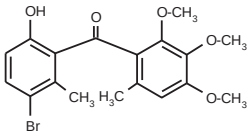
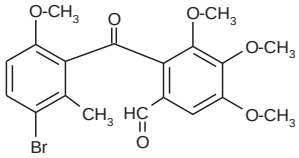
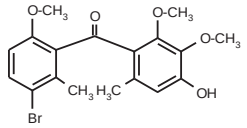
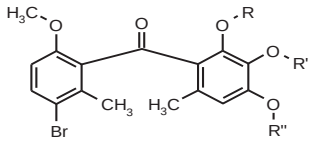


Metrafenone
(MW 409.3)

The following abbreviations are used for the major metabolites discussed below:

Table 1 Major metrafenone metabolites identified in plant, animal and soil matrices

Code	Structure	Chemical Name	Occurrence
CL 1023363		3-(3-bromo-6-methoxy-2-methylbenzoyl)-6-hydroxy-2-methoxy-4-methylphenyl β-D-glucopyranosiduronic acid Mono-O-glucuronide of Methanone, (3-bromo-6-hydroxy-2-methylphenyl)(3,4-dihydroxy-2-methoxy-6-methylphenyl-	goat
CL 1500698		3-(3-bromo-6-methoxy-2-methylbenzoyl)-2,6-dimethoxy-4-methylphenyl β-D-glucopyranosiduronic acid Methanone, (3-bromo-6-methoxy-2-methylphenyl)[3-beta-D-glucopyranuronosyloxyl)-2,4-dimethoxy-6-methylphenyl-	rat, goat
CL 1500836		3-methoxy-2-(2,3,4-trimethoxy-6-methylbenzoyl) benzaldehyde	wheat grape
CL 197675		Methanone, (3-bromo-6-methoxy-2-carboxyl)(2,3,4-trimethoxy-6-methylphenyl)-	grape

Code	Structure	Chemical Name	Occurrence
CL 3000402		7-bromo-4-methoxy-3-(2,3,4-trimethoxy-6-methylphenyl)-2-benzofuran-1(3H)-one	rat wheat grape
CL 376991		Methanone, (3-bromo-6-methoxy-2-methylphenyl)(2,3,4-trimethoxy-6-methylphenyl)-	rat wheat
CL 379395		2-(3-bromo-6-methoxy-2-methylbenzoyl)-3,4,5-trimethoxybenzaldehyde	grape
CL 434223		Methanone, (3-bromo-6-methoxy-2-methylphenyl)(4-hydroxy-2,3-dimethoxy-6-methylphenyl)-	rat wheat
M560F06	 R = H, R' and R" = CH_3 or R' = H, R and R" = CH_3 or R" = H, R and R' = CH_3	Methanone, (3-bromo-6-methoxy-2-methylphenyl)(2-hydroxy-3,4-dimethoxy-6-methylphenyl)- or Methanone, (3-bromo-6-methoxy-2-methylphenyl)(3-hydroxy-2,4-dimethoxy-6-methylphenyl)- or Methanone, (3-bromo-6-methoxy-2-methylphenyl)(4-hydroxy-2,3-dimethoxy-6-methylphenyl)-	hen

Animal metabolism

The Meeting received information on the metabolism of ^{14}C-metrafenone, separately labelled at the bromophenyl and the trimethoxyphenyl groups, in rats, lactating goats and laying hens. As no cleavage of the molecule was observed in these metabolism studies, the results for both radiolabels are reported together.

The metabolism of metrafenone in rats was evaluated by the WHO Core Assessment Group of the 2014 JMPR. Absorption of metrafenone is rapid and complete (> 88%) at the low dose of 10 mg/kg bw, limited to 15–20% at the high dose of 1000 mg/kg bw suggesting saturation of the absorption processes. Metrafenone is widely distributed in the body, with highest residue levels mainly found in the gastro-intestinal (GI) tract, liver and fat. There is no evidence of accumulation. The labelled material is relatively rapidly excreted into the GI tract via the bile (85–90%) resulting in extensive excretion via faeces. Excretion via urine is relatively low (5–6% depending on radiolabel position), and even lower at the high dose level (approximately 1%). Metrafenone is extensively metabolised, with most of the radioactivity (approximately 80%) not identified, consisting of many (11–26) different components and totalling < 0.1 ppm at the low dose and < 1 ppm at the high dose. The identified metabolites, mostly < 1.0 mg eq./kg, included metrafenone and glucuronic acid conjugates in fat, liver and kidney.

Lactating goats

Lactating goats were orally dosed with ^{14}C-metrafenone at doses equivalent to about 10 ppm (8–13 ppm) and 70 ppm (60–87 ppm) in the feed for 5 consecutive days and sacrificed 23 hours after the last dose.

The majority of the radioactivity (76–86% AR) was excreted, mainly through the faeces. The highest residue levels were found in liver (0.21-0.23 mg eq./kg at the lower dose and 0.72–1.3 mg eq./kg at the higher dose) and kidney (0.05–0.06 mg eq./kg at the low dose and 0.16–0.33 mg eq./kg at the higher dose). Residues were significantly lower in fat (0.015–0.022 mg eq./kg at the high dose rate) and were ≤ 0.01 mg eq./kg in muscle and milk regardless of the dose rate. Residues reached a plateau in milk (0.01 mg eq./kg) within 3 days.

Residue characterization and identification was conducted on samples from the high dose groups with more than 95% TRR could be extracted from liver, kidney, milk and fat. Muscle samples were not investigated further because of the low TRR levels (< 0.01 mg eq./kg).

In fat, the predominant residue was metrafenone (0.01–0.02 mg/kg), making up 60–85% TRR and no other residues above 0.005 mg eq./kg (9% TRR) were found.

In liver and kidney, metrafenone made up about 3-4% TRR. The predominant residues were the glucuronide metabolites CL 1500698 and CL 1023363, not measured individually but together represented up to 15-21% TRR (0.27 mg eq./kg) in liver and up to 26–28% TRR (max 0.09 mg eq./kg) in kidney. An additional radiolabel fraction that included the glucuronide metabolites CL 1023361, CL1023362 and CL 1500702 totalled about 9–14% of TRR (max 0.17 mg eq./kg in liver and 0.03 mg.kg in kidney). About half the TRR was made up of a number of other unidentified metabolites, each present at < 5% TRR.

In milk, residues of metrafenone (24% TRR) and numerous metabolites, including one radiolabel fraction containing CL 1500698 and CL 1023363 (11% TRR), were all < 0.005 mg eq./kg.

Laying hens

In a poultry study laying hens were orally dosed with ^{14}C-metrafenone at doses equivalent to about 14 ppm in the feed for 12 consecutive days and sacrificed 22 hours after the last dose.

The majority (86-95%) of the administered dose was excreted, with about 0.25% AR (0.1 mg eq./kg) remaining in eggs, up to 0.09% AR (0.3-0.5 mg eq./kg) found in liver, < 0.01 % AR (0.06–0.08 mg eq./kg) present skin+fat and 0.003% AR (0.01 mg eq./kg) in muscle. Residues reached a plateau in eggs after 9 days.

Extraction was able to retrieve about 80% TRR from eggs, 60% TRR from skin+fat and about 30% TRR in liver and muscle. Characterisation and identification of residues in solvent-extracted samples indicated the presence of numerous polar and non-polar components. The one identified metabolite M560F06 was found in poultry skin+fat (6-11% TRR, < 0.01 mg eq,/kg) and was identified but not quantified in eggs.

Metrafenone was found only in eggs and skin+fat, making up about 2% TRR (0.001-0.002 mg/kg) and the metabolite M560F06 was also measured in skin+fat (6–11% TRR) and identified in eggs. With the exception of one unknown component in eggs (about 14% TRR, 0.015 mg eq./kg) all other metabolites were <10% TRR (< 0.01 mg eq./kg) in all tissues and eggs.

In summary, residues were rapidly eliminated in the excreta (76–95% of the dose) with up to 0.5% of the total administered dose remaining in liver, 0.25% remaining in eggs, and TRRs were up to 0.02 mg eq./kg in fat and ≤0.01 mg eq./kg in muscle, poultry skin+fat and in milk.

The proposed metabolic pathways include hydroxylation and demethylation of the methyl groups and the phase II glucuronidation of the hydroxylated metabolites to various mono-O-glucuronides, qualitatively similar to the metabolic pathway in the rat.

Metrafenone made up about 2–4% of the TRR in liver, kidney, eggs and poultry skin+fat, was the main component in fat and was about 24% TRR in milk, but at very low levels (< 0.005 mg eq./kg). Most of the residues in liver and kidney were the glucuronide conjugates of metrafenone (CL 1500698, CL 1023363) which together made up 15–30% TRR, and numerous unidentified components, each present at < 10% TRR.

Plant metabolism

The Meeting received information on the metabolism of ^{14}C-metrafenone, separately labelled at the bromophenyl and the trimethoxyphenyl groups, in grapes, cucumber and wheat.

Grape

In outdoor grapevines treated with five foliar applications of ^{14}C-metrafenone at a rate equivalent to 0.2 kg ai/ha, 10–11 days apart, TRRs in grapes immediately after the last application were 0.6–0.77 mg eq./kg, reducing to 0.28–0.44 mg eq./kg at maturity, 35 days later. In leaves sampled immediately after the last application, TRRs were 40–42 mg eq./kg, reducing to 25–38 mg eq./kg after 35 days.

In grape juice, pomace and in leaves, 77–100% TRR was able to be sequentially extracted with acetone, methanol:water and water, with about 39–45% of the TRR in leaves being present in the acetone surface wash. Whole grapes were not analysed.

Metrafenone was not found in juice, but was the major residue in pomace (23–25% TRR, 0.06–0.11 mg/kg), and made up about 11–15% TRR in mature leave (35 days after the last application).

Characterisation of the residues in grape juice and pomace indicated the presence of several chromatographic fractions more polar than the parent, not exceeding 0.05 mg eq./kg and not more than 17% TRR. These were not identified further except for CL197675, found in juice at about 9% TRR (0.006 mg eq./kg).

Cucumber

In cucumber plants (confined) treated with two foliar applications of ^{14}C-metrafenone, applied 17 and 3 days before harvest, at a rate equivalent to 0.2 kg ai/ha, TRR in mature fruit, sampled 3 days after the second application were about 0.05 mg eq./kg (TRR), with 0.013 mg eq./kg present in pulp and 0.26 mg eq./kg in peel. More than 89% TRR was able to be extracted with methanol.

Metrafenone was the only identified residue component, making up 42% of the TRR in the mature fruit (0.02 mg/kg), and mostly in the peel (61% TRR, 0.16 mg/kg). Metrafenone also made up about 80% TRR in vines (without roots and fruit) at harvest.

Numerous polar and medium polar metabolites, characterized by their HPLC retention times and elution profiles, were present in fruit and vines at low concentrations (each less than 9% TRR).

Wheat

In outdoor wheat plants treated with 3 foliar applications of ^{14}C-metrafenone at rates equivalent to 0.3, 0.3 and 0.2 kg ai/ha, applied at 13–14 day intervals and with the last application being 35 days before harvest, highest radioactive residues (up to 9 mg eq./kg) were found in hay and straw, with the lowest residues found in the grain (0.2–0.4 mg eq./kg). The TRR in foliage, 3 days after the first application were 5–8 mg eq./kg. Methanol:water extraction was able to release about 95% TRR in foliage, 78% TRR in hay, 61% TRR in straw and 35% TRR in grain. Additional extraction with hexane and acidified methanol was able to release a further 12–14% TRR in grain.

Metrafenone was the major component in all matrices, about 59–64% TRR in foliage, 13–26% TRR in hay, 8–14% in straw and 3–8% TRR in grain.

Other characterized or identified metabolites in foliage, hay and straw each represented less than 10% TRR. In grain, no identified metabolites were found above 0.004 mg eq./kg and although only about 50% of the radioactivity was extracted, further investigation showed that residues in the PES were made up of multiple minor components.

The proposed metabolic pathway involves oxidation of the methyl groups on the bromophenyl and trimethoxyphenyl rings to yield the corresponding aldehydes. In the case of the bromophenyl ring, the aldehyde can undergo further oxidation to the carboxylic acid, cyclization to form the lactone, and/or dehalogenation to form the des-bromo aldehyde.

In summary, metrafenone is the predominant residue in crops, with numerous minor metabolites or fractions present at low concentrations and generally more polar than the parent. While these were not all identified or quantified, individual peaks were < 10% TRR or < 0.01 mg eq./kg.

Environmental fate

The Meeting received information the environmental fate and behaviour of metrafenone, including hydrolytic stability, photolysis in aqueous solutions, aerobic metabolism and rotational crop metabolism studies.

Metrafenone was stable in sterile buffered solutions at pH 4, 7, and 9 but rapidly degraded in aqueous pH 7 solutions by photolysis (DT_{50} values of 2.6–3.1 days) with the formation of multiple degradation products, all found at < 10% of the applied radioactivity. DT_{90} values were 8.5 days (natural water) and 10.2 days (sterile water).

Aerobic soil metabolism

Metrafenone degraded slowly in loamy sand, sandy loam and clay loam soils treated with the equivalent of about 0.1 kg ai/ha [bromophenyl-label]-metrafenone or [trimethoxy-label]-metrafenone and incubated for up to 210 days under aerobic laboratory conditions. Metrafenone made up about 82% AR after 120-days and 66–69% AR after 210 days. Calculated half-lives (1st order kinetics) ranged from 182–365 days.

Residues in succeeding crops

In one outdoor rotational crop metabolism study, a leafy vegetable crop (lettuce), root crop (radish) and oil crop (canola) were planted back at various time intervals (30, 60, 90 and 365 days) after a single application of [trimethoxy-label]-metrafenone or [bromophenyl-label]-metrafenone to bare soil at a rate equivalent to 0.625 kg ai/ha.

The uptake of residues in these representative rotational crops (lettuce, radish, canola) was low, with TRRs at all plant-back intervals ranging from < 0.004 to 0.048 mg eq./kg (in canola pods), generally highest in the samples from the 30-day plant back interval. In soil, radioactive residues declined by about 50% after 90 days, and were mostly found in the top 10 cm of soil samples.

Total extractable residues ranged from 64% to 88% TRR in the majority of the samples (42–86% TRR in canola seed) and comprised mostly of multiple unidentified polar components, all present at < 0.02 mg eq./kg. Metrafenone accounted for 0.004 mg/kg of the TRR in lettuce (90 DAT) and radish roots (30 DAT) and was not found in canola or radish tops.

In summary, metrafenone is stable to hydrolysis, rapidly degraded by photolysis, slowly degraded in soil under aerobic conditions (remaining mostly in the top 10 cm) and not found at significant levels in rotational crops. The Meeting concluded that residues are not expected in rotational crops following treatments according to the GAPs under consideration.

Analytical methods

Several analytical methods have been reported and validated for the analysis of metrafenone in plant and animal commodities. One method has also been validated for measuring residues of the CL 300402, CL 434223 and CL 376991 metabolites. The basic approach employs extraction with methanol/water, aqueous acetone or n-heptane/acetone, SPE or GPC clean-up and analysis by GC-ECD, GC-MS, or LC-MS/MS. In some methods, an additional partition step is included, using dichloromethane, cyclohexane, acetone, ethyl acetate, singly or sequentially.

For plant and processed plant commodities, the DFG S19 (GC-ECD or GC-MS) or the QuEChERS (LC-MS/MS) methods were used in most of the supervised residue field trials, with the RLA 12619 (LC-MS/MS) method used to measure parent and metabolites in some cereal trials. These methods were validated in a range of matrices (wheat, barley, grapes, cucumber, summer squash, melon, tomato, pepper, lemon, dry bean, oilseed rape and hops). The LOQ is 0.1 mg/kg for mushrooms and cereal forage and straws and 0.01 mg/kg for all other matrices.

For animal commodities, the DFG S19 (GC-MS) method was validated for the analysis of metrafenone in muscle, milk and eggs. After extraction with aqueous acetone, extracts are partitioned into ethyl acetate/cyclohexane before SPE clean-up and analysis. The LOQ is 0.01 mg/kg for milk and 0.05 mg/kg for muscle and eggs.

Stability of pesticide residues in stored analytical samples

Metrafenone residues were stable in analytical samples stored frozen (-18 to -20 °C) for up to 24 months in representative substrates with a high water content (lettuce, tomato), a high starch content (carrot, wheat grain), a high protein content (dry peas), a high oil content (soya bean) (grape, wine) and in , wheat forage and straw residues were stable for at least 31 months. In general, residues in the stored samples were greater than 80% of the spiked levels.

Definition of the residue

In animal commodities, metrafenone was the main identified component in goat fat (60–85% TRR) and poultry eggs and skin+fat (2% TRR) but made up about only 3–4% of the TRR in goat liver and kidney. Most of the identified residues in goat liver and kidney were in the radiolabel fraction that included CL 1500698 and CL 1023363 (totalling 15-30% TRR), with numerous unidentified minor fractions found at lower levels, each generally < 5% TRR. In muscle, TRRs were not found above 0.01 mg eq./kg in the highest dose groups of 65–87 ppm (goat) and 14 ppm (hen) and the TRR in milk was also < 0.01 mg eq./kg.

Based on the metabolism studies, a residue definition for animal commodities that includes metrafenone and the CL 1500698 and the CL 1023363 glucuronides could be considered. However the Meeting noted that these metabolites were not found in hens and only present in goats at low levels (totalling up to 0.27 mg eq./kg in liver and 0.09 mg eq./kg in kidney) following dosing at levels more than 7 times higher than the anticipated maximum livestock dietary burdens. CL 1023363 is structurally similar to rat metabolites and CL 1500698 was found in the rat metabolism study. Both metabolites are accommodated in the ADI. The Meeting therefore concluded that they need not be included in the residue definitions.

Based on the anticipated dietary exposure, the Meeting concluded that significant residues of the CL 1500698 and CL 1023363 metabolites are not expected in animal commodities and as a multi-residue method existed to measure the parent compound in animal commodities, a suitable residue definition for MRL-compliance and dietary intake estimation was metrafenone. Based on the Log P_{ow} of 4.3 and since residues of metrafenone were only found in fat and milk, the Meeting concluded that metrafenone is fat-soluble.

In plant commodities from treated crops, the metabolism studies indicated that metrafenone was the major residue in grape (up to 25% TRR in grape pomace), cucumber (up to 42% TRR) and

wheat matrices (up to 8% TRR in grain and 14-64% TRR in foliage and straw), with numerous minor metabolites or radiolabel fractions present at low concentrations and generally more polar than the parent. While these were not all identified or quantified, individual peaks were < 10% TRR or < 0.01 mg eq./kg.

Metabolite CL 3000402 was occasionally found in grain at levels up to about 10% of the parent concentration but were < 0.02 mg/kg. Metabolites CL 3000402, CL 434223 and CL 376991, found in the wheat metabolism study at up to 7% TRR in foliage and straw, were also measured in the foliage, straw and hay from a number of wheat and barley field trials, generally at levels less than 10% of the parent residue. These three metabolites were also found in the rat metabolism study and are accommodated in the ADI.

The Meeting noted that multiresidue methods exists to measure parent residues and agreed that for MRL-compliance and dietary intake estimation, the residue definition for plant commodities should be metrafenone.

Proposed definition of the residue (for compliance with the MRL and estimation of dietary intake for plant commodities): *metrafenone.*

Proposed definition of the residue (for compliance with the MRL and estimation of dietary intake for animal commodities: *metrafenone.*

Metrafenone is fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for foliar applications of metrafenone on a range of berries and other small fruits, fruiting vegetables, cereals and hops. These trials were conducted mainly in Europe and/or North America.

Where residues have been reported as ND (< LOD) the values have been considered as < LOQ (< 0.01 mg/kg) for the purposes of MRL setting. If a higher residue level was observed at a longer PHI than the GAP, the higher value has been used in MRL setting.

The Meeting noted that GAP has been authorised for the use of metrafenone in more than 50 countries in Europe, the Americas, Asia and the Pacific and that product labels were available from many of these countries. Supervised trial data were also provided for pome fruit, stone fruit and hops, but no GAP information was available to support maximum residue level estimations for these commodities.

Berries and small fruit

Results from supervised trials on grapes conducted in USA and strawberries conducted in Europe were provided to the Meeting.

Grape

The critical GAP for metrafenone on grapes is in Canada, up to 6 foliar applications of 0.225 kg ai/ha applied at least 14–21 days apart with a PHI of 14 days and with a total of 1.35 kg ai/ha/season. In trials from USA conducted at about 1.5 time higher rate than the Canadian GAP, metrafenone residues in grapes were: 0.11, 0.17, 0.18, 0.27, 0.32, 0.62, 2.1, 2.3, 2.4, 3.0, and 3.2 mg/kg. When proportionally adjusted to the Canadian GAP (scaling factors (S_f) of 0.64–0.68), metrafenone residues in these trials are: 0.08, 0.11, 0.12, 0.17, 0.22, 0.42, 1.1, 1.4, 1.5, 1.6, 2.0 and 2.2 mg/kg (n=12).

The Meeting estimated an STMR of 0.74 mg/kg and a maximum residue level of 5 mg/kg for metrafenone on grapes.

Strawberry

The critical GAP for metrafenone on strawberries is on protected crops in the Netherlands, up to 2 foliar applications of 0.15 kg ai/ha, applied at least 7 days apart with a PHI of 3 days. In trials on protected strawberries matching this GAP in Netherlands, metrafenone residues in fruit were: 0.05, 0.06, 0.08, 0.1, 0.16, 0.23, 0.28 and 0.34 mg/kg (n=8).

The Meeting estimated an STMR of 0.13 mg/kg and a maximum residue level of 0.6 mg/kg for metrafenone on strawberries.

Fruiting vegetables, Cucurbits

Results from supervised trials on cucumbers, summer squash (zucchini) and melons (cantaloupes) conducted in Europe and North America were provided to the Meeting. However no GAP information was available from North America

Cucumber

The critical GAP for metrafenone on cucumbers is in France, up to 2 foliar applications of 0.1 kg ai/ha, applied at least 7–10 days apart with a PHI of 3 days. This GAP applies to both outdoor and protected crops.

In trials on outdoor cucumbers in Europe matching this GAP in France, metrafenone residues in cucumbers were: 0.01, 0.02, 0.02, 0.02, 0.02, 0.02, 0.03 and 0.04 mg/kg (n=8).

In trials on protected cucumbers matching this GAP in France, metrafenone residues in cucumbers were: 0.02, 0.04, 0.04, 0.05, 0.05, 0.06, 0.06, 0.07 and 0.09 mg/kg (n=9).

Based on the data set for protected cucumbers, the Meeting estimated an STMR of 0.05 mg/kg and a maximum residue level of 0.2 mg/kg for metrafenone on cucumber.

The Meeting also agreed to extrapolate these estimations to gherkins.

Summer squash

The critical GAP for metrafenone on summer squash is in France, up to 2 foliar applications of 0.1 kg ai/ha, applied at least 7–10 days apart with a PHI of 3 days. In trials on summer squash in Europe matching this GAP in France, metrafenone residues in summer squash were: 0.01, 0.01, 0.01, 0.01, 0.02, 0.02, 0.02 and 0.04 mg/kg (n=8).

The Meeting estimated an STMR of 0.015 mg/kg and a maximum residue level of 0.06 mg/kg for metrafenone on summer squash.

Melons (except watermelon)

The critical GAP for metrafenone on melons is in France, up to 2 foliar applications of 0.1 kg ai/ha, applied at least 7–10 days apart with a PHI of 3 days. In trials on melons in Europe matching this GAP in France, metrafenone residues in melons were: < 0.01, 0.01, 0.01, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.03, 0.05, 0.06 and 0.07 mg/kg (n=13).

However the Meeting noted that in these trials, the melons had been quartered in the field and although the subsamples had been frozen within 12 hours after sampling, no information was available on residue stability in chopped or sliced samples.

The Meeting was unable to estimate a maximum residue level for metrafenone on melons.

Fruiting vegetables, other than Cucurbits

Results from supervised trials on tomatoes and peppers (sweet, bell and non-bell) conducted in Europe and North America and from trials on mushrooms in Europe were provided to the Meeting.

Mushrooms

The critical GAP for metrafenone on mushrooms is in France, one broadcast treatment of 0.05 kg ai/15 litres water/100 square metres of compost, applied up to 10 days before harvest. In trials on mushrooms in Europe matching this GAP in France, metrafenone residues in mushrooms were: 0.1, 0.1, 0.11 and 0.19 mg/kg (n=4)

The Meeting estimated an STMR of 0.105 mg/kg and a maximum residue level of 0.5 mg/kg for metrafenone on mushrooms.

The Meeting noted that the OECD MRL-calculator proposed a maximum residue level of 0.4 mg/kg, but agreed that a higher value on 0.5 mg/kg was more appropriate due to the small data set and because the relatively close spread of results may not reflect the residue variability arising from different composts used in mushroom production.

Pepper, Sweet

The critical GAP for metrafenone on peppers is in France for protected crops, up to 2 foliar applications of 0.15 kg ai/ha, applied at least 7–10 days apart with a PHI of 3 days. In trials on protected sweet peppers matching this GAP in France, metrafenone residues in peppers were: 0.07, 0.08, 0.1, 0.11, 0.12, 0.2, 0.21 and 1.3 mg/kg (n=8).

The Meeting estimated an STMR of 0.115 mg/kg and a maximum residue level of 2.0 mg/kg for metrafenone on peppers, sweet and agreed to extrapolate these estimations to chili pepper.

For dried chili peppers, applying the default processing factor of 10 to the STMR and the maximum residue level estimated for peppers, the Meeting estimated an STMR-P of 1.15 mg/kg and a maximum residue level of 20 mg/kg for metrafenone on dried chili peppers.

Tomato

The critical GAP for metrafenone on tomatoes is in Spain, up to 2 foliar applications of 0.015 kg ai/hL with a PHI of 3 days. This GAP applies to both outdoor and protected crops.

In trials on outdoor tomatoes in Europe matching this GAP in Spain, metrafenone residues in tomatoes were: 0.02, 0.05, 0.05, 0.06, 0.06, 0.07, 0.08 and 0.15 mg/kg (n=8).

In trials on protected tomatoes matching this GAP in Spain, metrafenone residues in tomatoes were: 0.06, 0.09, 0.09, 0.1, 0.1, 0.1, 0.16 and 0.17 mg/kg (n=8).

Based on the data set for protected tomatoes, the Meeting estimated an STMR of 0.1 mg/kg and a maximum residue level of 0.4 mg/kg for metrafenone on tomato.

Cereal grains

Results from supervised trials on wheat and barley conducted in Europe were provided to the Meeting.

Wheat

The critical GAP for metrafenone on wheat is in Poland, up to 2 foliar applications of 0.15 kg ai/ha with a PHI of 35 days. In trials in Europe matching this GAP in Poland, metrafenone residues in wheat grain were: < 0.01 (9), 0.01 (4), 0.02, 0.03, 0.03, 0.04 and 0.04 mg/kg (n=18).

The Meeting estimated an STMR of 0.01 mg/kg and a maximum residue level of 0.06 mg/kg for metrafenone on wheat.

The Meeting also agreed to extrapolate these estimations to rye and triticale.

Barley

The critical GAP for metrafenone on barley is in Poland, up to 2 foliar applications of 0.15 kg ai/ha with a PHI of 35 days. In trials in Europe matching this GAP in Poland, metrafenone residues in barley grain were: < 0.01, 0.02 (3), 0.03, 0.04, 0.05 (3), 0.06, 0.06, 0.07, 0.08, 0.09, 0.11, 0.13, 0.15, 0.16, 0.23 and 0.4 mg/kg (n=20).

The Meeting estimated an STMR of 0.06 mg/kg and a maximum residue level of 0.5 mg/kg for metrafenone on barley.

The Meeting also agreed to extrapolate these estimations to oats.

Animal feeds

Cereal forages

Wheat and barley plant or foliage samples were collected in many of the European trials matching the GAP in Hungary/Poland (up to 2 foliar applications of 0.15 kg ai/ha).

Wheat forage

In wheat trials matching the GAP in Poland, metrafenone residues in plant (forage) samples taken 0-days after the last application were: 1.8, 2.0, 2.0, 2.6, 2.6, 2.6, 2.8, 3.3, 3.7, 3.8, 4.3 and 4.8 mg/kg (fresh weight).

The Meeting estimated a median residue of 2.7 mg/kg (fresh weight) and a highest residue of 4.8 mg/kg (fresh weight) for wheat forage and agreed to extrapolate these estimations to rye and triticale.

Barley forage

In barley trials matching the GAP in Poland, metrafenone residues in plant (forage) samples taken 0-days after the last application were: 1.8, 2.3, 2.5, 2.5, 3.1, 3.4, 3.7, 3.8, 4.6, 5.0, 5.8 and 5.9 mg/kg (fresh weight).

The Meeting estimated a median residue of 3.75 mg/kg (fresh weight) and a highest residue of 5.9 mg/kg (fresh weight) for barley forage and agreed to extrapolate these estimations to oats.

Cereal and grass straws and hays

Wheat and barley straw samples were collected in many of the European trials matching the GAP in Poland (up to 2 foliar applications of 0.15 kg ai/ha).

Wheat straw

In trials in Europe matching this GAP in Poland, metrafenone residues in wheat straw (fresh weight) were: 0.67, 0.67, 0.98, 1.1, 1.3, 1.4, 1.6, 1.7, 1.8, 2.0, 2.1, 2.3, 3.1, 3.1, 3.5, 3.6, 3.6 and 6.7 mg/kg (n=18). After correction for an average 88% dry matter content, residues (dry weight) were: 0.76, 0.76, 1.1, 1.3, 1.5, 1.6, 1.8, 1.9, 2.1, 2.3, 2.4, 2.6, 3.5, 3.5, 4.0, 4.1, 4.1 and 7.6 mg/kg.

The Meeting estimated a median residue of 1.9 mg/kg (fresh weight), a highest residue of 6.7 mg/kg (fresh weight) and a maximum residue level of 10 mg/kg (dry weight) for metrafenone in wheat straw.

The Meeting also agreed to extrapolate these estimations to rye and triticale.

Barley straw

In trials in Europe matching the GAP in Poland, metrafenone residues in barley straw (fresh weight) were: < 0.01, 0.24, 0.41, 0.95, 1.0, 1.1, 1.1, 1.1, 1.2, 1.3, 1.3, 1.5, 1.7, 1.8, 1.9, 1.9, 2.0, 2.1, 3.6 and 3.9 mg/kg (n=20). After correction for an average 89% dry matter content, residues (dry weight) were: < 0.01, 0.29, 0.46, 1.1, 1.1, 1.2, 1.2, 1.24 1.4, 1.5, 1.5, 1.5, 1.9, 2.0, 2.1, 2.1, 2.3, 2.4, 4.0 and 4.4 mg/kg.

The Meeting estimated a median residue of 1.3 mg/kg (fresh weight), a highest residue of 3.9 mg/kg (fresh weight) and a maximum residue level of 6 mg/kg (dry weight) for metrafenone in barley straw.

The Meeting also agreed to extrapolate these estimations to oats.

Fate of residues during processing

The effect of processing on the nature of residues was investigated using radiolabelled metrafenone in buffer solutions incubated under conditions simulating pasteurisation (in pH 4 buffer at 90 °C for 20 minutes); baking, brewing, or boiling (in pH 5 buffer at 100 °C for 60 minutes); and sterilization (in pH 6 buffer at 120 °C for 20 minutes). Metrafenone was stable under these processing conditions with no significant changes in the radio-chromatograms.

The fate of metrafenone residues has been examined in a number of studies simulating household and commercial processing of apples, grapes, strawberries, tomatoes, barley, wheat and hops. Estimated processing factors and STMR-Ps for the commodities considered at this Meeting are summarised below.

Summary of selected processing factors and STMR-P values for metrafenone

RAC	Matrix	Metrafenone [a] Calculated processing factors	PF best estimate	STMR (mg/kg)	STMR-P (mg/kg)
Grape	grapes			0.76	
	must (red wine)	0.03, 0.15, < 0.18, 0.26, 0.57, 0.77, 0.78, 0.81, 1.17, 1.29	0.67		0.51
	wet pomace	2.8, 3.6	3.2		2.4
	wine	0.03, 0.07, < 0.17, < 0.18, < 0.19, < 0.19, < 0.21, < 0.26, < 0.38, < 0.71	0.19		0.14
	juice	0.04, 0.06	0.05		0.038
	raisins	< 0.63, < 0.71, 3.63, 3.94	3.75		2.85
Tomato	fresh			0.1	
	preserved	< 0.02, < 0.02, < 0.02, 0.02	< 0.02		< 0.002
	juice (raw)	0.26, 0.33, 0.35, 0.4	0.34		0.034
	wet pomace	3.3, 4.8, 6.2, 6.3	5.5		0.55
	paste	0.27, 0.3, 0.47, 0.53	0.385		0.039
	puree	0.65, 0.79, 0.83, 1.1	0.81		0.081
Mushroom	fresh			0.105	
	canned	0.16	0.16		0.017
Barley	grain			0.06	
	pearl barley	< 0.13, 0.13, < 0.2, 0.22	0.165		0.01
	abraded fraction	2.5	2.5		0.15
	malt	0.4	0.4		0.024
	brewers grain	0.3	0.3		0.018
	beer	< 0.1, < 0.13, < 0.17, < 0.33,	< 0.15		< 0.009
Wheat	grain			0.01	
	wholemeal flour	0.94, 1.1, 1.7, 1.9	1.4		0.014
	flour type 550	0.14, 0.17, 0.21, 0.29	0.19		0.002

RAC	Matrix	Metrafenone [a] Calculated processing factors	PF best estimate	STMR (mg/kg)	STMR-P (mg/kg)
	fine bran	2.6, 3.5, 4.9, 5.3	4.2		0.042
	whole grain bread	0.6, 0.64, 0.71, 1.0	0.675		0.007

[a] Each PF value represents a separate study where residues were above the LOQ in the RAC and is the ratio of the metrafenone residues in the processed item divided by the residues in the RAC.

The Meeting noted that in the studies available, metrafenone residues did not concentrate in food commodities during processing, except in dehydrated commodities such as raisins, bran and flour. Residues also increased in wet pomace (grape and tomato), tomato peel and barley abrasion fractions.

The Meeting estimated a maximum residue level for dried grapes of 20 mg/kg based on the maximum residue level estimated for grapes (5.0 mg/kg) and the median processing factor (3.75) from the USA processing studies.

The Meeting estimated a maximum residue level for wheat bran (processed) of 0.25 mg/kg based on the maximum residue level estimated for wheat (0.06 mg/kg) and a median processing factor of 4.2.

The Meeting estimated a maximum residue level for wheat wholemeal of 0.08 mg/kg based on the maximum residue level estimated for wheat (0.06 mg/kg) and a median processing factor of 1.4.

Residues in animal commodities

Farm animal dietary burden

The Meeting estimated the dietary burden of metrafenone in farm animals on the basis of the diets listed in Appendix IX of the 2009 edition of the JMPR Manual. Noting that fresh forage commodities are not significant in international trade, the Meeting only included the burden contributions from the cereal forages in the European dietary burden calculation, as metrafenone is not authorised for use on cereals in US-Canada, Australia or Japan.

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are presented in Annex 6 of the 2014 Report and are summarized below:

Estimated maximum and mean dietary burdens of farm animals

	Animal dietary burden, metrafenone, ppm of dry matter diet							
	US-Canada		EU		Australia		Japan	
	max	mean	max	mean	max	mean	max	mean
Beef cattle	0.8	0.26	5.9	3.8	9.3 [a]	4.9 [c]	0.07	0.07
Dairy cattle	0.8	0.25	5.9	3.8	9.2 [b]	4.9 [d]	0.42	0.14
Poultry – broiler	0.05	0.05	0.05	0.05	0.02	0.02	0.007	0.007
Poultry – layer	0.05	0.05	2.0 [e g]	1.3 [f h]	0.015	0.015	0.008	0.008

[a] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian tissues
[b] Highest maximum dairy cattle dietary burden suitable for MRL estimates for mammalian milk
[c] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian tissues.
[d] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.
[e] Highest maximum poultry dietary burden suitable for MRL estimates for poultry tissues.
[f] Highest mean poultry dietary burden suitable for STMR estimates for poultry tissues.
[g] Highest maximum poultry dietary burden suitable for MRL estimates for poultry eggs.
[h] Highest mean poultry dietary burden suitable for STMR estimates for poultry eggs.

For beef and dairy cattle, the calculated maximum dietary burden is 9.3 ppm dry weight of feed and for poultry, noting that in some countries, laying hens may also be consumed, the calculated maximum dietary burden suitable is 2.0 ppm dry weight of feed.

Farm animal feeding studies

No livestock feeding studies were provided.

Animal commodity maximum residue levels

The Meeting noted that in the goat metabolism study, up to 0.014 mg/kg metrafenone was found in the kidney from the high (87 ppm) dose group animals and by extrapolation, this would equate to a maximum level of 0.0015 mg/kg in kidney from animals exposed to the calculated maximum dietary burden of 9.3 ppm.

In liver, metrafenone residues were up to 0.025 mg/kg in the high (60 ppm) dose group animals and by extrapolation, this would equate to a maximum level of 0.004 mg/kg in liver from animals exposed to the calculated maximum dietary burden of 9.3 ppm.

In animals dosed with 10 ppm in the diet (approximating the maximum calculated dietary burden for beef and dairy cattle, radiolabel residues were < 0.005 mg eq./kg in muscle, milk and fat.

The Meeting estimated maximum residue levels of 0.01* mg/kg for metrafenone in meat (from mammals other than marine mammals), 0.01 mg/kg for edible offal (mammalian), 0.01* mg/kg for mammalian fat and 0.01* mg/kg for milks. Estimated STMRs for dietary intake estimation are 0 mg/kg for meat, 0.01 mg/kg for edible offal, 0 mg/kg for fat and 0 mg/kg for milk.

In the hen metabolism study, residues of metrafenone were up to 0.002 mg/kg in eggs and up to 0.001 mg/kg in skin+fat in hens dosed with 14 ppm in the diet (about 7-fold higher than the maximum calculated dietary burden for poultry). In muscle and liver, metrafenone residues were not detected.

The Meeting estimated maximum residue levels of 0.01* mg/kg for metrafenone in poultry meat, 0.01* mg/kg for poultry offal, 0.01* mg/kg for poultry fat and 0.01* mg/kg for eggs. Estimated STMRs for dietary intake estimation are 0 mg/kg for poultry fat, 0 mg/kg for poultry meat, 0 mg/kg for poultry offal and 0 mg/kg for 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 and for IEDI assessment.

Definition of the residue (for MRL-compliance and estimation of dietary intake, plant commodities): *metrafenone.*

Definition of the residue (for MRL-compliance and estimation of dietary intake, animal commodities): *metrafenone.*

The residue is fat soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intake (IEDI) for metrafenone was calculated for the food commodities for which STMRs or HRs were estimated and for which consumption data were available. The results are shown in Annex 3.

The International Estimated Daily Intakes of metrafenone for the 17 GEMS/Food cluster diets, based on estimated STMRs were 0% of the maximum ADI of 0.3 mg/kg bw (Annex 3). The Meeting concluded that the long-term intake of residues of metrafenone from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The 2014 JMPR decided that an ARfD was unnecessary. The Meeting therefore concluded that the short-term intake of metrafenone residues is unlikely to present a public health concern.