5.6 CYFLUMETOFEN (273)

TOXICOLOGY

Cyflumetofen is the ISO-approved common name for 2-methoxyethyl (*RS*)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-*o*-tolyl)propionate (IUPAC), with CAS number 400882-07-7. It is an acaricide and interferes with energy production (inhibition of complex II in mitochondria) on contact with spider mites. Cyflumetofen is a racemic mixture.

Cyflumetofen 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

The specific metabolism or degradation of the individual enantiomers of the racemic mixture was not investigated.

In a pharmacokinetic study performed in mice, peak concentrations of radiolabel in plasma were reached rapidly (0.5–1 hour after dosing) and were 2.4-fold higher in females than in males. Moreover, in females, a second C_{max} was observed at 2 or 8 hours after dosing, suggesting enterohepatic recirculation. Terminal half-lives were in the range of 22–60 hours. The absorption in mice represented by the AUC was up to 2-fold higher in females than in males and increased sublinearly with increasing doses.

Absorption of cyflumetofen in rats was approximately 68–78% at the low dose (3 mg/kg bw), based on urinary and biliary excretion, with saturation at the high dose (approximately 35–46% absorption at 250 mg/kg bw). There were no remarkable differences in absorption according to sex or the position of the radiolabels used. Saturation of oral absorption was also noted after repeated daily administration at the low dose. Peak concentrations in plasma after a single high-dose application occurred after 1–4 hours. Radioactivity in plasma decreased biexponentially, with a terminal half-life of 12–22 hours. The AUC increased less than proportionally with the dose, confirming saturation in absorption. The AUC was up to 2-fold higher in females than in males at the high dose.

The major route of elimination at the low dose was the urine (58–66%), with lower amounts in faeces (25–32%). At the high dose, the major route of excretion was the faeces (68–77%), with lower amounts in urine (14–22%). Studies in bile duct–cannulated rats showed that at least 30% and 18% were excreted in bile in males and females, respectively, regardless of dose level. A decrease in urinary excretion in cannulated compared with non-cannulated rats suggests that some reabsorption from the intestinal tract after biliary excretion might occur at low doses. Within 72 hours after administration, at least 95% of the absorbed dose was excreted.

In rats, the half-lives for elimination from tissues were in the range of 9–24.5 hours at the low dose and 14–42.5 hours at the high dose, with the longest half-lives in adipose tissue, followed by bone marrow. Highest residues were identified in the liver, followed by the kidney, regardless of sex, dose, label position and time point of measurement. Levels of radioactivity in tissues appeared to have reached steady state after 4 days of repeated daily dosing. Residues in erythrocytes and skin declined relatively slowly.

Cyflumetofen was extensively metabolized. The predominant metabolic pathway was cleavage of the *tert*-butylphenyl (A-ring) and trifluorotolyl (B-ring) moieties. Major reactions on the A-ring were cleavage of the methoxyethyl group, hydroxylation at the butyl group, and decarboxylation and glucuronidation at the butyl group. Major reactions on the B-ring were glutathione conjugation at the carboxyl group and further metabolism to mercapturic acid or thiolactic acid. In addition, hydroxylation and oxidation reactions at the butyl group and cleavage of the carboxylic ester moiety were observed on the parent structure (intact A- and B-ring).

Toxicological data

The oral LD_{50} was greater than 2000 mg/kg bw in female rats. The dermal LD_{50} was greater than 5000 mg/kg bw in rats, and the LC_{50} in an inhalation study in rats was greater than 2.65 mg/L. Cyflumetofen was not irritating to the skin of rabbits and was slightly irritating to the eyes of rabbits. Skin sensitization was observed in a maximization assay in guinea-pigs.

In repeated-dose toxicity studies in mice, rats or dogs, the main effects were on the adrenals (vacuolation and hypertrophy of cortical cells) and liver (e.g. hepatocellular hypertrophy). Leydig cell adenomas occurred at the highest doses in the rat carcinogenicity study.

In a 4-week mouse feeding study with dietary concentrations of 0, 100, 500, 1000 and 5000 ppm (equal to 0, 13.1, 67.2, 135 and 663 mg/kg bw per day for males and 0, 14.5, 74.9, 150 and 763 mg/kg bw per day for females, respectively), absolute and relative adrenal weights were increased at 5000 ppm. Histopathology showed an increased incidence of diffuse vacuolation and hypertrophy of adrenocortical cells at 5000 ppm. The NOAEL was 1000 ppm (equal to 135 mg/kg bw per day), based on increased adrenal weights and histopathological changes in the adrenals at 5000 ppm (equal to 663 mg/kg bw per day).

In a 13-week mouse feeding study with dietary concentrations of 0, 300, 1000, 3000 and 10 000 ppm (equal to 0, 35.4, 117, 348 and 1200 mg/kg bw per day for males and 0, 45.0, 150, 447 and 1509 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 117 mg/kg bw per day), based on histopathological changes in adrenocortical cells (e.g. increased incidence of diffuse vacuolation in females and of diffuse hypertrophy in males) at 3000 ppm (equal to 348 mg/kg bw per day).

In a 2-week rat feeding study with dietary concentrations of 0, 1000 and 10 000 ppm (equal to 0, 101 and 981 mg/kg bw per day for males and 0, 105 and 1000 mg/kg bw per day for females, respectively), the LOAEL was 1000 ppm (equal to 101 mg/kg bw per day), the lowest dose tested, based on changes in clinical chemistry, an increase in absolute and/or relative weight of liver and adrenals, hypertrophy of the adrenals in females and histopathological alterations, such as diffuse vacuolation of adrenocortical cells, at all doses. Moreover, all females showed vacuolation of interstitial cells in the ovary; vacuolation of corpora lutea was additionally observed at 10 000 ppm.

In a 4-week rat feeding study with dietary concentrations of 0, 100, 500, 1000 and 5000 ppm (equal to 0, 7.5, 37.6, 75.1 and 384 mg/kg bw per day for males and 0, 8.05, 40.8, 79.8 and 409 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 37.6 mg/kg bw per day), based on clinical chemistry changes (decreases in total cholesterol and triglycerides) and increases in organ weights (liver and adrenal) at 1000 ppm (equal to 75.1 mg/kg bw per day). In addition, histopathological effects, such as diffuse hypertrophy of hepatocytes (in all males at 1000 ppm and in both sexes at 5000 ppm), diffuse vacuolation of adrenocortical cells in both sexes accompanied by diffuse hypertrophy in all females and an increased incidence of vacuolation of interstitial cells in the ovaries, were observed at 1000 ppm and above. The results of lipid staining performed on the adrenals of both sexes and ovaries of females indicated that the vacuolation was due to the presence of lipid.

In a 4-week (26 days) rat feeding study with dietary concentrations of 0, 500, 1500, 4000 and 12 000 ppm (equal to 0, 43, 128, 339 and 1028 mg/kg bw per day for males and 0, 46, 132, 351 and 1039 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 43 mg/kg bw per day), based on increased adrenal weight in females and elevated incidences of adrenocortical cell vacuolation in both sexes at 1500 ppm (equal to 128 mg/kg bw per day).

In a 13-week rat feeding study with dietary concentrations of 0, 100, 300, 1000 and 3000 ppm (equal to 0, 5.4, 16.5, 54.5 and 167 mg/kg bw per day for males and 0, 6.28, 19.0, 62.8 and 193 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 16.5 mg/kg bw per day), based on increased adrenal weights and diffuse hypertrophy of adrenocortical cells in females, mild to moderate diffuse vacuolation of adrenocortical cells in males and vacuolation of ovarian interstitial cells at 1000 ppm (equal to 54.5 mg/kg bw per day).

In a 4-week dog study, cyflumetofen was administered in gelatine capsules to dogs at a dose of 0, 100, 300 or 1000 mg/kg bw per day. The NOAEL was 100 mg/kg bw per day, based on increases in adrenal weights in both sexes and increased incidences of fine vacuoles in adrenocortical cells (predominantly in the zona fasciculata and zona reticularis) at 300 mg/kg bw per day. Dark red foci (designated as capillary dilatation) on the right atrioventricular valve of the heart were observed in one female at 1000 mg/kg bw per day.

In a 13-week dog study, cyflumetofen was administered in gelatine capsules to dogs at a dose of 0, 30, 300 or 1000 mg/kg bw per day. The NOAEL was 300 mg/kg bw per day, based on a reduction in body weight gain (from day 1 to day 90), elevated absolute and relative adrenal weights in males, increased absolute and relative pituitary weights in females and an increase in absolute and relative testis weights at 1000 mg/kg bw per day. In addition, dark red foci (designated as capillary dilatation) on the right atrioventricular valve of the heart and dark red foci in the mucosa of the urinary bladder in one male and increased incidences of vacuolation of adrenocortical cells in females and males were observed at 1000 mg/kg bw per day.

In a 52-week dog study, cyflumetofen was administered in gelatine capsules at a dose of 0, 30, 300 or 1000 mg/kg bw per day. The NOAEL was 30 mg/kg bw per day, based on increased incidences of vacuolation in the adrenal cortex in both sexes, accompanied by degenerative processes (e.g. interstitial fibrosis and infiltration of brown pigment–laden macrophages) at 300 mg/kg bw per day.

In two 18-month feeding studies in mice, dietary concentrations were 0, 150, 500, 1500 and 5000 ppm (equal to 0, 15.5, 54.3, 156 and 537 mg/kg bw per day for males and 0, 14.3, 48.1, 144 and 483 mg/kg bw per day for females, respectively) in the first study and 0 and 10 000 ppm (equal to 0 and 1143 or 1132 mg/kg bw per day for males and females, respectively) in the second study. The overall NOAEL was 1500 ppm (equal to 144 mg/kg bw per day), based on increased adrenal weight (predominantly in females) and an increase in the incidence of diffuse vacuolation of adrenocortical cells in both sexes at 5000 ppm (equal to 483 mg/kg bw per day). There was no increased incidence of tumours in these studies.

In two 52-week feeding studies in rats, dietary concentrations were 0, 50, 150, 500 and 1500 ppm (equal to 0, 1.9, 5.6, 18.8 and 56.8 mg/kg bw per day for males and 0, 2.3, 6.9, 23.3 and 69.2 mg/kg bw per day for females, respectively) in the first study and 0 and 6000 ppm (equal to 0 and 250 or 319 mg/kg bw per day for males and females, respectively) in the second study. The overall NOAEL for systemic toxicity was 500 ppm (equal to 18.8 mg/kg bw per day), based on a reduction in total cholesterol and triglyceride concentrations in both sexes at several time points, increased liver weight (most pronounced early during the study period) in both sexes and increased adrenal weight in females at 1500 ppm (equal to 56.8 mg/kg bw per day). In addition, histopathology revealed an increased incidence of diffuse vacuolation of adrenocortical cells in males and an increased incidence of diffuse hypertrophy of adrenocortical cells in females at 1500 ppm. Moreover, vacuolation of interstitial gland cells in the ovaries was observed at 1500 ppm and above. In the second study, statistically significant increases in the incidence of hyperplasia of Leydig cells (19/20 versus 6/20 in controls) were observed at 6000 ppm at study termination.

In two 104-week studies in Fischer rats, dietary concentrations were 0, 150, 500 and 1500 ppm (equal to 0, 4.92, 16.5 and 49.5 mg/kg bw per day for males and 0, 6.14, 20.3 and 61.9 mg/kg bw per day for females, respectively) in the first study and 0 and 6000 ppm (equal to 0 and 220 or 287 mg/kg bw per day for males and females, respectively) in the second study. The overall NOAEL for systemic toxicity was 500 ppm (equal to 16.5 mg/kg bw per day), based on an increase in adrenal weights in both sexes, an increase in the incidence of epididymis atrophy and an increased incidence of diffuse hypertrophy and/or vacuolation of adrenocortical cells in both sexes at 1500 ppm (equal to 49.5 mg/kg bw per day). The overall NOAEL for carcinogenicity was 1500 ppm (equal to 49.5 mg/kg bw per day), the highest dose tested in the first study, based on a statistically significantly increased incidence of Leydig cell adenoma at 6000 ppm (equal to 220 mg/kg bw per day) in the second study, which was higher than the historical control incidence range. The increase in the incidence of Leydig

cell adenoma was associated with a statistically significant increase in mass of the testis, with an increase in absolute and relative testis weights at 6000 ppm. There was a slight, non-significant increase in the incidence of C-cell adenomas and adenocarcinomas of the thyroid in male rats. As these tumours are common in rats and there were no accompanying preneoplastic lesions, it was concluded that their occurrence was incidental.

The Meeting concluded that cyflumetofen is carcinogenic in male rats but not in female rats or male or female mice.

Cyflumetofen was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. A mouse lymphoma gene mutation assay was positive with and without liver enzyme activation at concentrations close to those at which precipitation occurred. Cyflumetofen was not genotoxic in an Ames test or in an in vitro chromosomal aberration assay. There was no evidence of genotoxicity in an in vivo micronucleus assay or in an in vivo unscheduled DNA synthesis assay in rat liver.

The Meeting concluded that cyflumetofen is unlikely to be genotoxic in vivo.

Five in vitro mechanistic studies were performed to identify a possible mode of action for the Leydig cell adenomas via perturbation of the estrogen or androgen system. Cyflumetofen was not a significant aromatase inhibitor in a human recombinant cell system, did not significantly interact with androgen or estrogen receptor binding using rat prostate or uterine cytosol protein preparations, respectively, and was not an agonist in a human estrogen receptor transcriptional activation system. In a steroidogenesis assay, cyflumetofen induced estrogen production at and above 5 µmol/L (maximally 1.64-fold) and inhibited testosterone production at and above 1 µmol/L (maximally 0.63-fold) in human adrenocarcinoma cells. Fischer rats are very sensitive to decreased testosterone levels, which lead to a compensatory increase in luteinizing hormone production and subsequent Leydig cell proliferation and Leydig cell adenoma progression. It cannot be excluded that this mode of action is relevant to humans. However, as an increased incidence of Leydig cell adenomas was observed only at high doses in rats, cyflumetofen can be considered as not likely to be carcinogenic to humans at levels occurring in the diet.

In view of the lack of genotoxicity, the absence of carcinogenicity in mice and the fact that only Leydig cell adenomas were observed in a particularly sensitive strain of rat at the highest dose tested, the Meeting concluded that cyflumetofen is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation study of reproductive toxicity in rats at dietary concentrations of 0, 150, 500 and 1500 ppm (equal to 0, 10.4, 34.6 and 100.3 mg/kg bw per day for males and 0, 12, 39.7 and 121.6 mg/kg bw per day for females, respectively), the NOAEL for parental toxicity was 150 ppm (equal to 10.4 mg/kg bw per day), based on increased incidences of white/enlarged adrenals and increased adrenal weights in females and an elevated incidence of hypertrophy of adrenocortical cells in both sexes at 500 ppm (equal to 34.6 mg/kg bw per day). In addition, delayed vaginal opening and decreased follicle stimulating hormone and progesterone concentrations in serum were noted in females at 500 ppm. In offspring, the NOAEL was also 150 ppm (equal to 10.4 mg/kg bw per day), based on increased adrenal weights in both sexes and increased incidences of hypertrophy of adrenocortical cells in males at 500 ppm (equal to 34.6 mg/kg bw per day). The NOAEL for reproductive toxicity was 1500 ppm (equal to 100.3 mg/kg bw per day), the highest dose tested.

In a range-finding study on the developmental toxicity of cyflumetofen in rats administered 0, 100, 500 or 1000 mg/kg bw per day, no treatment-related developmental or maternal toxicity was observed. In the main study on the developmental toxicity of cyflumetofen in rats at dose levels of 0, 50, 250 and 1000 mg/kg bw per day, the NOAEL for maternal toxicity was 50 mg/kg bw per day, based on increased adrenal weights accompanied by an increased incidence of vacuolation of adrenocortical cells at 250 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 50 mg/kg bw per day, based on an increased incidence of incompletely ossified sternal centra at 250 mg/kg bw per day.

In a range-finding study on the developmental toxicity of cyflumetofen in rabbits at dose levels of 0, 10, 100, 500 and 1000 mg/kg bw per day, no treatment-related developmental or maternal toxicity was observed. In the main study on the developmental toxicity of cyflumetofen in rabbits at dose levels of 0, 50, 250 and 1000 mg/kg bw per day, the NOAEL for maternal toxicity was 50 mg/kg bw per day, based on slight body weight loss at gestation days 6–9 and decreased body weight gain relative to controls during the entire treatment period, which partly correlated with a decrease in feed consumption at 250 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was also 50 mg/kg bw per day, based on changes in the number of ossification sites in the vertebrae, ribs and sternum at 250 mg/kg bw per day. An increased incidence of total variations (per fetus and per litter) was noted at 1000 mg/kg bw per day. Skeletal variations were confined to increased fetal and litter incidences of incompletely ossified sterna centra (above the historical control range of the laboratory) and an increase in fetal and litter incidences of angulated hyoid alae at 1000 mg/kg bw per day.

The Meeting concluded that cyflumetofen is not teratogenic.

In an acute neurotoxicity study in rats administered cyflumetofen at a dose of 0, 125, 500 or 2000 mg/kg bw, the NOAEL for acute toxicity and neurotoxicity was 2000 mg/kg bw, the highest dose tested.

In a 13-week neurotoxicity study in rats administered cyflumetofen in the diet at a concentration of 0, 500, 1500 or 5000 ppm (equal to 0, 30, 89 and 293 mg/kg bw per day for males and 0, 41, 99 and 353 mg/kg bw per day for females, respectively), the NOAEL for systemic toxicity was 500 ppm (equal to 30 mg/kg bw per day), based on an increase in adrenal weights and an increased incidence and higher degree of severity of diffuse vacuolation of the adrenocortical cells (predominantly in the zona fasciculata) in both sexes at 1500 ppm (equal to 89 mg/kg bw per day). The NOAEL for subchronic neurotoxicity was 5000 ppm (equal to 293 mg/kg bw per day), the highest dose tested.

The Meeting concluded that cyflumetofen is not neurotoxic.

In a 4-week mouse immunotoxicity study with dietary concentrations of 0, 500, 1500 and 5000 ppm (equal to 0, 33, 107 and 349 mg/kg bw per day, respectively), the NOAEL for immunotoxicity was 5000 ppm (equal to 349 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity was 500 ppm (equal to 33 mg/kg bw per day), based on an increase in adrenal weights correlating with discoloration and enlargement of the adrenals and microscopic changes such as vacuolation of adrenocortical cells at 1500 ppm (equal to 107 mg/kg bw per day).

The Meeting concluded that cyflumetofen is not immunotoxic.

In a mechanistic study to further elucidate the effects on adrenal gland and ovary, male and female rats were fed diets containing 0, 100 or 5000 ppm (equal to 0, 7.44 and 378 mg/kg bw per day for males and 0, 7.59 and 347 mg/kg bw per day for females, respectively) for up to 28 days. Cyflumetofen caused enlarged and discoloured adrenals with increased weights. An increase in total cholesterol concentration, predominantly cholesterylesters, in adrenals correlated with vacuolation by lipid deposition within the adrenocortical and ovarian interstitial cells at 5000 ppm in both sexes. Furthermore, a slight inhibitory effect on hormone-sensitive lipase expression, which is involved in cholesterol catabolism and therefore might result in lipid deposition, was noted at 5000 ppm in both sexes. In addition, CYP11A1 (cholesterol side-chain cleavage enzyme) expression was slightly enhanced in both sexes at 5000 ppm, probably due to the elevated supply of lipids. No effects were seen on adrenocorticotrophic hormone or corticosterone levels in serum. In conclusion, catabolism of lipids might be inhibited at cyflumetofen doses above 7.44 mg/kg bw per day, leading to the formation of lipid droplets in adrenals and ovarian cells.

In a safety pharmacology study, respiratory rate, blood pressure and heart rate (including electrocardiogram) were measured in male dogs after a single-dose application of 0 or 2000 mg/kg bw. Cyflumetofen did not cause any treatment-related adverse effects on the respiratory or cardiovascular system in dogs under the conditions of the study.

Toxicological data on metabolites and/or degradates

Acute toxicity and genotoxicity studies were performed for B-1, a goat and plant metabolite and food processing hydrolysis product. B-1 is also a major metabolite in the rat (occurring at up to 28% of the applied dose).

B-1 was of low acute oral toxicity (LD50 > 2000 mg/kg bw per day).

The potential genotoxicity of B-1 was tested in an adequate range of in vitro and in vivo assays. A mouse lymphoma gene mutation assay was positive at 1000 µg/mL without liver enzyme activation and in one of two experiments at 333 µg/mL with enzyme activation. B-1 was not mutagenic in an Ames test and was not genotoxic in an in vitro chromosomal aberration assay. There was no evidence of genotoxicity in an in vivo unscheduled DNA synthesis assay in rat liver.

The Meeting concluded that metabolite B-1 is unlikely to be genotoxic in vivo.

B-3, a soil metabolite, and AB-13, an impurity, have not been identified as food residues and are therefore not relevant for dietary risk assessment.

For AB-1, a goat metabolite and food processing hydrolysis product, no toxicological data were provided. AB-1 is an intermediate in the metabolism of rats and occurs at less than 1% of the applied dose in bile. It is further transformed to AB-3 and AB-2. In sum, AB-1, AB-2 and AB-3 and their glucuronidated derivatives account for greater than 20% of the applied dose in rats. As AB-1 is structurally similar to the parent and is transformed to metabolites, which represent a large portion of the metabolism in rats, its toxicity can therefore be considered to be covered by that of the parent.

Human data

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

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

Toxicological evaluation

The Meeting established an ADI of 0–0.1 mg/kg bw per day on the basis of a NOAEL of 10.4 mg/kg bw per day in the two-generation rat feeding study, based on parental and offspring toxicity at 34.6 mg/kg bw per day. A safety factor of 100 was applied. The margin between the upper bound of the ADI and the LOAEL of 220 mg/kg bw per day for Leydig cell adenomas in rats is 2200.

The Meeting concluded that it was not necessary to establish an ARfD for cyflumetofen 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 cyflumetofen

Species	Study	Effect	NOAEL	LOAEL
Mice	Eighteen-month studies of toxicity and carcinogenicity[a,b]	Toxicity	1 500 ppm, equal to 144 mg/kg bw per day	5 000 ppm, equal to 483 mg/kg bw per day
		Carcinogenicity	5 000 ppm, equal to 483 mg/kg bw per day[c]	–
Rat	Ninety-day study of toxicity[a]	Toxicity	300 ppm, equal to 16.5 mg/kg bw per day	1 000 ppm, equal to 54.5 mg/kg bw per

Species	Study	Effect	NOAEL	LOAEL
				day
	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	500 ppm, equal to 16.5 mg/kg bw per day	1 500 ppm, equal to 49.5 mg/kg bw per day
		Carcinogenicity	1 500 ppm, equal to 49.5 mg/kg bw per day	6 000 ppm, equal to 220 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	1 500 ppm, equal to 100.3 mg/kg bw per day[c]	–
		Parental toxicity	150 ppm, equal to 10.4 mg/kg bw per day	500 ppm, equal to 34.6 mg/kg bw per day
		Offspring toxicity	150 ppm, equal to 10.4 mg/kg bw per day	500 ppm, equal to 34.6 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
		Embryo/fetal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
		Embryo/fetal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
Dog	One-year study of toxicity[e]	Toxicity	30 mg/kg bw per day	300 mg/kg bw per day

[a] Dietary administration.
[b] Two studies combined.
[c] Highest dose tested.
[d] Gavage application.
[e] Gelatine capsules.

Estimate of acceptable daily intake (ADI)

0–0.1 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 exposure

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 68% at low dose (3 mg/kg bw), 35% at high dose (250 mg/kg bw)

Dermal absorption	20% at low concentration (0.2 g/L), 27% at high concentration (200 g/L)
Distribution	Widely distributed, highest levels in liver, followed by kidney and bone marrow
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	≥ 95% within 72 hours, mainly in urine, partly in bile
Metabolism in animals	Extensive, primarily cleavage between tolyl and phenyl moieties, hydroxylation and conjugation
Toxicologically significant compounds in animals and plants	Cyflumetofen, metabolite B-1 (goat, rat, plant and food processing hydrolysis product) and AB-1 (goat, rat and food processing hydrolysis product)
Acute toxicity	
Rat, LD_{50}, oral	> 2 000 mg/kg bw per day
Rat, LD_{50}, dermal	> 5 000 mg/kg bw per day
Rat, LC_{50}, inhalation	> 2.65 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slightly irritating
Guinea pigs, dermal sensitization	Sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Adrenals: weight and histopathological changes (rat, mouse, dog)
Lowest relevant oral NOAEL	16.5 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, highest dose tested
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Adrenals: weight and histopathological changes; testis: Leydig cell adenoma at highest dose (rats)
Lowest relevant NOAEL	16.5 mg/kg bw per day (rat)
Carcinogenicity	Unlikely to pose a carcinogenic risk from the diet
Genotoxicity	
	Unlikely to be genotoxic
Reproductive toxicity	
Target/critical effect	No reproductive toxicity
Lowest relevant parental NOAEL	10.4 mg/kg bw per day
Lowest relevant offspring NOAEL	10.4 mg/kg bw per day
Lowest relevant reproductive NOAEL	100.3 mg/kg bw per day, highest dose tested
Developmental toxicity	
Target/critical effect	Skeletal variations
Lowest relevant maternal NOAEL	50 mg/kg bw per day (rat and rabbit)
Lowest relevant embryo/fetal NOAEL	50 mg/kg bw per day (rat and rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw per day, highest dose tested
Subchronic neurotoxicity NOAEL	293 mg/kg bw per day, higheset dose tested
Developmental neurotoxicity NOAEL	No data

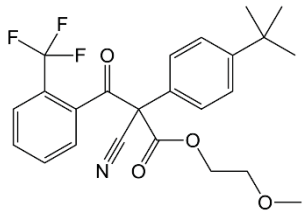
Other toxicological studies	
Mechanistic studies	Inhibition of cholesterol catabolism possibly due to a decrease in hormone-sensitive lipase expression in the adrenals of rats
	No significant effect on respiratory/cardiovascular systems in dogs
	No significant effect in vitro on the aromatase and estrogen/adrenal receptor system
	Induction of 17β-estradiol synthesis and inhibition of testosterone synthesis in an in vitro steroidogenesis assay
	Possible mode of action of Leydig cell adenoma: Testosterone level reduction and subsequent compensatory processes
Immunotoxicity NOAEL	349 mg/kg bw per day, highest dose tested
Studies on metabolites	B-1:
	LD_{50}: > 2 000 mg/kg bw
	Unlikely to be genotoxic in vivo
	Studies on B-3 and AB-13 were submitted, but these compounds are not relevant to a dietary risk assessment.
Medical reports	
	Three medical reports: No abnormal findings

Summary

	Value	Study	Safety factor
ADI	0–0.1 mg/kg bw	Two-generation reproductive toxicity study (rat)	100
ARfD	Unnecessary	–	–

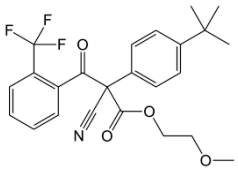
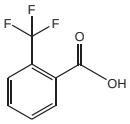
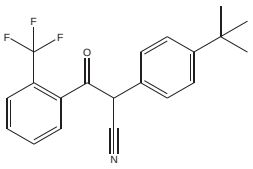
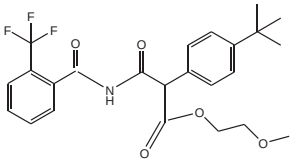
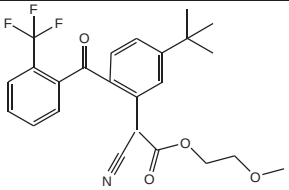
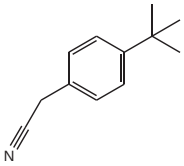
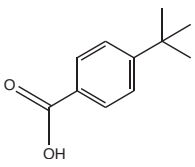
RESIDUE AND ANALYTICAL ASPECTS

Cyflumetofen (consisting of RS isomers) is a bridged diphenyl acaricide (miticide) for control of *Tetranychus* sp. and influences the mitochondrial electron transport chain by inhibiting the complex II substance in the cell. It has been registered in a number of countries.



The Meeting received information on physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, supervised trials, and processing. Cyflumetofen was scheduled by the Forty-fifth Session of the CCPR for review by the 2014 JMPR for the first time.

In this Appraisal, the following abbreviated names were used for referred metabolites.

Code (MW)	IUPAC name	Structure
Cyflumetofen (447.45)	2-methoxyethyl (*RS*)-2-(4-*tert*-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-*o*-tolyl)propionate	
B-1 (190.12)	2-(trifluoromethyl)benzoic acid	
AB-1 (345.36) Syn: M9210I003	(RS)-2-(4-tert-butylphenyl) -3-oxo-3-[2-(trifluoromethyl)phenyl] propanenitrile	
AB-6 (465.45)	2-methoxyethyl-2-((*R*,*S*)-4-*tert*-butylphenyl)-3-oxo-3-({[2-(trifluoromethyl)phenyl]carbonyl}amino)propanoate	
AB-7 (447.45)	2-methoxyethyl-((R,S)-4-tert-butyl-2-{[2-(trifluoromethyl)phenyl]carbonyl}phenyl (cyano)acetate	
A-2 (173.26) Syn: M9210I001	4-tert-butylphenyl-acetonitrile	
A-12 (178.23) Syn: M9210I002	4-tert-butylbenzoic acid	

Animal metabolism

The Meeting received information on the fate of orally-dosed cyflumetofen in lactating goats.

In metabolism studies, total radioactive residues are expressed in mg eq./kg cyflumetofen equivalents unless otherwise stated.

Metabolism of cyflumetofen in rat

Metabolism studies on laboratory animals including rats were reviewed in the framework of toxicological evaluation by the current JMPR.

In a rat metabolism study, highest residues were found in the liver followed by kidney regardless of sex, dose and label position and time point of measurement. Cyflumetofen was extensively metabolized. B-1, 2-trifluoromethylbenzoic acid, was the major metabolite (occurring up to 28% of the applied dose). The predominant metabolic pathway for cyflumetofen involves cleavage of the tert-butylphenyl and trifluorotolyl moieties. Major reactions on the tert-butylphenyl ring are cleavage of the methoxyethyl group, hydroxylation at the butyl group, decarboxylation and glucuronidation at the butyl group. Major reactions on the trifluorotolyl-ring are glutathione conjugation at the carboxyl group and further changes of the glutathione group to mercapturic acid or thiolactic acid. In addition, hydroxylation and oxidation reactions at the butyl group and cleavage of the carboxylic ester moiety are observed on the parent molecule.

Metabolism of cyflumetofen in lactating goat

Two lactating goats were orally administered [benzoyl-ring-U-^{14}C]-cyflumetofen (hereafter abbreviated as benzoyl-label) for 12 consecutive days at 0.27 or 0.30 mg/kg bw (12 or 15 ppm in feed). Other two goats were orally administered [t-butylphenyl-ring-U-^{14}C]-cyflumetofen (hereafter abbreviated as butylphenyl-label) for 10 consecutive days at 0.43 or 0.48 mg/kg bw (12 or 13 ppm in feed). The goats were sacrificed 18–24 hours after the last dose.

After radio-labelled cyflumetofen was administered orally to lactating goats, it was excreted in faeces and urine (total > 78% excreted during the testing periods) and only a small portion accounting for < 0.3% of the administered radioactivity (AR) remained in body tissues/organs. Radioactive residues were the highest in liver (0.29–0.40 mg eq./kg) followed by kidney (0.17–0.19 mg eq./kg) but low in fat (0.028–0.033 mg eq./kg) and muscle (0.009–0.020 mg eq./kg). Milk of each day contained < 0.02% AR and milk collected throughout the study period contained, in total, 0.008–0.19 mg eq./kg (0.03–0.14% AR).

The parent compound, cyflumetofen, was found only in fat but at low concentration of 0.003 mg/kg accounting for 20–21% of the total radioactive residues (TRR). The predominant metabolite in all tissues/organs and milk was B-1 at around 0.1 mg eq./kg in liver (32% TRR) and kidney (54% TRR), and lower than 0.01 mg eq./kg in muscle (46–51% TRR), fat (21–40% TRR) and milk (4.5% TRR).

The metabolism of cyflumetofen in goat involves extensive hydrolysis of formic acid ester and trifluoromethylbenzoyl moiety, decarboxylation, conjugation, hydroxylation and oxidation. In principle, excretion and distribution of cyflumetofen and its metabolism in goat are similar to those in rats with some differences in metabolites identified.

Plant metabolism

Satsuma mandarin

When Satsuma mandarin trees (grown outdoor) were sprayed with benzoyl- or butylphenyl-label cyflumetofen at a rate approximating 0.60 kg ai/ha (3× GAP rate of the USA), and Satsuma mandarin fruit samples were collected 1, 7 and 30 days after the treatment, the majority of the radioactivity was

recovered from the acetonitrile surface rinse of fruits: 95–96% TRR on 1DAT; 91–93% TRR on 7DAT; and 88–89% on 30 DAT.

The predominant radioactive residue on/in mandarin fruits (total) was cyflumetofen at 0.52–0.55 mg/kg (88-90% TRR) on 1DAT, decreasing to 0.33–0.37 mg/kg (79–83% TRR) on 7 DAT and further to 0.25–0.31 mg/kg (44–54% TRR) on 30 DAT.

Other than the parent, metabolites AB-6, AB-7, A-12 and B-1 were formed. B-1 increased over time from 0.028 mg eq./kg (4.7% TRR) on 1 DAT to 0.064 mg eq./kg (11% TRR) on 30 DAT. None of AB-6, AB-7 and A-12 exceeded 10% TRR. A number of unknown peaks were observed but none of them exceeded 10% of TRR.

The radioactive residues on/in leaves showed similar profile as those on/in fruits with the majority (>87% TRR) of radioactive residues found in the surface rinse, although the TRR was >50 times that on/in fruits. A majority of radioactive residues remaining in leaves were extracted. Cyflumetofen was also the predominant identified fraction in leaves: > 73% TRR in rinse and extract together. Four metabolites were identified but none exceeded 10% TRR.

Apple

When an apple tree (grown outdoor) was sprayed with benzoyl- or butylphenyl-label cyflumetofen at a rate approximating 0.60 kg ai/ha (3× GAP rate of the USA), and apple fruit samples were collected 1, 7 and 30 days after the treatment, the majority of the radioactivity was recovered from the acetonitrile surface rinse of fruits: 95–96% TRR on 1 DAT, 82–89% TRR on 7 DAT; and 67–71% TRR on 30 DAT.

The predominant radioactive residue on/in apple fruits was cyflumetofen at 0.061–0.066 mg/kg (58–61% TRR) in rinse while pulp extract contained too little radioactivity for further analysis on 1DAT, 0.061–0.14 mg/kg (78–84% TRR) on 7 DAT and decreased to 0.037–0.042 mg/kg (53–65% TRR) on 30 DAT.

There were minor metabolites identified. However, none of them exceeded 5% TRR.

The radioactive residues on/in leaves showed similar profile as those on/in fruits with the majority (>72% TRR) of radioactive residues found in the surface rinse, although the TRR was > 50 times that on/in fruits. Cyflumetofen was also the predominant identified fraction in leaves: > 44% TRR in rinse and extract together. No metabolites exceeded 10% TRR.

Eggplant

When eggplants (grown outdoor) were sprayed with benzoyl- or butylphenyl-label cyflumetofen at a rate approximating 0.60 kg ai/ha (3× GAP rate of the USA), and eggplant samples were collected 1, 7 and 14 days after the treatment, the majority of the radioactivity was also recovered from the acetonitrile surface rinse of fruits: 87–92% TRR on 1 DAT, 79–86% TRR on 7 DAT; and 56–81% TRR on 30 DAT.

The predominant radioactive residue on/in eggplant fruits was also cyflumetofen accounting for 0.31–0.44 mg/kg (91–95% TRR) on 1 DAT, decreased to 0.25–0.39 mg/kg (67–71% TRR) on 7 DAT and then to 0.18–0.20 mg/kg (42–62% TRR) on 14 DAT.

Metabolites B-1, AB-6 and AB-7 were identified from the fruit rinse and/or extracts. B-1 was found at 0.059 mg eq./kg (11% TRR) on 7 DAT and at 0.061 mg eq./kg (15% TRR) on 14 DAT on/in fruits. AB-6 and AB-7 did not exceed 10% TRR.

The tentatively identified U1/U2 (likely to be acid labile conjugates of B-1) and U4 were also found in 7 DAT and 14 DAT fruits but not in 1 DAT fruits. U1 was present at 0.067 mg eq./kg (16% TRR) on 14 DAT but < 10% TRR on 1 DAT and 7 DAT. No other metabolites exceeded 10% TRR.

U1 and U2 were found only in the fruit extracts but not in rinse, indicating that they were formed in the fruits.

The radioactive residues on/in leaves showed similar profile as those on/in fruits with the majority (> 69% TRR) of radioactive residues found in the surface rinse, although the TRR was > 50 times that on/in fruits. Cyflumetofen was also the predominant identified fraction in leaves: > 47% TRR in rinse and extract together. No metabolites exceeded 10% TRR.

Summary of plant metabolism

The metabolism of cyflumetofen in these crops involves hydrolysis, acyl migration, oxidation and conjugation (U1 and U2 are the conjugate of B-1).

The plant metabolism studies on Satsuma mandarin, apple and eggplant showed similar pattern. Applied radioactively was mostly recovered from surface rinse with decreasing trend over time. Fruit flesh contained far less radioactivity. The predominant radioactive residue was cyflumetofen accounting for > 42% TRR at all time points. The next important radioactive residue was B-1 but at the maximum 15% TRR (around 0.06 mg eq./kg). B-1 may be present in fruits in conjugated forms.

Environmental fate

Aerobic soil metabolism

The studies on aerobic soil degradation of cyflumetofen indicate that cyflumetofen sprayed on soil was rapidly and completely degraded with DT_{50} around 1.8–4.3 days in various soils at 20–25 °C. Additionally, aerobic soil degradation of B-1 and AB-1 was studied resulting in DT50 of 6–36 days and 0.07–0.11 days respectively at 20 °C.

Photolysis on soil surface

Photolysis of cyflumetofen on soil was found to be insignificant as the degradation rates of cyflumetofen with and without light irradiation were not significantly different.

Hydrolysis in aquatic system

Cyflumetofen is susceptible to hydrolysis and was hydrolyzed faster at higher pH in aqueous buffer solutions at 25 °C. DT_{50} was calculated using first order kinetics to be 7.7 days at pH 4, 6.0 days at pH 5, 9.8 h at pH 7 and 10.3 min at pH 9.

At slightly acidic to neutral pH, a number of degradates exceeded 10% of the applied dose: A-1 (max. 14% at 8 h), A-2 (max. 44% at 720 h), A-18 (max. 36% at 120 h), B-1 (max. 53% at 48 h) and AB-1 (maximum 45%, at 120 h).

Residues in succeeding crops

A confined rotational crop study was conducted to examine the nature and level of residues of cyflumetofen in three succeeding crops (white radish, lettuce and wheat) using benzoyl- and butylphenyl-label cyflumetofen. A single application of radio-labeled cyflumetofen was made on bare soil in plastic containers at a rate of 0.40 kg ai/ha (2× GAP rate of the USA). After plant back interval (PBI) of 30, 120 and 365 days, lettuce, white radish and spring wheat were sown into the treated soil.

Following the application of cyflumetofen to soil, uptake of radioactivity from soil into rotational crops was observed, in particular, with benzoyl-label (up to 1.25 mg eq./kg in wheat chaff of 30 PBI but much lower in edible portions with the maximum of 0.17 mg eq./kg in wheat grain of 30 PBI). With butylphenyl-label, uptake of radioactivity into rotational crops were lower (maximum

of 0.11 mg eq./kg in wheat straw+chaff of 120 PBI). Uptake was, in general, less with longer plant back interval.

The only major radioactive residue identified from benzoyl-label treatment was trifluoroacetic acid in all crops (39–100% TRR). B-1, specific to this label, was detected as a minor component.

With the butylphenyl-label, a series of label-specific metabolites were observed in the extracts but all of them were less than 0.01 mg eq./kg.

No parent compound was detected from any of the crop extracts.

No significant residues of cyflumetofen, other than trifluoroacetic acid, were expected to be found in rotational crops on the basis of aerobic soil degradation studies and confined succeeding crop study.

Methods of analysis

Analytical methods for determination of residues of cyflumetofen and its metabolites B-1, AB-6 and AB-7 were developed for a wide range of matrices of plant and animal origin.

In general, the method for data generation and enforcement for plant matrices employ extraction by shaking with acetonitrile and then a mixture of acetonitrile:water (75:25, v/v) or only with acetonitrile:water, cleanup by partitioning with a mixture of ethyl acetate:cyclohexane (75:25, v/v) and determination of the analytes using LC-MS/MS.

The method for plant matrices was validated for cyflumetofen, B-1, AB-6 and AB-7 resulting in acceptable recoveries and relative standard deviations (RSDs) with the LOQ of 0.01 mg/kg for various plant matrices.

The analytical method developed for cyflumetofen and B-1 in animal matrices was similar to the one for plant matrices but employs acetonitrile:water (50:50, v/v) for extraction instead of acetonitrile:water (75:25, v/v) and different clean-up procedure. This method was validated for cyflumetofen and B-1 resulting in acceptable recoveries and RSDs with the LOQ of 0.01 mg/kg for bovine liver, and meat and 0.001 mg/kg for bovine milk. The mean recoveries for cyflumetofen in poultry eggs were low at 65–67% (RSD, 3–6%) while those for B-1 were acceptable between 86–95%.

A number of scientific papers report the validation of the QuEChERS multi-residue method with GC-MS/MS for cyflumetofen in various plant commodities with the LOQ around 0.01 mg/kg.

Stability of pesticide residues in stored analytical samples

The stability of cyflumetofen, B-1, AB-6 and AB-7 in homogenates of almond, apple (fruit, juice) kidney bean, lettuce, orange (fruit, juice, oil), radish and wheat grain at -20 to -10 °C was tested at a spike level (each analyte separately spiked) of 0.1 mg/kg for 743–910 days (24–30 months).

Cyflumetofen was stable when stored frozen for 25 months, the longest storage time tested, in almond, apple fruit, apple juice, orange fruit (24 months), orange juice, orange oil and wheat grain. However, it was stable only up to 9 months in kidney bean and lettuce, and 3 months in radish root.

B-1 was stable when stored frozen for 30 months, the longest storage time tested, in almond, kidney bean, lettuce, orange fruit, orange juice and wheat grain. It was stable up to 22 months in apple fruit, apple juice and radish root, and 6 months in orange oil.

AB-6 was stable when stored frozen for 28 months, the longest storage time tested, in almond, apple fruit, kidney bean, orange juice, orange oil, radish root, and wheat grain. It was stable up to 21 months in apple juice, lettuce and orange fruit.

AB-7 was stable when stored frozen for 26 months, the longest storage time tested, in almond, apple fruit, apple juice, kidney bean, orange juice, and orange oil. It is stable up to 19 months in orange fruit and wheat grain, 12 months in lettuce, but one month in radish root.

Definition of the residue

In goat metabolism studies, radioactive residues were highest in liver followed by kidney. Far less radioactive residues were found in fat, muscle and milk.

When benzoyl-label cyflumetofen was administered, metabolite B-1 (2-trifluoromethylbenzoic acid) was the predominant residue in all edible tissues/organs (21–54% TRR) and milk (4.5% TRR). B-1 was also found in rat metabolism and is considered to be no more toxic than the parent and, therefore, toxicologically covered by the ADI for parent compound. Cyflumetofen was detected only in fat at 0.003 mg/kg (20% TRR). No other metabolites were detected above 10% TRR.

When butylphenyl-label cyflumetofen was administered, cyflumetofen was not detected in any of edible tissues/organs or in milk. None of all the identified metabolites exceeded 0.01 mg eq./kg and 10% TRR.

There is a validated LC-MS/MS method for cyflumetofen and B-1 in bovine matrices. The Meeting considered that both cyflumetofen and B-1 were suitable residues for enforcement of MRLs and for estimating dietary intake.

LogPow of cyflumetofen is 4.3 at 25 °C and cyflumetofen was found only in the fat at < 0.01 mg/kg in the goat metabolism study. B-1 is a carboxylic acid and was present at 0.13 mg eq./kg in liver and 0.10 mg eq./kg in kidney but < 0.01 mg eq./kg in fat. The Meeting considered that, over all, residues (cyflumetofen and B-1) were not fat soluble.

In the plant metabolism studies on Satsuma mandarin, apple and eggplant, cyflumetofen was the predominant residue and accounted for > 42% TRR in the fruits of these crops at any time points. The Meeting considered that for enforcement of MRLs, cyflumetofen was a suitable residue.

The next important radioactive residue was B-1 but at the maximum 15% TRR (around 0.06 mg eq./kg). In the supervised residue trials, the concentrations of B-1 were mostly 1/10–1/2 of those of cyflumetofen. Conjugated forms of B-1 (U1 and U2) were found in the eggplant metabolism at slightly higher concentrations (in total) than B-1 itself but they were not found in the studies on Satsuma mandarin or apple. A validated LC-MS/MS method is available and used in the trials for quantification of cyflumetofen and B-1 but it determines the free form of B-1.

The Meeting considered that, for calculating dietary intake from commodities of plant origin, cyflumetofen and B-1 were suitable residues.

Based on the above, the Meeting recommended the following residue definition for plant and animal commodities:

Definition of the residue for plant commodities (for compliance with the MRL): *Cyflumetofen.*

Definition of the residue for plant commodities (for estimation of dietary intake): *Sum of cyflumetofen and 2-trifluoromethylbenzoic acid, expressed as cyflumetofen.*

Definition of the residue for animal commodities (for compliance with the MRL and estimation of dietary intake): *Sum of cyflumetofen and 2-trifluoromethylbenzoic acid, expressed as cyflumetofen*.

Residue is not fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for cyflumetofen on citrus fruits, pome fruits, grapes, strawberry, tomato, eggplant and tree nuts conducted outdoor (except trials on eggplant in Japan) using foliar spray of 20% SC formulation of cyflumetofen (except in a trial on apple in Italy).

Residues were expressed in cyflumetofen equivalents. The analytical results for B-1 were converted to cyflumetofen equivalents by multiplying the analytical results for B-1 with a factor of 2.35 based on the molecular weights of cyflumetofen (447.45) and B-1 (190.12).

For the estimation of a sum of cyflumetofen and B-1, where B-1 was below the LOQ, it was regarded as 0.02 mg eq./kg (LOQ of 0.01 mg/kg for B-1 is converted to 0.02 mg eq./kg) as B-1 was sometimes present at concentrations comparable to those of parent in trials.

Although in a number of trials conducted in the USA the water volume was smaller than the minimum water volume on the label, the Meeting agreed to use the results of these trials in estimating maximum residue levels as the purpose of the minimum requirement was for better efficacy and not for safety, and residues from lower water volume trials were not always lower than those from higher water volume trials.

Citrus fruits

A total of 23 supervised trials were conducted on orange (12), grapefruit (6) and lemon (5) in the USA in 2009 and 2010. GAP in the USA for citrus fruits allows 2 applications at a maximum rate of 0.2 kg ai/ha (at least 935 L/ha) with a PHI of 7 days.

Residues of cyflumetofen from 11 independent orange trials matching GAP for citrus fruits in the USA were: 0.01, 0.01, 0.03, 0.06, 0.06, 0.08, 0.09, 0.10, 0.10, 0.11 and 0.12 mg/kg (median 0.08 mg/kg).

The information on four trials conducted on orange in Brazil in 2007 was also provided but they did not match the GAP in Brazil (one application at a spray concentration of 0.08 kg ai/hL).

Residues of cyflumetofen from six grapefruit trials matching GAP for citrus fruits in the USA were: < 0.01, 0.02, 0.04, 0.04, 0.04 and 0.07 mg/kg (median 0.04 mg/kg).

Residues of cyflumetofen from five lemon trials matching GAP for citrus fruits in the USA were: < 0.01, 0.02, 0.02, 0.08 and 0.14 mg/kg (median 0.02 mg/kg).

As the GAP in the USA is established for the group of citrus fruits, the median values from the trials conducted in the USA on these three commodities were not different more than 5-fold, and Kruskal-Wallis test indicated that the residues of orange, grapefruits and lemon were not statistically different, the Meeting decided to estimate a group maximum residue level. The residues of orange, grapefruits and lemon were combined for estimating a maximum residue level for estimating a maximum residue level for citrus fruits (n=22): < 0.01, < 0.01, 0.01, 0.01, 0.02, 0.02, 0.02, 0.03, 0.04, 0.04,0.04, 0.06, 0.06, 0.07, 0.08, 0.08, 0.09, 0.10, 0.10, 0.11, 0.12 and 0.14 mg/kg.

The Meeting estimated a maximum residue level of 0.3 mg/kg for citrus fruit.

For the estimation of STMR, the sum of cyflumetofen and B-1 was calculated: < 0.03, < 0.03, 0.03, 0.03, 0.04, 0.04, 0.04, 0.05, 0.06, 0.06, 0.06, 0.08, 0.08, 0.09, 0.10, 0.10, 0.11, 0.12, 0.12, 0.13, 0.14 and 0.16 and mg eq./kg.

The Meeting estimated an STMR of 0.07 mg/kg expressed as cyflumetofen.

Pome fruits

A total of 17 supervised trials were conducted in the USA in 2009 and 2010 on apples (12) and pear (5). One trial was conducted in Italy in 2006 on apples. The GAP in the USA for pome fruits allows 2 applications at a maximum rate of 0.2 kg ai/ha (at least 935 L/ha) with a PHI of 7 days.

The trial from Italy was provided but did not match any GAP and was not considered.

Residues from 12 apple trials matching US GAP were: 0.01, 0.04, 0.06, 0.07, 0.10, 0.10, 0.13, 0.16, 0.18, 0.18, 0.19 and 0.20 mg/kg (median 0.115 mg/kg).

Residues from five pear trials matching US GAP were: 0.06, 0.08, 0.11, 0.12 and 0.20 mg/kg (median 0.11 mg/kg).

As the GAP in the USA is established for the group of pome fruits, and the median values from the trials conducted in the USA on the two commodities did not differ by more than 5-fold, the Meeting decided to estimate a group maximum residue level.

Man-Whitney test indicated that the residues from apple trials and those from pear trials were not statistically different. The Meeting decided to combine the data for estimating a maximum residue level. Residues were (n=17): 0.01, 0.04, 0.06, 0.06, 0.07, 0.08, 0.10, 0.10, 0.11, 0.12, 0.13, 0.16, 0.18, 0.18, 0.19, 0.20 and 0.20 mg/kg.

The Meeting estimated a maximum residue level of 0.4 mg/kg for pome fruits.

The sum of cyflumetofen and B-1 was calculated: 0.03, 0.06, 0.08, 0.08, 0.09, 0.10, 0.12, 0.12, 0.13, 0.14, 0.15, 0.18, 0.20, 0.20, 0.21, 0.22 and 0.22 mg eq./kg.

The Meeting estimated an STMR of 0.13 mg/kg expressed as cyflumetofen.

Berries and Other Small Fruits

Grapes

A total of 12 supervised trials were conducted on grapes in the USA in 2009. The GAP in the USA for grapes allows 2 applications at a maximum rate of 0.2 kg ai/ha (at least 468 L/ha) with a PHI of 14 days.

Residues from 11 independent trials matching US GAP were: 0.02, 0.09, 0.10, 0.12, 0.15, 0.16, 0.19, 0.22, 0.22, 0.27 and 0.42 mg/kg.

The Meeting estimated a maximum residue level of 0.6 mg/kg for grapes.

The sum of cyflumetofen and B-1 was calculated: 0.04, 0.12, 0.12, 0.16, 0.20, 0.22, 0.23, 0.25, 0.25, 0.33 and 0.46 mg eq./kg.

The Meeting estimated an STMR of 0.22 mg/kg expressed as cyflumetofen.

Strawberry

A total of eight supervised trials were conducted on strawberry in the USA in 2009 and 2010. The GAP in the USA for strawberry allows 2 applications at a maximum rate of 0.2 kg ai/ha (at least 468 L/ha) with a PHI of 1 day.

Residues from eight trials matching US GAP were: 0.04, 0.10, 0.14, 0.14, 0.16, 0.20, 0.23 and 0.36 mg/kg.

The Meeting estimated a maximum residue level of 0.6 mg/kg for strawberry.

The sum of cyflumetofen and B-1 was calculated: 0.06, 0.13, 0.16, 0.16, 0.20, 0.24, 0.28 and 0.40 mg eq./kg.

The Meeting estimated an STMR of 0.18 mg/kg expressed as cyflumetofen.

Fruiting Vegetables, Other Than Cucurbits

Tomato

A total of 16 supervised trials were conducted on tomato in the USA in 2009 and 2010. The GAP in the USA for tomato allows 2 applications at a maximum rate of 0.2 kg ai/ha (at least 468 L/ha) with a PHI of 3 days.

Residues from 14 independent trials matching US GAP were: 0.01, 0.02, 0.02, 0.04, 0.04, 0.04, 0.04, 0.06, 0.06, 0.06, 0.07, 0.09, 0.12 and 0.15 mg/kg.

The Meeting estimated a maximum residue level of 0.3 mg/kg for tomato.

The sum of cyflumetofen and B-1 was calculated: 0.03, 0.04, 0.06, 0.06, 0.06, 0.06, 0.06, 0.08, 0.08, 0.09, 0.09, 0.11, 0.17 and 0.18 mg eq./kg.

The Meeting estimated an STMR of 0.07 mg/kg expressed as cyflumetofen.

Eggplant

Two supervised trials were conducted on eggplant, green house grown, in Japan in 2004 with the analysis of cyflumetofen and the conjugates of B-1. The current GAP of Japan allows 2 applications at a spray concentration of 0.02 kg ai/hL with a PHI of 1 day. A spray volume of 1000–3500 L can be sprayed per ha. In the trials, spray concentration was 0.02 kg ai/hL and spray volume was 1996 L/ha, matching Japanese GAP. Residues from these trials were: 0.34 and 0.46 mg/kg.

The Meeting concluded that the data were insufficient for estimating a maximum residue level for eggplant.

Tree nuts

Five trials were conducted on almonds and five other trials were conducted on pecans in the USA in 2009. The GAP in the USA for tree nuts allows 2 application at a maximum rate of 0.2 kg ai/ha (in at least 935 L/ha) with a PHI of 7 days.

Residues in almond nutmeat from five trials matching US GAP were: < 0.01 (5) mg/kg.

Residues in pecan nutmeat from five trials matching US GAP were: < 0.01 (5) mg/kg.

As the GAP in the USA is established for the group of tree nuts and as the residues were all < 0.01 mg/kg, the Meeting agreed to estimate a maximum residue level for the tree nuts group at 0.01* mg/kg.

As the B-1 was also below the LOQ in all the trials, and the nutmeat is protected by the hull and not exposed to cyflumetofen foliar spray, the Meeting estimated an STMR to be 0.01 mg/kg expressed as cyflumetofen.

Animal feeds

Almond hulls

Five trials were conducted on almond in the USA in 2009. The GAP is the USA allows 2 application at a maximum rate of 0.2 kg ai/ha (in at least 935 L/ha) with a PHI of 7 days.

Residues in almond hull from five trials matching US GAP were: 0.35, 0.53, 0.56, 0.86 and 1.87 mg/kg.

The Meeting estimated a maximum residue level of 4 mg/kg.

The sum of cyflumetofen and B-1 was calculated: 0.38, 0.63, 0.67, 0.98 and 2.11 mg/kg. The Meeting estimated a median residue of 0.67 mg/kg.

Fate of residues during processing

High temperature hydrolysis

To simulate the degradation of cyflumetofen during pasteurization, baking, brewing, boiling and sterilization, the hydrolysis of radio-labelled cyflumetofen was investigated in sterile buffered aqueous solutions.

After incubation at 90 °C (pH 4) for 20 minutes, 100 °C (pH 5) for 60 minutes or 120 °C (pH 6) for 20 minutes, no loss of radioactivity occurred. After heating at 90 °C for 20 minutes, about 69–71% of cyflumetofen remained with 14–23% of the applied radioactivity degraded to B-1. At 100 °C for 60 minutes, only 5–18% of cyflumetofen remained with the formation of B-1 (53–59% AR) and AB-1 (32–40% AR). At 120 °C, cyflumetofen was completely hydrolyzed with the formation of B-1 (44–75% AR) and AB-1 (39–49% AR). Cyflumetofen was susceptible to hydrolysis at high temperature.

Processing

The Meeting received information on processing of orange, apple, grape and tomato.

Processing factors calculated for the processed commodities of the above raw agricultural commodities are shown in the table below. STMR-Ps were calculated for processed commodities of orange, apple and grape for which maximum residue levels were estimated.

Processed Orange Product	Cyflumetofen		Cyflumetofen and B-1*		STMR-P or median
	Processing factor	Best estimate	Processing factor	Best estimate	
Orange					0.07
Juice	< 0.02, < 0.08	0.05	< 0.04, < 0.022	0.031	0.0022
Oil	102, 137	120	88.0, 133	111	7.77
Marmalade	0.026, < 0.08	0.026	0.059, < 0.22	0.14	0.0098
Peel	2.86, 2.97	2.92	2.56, 2.67	2.6	0.18
Molasses	< 0.02, < 0.08	< 0.06	< 0.075, < 0.502	0.28	0.020
Dried pulp	0.44, 0.584	0.51	0.759, 0.958	0.86	0.060
Apple					0.13
Applesauce	2.54, 2.91	2.7	3.16, 3.73	3.4	0.44
Juice	0.197, 0.268	0.23	0.224, 0.299	0.26	0.033
Dried apples	0.17, 0.825	0.50	0.21, 0.876	0.54	0.070
Canned apples	0.035, 0.175	0.10	0.076, 0.202	0.14	0.018
Wet pomace	0.937, 1.59	1.3	0.939, 1.57	1.3	0.17
Grape					0.22
Dried grape	0.65, 0.93, 1.88, 4.64	2.0	0.86, 1.1, 2.47, 4.65	2.3	0.506
Juice	0.064,0.11, 0.2, 0.25	0.16	0.10, 0.16, 0.28, 0.32	0.22	0.0484
Wine (Young)	< 0.006, 0.029, 0.04, 0.04	0.04	0.04, 0.083,0.17, 0.2	0.12	0.0264
Must	0.02, 0.18, 0.41, 0.53	0.29	0.04, 0.22, 0.44, 0.59	0.32	0.071
Wet Pomace	1.1, 0.02, 3.39, 4.22	2.2	1.1, 3.03, 3.3, 3.97	2.9	0.638
Tomato					0.07
Juice	< 0.06, 0.2	0.2	< 0.2, 0.38	0.38	0.027
Canned Tomato	< 0.04, 0.2	0.2	< 0.1, 0.3	0.3	0.021
Puree	0.3, 0.88	0.59	0.4, 0.79	0.60	0.042
Paste	0.2, 0.4	0.3	1.2, 2.2	1.7	0.12
Wet Pomace	1.3, 5.5	3.4	1.4, 5.0	3.2	0.22

* expressed as cyflumetofen.

As the residue concentration is higher in orange oil than in fresh orange, the Meeting estimated a maximum residue level of 36 mg/kg for citrus oil by multiplying the maximum residue level of citrus fruits (0.3 mg/kg) by 120.

As the residue concentration is higher in dried grapes than in fresh grapes, the Meeting estimated a maximum residue level of 1.5 mg/kg by multiplying the maximum residue level for grapes (0.6 mg/kg) by 2.0.

In processing of foods, heating in aquatic system at high temperature may be employed, and therefore it is likely that AB-1 is present at significant concentrations after processing at slightly acidic to neutral pH (≥ 5). It may also occur from cyflumetofen during the storage of processed foods with high water content at room temperature. However, the Meeting concluded that AB-1 is covered by the ADI for the parent compound.

Residues in animal commodities

Estimation of dietary burden

The maximum and mean dietary burdens were calculated using the median residues of cyflumetofen (sum of cyflumetofen and B-1 expressed as cyflumetofen, to cover the worst case) estimated at the current Meeting on a basis of the OECD Animal Feeding Table.

Summary of livestock dietary burdens (ppm of dry matter diet)

	US-Canada		EU		Australia		Japan	
	Max	Mean	max	Mean	Max	Mean	Max	mean
Beef cattle	0.007	0.007	0.085	0.085	0.934 [a]	0.934 [b]	0	0
Dairy cattle	0.117	0.117	0.049	0.049	0.934 [a]	0.934 [b]	0	0
Broilers	0	0	0	0	0	0	0	0
Layers	0	0	0	0	0	0	0	0

[a] Suitable for estimating maximum residue levels for milk, meat, fat and edible offal of cattle.
[b] Suitable for estimating STMRs for milk, meat, fat and edible offal of cattle.

Residues in milk and cattle tissues

In the goat metabolism studies, in which goats were administered benzoyl-label cyflumetofen at a dose equivalent to 12.8 ppm in feed for 12 consecutive days and sacrificed one day later, cyflumetofen was found only in fat at 0.003 mg/kg. B-1 were found in liver, kidney, muscle, fat and milk at 0.125, 0.102, 0.005, 0.006 and 0.001 mg eq./kg respectively. The sum of cyflumetofen and B-1 in fat is 0.009 mg/kg as cyflumetofen. Neither cyflumetofen nor metabolites were found above 0.01 mg/kg or 10% TRR when butylphenyl-label cyflumetofen was administered to goats.

These concentrations were multiplied by 0.934/12.8 for estimating STMRs. The Meeting estimated STMRs of 0.010, 0.008, 0, 0 and 0 mg/kg for liver, kidney, meat, fat and milk, respectively.

The Meeting estimated maximum residue levels of 0.02, 0.01*, 0.01* and 0.01* mg/kg expressed as cyflumetofen for edible offal (mammalian), meat (from mammals other than marine mammals), mammalian fats (except milk fat) and milks, respectively.

The dietary burden for poultry was calculated to be 0 ppm of dry matter diet. No metabolism or feeding studies on laying hens were conducted.

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 and IESTI assessment.

Definition of the residue for plant commodities (for compliance with the MRL): *Cyflumetofen*.

Definition of the residue for plant commodities (for estimation of dietary intake): *Sum of cyflumetofen and 2-trifluoromethylbenzoic acid, expressed as cyflumetofen.*

Definition of the residue for animal commodities (for compliance with the MRL and estimation of dietary intake): *Sum of cyflumetofen and 2-trifluoromethylbenzoic acid, expressed as cyflumetofen.*

Residue is not fat-soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Dietary Intakes (IEDIs) of cyflumetofen were calculated for the 17 GEMS/Food cluster diets using STMRs estimated by the current Meeting (Annex 3 of the 2014 Report). The ADI is 0–0.1 mg/kg bw and the calculated IEDIs were 0–1% of the maximum ADI. The Meeting concluded that the long-term intake of residues of cyflumetofen resulting from the uses considered by the current 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 residues of cyflumetofen is unlikely to present a public health concern.