5.4 BUPROFEZIN (173)

TOXICOLOGY

Buprofezin is the ISO approved name for (*EZ*)-2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one (IUPAC), CAS No. 69327-76-0. Buprofezin is an insecticide that acts by the inhibition of chitin synthesis.

Buprofezin was previously evaluated by the JMPR in 1991 when an ADI of 0–0.01 mg/kg bw per day was established based on a NOAEL of 0.9 mg/kg bw per day identified in a 2-year study in rats and with a safety factor of 100. The JMPR in 1999 considered that the establishment of an ARfD was unnecessary. Buprofezin was re-evaluated by the present Meeting as part of the CCPR periodic re-evaluation programme. New studies not previously evaluated by the Meeting included several studies of acute oral toxicity, irritation, sensitization and genotoxicity, metabolism studies in rats and a two-generation study in rats.

The more recent studies complied with GLP, but many of the older reported studies were performed before the widespread use of GLP.

Biochemical aspects

Studies with [phenyl-^{14}C]buprofezin showed that the radiolabel was absorbed with a C_{max} at 9 h and was rapidly excreted (> 60% in 24 h and > 80% in 48 h) in male and female rats given doses of 10 and 100 mg/kg bw. In males and females, urinary (22–25%) and faecal (70–74%) cumulative excretion at 10 and 100 mg/kg bw was similar after 4 days. In a study in bile-duct cannulated rats, oral absorption after 24 h was low (40–45%) in both sexes; of the administered dose, 30–38% was found in bile, 3–6% in the urine and about 5% in the liver and carcass (not including the gastrointestinal tract). The difference in urinary excretion between bile-duct cannulated and non-cannulated rats suggests that buprofezin excreted in the bile undergoes gastrointestinal re-circulation. The radiolabel was distributed within 2 h to the organs and tissues and after 7 days the highest concentrations were found in erythrocytes, the thyroid and the liver. The total amount of radiolabel recovered in the body accounted for less than 0.7% of the administered dose.

In a 24-week feeding study, no evidence for accumulation was observed. The metabolism of buprofezin was studied in rat liver homogenates and in vivo. Hydroxylation and subsequent methylation of the phenyl ring, oxidation of sulfur with subsequent ring-opening of the thiadiazinane ring and conjugation reactions with sulfate and glucuronic acid were the main metabolic routes. Buprofezin, 4-hydroxybuprofezin (BF2), tert-butylhydroxy-buprofezin (BF4), dione metabolite (BF9), buprofezin sulfoxide (BF10), phenylbiuret (BF11), isopropylphenylurea (BF12), 4-hydroxyisopropylphenylurea (BF13), dimethoxy buprofezin (BF20), 4-aminophenol (BF22), 4-hydroxyacetanilide (BF23), thiobiuret (BF25), hydroxy-methoxy-buprofezin (BF27), 2-[3-isopropyl-3-[methylsulfonylmethyl, (phenyl)carbamoyl]ureido]-2-methylpropionic acid (BF28) and dihydroxy buprofezin (C) were identified in the metabolism study in rats.

The results suggested that there are no significant differences between males and females in toxicokinetic parameters and metabolic profiles over a dose range of 10 to 100 mg/kg bw.

Toxicological data

Buprofezin was of low to moderate toxicity when administered orally, with an LD_{50} of 1635–3847 mg/kg bw in rats, LD_{50} > 5000 mg/kg bw in rabbits and LD_{50} > 10 000 mg/kg bw in mice and hamsters. By the dermal, subcutaneous and intraperitoneal routes, the LD_{50}s were > 10 000 mg/kg bw in mice and rats, and the inhalation LC_{50} was > 4.57 mg/L. In rabbits, buprofezin was not irritating to the skin and only very slightly irritating to the eye. In a Magnussen & Kligman maximization test in

guinea-pigs, buprofezin gave equivocal results suggesting a very slight potential for delayed contact hypersensitivity, while the results of a local lymph-node assay with buprofezin in mice were negative.

In short-term studies in rats and dogs, the main effects were liver-weight increases accompanied by histological changes; in dogs, behaviour was also affected.

In a 13-week feeding study in rats, the feed intake in males at 200 ppm and above and in females at 5000 ppm was low after 1 or 2 weeks, resulting in lower body weights in the groups at 5000 ppm at study termination. At 200 ppm and above, slight changes in clinical chemistry parameters, including decreased glucose and triglyceride concentrations and increased cholesterol, phospholipid, urea nitrogen and albumin and globulin concentrations were observed. In males and females at 5000 ppm, liver and thyroid weights were increased and spleen weights were decreased. The increases in liver weight were accompanied by hypertrophic and necrotic changes and, in the thyroid, by hypertrophic and hyperplastic changes. The NOAEL was 40 ppm, equal to 3.4 mg/kg bw per day, on the basis of changes in clinical chemistry parameters in rats at 200 ppm.

In a 13-week study in dogs fed capsules containing buprofezin, transiently subdued behaviour was observed 1 h after dosing at 50 mg/kg bw per day and above. This observation was predominantly made in the first few days of treatment, but also at other time-points throughout the study, although with a lower incidence. At 300 mg/kg bw per day, slight ataxia was shown by virtually all dogs 1 h after dosing and persisting for about 5 h. This effect was seen in females only in the first few days of the study, but persisted for 9 weeks in one male. Male and female dogs at the highest dose had significantly lowered body-weight gains, increased liver, kidney and thyroid weights and two- to three-fold increases in the activity of alkaline phosphatase (ALP). Increased liver weights were also seen in both sexes at 50 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day.

In a 2-year study in dogs given capsules containing buprofezin, which was performed before the 13-week study, no behavioural effects were reported at up to the highest dose of 200 mg/kg bw per day. Increased liver weights were seen in all females receiving buprofezin and in males at 200 mg/kg bw per day. Thyroid weights were high in males and females at 200 mg/kg bw per day. At 20 mg/kg bw per day and above, ALP activity was significantly increased from week 4 onwards and higher incidences of hepatocellular hypertrophy, bile-duct and mammary hyperplasia were found. The NOAEL was 2 mg/kg bw per day.

Since it was not clear whether the observation scheme used in the 2-year study could have detected putative behavioural changes 1 h after treatment, an overall NOAEL for behavioural changes could not be identified. The overall NOAEL for systemic toxicity in the 13-week and the 2-year studies in dogs was 10 mg/kg bw per day on the basis of hepatocellular hypertrophy and bile-duct and mammary hyperplasia at 20 mg/kg bw per day in the 2-year study in dogs.

The long-term toxicity and carcinogenicity of buprofezin has been investigated in mice and rats. The liver was identified as the main target of toxicity.

In the 2-year study in mice, body weights in both sexes were slightly (5–10% in females and about 5% in males) but statistically significantly reduced in the group at the highest dose at 5000 ppm from week 6 (males) and week 9 (females) onwards. A very slight trend towards reduced body weight was also observed at 2000 ppm in males and females. At the highest dose of 5000 ppm, males and females had higher platelet counts at study termination and females had transiently lower erythrocyte counts and lower concentrations of haemoglobin. In males and females, liver weights were increased at 2000 ppm and above at 52 weeks; liver weights were statistically significantly increased at study termination only in males at 5000 ppm. Histologically, higher incidences of hepatocellular hypertrophy were seen at 2000 ppm and above. Hyperplastic changes were increased in the livers of males at 5000 ppm and of females at 200 ppm and above, without a clear dose–response relationship in females. In females at 2000 or 5000 ppm, slightly increased incidences of liver adenoma were close to the upper bound of the range for historical controls but without a dose–response relationship. The NOAEL for toxicity was 200 ppm, equal to 17.4 mg/kg bw per day, on the basis of

hepatocellular hypertrophy at 2000 ppm. The NOAEL for carcinogenicity was 5000 ppm, equal to 481 mg/kg bw per day, the highest dose tested.

In the 2-year study in rats, terminal body weights were decreased in females at the highest dose of 2000 ppm. Males at 2000 ppm showed lowered activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Males and females at 2000 ppm had elevated liver weights at 26, 52 and 104 weeks. In the first year of the study, rats had higher thyroid weights that were statistically significant only in females. Treatment-related histological changes were restricted to the liver and the thyroid. Since the criteria for histopathological diagnosis had substantially changed since the release of the study report in 1982, the original histology slides for livers and thyroids were re-examined in 1995. At 2000 ppm, males and females had higher incidences for centrilobular hepatocellular and diffuse hypertrophy and females also had more eosinophilic foci than did the controls. A slight and statistically not significant increase in the incidence of liver adenoma was observed at 2000 ppm in females but not in males. Males at 200 ppm and males and females at 2000 ppm showed higher incidences of thyroid F-cell hypertrophy. The NOAEL for toxicity was 20 ppm, equal to 0.9 mg/kg bw per day, on the basis of higher incidences of thyroid F-cell hypertrophy at 200 ppm and the NOAEL for carcinogenicity was 2000 ppm, equal to 89.46 mg/kg bw per day, the highest dose tested.

Buprofezin was not carcinogenic in mice and rats.

Buprofezin was tested for genotoxicity in an adequate range of studies in vitro and for induction of micronucleus formation in vivo. In the submitted studies, there was no evidence for genotoxicity in vitro; however, in a published non-GLP study, micronucleus formation was induced in cultured cells by an aneugenic mechanism, rather than by chromosomal breakage. In assays for micronucleus formation in immature erythrocytes of mouse bone marrow in vivo, conflicting results have been obtained. One study reported statistically significantly increased incidences in two experiments, but the numerical results were very different and were not fully supported by equivocal results from an earlier study in which the administered doses were five times higher. Furthermore, the suggestion of an aneugenic effect in vitro in the published study was not confirmed in vivo.

The Meeting concluded that there was equivocal evidence that buprofezin might be genotoxic.

On the basis of clearly negative results in assays for genotoxicity in vitro and equivocal results in assays for genotoxicity in vivo and the absence of carcinogenicity, the Meeting concluded that buprofezin is unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of buprofezin has been investigated in two two-generation studies and one one-generation study in rats. In none of the three studies were there any effects on the fertility of males or females or on reproductive performance. In one study, minor increases in liver and kidney weights, without histological correlates, were seen in parental males at 1000 ppm. Pup body weights at birth were not affected by treatment with buprofezin in any of the three studies, but pup body-weight gains were lower at 1000 ppm from postnatal day 4 to postnatal day 21. At postnatal day 21, the body weights of pups at 1000 ppm were 10–18% lower than those of the controls. The NOAEL for parental toxicity was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of very slight changes in organ weights. The NOAEL for reproductive effects was 1000 ppm, equal to 66.0 mg/kg bw per day, the highest dose tested. The NOAEL for effects in offspring was 100 ppm, equal to 6.46 mg/kg bw per day, on the basis of reduced pup body-weight gain during lactation at 1000 ppm.

Developmental toxicity with buprofezin had been investigated in rats and rabbits. In rats at the highest dose of 800 mg/kg bw per day, clinical signs of intoxication were observed from day 10 of gestation onwards, dams had lower body-weight gains and lower feed intake from day 7 of gestation onwards and four total litter losses occurred. Additionally, postimplantation losses were increased and fetal body weights were low in this group. At this, the highest dose, there were also more fetuses with a space between body-wall and organs, subcutaneous oedema and retarded

ossification. The NOAEL for maternal toxicity and fetal toxicity was 200 mg/kg bw per day on the basis of lower body-weight gains and litter losses in dams and retarded ossification in fetuses at 800 mg/kg bw per day.

In the study of developmental toxicity in artificially inseminated rabbit dams given pooled semen, body-weight gain and feed intake were lowered from the first days of treatment in the group given the highest dose of 250 mg/kg bw per day. One rabbit at 50 mg/kg bw per day aborted and two rabbits at 250 mg/kg bw per day showed total litter losses. Since the frequency of total litter loss was within the range for historical controls, a relationship to treatment is questionable. One fetus from the group at 50 mg/kg bw per day and one fetus from the group at 250 mg/kg bw per day showed unilateral agenesis of one kidney. Because this finding is occasionally observed and might be related to carrier males, its toxicological significance is questionable. Additionally, enlarged aortic arches were observed in one fetus in each of two litters of the group at the highest dose. Although the incidence of this finding was very low it was above the range for historical controls and was thus considered to be treatment-related. The NOAEL for maternal and fetal toxicity was 50 mg/kg bw per day on the basis of lowered body-weight gain and feed intake in dams and increased incidence of enlarged aortic arches in fetuses.

The Meeting concluded that buprofezin was developmentally toxic only at doses that were maternally toxic and did not induce structural changes in fetuses.

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

The Meeting considered that buprofezin is not neurotoxic on the basis of the available data.

In mechanistic studies on thyroid function in rats, buprofezin at a dose of 1000 ppm, equivalent to 68.5 mg/kg bw per day, did not affect serum concentrations of triiodothyronine (T3) and thyroxine (T4). At higher doses, T4 was lowered at the beginning of dosing only and recovered thereafter. At doses of 500 mg/kg bw per day administered by gavage for 15–60 days, thyroid weights increased and concentrations of T4 decreased, but the activity of thyroid peroxidase did not change markedly. The Meeting concluded that the mechanistic studies did not explain the thyroid changes in studies in rats and dogs.

In studies with the rat metabolite BF4, no mortalities were observed in rats given a single oral dose at 300 mg/kg bw, but all rats died at 2000 mg/kg bw. BF4 gave negative results in the Ames test. In studies with the rat metabolite BF11, no mortalities were observed in rats given a single oral dose at 2000 mg/kg bw. BF11 gave negative results in the Ames test. In studies with the rat metabolite BF25, no mortalities were observed in rats given a single oral dose at 300 mg/kg bw, but all rats died at 2000 mg/kg bw. BF25 gave negative results in the Ames test. The plant metabolite BF26 did not induce mortalities in rats given a single oral dose at 50 mg/kg bw, but all animals died at 300 mg/kg bw. BF26 BF4 gave negative results in the Ames test.

No health effects related to exposure were reported among personnel involved in the synthesis and manufacture of buprofezin.

Toxicological evaluation

The Meeting established an ADI of 0–0.009 mg/kg bw based on a NOAEL of 0.9 mg/kg bw per day in the 2-year study in rats, identified on the basis of increases in the incidence of thyroid F-cell hypertrophy at 8.71 mg/kg bw per day. A safety factor of 100 was applied. The difference between the current ADI and the previous ADI of 0.01 mg/kg bw per day is due to rounding of the figures; both ADIs were based on the same NOAEL from the same study.

The Meeting established an ARfD of 0.5 mg/kg bw based on a NOAEL of 50 mg/kg bw identified on the basis of ataxia at 300 mg/kg bw per day in a 13-week feeding study in dogs. A safety factor of 100 was applied. This ARfD would also be protective against the finding of enlarged aortic arches in rabbit fetuses, although this effect is unlikely to be the result of a single dose.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	200 ppm, equal to 17.4 mg/kg bw per day	2000 ppm, equal to 190 mg/kg bw per day
		Carcinogenicity	5000 ppm, equal to 481 mg/kg bw per day[c]	—
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equal to 0.9 mg/kg bw per day	200 ppm, equal to 8.71 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 89.46 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a,d]	Reproductive toxicity	1000 ppm, equal to 66.0 mg/kg bw per day[c]	—
		Parental toxicity	100 ppm, equal to 6.46 mg/kg bw per day	1000 ppm, equal to 66.0 mg/kg bw per day
		Offspring toxicity	100 ppm, equal to 6.46 mg/kg bw per day	1000 ppm, equal to 66.0 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	200 mg/kg bw per day	800 mg/kg bw per day
		Embryo and fetal toxicity	200 mg/kg bw per day	800 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
Dog	13-week and two-year study of toxicity[b, e]	Toxicity	10 mg/kg bw per day	20 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

[d] The results for three studies were combined.

[e] The results for two studies were combined.

Estimate of acceptable daily intake for humans

0–0.009 mg/kg bw

Estimate of acute reference dose

0.5 mg/kg bw

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 buprofezin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 40–45%
Dermal absorption	—
Distribution	Extensive, highest levels in erythrocytes, thyroid, liver
Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	Rapid, > 80% within 48 h, mainly via bile
Metabolism in animals	Extensive, primarily via oxidations, thiadiazinane ring opening and conjugation
Toxicologically significant compounds (animals, plants and environment)	Buprofezin, rat metabolite BF25, plant metabolite BF26
Acute toxicity	
Rat, LD_{50}, oral	1635–3847 mg/kg bw
Rat, LD_{50}, dermal	> 10 000 mg/kg bw
Rat, LC_{50}, inhalation	4.57 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization (test method used)	Not a sensitizer (Magnusson & Kligman and local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Feed intake, clinical chemistry (rat) liver weight increase with histological changes (dog)
Lowest relevant oral NOAEL	3.4 mg/kg bw per day (13-week study in rats)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day, highest dose tested (24-day study in rats)
Lowest relevant inhalation NOAEL	No data
Genotoxicity	
	Equivocal evidence of genotoxicity
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increases in liver weight with histological changes (mouse, rat) and thyroid changes (rat)
Lowest relevant NOAEL	20 ppm, equal to 0.9 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects; reduced body-weight gain in pups during lactation
Lowest relevant reproductive NOAEL	1000 ppm, equal to 66 mg/kg bw per day (rat)

Lowest relevant offspring NOAEL	100 ppm; equal to 6.46 mg/kg bw per day (rat)
Developmental target/critical effect	Enlarged aortic arches (rabbit), retarded ossification (rat)
Lowest relevant developmental NOAEL	50 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	
	No evidence in conventional studies
Other toxicological studies	
	Rat metabolites BF4 and BF25 and plant metabolite BF26 had moderate acute oral toxicity; the rat metabolite BF11 was of low acute oral toxicity. All metabolites were not genotoxic.
Medical data	
	Medical surveillance of workers in a plant producing buprofezin did not reveal any adverse health effects.

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.009 mg/kg bw	Rat, 2-year study	100
ARfD	0.5 mg/kg bw	Dog, 13-week study	100

RESIDUE AND ANALYTICAL ASPECTS

The insecticide buprofezin was evaluated by the JMPR for residues in 1991, 1995 and 1999. Toxicology was reviewed in 1991. Buprofezin was listed within the periodic re-evaluation programme at the 40th Session of the CCPR for periodic review by the 2008 JMPR for toxicology and residues.

The Meeting received information on identity, metabolism, storage stability, residue analysis, use patterns, fate of residue during processing, livestock feeding studies and residues resulting from supervised trials on oranges, mandarins, lemons, grapes, apples, pears, persimmons, custard apples, mangoes, cucumbers, eggplants and tomatoes. The Meeting also received information on use patterns from Japan and Australia.

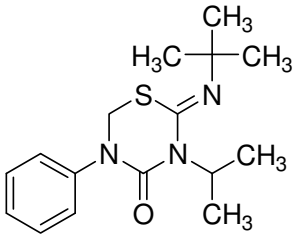
Chemical name

ISO common name: buprofezin

IUPAC: 2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one

CA: (Z)-2-[(1,1-dimethylethyl)imino]tetrahydro-3-(1-methylethyl)-5-phenyl-4H-1,3,5-thiadiazin-4-one

Structural formula:



Metabolites referred to in the appraisal were addressed by their common names, except reverse Schiff base which refers to 3-isopropyl-5-phenyl-1,3,5-thiadiazinan-2,4-dione and the allophanate degradate which refers to 2-amino-2-methylpropyl-2-methylethyl-4-phenyl-allophanate.

Animal metabolism

The Meeting received results of animal metabolism studies in a lactating cow and in laying hens. Experiments were carried out using uniformly [^{14}C]phenyl labelled buprofezin.

Metabolism in laboratory animals was summarized and evaluated by the WHO panel of the JMPR in 2008. Studies with [^{14}C]phenyl buprofezin showed that radioactivity was rapidly absorbed (C_{max} at 9 h) and rapidly excreted (> 60% in 24 h and > 80% in 48 h) in male and female rats at 10 and 100 mg/kg bw. The metabolism of buprofezin was studied in rat liver homogenates and in vivo. Hydroxylation with consecutive methylation of the phenyl ring, hydroxylation of the t-butyl moiety, oxidation of sulfur with consecutive ring opening of the thiadiazinane ring and conjugation reactions with sulfate and glucuronic acid were the main metabolic routes. Buprofezin, buprofezin sulfoxide, isopropylphenylurea, 4-hydroxybuprofezin, dihydroxybuprofezin, hydroxy-methoxybuprofezin, 4-aminophenol, 4-hydroxyacetanilide, dimethoxybuprofezin, reverse Schiff base, 4-hydroxyisopropylphenylurea, 2-[3-isopropyl-3-[methylsulfonylmethyl(phenyl)carbamoyl]ureido]-2-methylpropionic acid tert-butylhydroxy-buprofezin, biuret and thiobiuret were identified in the rat metabolism.

A lactating cow, orally treated twice daily for 7 consecutive days with [^{14}C]phenyl-buprofezin at a calculated dose rate of 27 ppm in the dry weight feed (equivalent to 0.38 mg ai/kg bw/d), was sacrificed 15 hours after the last dose. Most of the radioactivity was excreted: with 46% of the administered dose found in the faeces and 19% in the urine. Tissues contained only 1.6%, while milk contained 0.087% of the administered dose. The radioactivity in the tissues ranged from 1.21 mg/kg in the liver and 0.41 mg/kg in the kidney to 0.020 mg/kg in the fat and 0.018 mg/kg buprofezin equivalents in muscle. Radioactivity in milk reached a plateau on the fifth day of dosing at an average level of 0.026 mg/kg buprofezin equivalents. Radioactivity in the milk was distributed between cream and whey in a ratio of approximately 2:1.

Radioactivity was characterized in cow liver, kidney and milk. Metabolites were only identified after hydrolysis of the organic extracts with β-glucuronidase and sulfatase, indicating that the metabolites identified were conjugates.

No buprofezin was detected in the liver or kidney. The major metabolite in liver and kidney was 4-hydroxybuprofezin at 11% and 18% of the total radioactivity, respectively. Isopropylphenylurea, 4-hydroxyisopropylphenylurea and 4-hydroxyacetanilide were identified as minor metabolites at levels up to 8% of the total radioactivity. In milk 2.2% of the total radioactivity was identified as buprofezin. The principal milk metabolite was 4-hydroxyacetanilide at 14% of the total radioactivity with minor amounts of 4-hydroxybuprofezin and isopropylphenylurea at levels up to 4% of the total radioactivity. The major part of the residue in liver, kidney and milk remained unidentified (67–81% of the total radioactivity), but was characterized as organic soluble compounds (10–12% of the total radioactivity) comprising each less than 6% of the total radioactivity, as a mixture of highly polar metabolites (15–28% of the total radioactivity) or as unextractable residue

(20–55% of the total radioactivity). Most of the unextractable residue could be released by proteinase treatment (16–36% of the total radioactivity) indicating that residues were incorporated in animal tissues.

Six laying hens, orally treated twice daily for 14 consecutive days with [^{14}C]phenyl-buprofezin at a calculated dose rate of 12 ppm in the dry weight feed (equivalent to 0.80 mg ai/kg bw/d), were sacrificed 13–14 h after the last dose. The largest amount of radioactivity was found in the excreta, which contained 80% of the administered dose. Tissues contained only 0.2%, while eggs contained less than 0.1% of the administered dose. The radioactivity in the tissues ranged from 0.15 mg/kg in liver and 0.14 mg/kg in kidney to 0.035 mg/kg in fat and 0.019 mg/kg buprofezin equivalents in muscle. Radioactivity in egg yolks reached a plateau on the 12th day of dosing at an average level of 0.11 mg/kg buprofezin equivalents; radioactivity in egg whites reached a plateau on the 3rd day of dosing at an average level of 0.012 mg/kg buprofezin equivalents.

Radioactivity was characterized in hen liver, egg yolk and egg white. Metabolites could only be identified following hydrolysis of the organic extracts with dioxane/hydrochloric acid and sodium hydroxide under severe conditions, indicating that metabolites identified must be considered as conjugates.

Buprofezin was detected at trace amounts in liver, egg yolks and egg whites (0.3–0.9% of the total radioactivity). In addition, the reverse Schiff base, isopropylphenylurea, and 4-hydroxyisopropylphenylurea were identified as minor metabolites at levels up to 4% of the total radioactivity. The major part of the residue in liver, egg yolks and egg whites remained unidentified (90–95% of the total radioactivity), but was characterized as organic soluble compounds (9–31% of the total radioactivity) comprising each less than 9% of the total radioactivity, as aqueous soluble highly polar metabolites (11–39% of the total radioactivity) or as unextractable residue (45–56% of the total radioactivity). Residue released from solids was also characterized as a mixture of highly polar metabolites.

It was found that [^{14}C]buprofezin was efficiently eliminated from cattle and hens. The absorbed dose was extensively metabolized and conjugated as demonstrated by the wide range of solvent polarities required to extract and partition radioactivity from the tissues, the necessity for acid or enzyme hydrolysis to release identifiable metabolites and also the large amount of minor unidentifiable and bound metabolites.

The metabolic pathways have been found to be virtually identical for cattle and hens. Two basic metabolic pathways are proposed. The first is hydroxylation at the para position of buprofezin to form 4-hydroxybuprofezin, followed by cleavage of the thiadiazinane ring and loss of the $–CH_2$-S-C=N-C$(CH_3)_3$ group to leave 4-hydroxyisopropylphenylurea which is degraded to 4-hydroxyacetanilide. The second proposed route consists of a reverse Schiff base reaction followed by cleavage of the thiadiazinane ring with loss of $–CH_2$-S-C=O to form isopropylphenylurea which is hydroxylated and metabolized by multiple steps also to the 4-hydroxyacetanilide.

The metabolic pathway proposed for ruminants and hens is consistent with that for rats, though rats have some additional metabolic routes, i.e., double hydroxylation at the phenyl ring, hydroxylation of the tert-butyl moiety and formation of the buprofezin sulfoxide. These additional rat metabolic routes were evaluated in livestock, with the aid of reference standards, and were not found; the Meeting therefore concluded that these additional metabolic routes are specific to rats.

Plant metabolism

The Meeting received plant metabolism studies for buprofezin in fruit (citrus), fruiting vegetables (tomato), leafy vegetables (lettuce) and oilseeds (cotton). Experiments were carried out using uniformly [^{14}C]phenyl labelled buprofezin.

Greenhouse grown tomato plants were sprayed to run-off with [^{14}C]buprofezin four times at 14 day intervals at a dose rate of 0.0075 kg ai/hL. Tomato fruit were harvested 0, 1, 3 and 7 days after

the last application. Total radioactive residues declined from 0.49–0.67 mg/kg to 0.32–0.37 mg/kg buprofezin equivalents from 0 to 7 days. Washing with water released 16% to 4% of the total radioactivity from 0 to 7 days, while washing with ethanol released 61% to 26% of the radioactivity.

Fruit of greenhouse grown tomato plants were treated topically with [^{14}C]buprofezin at a dose rate equivalent to 0.062 kg ai/hL with the treated tomatoes harvested 0, 1, 3 and 7 days after the last application. The applied radioactivity on the day 7 tomato fruit was 36% surface residue, 33% in the peel and 11% in the fruit pulp, while 20% of the applied radioactivity was lost. Autoradiography of the day 7 tomato fruit indicated that a large part of the radioactivity was still present in the peel, although diffusion into the pulp had started. Total radioactive residues varied between 0.28 to 0.72 mg/kg buprofezin equivalents for day 0 to 7 samples. Of the total radioactivity in the day 7 tomato fruits, 93% was identified as unchanged buprofezin.

Fruit from greenhouse grown lemon trees, treated with [^{14}C]buprofezin, were harvested 75 days after a single foliar treatment of 1.0 kg ai/ha (0.05 kg ai/hL), 14 days after a double foliar treatment with a 75 day interval at 1.0 kg ai/ha (0.05 kg ai/hL) each or 30 days after a single foliar treatment of 3.5 kg ai/ha (0.17 kg ai/hL). Total radioactive residues were 0.40, 0.94 or 3.8 mg/kg buprofezin equivalents, respectively. The vast majority of the total radioactive residue from the fruit (90–98%) was recoverable by an ethanol surface wash and a solvent extraction of the peel, indicating surface residue, with 2–9% non-extractable. In the lemons treated two times at 1.0 kg ai/ha and a pre-harvest interval of 14 days, 79% of the total residue was identified with the aid of acid hydrolysis indicating conjugation of residues. Buprofezin was the major residue (66%) of which the majority remained on the surface of the fruit as unconjugated compound (64%). Reverse Schiff base (6.0%), isopropylphenylurea (1.7%), and allophanate degradate (5.7%) were identified as minor metabolites in the extractable and fibre bound residue with the aid of acid hydrolysis indicating conjugation. Levels of other unidentified metabolites individually did not exceed 3.6% of the total radioactivity (0.03 mg/kg eq). In the lemons treated once at 1.0 kg ai/ha and at a longer pre-harvest interval of 75 days, 68% of the total residue was identified. The metabolite profile was similar, but the levels of buprofezin had decreased (18% of total residue) and levels of conjugated metabolites had increased (7.3–34% of total residue).

Lemon twigs and leaves from greenhouse grown trees were treated topically with [^{14}C]buprofezin at 2.0 kg ai/ha (0.1 kg ai/hL) and adjacent fruits were harvested 28 days later. Fruits contained only 0.03–1.2% of the applied dose and total radioactive residues in the fruits were less than 0.01 mg/kg buprofezin equivalents. This translocation study indicates that buprofezin does not move systemically through the plant.

Field-grown leaf lettuce was sprayed with [^{14}C]buprofezin two times at an interval of 12 days at a dose rate of 0.86 kg ai/ha. The lettuce was then harvested 14 days after the last treatment. Average total radioactive residue was 43 mg/kg eq. The majority of the radioactive residues were removable by ethanol surface wash (89%) indicating that the residue resides primarily on the surface. The remainder of the residue was extractable with organic solvents and water (10%), while 1.1% was non-extractable. Buprofezin was the major component of the residue (89%) with the majority remaining on the leaf surface as unconjugated compound (89%). Reverse Schiff base (0.2%), isopropylphenylurea (0.4%), and allophanate degradate (0.6%) were identified as minor metabolites in the extractable and fibre bound residue with the aid of acid and base hydrolysis indicating conjugation. Levels of other unidentified metabolites individually did not exceed 1% TRR (0.52 mg/kg eq).

Field-grown cotton was sprayed with [^{14}C]buprofezin twice at an interval of 42 days at a dose rate of 0.85 kg ai/ha. The cotton was harvested 27 days after the last treatment and separated into seeds and gin trash. Average total radioactive residue was 16 and 0.37 mg/kg buprofezin equivalents in gin trash and cotton seeds, respectively. A large part of the radioactive residues were removable by ethanol surface wash (45–68%) indicating surface residues. The remainder of the residue was extracted with organic solvents and water (17–44%), while 13–14% was non-extractable. Approximately 76% and 62% of the total radioactive residue was identified in gin trash and cotton

seeds, respectively. Buprofezin was the major residue (59%) of which the majority remaining as unconjugated compound on the surface of the gin trash (46%) or cotton seeds (53%). With the aid of acid hydrolysis reverse Schiff base (1.4–5.8%), isopropylphenylurea (1.5–5.7%), and allophanate degradate (0.4–6.1%) were identified as minor metabolites in the extractable and fibre bound residue. Levels of other unidentified metabolites individually did not exceed 7.5% TRR (1.1 mg/kg eq).

In each commodity tested, buprofezin was found to be the major residue (59–89% of the total radioactivity), staying primarily on the surface of the treated crop as unconjugated compound. The remainder of the residue was identified as reverse Schiff base, isopropylphenylurea, and allophanate degradate as free or conjugated compound albeit at very low levels (less than 7% of the total residue). No single unidentified metabolite comprised more than 7.5% TRR in either crop tested (0.03–1.1 mg/kg eq, depending on the commodity).

Two metabolic pathways are proposed for buprofezin residues that penetrate the surface of plants. The first proposed route consists of a reverse Schiff base reaction followed by cleavage of the thiadiazinane ring with loss of $–CH_2$-S-C=O to form isopropylphenylurea. The second proposed route consists of chemical rearrangements and cleavage of the thiadiazinane ring to form allophanate degradate followed by formation of isopropylphenylurea or formation of reverse Schiff base followed by formation of isopropylphenylurea.

Other than the allophanate degradate, plant metabolites were also found in the rat. The unconjugated form of the allophanate degradate was only found in cotton seeds at trace levels (0.4% of the total residue). Conjugated forms of the allophanate degradate were found at levels of up to 5.7% of the total residue in lemons treated 14 days prior to harvest, while the levels increased to 34% of the total residue in lemons treated 75 days prior to harvest. Only trace levels of the allophanate degradate could be released from lemon by mild enzyme hydrolysis (ß-glucuronidase, ß-glucosidase, cellulase, 20 h at 37 °C), quantifiable amounts were released by a more forcing acid hydrolysis (dioxane:concentrated HCl, 5:2, v/v, 16 h at 50 °C). Additional analysis of the lemon residues indicated that the allophanate degradate most likely originated from hydrolysis of tert-butylhydroxy-buprofezin linked to a non-glucose hexose. The unconjugated tert-butylhydroxy-buprofezin could not be isolated from the lemon residues, but acid hydrolysis of a solution of tert-butylhydroxy-buprofezin (1 M HCl, 90 °C, 1 h) resulted in the formation of allophanate degradate, isopropyl-phenylurea and reverse Schiff base, which were the same metabolites as found in plants.

Environmental fate

The Meeting received information on the hydrolysis and photolysis of buprofezin in sterile water. Experiments were carried out using uniformly [^{14}C]phenyl labelled buprofezin.

Buprofezin is hydrolytically stable in sterile water at pH 7 and 9 but hydrolyses at pH 5 with a half life of 51 days. The proposed route for hydrolysis in water involves opening of the thiadiazinane ring to form thiobiuret followed by amide cleavage to produce isopropylphenylurea or replacement of the sulfur with oxygen to form biuret followed by amide cleavage to produce isopropylphenylurea.

The hydrolysis products thiobiuret and biuret were not found in or on crops treated with a foliar spray of buprofezin.

Three photolysis studies were conducted involving either artificial sunlight or natural sunlight, with, in each case, the light equivalent to 30 days of natural sunlight. Half lives ranged from 33 days (Study 2) and 38 days (Study 3) to 106–140 days (Study 1) for natural sunlight in summer. The major route is either a reverse Schiff base reaction or cleavage of the thiadiazinane ring to form thiobiuret followed by further degradation to isopropylphenylurea, phenylurea and the major photodegradate formanilide or formation of biuret. Minor photodegradation products found were des-isopropyl buprofezin, buprofezin sulfoxide, and 4-hydroxybuprofezin.

The photodegradation products 4-hydroxybuprofezin, des-isopropyl buprofezin, thiobiuret, biuret, phenylurea, formanilide and buprofezin sulfoxide were not found in or on crops treated with a foliar spray of buprofezin, despite buprofezin persisting for a long time on plant surfaces. Reference standards for phenylurea and formanilide were not available in the metabolism study on lettuce.

Methods of analysis

The Meeting received description and validation data for analytical methods for enforcement-monitoring of buprofezin and residue analytical methods used in the various study reports for buprofezin and its metabolites.

Multi-residue method DFG S19 is a post-registration monitoring and enforcement method for parent buprofezin in crops. The Meeting considered the method sufficiently validated for commodities with high water content, commodities with high acid content, commodities with high fat content and dried commodities. No enforcement-monitoring method was available for animal commodities.

The methods reported to the Meeting and used in the supervised residue trials, processing studies and storage stability studies on crops, determined parent buprofezin and in some cases also the metabolite 4-hydroxybuprofezin or the metabolites reverse Schiff base and isopropylphenylurea. Macerated samples were extracted with acetone or ethyl acetate. The extract was cleaned up by solvent partition and/or column chromatography and/or solid phase extraction, if necessary. The final residue could then be determined by GC-NPD, GC-MS, HPLC-UV or HPLC-MS-MS. Determination of 4-hydroxybuprofezin generally required acetylation. LOQs were in the 0.005–0.1 mg/kg range for buprofezin and 4-hydroxybuprofezin, and in the 0.01–0.05 mg/kg range for reverse Schiff base and isopropylphenylurea.

The methods reported to the Meeting and used in the feeding studies and storage stability studies on animal commodities, determined parent buprofezin and/or the metabolites 4-hydroxybuprofezin, isopropylphenylurea or 4-hydroxyacetanilide. Macerated samples were typically extracted with acetonitrile. The extract was cleaned up by solvent partition and/or solid phase extraction. The final residue could then be determined by GC-NPD or GC-MS. The analytical method has a reported limit of quantification of 0.01 mg/kg in milk and 0.05 mg/kg in tissues for each analyte, but suffers from matrix interferences, thereby increasing the valid limit of quantification to levels of 0.04 mg/kg for 4-hydroxyacetanilide in milk, 0.07 mg/kg buprofezin in beef fat, and 0.1 mg/kg in beef liver.

The Meeting noted that conjugated forms of buprofezin and its metabolites are unlikely to be detected by the analytical methods described for plant and animal commodities because of the simple extraction methods used.

Stability of pesticide residues in stored analytical samples

The Meeting received information on the stability of buprofezin, 4-hydroxybuprofezin, reverse Schiff base and isopropylphenylurea in samples stored frozen.

Parent buprofezin was stable when stored frozen for up to 32 months in crops with high water content (32 months lettuce, 30 months tomatoes, 5 months cucumbers), up to 12 months in crops with high acid content (12 months citrus, 4 months grapes), up to 6 months in dry tomato pomace and tomato juice, and 6 months in tomato paste.

Metabolites reverse Schiff base and isopropylphenylurea were stable when stored frozen for 32 months in crops with high water content (32 months lettuce, 30 months tomatoes), 12 months in crops with high acid content (citrus), and 6 months in dry tomato pomace, tomato juice, and tomato paste. 4-Hydroxybuprofezin is stable when stored frozen for 12 months in crops with high acid content (12 months citrus) and 5 months in crops with high water content (5 months tomato and 3 months cucumber).

Parent buprofezin was stable when stored frozen at –10 °C for 10 months in beef fat and 10 months in milk (degraded at 12 months). Storage stability results for beef liver could not be interpreted because of the high variability in the analytical results.

The Meeting extrapolated 32 months of storage stability for apple, pear, persimmon, custard apple, mango and eggplant samples from crops with high water content. Samples in selected supervised residue trials on mandarins, oranges were stored for periods up to 3 years (mandarin) or 2 years (oranges) which is longer than the maximum storage period tested of 12 months for crops with high acid content. Because pH for tomatoes (4.3–4.5) is similar to pH for oranges (3.7–4.3) or mandarins, the Meeting considered the storage stability for citrus to be sufficiently covered. Storage stability data for orange juice, orange pomace, wine, grape juice and raisins were not available, although the samples were stored for a period of up to 2 years. Processed tomato samples were stored for periods of up to 8 months, longer than the maximum storage period tested at 6 months for tomato juice and 6 months for tomato paste. The Meeting considered the storage stability for processed commodities to be adequately covered by the storage stability data on the raw commodities.

Residue definition

In the metabolism studies [^{14}C]buprofezin was efficiently eliminated from cattle and hens. The absorbed dose was extensively metabolized and conjugated as indicated by the wide range of solvent polarities required to extract and partition radioactivity from the tissues, the necessity for acid or enzyme hydrolysis to release identifiable exocons and also the large amount of minor unidentifiable and bound metabolites. Based on the metabolism studies significant residues were not identified in animal commodities.

However, the Meeting noted that in a feeding study on lactating cows, where the dose rate was 6 times higher than in the metabolism study, residues of up to 0.02 and 0.12 mg/kg buprofezin were found in milk and beef fat, respectively. Taking into account the residues found in this feeding study, the residue is defined as buprofezin (no metabolites) for enforcement in animal products as well as for dietary risk assessment.

The log K_{ow} of 3.7 for buprofezin suggests fat solubility. The fat solubility of buprofezin was indicated in a poultry metabolism study where buprofezin levels (as mg/kg) in egg yolk were higher by a factor of 2 than in egg whites and by a feeding study in cows where buprofezin concentrated by a factor of 4 in cream as compared to whole milk. Buprofezin, however, was not detected in the fat of cows or poultry and could be removed easily by washing with water from plants surfaces. As a consequence, the Meeting considers the residue to be not soluble in fat.

Based on the available comparative plant metabolism studies, parent buprofezin is the major component (59–89% of the total residue) of the crops tested at short pre-harvest intervals (14–27 days). The Meeting concluded that parent buprofezin is a suitable analyte in plant commodities for enforcement purposes.

The remainder of the residue was identified, principally after hydrolysis, as reverse Schiff base, isopropylphenylurea, and the allophanate degradate, albeit at very low levels (less than 7% of the total residue). The reverse Schiff base and isopropylphenylurea were found in rat, but the allophanate degradate was not. Based on toxicological data the Meeting considered the allophanate degradate toxicologically relevant.

Since unconjugated forms of allophanate degradate were only available at trace levels in cotton seeds, and the allophanate degradate could only be identified using strong acid hydrolysis conditions (dioxane:concentrated HCl, 5:2, v/v, 16 h 50 °C). The Meeting considered the allophanate degradate an artefact resulting from strong hydrolysis. No analytical methods were available to quantify free or conjugated allophanate degradate.

Although the allophanate degradate is, and some of the other metabolites might be, of toxicological relevance, the levels are so low that the Meeting agreed that they should be excluded from the residue definition for risk assessment.

The Meeting recommended the following as the residue definition for buprofezin:

Definition of the residue for compliance with the MRL or for estimation of the dietary intake for plants and animals: *buprofezin*

Results of supervised residue trials on crops

The Meeting received supervised residue trial data for buprofezin on lemons, mandarins, oranges, apples, pears, grapes, persimmons, custard apples, mangoes, cucumbers, egg plants and tomatoes.

Citrus fruits

Supervised field trials involving lemons were performed in New Zealand. GAP for citrus in New Zealand is for 2–4 applications at 0.013 kg ai/hL (PHI 14 days). In trials matching New Zealand GAP (2× 0.012 kg ai/hL, PHI 14 days), buprofezin residues in whole fruit were 0.22 mg/kg (n = 1). Buprofezin residues in lemon pulp were not available.

GAP for citrus in Australia is for 1–2 spray applications at 0.013–0.026 kg ai/hL (PHI 28 days) and in trials from New Zealand matching this GAP (2× 0.025 kg ai/hL, PHI 28 days), buprofezin residues in whole fruit were 0.40 mg/kg (n = 1). Buprofezin residues in lemon pulp were not available.

The Meeting agreed that the data corresponding to the New Zealand and Australian GAP were insufficient to estimate a maximum residue level for lemon.

Field trials involving mandarins were performed in Spain, Australia and Japan.

GAP for citrus in Spain is for 1 spray application at 0.013–0.025 kg ai/hL (PHI 7 days). In trials from Spain matching this GAP (1× 0.025 kg ai/hL, PHI 6–7 days), buprofezin residues in whole fruit were 0.11, 0.22, 0.23 (3), 0.41, 0.45, 0.46 mg/kg (n = 8). Buprofezin residues in mandarin pulp in these trials were < 0.01, 0.03, 0.04, 0.04, 0.05, 0.06 (3) mg/kg (n = 8).

GAP for citrus in Australia is for 1–2 spray applications at 0.013–0.026 kg ai/hL (PHI 28 days) and in trials from Australia matching this GAP (1–2× 0.024–0.026 kg ai/hL, PHI 28 days), buprofezin residues in whole fruit were 0.05, 0.33, 0.69 mg/kg (n = 3). Buprofezin residues in mandarin pulp in these trials were < 0.01 (2), 0.084 mg/kg (n = 3).

GAP for mandarin in Japan is for 1–3 spray applications at 0.017-0.025 kg ai/hL (PHI 14 days). In trials from Japan matching this GAP (2–3× 0.025 kg ai/hL, PHI 14 days), buprofezin residues in whole fruit were 0.07 (3) and 0.08 mg/kg (n = 4). Buprofezin residues in mandarin pulp in these trials were < 0.01 (3) and 0.02 mg/kg (n = 4).

The Meeting noted that the datasets from Australia and Japan were too small to estimate a maximum residue level. The Meeting agreed to use the dataset from Spain.

Field trials involving oranges were performed in Spain, USA, and Australia.

GAP for citrus in Spain is for 1 spray application at 0.013–0.025 kg ai/hL (PHI 7 days). In trials from Spain matching this GAP (1× 0.025 kg ai/hL, PHI 7–8 days), buprofezin residues in whole fruit were 0.17 (2), 0.19, 0.21 (2), 0.23, 0.32, 0.37 mg/kg (n = 8). Buprofezin residues in orange pulp in these trials were 0.03, 0.04 (4), 0.05, 0.10 mg/kg (n = 7).

Trial data from the USA did not match the available GAP for that country.

GAP for citrus in Australia is for 1–2 spray applications at 0.013–0.026 kg ai/hL (PHI 28 days) and in trials from Australia matching this GAP (2× 0.025 kg ai/hL, PHI 29 days), buprofezin

residues in whole fruit were 0.05, 0.067, 0.12 mg/kg (n = 3). Buprofezin residues in orange pulp in these trials were < 0.01, 0.011, 0.021 mg/kg (n = 3).

GAP for citrus in New Zealand is for 2–4 spray applications at 0.013 kg ai/hL (PHI 14 days) and in trials from Australia matching this GAP (2× 0.012 kg ai/hL, PHI 14 days), buprofezin residues in whole fruit were 0.051 and 0.11 mg/kg (n = 2). Buprofezin residues in orange pulp in these trials were 0.011 and 0.030 mg/kg (n = 2).

The Meeting noted that the individual datasets from Australia and New Zealand were too small to estimate a maximum residue level. The Meeting agreed to use the dataset from Spain.

The Meeting noted that GAPs for mandarin and orange were the same and that the datasets were from similar populations and could be combined. Buprofezin residues in whole fruit in ranked order were: 0.11, 0.17, 0.17, 0.19, 0.21, 0.21, 0.22, 0.23, 0.23, 0.23, 0.23, 0.32, 0.37, 0.41, 0.45, 0.46 mg/kg (n = 16). Buprofezin residues found in the pulp were: < 0.01, 0.03 (2), 0.04 (6), 0.05 (3), 0.06 (3), 0.10 mg/kg (n = 16).

The Meeting agreed that the mandarin and orange data could be used to support a citrus fruit commodity group maximum residue level and estimated a maximum residue level of 1 mg/kg for buprofezin on citrus fruit and estimated an STMR of 0.04 mg/kg and an HR of 0.10 mg/kg for buprofezin in the edible portion of citrus fruit. For purposes of calculating residues in processed citrus commodities an STMR of 0.23 mg/kg and an HR of 0.46 mg/kg was estimated based on whole fruit orange.

The Meeting withdrew its previous recommendation of 0.5 mg/kg for oranges, sweet and sour.

Pome fruits

Field trials involving apples were performed in New Zealand. Trials performed in New Zealand did not match with the available GAP for New Zealand or Australia.

The Meeting agreed that there was insufficient data to estimate a maximum residue level for apples.

Field trials involving pears were performed in Australia.

GAP for pears in Australia is for 2 spray applications at 0.013–0.026 kg ai/hL (PHI 56 days) and in trials from Australia matching this GAP (2–3× 0.026 kg ai/hL, PHI 52–62 days), buprofezin residues in whole fruit were 0.02, 0.04, 0.05, 0.05 mg/kg (n = 4).

GAP for pome fruit in New Zealand is for 3 spray applications at 0.013 kg ai/hL (PHI 56 days) and in trials from Australia matching this GAP (2–3× 0.013 kg ai/hL, PHI 52–62 days), buprofezin residues in whole fruit were < 0.01 (2), 0.02, 0.03 mg/kg (n = 4).

The Meeting noted that the individual datasets from Australia and New Zealand were too small to estimate a maximum residue level. As the GAPs were different, data could not be combined. The Meeting agreed that there was insufficient data to estimate a maximum residue level for pears.

Berries and other small fruits

Trials involving field-grown grapes were performed in Germany, France, Italy, USA, and Australia. Trials involving greenhouse-grown grapes were performed in Japan.

Trials performed in Germany, France and Italy did not match available GAPs for Switzerland or Italy.

Trials performed in the USA did not match the available GAP for the USA.

GAP for grapes in Australia consists of 2 applications at 0.013–0.026 kg ai/hL (PHI 56 days) and in field trials from Australia matching this GAP (2–3× 0.026 kg ai/hL, PHI 56–57 days), buprofezin residues in grapes were 0.02, 0.03, 0.07, 0.09, 0.19 mg/kg (n = 5).

GAP for grapes in New Zealand is for 2 spray applications at 0.013 kg ai/hL (no PHI, pre-flowering applications only) and in field trials from Australia matching this GAP (3× 0.013 kg ai/hL, pre-flowering), buprofezin residues found in grapes were < 0.01 (2) mg/kg (n = 2).

GAP for grapes in Japan is for 1–2 spray applications at 0.007–0.020 kg ai/hL (PHI 30 days) and in greenhouse trials from Japan matching this GAP (2× 0.013-0.020 kg ai/hL, PHI 30–31 days), buprofezin residues found in grapes were 0.18, 0.22, 0.28, 0.29 mg/kg (n= 4).

The Meeting noted that the individual datasets from Australia, New Zealand and Japan were too small to estimate a maximum residue level. As the GAPs were substantially different, the meeting decided that the data sets could not be combined. The Meeting therefore agreed that there was insufficient data available to estimate a maximum residue level for grapes.

Persimmons

Field trials involving persimmons were performed in Australia. GAP for persimmons in Australia is for 1–2 applications at 0.026 kg ai/hL (PHI 28 days) and in field trials from Australia matching this GAP (2× 0.026 kg ai/hL, PHI 28 days), buprofezin residues in whole fruits were 0.44 and 0.46 mg/kg (n = 2). The analytical method used to determine the residue values was insufficiently described and validated.

The Meeting agreed that there was insufficient data available to estimate a maximum residue level for persimmons.

Assorted tropical and subtropical fruits – inedible peel

Custard apple

Field trials involving custard apples were performed in Australia. GAP for custard apples in Australia is for 1–2 spray applications at 0.013–0.026 kg ai/hL (PHI 14 days) and in field trials from Australia matching this GAP (2× 0.024 kg ai/hL, PHI 14 days, buprofezin residues in whole fruits were 0.04 and 0.05 mg/kg (n = 2). Buprofezin residues in custard apple pulp were not available.

The Meeting agreed that there was insufficient data available to estimate a maximum residue level for custard apples.

Mangoes

Field trials involving mangoes were performed in Australia. GAP for mangoes in Australia is for 1–2 applications at 0.026 kg ai/hL (PHI 28 days) and in field trials from Australia matching this GAP (2× 0.025 kg ai/hL, PHI 28 days), buprofezin residues in whole fruits were < 0.01, < 0.01, 0.01, 0.03, 0.045 mg/kg (n = 5). Buprofezin residues determined in mango pulp from three of these trials were < 0.01, < 0.01, < 0.01 mg/kg (n = 3).

The Meeting estimated a maximum residue level of 0.1 mg/kg for buprofezin in mango whole fruit and estimated an STMR of 0.01 mg/kg and HR of 0.01 mg/kg for buprofezin in mango pulp.

Cucumbers

Trials involving field-grown cucumbers were performed in Spain, Greece and the USA. Trials involving indoor-grown cucumbers were performed in the UK, France, Italy, Spain, Australia and Japan.

Trials on field-grown cucumbers performed in Spain and Greece did not match the available GAPs from Spain, Greece, Italy and Portugal.

Trials on field-grown cucumbers performed in the USA did not match the available GAP from the USA.

GAP for cucumbers in Hungary is for applications at 0.25 kg ai/ha (PHI 3 days) and in indoor trials from the UK, France, Italy, and Spain matching this GAP (2× 0.20–0.28 kg ai/ha, PHI 3 days), buprofezin residues in whole fruit were < 0.01, 0.03 (3), 0.04 (2), 0.06, 0.09 mg/kg (n = 8) for SC formulations, and < 0.01, 0.03, 0.04, 0.10 mg/kg (n = 4) for WP formulations at the same locations. The Meeting noted that the residue populations corresponding to SC and WP formulations were from similar populations and as they were from the same location should be treated as replicates, i.e., only one residue should be selected per location. The Meeting therefore agreed to use only the dataset corresponding to the highest residue from each location. This resulted in the following dataset: < 0.01, 0.03, 0.03, 0.03, 0.04, 0.04, 0.06, 0.10 mg/kg (n = 8).

GAP for glasshouse cucumbers in Australia and New Zealand did not match with the indoor trials performed in Australia.

GAP for cucumbers in Japan is for 1–3 spray applications at 0.025 kg ai/hL (PHI 1 day) and in indoor trials from Japan matching this GAP (3× 0.020 kg ai/hL, PHI 1 day), buprofezin residues in whole fruit were 0.34 and 0.44 mg/kg (n = 2).

The Meeting noted that the dataset from Japan was too small to estimate a maximum residue level and agreed to use the dataset corresponding to Hungarian GAP.

The Meeting estimated a maximum residue level of 0.2 mg/kg for buprofezin in cucumber and estimated an STMR of 0.035 mg/kg and HR of 0.10 mg/kg for buprofezin in cucumber.

The Meeting withdrew its previous recommendation of 1 mg/kg for cucumber.

Fruiting vegetables other than cucurbits

Eggplant

Trials involving indoor-grown eggplants were performed in Spain.

GAP for eggplants in France is for spray applications at 0.13 kg ai/ha (PHI 5 days) and in trials from Spain matching this GAP (1× 0.14 kg ai/ha, PHI 4 days), buprofezin residues in whole fruit were 0.05 mg/kg (n = 1).

The Meeting agreed that the data were insufficient to estimate a maximum residue level for egg plants.

Tomatoes

Trials involving field-grown tomatoes were performed in Spain, Greece, France and the USA. Trials involving indoor-grown tomatoes were performed in the UK, France, Italy, Spain, New Zealand, and Japan.

Trials on field-grown tomatoes performed in Spain, Greece and France did not match the available GAPs from Spain, Greece, France, Italy and Portugal.

The GAP for tomatoes in the USA is for 1–2 applications at 0.28–0.43 kg ai/ha (PHI 7 days) and in trials on field-grown tomatoes from the USA matching this GAP (2× 0.42-0.43 kg ai/ha, PHI 7 days), buprofezin residues in whole fruit were 0.031 mg/kg (n = 1).

The GAP for tomatoes in Hungary is for spray applications at 0.13–0.25 kg ai/ha (PHI 3 days) and the GAP for Poland is for 2–4 spray applications at 0.012–0.025 kg ai/hL (PHI 3 days). In indoor trials from UK, France, Italy, and Spain matching this GAP (3× 0.23–0.27 kg ai/ha = 3×

0.025 kg ai/hL, PHI 3 days), buprofezin residues in whole fruit were 0.05, 0.12, 0.16, 0.17, 0.30, 0.35, 0.52 (2) mg/kg (n = 8) for the SC formulations and 0.13, 0.17, 0.24 mg/kg (n = 3) for the WP formulations applied at the same locations. The Meeting noted that the residue populations corresponding to SC and WP formulations were from similar populations and as they were from the same location should be treated as replicates, i.e., only one residue should be selected per location. The Meeting therefore agreed to use only the dataset corresponding to the highest residue from each location. This resulted in the following dataset: 0.05, 0.12, 0.16, 0.17, 0.30, 0.35, 0.52 (2) mg/kg (n = 8).

GAP for glasshouse tomatoes in New Zealand is for 1–2 applications at 0.013 kg ai/hL (PHI 3 days) and in indoor trials from New Zealand matching this GAP (0.012 kg ai/hL, PHI 4 days), buprofezin residues in whole fruit were 0.14 mg/kg (n = 1).

GAP for tomatoes in Japan is for 1–3 spray applications at 0.013–0.025 kg ai/hL (PHI 1 day) and in indoor trials from Japan matching this GAP (3× 0.025 kg ai/hL, PHI 1 day), buprofezin residues in whole fruit were 0.31 and 0.40 mg/kg (n = 2).

The Meeting noted that the individual datasets from New Zealand and Japan were too small to estimate a maximum residue level and agreed to use the dataset corresponding to Hungarian and Polish GAP.

The Meeting estimated a maximum residue level of 1 mg/kg for buprofezin in tomatoes and estimated an STMR of 0.24 mg/kg and HR of 0.52 mg/kg for buprofezin in tomatoes.

The Meeting confirmed its previous recommendation of 1 mg/kg for tomatoes.

Fate of residues in storage

Not applicable.

Fate of residues during processing

The Meeting received information on the fate of buprofezin under simulated processing conditions and on the fate of incurred residues of buprofezin during the processing of oranges, grapes, and tomatoes.

An aqueous solution of [phenyl-^{14}C]buprofezin was treated for 20 min at 90 °C at pH 4 (pasteurization), 60 minutes at 100 °C at pH 5 (brewing/baking/boiling), or for 20 minutes at 120 °C at pH 6 (sterilization). Degradation proceeded in the order pH 4 > pH 5 > pH 6 and 28.2%, 30.5%, 76% of the applied radioactivity remained as unchanged buprofezin after processing. Degradation proceeded via opening of the thiadiazinane ring to form thiobiuret (6.6–43%) followed by amide cleavage to produce isopropylphenylurea (5.3–31%) and aniline (7.2–18.9%) or replacement of the sulfur with oxygen to form biuret (< 4%).

The degradation products formed during simulated processing conditions are identical to the degradation products formed during hydrolysis in sterile water at low pH (pH 5), except that hydrolysis during simulated processing conditions proceeds further to aniline.

The degradation products isopropylphenylurea, biuret and thiobiuret were found in the rat metabolism study, but the degradation product aniline was not. Aniline is considered toxicologically relevant, but can come from sources other than buprofezin and could not therefore be included in a residue definition for risk assessment. Additional toxicological studies were available for biuret and thiobiuret and the Meeting considered these degradates toxicologically relevant.

In a processing study on tomatoes, where tomatoes were treated at 1× and 3× rate (3× 0.25 and 3× 0.75 kg ai/ha), biuret was not be detected in any samples. Thiobiuret was not be detected in the majority of samples, but was found in the 3× rate treatment at 0.02 mg/kg in two juice and two puree samples and at 0.01 mg/kg in one wet pomace and one canned tomato sample. The

concentration ratios buprofezin: isopropylphenylurea: thiobiuret were 14:0.5:1.0 and 6.5:1.0:1.0 for the juices sample, 26:10:1.0 and 20:13:1.0 for the puree samples, 110:14:1.0 for the wet pomace sample and 11:1.0:1.0 for the canned tomato sample. The ratios in juice and canned tomatoes resemble the ratios found in the simulated processing study at pH 6. Since biuret was not detected and the concentration level of thiobiuret was at maximum six times lower than the parent compound, the Meeting concluded that the residue definition for plant commodities is also suitable for the residues in processed plant commodities.

In the processed tomato commodities the isopropylphenylurea degradate was always present at levels lower than the parent. This was not the case in the processing study provided on grapes, where isopropylphenylurea was found at levels higher than the parent in white wine and grape juice. Since the level of isopropylphenylurea might be indicative for increased levels of thiobiuret, the Meeting considers additional quantitative data on thiobiuret in other processed commodities desirable.

Two processing studies were undertaken in which field treated oranges were processed into juice and wet or dry pomace. Calculated processing factors for buprofezin parent were 0.56, 0.58 for orange juice, 1.5 and 2.0 for wet pomace and 4.5 and 6.0 for dry pomace.

Fifteen processing studies were undertaken in which field treated grapes were processed into juice, white wine, red wine and raisins. Several processing studies were disregarded because residue levels in the raw agricultural commodity were near or below the LOQ and relevant processing factors could not be calculated. Calculated processing factors for buprofezin parent were 0.31 and 0.35 for grape juice, 0.51, 0.56, 0.69 and 0.78 for white wine, 0.52 for red wine, 1.0 and 1.7 for raisins.

Four processing studies were undertaken in which field or indoor treated tomatoes were processed into juice, canned tomatoes, puree, ketchup and wet/dry pomace. Calculated processing factors for buprofezin were 0.18, 0.2, 0.2, 0.21, 0.22, 0.22, 0.31, 0.42, 0.38, 0.75 for pasteurized tomato juice, 0.03, 0.09, 0.1, 0.11, 0.17, 0.19, 0.19, 0.2, 0.26, < 0.3 for canned whole tomatoes, 0.5, 0.71, 0.8, 0.81, 0.89, 0.9, 0.91, 0.95, 0.96, 1.0, 2.0 for tomato puree, 0.45, 0.47, 0.5, 0.5, 0.52, 0.67, 0.67, 0.69, 0.88, 1.2 for tomato ketchup, 2.3, 3.8, 4.1, 4.2, 5.2, 7.5 for wet tomato pomace, and 9.0, 15, 19, 20, 24, 40 for dry tomato pomace.

The Meeting considered the appropriate HR-P and STMR-P to be used in the livestock dietary burden calculation or dietary intake calculation. In the table below, relevant processing factors for citrus and tomatoes are summarized. The Meeting decided to extrapolate the processing factor for orange juice to citrus juice, to extrapolate the processing factor for canned tomatoes to peeled tomatoes and to extrapolate the processing factor for tomato puree to tomato paste.

Using the HR for tomatoes (0.52 mg/kg), the Meeting estimated HR-Ps for their processed commodities as listed below. Furthermore, using the STMRs for citrus whole fruit and tomatoes (0.23, 0.24 mg/kg, respectively), the Meeting estimated STMR-Ps for these commodities as listed below.

Codex Code	Commodity	Processing factors	Processing factor (median or best estimate)	STMR-P	HR-P
-	Citrus juice	0.56, 0.58	0.57	0.13	not applicable
AB0001-	Citrus pulp, dry	4.5, 6.0	5.25	1.2	not applicable
JF0448	Tomato juice	0.18, 0.2, 0.2, 0.21, 0.22, 0.22, 0.31, 0.42, 0.38, 0.75	0.22	0.053	not applicable
-	Tomato paste	0.5, 0.71, 0.8, 0.81, 0.89, 0.9, 0.91, 0.95, 0.96, 1.0, 2.0	0.9 [a]	0.22	not applicable

Codex Code	Commodity	Processing factors	Processing factor (median or best estimate)	STMR-P	HR-P
-	Tomato, peeled	0.03, 0.09, 0.1, 0.11, 0.17, 0.19, 0.19, 0.2, 0.26, < 0.3	0.17 [b]	0.041	0.088

[a] extrapolated from tomato puree

[b] extrapolated from canned tomatoes

The Meeting estimated an MRL of 2 mg/kg on a dry weight basis for Citrus pulp, dry.

Farm animal dietary burden

The Meeting estimated the dietary burden of buprofezin residues in farm animals from the diets listed in the Table of OECD Feedstuffs as published in Annex 6 of the 2006 JMPR Report[36]. Orange dry pomace was the only feedstuff identified as relevant to cattle. Poultry were not exposed to buprofezin through pesticide treated feed that was evaluated by the Meeting. A mean and maximum dietary burden of 0.40 ppm of dry matter diet was estimated for beef and dairy cattle in Australia as is shown in the table below.

Animal dietary burden for buprofezin, expressed as ppm of dry matter diet

	US-Canada		EU		Australia	
	max	mean	max	mean	max	mean
beef cattle	0.13	0.13	0.07	0.07	0.40 [a]	0.40 [a]
dairy cattle	0.13	0.13	0.26	0.26	0.40 [a]	0.40

[a] Highest mean and maximum beef or dairy cattle dietary burden suitable for maximum residue level and STMR estimates for mammalian meat and maximum residue level and STMR estimates for milk.

Farm animal feeding studies

The Meeting received a feeding study on lactating cows. Four groups of three lactating Holstein cows were dosed twice daily via gelatin capsules at levels of 0.0–5.0–15–50 ppm dry weight feed for 28 consecutive days. Taking the average body weight of 544 kg, this dose was equivalent to 0.0–0.22–0.66–2.2 mg ai/kg bw/d. Milk was collected throughout the study on days 2, 4, 7, 10, 14, 17, 21, 24 and 28 and tissues were collected on day 29 within 24 h after the last dose.

Residues of up to 0.02 mg/kg buprofezin were found in milk and residues of up to 0.12 mg/kg buprofezin were found in beef fat from cows dosed at the highest level.

Animal commodity maximum residue levels

In a feeding study where lactating cows were dosed at 5.0 and 15 ppm dry feed, no parent buprofezin residues were detected in tissues and milk. Therefore, no residues are to be expected in tissues and milk at the mean and maximum calculated dietary burden of 0.40 ppm.

The Meeting estimated a maximum residue level for buprofezin of 0.01* mg/kg for milks and 0.05* mg/kg for meat from mammals other than marine mammals and mammalian edible offal. The Meeting estimated STMRs and HRs of 0 mg/kg in milk, muscle, and edible offal of mammals.

[36] identical to OECD, series on testing and assessment number 64, series on pesticides number 32, ENV/JM/MONO(2006)32

FURTHER WORK OR INFORMATION

None required.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intakes (IEDI) for buprofezin was calculated from recommendations for STMRs for raw commodities in combination with consumption data for corresponding food commodities. The results are shown in Annex 3.

The International Estimated Daily Intakes (IEDI) of in the 13 GEMS/Food cluster diets, based on the estimated STMRs were in the range 0–9% of the maximum ADI of 0.009 mg/kg bw. The Meeting concluded that the long-term intake of residues of buprofezin from uses considered by the Meeting is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-term Intake (IESTI) for buprofezin was calculated for the food commodities for which STMRs or HRs were estimated and for which consumption data were available. The residue value for citrus was entered separately for orange, lemon, mandarin, and grapefruit. The results are shown in Annex 4.

The International Estimated Short-term Intake (IESTI) varied from 0–1% of the ARfD (0.5 mg/kg bw) for the general population. The IESTI varied from 0–3% of the ARfD for children 6 years and below. The Meeting concluded that the short-term intake of residues of buprofezin from uses considered by the Meeting is unlikely to present a public health concern.