BUPROFEZIN (173)

EXPLANATION

Buprofezin was first evaluated by the 1991 JMPR which established an ADI of 0-0.01 mg/kg bw and recommended temporary MRLs for oranges, cucumber and tomato pending the delivery of required information by 1995.

The 1995 JMPR reviewed the submitted information and concluded that the data were adequate to recommend MRLs for cucumbers and tomatoes but inadequate for citrus fruits, and recommended that the existing temporary MRL for oranges be withdrawn. It was further concluded that if citrus MRLs were contemplated in a future submission a citrus processing study, including analyses for the main residues identified in the metabolism study (e.g. buprofezin, metabolite A and the thiobiuret derivative unless it had been shown not to be formed during citrus metabolism) would be required, and experimental evidence that the thiobiuret does not occur during citrus metabolism would be desirable.

The 1995 JMPR also listed the following items as desirable.

1. Analysis of reserve cow liver and kidney samples from the ruminant metabolism studies for the presence of dihydroxybuprofezin, hydroxymethoxybuprofezin and the thiobiuret metabolite.

2. A conventional animal processing study to determine residues of buprofezin, *p*-hydroxybuprofezin and (in milk) *p*-acetamidophenol.

The 1998 Session of the CCPR noted that buprofezin would be reviewed by the 1999 JMPR and that data from additional residue trials on oranges would be submitted (ALINORM 99/24, para 76).

The Meeting received information on follow-up studies on metabolism in a lactating dairy cow and in lemons, GAP for fruits, vegetables and almonds, residue trials on oranges, a feeding study on dairy cows and a processing study on oranges. Further information was provided by the governments of Germany, The Netherlands, Poland and the UK.

METABOLISM AND ENVIRONMENTAL FATE

Animal metabolism

Huang and Smith (1997) re-examined residues in tissues from a previously reported study of metabolism in a lactating cow (Huang and Smith, 1995) in an effort to identify more of the residue in the liver, kidneys and milk. Despite extensive additional clean-up and attempts at identification no new metabolites were identified. The measured levels of metabolites differed slightly from the original (Table 2, page 28 of the 1995 Residue Evaluations). The large amount of unextractable and polar residue was taken as evidence of extensive conjugation.

Despite the extensive efforts no more than about 20-30% of the residue in the liver, kidneys and milk could be identified (Table 1) but the levels of individual unknown compounds were low, the highest being 0.07 mg/kg buprofezin equivalents in liver. An additional 13 compounds synthesised as possible metabolites were available as standards for identification, including 1-*tert*-butyl-3-isopropyl-5-phenyl-2-thiobiuret (BF-25, the ‘thiobiuret’ hydrolysis product) and 2-*tert*-butylimino-5-(4-

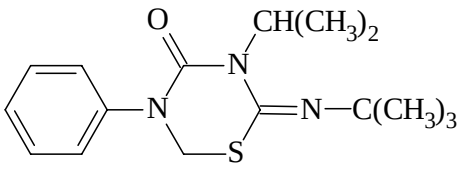
hydroxy-3-methoxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one (BF-27, the 'hydroxymethoxy-buprofezin' metabolite identified in rats). Neither was detected in the milk or tissues of the cow.

Table 1. Levels of [^{14}C]buprofezin and its metabolites in tissues and milk from a lactating cow (Huang and Smith, 1997).

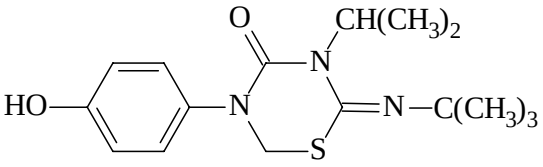
Compound	Liver		Kidneys		Milk	
	^{14}C as buprofezin, mg/kg	^{14}C as % of total in sample	^{14}C as buprofezin, mg/kg	^{14}C as % of total in sample	^{14}C as buprofezin, mg/kg	^{14}C as % of total in sample
Buprofezin		nd		nd	0.0007	2.2
BF2	0.13	10.9	0.07	18	0.0007	2.4
BF12	0.04	3.5	0.02	3.9	0.0011	3.6
BF13	0.03	2.5	0.01	3.1		nd
BF23	0.03	2.2	0.03	7.7	0.0041	13.7
Largest unknown	0.071	5.9	0.019	4.5	0.0009	2.9
Total identified	0.23	19.1	0.13	32.7	0.0066	21.9
TOTAL ^{14}C	1.21		0.41		0.03	

nd: not detected

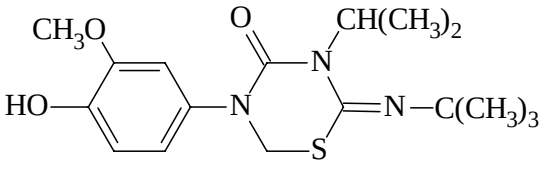
The structures of buprofezin, the bovine metabolites, and the comparison compounds BF-25 and BF-27 are shown below.



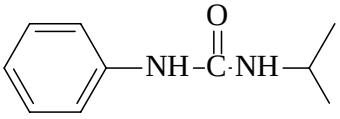
buprofezin



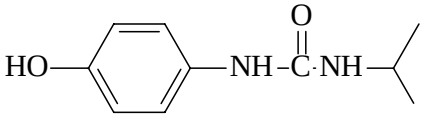
p-hydroxybuprofezin (BF-2)



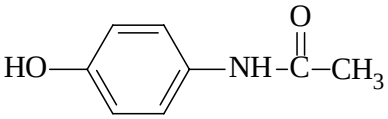
2-*tert*-butylimino-5-(4-hydroxy-3-methoxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one (BF-27)



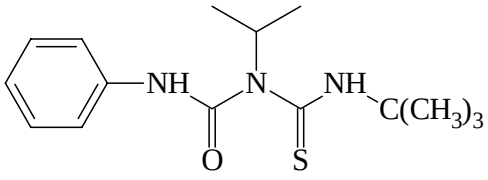
isopropylphenylurea (BF-12)



hydroxy-IPU (BF-13)



p-hydroxyphenylacetamide (BF-23)



1-*tert*-butyl-3-isopropyl-5-phenyl-2-thiobiuret (BF-25)

Plant metabolism

Smith (1997) re-examined the metabolites in extracts from the study of metabolism in lemons (Rieser and Smith, 1995) reviewed by the 1995 JMPR. The purpose was to determine the identity of the residue that produced 2-amino-2-methylpropyl 2-isopropyl-4-phenylallophanate (BF-26) on acid hydrolysis and to examine the extracts for the thiobiuret BF-25.

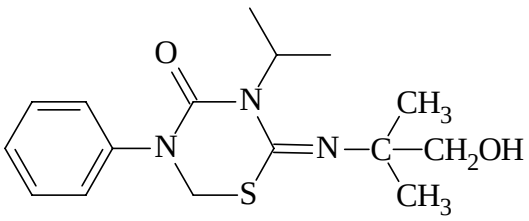
Extracts containing conjugates were cleaned up by preparative HPLC and examined by LC-MS. The molecular weight of the major conjugate was 484, which is consistent with 2-(2-hydroxy-1,1-dimethylethylimino)-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one (BF-4) linked to a hexose. After acetylation with acetic anhydride in pyridine the molecular weight of the conjugate was 652, demonstrating the incorporation of 4 acetyl groups, again consistent with a hexose conjugate of BF-4.

Hydrolysis of fractions containing conjugates was attempted with mild base and ∃-glucosidase but this released only 16-17% of the ^{14}C from which small amounts of BF-26 and BF-12 were identified. Enzymatic hydrolysis with ∀-glucosidase again released only a little of the ^{14}C from the conjugate. Metabolite BF-26 was identified in this hydrolysate. The original study had shown that strong acid hydrolysis of the main residue produced BF-26, BF-9 and BF-12, and mild acid hydrolysis of synthetic BF-4 produced BF-26 and BF-9.

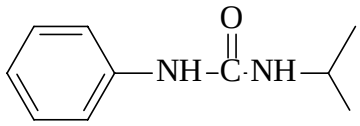
The evidence suggests that the main metabolite of buprofezin in lemons is a non-glucose hexose conjugate of BF-4. The BF-4 cannot be liberated from the conjugate without further degradation to BF-26, BF-9 and BF-12.

Unhydrolysed extracts from the lemons were examined for BF-25 but none was detected.

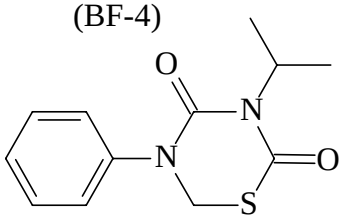
The structures of BF-4, the hydrolysis products, and BF-25 are shown below.



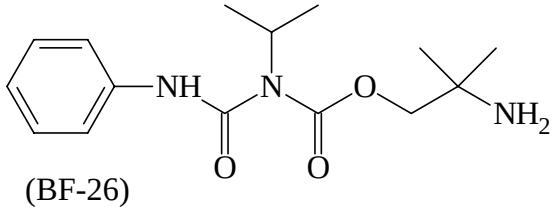
(BF-4)



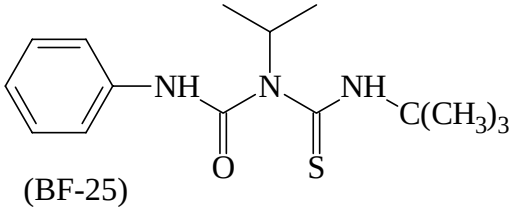
isopropylphenylurea (BF-12)



(BF-9)



(BF-26)



(BF-25)

METHODS OF RESIDUE ANALYSIS

Analytical methods

The Meeting received information on the GLC methods used to determine buprofezin and some metabolites in oranges and orange commodities in supervised trials, processing studies and animal feeding studies, and in kidneys, liver, fat, muscle and milk.

Barnard (1998) used analytical procedure NHH/089-01R to determine buprofezin, BF-9 and BF-12 in orange homogenate during freezer storage stability trials. The residues were extracted with acetone, the extract was acidified with hydrochloric acid and the acetone evaporated to leave an aqueous phase. This was washed with hexane, then neutralised with sodium hydroxide and pH 7 buffer, and the residues were extracted into dichloromethane. The dichloromethane was evaporated and the residue taken up in hexane and cleaned up on an aminopropyl solid-phase extraction cartridge to yield a fraction which contained the buprofezin for determination by GLC with an NPD. A separate portion of the original acetone extract was analysed for BF-9 and BF-12. The extract was diluted with pH 7 buffer and evaporated to leave an aqueous phase from which the residues were extracted with dichloromethane. The dichloromethane was evaporated and the analysis completed as above. Procedural recoveries at a fortification level of 0.1 mg/kg of 4 replicates of each analyte were buprofezin mean 80.5%, range 69-90%, BF-9 mean 92.3%, range 88-96%, and BF-12 mean 88.5%, range 78-97%.

Wilson (1997) used the same procedure for oranges, with an LOD for the three analytes of 0.01 mg/kg. Procedural recoveries at fortification levels of 0.1-0.5 mg/kg for each analyte (12 replicates) were buprofezin mean 85%, range 70-101%, BF-9 mean 87%, range 70-98%, and BF-12 mean 96%, range 75-109%.

Tymoschenko and Williams (1997) determined buprofezin residues in cattle tissues and milk. They extracted buprofezin and BF-12 from beef tissues with acetonitrile. This was diluted with hydrochloric acid and the residues partitioned into dichloromethane. The extract was evaporated and the residue dissolved in toluene for clean-up on an aminopropyl solid-phase extraction column. The residues in the eluate were determined by GLC with an MSD. The LODs for both buprofezin and BF-12 were 0.05 mg/kg. Recoveries from beef liver, kidneys, muscle and fat fortified at 0.05-0.20 mg/kg were buprofezin mean 96%, range 83-132%, and BF-12 mean 98%, range 83-135% (9 replicates). BF-2 was extracted with acetonitrile, the extract was washed with hexane, and the acetonitrile evaporated to dryness. The residue was taken up in pH 7 buffer and extracted with ethyl acetate. The ethyl acetate solution was evaporated to dryness, the residue was taken up in toluene, and the analysis completed as for buprofezin and BF-12. The LOD for BF-2 was 0.05 mg/kg. Recoveries from beef liver, kidneys, muscle and fat fortified at 0.05-0.20 mg/kg were mean 106%, range 82-118% (10 replicates).

Buprofezin and BF-12 were extracted from milk by mixture with acetonitrile, filtration, concentration, dilution with hydrochloric acid and partitioning into dichloromethane. The solvent was evaporated and the residue dissolved in toluene for clean-up and analysis as before. The LODs for buprofezin and BF-12 in milk were 0.01 mg/kg. Recoveries from milk fortified at 0.01 and 0.05 mg/kg were buprofezin mean 90%, range 69-112%, and BF-12 mean 91%, range 81-119% (16 replicates). To determine BF-23 in milk the mixture with acetonitrile was filtered, concentrated, diluted with sodium chloride, washed with hexane and partitioned into ethyl acetate. The ethyl acetate was evaporated, and the residue dissolved in toluene and cleaned up on a C-18 extraction column. The residue in the eluate was determined by GLC as before. The LOD for BF-23 was 0.01 mg/kg. Recoveries from milk fortified with BF-23 at 0.01 and 0.05 mg/kg were mean 98%, range 94-110% (4 replicates).

Neal (1997) described the method used for the determination of buprofezin, BF-9 and BF-12 in oranges, juice, oil and dry pulp. Oranges were extracted with acetone and the acetone evaporated to

leave an aqueous mixture which was acidified with hydrochloric acid. Hexane extracted BF-9 from the aqueous layer and the extract was cleaned up by Florisil column chromatography. Buprofezin and BF-12 were extracted from the remaining aqueous mixture with dichloromethane. The extract was combined with the cleaned-up BF-9 extract, the solvents evaporated and the residue dissolved in toluene before further clean-up on a solid-phase amino extraction column. The eluate was evaporated and the residue dissolved in toluene for analysis by GLC; the three analytes were readily separated. The initial extraction was modified for samples of juice, oil, and dry pulp. The LODs were 0.01 mg/kg for fruit and juice, 0.05 mg/kg for oil, and 0.1 mg/kg for dry pulp. Recoveries from whole oranges, oil, juice and dry pulp at spiking levels from 0.01 to 20 mg/kg were buprofezin mean 76%, range 41-103%, BF-9 mean 68%, range 46-88%, and BF-12 mean 84%, range 69-97% (9 replicates of each).

In the official method of The Netherlands (Ministry of Health, Welfare and Sport, 1996) buprofezin is determined in a multi-residue procedure by GLC with an ion-trap detector. The LOD is 0.05 mg/kg. The method produced good recoveries from various crop samples.

Stability of pesticide residues in stored analytical samples

Barnard (1998) determined the stability of buprofezin, BF-9 and BF-12 added to orange homogenate at 0.10 mg/kg in separate vials (10 g samples) and stored for 6 months at about -18°C (recorded daily maximum and minimum temperatures were mainly in the range -19°C to -11°C). Duplicate stored samples, a control and a procedural recovery sample were analysed by Method NHH/089-01R at each sampling.

The residues were apparently stable during the 6 months of storage (Table 2), but with the analytical error of the method at 0.1 mg/kg a 20-30% decrease would probably be needed to be noticeable.

Table 2. Percentage of buprofezin and metabolites remaining in fortified orange homogenate after storage at about -18°C for 6 months (Barnard, 1998). Results are not adjusted for recovery or control values.

Storage period	buprofezin		BF-9		BF-12	
	Stored sample, % of initial	Procedural recovery, %	Stored sample, % of initial	Procedural recovery, %	Stored sample, % of initial	Procedural recovery, %
0	86, 78	90	93, 84	92	93, 94	87
1 month	72, 71	69	92, 78	88	93, 89	78
3 months	75, 70	80	83, 99	96	97, 94	97
6 months	107, 77	91	74, 84	93	70, 75	92

Definition of the residue

The residue is defined as buprofezin, both for compliance with MRLs and for the estimation of dietary intake.

The log P_{ow} of 4.3 for buprofezin (JMPR residue evaluations, 1991) and the presence of buprofezin in body fat and milk fat but not in muscle or skimmed milk in a feeding study with dairy cows suggest solubility in fat. The Meeting agreed that buprofezin should be described as fat-soluble.

USE PATTERN

Details of the registered uses of buprofezin on citrus fruits in many countries were provided by the basic manufacturer, with copies of registered labels in some cases. Registered uses on vegetables in Europe, mainly glasshouse, were reported by national governments.

Table 3. Registered uses of buprofezin on citrus and other fruits, vegetables, and almonds.

Crop	Country	Form	Application				PHI, days
			Method	Rate, kg ai/ha	Spray conc. kg ai/hl	Number	
Almond	Greece	25% WP	foliar		0.018		28
Aubergine	UK	250 g/l SC	foliar to run-off		0.0075	g 2	3
Citrus	Argentina	25% WP	foliar		0.013		14
Citrus	Brazil	25% WP	foliar		0.025-0.05		7
Citrus	China	25% WP	foliar		0.013-0.025	2	35
Citrus	Greece	25% WP	foliar		0.013-0.050		28
Citrus	Guatemala	25% WP	foliar	0.6			14
Citrus	Italy	40% SC	foliar		0.024-0.032		7
Citrus	Italy	25% WP	foliar		0.025-0.038		7
Citrus	Jordan	25% WP	foliar		0.013-0.038		14
Citrus	Lebanon	25% WP	foliar		0.013-0.038		14
Citrus	Portugal	25% WP	foliar		0.013		7
Citrus	South Africa	50% WP	foliar		0.015	2	45
Citrus	Spain	25% WP	foliar		0.01-0.013		7
Citrus	UAE	25% WP	foliar		0.013-0.038		14
Citrus	Uruguay	25% WP	foliar	0.25-1.0			14
Citrus except mandarin	Japan	25% WP	foliar		0.017-0.025	3	45
Courgette	Netherlands	250 g/l EC	foliar	0.037-0.11	0.0075	g 2	3
Courgette	Netherlands	250 g/l EC	foliar	0.030-0.060	0.0075	2	3
Cucumber	Belgium	250 g/l SC	foliar		0.0075	g	3
Cucumber	Germany	250 g/l SC	foliar	0.045-0.09	0.0075	g 3	3
Cucumber	Greece	25% WP	foliar		0.010-0.015	g	7
Cucumber	Netherlands	250 g/l EC	foliar	0.037-0.11	0.0075	g 2	3
Cucumber	Poland	25% WP	foliar	0.036-0.50	0.012-0.025	2-4	3
Cucumber	Switzerland	25 % WP	foliar		0.013	g	3
Cucumber	UK	250 g/l SC	foliar to run-off		0.0075	g 8	3
Egg plant	Netherlands	250 g/l EC	foliar	0.037-0.11	0.0075	g 2	3
Egg plant	Poland	25% WP	foliar	0.036-0.50	0.012-0.025	2-4	3

Crop	Country	Form	Application				PHI, days
			Method	Rate, kg ai/ha	Spray conc. kg ai/hl	Number	
Gherkin	Germany	250 g/l SC	foliar	0.045-0.09	0.0075	g 3	3
Gherkin	Netherlands	250 g/l EC	foliar	0.030-0.060	0.0075	2	3
Gherkin	Netherlands	250 g/l EC	foliar	0.037-0.11	0.0075	g 2	3
Grapes	See Vines						
Mandarin	Japan 🗎	25% WP	foliar		0.017-0.025	3	14
Melons	Netherlands	250 g/l EC	foliar	0.037-0.11	0.0075	g 2	3
Melons	Switzerland 🗎	25 % WP	foliar		0.013	g	3
Olive	Greece 🗎	25% WP	foliar		0.019-0.025		40
Peach	Greece 🗎	25% WP	foliar		0.025		14
Peppers	Switzerland 🗎	25 % WP	foliar		0.013	g	3
Peppers, sweet	Netherlands	250 g/l EC	foliar	0.037-0.11	0.0075	g 2	3
Peppers, sweet	Poland	25% WP	foliar	0.036-0.50	0.012-0.025	2-4	3
Peppers, sweet	UK 🗎	250 g/l SC	foliar to run-off		0.0075	g 2	3
Tomato	Belgium 🗎	250 g/l SC	foliar		0.0075	g	3
Tomato	Germany	250 g/l SC	foliar	0.045-0.09	0.0075	g 3	3
Tomato	Greece 🗎	25% WP	foliar		0.010-0.015	g	7
Tomato	Netherlands	250 g/l EC	foliar	0.037-0.11	0.0075	g 2	3
Tomato	Poland	25% WP	foliar	0.036-0.50	0.012-0.025	2-4	3
Tomato	Switzerland 🗎	25 % WP	foliar		0.013	g	3
Tomato	UK 🗎	250 g/l SC	foliar to run-off		0.0075	g 8	3
Vines	Switzerland 🗎	25 % WP	foliar	0.25			

g: glasshouse use
🗎: label (and English translation) provided.

RESIDUES RESULTING FROM SUPERVISED TRIALS

The Meeting received information on field trials on oranges in Italy and Spain and on a feeding study with lactating dairy cows.

Where residues were not detected they are recorded in the Tables as below the limit of determination (LOD), e.g. <0.01 mg/kg. Residues, application rates and spray concentrations have generally been rounded to 2 significant figures or, for residues near the LOD, to 1 significant figure. Although trials included control plots, no control data are recorded in the tables except where residues in control samples exceeded the LOD. Residues are not corrected for recoveries.

Buprofezin sprays were applied to orange orchards in Italy and Spain with backpack lance sprayers that were calibrated before each application. Plot sizes were 340-450 m^2 or 3 rows each of 5 trees. Field samples consisted of 12-15 oranges (>2 kg) taken from the middle 3 trees of the central row. Samples were stored frozen until analysis for about 120-130 days.

Table 4. Buprofezin and metabolite residues in oranges resulting from supervised trials in Italy and Spain. Residues in field samples from replicate plots in one trial are shown separately. Whole oranges analysed. Double-underlined residues are from treatments according to GAP and were used to estimate a maximum residue level.

Country (location), year (variety)	Form	kg ai/ha	kg ai/hl	No.	PHI, days	Residue, mg/kg			Ref
						buprofezin	BF-9	BF-12	
Spain (Ayamonte), 1997 (Salustiano)	WP	0.26	0.013	1	7	0.06 0.04	<0.01 (2)	<0.01 (2)	NHH/089-01
Spain (Ayamonte), 1997 (Salustiano)	WP	0.52	0.026	1	7	0.13	<0.01	<0.01	NHH/089-01
Spain (Villaverde del Rio), 1997 (Navelino)	WP	0.26	0.013	1	7	0.07 0.06	<0.01 (2)	<0.01 (2)	NHH/089-02
Italy (Lentini), 1997 (Moro)	WP	0.51	0.025	1	7	0.26 0.26	<0.01 (2)	<0.01 (2)	NHH/089-03
Italy (Lentini), 1997 (Moro)	WP	1.0	0.051	1	7	0.43	<0.01	<0.01	NHH/089-03
Italy (Catania), 1997 (Tarocco)	WP	0.51	0.025	1	7	0.24 0.18	<0.01 (2)	<0.01 (2)	NHH/089-04

Groups of 3 lactating Holstein dairy cows (each animal weighing 370-699 kg) were dosed twice daily by gelatin capsule with 119, 357 or 1190 mg buprofezin per cow per day, equivalent to 5, 15 or 50 ppm in the feed, for 28 days (Tymoschenko and Williams, 1997). The animals consumed 17.4-28.2 kg feed/day (range of means), of which 85.6% was dry matter. Milk was collected regularly for analysis. On day 29 all the animals were slaughtered and liver, kidneys, perirenal fat and hindquarter muscle were analysed. Muscle and kidneys were dissected free from fat, and fat free from connective tissue, before analysis (Helsten, 1997).

The residues in milk are shown in Table 5. Buprofezin itself was detected only at the highest feeding level and in only 1 of the 3 animals, with the first detection on day 2. When day 28 milk was separated into skimmed milk and cream no residues were detected in the skimmed milk, but buprofezin was found in the cream from cows in the two higher dose groups.

BF-23 was detected in milk from control animals on days 24 and 28 as well as from cows in the 15 and 50 ppm groups. The residues were all 0.01 mg/kg, suggesting possible contamination. BF-23 is the analgesic acetaminophen or paracetamol, so contamination from other sources is possible. BF-12 was not detected in milk, skimmed milk or cream.

The residues in the tissues are shown in Table 6. BF-12 and BF-2 were not detected in any sample. Buprofezin was detected in the liver of one animal from the 50 ppm dose group at the LOD and in the fat of the 3 animals from the same group at 0.07, 0.11 and 0.12 mg/kg.

The intervals between sampling and analysis were 120 days for milk, 160 days for tissues, and 220 days for cream and skimmed milk. Information on the freezer storage stability of buprofezin

and its metabolites in animal commodities is needed to validate the study. Tymoschenko and Williams (1997) refer to such a study, but it was not available to the Meeting.

Table 5. Residues of buprofezin, BF-12 and BF-23 in milk collected during 28 days from cows dosed at 119, 357 or 1190 mg buprofezin per cow per day, equivalent to 5, 15 or 50 ppm in the diet (Tymoschenko and Williams, 1997). Residues are recorded for individual animals. Blank cells in the table denote no analyses.

Day	Residues, mg/kg								
	Buprofezin			BF-12			BF-23		
	5 ppm	15 ppm	50 ppm	5 ppm	15 ppm	50 ppm	5 ppm	15 ppm	50 ppm
1			<0.01 (3)			<0.01 (3)			
2			<0.01 (2) 0.02			<0.01 (3)			
4			<0.01 (2) 0.01			<0.01 (3)			
7			<0.01 (2) 0.01			<0.01 (3)			
10			<0.01 (2) 0.02			<0.01 (3)			
14			0.01 <0.01 0.02			<0.01 (3)			
17			<0.01 (2) 0.01			<0.01 (3)			
21			<0.01 (2) 0.02			<0.01 (3)			
24	<0.01 (3)	<0.01 (3)	<0.01 (2) 0.01	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3) c	0.01 (2) <0.01 c	0.01 <0.01 (2) c
28	<0.01 (3)	<0.01 (3)	<0.01 (2) 0.01	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3) c	<0.01 (3) c	0.01 <0.01 (2) c
Skimmed milk 28 days	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)	<0.01 (3)			
Cream, 28 days	<0.01 (3)	0.01 (3)	0.03 (2) 0.05	<0.01 (3)	<0.01 (3)	<0.01 (3)			

c: residues of BF-23 at 0.01 mg/kg were reported in the milk from control animals in all groups on days 24 and 28

Table 6. Residues of buprofezin and BF-12 and BF-23 in tissues from the cows of Table 5 slaughtered on day 29 (Tymoschenko and Williams, 1997). Residues are recorded for individual animals.

Sample	Residues, mg/kg, of buprofezin and metabolites in tissues								
	Buprofezin			BF-12			BF-2		
	5 ppm	15 ppm	50 ppm	5 ppm	15 ppm	50 ppm	5 ppm	15 ppm	50 ppm
Liver	<0.05 (3)	<0.05 (3)	<0.05 (2) 0.05	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)
Kidneys	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)
Fat, perirenal	<0.05 (3)	<0.05 (3)	0.11 0.07 0.12	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)
Muscle	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)	<0.05 (3)

Table 7. Interpretation table for buprofezin residues in oranges from the trials in Table 4 and from the 1991 and 1995 evaluations. GAP and trial conditions are compared for treatments considered valid for maximum residue level and STMR estimations.

Crop	Country	Use pattern				Trial[1]	buprofezin
		kg ai/ha	kg ai/hl	No of appl.	PHI days		mg/kg
Citrus	Italy GAP		0.025-0.038		7		
Orange	Italy trial	1.0	0.051	1	7	NHH/089-03	0.43
Orange	Italy trial	0.51	0.025	1	7	NHH/089-03	0.26
Orange	Italy trial	0.51	0.025	1	7	NHH/089-04	0.24
Orange	Spain trial	1	0.025	1	7	JMPR 1995	0.06
Orange	Spain trial	1	0.025	1	7	JMPR 1995	0.03
Orange	Spain trial	1	0.025	1	7	JMPR 1995	0.03
Orange	Spain trial	0.52	0.026	1	7	NHH/089-01	0.13
Citrus	Spain GAP		0.010-0.013	1	7		
Orange	Spain trial	0.26	0.013	1	7	NHH/089-01	0.06
Orange	Spain trial	0.26	0.013	1	7	NHH/089-02	0.07
Citrus	South Africa GAP		0.015	2	45		
Orange	South Africa trial	2.25 g/tree	0.015	2	42	JMPR 1991	0.02

[1]JMPR 1995: Residue Evaluations, 1995, Buprofezin Table 8.
JMPR 1991: Residue Evaluations, 1991, Buprofezin Table 4.

Residues in animal commodities

When treated oranges are processed buprofezin residues find their way into orange pulp, which is used as animal feed. The Meeting estimated the dietary burden of residues for cattle using the diets in Appendix IX of the FAO Manual. The estimated dietary burden for beef and dairy cattle calculated from the maximum residue level in the feed item is equivalent to 0.45 ppm in the diet (Table 8) and is suitable for estimating maximum residue levels for the animal commodities. A similar calculation from the STMR for processed dry orange pulp (0.27 mg/kg) produces a dietary level of 0.059 ppm and is suitable for estimating STMR levels for animal commodities.

Table 8. Estimated dietary burden for beef and dairy cattle calculated from maximum residues in processed dry orange pulp and standard animal diets. DM is dry matter. MaxRes/DM is the maximum residue expressed on a dry-matter basis.

Commodity	MaxRes, mg/kg	DM, %	MaxRes/DM, mg/kg	% in diet		Residue in diet, ppm	
				Beef cattle	Dairy cattle	Beef cattle	Dairy cattle
Processed dry orange pulp	2.05	91	2.25	20%	20%	0.45	0.45

FATE OF RESIDUES IN STORAGE AND PROCESSING

In processing

Information was provided on the effects of processing oranges to juice, oil and dry pulp on the residue levels of buprofezin and its metabolites.

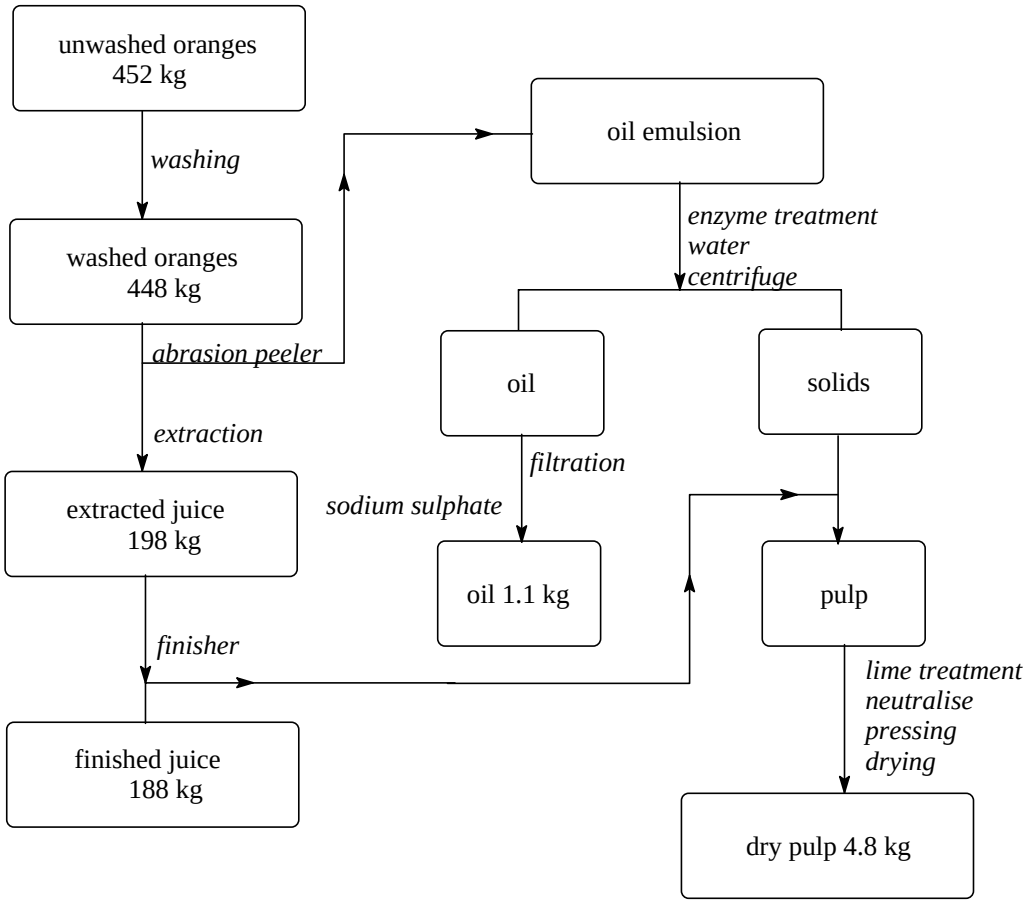
Neal (1997) treated Valencia orange trees twice (60 days interval) with buprofezin (Applaud® 70WP) at an exaggerated rate of 11 kg ai/ha (5 times the proposed maximum GAP rate) in California, USA, and harvested 460 kg oranges 66 days after the final treatment for processing.

The oranges were washed, abrasion peeled, and juiced, and the oil was extracted and the pulp dried in a simulated commercial process (Figure 1). The results are shown in Table 9, in which 'grower' refers to fruit sent directly from the orchard for analysis, and 'processor' to fruit sampled from the bulk delivered to the processor and representing the unwashed fruit entering the process. Freezer storage periods before analysis were fruit 149 days, oil 455 days, juice 147 days, and pulp 464 days. The freezer storage study on orange homogenate (Barnard, 1998) demonstrated adequate storage stability for 6 months but longer testing (possibly on related commodities) is needed to validate the processing study for oil and pulp.

Table 9. Residues of buprofezin and metabolites in oranges treated at an exaggerated rate of 11 kg ai/ha and in fractions produced during simulated commercial processing to oil, juice and dry pulp (Neal, 1997).

Commodity	Buprofezin, mg/kg, mean and (replicates)	BF-9, mg/kg, mean and (replicates)	BF-12, mg/kg, mean and (replicates)
Whole fruit (grower)	0.45 (0.59 0.43 0.35)	<0.01 (<0.01 (3))	0.01 (0.013 <0.01 0.016)
Whole fruit (processor)	0.27 (0.29 0.24 0.28)	<0.01 (<0.01 (3))	<0.01 (<0.01 (3))
Oil	11.6 (12.2 11.4 11.1)	0.17 (0.17 0.17 0.17)	<0.05 (<0.05(3))
Juice	0.049 (0.061 0.036 0.050)	0.01 (0.029 <0.01 <0.01)	0.01 (0.022 <0.01 <0.01)
Dry pulp	1.11 (0.98 1.11 1.23)	<0.1 (<0.1 (3))	0.14 (0.16 0.14 0.13)

Figure 1. Processing of oranges (Neal, 1997).



Residues in the edible portion of food commodities

Buprofezin residues in orange pulp, peel and whole fruit were recorded in a number of trials in the 1991 and 1995 residue evaluations, from which the ratio of the residues in the pulp to those in the whole fruit can be calculated. The mean ratio is 0.17 (Table 10).

Table 10. Ratio of residues in pulp to those in whole oranges from data recorded in the 1991 and 1995 JMPR residue evaluations.

Buprofezin residues in oranges, mg/kg		Ratio pulp/fruit	Trial	Ref.
whole fruit	pulp			
0.06	0.02	0.33	Spain 1994	JMPR 1995
1.12	0.0675	0.06	South Africa 1989	JMPR 1991
0.33	0.04	0.12	South Africa 1989	JMPR 1991
0.44	0.07	0.16	South Africa 1989	JMPR 1991
0.1975	0.0375	0.19	South Africa 1989	JMPR 1991
0.205	0.05	0.24	South Africa 1989	JMPR 1991
0.84	0.12	0.14	Portugal, 1987	JMPR 1991
0.76	0.12	0.16	Portugal, 1987	JMPR 1991
0.25	0.02	0.08	Portugal, 1987	JMPR 1991
		Mean 0.17		

NATIONAL MAXIMUM RESIDUE LIMITS

The Meeting was informed that the following national MRLs had been established for buprofezin.

Country	MRL, mg/kg	Commodity
Netherlands[1]	0.2	Cucurbits (with edible peel)
Netherlands	0.2	Cucurbits (with inedible peel)
Netherlands	0*(0.05)	other food commodities
Poland	0.5	fruits and vegetables
Poland	0.1	other plant commodities

[1]Netherlands residue definition: buprofezin, parent compound, expressed as buprofezin

APPRAISAL

Buprofezin was first evaluated by the 1991 JMPR, which recommended a temporary MRL for oranges pending the delivery of required information by 1995.

The 1995 JMPR concluded that the available data were inadequate for citrus fruits and recommended that the existing temporary MRL for oranges be withdrawn. The 1995 Meeting also concluded that if citrus MRLs were contemplated in a future submission a citrus processing study, including analyses for the main metabolites, would be required, and experimental evidence that the thiobiuret metabolite does not occur during citrus metabolism would be desirable.

The 1995 JMPR also listed the following items as desirable.

1. Analysis of reserve cow liver and kidney samples from the ruminant metabolism studies for the presence of dihydroxybuprofezin, hydroxymethoxybuprofezin and the thiobiuret metabolite.

2. A conventional animal processing study to determine residues of buprofezin, *p*-hydroxybuprofezin and (in milk) *p*-acetamidophenol.

The Meeting received follow-up studies on metabolism in a lactating dairy cow and in citrus fruit, information on GAP and residue trials on citrus fruits, a feeding study on dairy cows and a processing study on citrus fruits. Further information was provided by Germany, The Netherlands, Poland and the UK.

Liver, kidney and milk samples from the previously reported study of metabolism in a lactating dairy cow were re-examined to identify more of the residue. Despite extensive additional clean-up and identification work no new metabolites were identified. The large amount of unextractable and polar residue was taken as evidence of extensive incorporation. No more than about 20-30% of the residue in the liver, kidneys and milk could be identified, but only in the liver did an unknown (at 0.07 mg/kg) exceed 0.05 mg/kg in a tissue, i.e. the levels of individual unknowns were low.

Additional standard compounds were available in the follow-up study, including 1-*tert*-butyl-3-isopropyl-5-phenyl-2-thiobiuret, the ‘thiobiuret’ hydrolysis product BF-25, and 2-*tert*-butylimino-5-(4-hydroxy-3-methoxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one, the ‘hydroxymethoxybuprofezin' metabolite BF-27 which was identified in rats. Neither was detected in the cow tissues or milk. The remaining possibility, the ‘dihydroxybuprofezin’ metabolite, was not included in the study but it is closely related to metabolite BF - 27, so desirable information point 1 (*analysis of reserve cow*

liver and kidney samples from the ruminant metabolism trials on the presence of the dihydroxybuprofezin, hydroxymethoxybuprofezin and the thiobiuret metabolites) is substantially satisfied.

Metabolites in extracts from the study of metabolism in lemons were re-examined to determine the identity of the residue that produced 2-amino-2-methylpropyl 2-isopropyl-4-phenylallophanate (BF-26) on acid hydrolysis and to check primary extracts for the presence of 1-*tert*-butyl-3-isopropyl-5-phenyl-2-thiobiuret (BF-25). Various enzyme hydrolyses were tried but released little of the bound ^{14}C. The evidence strongly suggests that the main metabolite is a non-glucose hexose conjugate of 2-(2-hydroxy-1,1-dimethylethylimino)-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one (BF-4). The BF-4 cannot be liberated from the conjugate without further degradation to BF-26, 3-isopropyl-5-phenyl-1,3,5-thiadazinane-2,4-dione (BF-9) and 1-isopropyl-3-phenylurea (BF-12). Unhydrolysed extracts from the lemons were examined by TLC for metabolite BF-25 (the 'thiobiuret' metabolite), but none was detected. This satisfies the request from the 1995 JMPR for *experimental evidence that the thiobiuret metabolite does not occur during citrus metabolism.*

Analytical methods for residues of buprofezin and some metabolites in oranges, orange commodities and kidney, liver, fat, muscle and milk were reported. The methods were used in the supervised trials, processing studies and animal feeding studies.

Samples were extracted and the extracts cleaned up by solvent partition and an aminopropyl solid-phase extraction cartridge, and analysed by GLC with an NPD. The exact procedure was tailored to the sample. LODs were in the range 0.01 to 0.1 mg/kg. Recoveries were usually in the 70-100% range, but individual recoveries dropped below 50% for residues in orange processing fractions.

Buprofezin, BF-9 and BF-12 added separately at 0.1 mg/kg to orange homogenate did not decrease perceptibly when stored for 6 months at approximately -18°C, but with the analytical error at levels of 0.1 mg/kg, a decrease of 20-30% would be necessary to be discernible.

The Meeting was informed that the results of a 1-year freezer storage stability study for residues in milk, fat and liver would be available in the year 2000.

The Meeting received information on registered uses of buprofezin on citrus fruits in 14 countries. It is usually applied as a foliar spray in the concentration range of 0.013-0.038 kg ai/hl, with typical intervals of 7-14 days specified before harvest, although South Africa has a 45 days PHI. Labels for uses on citrus were available from Italy, South Africa and Spain.

Supervised residue trials with buprofezin on oranges were reported from Spain and Italy, which included analyses for BF-9 and BF-12 as well as buprofezin.

In Spain buprofezin is registered for application to citrus trees with a spray concentration of 0.010-0.013 kg ai/hl and harvest 7 days later. Buprofezin residues were 0.06 and 0.07 mg/kg in oranges from 2 Spanish trials complying with GAP.

Buprofezin is registered for use on citrus trees in Italy at a spray concentration of 0.025-0.038 kg ai/hl. A ±30% tolerance on 0.038 kg ai/hl extends from 0.026 to 0.049 kg ai/hl so the trials, at 0.025 and 0.051 kg ai/hl, were at the margins of the allowable range of application rates. Trials on oranges in Italy and Spain complying with Italian GAP, including 3 trials reported in the 1995 Residue Evaluations, produced residues of 0.03, 0.03, 0.06, 0.13, 0.24, 0.26 and 0.43 mg/kg.

An orange trial in South Africa, reported in the 1995 Residue Evaluations, where buprofezin was used according to South African GAP (2 applications of 0.015 kg ai/hl, 45 days PHI) produced residues of 0.02 mg/kg.

In summary, buprofezin residues in 10 trials according to GAP in Italy, Spain and South Africa in rank order, median underlined, were 0.02, 0.03, 0.03, 0.06, 0.06, 0.07, 0.13, 0.24, 0.26 and 0.43 mg/kg. The STMR for whole oranges is 0.065 mg/kg.

The mean processing factor for orange pulp was 0.17, calculated from data in the 1991 and 1995 Residue Evaluations. The estimated STMR for buprofezin in the edible portion of oranges then becomes $0.065 \times 0.17 = 0.011$ mg/kg.

The Meeting estimated a maximum residue level of 0.5 mg/kg and an STMR of 0.011 mg/kg for buprofezin in oranges.

In a farm animal feeding study, dairy cows were dosed with buprofezin at the equivalent of 5, 15 and 50 ppm in the feed for 28 days. Buprofezin itself was detected in milk only at the highest feeding level and in only 1 of the 3 animals with the first detection occurring on day 2 and continuing only in this animal throughout the study. When day 28 milk was separated into skimmed milk and cream no residues were detected in the skimmed milk, but buprofezin residues were present in the cream from cows in the 15 and 50 ppm feeding groups. Metabolite BF-12 was not detected in milk, skimmed milk or cream. Metabolite BF-23 (acetaminophen or paracetamol) was detected in milk on days 24 and 28 both in samples from some treated groups and control samples, all at 0.01 mg/kg, suggesting contamination.

Buprofezin was detected at the LOD in the liver of one animal from the 50 ppm feeding group and at 0.07, 0.11 and 0.12 mg/kg in the perirenal fat of the 3 animals of the 50 ppm feeding group. The metabolites BF-12 and 2-*tert*-butylimino-5-(4-hydroxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one (*p*-hydroxy-buprofezin, BF-2) were not detected in any tissue. This provides the desirable information item 2 from the 1995 JMPR *a conventional animal transfer study in which residues of buprofezin, p-hydroxybuprofezin and (in milk) p-acetamidophenol are determined*.

The residue is defined as buprofezin, which is suitable both for compliance with MRLs and for the estimation of dietary intake. The buprofezin log P_{ow} of 4.3 (JMPR Residue Evaluations, 1991) and the presence of buprofezin in tissue fat and milk fat but not in muscle or skimmed milk in the dairy cow feeding study imply fat-solubility.

The Meeting agreed that buprofezin should be described as fat-soluble.

The Meeting received information on the fate of buprofezin and metabolites BF-9 and BF-12 during the processing of oranges to juice, oil and dry pulp. Oranges were harvested 66 days after treatment with buprofezin at an exaggerated rate (11 kg ai/ha). Fruit and juice were stored frozen for approximately 5 months before analysis, a period covered by the storage stability study on orange homogenate. Oil and dry pulp were stored for approximately 15 months before analysis without supporting evidence of stability for this period.

The calculated processing factors for buprofezin residues were oil 43, juice 0.18, and dry pulp 4.1. The residues of the metabolites were below or about the LOD (0.01 mg/kg) in the fruit so it is not possible to estimate processing factors, but BF-9 tended to be concentrated in the oil, while BF-12 was concentrated in the dry pulp.

The orange processing study meets the requirement of the 1995 JMPR for a citrus processing study that includes the main residues identified in the metabolism study.

From these processing factors and the STMR for whole oranges (0.065 mg/kg) the Meeting estimated an STMR for orange juice of 0.012 mg/kg and for dry orange pulp of 0.27 mg/kg.

Dry processed orange pulp is an animal feeding material that may represent 20% of the diet for dairy and beef cattle. The estimated maximum dietary burden of buprofezin for beef and dairy

cattle (on the basis of the estimated maximum residue level for oranges, 0.5 mg/kg, and the processing factor for dry pulp, 4.1) was equivalent to 0.45 ppm in the diet. The lowest feeding level in the dairy cow study was 5 ppm, which did not produce detectable levels of buprofezin in the tissues or milk, so the Meeting estimated maximum residue levels at or about the LOD for buprofezin residues in cattle milk (0.01* mg/kg), cattle meat (0.05* mg/kg), cattle kidney (0.05* mg/kg) and cattle liver (0.05* mg/kg), but could not recommend these maximum residue levels as being suitable for use as MRLs until the stabilities of the residues during freezer storage are confirmed.

The STMR for dry processed orange pulp is 0.27 mg/kg and the corresponding dietary burden for cattle, 0.059 ppm, is suitable for estimating STMRs for animal commodities.

The residues were below LOD in the muscle and kidney at the 5, 15 and 50 ppm feeding levels, and in the liver, fat and milk at the 5 and 15 ppm levels. Residues of buprofezin were detected in the fat and liver at the 50 ppm level and in milk fat at the 15 and 50 ppm levels. The Meeting noted that the dietary burden of 0.059 ppm was much less than the lowest feeding level where no residues were detected and, as an approximation for extrapolation, assumed proportionality between tissue level and dietary intake.

STMR (animal commodity) = LOD × (STMR dietary burden) ÷ (feeding level)

STMR for meat = 0.05 × 0.059 ÷ 50 = 0.00006 mg/kg (no detections at 50 ppm feeding level)

The same applies to kidney. For liver and milk there were no detections at the 15 ppm feeding level, so calculated STMRs are 0.0002 and 0.00004 mg/kg respectively. The Meeting regarded these calculated values as effectively zero and estimated STMRs of 0 mg/kg for meat, kidney, liver and milk, but the STMRs would not apply until MRLs are recommended.

RECOMMENDATIONS

On the basis of the data from supervised trials the Meeting estimated the maximum residue and STMR levels listed below. The maximum residue level is recommended for use as MRLs.

Definition of the residue (for compliance with MRLs and for estimation of dietary intake): buprofezin.

The residue is fat-soluble.

Commodity		MRL, mg/kg		STMR
CCN	Name	New	Previous	mg/kg
JF 0004	Oranges, Sweet, Sour	0.5	0.3 T[1]	0.011
	Orange juice			0.012
	Orange pulp, dry			0.27

[1]Withdrawal recommended by 1995 JMPR

FURTHER WORK OR INFORMATION

Desirable

Information is needed on the freezer storage stability of residues in animal commodities to validate the dairy cow feeding study. The Meeting was informed that the results of a 1-year freezer storage stability study for milk, fat and liver would be available in the year 2000.

DIETARY RISK ASSESSMENT

Chronic intake

A revised MRL for buprofezin in oranges has been recommended in addition to previous recommendations. STMR levels have been estimated for oranges and some processed commodities. The other values (2) used for the intake estimation are previously established CXLs.

The dietary intake of buprofezin is presented in Annex III. Estimated dietary intakes for buprofezin for the 5 GEMS/Food regional diets were in the range of 2-10% of the ADI. The Meeting concluded that intake of buprofezin resulting from its uses that have been considered by the JMPR is unlikely to present a public health concern.

Acute intake

The Meeting concluded that an acute RfD for buprofezin is unnecessary. This conclusion was based on a determination that the pesticide is unlikely to present an acute toxicological hazard, and residues are therefore unlikely to present an acute risk to consumers.

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