

BUPROFEZIN (173)

EXPLANATION

Buprofezin was first evaluated by the 1991 JMPR, which allocated an ADI of 0-0.01 mg/kg bw and recommended TMRLs for cucumber, tomato and oranges with 8 required and 4 desirable items of further work or information. Review was postponed at the request of the manufacturer until 1995, with the likely prospect of withdrawal of the TMRLs if analytical data were not available. The Meeting received and reviewed submissions intended to provide most of the required information.

METABOLISM AND ENVIRONMENTAL FATE

The 1991 JMPR monograph described the fate of residues in animals, plants, soil, water and water/sediment systems and listed the structures of buprofezin metabolites and related compounds. In response to requirements of the 1991 JMPR additional information was provided to the present Meeting on metabolism in animals and plants and fate in water (hydrolysis).

Animal metabolism

The 1991 JMPR reviewed metabolism studies on rats and chickens. It noted that these studies suggested that hydroxylation of the phenyl ring, oxidation at the sulfur atom and cleavage of the thiadiazinane ring are the major routes of metabolism. However, it concluded that on the basis of these studies alone the fate of residues in animals was not adequately understood and required submission of a ruminant metabolism study. The present Meeting received a study of metabolism in a cow (Haung and Smith, 1995), reportedly conducted according to US GLP.

A 420 kg lactating Jersey cow was dosed orally by gelatin capsules twice daily (after the morning and evening milkings) at a daily rate of 163 mg [¹⁴C]buprofezin uniformly labelled in the phenyl ring (equivalent to 24.4 ppm wet weight or 26.6 ppm dry weight in the diet, or 0.38 mg/kg bw). Milk, urine and faeces were collected twice daily during treatment, and liver, kidney, muscle, fat and blood were collected after slaughter 15 hours after the last dosing. Samples were shipped frozen the same day to the test facility where they were kept frozen until analysis.

Samples were subjected to a number of extraction, hydrolysis and partitioning steps for analysis. For example liver, kidney and muscle samples were lyophilized and Soxhlet-extracted sequentially with solvents of increasing polarity (hexane, acetonitrile, ethanol and water). Organic and aqueous extracts were incubated with β -glucuronidase and sulfatase before chromatography. Liver and kidney solids remaining after the exhaustive extraction ("bound" residues above 0.05 mg/kg) were subjected to acid (0.1M HCl) then base (0.1 M NaOH) hydrolysis, followed sequentially by incubations with proteinase, glucuronidase and 6 M HCl. These treatments released respectively 2.1%, 7.7%, 36.2%, 1%, and 6.7% of the ¹⁴C in the liver. It can be seen that the proteinase released the highest proportion of the bound residue.

Samples were subjected to liquid scintillation counting and combustion analysis to determine the distribution of residues. Components of the residues were identified by TLC (normal, reverse-phase, and two-dimensional) and HPLC; separated fractions were compared with reference standards of known and likely metabolites (not including the thiobiuret derivative formed by hydrolysis).

The distribution of total ^{14}C - residues, expressed as buprofezin equivalents, is shown in Table 1.

Table 1. Distribution of ^{14}C residues in a cow dosed with [^{14}C]buprofezin (Haung and Smith, 1995).

Sample	^{14}C distribution		^{14}C in extract			
			hexane ⁴	CH ₃ CN + EtOH	aqueous	unextractable
	mg/kg as buprofezin	% of total dose	% of total in sample (mg/kg ⁵)	% of total in sample (mg/kg ⁵)	% of total in sample (mg/kg ⁵)	% of total in sample (mg/kg ⁵)
Liver	1.21	0.66	0.02 (<0.001)	29.2 (0.35)	15 (0.18)	55 (0.66)
Kidney	0.41	0.04	0.002 (<0.001)	43.9 (0.18)	26.8 (0.11)	30 (0.12)
Muscle	0.018	0.24	--	44.4 (0.008)	16.7 (0.003)	44.4 (0.008)
Milk	0.028 ¹	0.087	0.004 (<0.001)	42.9 (0.012)	28.6 (0.008)	21.4 (0.006)
Fat	0.02	0.15	0.15 (<0.001)	55 (0.01)	5 (0.001)	25 (0.005)
Blood	0.23	0.49	--	--	--	--
Faeces	5.4-12 ²	45.56	--	--	--	--
Urine	4.9-10.5 ³	18.84	--	--	--	--

¹ Highest level reached in whole milk (day 5 at plateau). Residues in cream about 1.5 times those in skimmed milk

² After day 3

³ After day 2

⁴ Residue in hexane after back wash with ethanol/water or acetonitrile

⁵ Expressed as buprofezin

The distribution of identified and characterized metabolites is shown in Table 2.

Table 2. Identified and characterized compounds¹ in milk, tissues and excreta of a cow dosed with [^{14}C]buprofezin (Huang and Smith, 1995).

Sample	buprofezin	BF-9 = J ("Dione")	BF-2 = B		BF-12 = G		BF-13 = H		BF-23 = L		Largest unknown	
	% of TRR ²	% of TRR	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR (no. of unknowns)	mg/kg
Liver	ND ³		10.9	0.13 ⁴	3.5	0.042	2.5	0.030	2.2	0.027	5.9 (16)	0.07
Kidney	ND		18	0.074	3.9	0.016	3.1	0.013	7.7	0.032	4.5 (8)	0.02
Milk	ND		1	<0.001	2.1	0.001	2.6	<0.001	9.2	0.003	4.9 (6)	0.001
Faeces	12.6		48.4		--	--	--	--	--	--	11 (2)	
Urine 30 after min. reflux ⁵	--	--	--	--	16.6		--	--	4.9		9	--
Urine after overnight digest ⁶	--	1.3	7.7		14.5		5.4		6.4		13 (6)	

¹ Identification:

1991 monograph code Huang and Smith code Chemical name

B BF-2 2-*tert*-butylimino-5-(4-hydroxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one
 G BF-12 1-isopropyl-3-phenylurea
 H BF-13 1-(4-hydroxyphenyl)-3-isopropylurea
 J BF-9 3-isopropyl-5-phenyl-1,3,5-thiadiazinan-2,4-dione
 L BF-23 *N*-(4-hydroxyphenyl)acetamide

² Total radioactive residue

³ Not detectable

⁴ Calculation: 1.2 mg/kg from Table 1 x 10.9% TRR in liver = 0.13 mg/kg

⁵ In 0.5M/HCl

⁵ In dioxane/HCl 50°C

These findings led the author to propose the metabolic profile for buprofezin in ruminants presented in Figure 1 and confirmed the metabolic profile proposed for animals in the 1991 monograph, which is repeated for reference in Figure 2. The structures in Figures 1 and 2 are identified in the list below.

Identification codes, chemical names, and common or trivial names of compounds in Figure 1 and Figure 2

Code used in Fig. 1	Code use in Fig. 2 & Fig. 1 of 1991 monograph	Chemical, common and trivial names
BF-1	A	buprofezin
BF-2	B	2- <i>tert</i> -butylimino-5-(4-hydroxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one ("p-hydroxybuprofezin")
	C	2- <i>tert</i> -butylimino-5-(3,4-dihydroxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one
	D	2- <i>tert</i> -butylimino-5-(4-hydroxy-3-methoxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one
BF-10	E	2- <i>tert</i> -butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one 1-oxide (buprofezin oxide)
BF-11	F	1- <i>tert</i> -butyl-3-isopropyl-5-phenylbiuret
BF-12	G	1-isopropyl-3-phenylurea (IPU)
BF-13	H	1-(4-hydroxyphenyl)-3-isopropylurea (hydroxy-IPU)
BF-9	- ¹	3-isopropyl-5-phenyl-1,3,5-thiadiazinane-2,4-dione (the "dione")
	K	4-aminophenol (<i>p</i> -aminophenol)
BF-23	L	<i>N</i> -(4-hydroxyphenyl)acetamide

Figure 1. Metabolic profile of buprofezin in ruminants.

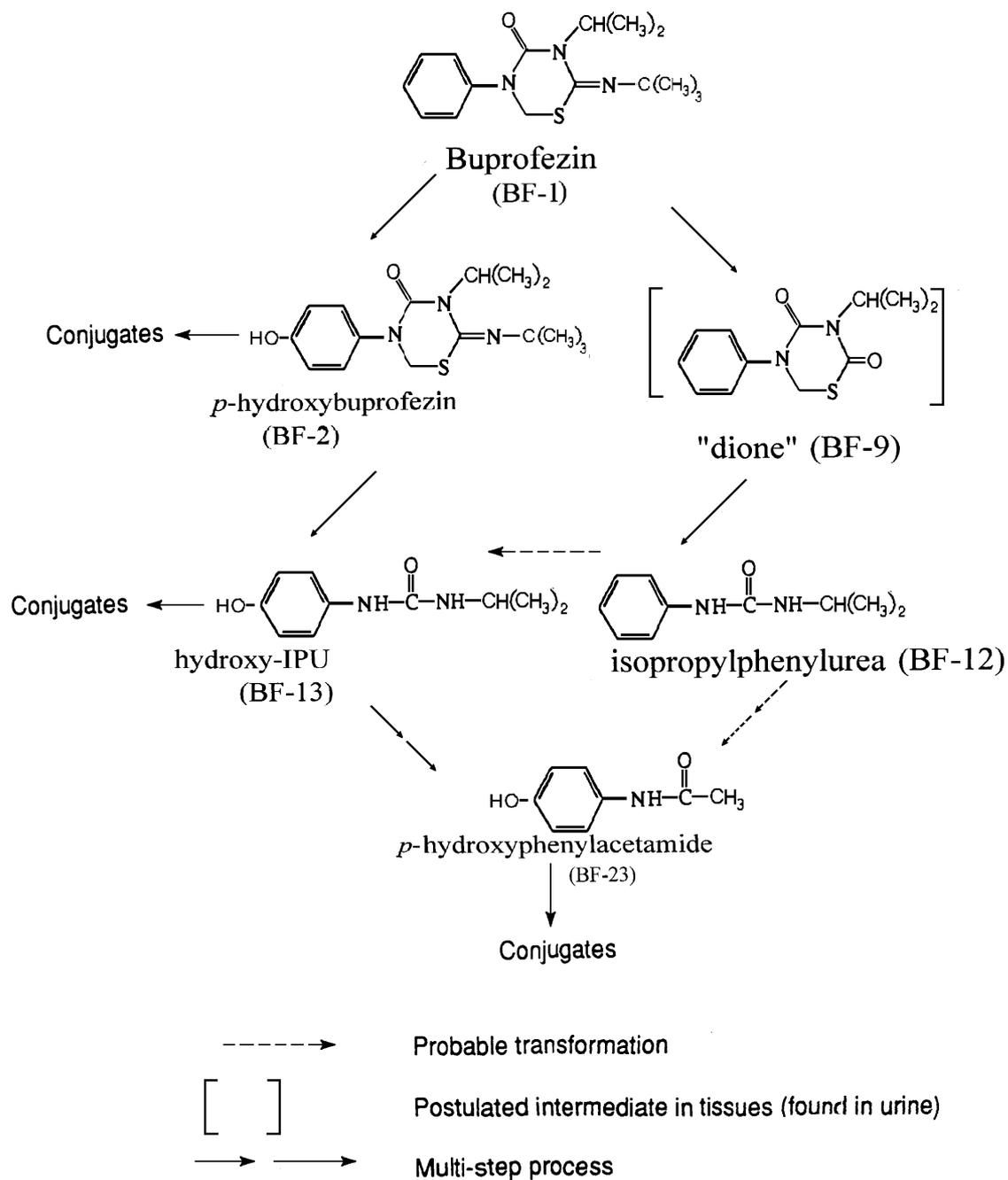
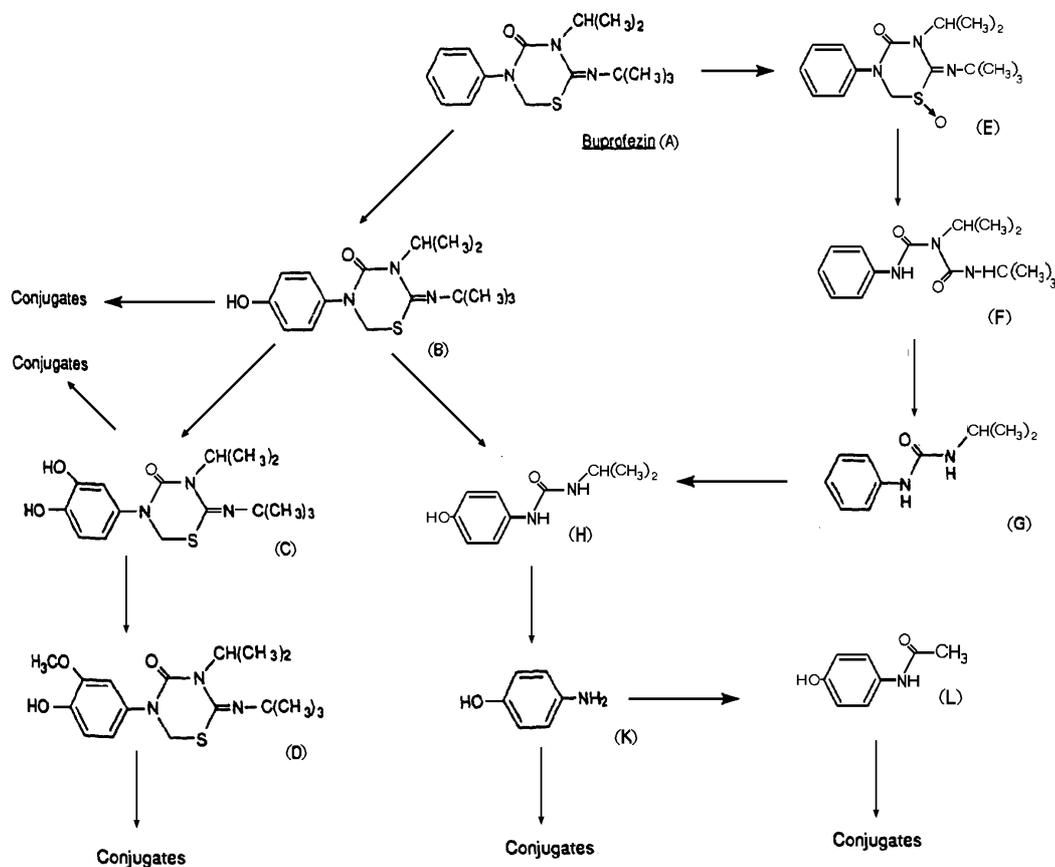


Figure 2. Proposed metabolic pathways of buprofezin in animals.

¹ Shown as J in Fig. 3 of 1991 monograph

Figure 2. Proposed metabolic pathways of buprofezin in animals.



Plant metabolism

The 1991 JMPR reviewed information on the metabolism in plants. More than 90% of the residue 7 days after application to tomato fruits was unchanged buprofezin. In geponic- or hydroponically-grown rice plants residues were taken up by the roots and translocated to other plant parts, the major residues being unchanged buprofezin and *p*-hydroxybuprofezin. In several other hydroponically-grown plants buprofezin was again the main residue, but the major metabolite was buprofezin sulfoxide. Because of these differences the 1991 Meeting concluded that a study of metabolism in a crop representing a major use was needed and required information on the fate in citrus fruits.

A study of citrus metabolism was completed, reportedly in compliance with US GLP requirements (Rieser and Smith, 1995). [*Phenyl*-¹⁴C]buprofezin in an SC formulation was applied to different lemon trees, grown in pots in a glasshouse, according to three regimens. In the first, two applications were made at the equivalent rate of 1 kg ai/ha and 50 g ai/hl, the first 75 days and the second 14 days before harvest in accordance with the "normal" GAP. The rate and the 14-day PHI are consistent with most GAP reported to the 1991 JMPR.

The actual application was to fruit approaching maturity with a micropipette in approximately 200 ÷ 1 (0.46 mg ai/ml = 0.05%) estimated to simulate applications to run-off. In the second regimen only the first 75-day treatment was applied, and in the third the treatment was at 3.5 kg ai/ha 30 days before

harvest in order to facilitate the identification of metabolites.

The potential for residue translocation was investigated by application at an equivalent of 2 kg ai/ha to the twigs and leaves of greenhouse trees with immature fruit, with harvest after 28 days. The proportions of the ¹⁴C translocated to immature fruit were 0.6-1.2% (0.005-0.006 mg/kg buprofezin equivalents) from twigs and 0.07-0.12% (0.002-0.009 mg/kg) from leaves.

For the metabolism part of the study, surface residues were removed by washing with ethanol. The washed fruits were separated into peel and pulp which were separately extracted with successively more polar solvents (acetonitrile, 1:1 acetonitrile/water and (by Soxhlet) water). These extracts were combined and extracted with ethyl acetate without adjustment of pH and at pH 2 and pH 10. The ethyl acetate extracts and the remaining fibre containing >10% of the residue (or >0.05 mg/kg) were hydrolyzed with HCl in dioxane. The hydrolysates were again extracted with ethyl acetate (pH unadjusted, pH 7 and pH 10). Fractions were analysed by one- or two-dimensional TLC and HPLC. The identity of metabolite A was also confirmed by tandem MS (HPLC-MS-MS). The total radioactivity in individual fractions was determined by combustion analysis and scintillation counting.

On day 0 essentially all of the radioactivity was in or on the peel and 93-97% was in the surface wash. The distribution of radioactivity after other intervals is shown in Table 3.

Table 3. Radioactivity in lemons treated with buprofezin labelled in the phenyl ring (Rieser and Smith, 1995).

Treatment		PHI (days)	Mean residue, mg/kg buprofezin equiv. or % of total ¹⁴ C						Recov., % of applied
			Total (mg/kg)	Surface wash mg/kg (% of total)	Peel		Pulp		
No.	Rate (kg ai/ha)					Extractable mg/kg (% of total)	Non-extractable mg/kg (% of total)	Extractable mg/kg (% of total)	Non-extractable mg/kg (% of total)
1	1	75	0.4	0.06 (15.8)	0.3 (74)	0.04 (8.9)	0.006 (1.2)	<0.001 (0.1)	41.8
2	1	14	0.9	0.6 (65)	0.3 (31.8)	0.03 (2.8)	0.003 (0.3)	<0.001 (<0.1)	65
1	3	30	3.8	3.0 (78.7)	0.7 (19.2)	0.06 (1.6)	0.02 (0.5)	0.001 (<0.1)	95

The distribution of the compounds identified in extracts from the lemons treated once at 1 kg ai/ha (75-day PHI) is shown in Table 4 and that from those treated twice (14-day PHI) in Table 5.

Table 4. Residues in glasshouse-grown lemons from a single treatment with buprofezin at 1 kg ai/ha after a 75-day PHI (Rieser and Smith, 1995).

Sample	Buprofezin % of TRR (mg/kg)	"Dione", BF-9 % of TRR (mg/kg)	IPU, BF-12 % of TRR (mg/kg)	Metabolite A ¹ % of TRR (mg/kg)	Metabolite B ² % of TRR (mg/kg)	Remainder ³ % of TRR (mg/kg)
Peel						
Wash	13.9 (0.06)	--	--	--	--	0.4 (0.001)
Organic hydrolysis, organic extract	2.9 (0.012)	3.4 (0.014)	2 (0.008)	1.5 (0.006)	1.0 (0.004)	2.1 (0.008)
Organic hydrolysis, aqueous extract	NA ⁴	NA	NA	NA	NA	<0.1 (<0.001)

Sample	Buprofezin % of TRR (mg/kg)	"Dione", BF-9 % of TRR (mg/kg)	IPU, BF-12 % of TRR (mg/kg)	Metabolite A ¹ % of TRR (mg/kg)	Metabolite B ² % of TRR (mg/kg)	Remainder ³ % of TRR (mg/kg)
Aqueous hydrolysis, organic extract	0.8 (0.003)	3.2 (0.013)	4.5 (0.018)	31.3 (0.126)	6.9 (0.028)	7.3 (0.03)
Aqueous hydrolysis, aqueous extract	NA	NA	NA	NA	NA	6.2 (0.025)
Fibre hydrolysis, organic extract	0.8 (0.003)	0.7 (0.003)	1.6 (0.006)	1.2 (0.005)	1.2 (0.005)	1.5 (0.006)
Fibre hydrolysis, aqueous extract	NA	NA	NA	NA	NA	1.8 (0.007)
Hydrolysd fibre	NA	NA	NA	NA	NA	1.9 (0.008)
Pulp						
Pulp	NA	NA	NA	NA	NA	2 (0.012)
Totals	18.4 (0.08)	7.3 (0.03)	8.1 (0.03)	34 (0.14)	9.2 (0.04)	23.2 0.10

¹ Metabolite A = 2-amino-2-methylpropyl 2-isopropyl-4-phenylallophanate (see (Q), Figure 3, for structure)

² Unidentified

³ No single unidentified peak was greater than metabolite B

⁴ NA = not analysed

Table 5. Residues in lemons from two treatments of glasshouse-grown trees with buprofezin at 1 kg ai/ha with a 14-day PHI (Rieser and Smith, 1995).

Sample	Buprofezin % of TRR (mg/kg)	"Dione" % of TRR (mg/kg)	IPU % of TRR (mg/kg)	Metabolite A ¹ % of TRR (mg/kg)	Metabolite B ² % of TRR (mg/kg)	Remainder ³ % of TRR (mg/kg)
Peel						
Wash	63.8 (0.533)	--	--	--	--	0.4 (0.004)
Organic hydrolysis, organic extract	2.0 (0.016)	5.2 (0.043)	0.5 (0.004)	0.8 (0.007)	1.3 (0.011)	2.4 (0.022)
Organic hydrolysis, aqueous extract	NA ⁴	NA	NA	NA	NA	0.2 (0.002)
Aqueous Hydrolysis, organic extract	0.2 (0.002)	0.8 (0.006)	1.2 (0.010)	4.9 (0.041)	2.3 (0.019)	4.7 (0.039)
Aqueous hydrolysis, aqueous extract	NA	NA	NA	NA	NA	6.9 (0.058)
Fibre	NA	NA	NA	NA	NA	2.2 (0.018)
Pulp						
Pulp	NA	NA	NA	NA	NA	0.3 (0.003)
Totals	66 (0.55)	6 (0.05)	1.7 (0.014)	5.7 (0.05)	3.6 (0.03)	17.1 (0.15)

¹ Metabolite A = 2-amino-2-methylpropyl 2-isopropyl-4-phenylallophanate (see (Q), Figure 3, for structure)

² Unidentified

³ No single unidentified peak was greater than metabolite B

⁴ NA = not analysed

Separate aliquots of unhydrolysed aqueous fractions from the lemons treated once at 1 kg ai/ha (75-day PHI) were also incubated with β -glucuronidase, β -glucosidase or cellulase. The proportions of the radioactivity released were 34.1%, 16.6% and 21.1% respectively. Most of this was associated with unresolved polar fractions, and all three incubation systems contained small amounts of the dione metabolite and more of the allophanate (metabolite A) and IPU.

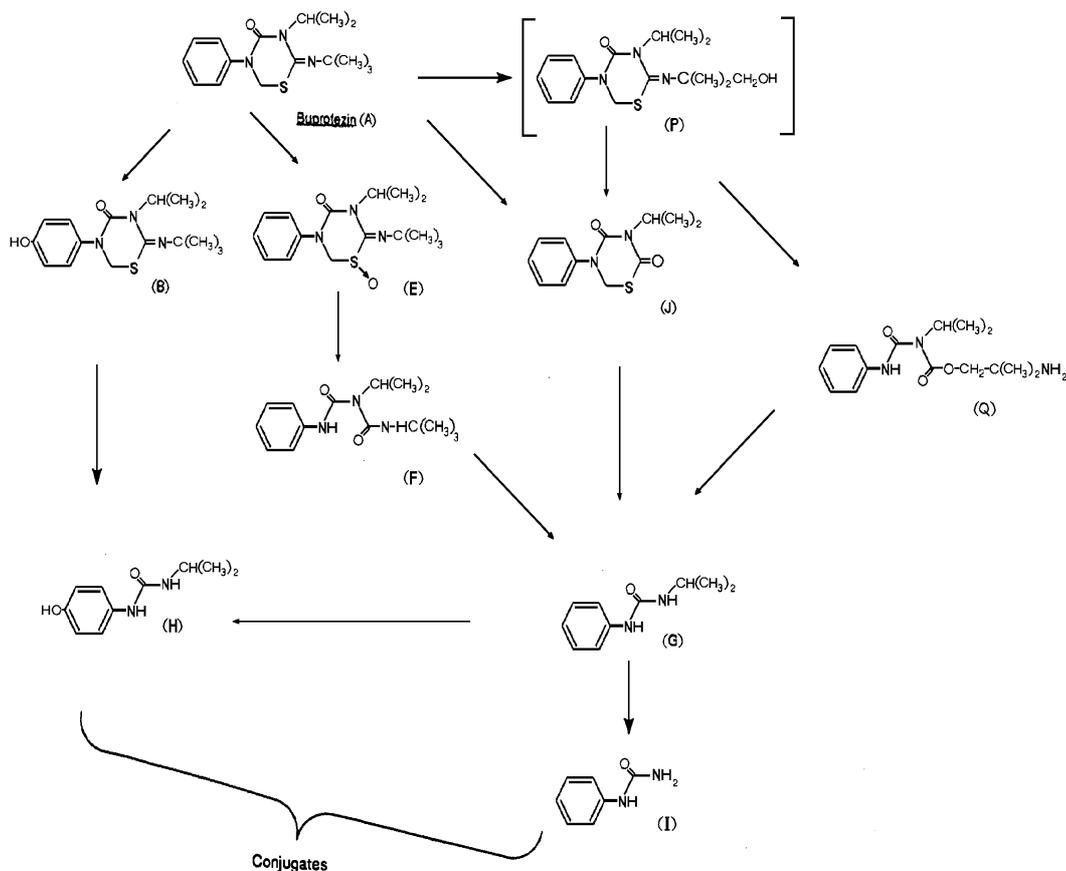
In the translocation experiment $\leq 1.2\%$ of the radioactivity applied the stems and $< 2\%$ of that applied to the leaves was translocated into immature fruit.

The manufacturer reported that there was no evidence of the thiobiuret (O, BF-25) in the citrus metabolism study, nor of buprofezin sulfoxide (E, BF-10) or 1-*tert*-butyl-3-isopropyl-5-phenylbiuret (F, BF-11) (Nokata, 1995).

Previous work had shown that a compound with similar chromatographic properties to metabolite A is formed by the acid degradation of BF-4. The preparation and purification of this product from the large-scale degradation of BF-4 allowed the structure of metabolite A to be confirmed by MS. Compound BF-4 was postulated to be an intermediate metabolite in citrus, although it was not actually detected.

On the basis of these findings the authors proposed the metabolic pathway for plants shown in Figure 3.

Figure 3. Proposed metabolic pathways of buprofezin in plants



- B = BF-2 2-*tert*-butylimino-5-(4-hydroxyphenyl)-3-isopropyl-1,3,5-thiadiazinan-4-one
 E = BF-10 2-*tert*-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one 1-oxide
 F = BF-11 1-*tert*-butyl-3-isopropyl-5-phenylbiuret
 G = BF-12 1-isopropyl-3-phenylurea
 H = BF-13 1-(4-hydroxyphenyl)-3-isopropylurea
 I = BF-16 phenylurea
 J = BF-9 3-isopropyl-5-phenyl-1,3,5-thiadiazinan-2,4-dione
 P = BF-4 tetrahydro-2-[(2-hydroxy-1,1-dimethylethyl)imino]-3-isopropyl-5-phenyl-1,3,5-thiadiazin-4-one (hydroxy-*tert*-butyl-buprofezin)
 Q = metabolite A 2-amino-2-methylpropyl 2-isopropyl-4-phenylallophanate

Environmental fate in soil and water/sediment systems

Hydrolysis in water

The 1991 JMPR reviewed a hydrolysis study conducted in the dark at pH 4 in which the major degradation product (41.7%) after 11 days was reported as 1-*tert*-butyl-3-isopropyl-5-phenyl-2-thiobiuret (hereafter referred to as "the thiobiuret") Buprofezin (55%) and 1-isopropyl-3-phenylurea (IPU, 14.7%) were also reported. Evidence suggested to the Meeting that IPU resulted from degradation of the thiobiuret under the acidic conditions. The thiobiuret was also reported at much lower levels from the exposure of buprofezin in sterile dionized water to natural sunlight.

Since biological systems may be substantially acidic the 1991 Meeting questioned why the thiobiuret was not reported in the submitted metabolism studies, to which the manufacturer responded that radiograms from these studies "did not show evidence of this compound". Because the studies did not provide proof of the identity of the thiobiuret the 1991 JMPR required such proof, and requested that samples from any future metabolism studies or field trials should be analysed for this compound if it was shown to have occurred at significant levels.

In response to the 1991 requirements the Meeting received confirmation of the identity of the thiobiuret formed during hydrolysis under acidic conditions (Kimura and Nishizawa, 1994) and comments on its relevance to residue levels (Nokata, 1995). In the 1994 confirmatory hydrolysis study buprofezin was stored for 6 days in the dark in water buffered at pH 5 at 45°C, as compared with the incubation for 11 days at pH 4 and 35°C in the study reviewed by the 1991 JMPR.

The thiobiuret was isolated from the hydrosylate by silica gel chromatography and further purified by gel permeation chromatography. The hydrosylate was reported to contain unchanged buprofezin, the thiobiuret and IPU, although the report did not list the relative amounts. 41.4 mg of the purified thiobiuret was obtained from the initial 480 mg of buprofezin, which suggests that about 9% of the buprofezin was degraded to the thiobiuret under these conditions. The purified degradation product was subjected to MS and NMR analyses. MS indicated a molecular weight of 293, as required for the thiobiuret. NMR indicated the loss of the methylene protons in the thiadiazine ring and the appearance of an amino proton, both of which indicate cleavage of the ring. This, together with the appearance of a thiocarbonyl carbon (C=S) in the degradation product, the mass spectrum and other observations, confirmed the identity of the compound as the thiobiuret.

The comments by Nokata (1995) cite the absence of the thiobiuret as a metabolite in the citrus metabolism study as evidence that there is no need to regulate this compound in plants.

METHODS OF RESIDUE ANALYSIS

Analytical methods

The analytical method PPRAM 82, which had been used for most of the residue trials reviewed by the 1991 JMPR, was received too late for review at that Meeting and lacked validation for fruiting vegetables. The 1991 JMPR required validations of the method. Several studies relevant to the analysis of buprofezin or its metabolites were provided.

The first describes a clean up procedure for the determination of buprofezin and *p*-hydroxybuprofezin in crops (Nishizawa *et al.*, 1994). Samples are extracted with acetone or methanol, partitioned between hexane and 1N HCl, neutralized and extracted with hexane for determination by GLC with an AFID. Hydroxybuprofezin is acetylated with acetic anhydride before analysis. Reported "limits of detection" were 0.005 mg/kg in hulled rice, citrus pulp and tomato and 0.01 mg/kg in rice straw and citrus peel. Reported recoveries were 75-97% at 0.1 or 0.2 mg/kg fortification levels.

The few published chromatograms from hulled rice and tomato samples suggest that reasonable limits of determination would be 0.01 and 0.02 mg/kg for buprofezin and *p*-hydroxybuprofezin respectively in hulled rice and 0.02 mg/kg for both in tomatoes. However, the method was not validated below 0.1 mg/kg. Chromatograms from citrus were not included. The extraction of buprofezin from

hulled rice by acetone or methanol was shown to be acceptable.

In the second study, three methods were assessed for the extraction of buprofezin residues from peppers, beans and egg plants (García *et al.*, 1993). In the first (Mills *et al.*, 1963) the chopped sample is extracted with acetonitrile and partitioned into hexane after diluting with water. In the second (Luke *et al.*, 1975, 1981) samples are extracted with acetone and partitioned with petroleum ether and methylene chloride. In the third (Leary, 1971) extraction is with ethyl acetate, with clean-up on a short Florisil column. Recoveries of buprofezin were >81% for all three extraction procedures at 0.1 mg/kg fortification levels from each of the crops tested, but were better with the Leary extraction (>89% in all crops at 0.1 mg/kg). The Leary extraction was also tested at 0.02 mg/kg fortification levels and gave >93% recoveries from all the crops.

Another procedure is based on extraction with acetone, concentration to an aqueous solution, partitioning into dichloromethane under basic conditions and determination of buprofezin by GLC with a nitrogen detector (Dick and Rounds, 1984). This appears to be the method used to produce most of the data provided to the 1991 JMPR (except in the Japanese trials) and referred to in the 1991 monograph as ICI method PPRAM 82. The reported limit of determination was 0.005 to 0.01 mg/kg. The only reported recoveries were 101% at 0.1 mg/kg and 95% at 0.5 mg/kg fortification levels. The few chromatograms provided suggest that residues may be quantified at 0.01 mg/kg in tomatoes, although the only two controls were reported as <0.01 and 0.6 mg/kg.

A study of the extractability of weathered buprofezin residues from peaches by various solvent systems (Roberts-McIntosh, 1991) was supplied in response to the 1991 JMPR requirement for validation of analytical method PPRAM 82. A sample of peaches which had been treated with buprofezin at 60 g ai/ha and harvested after 7 days "and analysed in June 1990 (ref. 3) using ICI Plant Protection Division Analytical Method (PPRAM) 82 was found to contain a residue of 0.66 mg/kg". The peaches were stored at -20°C until selected for the study in 1991. Five extraction systems were investigated and the results of analyses by PPRAM 82 are summarized in Table 6. The "ref. 3" quoted was not provided to the present Meeting.

Table 6. Extractability of buprofezin in weathered peaches harvested 7 days after treatment at 60 g ai/ha (Roberts-McIntosh, 1991).

Extraction method	Mean residue ¹ (mg/kg)		% Recovery ²
	Treated	Control	
Cold acetone	0.65	ND ³	83
Cold methanol/water (90:10)	0.68	ND	87
Cold acetone/hexane (80:20)	0.78	ND	86
Acetone reflux	0.67	ND	68
Methanol/water reflux (90:10)	0.52	ND	80
Mean	0.66	--	80.1

¹ Mean of 3 assays, uncorrected for recovery

² From 0.5 mg/kg fortification

³ ND = not detected (<0.005 mg/kg)

Summary recovery data for cucumbers and gherkins (74-106%) and tomatoes (79-91%) were also provided to the Meeting (Olthof, 1995). Although the LOD was reported to be 0.01 or 0.02 mg/kg for each vegetable, no fortification levels, controls or chromatograms were provided.

A recent analytical method (RAM No. BF/06/94) has been described for the determination of buprofezin, BF-12 (1-isopropyl-3-phenylurea) and BF-9 (the "dione" or 3-isopropyl-5-phenyl-1,3,5-

thiadiazinane-2,4-dione) in tomatoes (Neal, 1994). The metabolites had been identified in lettuce. Samples are extracted with acetone, the acetone is evaporated and the aqueous remainder acidified. BF-9 is extracted with hexane, the aqueous solution is neutralized and buprofezin and BF-12 are extracted with ethyl acetate/hexane. The BF-9 fraction is cleaned up on a Florisil column, all the extracts are combined and concentrated, and the three compounds are determined by GLC with an NPD.

During development of the method recoveries were 94% for buprofezin and 79 and 91% for BF-12 and BF-9 respectively at 0.01 mg/kg, reported to be the limit of determination. The lowest validated levels in the processing study were 0.05 mg/kg. Mean buprofezin recoveries at this level were fruit 86%, wet pomace 80%, dry pomace 79%, juice 108%, purée 101 % and paste 123%, with similar recoveries of the metabolites. A limit of determination of 0.05 mg/kg for each compound in each tomato product is reasonable.

Other analytical methods for buprofezin or *p*-hydroxybuprofezin are described in the 1991 monograph.

Stability of pesticide residues in stored analytical samples

Information already reviewed by the 1991 JMPR (Bioanalytical Research, 1991) was re-submitted. From this study the 1991 JMPR reported no significant loss of buprofezin from apples, peaches or courgettes and only 13% from kiwifruit after storage up to a year at -20°C. Information on the stability of buprofezin and *p*-hydroxybuprofezin in stored analytical samples of citrus (Iwamoto and Matano, 1993) and buprofezin in cucumbers (Iwamoto and Nishizawa, 1993) and tomatoes (Iwamoto and Kanauchi, 1994) was provided to the present Meeting and is discussed later under "Residues resulting from supervised trials".

Mean recoveries of 80-106% of both buprofezin and *p*-hydroxybuprofezin were attained after storage of citrus pulp for 56-58 days and citrus peel for 91-93 days at -20°C when fortified at 0.5 mg/kg.

In cucumbers fortified at 0.2 mg/kg, 90% of the residue was reported to remain after 130 days at -20°.

Mean recoveries from tomatoes fortified at 0.05 mg/kg and stored at four sites for periods of 53-94 days ranged from 100 to 114%.

Residue definition

On the basis of a tomato metabolism study indicating that over 90% of the residue in tomatoes is unchanged buprofezin the 1991 JMPR defined the residue as buprofezin. That Meeting also required additional information on the fate of residues in animals, water, and citrus, which were supplied and are described above.

The citrus metabolism study confirms unchanged buprofezin to be the main residue (66% of the total ¹⁴C) after 14 days and the second most abundant (18%) even after 75 days. After 75 days the main residue was (Q) or metabolite A (2-amino-2-methylpropyl 2-isopropyl-4-phenylallophanate). No significant residues of buprofezin sulfoxide (reported in hydroponic metabolism studies) or the

phenylbiuret metabolite were reported, nor was there any evidence of the thiobiuret known to be formed under acidic conditions in water. In the data on supervised trials submitted to the Meeting no residues of *p*-hydroxybuprofezin were reported in citrus or tomatoes.

These findings support the 1991 JMPR's conclusion that buprofezin *per se* is the appropriate definition of the residue, at least for regulatory purposes for cucumbers, tomatoes and oranges. This definition may need to be re-assessed if MRLs are recommended in the future for additional crops, since metabolism varies among plants of different types.

As discussed in the appraisal, the definition of the residue in animal products will have to be determined if it is decided in the future that limits are needed for them.

USE PATTERN

Information on approved uses of buprofezin provided to the Meeting is summarized in Table 7.

Table 7. Approved uses of buprofezin on crops.

Crop, country	Application				PHI, days	Notes
	Form.	kg ai/ha	kg ai/hl	No.		
Citrus fruits						
Spain ¹	WP?	0.4-1	0.01-0.025	1	7	
New Zealand	WP	0.375	0.0125	2-6	14	high vol. to run-off
Cucumbers						
Germany	SC	0.5-0.9	0.0075	1	--	At infestation
Netherlands	EC	0.04-0.11	0.007	2	3	Glasshouse
UK	SC	0.075-0.375	0.0075	8*	3	Glasshouse. * max. 2 treatments, 45 day interval
Egg plants						
UK	SC	0.075-0.375	0.0075	2	3	Glasshouse
Grapes (wine and table)						
New Zealand	WP	0.125	0.0125	2	*	*pre-flower, high vol. to run-off
Gherkins						
Netherlands	EC	0.04-0.11 (0.05-0.08)*	0.007	2	3	Glasshouse *Field use
Kiwifruit						
New Zealand	WP	0.25	0.0125	1-2	*	*pre-flower, high vol. to run-off
Melons						
Netherlands	EC	0.04-0.11	0.007	2	3	Glasshouse
Persimmons						
New Zealand	WP	0.25	0.0125	2	*	*pre-flower, high vol. to run-off
Peppers (sweet)						
Germany ("peppers")	SC	0.5-0.9	0.0075	1	--	at infestation
Netherlands	EC	0.04-0.11	0.007	2	3	Glasshouse
UK	SC	0.075-0.375	0.0075	2	3	Glasshouse
Pome fruit						
New Zealand	WP	0.375 (pear to 0.625)	0.0125	2	*	*pre-flower, high vol. to run-off

Crop, country	Application				PHI, days	Notes
	Form.	kg ai/ha	kg ai/hl	No.		
Summer squash						
Netherlands	EC	0.04-0.11 (0.05-0.08)*	0.007	2	3	Glasshouse *Field use
Tamarillos						
New Zealand	WP	0.275	0.0125	2	7	high vol. to run-off
Tomatoes						
Germany	SC	0.5-0.9	0.0075	1	--	at infestation
Netherlands	EC	0.04-0.11	0.007	2	3	Glasshouse
UK	SC	0.075-0.375	0.0075	8	3	Glasshouse. 2 treatments max. in 65-day period

¹ Application rate not supported by label, but reported in manufacturer's working paper. Number of applications from 1991 JMPR monograph.

RESIDUES RESULTING FROM SUPERVISED TRIALS

The 1991 JMPR required additional data on outdoor supervised trials on cucumbers and tomatoes if outdoor uses were shown to be GAP, and additional data on supervised trials on oranges, including the final report on the Brazilian trials on which only a draft report had been provided. For the additional trials the 1991 Meeting required analyses for *p*-hydroxybuprofezin (the main metabolite in geponic and hydroponic metabolism studies on rice), the thiobiuret derivative (formed by hydrolysis under acidic conditions), buprofezin sulfoxide and the phenylbiuret metabolite (the major metabolites in hydroponic studies on several crops). Unchanged buprofezin had been the only significant residue found in a study of tomato metabolism. Data were provided on residue trials on cucumbers, tomatoes and citrus.

No MRLs were recommended by the 1991 JMPR for animal products. Although that Meeting reviewed a conventional dairy cow feeding study, no study of ruminant metabolism had been provided. The 1991 Meeting drew tentative conclusions, but recommended reconsideration when the required studies on the fate of residues during processing and on animal metabolism had been reviewed. These studies were provided to the present Meeting.

Plants

Citrus. The 1991 JMPR recommended a TMRL of 0.3 mg/kg for oranges, based trials in Japan, South Africa and Portugal, but mainly Japan because the others did not closely reflect maximum GAP conditions. The present Meeting received data on natsudaidais from Japan and on oranges from Spain and Brazil, the last being the final report required by the 1991 JMPR. The results are shown in Table 8.

In the Brazilian trials 3 knapsack mistblower applications were made to "Pera Natal" orange trees in 1000 m² plots (within a 5000 m² grove). The trials were conducted according to FAO guidelines. Samples were received at the laboratory within 6 hours of harvest and stored at -15°C until analysis (<2 weeks). Analyses were by the method of Nihon Nohyaku (1985) described in the 1991 evaluation. The limit of "detection" was reported as 0.01 mg/kg, although the chromatograms provided were not sufficiently legible for an independent assessment of a limit of determination. Reported recoveries (not documented) from peel, juice and bagasse fortified at 0.1 mg/kg were 85-95%. The results were provided uncorrected, but are shown corrected for recoveries in Table 8.

The Spanish trials (3 locations, 128-200 m² plots) were reported to be conducted according to OECD GLP. The report was well documented. Samples were frozen shortly after harvest and stored at -20°C until analysis (approximately 80 days after field sampling) by HPLC for both buprofezin and *p*-

hydroxybuprofezin (separate peaks). Extraction was with acetone and clean-up by successive hexane partitions under acidic and neutral conditions (similar to Nishizawa *et al.*, 1994). Recoveries of buprofezin and *p*-hydroxybuprofezin from whole oranges were about 90-100% and 75-84% respectively at 0.02 and 0.5 mg/kg fortification levels. No residues (<0.015 mg/kg) of the metabolite were found in any sample. Representative chromatograms suggest that a limit of determination of 0.02 mg/kg is possible for both compounds in whole oranges.

In the Japanese 2-tree plot trial buprofezin (WP) was applied by knapsack sprayer at 25g ai/hl and 5000 l/ha (25 g ai/hl, with a 14-day PHI, is confirmed GAP). Samples were sent to the laboratory "shortly after harvest" for analysis for buprofezin and *p*-hydroxybuprofezin. They were stored at -10°C until analysis, although the handling and storage conditions before receipt at the laboratory and the interval from field sampling to analysis were not stated. The report was completed in December 1993. Mean recoveries of both compounds at fortification levels of 0.5 mg/kg were 80-106% after storage of pulp for 56-58 days and peel for 91-93 days at -20°C. Although the sampling-to-analysis intervals for the field-treated samples were not stated, the storage stability study was completed in April-July 1988.

Samples for analysis were extracted with acetone. The extract was adjusted to pH 7-8, extracted with hexane and partitioned with acetonitrile. The last extract was concentrated and cleaned up on a silica gel column, which was also used to separate the parent compound from the metabolite, and both compounds were determined by GLC with an NPD. Recoveries of 91 and 100% were reported for buprofezin and *p*-hydroxybuprofezin respectively at 0.1 mg/kg fortification levels. The limit of determination was reported as 0.01 mg/kg, although chromatograms suggest that 0.02-0.05 mg/kg might be more realistic for routine analyses, especially since recoveries were only verified at 0.1 mg/kg. All controls were reported as <0.01 mg/kg for both compounds.

Table 8. Buprofezin residues in citrus fruit resulting from supervised trials. Underlined residues are from treatments according to GAP.

Country, year	Application/treatment			Sample	Residues (mg/kg) at PHI (days)						Ref.
	Form.	No.	kg ai/ha (kg ai/hl)		7	28	63	91	105		
Oranges											
Brazil 1990	25% WP	3	0.5 (0.03)	Peel		0.2 0.1	0.10 0.19	0.03 0.01	0.02 0.01		1
				Juice		<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01		
				Finisher pulp		<0.01 <0.01	<0.01 <0.01	<0.01 <0.01	<0.01 <0.01		
				Whole fruit ¹		0.04 0.02	0.02 0.04	<0.01 <0.01	<0.01 <0.01		
Spain 1994	25% WP	1	1 (0.025)	Peel	<u>0.05</u>						2
				Pulp	<u>0.02</u>						
				Whole fruit	<u>0.06</u> ²						
	25% WP	1	1 (0.025)	Peel	<u>0.07</u>						
				Pulp	<u><0.02</u>						
				Whole fruit	<u>0.03</u> ²						
	25% WP	1	1 (0.025)	Peel	<u>0.11</u>						
				Pulp	<u><0.02</u>						
				Whole fruit	<u>0.03</u> ²						

Country, year	Application/treatment			Sample	Residues (mg/kg) at PHI (days)						Ref.
	Form.	No.	kg ai/ha (kg ai/hl)		7	28	63	91	105		
Natsudaidais											
Japan 1987-88	25% WP	5	1.25 (0.025)	Peel	Residue (mg/kg) at PHI (days)						3
					21	30	45	90	120	150	
					<u>1.9</u> <u>1.7</u>	<u>1.2</u> <u>1.1</u>	<u>0.7</u> <u>0.6</u>	<u>0.4</u> <u>0.4</u>	<u>0.5</u> <u>0.5</u>	<u>0.2</u> <u>0.2</u>	
				Pulp	<u>0.3</u> <u>0.3</u>	<u>0.2</u> <u>0.2</u>	<u>0.07</u> <u>0.07</u>	<u>0.03</u> <u>0.03</u>	<u>0.02</u> <u>0.02</u>	<u>0.01</u> <u>0.01</u>	
					Whole fruit ³	<u>0.7</u>	<u>0.4</u>	<u>0.2</u>	<u>0.2</u>	<u>0.2</u>	

¹ Residues in whole fruit estimated on basis of 20.3% peel weight. All results corrected for recoveries. Duplicate results are from separate plots.

² *p*-hydroxybuprofezin was not detected (<0.015 mg/kg) in pulp, peel or whole oranges. All results are means of duplicate injections. Residues in whole fruit are from analyses of whole oranges.

³ *p*-hydroxybuprofezin was not detected (<0.005 mg/kg) in pulp, peel or whole fruit. Residues in whole fruit estimated on basis of pulp/peel weight ratio of 2.2:1.

References

1. Salgado, 1990
2. Melkebeke and Genijen, 1995a
3. Iwamoto and Matano, 1993

Cucumbers. The temporary MRL of 0.3 mg/kg recommended by the 1991 JMPR was based on data (mainly from indoor trials) from The Netherlands, the UK, Greece and Japan. Maximum residues representing GAP were 0.06 mg/kg from The Netherlands (3-day PHI) and Greece (7-day PHI) and 0.21 mg/kg from proposed UK GAP (3-day PHI). The highest residues from trials according to GAP in a Japanese trial were 0.13 mg/kg after three days (the GAP PHI is 1 day), but at only 0.6 times the maximum permitted rate. Residues were 0.6 mg/kg at 1 day from twice the maximum GAP rate. Because only the trials in Greece were outdoor the 1991 Meeting required additional data from outdoor trials if outdoor uses were confirmed to be GAP.

All GAP for buprofezin uses on cucumbers reported to the present Meeting (see Table 7) were for glasshouse treatments, although GAP for gherkins in The Netherlands also included field uses. Current GAP in The Netherlands and the UK (now authorized) essentially confirms that reported in 1991. GAP was also reported for German glasshouse uses, in which the rate of 0.0075 kg ai/hl is essentially the same as in The Netherlands and the UK

Additional data were received from Japanese supervised trials with 3 applications at 0.025 kg ai/hl (0.6-0.75 kg ai/ha) and PHIs of 1, 3 and 7 days.

The application rates in terms of kg ai/ha were in accordance with Japanese GAP reported in the 1991 monograph, as was the 1-day PHI. Trials were conducted at 4 sites, in which the plots were 14-22 m² and application was by knapsack power sprayer. Samples were sent to the test facility "just after harvest" where they were stored at -20°C until analysis for buprofezin (only) ≤1 month after field sampling.

Analysis was by the method of Nishizawa *et al.* (1994), with 98% recoveries from samples fortified at 0.2 mg/kg. The reported limit of determination was 0.01 mg/kg. Chromatograms of treated samples containing 0.05 mg/kg and controls suggest that this is an achievable level, although it was not validated below 0.2 mg/kg. When samples fortified at 0.2 mg/kg were stored for 130 days at -20°C, the recovery was reported to be 90%.

The results are shown in Table 9.

Table 9. Buprofezin residues in greenhouse-grown cucumbers resulting from supervised trials in Japan in 1992 with a 25% WP Formulation (Iwamoto and Nishizawa, 1993). Underlined residues are from treatments at GAP application rates in terms of ai/ha.

Application			Site	PHI, days	Residues, mg/kg
No.	kg ai/ha	kg ai/hl			
3	0.55-0.75	0.025	1	1	<u>0.8, 0.7</u>
				3	<u>0.25, 0.25</u>
				7	<u>0.09, 0.08</u>
3	0.75	0.025	2	1	<u>0.8, 0.6</u>

Application			Site	PHI, days	Residues, mg/kg
No.	kg ai/ha	kg ai/hl			
			3		<u>0.4, 0.4</u>
			4		<u>0.4, 0.4</u>
			2	3	<u>0.3, 0.3</u>
			3		<u>0.2, 0.2</u>
			4		<u>0.1, 0.09</u>
			2	7	<u>0.09, 0.09</u>
			3		<u>0.09, 0.09</u>
			4		<u>0.05, 0.05</u>

Tomatoes. The temporary MRL of 0.5 mg/kg estimated by the 1991 JMPR was based on trials in The Netherlands, the UK, Greece and Japan, with maximum residues from treatments according to GAP of 0.2 mg/kg in The Netherlands (0.3 mg/kg from 1.3 times GAP rate; GAP is 0.0075 kg ai/hl, 3-day PHI); 0.3 mg/kg in the UK (proposed GAP the same as The Netherlands) and 0.4 mg/kg in Japan (GAP 0.19-1 kg ai/ha or 0.0125-0.025 kg ai/hl). As with cucumbers, additional data were required if field uses were confirmed to be GAP.

No GAP for field uses was reported to the present Meeting, but the application rates cited by the 1991 JMPR for The Netherlands and the UK were confirmed to be authorized GAP in both countries and the same spray concentration, 0.0075 kg ai/hl, was reported as GAP in Germany. Additional data were provided from Italy on field trials and from Japan on glasshouse trials.

The Italian outdoor tomato trials were at three locations in Northern Italy, all on 30 m² plots and reportedly according to OECD GLP. Samples were stored in an acceptable manner and analyses were within 4 months of sampling. The analytical method was similar to that for cucumbers, but the determination of buprofezin and *p*-hydroxybuprofezin was by HPLC instead of GLC. Mean recoveries were 102% for buprofezin and 106% for *p*-hydroxybuprofezin at 0.02 mg/kg and 86 and 94% respectively at 0.5 mg/kg. Chromatograms from controls and samples spiked at 0.02 mg/kg suggest that an LOD of 0.01 to 0.02 mg/kg is reasonable.

The Japanese glasshouse trials were at four sites, on plots of 5, 8.1, 50 and 90 m², with applications by knapsack power sprayer. Samples were stored at -20°C until analysis within approximately 3 months of harvest. The analytical procedure was similar to that for cucumbers, except a partition into dichloromethane preceded addition of the acid for the acidic hexane extraction. Average recoveries were 94% at 0.1 mg/kg and the reported limit of "determination" was 0.005 mg/kg, although this appears to be a limit of detection. Controls ranged from <0.005 to 0.04 mg/kg. Mean recoveries after storage at the four sites for periods of 53-94 days ranged from 100 to 114% at 0.05 mg/kg fortification levels. An LOD of 0.05 mg/kg would appear to be reasonable on the basis of the chromatograms provided. The results are shown in Table 10.

Table 10. Residues of buprofezin in tomatoes resulting from indoor and outdoor supervised trials with WP formulation. Underlined residues are from applications according to GAP.

Country, year	Application			Site ¹	PHI, days	Residues, mg/kg	Ref.
	No.	kg ai/ha	kg ai/hl				
Outdoor field trials (buprofezin and <i>p</i> -hydroxybuprofezin) ²							
Italy 1994	2	0.25	0.025		0	0.10	Melkebeke and Genijen, 1995b

Country, year	Application			Site ¹	PHI, days	Residues, mg/kg	Ref.			
	No.	kg ai/ha	kg ai/hl							
				1						
				2		0.08				
				7		0.09				
				14		0.03				
	2	0.25	0.025	2	2	0.08				
2	0.25	0.025	3	2	0.17 Tomatoes 0.03 Juice 0.15 Purée					
Glasshouse trials (analysis for buprofezin only)							Iwamoto and Kanauchi, 1994			
Japan 1993/94	3	0.625	0.025	1	1	<u>0.7, 0.7</u>				
					3	<u>0.6, 0.6</u>				
					7	<u>0.4, 0.4</u>				
	3	0.75	0.025	2	1	<u>0.4, 0.4</u>				
						3		<u>0.3, 0.3</u>		
						4		<u>0.3, 0.3</u>		
				2	3	<u>0.3, 0.3</u>				
						3		<u>0.2, 0.1</u>		
						4		<u>0.3, 0.3</u>		
				2	7	<u>0.2, 0.3</u>				
						3		<u>0.1, 0.1</u>		
						4		<u>0.3, 0.3</u>		
				Controls <0.005-0.04 mg/kg ³						

¹ Site 1: Chiba, 50 m² plot. Site 2: Fukushima, 90 m² plot. Site 3: Iwate, 8.1 m² plot. Site 4: Nagano, 5 m² plot.

p-hydroxybuprofezin was not detected (<0.015 mg/kg)

² High control was at Site 1. 0.186 ng buprofezin/5 mg sample = 0.04 mg/kg. Submission erroneously recorded 0.01 mg/kg.

Animals

Cows. The 28-day feeding study on dairy cows reviewed by the 1991 JMPR included two feeding levels, 20 and 200 ppm in the diet. The 1991 monograph reported as follows.

No residues of buprofezin (<0.01 mg/kg) were detected in milk from the low-dose cows. In milk from one of the high-dose cows, buprofezin peaked at 0.04 mg/kg after 21 days, declining to <0.01 mg/kg after a 3-day withdrawal period. No residues (<0.01 mg/kg) from either dose were detected in kidney, liver or muscle. Residues were up to 0.14 and 0.2 mg/kg in subcutaneous and peritoneal fat respectively from the high dose, but 0.02 mg/kg in fat from the low dose.

On the basis of these findings and the temporary limits of 0.5 and 0.3 mg/kg recommended for tomatoes and oranges respectively the 1991 Meeting tentatively concluded that residues of buprofezin *per se* were unlikely in the muscle, kidney, liver or milk of cattle, but concluded that reconsideration might be needed when the required information on processing and studies of animal metabolism were reviewed. These have now been provided and are described below under "Fate of residues in storage and processing" and above under "Animal metabolism".

The 7-day metabolism study on a dairy cow (see above) was with the equivalent of 27 ppm in the diet, a level similar to the 20 ppm low-dose feeding study reviewed by the 1991 JMPR. The metabolism study supports the finding in the feeding trial that residues of buprofezin are unlikely to be found in the muscle, offal or milk of cattle at a dietary intake of 20 ppm. However, it also reveals that the main residue in animal products is *p*-hydroxybuprofezin in liver and kidney and *p*-acetamidophenol in milk, not the parent compound determined in the feeding study. At the 27 ppm feeding level *p*-hydroxybuprofezin occurred at 0.13 mg/kg in liver and 0.07 mg/kg in kidney, with lower levels of other metabolites. In milk the highest residue was *p*-acetamidophenol at 0.002 mg/kg. Residues in muscle (≤ 0.02 mg/kg buprofezin equivalent) could not be identified.

No information on processing has been provided for citrus, but the tomato processing study showed buprofezin concentrations of 23- and 34-fold in wet and dry pomace respectively. If it is assumed that dry tomato pomace is fed to beef cattle at 25% of the diet (or to dairy cattle at 10%) and residues are at the proposed MRL of 1 mg/kg in the tomato fruit a theoretical worst-case dietary intake of buprofezin from the feeding of dry pomace would be 8.5 ppm in beef cattle and 3.4 ppm in dairy cattle. A similar level would be expected from feeding "citrus pulp" (i.e. a commercial process fraction including extractor residue and peel) if a similar concentration of the residue occurs. Concentration in citrus pulp is likely since most of the residue has been shown to be in the peel.

With these gross assumptions it can be estimated from the metabolism study that residues of the main residue (*p*-hydroxybuprofezin or *p*-acetamidophenol) in cattle could occur at approximately 0.04 mg/kg in liver, 0.02 mg/kg in kidney and <0.001 mg/kg in milk. The significance of this is considered below in the Appraisal.

FATE OF RESIDUES IN STORAGE AND PROCESSING

In storage

No information was provided.

In processing

The 1991 JMPR required "information on the fate of buprofezin in commodities in processing (e.g. tomato processing into pulp, juice, ketchup or purée and the Brazilian citrus pulp data cited". In a 1994 study conducted in the USA in accordance with US GLP, a 0.15 ha plot of tomatoes was treated four times by a tractor-mounted high-cycle sprayer with a 40 SC formulation at 1 kg ai/ha (2.4 times the proposed rate) and the tomatoes harvested after 7 days. They were subsequently processed into wet pomace, dry pomace, juice, purée and paste, which were analysed for buprofezin and the metabolites BF-12 (1-isopropyl-3-phenylurea) and BF-9 (the "dione" or 3-isopropyl-5-phenyl-1,3,5-thiadiazinane-2,4-dione) (Neal, 1995).

Simulated commercial processing involved washing, crushing, heating to 196°F and screening (0.033" screen) to yield tomato juice and wet pomace. Dry pomace was produced by overnight drying to 99% dry solid in a dehydrator on trays at approximately 147°F. Juice was canned after heating at 240°F for 51 minutes. Purée was prepared from juice by vacuum evaporation to approximately 13% solids. To produce paste, purée was further evaporated to approximately 26% solids.

The analytical method employed and appended to the study (Neal, 1994) is described above under Analytical methods. An LOD of 0.05 mg/kg should be reasonable for the routine analysis of tomato products by this method, although lower levels may be possible. The residues found are shown in Table 11.

Table 11. Residues of buprofezin and metabolites in processed tomato fractions from tomatoes treated with a 40SC formulation at 1 kg ai/ha and harvested after 7 days (Neal, 1995).

Sample	Residues, mg/kg ¹			Buprofezin concentration/ reduction factor ⁴
	Buprofezin (control)	BF-9 ² (control)	BF-12 ³ (control)	
Unwashed fruit	0.55 (ND) ⁵	ND (ND)	ND (ND)	1.0
Wet pomace	12.7 (0.01)	0.02 (ND)	0.06 (ND)	23.1
Dry pomace	18.6 (0.01)	0.04 (0.02)	0.09 (ND)	33.8
Juice	0.05 (ND)	0.01 (0.01)	0.05 (ND)	0.09
Purée	0.35 (ND)	0.0 (ND)	0.02 (ND)	0.6
Paste	0.68 (ND)	ND (0.01)	0.04 (ND)	1.2

¹ Averages of replicates corrected for recoveries of 93% for buprofezin, 95% for BF-9 and 85% for BF-12. All values below 0.05 mg/kg are below the routine limit of determination.

² 3-isopropyl-5-phenyl-1,3,5-thiadiazinan-2,4-dione

³ 1-isopropyl-3-phenylurea

⁴ Residue in sample divided by residue in unwashed fruit

⁵ Not detected

Residues in the edible portion of food commodities

Data reviewed by the 1991 JMPR indicated that residues in whole citrus fruit are about 3-10 times the level in the pulp. The trials reviewed by the present Meeting show similar ratios of 3-8 (see Table 8). The above processing study on tomatoes shows that residues are lower in juice and purée than in the original fruit, and at about the same level in tomato paste.

RESIDUES IN FOOD IN COMMERCE OR AT CONSUMPTION

No information was provided.

NATIONAL MAXIMUM RESIDUE LIMITS

The only MRLs provided that were not recorded by the 1991 JMPR were New Zealand national MRLs for citrus, 0.5 mg/kg and tamarillos, 0.1 mg/kg.

APPRAISAL

Buprofezin was first evaluated by the 1991 JMPR, which estimated an ADI of 0-0.01 mg/kg bw and recommended TMRLs for cucumbers, tomatoes and oranges with 8 required and 4 desirable items of further work or information. Data were provided in response to the 1991 requirements on the fate of residues in water (acidic conditions), metabolism in ruminants and plants, additional supervised trials on citrus, cucumbers and tomatoes, and other items.

Data provided by the manufacturer allowed the Meeting to conclude that the identity of the thiobiuret metabolite formed in water under acidic conditions had been confirmed.

The 1991 JMPR reviewed data on the metabolism of buprofezin in rats and reports of the metabolic products found in the excreta of hens and proposed a tentative metabolic pathway for animals, but required a ruminant metabolism study. Such a study on a lactating Jersey cow fed the equivalent of 27 ppm in the diet of [¹⁴C]phenyl-labelled buprofezin for 7 days was reviewed by the Meeting. More than 64% of the total radioactive dose was excreted in the faeces and urine, over twice as much in faeces as in urine. Tissues and milk accounted for 1.2% of the administered dose, the highest proportion in the liver (0.7%), the next highest in muscle (0.2%) and the lowest in the kidneys (0.04%).

Unchanged buprofezin and *p*-hydroxybuprofezin were the only identified residues found in the faeces, accounting for 61% of the total radioactive residue (TRR). These two, with isopropylphenylurea (IPU), hydroxy-IPU, acetamidophenol and low levels of the dione metabolite were found in urine (35% of the TRR was identified). No unchanged buprofezin was detected in the liver, kidneys, or milk. None of the several metabolites in the muscle and fat could be identified owing to the low levels present (≤ 0.02 mg/kg buprofezin equivalent).

In liver the predominant residue was *p*-hydroxybuprofezin (11% of the TRR), with lesser amounts of IPU, hydroxy-IPU and *p*-acetamidophenol (19.1% of the TRR was identified). A similar profile of the same metabolites was found in the kidneys (33% of the TRR identified). In milk (15% of the TRR identified) the same compounds were found, but *p*-acetamidophenol was the predominant residue (9.2% of the TRR). Several unidentified metabolites were also observed in each sample, the major one constituting 5.9, 4.5 and 4.9% of the TRR in the liver, kidneys and milk respectively.

From these findings two basic metabolic pathways are proposed for ruminants. The first is hydroxylation at the para position of buprofezin, followed by cleavage of the thiadiazinane ring and loss of the $-\text{CH}_2\text{-S-C=N-C}(\text{CH}_3)_3$ group to leave hydroxy-IPU which is degraded to *N*-hydroxyphenylacetamide (*p*-acetamidophenol). The second proposed route involves formation of the dione metabolite (found in urine as well as in citrus metabolism) as an intermediate before cleavage of the thiadiazinane ring with loss of $-\text{CH}_2\text{-S-C=O}$ to form IPU which is hydroxylated and metabolized by multiple steps also to the acetamidophenol. The dione was not reported in the hen study, but was found in the degradation of buprofezin in soil and water.

The metabolic pathway proposed for ruminants is consistent up to a point with that proposed by the 1991 JMPR for animals on the basis of the data on rats and hens. The difference is an additional hydroxylation of the phenyl ring in rats to form dihydroxyphenyl-buprofezin, followed by methylation of one of the hydroxy groups to form hydroxy-methoxy-buprofezin. Neither of these compounds nor the thiobiuret metabolite were among those used as reference standards in the cow metabolism study. It is possible that unidentified metabolites found in the cow study could have included them.

From these studies the Meeting concluded that the metabolism in ruminants is reasonably well understood. Even so, it would have preferred to see a higher proportion of the TRR identified in liver and kidney, since some of the unknown metabolites occurred at levels near or above some of those which were identified. For this reason and for further confirmation of proposed metabolic pathways, the Meeting concluded that a desirable extension to the work already done would be the analysis of any reserve (or future) cow liver and kidney samples for the two additional metabolites found in rats and for the thiobiuret (the major product formed in water under acidic conditions).

The Meeting confirmed the view of the 1991 JMPR that any future uses of buprofezin on major poultry feed items may require a more definitive poultry metabolism study.

The 1991 JMPR reviewed information on the metabolism in plants. On tomatoes buprofezin *per se* accounted for >90% of the residue after 7 days. In geponic- or hydroponically-grown rice plants residues were taken up by the roots and translocated to other plant parts, the main residue being unchanged buprofezin and the major metabolite *p*-hydroxybuprofezin. In several other hydroponically-grown plants buprofezin was again the main residue, but the major metabolite was buprofezin sulfoxide, followed by the phenylbiuret. Because of these differences, the 1991 Meeting concluded that a study of metabolism by a major crop on which there was extensive use was needed, and required a citrus metabolism study. The 1991 JMPR also requested analysis for buprofezin sulfoxide, the phenylbiuret and *p*-hydroxybuprofezin in future field trials and for the thiobiuret in future metabolism or residue studies if it was found as a metabolite in citrus.

A citrus metabolism study was completed on glass-grown lemon trees at rates approximating GAP. Little translocation was found in immature fruit after applications of [¹⁴C]buprofezin to leaves or stems. The manufacturer reported that the thiobiuret (BF-25), phenylbiuret (BF-11) and buprofezin sulfoxide (BF-10) were all used as reference standards in the study and were not detected. The one and two-dimensional TLC and HPLC analyses supported this with respect to buprofezin sulfoxide and the phenylbiuret, but none of the chromatograms of samples or standards provided confirmed analyses for the thiobiuret.

The Meeting concluded that there was little likelihood that significant levels of the phenylbiuret or buprofezin sulfoxide would be formed from topical applications to citrus and that this conclusion could reasonably be extended to other commodities for which temporary limits had been proposed, when taking into account the previously reviewed study on tomatoes. In view of the presence of significant levels of unidentified metabolites, proof that the thiobiuret was formed under acidic conditions and the lack of firm experimental evidence that it was not present in the citrus metabolism study, the Meeting had no basis to conclude that this compound is not formed during citrus metabolism.

As for other aspects of citrus metabolism, on day zero essentially all of the radioactivity was in or on the peel and 93-97% in the surface wash. After 14 days the proportion in the surface wash was reduced to 65% of the total and after 75 days to 16%. After these two periods the total residues in the peel (extractable + non-extractable) were 34.6 and were 82.9% respectively, indicating penetration from the surface into the peel with time. This was confirmed by the increase in the low pulp residue (from <0.4 to 1.3% of the total) over the same period.

After 14 days 66% of the TRR was unchanged buprofezin, 6% the dione metabolite (3-isopropyl-5-phenyl-1,3,5-thiadiazinane-2,4-dione), 5.7% 2-amino-2-methylethyl-2-methylpropyl-4-phenylallophanate (designated as O or metabolite A), 3.6% unidentified metabolite B and 1.7% 1-isopropyl-3-phenylurea (IPU). After 75 days the levels were 18% buprofezin, 34% metabolite A, 9% metabolite B, 8% IPU and 7% dione.

On the basis of these findings the proposed metabolic pathway for plants is similar to, but more complex than, that outlined in the 1991 monograph. One route involves oxidation at the para position on the phenyl group to form hydroxybuprofezin, followed by cleavage of the heterocyclic ring to form hydroxy-IPU. A second route involves oxidation of the sulfur followed by ring cleavage to form the phenylbiuret, which is further degraded to IPU and oxidized to hydroxy-IPU.

In a third route oxidation of the *tert*-butylimino group to form the dione is followed by ring cleavage and formation of IPU, which is again oxidized to hydroxy-IPU. In the fourth route postulated hydroxylation of the *tert*-butyl group to an intermediate designated as BF-4 is followed by ring cleavage to give metabolite A, which is degraded to IPU and this again may be oxidized to hydroxy-IPU. In all cases the IPU may also be degraded to phenylurea.

Because of the multiple metabolic routes shown to occur in plants, future submissions of data on commodity groups other than fruiting vegetables and citrus should be accompanied by geponic metabolism studies for the groups in question. The metabolism studies should be conducted before the field trials. If significant residues of additional metabolites are identified, field trials may need to include analyses for these as well.

Several extractability studies and analytical methods for buprofezin (including ICI method PPRAM 82) were provided in response to the requirement for validation of PPRAM 82, which was used for most of the trials reviewed by the 1991 JMPR. Several of the studies submitted to the present Meeting demonstrated that acetone (used in method 82) efficiently extracts buprofezin from fortified samples.

The primary response to the 1991 requirement was re-analysis of peaches which were found by PPRAM 82 in 1990 to contain 0.66 mg/kg buprofezin. The extraction procedures tested in the re-analysis were cold acetone, cold acetone/water, acetone reflux, cold methanol and methanol/water reflux. Results were similar in all cases, ranging from 0.52 to 0.78 mg/kg (mean 0.66 mg/kg), with 68-87% recoveries from 0.5 mg/kg fortifications.

The Meeting concluded that the available information sufficiently validated method 82 for the MRL levels recommended in 1991 (0.3 to 0.5 mg/kg), but agreed that additional validation was still desirable to allow an accurate estimate of a limit of determination. Full validation is needed for any future data developed with this method or others.

Another GLC method described for the determination of buprofezin and its dione and isopropylphenylurea metabolites has a limit of determination of 0.05 mg/kg in tomato products.

The 1991 JMPR required additional data from outdoor supervised trials on cucumbers and tomatoes if such uses were shown to be GAP, and additional data on oranges with analyses for *p*-hydroxybuprofezin, the thiobiuret, buprofezin sulfoxide and phenylbiuret in addition to buprofezin. Unchanged buprofezin had been the only significant residue in a tomato metabolism study. Data were provided for cucumbers, tomatoes and citrus.

Citrus. The temporary MRL of 0.3 mg/kg recommended for oranges by the 1991 JMPR was based mainly on Japanese trials, since other trials did not reflect maximum GAP conditions (trials in South Africa showed <0.05 mg/kg after 127 days but the GAP PHI is 90 days) or otherwise did not comply with GAP (too many applications or excessive rates). The 1991 Meeting considered maximum residues in the Japanese trials according to GAP to be approximately 0.3 mg/kg on a whole fruit basis, from a GAP application rate of 2.5 kg ai/ha (50 g ai/hl) and a 14-day PHI. Additional data from trials reflecting GAP were required.

The Meeting was informed that current Japanese GAP involves 5 applications at 25 g ai/hl and a 14-day PHI. Application rates are not on a kg ai/ha basis because that is volume-dependent and the volume varies according to the size of the trees. Therefore, it follows that the Japanese trials on oranges reviewed in 1991 were at twice GAP rates.

Additional citrus data from Brazil, Spain and Japan were provided to the Meeting in response to the 1991 requirement. In Brazilian trials the maximum residues in oranges were 0.04 mg/kg after 28 or 63 days and <0.01 mg/kg after 91 days from applications at 0.5 kg ai/ha (30 g ai/hl). Although the trials were according to FAO guidelines, buprofezin was reported not to be registered in Brazil and the data could not be related to the known GAP of other countries. The application rate was reported to be twice the "recommended" dosage, but it is within the range of GAP reported by some countries (either as g ai/ha or g ai/hl, not always both). Samples were adequately stored for the short period before analysis. The Meeting concluded that the Brazilian data were not sufficiently related to available GAP to estimate a maximum residue level.

In three Spanish trials maximum residues in whole oranges were 0.06 mg/kg after the Spanish 7-day GAP PHI, from an application rate of 1 kg ai/ha (25 g ai/hl), which was reported to be current Spanish GAP. This is an increase from the 10-12.5 g ai/hl rate reported as Spanish GAP in the 1991 JMPR monograph. The limit of determination for the HPLC determination was approximately 0.02 mg/kg in whole oranges for both buprofezin and *p*-hydroxybuprofezin. No residues (<0.02 mg/kg) of the latter were detected.

In a Japanese trial maximum residues on natsudaidais from GAP application rates were 0.7 and 0.4 mg/kg after 21 and 30 days respectively, the GAP PHI being 14 days. Five knapsack applications at GAP rates were made, each at 25 g ai/hl (1.25 kg ai/ha), to 2-tree plots. The trials were therefore within GAP but did not reflect the shortest GAP PHI. The reported limit of determination is 0.01 mg/kg for buprofezin and *p*-hydroxybuprofezin, but chromatograms suggest that 0.02-0.05 mg/kg may be more realistic.

Analytical samples from the Japanese trials stored at -20°C showed 80-106% recoveries of parent and *p*-hydroxybuprofezin after 58 days (pulp) or 90 days (peel). The field samples were stored at -10°C for periods ranging from 60 to 90 days for the shorter PHI samples to over twice that period for samples taken at longer intervals. Taken together with the good stability reported in 1991 of residues in apples, peaches and kiwifruit stored up to a year at -20°C, the Meeting concluded that the stability of stored samples in the Japanese trials was reasonably validated.

In the data submitted to the Meeting buprofezin residues in whole fruit were approximately 3 to 10 times those in the pulp, depending on the interval, which is consistent with the 3 to 8 times reported in the 1991 monograph. No residues of *p*-hydroxybuprofezin (<0.02 mg/kg) were detected in the peel, pulp or whole oranges in either the Spanish or Japanese trials. This is consistent with the metabolism study for this PHI and at the relative residue levels of buprofezin.

Although results have been provided from 14 supervised trials in 1991 and 1995 in 4 countries, few of them reflect GAP. The Japanese trials are the most significant because they most closely reflected maximum GAP conditions. The results from trials according to relevant GAP were South Africa, 1 trial, <0.05 mg/kg (4 results); Spain, 3 trials, 0.06 mg/kg, 2 x 0.03 mg/kg; Japan, 1 trial, 0.7, 0.4, 3 x 0.2, 0.08 mg/kg, giving altogether 0.7, 0.4, 0.2(3), 0.08, 0.06, <0.05(4) and 0.03(2) mg/kg.

The Meeting concluded that the available data were still insufficient to recommend an MRL for such a major commodity as citrus and recommended that the current temporary limit of 0.3 mg/kg be withdrawn. For future consideration of a citrus limit additional data reflective of GAP (including maximum application rates and shortest PHIs) need to be provided, together with confirmation of the current GAP, with labels in English or with an English translation, and all critical supporting information including a citrus processing study.

Cucumbers. The temporary MRL of 0.3 mg/kg estimated by the 1991 JMPR was based on data (mainly indoor) from The Netherlands, the UK, Greece and Japan. Maximum residues representing GAP were 0.06 mg/kg from The Netherlands (3-day PHI) and Greece (7-day PHI) and 0.21 mg/kg from trials according to proposed UK GAP (3-day PHI). Maximum residues reflecting GAP in a Japanese trial were 0.13 mg/kg after three days (the GAP PHI is 1 day), at 0.6 times the maximum permitted rate and 0.6 mg/kg at 1 day from a double rate. Residues were roughly proportional to the application rate. Because only the trials in Greece were outdoor, the Meeting required additional data from outdoor trials if outdoor uses are confirmed to be covered by GAP. Most of the GAP for cucumbers reported to the 1991 JMPR did not distinguish between field and glasshouse uses, but most of the trials were glasshouse.

No confirmation was received of non-glasshouse GAP for buprofezin uses on cucumbers, although uses listed by the 1991 JMPR for The Netherlands, the UK and Japan were confirmed (The Netherlands and UK confirmed as glasshouse uses). Accordingly there were no data from supervised field trials. However, additional data from trials according to GAP at four glasshouse sites in Japan were received. Residues at the 1-day Japanese GAP PHI were 0.4 to 0.8 mg/kg (mean 0.6 mg/kg), decreasing to a mean residue of 0.08 mg/kg after 7 days. On the basis of these new results, together with data reviewed by the 1991 JMPR, the Meeting recommended that the previous temporary MRL of 0.3 mg/kg should be replaced by an MRL of 1 mg/kg.

Tomatoes. The temporary MRL of 0.5 mg/kg recommended by the 1991 JMPR was based on trials in The Netherlands, the UK, Greece and Japan, with maximum residues reflecting GAP in The Netherlands of 0.2 mg/kg (0.3 mg/kg from a 1.3-fold rate) (GAP 0.075 kg ai/hl, 3-day PHI); UK 0.3 mg/kg (proposed GAP the same as The Netherlands) and Japan 0.4 mg/kg (GAP 1-1.9 kg ai/ha or 0.025 kg ai/hl). As with cucumbers, additional data would be required if field uses are confirmed to be GAP. GAP reported by the 1991 JMPR did not generally make a distinction, although most of the trials reviewed were glasshouse.

No specific information on GAP for field uses on tomatoes was provided to the Meeting, but additional data from Italy (field) and Japan (glasshouse) were provided. GAP in Germany, The Netherlands and the UK, and indirectly in Japan (where trials were reported as being according to GAP) was confirmed as applying to glasshouse uses. No GAP was provided for Italy, although the trials were within reported Japanese GAP. After 2 days (compared to the Japanese 1-day PHI) residues at the three Italian field locations ranged from 0.08 to 0.2 mg/kg, with no concentration reported in the juice and purée, although details of the processing were not given. No residues (<0.015 mg/kg) of *p*-hydroxybuprofezin were detected in any sample. Samples were stored appropriately to ensure their integrity.

The two applications in the Italian trials were also within the total seasonal application permitted in German GAP, although German GAP allows only one application. The rates expressed as kg ai/hl are also compatible with GAP rates reported in 1991 for Bulgaria, former Czechoslovakia, Jordan and Poland (Jordan and Poland have a 3-day PHI; the Italian results were at 2 or 7 days). The available results show a slow decrease in residues during the first 3 days. Although the Italian results cannot be strictly related to the GAP provided, they can be considered supplementary supportive information. Residues were generally lower than in the Japanese trials (see next para.), but the application rate expressed as kg ai/ha was higher in the latter, although the kg ai/hl rate is the same. A reasonable limit of determination for the HPLC method used would be 0.02 mg/kg.

Residues in the Japanese glasshouse trials according to GAP ranged from 0.3 to 0.7 mg/kg after 1 day, decreasing to 0.1 to 0.3 mg/kg after 7 days. Controls ranged from <0.005 to 0.04 mg/kg. An LOD of 0.05 mg/kg would appear to be reasonable for the method used (GLC with NP detection), according to chromatograms provided.

Taking into account residues from GAP applications up to 0.3 mg/kg in trials reviewed by the 1991 JMPR and up to 0.7 mg/kg in the new trials, the Meeting recommended that the previously recommended temporary MRL of 0.5 mg/kg should be replaced by an MRL of 1 mg/kg.

Processing tomatoes with field-incurred residues from exaggerated application rates revealed concentration factors of 23 and 34 from unwashed fruit to wet and dry pomace respectively. No significant concentration was observed in juice, purée or paste and residues of the dione metabolite did not exceed 0.02 mg/kg in dry pomace, even from more than twice the field application rate. No residues of the isopropylphenylurea metabolite were observed.

The JMPR reported no significant loss of buprofezin from apples, peaches and courgettes and only 13% from kiwi fruit after storage up to a year at -20°C. New information showed mean recoveries of 80-106% of both buprofezin and *p*-hydroxybuprofezin from 0.5 mg/kg fortification levels after storage of citrus for 56-58 days (pulp) or 91-93 days (peel) at -20°C. In cucumbers stored for 130 days at -20°C the recovery of buprofezin at 0.2 mg/kg fortification was reported as 90%. Mean recoveries of buprofezin added to tomatoes at 0.05 mg/kg ranged from 100 to 114% at four sites after storage for periods ranging from 53 to 94 days.

Residues in animals. The 1991 JMPR reviewed a conventional 28-day dairy cow feeding study which included feeding levels of 20 and 200 ppm in the diet. No residues of buprofezin (<0.01 mg/kg) were reported in muscle, kidney, liver, fat or milk from the low dose. On the basis of these results and the 0.5 and 0.3 mg/kg temporary limits recommended for tomatoes and oranges respectively, the 1991 Meeting tentatively concluded that residues of buprofezin *per se* were unlikely to occur in the muscle, kidneys, liver or milk of cattle, but recommended reconsideration of this conclusion in the light of required processing information and animal metabolism studies, which were provided to the present Meeting.

A 7-day metabolism study on a dairy cow was conducted at the equivalent of 27 ppm in the diet, a similar level to the 20 ppm feeding study reviewed by the 1991 JMPR. The metabolism study supports the finding in the feeding trial that residues of buprofezin are unlikely in the muscle, offal or milk of cattle at 20 ppm feeding levels. However, it also reveals that the main residue in animal products is *p*-hydroxybuprofezin (in the liver and kidneys) or *p*-acetamidophenol (in milk), not the parent compound determined in the feeding study. In the metabolism study, *p*-hydroxybuprofezin occurred at 0.13 mg/kg in liver, 0.07 mg/kg in kidney and <0.001 mg/kg in milk with lower levels of other metabolites. In milk the highest residue was *p*-acetamidophenol at 0.002 mg/kg. Residues in muscle were ≤ 0.02 mg/kg buprofezin equivalent and could not be identified.

The tomato processing study showed concentration of buprofezin by factors of 23 and 34 in wet and dry pomace respectively. With worst-case assumptions (e.g. residues at the proposed MRL level of 1 mg/kg in fruit, 34-fold concentration in dry tomato pomace, feeding levels of dry pomace of 25% of the diet in beef and 10% in dairy cattle) it can be estimated from the metabolism study that the main residue *p*-hydroxybuprofezin in cattle could occur at approximately 0.04 mg/kg in liver, 0.02 mg/kg in kidney and <0.001 mg/kg in milk, and *p*-acetamidophenol also <0.001 mg/kg in milk. Although no maximum residue level has been estimated for citrus, similar levels might be expected from the feeding of dry citrus pomace if the concentration factors are similar. Concentration in citrus pomace would be expected since most of the buprofezin residue has been shown to be in the peel. This observation and the concentration found in tomato pomace support the need for a citrus processing study.

Therefore, while the 1991 finding that no residues of buprofezin *per se* would be expected in meat, offal and milk was confirmed, there is a potential for low residues of *p*-hydroxybuprofezin in liver and kidney. Although a conventional feeding trial has been conducted it was less useful than it might have been because only buprofezin was determined, not the residues likely to occur, mainly hydroxybuprofezin.

The guidance on the need for conventional feeding studies in the 1993 JMPR report requires feeding trials if detectable residues (>0.1 mg/kg) occur in feeds and metabolism studies indicate that residues may occur at levels >0.01 mg/kg. Even if it is assumed that residues in the whole fruit before processing into pomace are likely to be ≤ 50% of the MRL (generally true for tomatoes and citrus), the information on buprofezin indicates that an adequate conventional animal transfer study is required.

The Meeting therefore concluded that the available data were insufficient to estimate reliable maximum residue levels for animal products. The information required would include a conventional feeding trial in which the residues determined would include at least buprofezin and *p*-hydroxybuprofezin, and preferably also *p*-acetamidophenol in milk, with details of analytical methods. A suitable definition of the residue in animal products can be determined when these data are available, should it be decided that MRLs for animal products are needed.

Because a new metabolism study on citrus and new residue data on plants were available, the Meeting reconsidered the 1991 JMPR definition of the residue for regulatory purposes as buprofezin. The 1991 conclusion was based to a large extent on the tomato metabolism study showing over 90% of the residue in tomatoes to be unchanged buprofezin after 7 days.

The citrus metabolism study showed unchanged buprofezin to account for 66% of the residues after 14 days and 18% even after 75 days. After 75 days the main residue was shown to be metabolite A (2-amino-2-methylethyl-2-methylpropyl-4-phenylallophanate). No significant residues of buprofezin sulfoxide (reported in hydroponic metabolism studies) or the phenylbiuret or the thiobiuret metabolites were reported. However, as noted earlier, there was no experimental evidence provided to demonstrate that residues of the thiobiuret metabolite did not occur in citrus. In supervised trials data submitted to the Meeting no residues of *p*-hydroxybuprofezin were reported in citrus or tomatoes.

On the basis of these findings the Meeting confirmed the 1991 JMPR recommendation that the definition of the residue for regulatory purposes in cucumbers, tomatoes and oranges should be buprofezin. The Meeting was informed by the manufacturer that the definition of the residue for human foods of plant origin is buprofezin *per se* in Spain, The Netherlands, Belgium, Switzerland and Japan. The definition may need to be re-assessed if MRLs are proposed in the future for additional crop types (since metabolism varies among crops) or if the need is indicated when the desirable information listed below is provided.

buprofezin

RECOMMENDATIONS

The temporary maximum residue levels estimated by the 1991 JMPR are revised as shown below. The maximum residue levels estimated for cucumber and tomato are recommended for use as MRLs.

Definition of the residue: buprofezin

Commodity		Recommended MRL (mg/kg)		PHI (days) on which based
CCN	Name	New	Previous (temporary)	
VC 0424	Cucumber	1	0.3	1
VO 0448	Tomato	1	0.5	1
FC 0004	Oranges, sweet, sour	W ¹	0.3	21

¹ Withdrawn

FURTHER WORK OR INFORMATION

Desirable

1. Analysis of any reserve cow liver and kidney samples from the ruminant metabolism study for the presence of the dihydroxybuprofezin, hydroxymethoxybuprofezin and the thiobiuret metabolites.
2. Further validation of PPRAM method 82 with sufficient chromatograms, recoveries and controls to permit an accurate estimate of the limit of determination.
3. Information on buprofezin and *p*-hydroxybuprofezin residues in food and commerce or at consumption, especially on buprofezin residues in commodities for which buprofezin uses are approved.
4. A conventional animal transfer study in which residues of buprofezin, *p*-hydroxybuprofezin and (in milk) *p*-acetamidophenol are determined, with suitable and validated analytical methods. Alternatively, reserve samples from the original transfer study can be analysed for these compounds if it can be convincingly demonstrated that such analyses would still be valid after prolonged storage. These studies are highly desirable, and would be required before maximum residue levels could be estimated for animal products.
5. Further information on national definitions of the residue for MRLs for crop and animal commodities.
6. Should citrus MRLs be contemplated in a future submission, the following further work or information would be:

Desirable

Experimental evidence that the thiobiuret metabolite does not occur during citrus metabolism.

buprofezin

Required 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 has been shown not to be formed during citrus metabolism).

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