CARBOSULFAN (145)

EXPLANATION

Carbosulfan was first evaluated by the 1984 JMPR which allocated a temporary ADI of 0.005 mg/kg bw and recommended a temporary MRL of 2 mg/kg for the sum of carbosulfan, carbofuran, 3-hydroxy-carbofuran in citrus fruits. The temporary ADI was replaced by an ADI of 0-0.01 mg/kg bw by the 1986 JMPR.

The 1991 JMPR considered a request from the CCPR to harmonize the definition of the residue with that of carbofuran (which did not include 3-keto-carbofuran). It recommended that MRLs for carbosulfan should refer to a residue defined as carbosulfan and that MRLs for carbofuran should be for the sum of carbofuran and 3-hydroxy-carbofuran expressed as carbofuran, whether from the use of carbofuran or carbosulfan, with a clear indication of which use was the basis for the MRL. It further recommended that analyses of samples from supervised trials should continue to include the determination of 3-keto-carbofuran.

Because information required by the 1991 Meeting was not provided, the 1993 JMPR recommended withdrawal of the proposed limits for carbofuran and carbosulfan in citrus fruits. It was informed that additional studies were under way.

Both carbofuran and carbosulfan were considered as candidates for review as part of the CCPR periodic review programme. As a result, the 1996 JMPR estimated an ADI of 0-0.002 mg/kg bw for carbofuran. Carbosulfan has not yet been scheduled for periodic review of its toxicology. The residue and analytical aspects of both carbofuran and carbosulfan were subjected to periodic review at the present Meeting.

IDENTITY

Common name: Carbosulfan (BSI, ANSI, E-ISO, F-ISO)

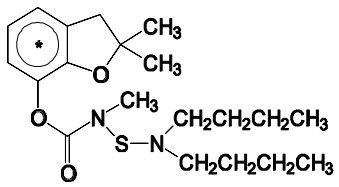
Chemical name:
IUPAC: 2,3-dihydro-2,2-dimethylbenzofuran-7-yl(dibutylaminothio)methylcarbamate
CA: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl [(dibutylamino)thio]methylcarbamate

CAS Registry No.: 55285-14-8

CIPAC No: 417

Synonyms: FMC 35001, Marshal®, Advantage®

Structural formula:



Molecular formula: $C_{20}H_{32}N_20_3S$

Molecular weight: 380.5

Physical and chemical properties (Alvarez,1995)

Pure active ingredient

Vapour pressure: 2.69×10^{-7} mm Hg at 25°C; nitrogen saturation method; 97.1% pure

Melting point: Carbosulfan is a liquid. It decomposes at elevated temperature (boiling point not determinable)

Octanol/water partition coefficient (25°C, pH 9, 97.1% pure):

Log K_{OW} = 5.4; $K_{OW} = 2.8 \times 10^5$
(carbosulfan is hydrolysed at lower pH)

Solubility: available only for technical material

Specific gravity: available only for technical material

Hydrolysis Hydrolyzes at <pH 9
Photolysis: mainly to carbofuran and dibutylamine in aqueous solutions, half-life 1.4 days at pH 7 and 4-8 days in distilled water. Half-life <10 minutes but greater at 70% field moisture (Capps, 1981, in Alvarez, 1995). See also environmental fate in soil and water below.

Technical material

Purity: Not included in cited reference (1984 JMPR reported 93% for technical and 86-91% for manufacturing-use product).

Solubility:

water (pH 9, 25°C) 0.3 mg/l
acetone, acetonitrile, toluene, hexane (25°C) miscible at carbosulfan to solvent weight ratios from 0.02 to 2
Exxon Aromatic 100 (23°C) 1 g completely miscible in 4 ml solvent

Specific gravity (25°C):
Technical 1.054 g/ml
Manufacturing-use product1.052
Melting range: carbosulfan is a liquid. It is degraded at elevated temperature

Stability (10 days at 23 and 50)
Stainless steel and aluminum surfaces: no instability during

Hydrated ferric, manganous and cupric sulfates (10 days)
Manganous sulfate: no significant decrease (92.8% remained)
Ferric sulfate: significant decrease at 23 or 50°C (9.6% remained at 23 , 5.7% at 50°C)
Copper: significant decrease at 50°C (3.75% remained)

Oxidizing and reducing agents: exposure to zinc and potassium permanganate showed no tendency for oxidation or reduction.

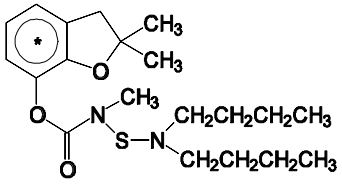
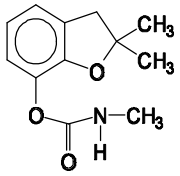
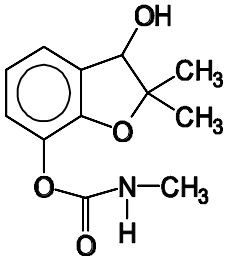
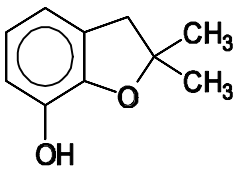
Formulations

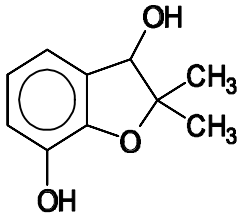
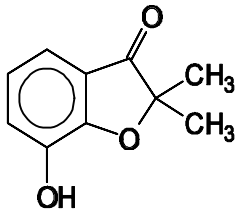
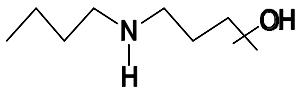
Liquid emulsion 26.1% active ingredient
Liquid emulsion 250 g ai /l

METABOLISM AND ENVIRONMENTAL FATE

The fate of carbosulfan has been investigated in rats, goats and oranges. In each case [^{14}C]carbosulfan was labelled (in separate experiments) both uniformly in the phenyl ring and at the C-1 carbons of the dibutylamine (DBA) group. The structures and chemical names of the major metabolites identified are shown in Table 1.

Table 1. Structures and chemical names of carbosulfan and some major identified metabolites.

Common or abbreviated name Abbreviation FMC no.	Chemical name	Chemical structure
carbosulfan	2,3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio)methylcarbamate	
carbofuran CF FMC 10242	2,3-dihydro-2,2-dimethylbenzofurany-7-yl methylcarbamate	
3-hydroxy-carbofuran 3-OH-CF FMC 18209	2,3-dihydro-3-hydroxy-2,2-dimethylbenzofuran-7-yl methylcarbamate	
7-phenol 7-P FMC 10272	2,3-dihydro-2,2-dimethylbenzofuran-7-ol	

Common or abbreviated name Abbreviation FMC no.	Chemical name	Chemical structure
3-hydroxy-7-phenol 3-OH-7-P FMC 16497	2,3-dihydro-2,2-dimethylbenzofuran-3,7-diol	
3-keto-7-phenol 3-K-7-P FMC 16490	2,3-dihydro-2,2-dimethyl-3-oxobenzofuran-7-ol	
Dibutylamine DBA FMC 65387	dibutylamine	$[CH_3(CH_2)_3]_2NH$
Various hydroxylated dibutylamines	Name depends on position of OH substitutent	

Other test substances and reference compounds used but not listed above included 5-hydroxy-carbofuran, *N*-hydroxy-carbofuran, 3-hydroxy-carbosulfan, 3-keto-carbosulfan, carbosulfan sulfone, 3-keto-carbosulfan sulfone, 4-aminobutanol, 1-amino-2-butanol, butylamine, l-henylalanine, l-tyrosine, dl-tryptophan, and C_4 to C18 fatty acids (Curry and Weintraub, 1996).

The major compounds (>10% of the TRR) found in rats, goats and oranges are shown in Table 2..

Table 2. Major components (>10% of the TRR) of the residues from [^{14}C]carbosulfan found in plants and animals.

Compound	RAT		GOAT (MILK)		ORANGE[1]	
	Phenyl label	DBA label	Phenyl label	DBA label	Phenyl label	DBA label
carbosulfan					X	X
carbofuran					X	
3-hydroxy-carbofuran	X		X			
3-keto-7-phenol	X		X			
3-hydroxy-7-phenol			X			
7-phenol	X		X			

Compound	RAT		GOAT (MILK)		ORANGE[1]	
	Phenyl label	DBA label	Phenyl label	DBA label	Phenyl label	DBA label
dibutylamine		X				X
hydroxylated dibutylamines		X				
aminobutanols				X		
Fatty acids				X		
amines				X		

[1] Surface rinses and extracts of peel

Animal metabolism

The fate of carbosulfan has been investigated in rats (Fang and ElNaggar, 1995) and goats (Curry and Weintraub, 1995).

Rats. In the definitive phase of the study, conducted according to US EPA GLP, 60 male or female Hsd:Sprague Dawley rats were dosed orally in groups by syringe with either phenyl- or DBA-labelled [^{14}C]carbosulfan, according to one of three treatment regimens. Four additional animals were used as controls. The dosing solutions (pure radio-labelled standards diluted with unlabelled carbosulfan) were administered at approximately 4.3 mg/kg bw (about 22 uCi/mg specific activity) for low doses and 29 mg/kg (*c.* 3.1 uCi/mg specific activity) for high doses. Dosing was based on animal weights which were about 180 to 240 g for males and 180 to 207 g for females. The dosing regimens (5 female and 5 male rats each for phenyl and DBA labels in each regimen) are designated in Tables 3 and 4 as shown below.

SLD single low dose
MLD multiple low doses (single unlabelled dose followed by labelled dose)
SHD single high dose

Samples of urine, faeces and cage rinses were collected at intervals up to 48 hours and daily thereafter for a total of 168 hours. In the experiment with the DBA label CO_2 and other volatiles were collected at intervals based on expectations from preliminary studies. After 168 hours the rats were killed and samples including fat, muscle, skin, organs and brain were taken for analysis. Solid samples were combusted and ^{14}C measured as carbosulfan equivalents in all samples by liquid scintillation counting (LSC). The distribution and recovery of radioactivity is shown in Table 3.

Table 3. Distribution and total recoveries of administered radioactivity in dosed rats.

Group, Label	Distributions and Total Recoveries of Administered Radioactivity											
	% of Dose											
	Urine		Faeces		CO2		Tissue		Carcase		Total	
	M	F	M	F	M	F	M	F	M	F	M	F
SLD												
Phenyl	76	81	22	16	NA	NA	<0.01	<0.01	0.20	0.79	98	98
DBA	66	65	13	17	12	10	0.20	0.13	1.07	0.98	92	93
MLD												
Phenyl	79	88	15	5	NA	NA	<0.01	<0.01	ND	0.27	93	95
DBA	71	71	8	8	13	15	0.02	0.13	1.01	0.93	94	94
SHD												
Phenyl	83	72	10	17	NA	NA	ND	ND	0.52	1.92	94	91
DBA	66	66	8	12	17	10	0.23	0.09	1.42	1.58	93	90

NA not applicable; ND none detected

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About 80-90% of the low dose of both labels was excreted within 24 to 48 hours of dosing by both sexes. With the high dose about 72 hours or longer was required for this level of excretion.

Samples of urine and faeces were analysed separately for the identification and characterization of metabolites mainly by HPLC and TLC with enzymatic hydrolyses as needed. The urine metabolites were 4-9% nonconjugates, 16-27% glucuronide conjugates and 49-57% sulfate conjugates. The identities of the major metabolites were confirmed by electron-impact GC-MS or LC-MS of dansyl, 4-chlorobenzoyl, acetyl or trimethylsilyl (TMS) derivatives. The results are shown in Table 4.

Table 4. Compounds identified in rat excreta (combined faeces and urine).

Compound	Distribution of ^{14}C-Residues as % of Dose					
	SLD		MLD		SHD	
	Male	Female	Male	Female	Male	Female
Phenyl label						
3-keto-7-phenol	20.7	26.7	14.6	20.8	25.9	24.3
3-hydroxy-7-phenol	15.4	13.4	26.3	26	18.5	18.4
7-phenol	23.9	23.1	8.9	11.7	7.4	5.1
3-hydroxy-carbofuran	17.3	13.9	22.1	21.1	17.2	13.4
carbosulfan	2.5	3.4	0.9	0.4	1.7	4.6
Minor metabolites (6)*	6	5.2	6	3.5	6	7.8
Total identified	85.9	85.6	78.8	83.5	76.8	73.6
DBA label						
dibutylamine	38.3	42.8	40.9	44.7	39	50.8
hydroxy-dibutylamine	23.8	22.5	25.8	24.7	23.5	17.1
CO_2	10.7	8.7	12.1	12.5	14.6	9
carbosulfan	6.3	8.3	3.5	4.2	4.1	6.9
Total identified	79.1	82.3	82.2	86.1	81.1	83.8

* Minor metabolites (each <5% of the dose): 5-hydroxy-carbofuran, 3-keto-carbofuran, carbofuran, 3-keto-carbosulfan sulfone, 3-hydroxy-carbosulfan, 3-keto-carbosulfan

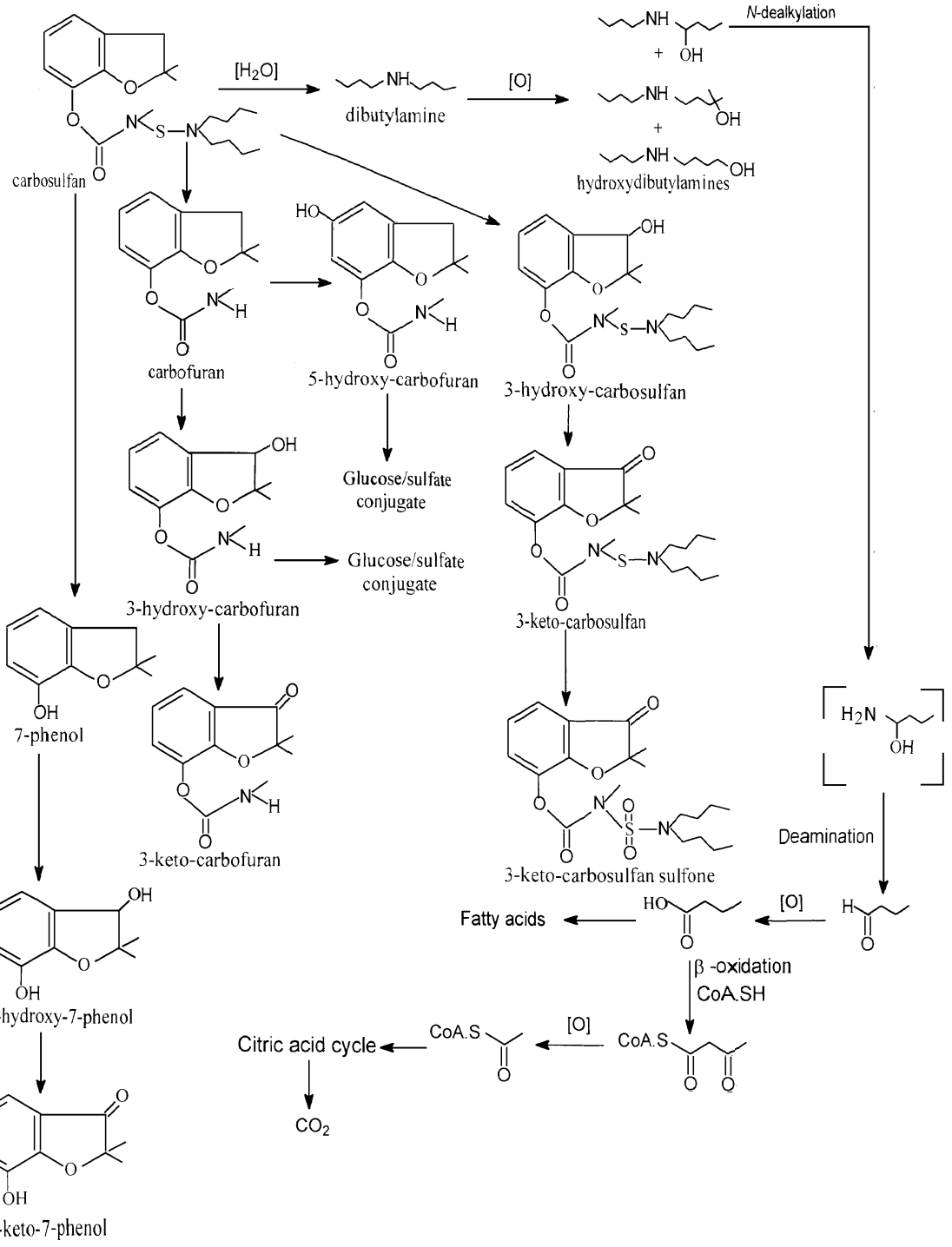
In carcase tissues the radioactivity ranged from undetectable to 1.9% of the applied dose. As an example, the mean residues ^{14}C expressed as mg/kg carbosulfan in the tissues of males rats from single doses were:

	DBA label		Phenyl label
	Low dose	High dose	Low dose
Liver	0.1 ± 0.005	0.87	ND
fat (reproductive)	0.08± 0.002	0.74	ND
skin	0.04± 0.006	0.57	ND
carcass (residual)	0.04± 0.007	0.40	0.008±0.008
kidney	0.03± 0.002	0.34	0.008±0.016
muscle (thigh)	0.01±0.002	0.16	ND

The residue levels from the DBA label were not appreciably different between males and females or between multiple-dose and the single low-dose samples. Residues from single doses of the phenyl label in some tissues of females were 3-4 times as high as in males. The residue levels were too low for further characterization or identification of the tissue residues..

The metabolic pathways proposed for carbosulfan in rats are shown in Figure 1.

Figure 1. Proposed metabolic pathways of carbosulfan in rats (Fang and El Naggar, 1995).



Goats. In this study, again according to US EPA GLP, 4 two year-old lactating Nubian goats were dosed orally once daily with either phenyl- or dibutylamine-labelled carbosulfan by balling gun for 7 consecutive days, two goats dosed with each label and with one control. Doses were prepared from 99.2% pure phenyl- and 98.8% DBA-labelled carbosulfan by dilution with unlabelled material. The phenyl labelled dose was approximately 44.7 mg/goat/day (1.75 mCi/day = 39.2 μCi/mg specific activity) corresponding to approximately 23 ppm in the diet based on average feed consumption of

approximately 1.9 kg/day. The DBA-labelled dose was approximately 40.9 mg/goat/day (1.91 mCi/day = 46.7 μCi/mg specific activity), about 25 ppm in the diet.

Urine and faeces were collected daily and milk in the afternoon and in the morning before dosing. They were stored separately. Blood was sampled just before slaughter 22 hours after the last dose and samples of omental and peripheral fat, liver, kidney and leg and lumbar muscle were taken for analysis. Storage was at ≤-20°C until analysis, generally within 6 months of sample collection.

Organic and aqueous extracts were analysed separately as such or after further clean-up. Some aqueous phases were subjected to enzymatic or acid hydrolysis and further partitions and separations. As an example, milk containing the phenyl label was shaken with acetone and extracted with acetonitrile. The acetonitrile extracts were combined and concentrated, and partitioned with hexane. Aliquots of the acetonitrile fraction were hydrolised with β-glucosidase of sulfatase and cleaned up on a C-18 solid-phase extraction column. The remaining unextractable fractions were analysed by combustion.

Milk samples containing the DBA label were also extracted with acetone and acetonitrile. The combined and concentrated aqueous acetonitrile extract was basified with ammonium hydroxide and partitioned with hexane, yielding a hexane fraction and an aqueous acetonitrile fraction. The hexane fraction after washing with 0.01 N HCl was saponified and partitioned with ethyl ether, and the resulting aqueous fraction acidified and again partitioned with ethyl ether. The acidic hexane wash was saponified and partitioned with ethyl ether, and the resulting aqueous fraction acidified and again partitioned with ethyl ether. The acidic hexane wash was basified and extracted with ethyl ether, to yield acqueous and ethyl ether fractions for analysis.

The aqueous acetonitrile fraction from the first hexane partition was concentrated, adjusted to pH 12 and partitioned with dichloromethane. The aqueous fraction was hydrolysed with β-glucuronidase and sulfatase and the hydrolysate passed through a solid-phase extraction column from which methanolic and aqueous fractions were eluted. The methanolic fraction was hydrolysed with HCl. The unextractable fractions containing the DBA label were tested for association with carbohydrates (phenylhydrazone derivatization) or proteins (pepsin/pronase digestion), or characterized by size-exclusion chromatography (to detect highly polar residues in protein fractions).

Similar separative steps were used for other samples, although extraction solvents varied and blending was required. Fats were dissolved in hexane before extraction with acetonitrile (the analysis included saponification with ethanolic KOH and esterification). Liver samples were initially blended with methanol buffered at pH9 and kidney and muscle samples with methanol buffered at pH 6.

The analytical procedures included combustion and liquid scintillation counting, normal and reversed-phase TLC, HPLC, GC-MS (quadrupole MS), LC-MS and chemical derivatization (dansyl, *p*-chlorobenzoyl, diazomethane, *p*-bromophenacyl and trimethylsilyl (TMS) derivatives. Components of the residues were identified in samples with the highest residues, namely milk at 7 days for the phenyl label and 5 days for the DBA label, lumber muscle and omental fat for the DBA label, and liver and kidney for both labels. The residues in phenyl-labelled muscle and fat were too low for identification. The total cumulative percentages of the dose recovered are shown in Table 5 and the total radioactivity in milk and tissues in Table 6.

Table 5. Percentages of administered ^{14}C recovered from urine, faeces, milk, tissues and cage rinses of goats dosed with [^{14}C]carbosulfan (Curry and Weintraub, 1996).

SAMPLE	% OF DOSE RECOVERED			
	PHENYL LABEL		DBA LABEL	
	GOAT 1	GOAT 2	GOAT 1	GOAT 2
URINE	80.77	84.37	70.02	66.19
Faeces	6.50	7.41	4.02	2.54
Cage rinse	1.40	1.30	0.35	0.31
Milk	0.16	0.17	1.97	2.66
Liver	0.02	0.02	0.37	0.31
Kidney	0.01	<0.01	0.03	0.04
Rear leg muscle	<0.01	<0.01	0.08	0.07
Lumbar muscle	<0.01	<0.01	0.05	0.04
Omental fat	<0.01	<0.01	0.18	0.13
Peripheral fat	<0.01	<0.01	0.06	0.05
Total	88.86	93.27	77.13	72.34
Mean total	91.07		74.74	

Table 6. Total radioactive residues (TRR) in milk and tissues of goats dosed with phenyl- and DBA-labelled carbosulfan (Curry and Weintraub, 1996).

^{14}C expressed as carbosulfan, mg/kg						
Label	Milk (pm sample)	Liver	Kidney	Muscle	Peripheral fat	Omental fat
Control	ND	ND	ND	ND	ND	ND
Phenyl	0.04-0.09	0.06	0.18	<0.01	0.01	0.009
DBA	0.3 - 0.94	1.13	0.75	0.18	0.74	1.2

The distribution and identity of residues from the phenyl label are shown in Table 7 and the DBA-label in Table 8.

Table 7. Identity and distribution of carbosulfan metabolites from feeding of phenyl-labelled carbosulfan to goats for 7 days (Curry and Weintraub, 1996).

Metabolite	% of TRR		
Total residue, mg/kg, as carbosulfan	Milk[1]	Liver	Kidney
3-hydroxycarbofuran	34.20	9.50	21.50
3-oh-7-phenol	21.10	15.60	13.30
3-keto-7-phenol	29.90	3.00	8.30
7-phenol	9.20	4.60	8.90
Minor components(<6)	1.20	4.40[2]	7.60[3]
Characterized organosolubles	2.30	17.30	17.40
Protein-associated metabolites	-	22.60	2.50
Polar aqueous metabolites	0.70	10.40	18.40
Unextractable residue	1.40	12.70	2.10
Total Residue (% of the TRR)	100.00	100.00	100.00
Total residue (mg/kg as carbosulfan)	0.09	0.06	0.154

[1]Day 7, pm. Metabolites identified in hydrolysed organo-extractable fraction containing 96.8% of the milk radioactivity. 3-keto-carbofuran (1.2% of the TRR) was also identified.

[2]Comprising 5-hydroxy-carbofuran (1.1%), 3-keto-carbofuran (1.5%), *N*-hydroxy-carbofuran (0.5%), carbofuran (0.2%), carbosulfan (0.1%) and a combination of 3-keto-carbosulfan sulfone, 3-hydroxy-carbosulfan and carbosulfan sulfone (1%, unresolved by the HPLC system).
[3]Comprising 5-hydroxycarbofuran (1.7%), 3-keto-carbofuran (3.7%), *N*-hydroxy-carbofuran (1%), carbofuran (0.8%), carbosulfan (0.1%) and a combination of 3-keto-carbosulfan sulfone, 3-hydroxy-carbosulfan and carbosulfan sulfone (0.3%, not resolved by the HPLC system).

Table 8. Identity/characterization and % of the TRR of carbosulfan metabolites from feeding of DBA-labelled carbosulfan to goats for 7 days (Curry and Weintraub, 1996)

METABOLITE	PERCENT OF TRR				
Total residue, mg/kg, as carbosulfan	MILK[1]	FAT	LIVER	KIDNEY	MUSCLE
Aminobutanols[1]	29.7	0.8	8.1	11.9	ND
Dibutylamine + related cpds [2]	6.7	0.6	13.4	10.5	9.6
Natural constituents[3]	30.2	87.3	29.1	13.8	32.0
Unconjugated amines[4]	11.8	ND	6.3	24.3	5.9
Conjugated or bound amines	10.5	ND	18.0	12.3	14.7
Lipophilic metabolites	0.6	0.5	1.3	4.5	1.2
Polar aqueous metabolites	7.6	0.2	16.6	18.5	26.5
[Un]extractable residues	2.9	10.5	7.2	4.2	10.0
Total residue (% of the TRR)	100.0	100.0	100.0	100.0	100.0
Total residue (mg/kg as carbosulfan)	0.680	1.286	0.986	0.823	0.193

[1]Day 5, pm. Metabolites identified in 4-aminobutanol; 1-amino-2-butanol.
[2]e.g. dibutylamine; hydroxydibutylamine; butylamine. In liver carboxy-DBA (10% of the TRR).
[3]e.g. in milk, fatty acids (13.4% of the TRR), amino acids (5.5%), carbohydrates (10.3%) and triglycerides (1.1%). In omental fat, fatty acids (82%) and triglycerides (5.3%). In lumbar muscle 20.6% of the TRR was associated with conjugated, unconjugated or bound amines and 32%of the TRR with amino acids.
[4]e.g. in milk 7 unknowns, each ≤0.024 mg/kg carbosulfan equivalent in omental fat 0.6% of the TRR, in liver 6.3% , in kidney (6 unknowns, each ≤0.06 mg/kg) 24.3% of the TRR (5 unknowns).

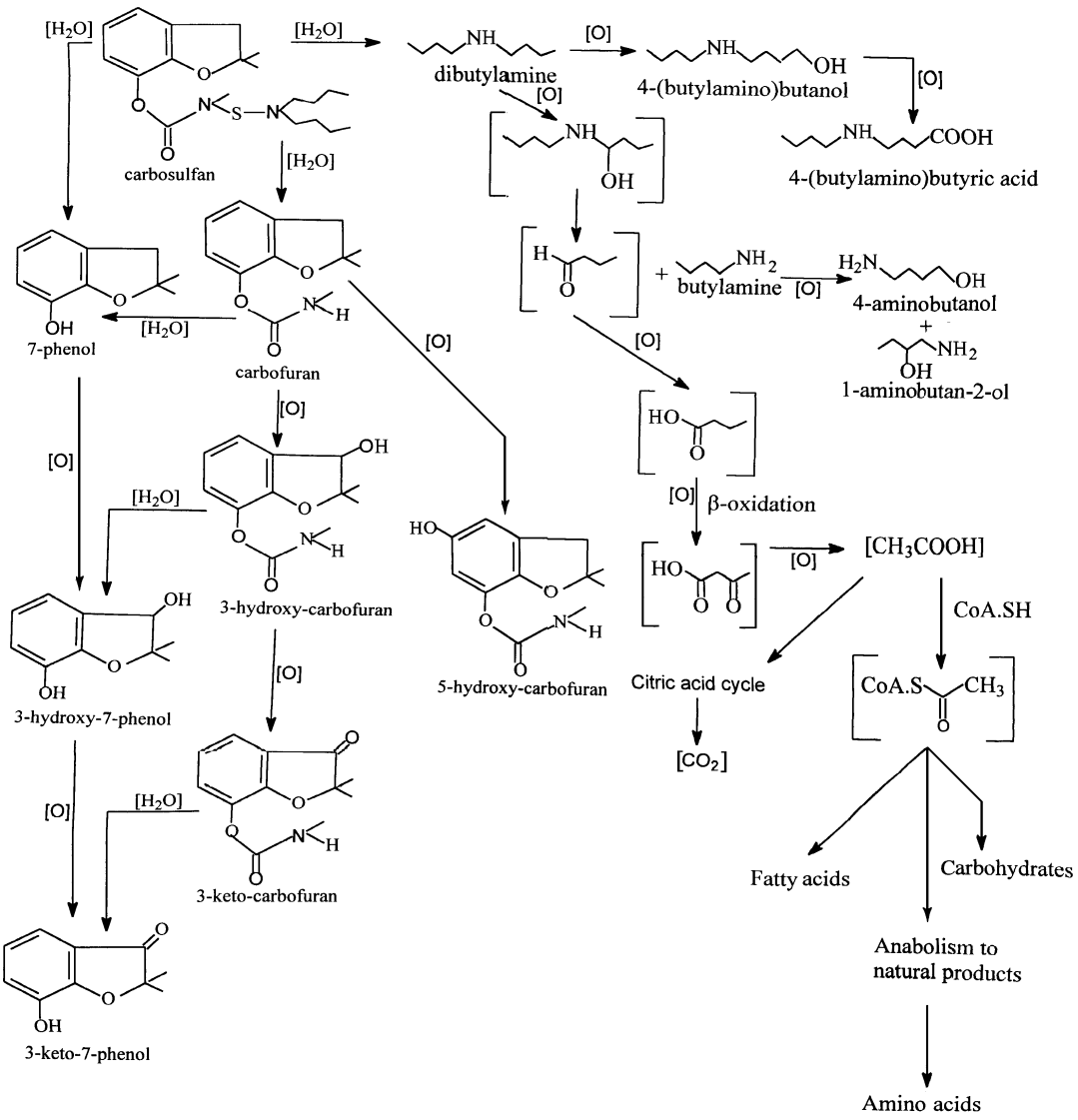
The proposed metabolic pathways in goats are presented in Figure 2.

Plant metabolism

A plant metabolism study was conducted according to US EPA GLP on three separate 21-year-old California commercial Navel orange trees in the field. One was treated with phenyl-labelled carbosulfan (99.3% purity, isotopic dilution 13.9 mCi/mmole), one with DBA-labelled carbosulfan (97.4% purity, isotopic dilution 15.08 mCi/mmole) and the third with a formulation blank as a control. Both labelled compounds were applied as 250 EC formulations at a nominal concentration of 0.5 g ai /l (*c.*450 ml/tree or an estimated 123 l/ha), with the trees protected from rain by plastic sheeting after the application. The trees were not irrigated during the study. Mature oranges were individually sprayed twice with a spray bottle with minimum run off (*c.*1 ml/orange), and the foliage was sprayed with a nitrogen-pressurized spray wand.

Ten to 20 mature fruit were collected for analysis on days 0, 7, 15 and 30, and leaves on days 0 and 30. Precautions were taken during shipment and storage to preserve the residues. Individual fruits from each sampling were processed and the fractions combined. Surface residues were removed by mild agitation in methanol/methylene chloride (1/1 v/v). The rinsed fruits were peeled and the peels homogenized under dry ice, as were leaves. The peeled oranges were puréed and centrifuged, and the juice was decanted.

Figure 2. Proposed metabolic pathways of carbosulfan in goats (Curry and Weintraub, 1996).



The total ^{14}C was determined in the processed fractions by combustion counting of $^{14}CO_2$ and in the rinses directly by LSC. Peels containing the phenyl label were extracted with KH_2PO_4/NaOH buffer and methanol. Non-polar fractions were separated by methylene chloride partition, and polar and conjugated fractions on a C18-solid phase extraction column (SPE) with methanol as eluant after hydrolysis with HCl and adjustment to pH 1. Post-extraction solids were refluxed with HCl and separated by SPE chromatography, yielding released extractable, released unextractable and bound residues.

Samples containing the DBA label were extracted and separated in a similar manner, except that the polar fractions were not adjusted to pH 1 before SPE chromatography. The remaining aqueous solution was adjusted to pH 14 before further SPE chromatography. Total ^{14}C was measured in the post-extraction solids, but they were not further characterized as they accounted for <5% of the TRR.

The separated fractions containing the phenyl label were analysed primarily by gradient reverse-phase HPLC with multi-wavelength UV detection at 230, 225.4, and 280 nm, and those with the DBA label primarily by reverse-phase TLC. Both groups were analysed by normal-phase TLC as

a secondary mode. Isolated residues of carbosulfan, carbofuran, dicarbofuran sulfide and carbosulfan sulfone from phenyl-labelled treatments were collected for HPLC-MS (CI) analysis, although the identities of the last two were confirmed only by normal-phase TLC. DBA-labelled compounds were derivatized with dansyl chloride before electron-impact GC-MS. Carbosulfan was unstable under the TLC isolation procedure. Peel rinses were analysed within 2 months, non-polar fractions of peel extracts within 6 months and polar, including conjugated, fractions within 12 months. Analyses by methods used in the field trials gave comparable results for carbosulfan and DBA as determined in the metabolism study. Analyses of fractions from stored 15-day peel samples late in the study gave similar results to those of analyses early in the study. This was adduced by the manufacturers as evidence that residues in the peel were stable under the frozen storage conditions for the duration of the study.

Residues of ^{14}C expressed as carbosulfan in the leaves were 16 mg/kg and 12.8 mg/kg after 0 and 30 days respectively from the phenyl label and 9.3 and 4.6 mg/kg from the DBA label. The distribution of ^{14}C in whole oranges and orange components is shown in Table 9, and in analytical fractions in Table 10. The compounds identified from both labels are shown in Table 11.

Table 9. Distribution of ^{14}C in navel oranges and orange components following treatment at nominal rates of 0.5g ai/l with phenyl- or DBA-labelled [^{14}C]carbosulfan (Weintraub, 1996).

Days after treatment	Label	TRR in whole fruit, mg/kg as carbosulfan	% of the TRR in			
			Peel rinse	Peel	Pulp	Juice
0	Phenyl	0.81	95.8	4.1	0.1	0.0
	DBA	0.72	93.9	5.7	0.3	0.2
7	Phenyl	0.85	86.6	13.2	0.1	0.0
	DBA	0.68	86.8	13	0.1	0.0
15	Phenyl	0.81	75.6	23.9	0.2	0.2
	DBA	0.56	75.1	24.5	0.2	0.2
30	Phenyl	0.78	53.7	45.9	0.1	0.3
	DBA	0.59	58.0	41.5	0.2	0.3

Table 10. Distribution of ^{14}C in polar, non-polar and bound fractions of rinsed peels of Navel oranges treated at nominal rates of 0.5g ai /l with phenyl- or DBA-labelled [^{14}C]carbosulfan (Weintraub, 1996).

DAY	Label	Whole Fruit	Rinsed peel	^{14}C fractions from rinsed peel					
				Extractable					
				Non-polar		Polar/conjug.		Bound	
				mg/kg[1]	% of TRR[2]	mg/kg[1]	`% of TRR[2]	mg/kg[1]	% of TRR[2]
0	Phenyl	0.809	0.033	0.029	3.6	0.002	0.2	0.003	0.3
0	DBA	0.702	0.040	0.026	3.7	0.013	1.9	0.001	1.1
15	Phenyl	0.808	0.193	0.153	19.0	0.024	3.0	0.016	2.0
15	DBA	0.563	0.138	0.054	9.6	0.078	13.9	0.006	1.1
30	Phenyl	0.775	0.356	0.274	35.4	0.052	6.7	0.029	3.7
30	DBA	0.588	0.244	0.085	14.5	0.147	25.2	0.012	2.0

[1]carbosulfan equivalent
[2]in whole fruit

Table 11. Compounds identified in 30-day rinses and extracts from of peel oranges of trees 30 days after treatment at nominal rates of 0.5g ai /l with phenyl- or DBA-labelled [^{14}C]carbosulfan (Weintraub, 1996).

Label	Compound	% of the TRR	mg/kg as carbosulfan
Phenyl	Carbosulfan	40.1	0.311
	Carbofuran	33.9	0.263
	Carbosulfan sulfone	3.1	0.025
	3-hydroxy carbofuran	2.0	0.016
	3-keto-carbofuran	2.0	0.016
	N-hydroxymethyl-carbofuran	1.2	0.01
	Dicarbofuran sulfide	1.0	0.007
	7-Phenol	0.4	0.003
	Total identified	83.7	0.63
DBA	Carbosulfan	31.2	0.183
	Dibutylamine	58.2	0.342
	Total Identified	89.4	0.525

The proposed metabolic pathways in oranges are shown in Figure 3.

Environmental fate in soil

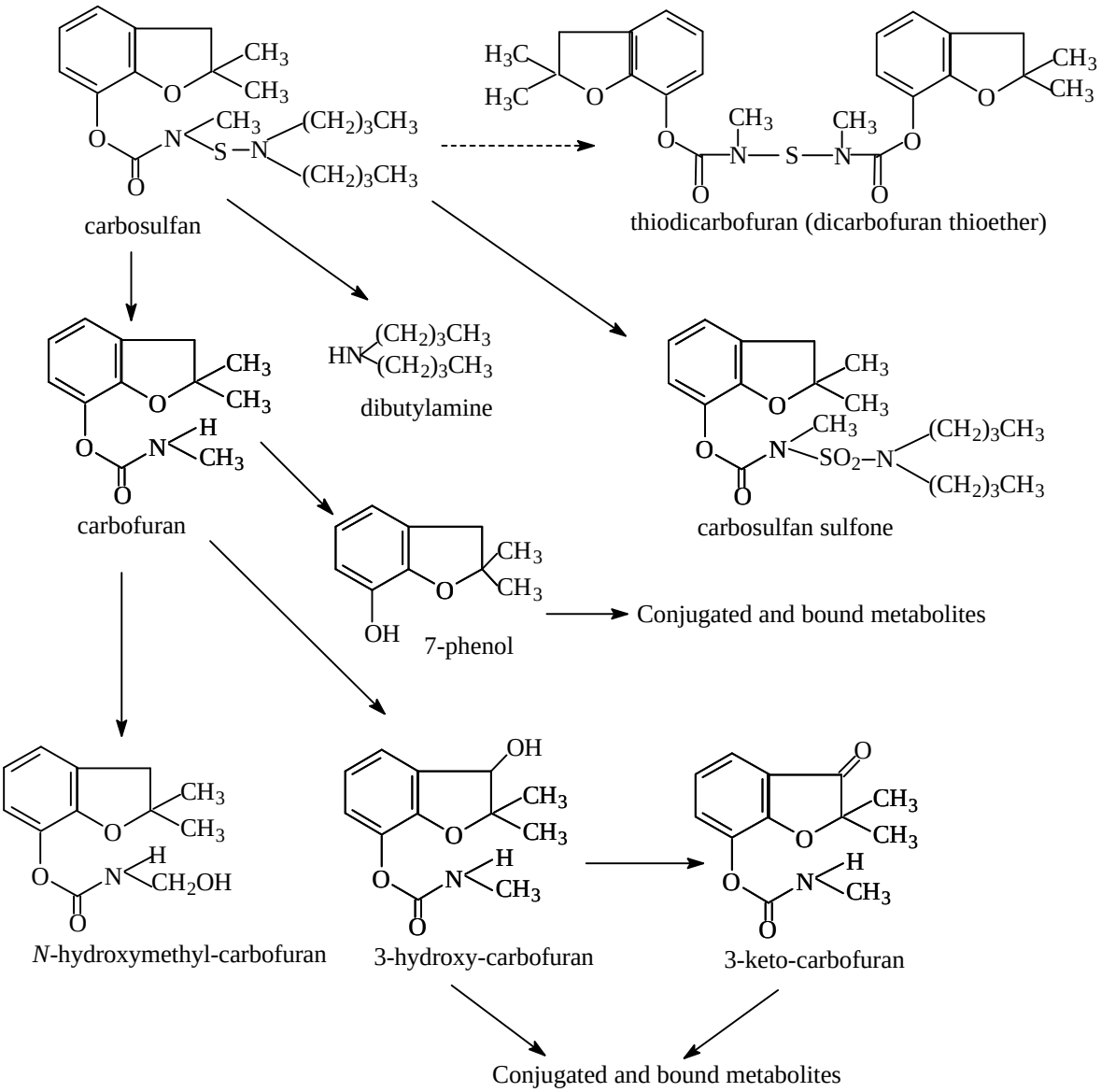
Photodecomposition. The photodecomposition of phenyl- and DBA-labelled carbosulfan was investigated on air-dried (apparently non-sterile) USA New York Dunkirk silt loam soil coated on watch glasses (25 µg ai/g soil) as a thin layer (250µm) (Capps, 1981). The soil characteristics were pH 9, 3.9% organic matter, 51.6% silt, 25.2% sand and 23.2% clay. Irradiation was at 2500 µW/cm^2 with a sun lamp and samples were removed after 10, 20 and 30 minutes and 1, 5, and 8 days of exposure for analysis by TLC, HPLC, LSC, GLC or in some cases GC-MS. The main compounds identified are shown in Table 12.

Table 12. Compounds identified after irradiating dry soil treated with phenyl- or DBA-labelled carbosulfan for up to 8 days with a sun lamp (Capps, 1981).

Compound	Phenyl label[1], % of TRR				DBA label[1] of TRR			
	10 min.		8 days		10 min.		8 days	
	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control
carbosulfan	1.2	13.4	-	-	11.4	20.1	2.1	0.8
carbofuran	86.4	77	54.5	77.2				
3-hydroxycarbofuran	-	-	3.5	0.7				
carbosulfan sulfone	4.5	3.6	2.6	3.8				
7-phenol	2.6	2.0	1.0	0.5				
3-keto-7-phenol	0.5	-	1.0	-				
N-hydroxymethyl-3-hydroxycarbofuran	-	-	0.8	-				
3-keto-carbofuran	-	-	0.7	0.3				
3-keto-carbosulfan sulfone	-	-	0.6	0.4				
dibutylamine					38.6	43.4	1.8	7.7
N-formyl-dibutylamine					6.4	2.5	4.2	3.1
N-acetyl-dibutylamine					1.1	0.9	1.1	2.0

[1] % of TRR

Figure 3. Proposed metabolic pathways of carbosulfan in oranges (Weintraub, 1996).



The extractability of ^{14}C from the phenyl label in irradiated samples ranged from 96.1% TRR after 10 minutes to 73.3% after 8 days. Decomposition was very rapid. After 10 minutes only 1.2% of the residue was intact carbosulfan, with its sulfone at 4.5%. The main component was carbofuran at 86.4%. After 5 days no carbosulfan was detected, although the sulfone was still 2.7%. After 8 days the identified residues were carbofuran, 3-hydroxycarbofuran, carbosulfan sulfone, the 7-phenol, 3-keto-7-phenol, *N*-hydroxymethyl-carbofuran, 3-keto-carbofuran and 3-keto-carbosulfan sulfone.

A similar rapid degradation was observed with the DBA label, but the extractability was lower (71.2% of the TRR after 10 minutes and 38% after 8 days). After 10 minutes the identified residues were DBA 38.6% of the TRR, carbosulfan 11.4%, *N*-formyldibutylamine 6.4% and *N*-acetyldibutylamine 1.1%. After 8 days these residues were 1.8%, 2.1%, 4.2% and 1.1% of the TRR respectively.

In the same study the author irradiated soil at 70% field moisture, instead of dry soil, treated with DBA-labelled carbosulfan in a similar manner for 48 hours. Intact carbosulfan and DBA accounted respectively for 85.9% and 6.5% of the TRR in irradiated soil and 83% and 10.2% in control soil after 3 hours. After 48 hours the corresponding figures were 76.2 and 86% carbosulfan and 6.7 and 3% DBA.

Environmental fate in water/sediment systems

Water. The photolysis of phenyl- and DBA-labelled carbosulfan was investigated in distilled water, buffered at pH 7 and unbuffed, at concentrations of 5 mg/l with 1% acetonitrile as co-solvent in pyrex flasks (Capps, 1981). The solutions were irradiated at 1500 $\mu W/cm^2$ with a sunlamp and samples were withdrawn for analysis initially and after 1, 4, and 8 days.

About 90% or more of the ^{14}C from both labels was extractable from both buffered and unbuffered water throughout the study, and on 0 day carbosulfan accounted for over 98% of the TRR in all the samples. The major compounds identified in the phenyl- and DBA-label experiments are shown in Tables 13 and 14 respectively.

Table 13. Major compounds identified after irradiating phenyl-labelled carbosulfan in pH 7 buffered and distilled water for 8 days with a sun lamp (Capps, 1981).

Compound	pH 7 buffered water[1]				Distilled water[1]			
	1 day		8 days		1 day		8 days	
	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control
Carbosulfan	72.4	87.6	1.8	46.3	79.7	91.7	22.4	73.4
Carbofuran	12.6	6.1	59.7	37.6	8.6	4.4	38.8	15.8
carbosulfan sulfone	1.2	0.3	4.5	0.4	0.7	0.4	3.1	0.5
7-phenol	1.4	0.3	5.6	2.0	0.9	-	4.3	0.5
3-keto-7-phenol	2.8	0.2	4.5	0.4	2.7	-	1.9	0.3

[1] % of TRR

In buffered water after 1 day of exposure carbosulfan was still the predominant compound, followed by carbofuran and the 3-keto-7-phenol. After 8 days the main compound (60% of the ^{14}C was carbofuran, with the 7-phenol, carbosulfan sulfone, the 3-keto-7-phenol and carbosulfan all below 6%. In distilled water however carbosulfan still accounted for 22.4% of the TRR after 8 days.

Table 14. Major identified compounds after irradiating phenyl-labelled carbosulfan in pH 7 buffered and distilled water for 8 days with a sun lamp (Capps, 1981).

Compound	pH 7 Buffered Water				Distilled Water			
	1 day		8 days		1 day		8 days	
	Irradiated	Control	Irradiated	Control	Irradiated	Control	Irradiated	Control
carbosulfan	76.5	89.2	2.1	57	88.2	96.2	47.3	81.7
dibutylamine	16.6	0.7	8.5	33.8	7.4	-	36.6	12.7
N-formyldibutylamine	1.1	0.3	3.4	2.5	-	3.0	-	-
N-acetyldibutylamine	-	-	-	-	1.5	-	3.4	1.3

In buffered water most of the labelled material in the irradiated sample was not identified after 8 days, but in distilled water carbosulfan and dibutylamine accounted for 47.3 and 36.6% of the TRR respectively.

The degradation of carbosulfan was rapid in buffered water with a half-life of about 1.4 days, but slower in distilled water with a half-life of about 4-8 days.

METHODS OF RESIDUE ANALYSIS

Analytical methods

Two analytical methods were available from the manufacturer for the determination of carbosulfan and metabolites in oranges (one independently validated), one for the determination of residues in processed fractions of oranges and one for the determination of residues in bovine meat and milk (independently validated). A multiresidue method, and methods provided by the government of The Netherlands were also reported.

Method P-2964M (Barros, 1995) was used for the determination of residues in Mexican and Brazilian trials on oranges. The development and application were consistent with GLP principles. It is capable of measuring residues of carbosulfan, its carbamate metabolites carbofuran, 3-keto-carbofuran and 3-hydroxycarbofuran, the 7-phenol, 3-keto-7-phenol, and 3-hydroxy-7-phenol metabolites, and dibutylamine. Whole oranges are homogenized in a Hobart chopper with liquid nitrogen. To determine carbosulfan the sample is extracted with dichlormethane, and the extract is filtered, concentrated, eluted from an aminopropyl solid-phase extraction (SPE) column and concentrated for analysis by reverse-phase HPLC. The HPLC configuration includes a post-column reactor to hydrolyse carbosulfan to carbofuran and a second basic hydrolysis reactor in which carbofuran is derivatized with *o*-phthalaldehyde + *N*,*N*-dimethyl-2-mercaptoethylamine before detection with a fluorescence detector operated at 330 nm excitation and 465 nm emission. The method for the carbamate metabolites is similar, except the sample is hydrolysed with boiling HCl before clean-up directly on C18 and aminopropyl SPE cartridges before HPLC determination. In this case only the second post-column reactor (basic hydrolysis and derivatization) is used.

The determination of phenolics also begins with hydrolysis in boiling HCl, followed by C18-SPE and liquid/liquid partition clean-up before derivatization with pentafluorobenzyl bromide and ethylation of the 3-hydroxy7-phenol. Dibutylamine is extracted with methanol/water (2:1). The extract is cleaned up by organic solvent/water partitions and the dibutuylamine derivatized with dansyl chloride. The derivatized phenolics and dibutylamine are determined by GC-MS (electron ionization) with single-ion monitoring.

The method was validated by six analytical recovery experiments with each compound, 5 at 0.03 mg/kg (the reported limit of determination) and one at 0.1 mg/kg for the carbamates and phenols, 4 at 0.05 mg/kg (the limit of determination) and 2 at 0.1 mg/kg for dibutylamine. The results at 0.1 mg/kg were not significantly different from those at the lower levels. In all cases the limit of detection was <0.01 mg/kg. The reported average percentage recoveries with their standard deviations were carbosulfan 85 $\pm$ 21, carbofuran 92 $\pm$ 11, 3-keto-carbofuran 89 $\pm$ 15, 3-hydroxycarbofuran 100 $\pm$ 9, 7-phenol 78 $\pm$ 9, 3-keto-7-phenol 81 $\pm$ 6, 3-hydroxy7-phenol 76 $\pm$ 3, and dibutylamine 89 $\pm$ 22. The high variability of the recovery of carbosulfan was attributed to the acidity of oranges, since carbosulfan is unstable at a pH of 4 or lower.

The method P-2964M was independently validated for carbosulfan at 0.03 and 0.1 mg/kg in oranges in two trials (Wood, 1996). The first was not successful at the 0.03 mg/kg fortification level (38.3 and 53.7% recovery) and only marginally so at 0.1 mg/kg (67.6 and 73.4% mg/kg), but the second was satisfactory with 72 and 98.3% recovery at 0.03 mg/kg and 93.8 and 97.6% at 0.1 mg/kg).

Method FCC 0193 (Gill, 1995a) was used for the determination of carbosulfan residues in supervised trials on oranges in Spain. It was developed in accord with GLP principles and measures residues of carbosulfan, carbofuran and 3-hydroxycarbofuran. It actually consists of two methods,

one for the first two and the other for 3-hydroxycarbofuran. In analyses for carbosulfan and carbofuran the chopped or homogenized sample is blended with hexane/propan-2-ol (2:1) and the extract is cleaned up by partition between hexane and water, followed by Florisil column chromatography with ethyl acetate/hexane elution. Determination is by GLC with a nitrogen/phosphorus detector. The 3-hydroxycarbofuran is hydrolysed and extracted by HCl reflux, cleaned up by partition with dichloromethane, and derivatized by ethoxylation in acidified ethanol. After liquid/liquid partition and Florisil column clean-up the derivative is determined by GLC with NP detection.

Recoveries of each of the three compounds were determined at 0.01, 0.05 and 0.1 mg/kg (6 replicates at each level). The average percentage recoveries and their SDs were as shown below.

	0.01 mg/kg	0.05 mg/kg	0.1 mg/kg	Controls
carbosulfan	78.5 ±18.5	75.3±3.8	77.0±5.4	<0.01
carbofuran	81.0±13.6	78.7±5.3	85.5±9.7	<0.01
3-hydroxy-carbofuran	86.5±12.2	110.±5.3	109.7±7.1	<0.01

The report showed that carbosulfan was not converted to carbofuran during the procedure. The limit of determination was reported as 0.01 mg/kg for all three compounds (signal to noise ratio 3:1). The limit of "detection" was 50 pg analyte and a "reporting limit" of 0.05 mg/kg was adopted.

Older analytical procedures used for the determination of carbosulfan, carbofuran and 3-hydroxycarbofuran in processed products of citrus (Leppert, 1981) were somewhat similar in principle to those described above (including GLC with NP detection), but there were differences in the extraction and clean-up.

For the determination of carbosulfan and carbofuran macerated frozen samples of juice, finisher pulp or whole fruit are extracted with 2:1 hexane: 2-propanol and cleaned up on an attaclay-aluminum oxide column, concentrated and cleaned up further on a Florisil column. Molasses and dried pulp are extracted with methanol buffered at pH 8 and again cleaned up on attaclay-aluminum oxide and Florisil columns. Oil is partitioned between hexane and acetonitrile (carbosulfan going into hexane and carbofuran to acetonitrile), and the two solutions are cleaned up on the same two columns.

For 3-hydroxycarbofuran determinations samples of whole oranges and processed fractions are hydrolysed by refluxing with HCl (ethanolic HCl for oil), partitioned with methylene chloride and cleaned up on an attaclay-aluminum oxide column with a Florisil column also for finisher pulp and oil. The percentage recoveries from oranges and grapefruit, at the 0.05 mg/kg fortification level unless otherwise indicated, were as shown below.

	carbosulfan	carbofuran	3-hydroxycarbofuran
Unwashed grapefruit	96	74-97/0.2 mg/kg	93/0.2 mg/kg
Unwashed oranges	86/0.2 mg/kg		
Washed oranges	78/0-1 mg/kg	90/10-1mg/kg	88/0.1mg/kg
Washed grapefruit	69/10.1mg/kg		
Orange juice	86	86	
Grapefruit juice	92	94	
Orange molasses	82	100	72
Dried orange pulp (peel)	80	--	
Dried grapefruit pulp			78
Orange finisher pulp			74
Grapefruit finisher pulp	88	94	
Grapefruit oil	78	--	66/0.1 mg/kg

Limits of determination ("sensitivities") were reported as 0.05 mg/kg and limits of detection as 0.01 mg/kg in all samples, except oil where the limits of determination and detection were respectively reported as 0.05 and 0.01 mg/kg for carbosulfan, 1.0 and 0.50 mg/kg for carbofuran and 0.1 (0.01) mg/kg for 3-hydroxycarbofuran.

Animal products

Analytical method P-3065M, developed according to GLP, is available for the determination of carbosulfan, its carbamate metabolites carbofuran, 3-keto-carbofuran, 3-hydroxycarbofuran, the 7-phenol, 3-keto-7-phenol and 3-hydroxy7-phenol metabolites, and dibutylamine in bovine milk, meat and meat byproducts (Chen, 1995a). It was used for the cow feeding study reported below. It is generally similar to method P-2964M. Carbosulfan is extracted from tissues and milk by shaking with acetone (instead of dichloromethane) and partitioned sequentially with dichloromethane and ethyl acetate which are combined, concentrated and analysed by HPLC. Fat and cream extracts are partitioned with acetonitrile before analysis (there is no SPE column clean-up). The HPLC configuration is essentially that used for Method P-2964M. Analyses for the carbamate metabolites also follows Method P-2964M, including the HCl reflux and sequential chromatography on C18- and aminopropyl-SPE columns (cream and fat samples are partitioned with acetonitrile before HPLC). The phenols are extracted by acid reflux as in P-2964M followed by clean-up on a C18-SPE column for milk and C18- and SPE columns for tissues, fat and cream. Fat and cream samples are basified, partitioned with hexane, and the aqueous layer re-acidified before application to the SPE columns. Phenolic samples are derivitized with PFBBr and ethylated. Tissue, fat and cream samples are further eluted through an SI SPE cartridge before concentration for analysis. The phenolic derivatives are determined by single-ion GC-MS as before. The determination of dibutylamine also follows P-2964M, but extraction is with acetone.

Analytical recoveries were determined by analysing 14-19 fortified samples for each analyte of milk and 12-15 of tissues and cream (only 8 for DBA), mainly at low levels. Control levels of all compounds except DBA were <5 in milk and <10 in tissues and cream. Apparent DBA residues ranged from <5 to 37 in milk and <10 to 34 in kidney, fat and muscle. The recoveries were as shown below.

	Milk			Tissues and cream		
	Fortification,	Recovery, %		Fortification,	Recovery, %	
	µg/kg	Mean	± SD	µg/kg	Mean	± SD
carbosulfan	25	87	13	25-100	88	14
carbofuran (CF)	25, 50	93	13	50, 200	85	9
93-keto-CF	25, 50	92	11	50, 200	92	11
3-hydroxy-CF	25, 50	84	13	50, 200	79	15
7-phenol	25, 50	88	12	50, 500	79	24
3-keto-7-phenol	25, 50	99	11	50, 500	114	21
3-hydroxy-phenol	25, 50	104	15	50, 500	97	20
DBA	25	95	21	50-1000	78	12

The limits of determination of all compounds were 25 µg/kg in milk and 50 µg/kg in tissues and cream. The limits of detection were 5 and 10 µg/kg respectively.

The method was validated by an independent laboratory (Burton, 1996a) for carbosulfan in milk at 25 and 50 µg/kg (recovery 92%, 7.2 SD) and (Burton, 1996b) for carbofuran (97.7% ±7.1 SD), 3-keto-carbofuran (94.2% ±17.9 SD) and 3-hydroxycarbofuran (99.1% ±4.3 SD) in milk at 25 and 100.

Multi-residue methods

Methods in the US EPA's PAM testing protocols were tested for the detection and recovery of carbosulfan (Mayer, 1995). Although carbosulfan was successfully eluted from Florisil columns under some PAM conditions, the sensitivity of the detection systems (including the most sensitive GLC N/P detection) was not considered sufficient at the 0.05 mg/kg level in fatty or non-fatty foods (≥25 ng required for half-scale deflection compared with 1.5 ng for chlorpyrifos, and 1.5 µg/ml considered to be the limit of quantification, defined as average noise +10 x SD).

The government of The Netherlands official multi-residue method Part 1, Method 1 was provided as a method for carbosulfan (Olthof, 1997). Samples are extracted with acetone/dichlormethane/petroleum ether. There is no clean-up and an EC or ion trap detector is used. No validation data were provided for carbosulfan in specific types of sample.

Stability of pesticide residues in stored analytical samples

Several studies have been carried out on the stability of carbosulfan and/or its metabolites in crops, soils or animal products during frozen storage. The studies which include carbosulfan are described here. Other studies on the stability of carbofuran and its metabolites are discussed in the monograph on carbofuran.

`Markle (1980) studied the stability of carbosulfan in alfalfa, oranges and three types of soil stored for a year at 0° (-18°C). Samples of treated oranges (two applications at 1.7 kg ai/ha) and alfalfa (1.12 kg ai/ha) were macerated and mixed before storage. Air-dried soils were treated at 1 mg ai/kg soil. The soils and dry alfalfa were extracted with methanol buffered at pH 8, and green alfalfa and oranges with hexane/propanol (as in the method of Leppert, 1981). Clean-up was by liquid/liquid partition and Florisil column chromatography and carbosulfan was determined by GLC with NP detection. Samples were analysed on the day of application and at intervals during storage for one year. The residue levels after selected intervals are shown below in Table 15.

Table 15. Residues of carbosulfan and carbofuran in carbosulfan-treated crops and soils at intervals after storage at -18°C (Markle, 1980).

Sample	Carbosulfan, mg/kg[1]			Carbofuran, mg/kg[1]		
	Day 0	Day 179	Day 365	Day 0	Day 179	Day 365
Oranges	1.5	1.6	1.5	0.31	0.4	0.53
Green alfalfa	32	34	30	2.9	3.8	3.8
Alfalfa hay	44	50 [2]	45	10	13	12
	Day 0	Day 144	Day 357	Day 0	Day 144	Day 357
Silt loam pH 4.8	Carbosulfan was almost completely degraded after 3 hours. This was attributed to the soil acidity.					
Silty clay loam pH 6.0	0.96	0.51	0.40	0.41	0.47	0.61
Sandy loam pH 6.8	0.63	0.3	0.24	0.15	0.37	0.33

[1] Average of three replicate samples at each sampling[2] Day 185

In the silty clay loam and sandy loam soils the combined residues of carbosulfan and carbofuran expressed as carbosulfan were fairly constant during the storage period.

In an interim report Pearsall (1996) described studies of the stability of carbosulfan, carbofuran, 3-hydroxycarbofuran, 3-keto-carbofuran, the 7-phenol, 3-keto-7-phenol, and 3-hydroxy-7-phenol and dibutylamine in laboratory-fortified oranges and their processed products stored for up to a year at -18°C. Whole oranges, dried pulp, juice, molasses and oil were fortified at 0.25 mg/kg and samples of most of these were taken for analysis on day 0 however, because degradation was rapid, and after approximately 3, 6, and 12 months. Analyses of juice, molasses and oil for carbosulfan were discontinued after day 0 and orange oil was analysed for the metabolites only on day 0 and after 12 months. The reported results were the averages of analyses of triplicate samples. The limits of detection and determination were reported to be 0.025 and 0.125 mg/kg respectively for all compounds in all samples. The results obtained with pulp, juice, molasses and oil are shown in Table 16.

Table 16. Residues of carbosulfan, its carbamate and phenolic metabolites and dibutylamine in processed orange products, each fortified at 0.25 mg/kg and stored for up to one year at -18°C (Pearsall, 1996).

Compound	Residues, mg/kg[1], after storage			
	0 days	3 months	6 months	12 months
DRIED PULP				
carbosulfan	0.24	0.11	0.18	NA[2]
carbofuran	0.22	0.27	0.21	0.30
3-hydroxycarbofuran	0.24	--	0.19	0.24
3-keto-carbofuran	0.21	0.23	0.26	0.26
dibutylamine	0.20	0.21	0.26	0.25
7-phenol	0.22	0.26	0.22	0.29
3-keto-7-phenol	0.21	0.24	0.23	0.23
3-hydroxy-7-phenol	0.20	0.23	0.34	0.22
JUICE				
Carbosulfan	ND	NA	NA	NA
carbofuran	0.24	0.25	0.24	0.25
3-hydroxycarbofuran	0.23	0.21	0.19	0.25
3-keto-carbofuran	0.25	0.23	0.19	0.27
dibutylamine	0.31	0.33	0.21	0.23
7-phenol	0.23	0.28	0.23	0.28
3-keto-7-phenol	0.24	0.25	0.23	0.26
3-hydroxy -7-phenol	0.24	0.28	0.21	0.20
MOLASSES				
carbosulfan	0.08	NA	NA	NA
carbofuran	0.25	0.31	0.26	0.27
3-hydroxycarbofuran	0.21	0.31	0.25	0.27
3-keto-carbofuran	0.22	0.25	0.20	0.22
dibutylamine	0.20	0.28	0.32	0.24
7-phenol	0.25	0.20	0.23	0.27
3-keto-7-phenol	0.21	0.21	0.25	0.27
3-hydroxy-7-phenol	NA	0.21	0.34	0.20
OIL				
carbosulfan	0.12	NA	NA	NA
carbofuran	0.25	NA	NA	0.24
3-hydroxycarbofuran	0.21	NA	NA	0.29
3-keto-carbofuran	0.23	NA	NA	0.25
dibutylamine	NA	NA	NA	0.13
7-phenol	0.19	NA	NA	0.23[2]
3-keto-7-phenol	0.23	NA	NA	0.22[2]
3-hydroxy-7-phenol	0.22	NA	NA	0.22[2]

NA = Not analysed ND = not detected (<0.025 mg/kg)
[1] Average of 3 replicate samples
[2] Two samples only. Third not included because it was inconsistent with other samples

In a study of the stability of carbosulfan and DBA in bovine milk and tissues (Barrett, 1996), samples of milk, muscle and liver were laboratory-fortified at 0.25 mg/kg with either DBA or carbosulfan and stored for 8 months at -18°C. Samples were analysed initially and after 3, 6, or 8 months storage by methods in FMC report P-3065 described above under methods of analysis. Limits of detection and determination for all the compounds were 5 and 25 µg/kg in milk and 10 and 25 µg/kg in tissues. Results are shown in Table 17.

Table 17. Stability of carbosulfan and dibutylamine added to bovine milk, muscle and liver at 0.25 mg/kg and stored up to 8 months at -18°C (Barrett, 1996).

Compound/sample	Residues (mg/kg) at Storage Intervals (months)							
	0		3		6		8	
	Mg/kg	% change	mg/kg	% change	mg/kg	% change	mg/kg	% change
Carbosulfan Milk	0.21	-16	0.15	-40	0.09	-64	0.04	-84
Muscle	0.25	0.0	0.05	-80	0.02	-92	ND	-100
Liver	0.24	-4	0.07	-72	0.07	-72	0.05	-80
Dibutylamine Milk	0.26	+4	0.31	+24	0.23	-8	NA	NA
Muscle	0.31	+24	0.23	-8	0.38	+52	NA	NA
Liver	0.24	-4	0.16	-36	0.24	-4	NA	NA

ND = not detected (<0.01 mg/kg)
NA = not analysed

Because of the losses of carbosulfan mass balances were determined in an attempt to confirm a hypothesis that most of the losses could be attributed to degradation to carbofuran or phenols. Residues of carbosulfan and carbofuran were determined after 3 and 6 months (Table 18), and those of carbosulfan, carbofuran and the 7-phenol after 8 months (Table 19).

Table 18. Carbosulfan and carbofuran expressed as carbosulfan in bovine milk, muscle and liver which had been fortified with 0.25 mg/kg carbosulfan and stored at -18°C (Barrett, 1996).

Sample	Storage period, Months	Residue, mg/kg			Total residue as % of fortification level
		Carbosulfan	Carbofuran as carbosulfan	Total	
Milk	3	0.15	0.02	0.17	68
Muscle	6	0.02	0.2	0.22	88
Liver	6	0.07	0.04	0.11	0.44

Table 19. Carbosulfan, carbofuran and 7-phenol expressed as carbosulfan in bovine milk, muscle and liver which had been fortified with carbosulfan at 0.25 mg/kg carbosulfan (Barrett, 1996).

Sample	Storage period, months	Residue, mg/kg				Total residue as % of fortification level
		Carbosulfan	Carbofuran as carbosulfan	7-phenol	Total	
Milk	8	0.04	0.11	0.01	0.16	64
Muscle	8	ND	0.18	0.19	0.37	148
Liver	8	0.05	0.03	0.05	0.13	52

Definition of the residue

Previously the JMPR had recommended separate limits for carbosulfan and carbofuran to accommodate residues from uses of carbosulfan. This is consistent with the internationally accepted approach of recommending separate MRLs and definitions of residues when a metabolite of a pesticide is also a pesticide in its own right, as is carbofuran. Residues of the carbamate metabolites have been determined separately for the estimation of maximum residue levels of carbofuran, regardless of whether they result from the use of carbofuran or carbosulfan. The Meeting therefore concluded that residues of carbosulfan should be defined as carbofuran.

USE PATTERN

Reported information on GAP is shown in Table 20.

Table 20. GAP for the use of carbosulfan on citrus fruit.

Country Crop	Application				PHI, Days	Comments
	Form.	g ai/ha or [g ai/tree]/appl.	l/ha	No.		
Mexico Valencia oranges	26.1% LE	250	1000	4	7	Last broadcast applic. *c.*230 days after bloom. Ref. Report P-3182.
Other oranges	26.1%	250	1000	3	7	Last broadcast applic. *c.*230 days after bloom. Ref. Report P-3183
Brazil Oranges	CE 250 g ai /l	[0.93-1.69]	----	2	7	Last broadcast spray *c.*50 days after bloom. Ref. Report P-2964.
Spain Valencia oranges	LE 250 g ai /l	[2.83-3.14]	141-156 l/trial	2	112	Last broadcast foliar application *c.*28-30 days after bloom. Ref. Report P-3100.
Mandarin oranges	LE 250 g ai /l	[3.2-3.6]	160-180 l/trial	2	112	Last broadcast foliar application *c.*28 days after bloom. Ref. Report No. P-3101
Oranges	250EC	937.5	3000	2	123-147	
Mandarins	250EC	937.5	3000	2	110	
Clementines	250EC	937.5	3000	2	115	

RESIDUES RESULTING FROM SUPERVISED TRIALS

Plants

Oranges. Seven supervised trials were conducted in Mexico, 6 in Brazil and 17 in Spain. The analytical methods were P-2964 in Mexico and Brazil and FCC 0193in Spain, described above. Limits of detection and determination of 0.01 and 0.05 mg/kg respectively in citrus fruits and their products are reasonable for carbosulfan and its metabolites except dibutylamine, for which a limit of determination of 0.1 mg/kg would be more realistic because apparent DBA is frequently found at about 0.02 mg/kg in control samples. All the trials were in accordance with reported GAP. Generally spray was to run-off and 6 trees were treated in each trial. Precautions were taken during sampling shipment and storage to preserve the integrity of the samples. The results are shown in Tables 21 and 22. Duplicate samples were taken in most trials and the two results have been recorded. The results for carbosulfan and the carbamate metabolites were corrected for analytical recoveries where they were low.

Table 21. Residues of carbosulfan and metabolites from supervised trials on oranges and mandarins. Whole fruit analysed. Underlined results were used for estimation of maximum residue and STMR levels. Details of the trials follow the Table.

Trial ref.	PHI, days	Sample[1]	Residues, mg/kg[2]										
			Sulfan	Furan	CO-furan	HO-furan	Furan + HO-furan	Total carba-mate	7-phenol	CO-phenol	HO-phenol	Total phenols	DBA
Trial A1	7	NCOR	0.04	0.13	0.01	0.07	0.20	0.25	0.02	0.01	0.04	0.07	0.11
			0.01	0.19	0.04	0.11	0.30	0.35	0.02	0.01	0.08	0.11	0.11
		C	ND	ND	ND	ND		ND	ND	ND	ND	ND	0.02
		COR	0.08	0.17	0.01	0.09	0.26	0.35					
			0.02	0.25	0.04	0.14	0.39	0.45					
Trial A2	7	NCOR	0.01	0.08	0.01	0.03	0.11	0.13	0.01	0.01	0.02	0.04	0.14
			0.01	0.08	0.02	0.02	0.10	0.13	ND	ND	0.02	0.02	0.14
		C	ND	ND	ND	ND		ND	ND	ND	ND	ND	0.01
		COR	0.02	0.11	0.01	0.04	0.15	0.18					
			0.02	0.11	0.02	0.03	0.14	0.18					
Trial A3	7	NCOR	ND	0.2	0.04	0.11	0.31	0.35	0.02	0.03	0.07	0.12	0.02
			ND	0.12	0.02	0.05	0.17	0.19	0.01	0.01	0.03	0.05	0.02
		C	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND
		COR	ND	0.26	0.04	0.14	0.40	0.44					
			ND	0.16	0.02	0.06	0.22	0.24					
Trial A4	7	NCOR	0.02	0.11	ND	0.06	0.17	0.19	0.02	ND	0.04	0.06	0.10
			ND	0.15	0.01	0.10	0.16	0.26	0.03	0.01	0.06	0.07	0.09
		C	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND
		COR	0.04	0.14	ND	0.08	0.22	0.26					
			ND	0.20	0.01	0.13	0.33	0.34					
Mean analytical recoveries, %			51	76	93	80		--	64	74	57	--	65
Trial B1	7	NCOR	0.02	0.07	ND	ND	0.07	0.09	ND	ND	0.03	0.03	0.06
			0.01	0.07	0.02	0.02	0.09	0.12	0.01	ND	0.04	0.05	0.06
		C	ND	ND	ND	ND		ND	ND	ND	ND	ND	ND
		COR	0.03	0.08	ND	ND	0.08	0.11					
			0.02	0.08	0.03	0.03	0.11	0.16					
Trial B2	7	NCOR	ND	0.08	ND	0.05	0.13	0.13	ND	ND	0.02	0.02	0.07
			ND	0.07	ND	0.02	0.09	0.09	ND	0.01	0.03	0.04	0.07
		C	ND	ND	ND	ND		ND	ND	ND	ND	ND	0.01
		COR	ND	0.10	ND	0.07	0.17	0.17					
			ND	0.08	ND	0.03	0.11	0.11					
Trial B3	7	NCOR	ND	0.09	0.02	0.06	0.15	0.17	ND	ND	0.03	0.03	0.06
			ND	0.09	0.02	0.04	0.13	0.15	ND	ND	0.03	0.03	0.04
		C	ND	ND	ND	ND		ND	ND	ND	ND	ND	0.01
		COR	ND	0.11	0.03	0.08	0.19	0.22					
			ND	0.11	0.03	0.06	0.17	0.20					
Mean analytical recoveries, %			64	83	72	72			55	69	53		70

Trial ref.	PHI, days	Sample[1]	Residues, mg/kg[2]										
			Sulfan	Furan	CO-furan	HO-furan	Furan + HO-furan	Total carba-mate	7-phenol	CO-phenol	HO-phenol	Total phenols	DBA
Trial C1	7	NCOR	ND 0.02	0.04 0.02	ND ND	0.01 ND	0.05 0.02	0.05 0.04	ND ND	ND ND	ND ND	ND ND	0.07 0.06
Trial C2	7	NCOR	ND ND	0.05 0.06	ND ND	0.03 0.02	0.08 0.08	0.08 0.08	ND ND	ND ND	0.02 0.01	0.02 0.01	0.09 0.07
Trial C3	7	NCOR	ND ND	0.03 0.06	ND 0.02	0.02 0.03	0.05 0.09	0.05 0.11	ND ND	ND ND	ND 0.02	ND 0.02	0.05 0.04
Trial C4	7	NCOR	0.02 0.02	0.02 0.01	ND ND	0.01 0.01	0.03 0.02	0.05 0.04	ND ND	ND ND	0.01 ND	0.01 ND	0.07 0.03
Trial C5	7	NCOR	0.03 0.03	0.03 0.04	ND ND	0.02 0.02	0.05 0.06	0.08 0.09	ND ND	ND ND	0.01 0.02	0.01 0.02	0.13 0.15
Trial C6	7	NCOR	0.01 0.01	0.05 0.06	ND ND	0.02 0.03	0.07 0.09	0.08 0.10	ND ND	ND ND	ND 0.01	ND 0.01	0.05 0.04
Mean analytical recoveries, %			85	92	89	100			78	81	76	--	89
Trial D1	28	NCOR C	ND ND	0.61 0.40	ND ND	0.35 0.13	0.96 0.53	0.96 0.53	0.05 0.07	0.02 0.02	0.13 0.19	0.20 0.28	0.17 0.29 0.03
		COR	ND ND	0.82 0.54	ND ND	0.39 0.14	1.2 0.68	1.2 0.68					
	112	NCOR C	ND ND	0.07 0.27	ND 0.04	0.02 0.13	0.09 0.40	0.09 0.44	0.02 0.05	ND 0.03	0.05 0.17	0.07 0.25	0.12 0.14 0.02
		COR	ND ND	0.10 0.36	ND 0.05	0.02 0.14	0.12 0.50	0.12 0.55					
Trial D2	28	NCOR C	ND ND	0.11 0.14	ND ND	0.17 0.27	0.38 0.41	0.38 0.41	0.06 0.05	0.05 0.04	0.34 0.32	0.45 0.41	0.20 0.20 0.07
		COR	ND ND	0.15 0.19	ND ND	0.19 0.30	0.34 0.49	0.34 0.49					
	113	NCOR	ND ND	ND 0.02	0.01 ND	0.05 0.10	0.05 0.12	0.06 0.12	0.01 0.01	0.01 ND	0.06 0.06	0.08 0.07	0.09 0.11
		COR	ND ND	ND 0.03	0.01 ND	0.06 0.11	0.06 0.14	0.06 0.14					
Mean analytical recoveries, %			82	74	81	90			106	103	80		66
Trial E1	28	NCOR C	ND ND	0.23 0.16	ND ND	0.30 0.20	0.26 0.18	0.26 0.18	0.03 0.03	0.23 0.24	0.37 0.41	0.63 0.67	0.09 0.07 0.03
		COR		0.26 0.18	ND ND	0.34 0.23	0.34 0.23	0.60 0.41					
	112	NCOR	ND ND	ND ND	0.01 ND	0.09 0.06	0.09 0.06	0.10 0.06	ND ND	ND ND	0.10 0.08	0.10 0.08	0.06 0.05
		COR	ND ND	ND ND	0.01 ND	0.10 0.07	0.10 0.07	0.11 0.07					

Trial ref.	PHI, days	Sample[1]	Residues, mg/kg[2]										
			Sulfan	Furan	CO-furan	HO-furan	Furan + HO-furan	Total carba-mate	7-phenol	CO-phenol	HO-phenol	Total phenols	DBA
Trial E2	28	NCOR	ND	0.18	ND	0.13	0.31	0.31	0.01	ND	0.10	0.20	0.24
			ND	0.12	ND	0.14	0.26	0.26	ND	ND	0.10	0.20	0.29
		C											0.10
		COR	ND	0.21	ND	0.15	0.36	0.36					
			ND	0.14	ND	0.16	0.30	0.16					
	112	NCOR	ND	ND	ND	0.02	0.02	0.02	ND	ND	0.02	0.02	0.04
			ND	ND	ND	0.03	0.03	0.03	ND	ND	0.04	0.04	0.04
		COR	ND	ND	ND	0.02	0.02	0.03					
			ND	ND	ND	0.03	0.03	0.03					
Mean analytical recoveries, %			88	88	92	88			87	87	91		67

[1]C = control . COR = corrected for recovery. NCOR = not corrected for recovery
[2]Sulfan = carbosulfan; Furan = carbofuran; CO-furan = 3-keto-carbofuran; HO-furan = 3-hyrdroxy-carbofuran; CO-phenol = 3-keto-7-phenol; HO-phenol = 3-hydroxy-7-phenol; DBA = dibutylamine. All results below the limits of determination indicated for each reference (0.03 or 0.05 mg/kg) are estimated values. ND = < limit of detection of 0.01 mg/kg. In each trial duplicate field samples were analysed and both results are shown. Some individual field samples were analysed in duplicate. In these cases the means are recorded.

A trials
Mexico, 1995 (Barros, 1996a) Valencia oranges.25LE formulation, 4 x 250 g ai/ha.
LOD 0.05 mg/kg. Recoveries determined at 0.05-0.5 mg/kg, DBA 0.05-0.2 mg/kg. No appreciable difference between 0.05 and 0.5. 20-30 trees/plot, 6 trees per plot sprayed to run-off by backpack sprayer
Trial 1: Nuevo Leon, 979-1025 l spray/ha.
Trial 2: Tamaulipas, 940-1000 l spray/ha.
Trial 3: Veracruz, 966-1035 l spray/ha.
Trial 4: Sonora, 990-1030 l spray/ha.

B trials
Mexico 1995 (Ramsey and Barros, 1996). Other oranges. 25LE formulation, 3 x 250 g ai/ha.
LOD 0.05 mg/kg. Recoveries determined at 0.05-0.5 mg/kg, DBA 0.05-0.1 mg/kg. No appreciable difference between 0.05 and 0.5 except HO-furan 61% at 0.05 mg/kg
Trial 1: Nuevo Leon, 976-1025 l spray/ha.
Trial 2: Tamaulipas, 955-1000 l spray/ha.
Trial 3: Veracruz, 977-1020 l spray/ha.

C trials
Brazil 1993 (Shevchuk, 1996). Oranges, Pera Valencia and Pera Coroa. All trials Campinas region, 2 x 250CE formulation.
LOD 0.03 mg/kg except DBA 0.05 mg/kg. Recoveries determined at 0.03-0.1 mg/kg, DBA 0.05-0.1 mg/kg.
Trial 1 1.7 and 1.1 g ai/tree; 410 and 250 l spray/trial.
Trial 2 0.93 and 1.0 g ai/tree; 268 and 240 l spray/trial.
Trial 3 1.0 and 1.1 g ai/tree; 300 and 360 l spray/trial.
Trial 4 1.4 and 1.4 g ai/tree; 330 and 360 l spray/trial.
Trial 5 1.6 and 1.5 g ai/tree; 368 and 360 l spray/trial.
Trial 6 1.4 and 1.5 g ai/tree; 332 and 360 l spray/trial.

D trials
Spain 1994 (Barros, 1996b). Valencia oranges. 25LE formulation, 2 x 3.1 g ai/tree.
Method P-2719 for carbamates, P-0748 for DBA; 6 trees from 30-tree plot treated, 1st treatment post-bloom. LOD 0.03 mg/kg except DBA 0.05 mg/kg. Recoveries determined at 0.03-0.5 mg/kg except carbosulfan 0.03 mg/kg. Controls all ND except DBA where shown
Trial 1 Lepe, 153 and 156 l spray/trial.
Trial 2 Santiponce , 141 and 142 l spray/trial.

E trials
Spain, 1994 (Barros, 1996c). Mandarin oranges. 25LE formulation.

LOD 0.03 mg/kg. Controls ND except DBA where shown. Two applications (1st after full bloom and 2nd 28 days later) to 6 centre trees of 20-tree plots with broadcast spray to run-off. Recoveries determined at 0.03-0.5 mg/kg, except DBA 0.05-0.5 mg/kg and carbosulfan only 0.03 mg/kg. Recoveries not substantially affected by fortification levels.
Trial 1 Lepe. 3.3 and 3.6 g ai/tree; 160 and 180 l spray/trial.
Trial 2 La Algaba. 3.2 and 3.2 g ai/tree; 162 and 180 l spray/trial.

Table 22. Residues of carbosulfan, carbofuran and 3-hydroxycarbofuran in oranges and mandarins resulting from supervised trials in Spain. All 2 applications of 250 EC formulation at 937.5 kg ai/ha, 3000 l/ha. Underlined results were used for estimation of maximum residue and STMR levels.

Location, Variety	PHI, Days	Sample[1]	Residue, mg/kg[2]					Refs. & comments
			Sulfan	Furan	HO-furan	Furan + HO-furan	Total carba-mates	
Benifay — Clementines	0	Fruit						Gill, 1995b 1 trial in 1993
		C	0.03	0.06	0.24*			
		NCOR	2.6	0.94	0.45			
			1.8	0.78	0.46			
		COR	3.3	1.0	0.45	1.5	4.8	First applicn. At 5% final fruit size. Report states 1875 g ai/ha (presumably 2 x 937.5) .Summary reports 937.6 Controls decreasing with time suggests contamination of control plot. Plot diagrams support this. Results not corrected for 111% recovery at any PHI
			2.3	0.83	0.46	1.3	2.1	
	30	C	ND	ND	0.05*			
		NCOR	ND	0.08	0.29			
			ND	0.17	0.46			
		COR	ND	0.09	0.29	0.38	0.38	
			ND	0.18	0.46	0.64	0.64	
	60	C	ND	ND	ND*			
		NCOR	ND	ND	0.12			
			ND	ND	0.11			
		COR	ND	ND	0.12	0.12	0.12	
			ND	ND	0.11	0.11	0.11	
	115**	C	ND	ND	ND*			** Normal harvest
		NCOR	ND	ND	0.05			
			ND	ND	0.06			
		COR	<u>ND</u>	ND	0.05	<u>0.05</u>	0.05	
			<u>ND</u>	ND	0.06	<u>0.06</u>	0.06	
Mean recovery, %, at 0.05 mg/kg					111			
Trial 1 Satsuma mandarins Carlet	0	Fruit (peel + pulp						Gill, 1995c 2 trials in 1993
		C	0.01	ND	0.02			
		NCOR	1.0	0.81	0.92			Report states 1875 g ai/ha (presumably 2 x 937.5) .Summary of report states.2 x 1875. Summary in mfgr.'s evaluation states total of 937.5.
			0.84	0.56	0.76			
		COR	1.3	0.96	0.92	1.9	3.2	
			1.1	0.67	0.76	1.4	2.5	
	45	C	ND	ND	ND			
		NCOR	ND	0.05	0.60			
			ND	0.04	0.19			
		COR	ND	0.06	0.60	0.66	0.66	
			ND	0.05	0.19	0.24	0.66	
	110**	C	ND	ND	ND			** Normal harvest
		NCOR	ND	ND	0.11			
			ND	ND	0.12			
		COR	<u>ND</u>	ND	0.11	<u>0.11</u>	0.11	
			<u>ND</u>	ND	0.12	<u>0.12</u>	0.12	

Location, Variety	PHI, Days	Sample[1]	Residue, mg/kg[2]					Refs. & comments
			Sulfan	Furan	HO-furan	Furan + HO-furan	Total carba-mates	
Trial 2 Sueca	0	C NCOR	ND 1.9 1.0	ND 0.80 0.67	ND 0.76 0.88			
		COR	2.4 1.3	0.95 0.80	0.76 0.80	1.7 1.6	3.1 2.9	
	45	C NCOR	ND ND ND	ND 0.04 0.04	0.01 0.50 0.40			
		COR	ND ND	0.05 0.05	0.50 0.40	0.55 0.45	0.55 0.40	
	110	C NCOR	ND ND ND	ND ND ND	ND 0.11 0.13			
		COR	ND ND	ND ND	0.11 0.13	0.11 0.13	0.11 0.13	
Mean recovery, %, at 0.05 mg/kg			79	84	103			
Trial 1 Carlet Navel oranges	147	Fruit (Peel + pulp)* C Treated	 ND ND ND	 ND 0.03 0.02	 ND 0.04 0.07	 ND 0.07 0.09	 ND 0.07 0.09	Gill, 1995d 3 trials in 1993 Results corrected for recovery only for sulfan (86% at 0.05 mg/kg). Furan and HO-furan were ∃ 95% *Peel/pulp ratio about 40/60. Peel/pulp residues are means of duplicate samples
Trial 2 Sueca Newhall oranges	0	Fruit C Treated	 ND 0.49 0.81	 0.02 0.45 0.53	 ND 0.94 0.75	 ND 1.7 1.3	 0.02 2.2 2.1	
	45	C Treated	ND ND ND	ND 0.02 0.13	ND 0.21 0.17	ND 0.23 0.30	ND 0.25 0.43	
		peel*	ND	0.27	0.36	0.65	0.65	
		pulp*	ND	ND	0.01	0.01	0.01	
	105	C Treated	ND ND ND	ND 0.06 0.04	ND 0.05 0.04	ND 0.11 0.08	ND 0.11 0.12	
	123**	C Treated	ND ND ND	ND 0.04 0.04	0. 03 0.08 0.08	0.03 0.12 0.12	0.03 0.12 0.12	** Normal harvest
Trial 3 – Benifay Navel oranges	0	Peel + pulp C	ND 0.32 0.44	ND 0.37 0.45	ND 0.23 0.64	ND 0.60 1.1	ND 0.92 1.5	
	45	C Treated	ND ND ND	ND 0.13 0.17	ND 0.08 0.24	ND 0.21 0.41	ND 0.21 0.41	

Location, Variety	PHI, Days	Sample[1]	Residue, mg/kg[2]					Refs. & comments
			Sulfan	Furan	HO-furan	Furan + HO-furan	Total carba-mates	
		Peel	ND	0.37	0.36	0.73	0.73	
		Pulp	ND	ND	0.01	0.01	0.01	
	105	C Treated	ND ND ND	ND 0.04 0.04	ND 0.03 0.04	ND 0.07 0.08	ND 0.07 0.08	
	140	C Treated	ND ND ND	ND 0.02 0.03	ND 0.05 0.08	ND 0.07 0.11	ND 0.07 0.11	
Trial 1 Benifay – Clementines	0	Whole fruit C Treated	 0.01 0.50	 ND 0.69	 0.02 0.52	 0.02 1.2	 0.03 1.7	Gill, 1996a 2 trials in 1994 Results not corrected as all were ∃96% at 0.05 mg/kg. .
	30	C Treated	ND ND	ND 0.12	ND 0.35	ND 0.47	ND 0.59	
	60	C Treated	ND ND	ND 0.01	0.01 0.10	0.01 0.11	0.01 0.11	
	104*	C Treated	ND ND	ND ND	ND 0.06	0.11 0.06	0.11 0.06	* Normal harvest
Trial 2 Catadau Clementines	0	Whole fruit* C Treated	 <0..02 0.65	 ND 0.46	 <0.02 0.33	 <0.04 0.79	 <0.06 1.4	* Estimated from measured peel/pulp ratios at each sampling
		Peel	0.9	0.63	0.44	1.1	2.0	
		Pulp	ND	ND	0.03	0.03	0.03	
	30	C Treated	ND ND	ND <0.02	ND 0.21	ND <0.23	ND <0.23	
		Peel	ND	0.03	0.55	0.58	0.58	
		Pulp	ND	ND	0.02	0.02	0.02	
	60	C Treated	ND ND	ND ND	0.02 <0.06	0.02 <0.06	0.02 <0.06	
		Peel	ND	ND	0.22	0.22	0.22	
		Pulp	ND	ND	ND	ND	ND	
	104**	C Treated	ND ND	ND ND	ND <0.04	ND <0.04	ND <0.04	** Normal harvest
		Peel	ND	ND	0.14	0.14	0.14	
		Pulp	ND	ND	ND	ND	ND	

Location, Variety	PHI, Days	Sample[1]	Residue, mg/kg[2]					Refs. & comments
			Sulfan	Furan	HO-furan	Furan + HO-furan	Total carba-mates	
Trial 1 Carlet Satsumas	0	Whole fruit* C Treated	 0.02 0.31	 0.02 0.51	 0.04 0.39	 0.06 0.90	 0.08 1.2	Gill, 1996b 2 trials in 1994 * Estimated from measured peel/pulp ratios at each sampling Results not corrected for recoveries of ∃94%.
	45	C Treated	ND ND	ND ND	0.01 0.18	0.01 0.18	0.01 0.18	
	84	C Treated	ND ND	ND ND	0.03 0.04	0.03 0.04	0.03 0.04	
Trial 2 Sueca Satsumas	0	Whole fruit * C Treated	 <0.02 0.43	 ND 0.28	 <0.02 0.41	 <0.02 0.69	 <0.04 1.1	
		Peel	0.70	0.45	0.64	1.1	1.8	
		Pulp	ND	0.02	0.05	0.07	0.07	
	45	C Treated	ND <0.02	ND <0.03	ND 0.30	ND <0.33	ND <0.35	
		Peel	0.04	0.07	0.73	0.8	0.84	
		Pulp	ND	ND	0.03	0.03	0.03	
	92	C Treated	ND ND	ND ND	0.02 0.13	0.02 0.13	0.02 0.13	
		Peel	ND	ND	0.40	0.40	0.40	
		Pulp	ND	ND	0.02	0.02	0.02	
Trial 1 Benifay Naveline oranges	0	Whole fruit C NCOR COR	 ND 0.56 0.62	 0.14 0.83 1.0	 0.09 0.30 0.41	 1.4	 2.0	Gill, 1996c 3 trials in 1994
	45	C NCOR COR	ND ND ND	0.09 0.21 0.26	0.03 0.17 0.23	 0.49	 0.49	
	105	C NCOR COR	ND ND ND	0.03 0.07 0.09	0.01 0.11 0.15	 	 0.24	
	140	C NCOR COR	ND ND ND	0.02 0.03 0.04	0.01 0.05 0.07	 	 0.11	
Mean recovery, %, at 0.05 mg/kg			90	80	74			
Trial 2 Carlet Naveline oranges	0	Whole fruit * C NCOR COR	 <0.02 0.17 0.19	 ND 0.18 0.23	 ND 0.08 0.11	 0.34	 0.53	Estimated from peel/pulp. ratio at harvest of 27/73

Location, Variety	PHI, Days	Sample[1]	Residue, mg/kg[2]					Refs. & comments
			Sulfan	Furan	HO-furan	Furan + HO-furan	Total carba-mates	
		peel C NCOR	0.02 0.25	0.01 0.26	<0.01 0.12	0.38	0.63	
		Pulp C NCOR	ND ND	ND ND	ND 0.01	ND 0.01	ND 0.01	
	45	C NCOR COR	ND ND ND	ND <0.12 <0.15	ND <0.08 0.11	<0.26	<0.26	
		peel C NCOR	ND ND	ND 0.35	ND 0.24	ND 0.59	ND 0.59	
		pulp C NCOR	ND ND	ND ND	ND ND	ND ND	ND ND	
	105	C NCOR COR	ND ND ND	ND <0.05 <0.06	ND <0.04 <0.05	<0.11	<0.11	
		peel C NCOR	0.02 ND	ND 0.14	ND 0.11	ND 0.25	0.02 0.25	
		pulp C NCOR	ND ND	ND ND	ND ND	ND ND	ND ND	
	140	C NCOR COR	ND ND ND	ND <0.05 <0.06	ND <0.03 <0.04	<0.10	<0.10	
		peel C NCOR	ND ND	ND 0.14	ND 0.09	ND 0.23	ND 0.23	
		pulp C NCOR	ND ND	ND ND	ND ND	ND ND	ND ND	
Trial 3 Sueca Newhall oranges	0	Whole fruit * C NCOR COR	<0.05 0.40 0.44	ND 0.25 0.31	ND 0.14 0.19			* Estimated from peel/pulp. ratio at harvest of 28/72
		peel C NCOR	0.07 0.56	0.01 0.35	0.01 0.19	0.02 0.54	0.09 1.1	
		pulp C NCOR	ND ND	ND ND	ND ND	ND ND	ND ND	
	45	C NCOR COR	ND ND ND	ND <0.11 <0.14	ND <0.12 <0.16			
		peel C NCOR	ND ND	ND 0.29	ND 0.32	ND 0.61	ND 0.61	
		pulp C NCOR	ND ND	ND ND	ND ND	ND ND	ND ND	

Location, Variety	PHI, Days	Sample[1]	Residue, mg/kg[2]					Refs. & comments
			Sulfan	Furan	HO-furan	Furan + HO-furan	Total carba-mates	
	105	C	ND	ND	ND	ND	ND	
		NCOR	ND	<0.03	<0.04	<0.07	<0.07	
		COR	ND	<0.04	0.05	<0.09	<0.09	
		peel C	ND	ND	ND	ND	ND	
		NCOR	ND	0.09	0.12	0.21	0.21	
		pulp C	ND	ND	ND	ND	ND	
		NCOR	ND	ND	ND	ND	ND	
	140	C	ND	ND	ND	ND	ND	
		NCOR	ND	0.02	<0.03	<0.05	<0.05	
		COR	ND	0.03	<0.04	<0.07	<0.07	
		peel C	ND	ND	ND	ND	ND	
		NCOR	ND	0.07	0.08	0.15	0.15	
		pulp C	ND	ND	ND	ND	ND	
		NCOR	ND	ND	ND	ND	ND	
Mean recovery, %, at 0.05 mg/kg		90	80	74				

[1]Duplicate field samples where two values are given. C = control, ND = <limit of detection of 0.01 mg/kg; LOD = 0.05 mg/kg. Values between 0.01 and 0.05 mg/kg are estimates. NCOR = not corrected for recoveries, COR = corrected for recoveries. Because only one PHI per trial represents GAP, underlined values were used to estimate maximum residue and STMR levels.

Animals

In a feeding study (Chen, 1995b) three groups of 3 Holstein dairy cows were given carbosulfan daily after the morning milking by bolus at rates equivalent to about 1, 3, or 10 ppm in the diet on a dry weight basis for 28 days. Another group of three served as controls and a fifth group of 4 cows was given the equivalent of 50 ppm in the diet for the same period. These levels were chosen to represent 1, 3, 10 and 50 times the expected dietary intakes from feed items.

Milk was collected in the evening and before dosing the next morning at regular intervals and composited, with sampling from 0-33 days. Cream and skimmed milk were separated from some samples to study the propensity for residues to accumulate in the fat. All animals from the 1 and 3 ppm dosing groups and two from the 10 and 50 ppm groups were slaughtered within 24 hours of the last dose for sampling of kidneys, liver, muscle and fat. The remaining animal in the 10 ppm group and one of the two remaining in the 50 ppm group were slaughtered after a 3-day recovery period. The last cow from the 50 ppm group was slaughtered after a 6-day recovery period. The samples were stored frozen and analysis of milk and tissues completed within 4 and 6 months respectively. The analytical method was Chen (1995a). Because significant residues were found only at the higher feeding levels, the results are shown only for the 10 and 50 ppm groups (Tables 23 and 24).

Table 23. Residues ug/kg (ppb)[1] carbosulfan and metabolites in the milk and tissues of three cows dosed with carbosulfan for 28 days at the equivalent of 10 ppm carbosulfan in the diet (Chen, 1995b).

Sample	Residues, µg/kg							
	carbo-sulfan	Carbo-furan	3-keto-carbo-furan	3-0H-carbo-furan	7-phenol	3-keto-7-phenol	3-hydroxy-7-phenol	DBA, range and mean
Milk, day0	ND	ND	ND	ND	ND	ND	ND	(16)-28(21)

Sample	Residues, µg/kg							
	carbo-sulfan	Carbo-furan	3-keto-carbo-furan	3-0H-carbo-furan	7-phenol	3-keto-7-phenol	3-hydroxy-7-phenol	DBA, range and mean
1	ND	ND	ND	ND	ND	ND	ND	32-45/37
2	ND	ND	ND	ND	ND	ND	ND	ND-(23)/(16)
4	ND	ND	ND	**ND-(7)**/(4)	ND	ND	ND	26-3[2]30
7	ND	ND	ND	ND	ND	ND	ND	ND-(10)/(7)
14	ND	ND	ND	ND	ND	ND	ND	(5)-(12)/(9)
21	ND	ND	ND	ND	ND	ND	ND	NA
27	ND	ND	ND	ND	ND	ND	ND	32-54/42
30 (one cow)	ND	ND	ND	ND	ND	ND	ND	(7)
Skimmed milk, day 21	ND	ND	ND	ND	ND	ND	ND	NA
Cream, day 21	ND	ND	ND	ND	ND	ND	ND	NA
Kidney	ND	ND	ND	ND	48,57/53	ND	ND,(12)/(9)	52-106/79
Liver	ND	ND	ND	ND	ND	ND	ND	(39)-(45)/(42)
Muscle	ND	ND	ND	ND	ND	ND	ND	(14)-70/(42)
Fat	ND	ND	ND	ND	ND	ND	ND	(14)-(16)/(15)

ND = Not detectable = <5 µg/kg in milk and <10 µg/kg in tissues for all compounds
NA = not analysed
[1]Where there were finite residues in one or more of the three cows, the range and average is given. In these cases ND was added as 1/2 ND for calculation of the average, presented as range/average. Derivative values adjusted for molecular weight for parent molecule value. A 0.581 m.w. correction factor is required to convert from carbosulfan to carbofuran. The limits of determination for all compounds were 25 µg/kg in milk and 50 µg/kg in cream and tissues.. Data from Chen, 1995 Tables 2, 3 and 4.
[2]Values in parentheses are estimates where results are between the limits of detection and determination

Table 24. Residues of carbosulfan and metabolites in milk and tissues from feeding four cows carbosulfan for 28 days at 50 µg/kg carbosulfan in the diet (Chen, 1995).

Sample	Residues, :g/kg (range/mean)[1]									
	Carbo-sulfan	Carbo-furan	3-keto-carbo-furan	3-0H-carbo-furan	Total carbam-ates[2]	7-phenol	3-keto-7-phenol	3-hydroxy-7-phenol	Total Phenols[2]	DBA range/ average
Milk test day 0	ND/ND	ND	ND	ND	20	ND	ND	ND	(15)	ND-(9)/ ND
1	ND/ND	ND	ND	**(12)-(22)**/ (16)	31	ND-(6)/ (4)	(15)-38/ 25	(10)-(15) /(13)	(42)	(24)-36/ (29)
2	ND/ND	ND	ND	**ND-30**/ (17)	32	ND-(5)/ (3)	(15)-4[2] 27	(13)-26/ (19)	(49)	34-79/ 57
4	ND/ND	**ND-(6)**/ (3)	ND	**(12)-(21)**/ (17)	30	ND-(5)/ (3)	(16)-26/ (19)	(8)-(16)/ (11)	(33)	ND-(20)/ (11)
7	ND-(5)/ ND	**ND-(8)**/ (4)	ND	**(15)-(24)**/ (19)	33	ND	(16)-25/ (19)	(9)-(13)/ (11)	(35)	(24)-**119**/ 77
14	ND-(12)/ (7)	ND	**ND-(11)**/ (5)	**(12)-(20)**/ (15)	32	ND-(8)/ (4)	(11)-4[2] (23)	(8)-(13)/ (11)	(38)	60-105/ 73
21	ND-(11)/ (6)	ND	ND	**(7)-(17)**/ (14)	30	ND-(7)/ (4)	(12)-27/ (18)	(7)-(13)/ (11)	(33)	NA/NA

Sample	Residues, :g/kg (range/mean)[1]									
	Carbo-sulfan	Carbo-furan	3-keto-carbo-furan	3-0H-carbo-furan	Total carbam-ates[2]	7-phenol	3-keto-7-phenol	3-hydroxy-7-phenol	Total Phenols[2]	DBA range/ average
27	ND-(5)/ ND	ND	ND	**(10)-(13)**/ (11)	26	ND-(7)/ (4)	(14)-3[2] (20)	(7)-(11)/ (9)	(33)	38-7[1] 60
30 (2 cows)	ND/ND	ND	ND	ND	20	ND	ND	ND	(15)	(9)-25/ (17)
33 (1 cow)	NA/ND	ND	ND	ND	20	ND	ND	ND	(15)	(15)/NA
Skimmed milk, day 21	ND/ND	ND	ND	**ND-(20)**/ (10)	25	ND-(8)/ (6)	(12)-(39)/ (23)	(7)-(14)/ (12)	(41)	NA
Cream, day 21	(11)-(45)/ (28)	**ND-(16)/ (8)**	ND	ND	56	ND-(20)/ (11)	(10),(17)/ (14)	ND	(35)	NA
Kidney day 28 day 31[4] day34[5]	ND/ND	ND	ND	**90 133**/ 112 ND ND	142	**315 400**/ 358 ND (12)	58,74/ 66 ND ND[7]	**152 173**/ 163 ND ND	587	**290 890**/ 590 (26)/ (22)
Liver	ND/ND	ND	**ND, (23)** (14) NDND	**47 60**/ 54 19)/ ND	88	ND	ND	(29),(34) /(32) (14)/ND	52	149 294/ 222 (36)/ (24)
Muscle	ND/ND	ND	ND	**20 (30)**/ (25) ND ND	55	ND	ND	ND,(12)/ (9) ND ND	29	(38) 58/ (48) (23)/ (19)
Fat	(11), 76/ (44) (33)/ (10)	ND	ND	ND	74	ND,(14)/ 10 ND ND	ND	ND,(11)/ (8)	28	(25) (47)/ (36) (15)/ND

[1]ND = Not detectable = <5 μg/kg (all compounds) in milk and <10 μg/kg in other samples. Where there were finite residues in one or more of the three cows, the range and average is given. In these cases ND was added as 1/2 ND for calculation of the average, presented as range/average. Derivative values adjusted for molecular weight for parent molecule value. A 0.581 m.w. correction factor is required to convert from carbosulfan to carbofuran. The limits of determination for all compounds were 25 μg/kg in milk and 50 μg/kg in cream and tissues. Data from Chen, 1995, Tables 2, 3 and 4.
[2]Simple sum of mean values where ND = 5 for milk and skimmed milk, 10 for other samples without m.w. conversion.
[3]Parentheses indicate residue estimates between the limits of detection and determination.
[4]3-day recovery period
[5]6-day recovery period

FATE OF RESIDUES IN STORAGE AND PROCESSING

Storage

No information

Processing

Citrus fruit. Two processing studies were conducted. In one, Florida grapefruit were treated 4 times by a hydraulic sprayer with a 2.5 EC formulation at 1.1-4.2 kg ai/ha (total 10.5 kg ai/ha), and in the other Valencia oranges were treated 5 times at the same rates (total 11.5 kg ai/ha, 14025 l/ha). In both trials the fruit were harvested on the day of the last application and processed (Leppert, 1991).

Processing was in an FMC Corporation citrus processor according to the method of Kesterson and Braddock (1979). A summary of the processing method was provided. It includes a pre-wash rinse, a soap and water brush wash, water removal between foam rollers, juicing with an FMC modified Model 35 finisher and collection of unfinished juice in cold-wall cooling tanks from which finisher pulp is removed to leave "single" strength juice. The oil emulsion fraction from the rollers is screened and centrifuged for the collection of citrus oil. The peel, rag and seeds are chopped, soaked in 0.3% dehydrated lime slurry and passed through a reaction screw to remove press liquor which is boiled under vacuum and concentrated to produce citrus molasses. The solids are dried to about 8% moisture to yield dried pulp and meal.

The analytical method is described above (Leppert, 1981). Carbosulfan, carbofuran and 3-hydroxycarbofuran were determined. The results are shown in Table 25.

Table 25. Residues and processing factors for carbosulfan, carbofuran and 3-ydroxycarbofuran in whole fruit and processed fractions of oranges and grapefruit.

Sample	Residues, mg/kg (range/mean)[1]									
	Valencia oranges					Marsh grapefruit				
	Carbo-sulfan	Carbo-furan	3-hydroxy-carbo-furan	Total carba-mate	Carbo-furan + 3-hydroxy-carbo-furan	Carbo-sulfan	Carbo-furan	3-hydrox ycarbo -furan	Total carba-mate	Carbo-furan + 3-hydroxy-carbo-furan
Unwashed whole fruit	0.17	0.27	0.30	0.73	0.57	0.12	0.14	0.32	0.57	0.46
Washed whole fruit	0.08	0.34	0.40	0.82	0.74	(0.04)	(0.11)	0.30	0.45	0.41
Processing factor	0.47	1.31	1.3	1.1	1.3	0.33	0.79	0.94	0.79	0.89
Juice	ND	(0.01)[1]	ND	(0.01)	(0.01)	ND	(0.01)	ND	(0.01)	(0.01)
Processing factor	NF [2]	0.04	NF	0.01	0.02	NF	0.07	NF	0.02	0.02
Molasses	(0.02)	0.42	0.38	0.81	0.80	ND	0.24	0.94	1.18	1.18
Processing factor	0.12	1.6	1.3	1.1	1.4	NF	1.7	2.9	2.1	2.6
Dried pulp	0.14	0.31	1.67	2.11	1.98	0.10	0.15	1.28	1.53	1.43
Processing factor	0.82	1.2	5.6	2.9	3.5	0.83	1.1	4.0	2.7	3.1
Finisher pulp	ND	(0.01)	(0.02)	(0.03)	(0.03)	ND	ND	ND	ND	ND
Processing factor	NF	0.04	0.07	0.04	0.05	NF	NF	NF	NF	NF
Oil	1.22	3.94	(0.04)	5.20	3.98	2.42	1.58	(0.04)	4.04	1.62
Processing factor	7.2	14.6	0.13	7.1	7.0	20.2	11.3	0.13	7.1	3.5

NF = no processing factor could be calculated: ND = not detected
[1]Mean of duplicate results (whether duplicate samples or analyses is not indicated)
[2]Values in parentheses are estimates at levels below the limit of determination

Residues in the edible portion of food commodities

Nearly all of the residue in citrus fruit is in or on the peel (≦0.3% of the TRR was found in the pulp or juice). There is little likelihood of carbosulfan residues occurring in the edible portions of citrus fruits.

RESIDUES IN FOOD IN COMMERCE OR AT CONSUMPTION

No information.

NATIONAL MAXIMUM RESIDUE LIMITS

The following national MRLs in The Netherlands were reported to the Meeting.

Commodity	MRL, mg/kg	
	carbosulfan	carbofuran + 3-hydroxycarbofuran
Carrots	0.1	0.3
Parsnips	0.1	0.3
Tea	0.1*	0.2*
Other foods	0.05*	--

* At or about the limit of determination

APPRAISAL

Carbosulfan, 2,3-dihydro-2,2-dimethylbenzofuran-7-yl (dibutylaminothio)methylcarbamate is a broad-spectrum carbamate pesticide used on a variety of crops, but mainly on citrus fruits, and this use is the focus of the present evaluation. Carbosulfan was first evaluated by the 1984 JMPR, which recommended a temporary ADI and a temporary MRL of 2 mg/kg for citrus fruits. The temporary ADI was converted to an ADI of 0-0.01 mg/kg bw by the 1986 JMPR.

Because information required by the 1984 and 1991 Meetings had not been provided, the 1993 JMPR recommended withdrawal of the proposed TMRLs for carbofuran and carbosulfan in citrus fruits. The 1993 Meeting was informed that additional studies were under way. Carbosulfan was subsequently scheduled for periodic review by the FAO Panel in 1997. New studies on citrus fruit have been reviewed by the Meeting, together with supporting data.

Carbofuran is a major metabolite of carbosulfan as well as being itself a pesticide. The present periodic review of carbosulfan includes estimates of maximum residue levels, an STMR and STMR-Ps for carbosulfan *per se* resulting from its use on citrus fruit. The concurrent review of carbofuran includes estimates to accommodate residues of carbofuran and 3-hydroxycarbofuran resulting from the use of carbosulfan on citrus fruit.

Metabolism studies on rats and goats were available. The distribution, excretion and fate of carbosulfan was investigated in rats by oral gavage administration of dibutylamine- or phenyl-labelled [^{14}C]carbosulfan at low (4 mg/kg) or high (30 mg/kg) dosing levels. About 66-88% was eliminated in the urine, 5-22% in the faeces and 10 to 17% as CO_2 from the dibutylamine (DBA) label but none from the phenyl label. Up to about 2% remained in the carcase. Eighty to 90% was excreted within 24 to 48 hours of dosing at the lower dose and within 72 hours or so at the higher dose. The main excreted compounds identified from the phenyl label in decreasing order were the 7-phenol, 3-keto-7-phenol, 3-hydroxycarbofuran, 3-hydroxy-7-phenol and carbosulfan, with minor residues of 5-hydroxycarbofuran, 3-ketocarbofuran, carbofuran, 3-ketocarbosulfan sulfone, 3-hydroxycarbosulfan and 3-ketocarbosulfan. From the DBA label DBA, hydroxy-DBA, CO_2 and carbosulfan were found in decreasing order. No major sex differences were observed. Higher residues of ^{14}C were found in tissues from the DBA label than from the phenyl. This was attributed to incorporation of the DBA moiety into natural stored fat by oxidation, *N*-dealkylation or deamination and further oxidation to fatty acids.

The metabolites are consistent with metabolic routes which include a series of hydrolyses, oxidations and conjugations. A main indicated route includes hydrolysis to the 7-phenol, oxidation to 3-hydroxy-7-phenol and further to 3-keto-7-phenol, and conjugation as sulfates or glucuronides. Another route involves oxidation to 3-hydroxycarbosulfan which may be hydrolysed to 3-hydroxycarbofuran or oxidized further to 3-ketocarbosulfan, which in turn may be oxidized again to 3-ketocarbosulfan sulfone or hydrolysed to 3-ketocarbofuran. The 3-hydroxycarbofuran or 3-ketocarbofuran may be hydrolysed to their phenols before conjugation. Hydrolysis also results in the release of DBA which may be oxidized at different carbons to hydroxydibutylamines. The authors also postulate the *N*-dealkylation/deamination and oxidation to fatty acids which may be incorporated in natural fats as described above, or result in the release of CO_2 by the citric acid cycle as indicated by the detection of radiolabelled CO_2.

Lactating goats were dosed with either phenyl- or DBA-labelled carbosulfan for 7 days at levels corresponding to approximately 25 ppm in the diet. Samples of urine, faeces, milk and tissues were analysed. As in rats, most of the ^{14}C was eliminated in the urine, approximately 82% of the phenyl label and 68% of the DBA label. About another 7% and 3% respectively were eliminated in the faeces. Approximately 0.2% of the dose (0.04-0.09 mg/kg carbosulfan equivalent) was found in the milk, 0.02% (0.06 mg/kg) in liver and 0.01% (0.2 mg/kg) in kidney from the phenyl label, but less than 0.01% (≤0.01 mg/kg) in muscle or fat. Because of these low levels, the identification of ^{14}C residues in muscle and fat from the phenyl label was not attempted.

The residues were higher from the DBA label: 2.3% (0.3-0.94 mg/kg) in the milk, 0.34% (1.13 mg/kg) in the liver, 0.04% (0.75 mg/kg) in the kidneys, 0.08% (0.18 mg/kg) in the muscle, and 0.15% (1.2 mg/kg) in the omental fat. The higher levels were attributed to incorporation into natural body constituents such as carbohydrates and proteins. The detection of radioactivity in fatty acids, amino acids, triglycerides and amines was consistent with that hypothesis.

A series of extractions, partitions, pH adjustments and acid or enzymatic hydrolyses were used to separate metabolites for comparison with authentic standards by HPLC, TLC, GC-MS, HPLC-MS and size-exclusion chromatography. From the phenyl label 98.6% of the TRR was extractable from milk, 37.3% from liver and 62% from kidney.

The major metabolites identified in milk, liver and kidney from the phenyl label were 3-hydroxycarbofuran and the 3-keto-7-phenol, 3-hydroxy-7-phenol and 7-phenol, accounting for approximately 94.4% of the TRR in milk (reaching a plateau after about 2 days), 32.7% in liver (37.1% for all identified residues) and 52% in kidney (59.6% for all identified residues). 3-hydroxycarbofuran (34.2% of the TRR) and the 3-keto-7-phenol (29.9% of the TRR) predominated in the milk, 3-hydroxycarbofuran in kidney (21.5% of the TRR) and the 3-hydroxy-7-phenol in liver (15.6% of the TRR). Minor identified residues included 5-hydroxycarbofuran, 3-ketocarbofuran, *N*-hydroxymethyl-carbofuran, carbofuran, carbosulfan, 3-ketocarbosulfan sulfone, 3-hydroxycarbosulfan and carbosulfan sulfone. None of these exceeded 4% of the TRR in milk, liver or kidney. Carbosulfan and carbofuran were detected only at very low levels in the milk and tissues (0.001 mg/kg).

Although only 37.1% of the phenyl label radioactivity was extractable from the liver with the initial solvent extraction, enzymatic and HCl hydrolysis allowed further characterization. Unidentified radioactivity was characterized as very polar (10.4% of the TRR), protein-associated (22.6% of the TRR) or unextractable (12.7% of the TRR).

In the kidney very polar unidentified metabolites accounted for 18.4% of the TRR, with another 17.4% characterized, but not identified.

Residue levels were much higher from the DBA label and this was attributed largely to incorporation into natural products as in rats. Residues in the fat and muscle from the phenyl label were too low for identification or characterization, but were high enough with the DBA-labelled samples (1.3 and 0.2 mg/kg carbosulfan equivalent in fat and muscle respectively). Approximately 80.1% of the DBA TRR (0.6 mg/kg carbosulfan equivalent) in milk and 90% in fat was organo-extractable, but only 45% in liver, slightly more than with the phenyl label. Approximately 70% of the TRR was extractable from kidney and 52% from lumbar muscle.

Residues in milk and tissues from the DBA label consisted mainly of aminobutanols, dibutylamine-related compounds, material incorporated into natural constituents (fatty acids, amino acids, carbohydrates, triglycerides etc.), amines (conjugated , unconjugated and bound) and polar water-soluble metabolites. In milk aminobutanols accounted for approximately 30% of the TRR and another 30% was found in natural constituents. 87% of the TRR in fat, 32% in muscle and 30% in liver was found in natural constituents. In liver another 21% was in the form of aminobutanols or dibutylamine and related compounds. In kidney 24% of the TRR was characterized as unconjugated amines, approximately 19% as polar water-soluble metabolites, and 14% as natural constituents.

The main metabolic routes in goats are similar to those in rats, starting with hydrolysis either directly to the 7-phenol or to carbofuran and dibutylamine. The 7-phenol is oxidized progressively to the 3-hydroxy-7-phenol and 3-keto-7-phenol and carbofuran to 3-hydroxy- or 5-hydroxycarbofuran. The 3-hydroxycarbofuran may be oxidized to 3-ketocarbofuran and each of these hydrolysed to the corresponding phenol. Dibutylamine may be oxidized to 4-(butylamino)butanol and further to the corresponding butanoic acid, or undergo a series of reactions to form butylamines and butanols. The degradation of DBA may also lead to incorporation into fatty acids, amino acids and carbohydrates and presumably through the citric acid cycle to CO_2, but CO_2 was not trapped.

The minor residues derived from the phenyl-labelled compound also indicate a subsidiary metabolic route in which the carbamate structure is retained with either direct oxidation to the sulfone or by 3-hydroxycarbosulfan and 3-ketocarbosulfan to 3-ketocarbosulfan sulfone.

Plant metabolism. Metabolism studies with both phenyl- and DBA-labelled carbosulfan were conducted in the field on navel oranges with spray application at a nominal rate of 0.5 g ai/l. Orange samples were taken at 0, 7, 15 and 30 days and leaves at 0 and 30 days. Oranges were rinsed and samples of peel rinse, peel, pulp and juice were analysed by HPLC, TLC, MS and LSC. The TRR in whole oranges amounted to 0.81 and 0.7 mg/kg carbosulfan equivalent from the phenyl and DBA labels on day 0 to 0.78 and 0.59 mg/kg on day 30.

Nearly all of the residue in the whole fruit was in or on the peel (99.9% of the phenyl ^{14}C, 99.6 of the DBA) on day 0 and these proportions remained essentially unchanged even after 30 days. Almost all of the residue was on the peel surface on day 0 (95.8% of the phenyl TRR, 93.9% of the DBA), but by 30 days more of the residue had penetrated into the peel (45.9% of the ^{14}C from the phenyl label and 41.5% from the DBA). No more than 0.3% of the TRR (<0.01 mg/kg carbosulfan equivalent) from both labels was in the pulp or juice over the 30-day period. More than 90% of the TRR in rinsed peel from both labels was extractable throughout the 30 days with the proportion of polar and conjugated material increasing with time, especially that from the DBA label where it reached 57% by day 30.

The peel rinses and extracts were examined to identify the residues. Residues from the phenyl label after 30 days as a proportion of the TRR were carbosulfan 40.1%, carbofuran 33.9%, carbosulfan sulfone 3.1%, 3-hydroxycarbofuran and 3-keto carbofuran 2% each and *N*-hydroxymethyl-carbofuran, dicarbofuran sulfide and the 7-phenol less than 2% each, making a total of 83.7% of the TRR. From the DBA label carbosulfan and DBA accounted for 31.2 and 58.2% of the TRR respectively. This is consistent with the primary metabolic cleavage of the two N-S bonds to form carbofuran and DBA. Some oxidation to carbosulfan sulfone occurs before cleavage of these bonds and a minor route resulted in the formation of dicarbofuran sulfide. The rest of the metabolism

is effectively that of carbofuran. This includes direct oxidation to the 7-phenol or retention of the intact carbamate with oxidation at the *N*-methyl to form *N*-hydroxymethylcarbofuran or on the ring to form 3-hydroxycarbofuran which may be further oxidized to 3-ketocarbofuran.

The only metabolite found in oranges which was not also identified in goats was dicarbofuran sulfide at very low levels. The other notable difference between plants and animals is that only very low levels of intact carbamates were detected in animals (apart from 3-hydroxycarbofuran at exaggerated feeding levels), whereas they were the main residues in plants after 30 days.

Environmental fate. Although limited information was available on photolysis in soil and water, other environmental studies noted as being necessary in the 1995 JMPR report (Section 2.5.2) were not provided. The degradation of carbosulfan in dry soil and soil at 70% water capacity exposed to a sun lamp was investigated with phenyl- and DBA-labelled [^{14}C]carbosulfan. The spectral characteristics of the sun lamp were not reported, nor was the temperature.

The half-life of carbosulfan with both labels was less than 10 minutes in the dry soil. After 8 days the main residue from the phenyl label was carbofuran (54.5% of the TRR), with 3.5% of 3-hydroxycarbofuran, 2.6% of carbosulfan sulfone, and lesser amounts of phenols or oxidized carbamate metabolites of carbosulfan or carbofuran. The predominant residues from the DBA label after 10 minutes were dibutylamine (38.6% of the TRR), carbosulfan (11.4%), *N*-formyldibutylamine (6.4%) and *N*-acetyldibutylamine (1.1%). After 8 days the same compounds were detected, but at very low levels (the rest was unidentified). Degradation was substantially slower with the wet soil treated with DBA-labelled carbosulfan. After 48 hours carbosulfan was still 76.2% of the TRR.

The authors concluded that as the results from irradiated and control soils with both labels were so similar exposure to light had very little effect, suggesting that degradation resulted from soil contact rather than the effect of light. The Meeting could not draw such a firm conclusion from the data, although it is likely that the soil was the main contributor to the degradation.

Photolytic and hydrolytic degradation were also investigated in water buffered at pH 7 and distilled water with both DBA- and phenyl-labelled [^{14}C]carbosulfan (5 mg/l) and irradiation for up to 8 days with a sun lamp. Apart from specifying that the radiation was above 300 nm, the spectral characteristics of the sun lamp were not reported nor was the kept. The half-life was about 1.4 days in buffered water and 4-8 days in distilled water. Degradation was much more rapid in irradiated samples than in controls, although the identified products were the same. Degradation was mainly to carbofuran and dibutylamine. Other lesser products from the phenyl label were carbosulfan sulfone, the 7-phenol and the 3-keto-7-phenol. From the DBA label the main product was dibutylamine, with lesser amounts of *N*-formyldibutylamine and *N*-acetyldibutylamine.

No other studies on environmental fate were submitted to the Meeting.

Methods of analysis. A number of analytical procedures are available for the determination of carbosulfan, its carbamate and phenolic metabolites and dibutylamine in citrus and animal products. Recent methods used in some of the field trials with citrus fruit and animals are based on the extraction of carbosulfan with dichloromethane (from citrus) or acetone (from animals products) and clean-up on solid-phase extraction (SPE) cartridges before analysis. Carbamate and phenolic metabolites are hydrolysed with HCl before SPE column extraction and dibutylamine is extracted with methanol/water. Some procedures include liquid-liquid partitions.

The HPLC configuration for the determination of carbosulfan includes two post-column reactors, one with H_2SO_4 to hydrolyse carbosulfan to carbofuran and the other with *o*-phthalaldehyde + *N*,*N*-dimethyl-2-mercaptoethylamine to form a chromophore for fluorescence detection. The configuration is the same for carbamate metabolites, except that only the second reactor is used. Phenolic fractions are derivatized with pentafluorobenzyl bromide (PFBBr), and 3-hydroxy-7-

phenols also by ethylation, before analysis. Dibutylyamine fractions are derivatized with dansyl chloride for analysis. Both the phenolic and DBA derivatives are analysed by GC-MS with single ion monitoring.

In citrus a limit of determination of 0.05 mg/kg for all analytes would appear to be supported for this group of methods by adequate recoveries and sample chromatograms, but citrus controls consistently had apparent DBA levels up to 0.02 mg/kg. For this reason 0.1 mg/kg may be a more realistic limit of determination for DBA. For animal products limits of determination of 0.025 mg/kg in milk and 0.05 mg/kg in tissues also appear to be supported for all compounds on the basis of adequate recoveries and sample chromatograms, but DBA was again reported near the limit of determination in some milk samples (0.005-0.037 mg/kg). The methods were independently validated.

The methods used in some other citrus trials involved hexane/propanol extraction of carbosulfan and carbofuran, and HCl reflux extraction of 3-hydroxycarbofuran after ethoxylation, followed by liquid/liquid partition, Florisil column clean-up and GLC with NP detection. The reported limit of determination was 0.01 mg/kg and the methods were validated at that level. However sample chromatograms and corroborating information from multi-residue methods indicated that 0.05 mg/kg would appear to be a more practical limit of determination; it was also recommended as the reporting level.

Older methods used in citrus processing studies were similar to that just described, including GLC with NP detection, but with different clean-up columns. A limit of determination of 0.05 mg/kg is again reasonable, except perhaps for citrus oil where 0.1 mg/kg might be more realistic. Published multi-residue methods were not adequate for carbosulfan, mainly owing to low detector sensitivity.

Stability of residues in stored analytical samples. In a 1980 storage stability study no significant losses of carbosulfan were observed when orange and alfalfa samples fortified with carbosulfan were stored for one year at -18°C. Carbofuran was the predominant metabolite. However, in a pH 4.8 silt loam soil in this same study carbosulfan was almost completely degraded after only three hours at -18°C. This was attributed to the acidity, although carbosulfan was stable in the orange samples, also likely to be acidic. It was more stable in a pH 6 silty clay loam and a pH 6.8 sandy loam at -18°C, with half-lives of about 220 and 144 days respectively.

An interim report described studies of the stability of carbosulfan, its carbamate and phenolic metabolites and DBA in laboratory-fortified oranges and processed orange products stored for up to a year at -18°C. Samples of whole oranges, dried pulp, juice, molasses and oil were fortified at 0.25 mg/kg and most samples were taken for analysis on day 0 and after approximately 3, 6, and 12 months. On day 0 no residues were detected in the juice, and residues in molasses and oil were only 0.08 and 0.12 mg/kg respectively. In these cases carbofuran was the main product of carbosulfan degradation as demonstrated by mass balance investigations. Later samples of juice, molasses and oil were therefore analysed for carbosulfan. Orange oil was analysed for carbosulfan metabolites only on day 0 and after 12 months. There were no appreciable losses of the other carbamate or phenolic residues in any of the samples. It was reported that results of analyses for DBA after 18 and 24 months will be available at an unspecified future time.

The stability and mass balance of residues in bovine milk and tissues fortified at 0.25 mg/kg with carbosulfan and DBA were investigated after storage at -18EC for intervals up to 8 months. Carbosulfan was shown to be degraded rapidly in milk, muscle and liver with losses of 16 and 4% from milk and liver respectively in the first month and of 84% from milk, 100% from muscle and 80% from liver after 8 months. Losses of DBA from milk and liver were 8 and 4% respectively after 6 months, but DBA residues in muscle showed an apparent increase of 52% after 6 months. Overall the analyses for DBA were erratic over the test period. Mass balance studies showed that carbosulfan + carbofuran accounted for 68% of the fortification level in milk after 3 months and 88 and 44% in

muscle and liver respectively after 6 months. After 8 months carbofuran and the 7-phenol together accounted for over half of the fortification level in milk and liver and 148% in muscle.

These results confirm the instability of carbosulfan *per se* in animal products even under frozen storage conditions, and add confidence to the prediction that there is little likelihood of finding it in animal products as a result of using carbosulfan on citrus. The results also confirm that degradation is likely to be mainly to carbofuran and its metabolites. The trials did not include analyses for 3-ketocarbofuran, 3-hydroxycarbofuran, or other minor carbamate metabolites. On the basis of the results of the cow feeding study it is likely that 3-hydroxycarbofuran especially may constitute a significant proportion of the residue unaccounted for in these studies.

Citrus residue trials. 30 supervised trials were conducted in 1993-4 in Brazil, Mexico and Spain using the analytical methods described above. The six 1993 Brazilian trials on Valencia and Pera Coroa oranges with a CE formulation (*c*.1-1.7 g ai/tree) were typical. Six trees/trial were treated, and 4 oranges/tree or 24 oranges/trial sample were taken. Duplicate samples were analysed separately in each trial and both results are tabulated. All trials were according to GAP (a maximum of 2 foliar applications at 0.9-1.7 g ai/tree, the first after full bloom and the 2nd approximately 50 days later). Sprays were to run-off and the 7-day GAP PHI was observed. Sampling, transport and storage were adequate to provide confidence in sample integrity. Analytical results were not corrected for recoveries. At the 7-day GAP PHI the maximum residues were carbosulfan <0.01-0.03 mg/kg, carbofuran 0.02-0.06 mg/kg, and 3-hydroxycarbofuran <0.01-0.03 mg/kg. The 3-ketocarbofuran metabolite occurred in only one trial, at 0.02 mg/kg. Total phenols were up to 0.02 mg/kg and dibutylamine up to 0.15 mg/kg.

A 7-day GAP PHI was also observed in the 7 Mexican trials on Valencia and other oranges with an LE formulation (250 g ai/ha). Residues from application according to GAP, corrected for recoveries, were <0.01-0.08 mg/kg carbosulfan, 0.08-0.26 mg/kg carbofuran, <0.01-0.14 mg/kg 3-hydroxycarbofuran, <0.01-0.04 mg/kg 3-ketocarbofuran, and <0.01-0.14 mg/kg 3-hydroxycarbofuran. Residues of total phenols (uncorrected) were 0.01-0.12 mg/kg and of DBA up to 0.14 mg/kg.

In four 1994 Spanish trials according to GAP with an LE formulation (*c*.3 g ai/tree) both mature (112-day PHI) and immature (28-day PHI) Valencia oranges were analysed. No residues (<0.01 mg/kg) of carbosulfan were detected at either PHI. In the mature oranges carbofuran residues (corrected for recovery) were <0.01-0.36 mg/kg, 3-ketocarbofuran <0.01-0.05 mg/kg, and 3-hydroxycarbofuran 0.02-0.14 mg/kg. The residues of total phenols (uncorrected) were up to 0.25 mg/kg and of DBA up to 0.14 mg/kg. Not unexpectedly, residues (except of 3-ketocarbofuran) were substantially higher in the immature oranges with a corrected maximum of 0.82 mg/kg carbofuran, <0.01 mg/kg 3-ketocarbofuran, and 0.39 mg/kg 3-hydroxycarbofuran. Maximum (uncorrected) residues of total phenols were 0.63 mg/kg and of DBA 0.29 mg/kg.

Additional Spanish trials according to GAP were conducted in 1993 and 1994 with high-volume applications of an EC formulation (937.5 g ai/ha, 3000 l/ha). Sampling was not only at the harvest GAP PHI (84-147 days), but also at days 0, 30, 45, 60...147. Analyses were only for carbosulfan, carbofuran, and 3-hydroxycarbofuran. Because these compounds are those that the Meeting recommended for inclusion in the definitions of the residues arising from the use of carbosulfan and carbofuran (see below), the results can be used to estimate maximum residue levels and STMRs. The JMPR had previously recommended that all field trials should also include analyses for 3-ketocarbofuran. The Meeting upheld that recommendation with respect to future submissions of data on commodities other than citrus but concluded that, because a number of trials had demonstrated that the compound occurs at relatively low levels in citrus, the data on 3-ketocarbofuran were adequate for citrus fruit.

No residues of carbosulfan were detected at these extended PHIs (84-147 days), but residues of carbosulfan at day 0 (corrected for recoveries) were as high as 3.3 mg/kg. It was seldom detectable

after 30 days and even then only in the peel. Carbofuran and especially 3-hydroxycarbofuran were present in some cases after 30 days, and even at harvest after the lengthy PHIs. Generally 3-hydroxycarbofuran was the higher of the two at this stage.

In the six 1993 trials residues at harvest (110-147 days) were <0.01-0.04 mg/kg carbofuran (mostly <0.01) and 0.05-0.13 mg/kg 3-hydroxycarbofuran. Peel and pulp samples were also analysed at 45 days in two of the trials: carbosulfan was not detected in the peel or pulp (<0.01 mg/kg) and carbofuran residues were about 0.27 or 0.37 mg/kg in the peel and <0.01 mg/kg in the pulp, giving 0.02-0.17 mg/kg in whole oranges. 3-hydroxycarbofuran was at a similar level in the peel and 0.01 mg/kg in the pulp.

In the seven 1994 studies (PHIs 84-140 days) residues in whole oranges were calculated from those in the peel and pulp and the measured peel/pulp weight ratios (24/76, 27/73, 28/72 or 30/70 at harvest, depending on the type of orange). The calculated residues were <0.01 mg/kg carbosulfan, <0.01-0.06 mg/kg carbofuran and 0.04-0.13 mg/kg 3-hydroxycarbofuran. At day 0 the maximum residues in the peel and pulp (uncorrected for recovery) were 0.9 and <0.01 mg/kg carbosulfan, 0.63 and <0.01 mg/kg carbofuran and 0.64 and 0.05 mg/kg 3-hydroxycarbofuran.

The Meeting concluded that MRLs for citrus fruits should be established both for carbosulfan defined as carbosulfan and for carbofuran defined as the sum of carbofuran and 3-hydroxycarbofuran, expressed as carbofuran. The Meeting examined the distribution of data from trials complying with GAP according to these definitions in order to estimate MRLs and STMRs, and observed (not surprisingly) the absence of detectable carbosulfan residues in the Spanish trials with PHIs of 84-147 days compared with the measurable but low levels after 7 days in the Mexican and Brazilian trials. There is much less variation in the sum of carbofuran and 3-hydroxycarbofuran residues however, even with the wide divergence of national PHIs.

Duplicate samples were analysed in most of the trials and the results recorded separately. In order to avoid averaging problems in cases where one of two duplicate results was below the level of detection and to avoid the possibility of over-estimating the median residue, the Meeting decided to treat all the results separately for the estimation of MRLs and STMRs, and included estimated levels for residues between the limits of detection and determination.

The Meeting had two options for the estimation of STMRs for carbosulfan and carbofuran in oranges. One was to use the residues found in the pulp in four 1994 Spanish trials in which carbosulfan was undetected (<0.01 mg/kg) in all four trials and carbofuran + 3-hydroxycarbofuran was undetected in three and at a level of 0.02 mg/kg in the fourth. Since residues were detectable and estimated to be 0.01 mg/kg in each of two 45-day pulp samples in Spanish trials, the residue of 0.02 mg/kg found in the one trial according to GAP is not likely to be aberrant.

The second option was to estimate STMRs for residues of carbosulfan and carbofuran + 3-hydroxycarbofuran in whole oranges from the much larger database of 30 trials. Because of the greater uncertainty associated with the database of only four trials, the Meeting took the second option.

The low or undetectable residues found in the limited number of orange pulp samples and the results of the orange metabolism study described above which showed ≤0.2% and ≤0.3% of the TRR in the pulp and juice respectively give added assurance that residues of carbosulfan and carbofuran + 3-hydroxycarbofuran in the edible portions of oranges, if present, are likely to be very low.

The residues of carbosulfan from all the treatments according to GAP (counting duplicate samples separately) were 0.08, 0.04, 0.03 (3), 0.02 (7), 0.01 (2), and <0.01 mg/kg (39) in a total of 53 samples. If the Spanish trials are excluded, the residues of carbosulfan were 0.08, 0.04, 0.03 (3), 0.02 (7), 0.01 (2), and <0.01 mg/kg (12): 26 samples. From these results, the Meeting estimated a

maximum residue level of 0.1 mg/kg and an STMR of 0.01 mg/kg for carbosulfan in oranges. The STMR is at the limit of detection.

The residues of the simple sum of carbofuran + 3-hydroxycarbofuran were 0.5, 0.4 0.39, 0.33, 0.26, 0.22 (2), 0.19, 0.17 (2), 0.15, 0.14 (2), 0.13 (2), 0.12 (4), 0.11 (6), 0.10, <0.10, 0.09 (3), 0.08 (3), 0.07 (4), <0.07, 0.06 (4), 0.05 (4), 0.04, <0.04, 0.03 (3), and 0.02 (2) mg/kg (53 samples).

On the basis of this distribution, recognizing that the residues would be only very slightly lower if an adjustment were made for the molecular weight of 3-hydroxycarbofuran (about 7% higher than carbofuran), the Meeting estimated a maximum residue level of 0.5 mg/kg and an STMR of 0.1 mg/kg for the sum of carbofuran and 3-hydroxycarbofuran, expressed as carbofuran, in oranges. The Meeting concluded that the STMR for the total carbamate residues would be essentially the same as for carbofuran + 3-hydroxycarbofuran because of the low proportion of carbosulfan in the total carbamate residue.

The Meeting also received information on GAP (without labels) from Thailand for carbosulfan uses on rice, asparagus and watermelons together with what appeared to be an incomplete report of field trials. Although fairly detailed information was provided on the conduct of the trials, no analytical results were included. The information on GAP for these crops was recorded in the evaluation in case the results of the trials become available in the future and provided the GAP is confirmed by approved labels. However, the two trials apparently completed on each of these crops would not be sufficient to estimate maximum residue levels.

Summary information on GAP for German uses on rape, maize and hops was also received but no labels or residue data were provided. Official information on GAP for several commodities was also received from the UK, but again without data on residues. The information on GAP should be re-submitted, together with relevant labels, with any future reports of residue trials.

Feeding studies. Holstein dairy cows were dosed at levels equivalent to 0, 1, 3, 10 and 50 ppm carbosulfan in the diet for 28 days. Milk, kidney, liver, muscle and fat were analysed for carbosulfan, carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, the 7-phenol, 3-keto-7-phenol and 3-hydroxy-7-phenol metabolites and dibutylamine. Selected cows were held for an additional 3 or 6 days for recovery studies. The residues in the milk and tissues were generally in the decreasing order 3-hydroxycarbofuran, 3-ketocarbofuran, carbofuran and carbosulfan, although in some samples of milk carbosulfan residues were of the same order as those of carbofuran.

No carbamate residues, except one of 7 µg/kg 3-hydroxycarbofuran in the milk of one of three cows at the 10 ppm feeding level after four days, were detected in any samples at the 1, 3 or 10 ppm feeding levels. Phenols were detected at the 10 ppm level, but only in kidney (max. 57 µg/kg 7-phenol and 12 µg/kg 3-hydroxy-7-phenol). Dibutylamine was found at the 10 ppm feeding level up to 54 µg/kg in milk and in all the tissues (highest in kidney at 106 µg/kg). Carbamates were found in the milk and tissues from the 50 ppm feeding level. In summary, the maximum and mean residues at the 50 ppm feeding level were as shown below.

	Maximum/mean residue, µg/kg						
Compound	Milk	Skimmed milk	Cream	Kidney	Liver	Muscle	Fat
carbosulfan	12/7	ND	45/28	ND	ND	ND	76/44
carbofuran	8/4	ND	16/8	ND	ND	ND	ND
3-hydroxycarbofuran	30/19	20/10	ND	133/112	60/54	30/25	ND
3-ketocarbofuran	11/5	ND	ND	ND	23/14	ND	ND
7-phenol	8/4	8/6	20/11	400/358	ND	ND	14/10
3-keto-7-phenol	42/27	39/23	17/14	74/66	ND	ND	ND
3-hydroxy-7-phenol	26/19	14/12	ND	173/163	34/32	12/9	11/8
dibutylamine	119/77	---	---	890/590	294/222	58/48	47/36

where ND = 5 µg/kg in milk and 10 µg/kg in the other substrates, with limits of determination of 25 µg/kg and 50 µg/kg respectively.

At the 50 ppm feeding level carbosulfan was found in milk up to 12 μg/kg, cream up to 45 μg/kg, and fat up to 76 μg/kg, but not in kidney, liver or muscle. At this feeding level carbofuran was found only in milk (up to 8 μg/kg in one cow) and in cream up to 16 μg/kg in a different cow after a 3-day withdrawal period. 3-ketocarbofuran was detected only at the 50 ppm feeding level and then only in the milk and liver at maximum levels (in the same cow) of 11 and 23 μg/kg respectively. No residues were detected in either milk or liver after 3- or 6-day withdrawal periods.

Most of the carbamate residue at the 50 ppm level consisted of 3-hydroxycarbofuran, except in cream and fat. In milk the mean and maximum residues of 3-hydroxycarbofuran were 19 μg/kg after 7 days and 30 μg/kg after 2 days respectively, gradually decreased to 11 μg/kg after 27 days, and were undetectable after a 3-day withdrawal period. There was some reduction of carbosulfan and dibutylamine in fat during the 3- and 6-day recovery periods and no 7-phenol was detected in fat during these periods. The total carbamate residues in milk were fairly constant at approximately 30 μg/kg after the first day of sampling through the dosing period. The 3-hydroxycarbofuran metabolite was up to 133 μg/kg in kidney, ≤60 μg/kg in liver and ≤30 μg/kg in muscle. It was not detected in fat.

Both carbosulfan and carbofuran were found in cream (at mean levels of 28 and 8 μgkg respectively) but not in skimmed milk (<5 μg/kg). 3-ketocarbofuran was not found in either, and 3-hydroxycarbofuran in skimmed milk (mean level 10 μg/kg) but not in cream, not unexpectedly in view of the polarity afforded by the hydroxyl group.

In the milk, the residues of total phenols were fairly constant over the test period after the first day, with the 3-keto-7-phenol generally predominating. The highest average residues were 3-keto-7-phenol 27 μg/kg, 3-hydroxy-7-phenol 19 μg/kg and 7-phenol 4 μg/kg. These were all undetectable (<5 μg/kg) after 3 or 6 days withdrawal. The highest phenolic residues in the tissues were in the kidney with mean levels of the 7-phenol of 358 μg/kg, the 3-hydroxy-7-phenol of 163 μg/kg and the 3-keto-7-phenol of 66 μg/kg. In the liver and muscle only the 3-hydroxy-7-phenol was detected, at mean levels of 32 and 9 μg/kg respectively, and in fat only the 7-phenol (10 μg/kg) and 3-hydroxy-7-phenol (8 μg/kg).

Apparent dibutylamine was reported in most controls at maximum levels of about 50 μg/kg in both milk and tissue samples, and the residues in treated groups did not correlate well with the dose rates. Its apparent natural occurrence made reliable estimates of the DBA derived from carbosulfan difficult in all samples from animals at the 1 to 10 ppm feeding levels, and in muscle and fat at the 50 ppm level. The mean residues of 590 μg/kg dibutylamine in the kidneys and 222 μg/kg in the livers of the 50 ppm group clearly arose mainly from the treatment however.

Since the highest carbamate residues likely to result from the use of carbosulfan in an animal feed item would be about 2 mg/kg from dry citrus pulp with an STMR of 0.29 mg/kg and this is likely to constitute no more than 20-25% of a cattle diet, and since there were no significant residues at the 10 ppm feeding level and relatively low levels even at 50 ppm, the Meeting concluded that no MRL was required for carbosulfan or its metabolites in milk or tissues to accommodate the use of carbosulfan on citrus. Any residues that might occur would be covered by the maximum residue levels estimated for animal products to accommodate the use of carbofuran (see Section 4.5).

Processing

The Meeting examined reports of two processing studies, one on grapefruit and one on oranges, although data on supervised trials were available only for oranges.

Washing the fruit reduced residues of carbosulfan in grapefruit and oranges by about 67% and 53% respectively. Both carbofuran and total carbamates were reduced by about 21% in grapefruit,

but there was no reduction in oranges. The loss of carbosulfan from oranges appears to be offset by increases in carbofuran and 3-hydroxycarbofuran. This situation is analogous to the finding of low or undetectable residues of carbosulfan in harvest samples of oranges although total carbamate residues remain relatively constant over long periods. Because the oranges were processed on the day of the last application instead of after the normal pre-harvest interval, the Meeting decided to consider the total carbamate levels as a measure of the residue in evaluating the processing study. To omit carbosulfan, which would have been largely converted to carbofuran and 3-hydroxycarbofuran at harvest, would underestimate the residues.

No residues (<0.01 mg/kg) of carbosulfan were found in orange juice, so no processing factor could be calculated. Because the residue of 0.17 mg/kg carbosulfan in the whole unwashed oranges is similar to or slightly above the maximum residue found in field trials according to GAP, there would be no real expectation of finding carbosulfan in orange juice. An STMR-P of 0 for carbosulfan *per se* in orange juice would be reasonable. The residue of 0.73 mg/kg total carbamates is also slightly higher than the residues found from GAP applications in field trials. There was no concentration of any carbosulfan metabolite in the juice, although carbofuran was detected at low levels. A processing factor of about 0.01 for total carbamates applied to an STMR of 0.1 mg/kg for the sum of carbofuran and 3-hydroxycarbofuran would give an STMR-P of 0.001 mg/kg for carbofuran + 3-hydroxycarbofuran in orange juice. Although no MRL for either carbosulfan or the sum of carbofuran and 3-hydroxycarbofuran would appear to be needed since the residues would be expected to be below the limit of detection of 0.01 mg/kg, an MRL of 0.05 mg/kg, at the limit of determination, would be reasonable if one is needed.

The processing factor for carbosulfan on processing unwashed orange fruit to molasses was approximately 0.12. A worst-case STMR-P for carbosulfan in orange molasses would be the STMR for oranges, 0.01 mg/kg, x 0.12 = 0.0012 mg/kg. The processing factor for total carbamate residues was 1.1, and this multiplied by the STMR of 0.1 mg/kg for the sum of carbofuran and 3-hydroxycarbofuran in unwashed whole fruit gives an STMR-P of 0.11 mg/kg. If an MRL for carbosulfan in molasses is needed, a value of 0.05 mg/kg, at the limit of determination, would be appropriate (the maximum expected residue being 0.012 mg/kg). Because there is no significant concentration, the residues of carbofuran + 3-hydroxycarbofuran would not be expected to exceed the maximum residue level of 0.5 mg/kg estimated for whole fruit.

The processing factor for carbosulfan from unwashed oranges to dry pulp was 0.82, so the STMR-P = the STMR for oranges, 0.01 mg/kg, x 0.82 = 0.0082 mg/kg. The STMR-P for the sum of carbofuran and 3-hydroxycarbofuran = STMR 0.1 x processing factor 2.9 = 0.29 mg/kg. On the basis of the 0.82 processing factor and the recommended MRL of 0.1 mg/kg for carbosulfan in whole oranges, 0.1 mg/kg would also be sufficient as an MRL for carbosulfan in dry citrus pulp. The processing factor of 2.9 and the recommended MRL for carbofuran + 3-hydroxy-carbofuran in oranges of 0.5 mg/kg, indicate that 2 mg/kg should be the MRL for the sum of carbofuran and 3-hydroxycarbofuran in dry citrus pulp.

Orange oil. The processing factor for carbosulfan was 7.2 and that for the sum of carbofuran and 3-hydroxycarbofuran was 7.1. Since the STMR levels in oranges were 0.01 and 0.1 mg/kg respectively the corresponding STMR-Ps for orange oil would be 0.072 and 0.71 mg/kg. The same processing factors applied to the recommended MRLs of 0.1 mg/kg for carbosulfan and 0.5 mg/kg for carbofuran in oranges would lead to recommended MRLs of 1 and 5 mg/kg respectively for the oil.

The results of these estimates are shown below.

Sample	Carbosulfan				Carbofuran + 3-hydroxycarbofuran			
	Processing factor	Max. res. level[1], mg/kg	Orange STMR, mg/kg	Processed fraction STMR-P, mg/kg	Processing factor	Max. res. level[1], mg/kg	Orange STMR, mg/kg	Processed fraction STMR-P, mg/kg
Whole	--	0.1	0.01	--	--	0.5	0.1	--

Sample	Carbosulfan				Carbofuran + 3-hydroxycarbofuran			
	Processing factor	Max. res. level[1], mg/kg	Orange STMR, mg/kg	Processed fraction STMR-P, mg/kg	Processing factor	Max. res. level[1], mg/kg	Orange STMR, mg/kg	Processed fraction STMR-P, mg/kg
oranges								
Juice	NF[2]	<0.01 (0.05*)	0.01	0.0	0.01	0.005 (0.05*)	0.1	0.001
Molasses	0.12	0.012 (0.05*)	0.01	0.0012	1.1	0.55 (0.5)	0.1	0.11
Dry Pulp	0.82	0.08 (0.1)	0.01	0.0082	2.9	1.5 (2.0)	0.1	0.29
Oil	7.2	0.72 (1.0)	0.01	0.072	7.1	3.5 (5.0)	0.1	0.71

[1] The first number is the estimated maximum residue based on the processing factor and the maximum residue level for whole oranges. The numbers in parentheses are the recommended MRLs. If the estimated maximum residue level is less than the 0.05 mg/kg limit of determination, the limit of determination is recommended as the MRL.

[2] No factor could be estimated because no residues were detected in the juice

RECOMMENDATIONS

The Meeting estimated the maximum residues levels and STMR levels shown below. The maximum residue levels are recommended for use as MRLs.

Definition of the residue for compliance with MRLs and estimation of dietary intake: carbosulfan

Commodity		Recommended MRL, mg/kg		STMR, mg/kg
CCN	Name	New	Previous	
DM 001	Citrus molasses			0.0012 P[1]
AB 0001	Citrus pulp, dry	0.1	-	0.0082 P
JF 0004	Orange juice		-	0 P
FC 0004	Oranges, sweet, sour	0.1		0.01

[1]STMR-P

FURTHER WORK OR INFORMATION

Desirable

1. Information on residues of carbosulfan in food in commerce or at consumption

2. The final report on the studies of the stability of carbosulfan and its metabolites in oranges and their processed products during frozen storage (final version of Interim Report P-3154)

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