CARBOFURAN (096)

EXPLANATION

Carbofuran, a systemic acaricide, insecticide and nematicide, was first evaluated in 1967 and reviewed in 1979, 1991 and 1993. The Ad Hoc Working Group on Priorities of the CCPR in 1993 proposed carbofuran for re-evaluation, as the ADI was established in 1982 (ALINORM 93/24A para 251). It was scheduled for toxicological review in 1996 by the 1994 CCPR (ALINORM 95/24 Appendix VI) and for residue review in 1997 by the 1995 CCPR (ALINORM 95/24A, Appendix IV).

The toxicology of carbofuran was re-evaluated by the Joint Meeting in 1996. An ADI of 0-0.002 mg/kg bw was allocated on the basis of the NOAEL for erythrocyte acetylcholinesterase inhibition of 0.22 mg/kg bw per day in a four-week study in dogs and a safety factor of 100. The effect observed was reversible and acute. The previous ADI was 0-0.01 mg/kg bw.

Carbosulfan, the subject of a separate residue re-evaluation at the present Meeting, is metabolized to carbofuran and evaluations of carbofuran residues must account for carbofuran and its metabolites resulting from the use of carbosulfan according to GAP.

IDENTITY

ISO common name: carbofuran

Chemical name:

IUPAC: 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate

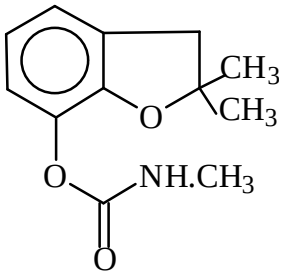
CA: 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate

CAS No.: 1563-55-2

CIPAC No.: 276

Synonyms: Furadan; Curraterr; Yaltox; FMC 10242

Structural formula:



Molecular formula: $C_{12}H_{15}NO_3$

Molecular weight: 221.26

Physical and chemical properties

Pure active ingredient

Vapour pressure: 6 x 10^{-7} mm Hg at 25°C (Alvarez, 1989)

Melting point: 153-154°C (USA Standard, 1968)

Octanol/water partition coefficient:

log P_{ow} 1.3 at 20°C (Brandau, 1975)

Solubility:
g/100 g at 25°C:

acetone	15	(USA Standard, 1968)
acetonitrile	14	
benzene	4	
cyclohexanone	9	
dichloromethane	12	
dimethylformamide	27	
dimethylsulfoxide	25	
ethanol	4	
water	0.035 g/100 ml	(Alvarez, 1987)
xylene	<1	

Specific gravity: 1.18 at 20°C

Hydrolysis:

pH	Temperature, °C	Half-life, h
25	>20,000	(Alvarez, 1987; Dziedzic 1987)
3.1	35	>20,000
3.1	45	>20,000
6.2	25	≥7,000
6.2	35	1400
6.2	45	320
7.0	25	670
7.5	25	220
8.0	25	65
9.1	25	15
9.1	35	3.2
9.1	45	0.76
9.9	25	2.2
9.9	35	0.55
9.9	45	0.16

Photolysis:

Half-life 150 hours in pH 7.0 buffered aqueous solution (5 mg/l) at 25°C when subjected to 300-400 nm radiation with a power of 150 μw/cm^2.

Technical material

Purity: 98%

Melting range: 150-152°C

Stability: Stable under neutral or acid conditions. Unstable in alkaline media.

Formulations

Formulated products containing carbofuran are listed in Table 1.

Table 1. Formulations of carbofuran.

Product	Form.	Active ingredient(s)	% ai
Furadan 75 DB	DP	carbofuran	75
Furadan 85 DB	DP	carbofuran	85
Furadan 3G (Carbo 3G)	GR	carbofuran	3
Furadan 5G or 50G (Carbosip 5G)	GR	carbofuran	5
Furadan 10G	GR	carbofuran	10
Furadan 20F	SC	carbofuran	20
Furadan 35 FS	FS	carbofuran	35
Furadan 4F or 40 F	SC	carbofuran	4
Furadan 47F	SC	carbofuran	47
Furadan 300ST	ST	carbofuran	30
Furadan 310ST (Furazin 310 TS)	ST	carbofuran	31
Furadan 35 or 350	FS	carbofuran	35
Furadan 360	FS	carbofuran	36
Furadan 350SC	SC	carbofuran	35
Curraterr 10G	GR	carbofuran	10
Curraterr 5G	GR	carbofuran	5
Furadan Combi	ST	carbofuran + carbendazim + thiram	27 5 5
Yaltox	ST	carbofuran	

METABOLISM AND ENVIRONMENTAL FATE

Animal Metabolism

The metabolism of [^{14}C]carbofuran has been studied in rats, houseflies, laying hens and lactating goats (Table 2). The carbofuran was uniformly labelled in the phenyl ring in all studies except on houseflies, where [*carbonyl*-^{14}C]carbofuran was used. Both labels were used in the rat study.

Table 2: Animal metabolism studies on [^{14}C]carbofuran.

Subject	Treatment	References
Rats Houseflies	4 mg/kg bw, single oral 0.05 µg/fly, topical	Dorough, 1968
Lactating goats	25 ppm for 7 days[1]	Hoffman and Robinson, 1994a
Laying hens	25 ppm for 7 days[1]	Hoffman and Robinson, 1994b

[1]Doses were daily by capsule, equivalent to 25 ppm in feed

Rats. Rats of 200 g each were treated orally with either 0.4 mg per kg bw [*carbonyl*-^{14}C]carbofuran or 4.0 mg per kg bw *phenyl*-^{14}C]carbofuran in a single dose. The urine and faeces were collected and assayed for the total radioactivity. ^{14}C was collected from the rats given the carbonyl label. The cumulative percentages of the administered doses found in air, urine and faeces are shown in Table 3. About 86-90% of the radiolabelled carbofuran was eliminated by 32 hours after treatment. Additional elimination was minimal. About 45% of the radiolabelled carbofuran was eliminated by cleavage of the carbamoyl moiety.

Table 3. Cumulative percentage of administered ^{14}C in air, urine and faeces from orally-dosed rats.

Time after dosing (h)	Cumulative % of administered dose				
	[carbonyl ^{14}C]carbofuran			[phenyl ^{14}C]carbofuran	
	CO_2	Urine	Faeces	Urine	Faeces
2	5.6	2.7	0.0	5.9	0.3
6	31	25	0.8	21	0.5
24	43	37	1.9	72	2.3
32	45	38	2.6	88	2.4
48	45	38	3.8	89	2.5
72	45	38	4.4	91	3.3
96	45	38	4.4	92	3.3
120	45	38	4.4	92	3.3

Urine from the [*phenyl*-^{14}C]carbofuran-treated rats from the 2-24 hour periods was extracted with an organic solvent. Less than 5% of the radioactivity was organosoluble. The major component identified by TLC in the 24-hour sample was 2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuran-7-yl-hydroxymethylcarbamate, 1.1% of the total radioactivity in the urine. Pooled urine collected for 72 hours from the [*phenyl*-^{14}C]carbofuran-treated rats was acidified to 0.5 N, boiled for 10 minutes and extracted with chloroform. About 95% of the water-soluble residue was converted to chloroform-soluble material. The compounds tentatively identified by TLC, with their percentages of the total radioactivity in the pooled sample, were 2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuran-7-yl-hydroxymethylcarbamate (3.8%), 3-hydroxy-carbofuran (14%), 2,3-dihydro-2,2-dimethylbenzofuran-3,7-diol (1.4%), 2,3-dihydro-2,2-dimethyl-3-oxobenzofuran-7-ol (48%) and 2,3-dihyrdro-2,2-dimethylbenzofuran-7-ol (20%). Control experiments showed that carbofuran, 2,3-

dihyrdro-2,2-dimethylbenzofuran-7-ol, 2,3-dihydro-2,2-dimethylbenzofuran-3,7-diol and 2,3-dihydro-2,2-dimethyl-3-oxobenzofuran-7-ol were not altered by the treatment.

Houseflies (6 days old) were treated topically with [*carbonyl*-^{14}C]carbofuran at 0.05 µg per fly and analysed in groups of 100 one hour after application. Surface radioactivity was removed with an acetone rinse. Internal radioactivity was extracted by homogenizing with acetone/water (1:1) and partitioning with chloroform. The vials were rinsed with acetone/water to collect excreted ^{14}C and the wash was partitioned with chloroform. Water-soluble fractions were hydrolysed with acid and extracted with chloroform. The organic extracts were analysed by TLC, with the results given in Table 4. Identities were not confirmed.

Table 4. Tentative identification of the radiolabelled residue from the topical application of [*carbonyl*-^{14}C]carbofuran to houseflies.

Compound	% of applied dose		
	Surface residue	Internal residue	Excretion
Carbofuran	23	12	7.1
3-hydroxy-carbofuran	0.3	5.7	0.4
3-hydroxy-carbofuran conjugated[1]	0	11	4.3
2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuran-7-yl hydroxymethylcarbamate	0.2	0.7	0.3
2,3-dihydro-2,2-dimethyl-3-hydroxybenzofuran-7-yl hydroxymethylcarbamate conjugated[1]	0	2.1	0.1
3-oxo-carbofuran	0.2	1.2	2.0
2,3-dihydro-2,2-dimethylbenzofuran-7-yl-N-hydroxymethylcarbamate conjugated[1]	0	1.8	0.2
Total[2]	24	34	14

[1]Released by mild acid hydrolysis
[2]An unknown (free and conjugated) accounted for 9% of the applied dose. Also, part of the dose may have been lost as $^{14}CO_2$ from hydrolysis of the carbamate group

Fifteen laying hens (1.34-1.68 kg, randomly divided into groups of 5) each received a capsule containing 3 mg of [^{14}C]carbofuran on each of 7 consecutive days. Eggs were collected on each day, separated into yolk and whites and pooled by group. Excreta were collected daily and pooled by group. Within 22 hours of the final dose, the hens were killed and samples of breast, thigh, fat with skin, liver and kidneys were collected from each hen and pooled by group.

Most of the administered dose was eliminated in the excreta, with the cumulative percentage of it ranging from an average of 71% on day 1 to 83% on day 7. The distribution of the radiocarbon in the eggs, excreta and tissues is shown in Table 5.

Table 5. Total radioactive residues as cumulative percentage of administered dose and as carbofuran equivalents.[1]

Sample	Day	% of applied dose	Total ^{14}C as carbofuran, mg/kg
Excreta	1	70.6	
	3	75.2	
	7	82.8	
Egg white	1	0.18	0.032
	3	0.21	0.069
	7	0.27	0.059
Egg yolk	1	0.07	0.027
	3	0.09	0.078

Sample	Day	% of applied dose	Total ^{14}C as carbofuran, mg/kg
	7	0.21	0.141
Liver	7	0.11	0.137
Kidneys	7	0.01	0.034
Breast muscle	7	0.02	<0.010
Thigh muscle	7	<0.01	<0.010
Skin and fat	7	<0.01	<0.010
Total recovery	7	83.4	

[1] Average of three groups.

The tissue samples containing >0.01 mg/kg total radioactive residue (TRR) were extracted sequentially with acetonitrile and methanol/water. Egg white was extracted with acetonitrile and egg yolk with a mixture of acetonitrile and hexane. The extractions removed the following percentages of the TRR: egg yolk 91%; egg white 91%; liver 16%; kidneys 41%. The post-extraction solids from the liver and kidneys were treated sequentially with protease, acid and base. Protease released 25% of the TRR from the liver and 19% from the kidneys. Acid and base treatments released an additional 48% from the liver and 28% from the kidneys.

The radiolabelled residues released by solvent extraction and enzyme, acid and base hydrolyses were investigated by normal-phase TLC and reverse-phase HPLC. The characterizations and identifications are shown in Table 6. The structures of the metabolites are given in Figure 1.

Table 6. Characterization and identification of the total radiolabelled residue from the administration of [^{14}C]carbofuran to hens.

Metabolite or characterization	Liver		Kidneys		Egg white		Egg yolk	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
3-hydroxy-carbofuran	--	-	-	-	-	-	12	0.019
2,3-dihydro-2,2-dimethylbenzofuran-7-ol	5.7[1]	0.008	4.9[1]	0.001	-	-	16	0.026
2,3-dihydro-2,2-dimethylbenzofuran-3,7-diol	-	-	-	-	-	-	39	0.062
2,3-dihydro-2,2-dimethyl-3-oxobenzofuran-7-ol	-	-	-	-	-	-	8.5	0.014
Phenolic conjugates	-	-	-	-	90	0.060	-	-
Enzyme digestion aqueous fraction	7.3	0.010	4.6	0.002	-	-	4.6	0.007
Mild acid hydrolysis aqueous fraction	3.1	0.004	5.8	0.002	-	-	-	-
Strong acid hydrolysis aqueous fraction	12	0.016	8.2	0.003	-	-	-	-
Mild base hydrolysis aqueous fraction	4.0	0.005	3.6	0.001	-	-	-	-
Polar residues from initial extractions	12	0.016	8.2	0.003	-	-	-	-

[1] conjugated, released by enzyme treatment.

The total radiolabelled residues in the muscle and fat with skin were negligible and the residues in the kidneys, liver and eggs ranged from 0.03 to 0.2 mg/kg. The parent compound was not detected. The metabolic pathway includes oxidation to 3-hydroxy- and 3-keto-carbofuran and hydroxylation to phenolic metabolites. See Figure 2.

[^{14}C]carbofuran, uniformly labelled in the phenyl ring, was administered orally to 2 goats for 7 consecutive days. The dose was equivalent to 25 mg/kg carbofuran in the feed. Urine, faeces and milk were collected twice daily and pooled. The goats were slaughtered within 24 hours of the final dose and samples of muscle (leg and loin), liver, kidney, omental fat and blood were taken. The distribution of the ^{14}C is shown in Table 7.

Table 7. Total radioactive residue as cumulative percentage of administered dose and as carbofuran equivalents.[1]

Sample	Day	% of applied dose	TRR as carbofuran, mg/kg
Milk	1	0.32	0.010
	3	0.29	0.14
	7	0.30	0.098
Urine	1	95	
	3	90	
	7	88	
Faeces	1	4.1	
	3	5.1	
	7	5.0	
Liver	7	0.025	0.11
Kidneys	7	<0.01	0.18
Leg muscle	7	<0.01	<0.01
Loin muscle	7	<0.01	0.01[2]
Omental fat	7	<0.01	<0.01
Total recovery	7	95	

[1]Average of 2 goats
[2]Goat B only. Goat A was <0.01 mg/kg

Milk (day 5 pm, containing 0.32 mg/kg carbofuran equivalents) was extracted with acetone. Muscle tissue (from goat B), liver and kidneys were sequentially extracted with chloroform and methanol/water. The percentages of the total radioactive residue extracted were milk 99%; muscle 30%; liver 27%; kidney 20%. The post-extraction liver and kidney samples were sequentially treated with protease, mild acid extraction and strong acid hydrolysis. Protease released 41% of the total radioactive residue from the liver and 49% from the kidneys. The mild acid extraction released 12% from the liver and kidneys.

The released radioactive residues were characterized and the components identified by normal-phase TLC and reverse-phase HPLC, with the results shown in Table 8. The structures of the metabolites are given in Figure 1.

Table 8. Characterization and identification of total radioactive residue from the administration of [^{14}C]carbofuran to lactating goats.

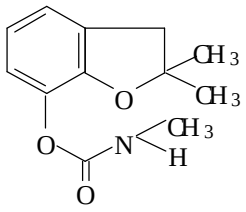
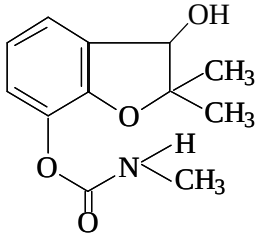
Metabolite or characterization	^{14}C, % of the TRR and mg/kg as carbofuran							
	Milk		Muscle		Liver		Kidney	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
carbofuran	0.41	0.001	-	-	-	-	-	-
3-hydroxy-carbofuran	10	0.032	-	-	4.0[3]	0.005	11[6]	0.029
2,3-dihydro-2,2-dimethylbenzofuran-7-ol	15	0.048	-	-	2.4[4]	0.003	-	-
2,3-dihydro-2,2-dimethylbenzofuran-3,7-diol	6.8[1]	0.021	-	-	12[5]	0.017	16[7]	0.042
2,3-dihydro-2,2-dimethyl-3-	32[2]	0.10	-	-	-	-	-	-

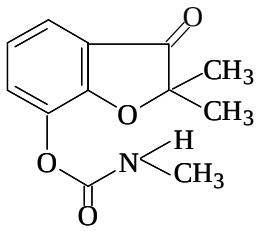
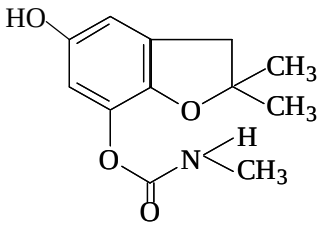
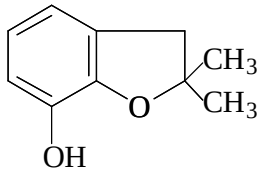
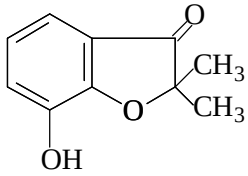
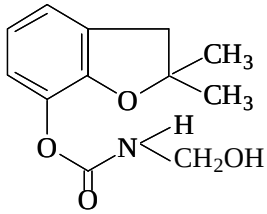
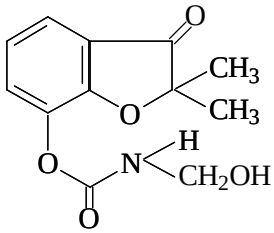
Metabolite or characterization	^{14}C, % of the TRR and mg/kg as carbofuran							
	Milk		Muscle		Liver		Kidney	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
oxobenzofuran-7-ol								
Aqueous fraction from initial extractions	6.3	0.020	28	0.003	5.0	0.007	3.5	0.009
Aqueous fraction from enzyme digestion	-	-	-	-	16	0.022	13	0.035
Aqueous fraction from mild acid hydrolysis	-	-	-	-	4.5	0.007	5.1	0.014
Aqueous fraction from strong acid hydrolysis	-	-	-	-	6.3	0.009	6.7	0.018
Polar residues (in initial extracts)	22	0.070	-	-	6.9	0.010	17	0.044

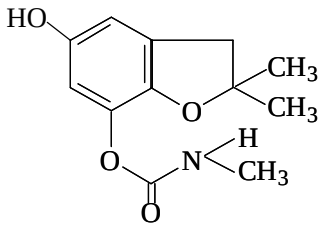
[1]Including 2% conjugated, released by sulfatase treatment
[2]Including 29% conjugated, released by sulfatase treatment
[3]Including 2.2% conjugated, released by protease treatment
[4]Conjugated, released by protease treatment
[5]Including 11% conjugated, released by protease treatment
[6]Including 8.2% conjugated, released by protease treatment
[7]Conjugated, released by protease treatment

The total radioactive residues in the tissues and fat were negligible (≤001 mg/kg) after the dietary equivalent of 25 mg/kg for 7 days. Residues in the kidneys, liver and milk ranged from 0.09 to 0.39 mg/kg. The identified metabolites are the same as those found in poultry, but the parent compound was also detected in milk. Figure 2 shows the probable metabolic pathways in poultry and ruminants. Two paths are indicated, in which oxidation at C-3 is followed or preceded by hydrolysis of the carbamate linkage. Oxidation of the carbamate methyl was not observed in goats or hens.

Figure 1. Names and chemical structures of carbofuran and its potential metabolites.

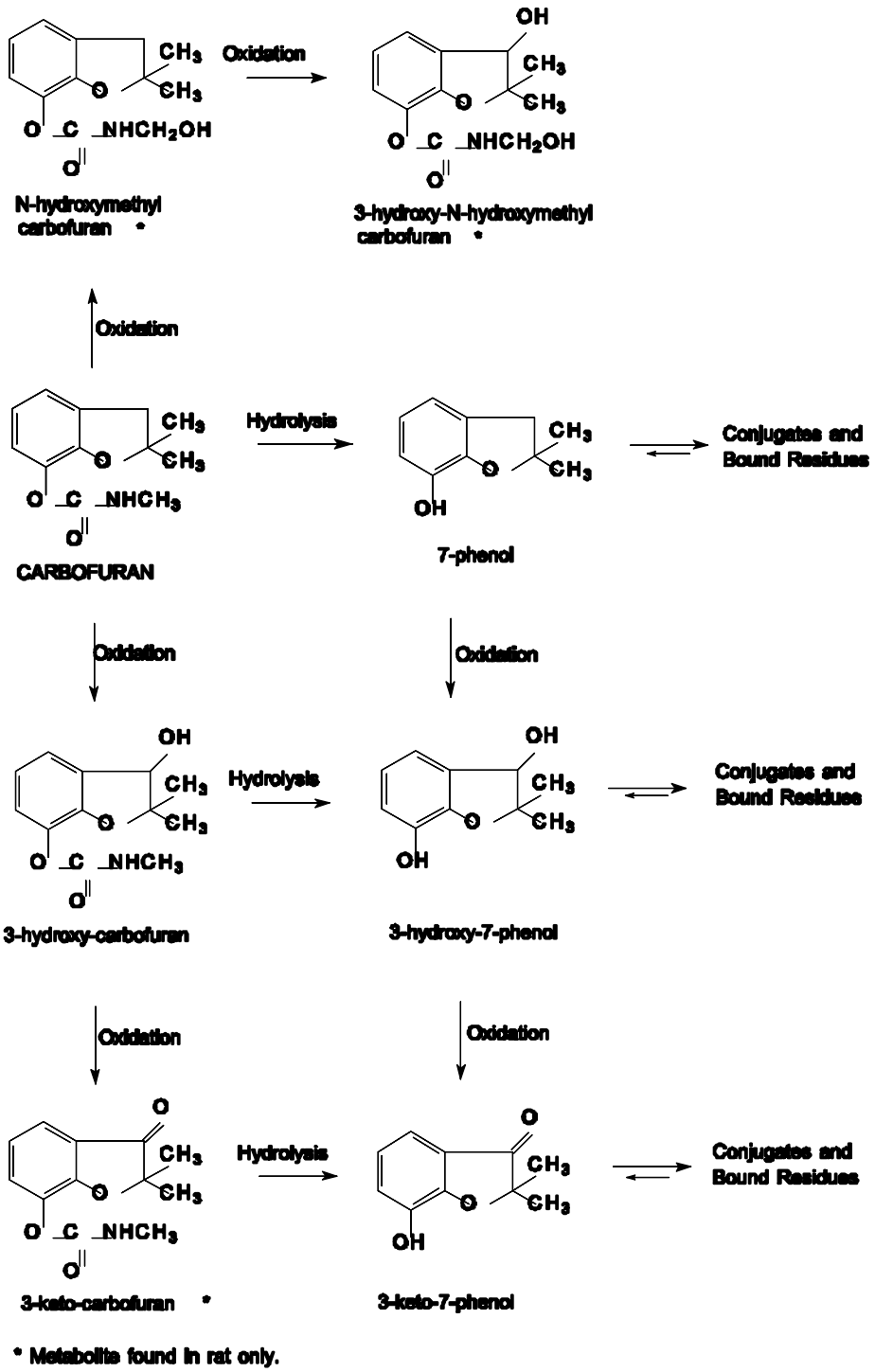
{PRIVATE }Common or derived[1] name Abbreviation used in Tables FMC number	Chemical name	Structure
Carbofuran CF FMC 10242	2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate	
3-hydroxy-carbofuran 3-OH-CF	2,3-dihydro-3-hydroxy-2,2-dimethylbenzofuran-7-yl methylcarbamate	

{PRIVATE }Common or derived[1] name Abbreviation used in Tables FMC number	Chemical name	Structure
3-keto-carbofuran 3-K-CF	2,3-dihydro-2,2-dimethyl-3-oxobenzofuran-7-yl methylcarbamate	
5-hydroxy-carbofuran 5-OH-CF FMC 27552	2,3-dihydro-5-hydroxy-2,2-dimethylbenzofuran-7-yl methylcarbamate	
7-phenol 7-P FMC 10272	2,3-dihydro-2,2-dimethylbenzofuran-7-ol	
3-keto-7-phenol 3-K-7-P FMC 16490	2,3-dihydro-2,2-dimethyl-3-oxobenzofuran-7-ol	
N-hydroxymethyl carbofuran N-CH_2OH CF FMC 53858	2,3-dihydro-2,2-dimethylbenzofuran-7-yl hydroxymethylcarbamate	
3-keto-N-hydroxymethyl carbofuran 3-K-N-CH_2OH CF FMC 53895	2,3-dihydro-2,2-dimethyl-3-oxobenzofuran-7-yl hydroxymethylcarbamate	

{PRIVATE }Common or derived[1] name Abbreviation used in Tables FMC number	Chemical name	Structure
{PRIVATE } 5-hydroxy-carbofuran 5-OH-CF FMC 27552	2,3-dihydro-2,2-dimethyl-5-hydroxybenzofuran-7-yl N-methylcarbamate	

[1]Names such as 3-hydroxy-carbofuran, 7-phenol etc., derived from the common name carbofuran

Figure 2. Proposed biotransformation pathways of carbofuran in poultry and ruminants.



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Plant metabolism

Metabolism studies were reported for potatoes, soya beans and maize (field corn). Supplementary information was submitted on the metabolism of radiolabelled carbofuran in several rotational crops.

Potatoes. Greenhouse-grown potato plants, height about 20 cm, were treated with [*phenyl*-^{14}C]carbofuran (2.65 mCi/mmol, 26,548 dpm/µg) in a single directed application to the soil surface at 7.4 kg ai/ha (Chang, 1994). The [^{14}C]carbofuran was formulated as a 0.5 kg ai/l flowable formulation (4F) and was diluted with water before application. Immature vines were sampled after 56 days and mature tubers harvested after 104 days. The total radioactive residues were 30.5 mg/kg as carbofuran in the vines and 0.80 mg/kg in the potatoes. Extraction of immature vine and mature potatoes with methanol/water followed by methylene chloride partition of the acidified and concentrated extract yielded 6.0% of the foliage and 22% of the tuber TRR. The aqueous from the methylene chloride partition, containing 87% of the foliage and 61% of the tuber TRR, was sequentially incubated with β-glucosidase (7.9% of the tuber and 51% of the foliage ^{14}C was organosoluble) and hydrolysed with 0.25 N HCl (32% of the tuber and 14% of the foliage TRR was organosoluble) and 2 N HCl (9.4% of the tuber and 13% of the foliage TRR was organosoluble). The parent compound and metabolites were identified or characterized by reverse-phase HPLC and normal-phase TLC. Tentative identifications were confirmed by GC-MS, both EI and CI. The major metabolite identified in the mature tubers was the 7-phenol (45% of the TRR) and in the foliage 5-hydroxy-carbofuran (34%). The results are shown in Table 9.

Table 9. Identification or characterization of radiolabelled residues in or on potatoes from the application of [*phenyl*-^{14}C]carbofuran to soil at 7.4 kg ai/ha after plant emergence.

Compound	Mature tuber (104-day PHI)		Immature foliage, 56-day PHI	
	%of TRR	mg/kg	%of TRR	mg/kg
Carbofuran	-	-	3.5%	1.071
3-OH-carbofuran	2.9%	0.023	22.6%	6.906
3-keto-carbofuran	-	-	1.1%	0.324
7-phenol	45.3%	0.361	6.7%	2.044
3-OH-7-phenol	13.4%	0.107	5.4%	1.658
3-keto-7-phenol	6.6%	0.052	9.4%	2.858
5-OH-carbofuran	-	-	34.4%	10.522
Total Identified[1]	68.2% (22% unconjugated)	0.543	83.1% (4.6% unconjugated)	25.383
Other	3.7%	0.029	2.6%	0.807
Polar residues	23.3%	0.185	11.0%	3.354
Unextractable	4.9%	0.039	3.3%	1.002
Total Residues[1]	100.0%	0.80	100.1%	30

[1]Results are normalized for recovery (91-101%).

Soya beans. Sandy loam soil in two 61 x 120 x 61 cm boxes was treated with carbofuran uniformly labelled with ^{14}C in the phenyl ring at 5.5 kg ai/ha in Watsonville, CA, USA. The treatment solution also contained carbofuran labelled with ^{13}C on one of the two gem-dimethyl groups and was prepared as a 0.5 kg ai/l flowable formulation (4F) in acetone/water. As applied the solution had a specific activity of 8.03 mCi/mmol. The test material was applied in a 15 cm band to a 1.3 cm deep furrow. Immediately after the application, soya bean seeds were sown in a single row down the middle of the furrow and covered with untreated soil. The soya beans were grown outdoors and samples of forage at 45 days PHI, beans at 139 days and hay at 139 days were collected.

Samples were assayed for the total radioactive carbon by oxidation and liquid scintillation counting. The forage contained 63 mg/kg carbofuran, the beans 0.32 mg/kg and the hay 36 mg/kg. Samples were then extracted with methanol/water (4:1 v/v) and subsamples of the extracts were concentrated and refluxed for one hour with 0.25 N HCl. The product mixtures were extracted with methylene chloride and the residual solids sequentially hydrolysed with 0.25 N HCl (60°C), cellulase, β-glucosidase, amyloglucosidase, pectinase, protease, 6N HCl (60°C) and 2N NaOH (65°C). The solid residues from the hay samples after solvent extraction were solubilized with dioxane/water (3/1 v/v) to release lignin (85°C, 48 hrs). After each hydrolysis the aqueous product solutions were adjusted to pH 2 and extracted with acetonitrile to recover organosoluble residues. The distribution and characterization of the radiolabelled residues are shown in Table 10.

The methanol/water and acid-refluxed methanol/water extracts were analysed by HPLC (reverse-phase) and fractions were collected for radioanalysis. Confirmation was by normal-phase (silica gel) TLC. The main metabolites were identified by GC-MS in both CI and EI modes. Unknown compounds separated by TLC or HPLC were investigated by HPLC-MS. The compounds identified are shown in Table 11.

Table 10. Distribution of the ^{14}C in hydrolysates and extracts of soya bean forage, beans and hay.

Sample	Fraction	[^{14}C]carbofuran equivalents, mg/kg	% of TRR
Forage (63 mg/kg)	Methanol/water extract	50	80
	0.25 N HCl treatment of PES[1], organosoluble	1.4	2.3
	Cellulase of PES, organosoluble	0.11	0.18
	B-glucosidase of PES, organosoluble	0.10	0.16
	Amyloglucosidase of PES, organosoluble	0.24	0.38
	Pectinase of PES, organosoluble	0.14	0.22
	Protease of PES, organosoluble	0.84	1.3
	6.0 N HCl treatment of PES, organosoluble	0.82	1.3
	2.0 N NaOH of PES, organosoluble	4.1	6.5
	Final Residual Solid	4.3	6.9
	TOTAL	62	99
Beans (0.32 mg/kg)	Methanol/water extract	0.19	59
	0.25 N HCl treatment of PES[1], organosoluble	0.030	9.3
	Cellulase of PES, organosoluble	0.011	3.6
	B-glucosidase of PES, organosoluble	0.003	0.92
	Amyloglucosidase of PES, organosoluble	0.006	1.8
	Pectinase of PES, organosoluble	0.014	4.3
	Protease of PES, organosoluble	0.022	6.9
	6.0 N HCl treatment of PES, organosoluble	0.013	4.0
	2.0 N NaOH of PES, organosoluble	0.019	5.9
	Final Residual Solid	0.019	5.9
	TOTAL	0.33	102
Hay [2](36 mg/kg)	Methanol/water extract	13	35
	0.25 N HCl treatment of PES organosoluble	5.4	15
	Cellulase of PES organosoluble	0.41	1.1
	B-glucosidase of PES organosoluble	0.26	0.72
	Amyloglucosidase of PES organosoluble	0.23	0.65

Sample	Fraction	[^{14}C]carbofuran equivalents, mg/kg	% of TRR
	Pectinase of PES organosoluble	0.13	0.36
	Protease of PES Organosoluble	0.17	0.48
	6.0 N HCl treatment of PES organosoluble	0.35	0.97
	2.0 N NaOH of PES organosoluble	0.40	1.1
	Dioxane (lignin release) of PES	2.1	5.9
	Final Residual Solid (before lignin release)	15	43
	TOTAL	35	98

[1]Post-extraction
[2] Moisture content of the hay was not determined: figures refer to undried hay

Table 11. Identification of carbofuran and metabolites in the radiolabelled residue isolated from methanol/water extracts of soya bean seed, forage and hay.

Compound	^{14}C, % of TRR and mg/kg as carbofuran											
	Extract		Acid-refluxed extract		Extract		Acid-refluxed extract		Extract		Acid-refluxed extract	
	% TRY	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
carbofuran	11.6	7.3	11.4	7.2	-	-	0.42	0.001	0.30	0.11	0.62	0.22
3-keto carbofuran	1.7	1.1	1.6	1.0	-	-	5.3	0.02	0.41	0.15	-	-
3-hydroxy-carbofuran	10.6	6.6	28	18	0.56	0.002	1.5	0.005	3.2	0.50	7.8	2.8
7-phenol	-	-	1.4	0.90	0.38	0.001	4.0	0.013	0.67	0.24	0.72	0.26
3-keto-7-phenol	1.6	1.0	13	8.1	0.71	0.002	9.2	0.030	4.3	1.6	9.8	3.5
O-glucoside conjugate of 3-hydroxy or 3-keto-7-phenol[1]	16	9.9	3.4	2.1	11	0.036	-	-	3.6	1.3	-	-
2-hydroxymethyl-3-keto carbofuran[2]	-	-	3.6	2.2	-	-	0.93	0.03	-	-	0.84	0.30
TOTAL identified	42		62		13		21		12		20	

[1]Identification by LC-MS. No comparison with reference standard
[2]Identification by GC-MS (EI and CI). No comparison with reference standard

Maize. A 1.5 x 1.5 m plot of tilled Crosby Loam soil in Ohio was treated with carbofuran uniformly labelled with ^{14}C in the phenyl ring at a rate of 8.3 kg ai/ha treated area, equivalent to 3.0 kg ai/ha broadcast (Curry, 1994). The radiolabelled material was isotopically diluted with [^{13}C]carbofuran labelled in one of the gem-dimethyl groups and with unlabelled carbofuran to a specific activity of 2.65 mCi/mmol or 26548 dpm/µg. The carbofuran was prepared as a 0.5 kg ai/l flowable formulation (4F) and was mixed with water before application. The test material was sprayed in a 15 cm band on the soil and incorporated to a depth of about 5 cm before planting maize seed (Pioneer Hybrid 3394).

Maize samples were taken at three growth stages: forage (immature stage, 47 days PHI), silage (reproductive stage, 99 days PHI) and stover and grain (mature stage, kernels without cob and husk, 158 days PHI). The samples were assayed for total ^{14}C by combustion and liquid scintillation counting. Each sample was extracted with methanol/water (1:1 v/v), and the extracts acidified to pH 1 and partitioned with methylene chloride/ether (3:1 v/v). The aqueous fractions from the methylene chloride/ether partitions of the forage and silage samples were divided into two equal portions: one was treated with ß-glucosidase and the other was acidified to 0.25 N, refluxed for one hour, and

extracted with methylene chloride/ether. The aqueous layer from this extract of the silage samples was acidified to 1 N, refluxed for one hour, and extracted with methylene chloride/ether.

The post-extraction solids (PES) from the initial methanol/water extractions were refluxed with 0.25 N HC1 for one hour. The hydrolysate from the grain was tested to determine the presence of reducing sugars with Benedict's solution and by osazone formation. Both tests indicated reducing sugars. The residue after acid hydrolysis was treated with a surfactant, sodium dodecyl sulfate. The distribution of radioactivity in the various fractions, as determined by liquid scintillation counting, is shown in Table 12.

Table 12. Distribution of the radiolabelled residue in the extracts and hydrolysates of maize grain, forage, silage and stover (fodder) from the pre-plant application of [^{14}C]carbofuran

Fraction	Grain (0.023 mg/kg)		Forage (0.81 mg/kg)		Stover (0.075 mg/kg)		Silage (0.14 mg/kg)	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
Methylene chloride/ether (non-conjugates)	5.8[1]	0.001	42	0.34	4.4	0.003	4.6	0.006
Acid-released (0.1 n), methylene chloride/ether (aglycones)	-	-	32	026	-	-	20	0.028
Glucosidase-released (aglycones)	-	-	19	0.15	-	-	23	0.032
Residual acid aqueous	-	-	8.1	0.066	22	0.016	31[2]	0.036
Acid-released from PES	48[3]	0.011	3.5 (1.0 organo-soluble)	0.028	13 (4.2% organo-soluble)	0.010	9.9 (2.8% organo-soluble)	0.014
Surfactant-released from PES	-	-	1.6	0.013	4.7	0.004	5.1	0.007
Total released residue	48		87		44		71	

[1]Methanol/water extract
[2]1 N HCl treatment of the residual 0.25 N aqueous fraction generated an additional 7.9% of the TRR (0.011mg/kg) of organosoluble residue
[3] <1% partitioned into methylene chloride.

The organosoluble fractions from the forage and silage, i.e. the methylene chloride/ether extracts of the acidified methanol/water extract and of the 0.25 N HCl hydrolysate, were analysed by HPLC, TLC and GC-MS. The methylene chloride/ether extract of the 1 N HCl hydrolysate of silage was also analysed. Because of the relatively low levels of radiolabelled residue, the extracts from the grain and stover were not analysed. The identified compounds are shown in Table 13.

Table 13. Carbofuran and its metabolites in organosoluble extracts of maize silage and forage.

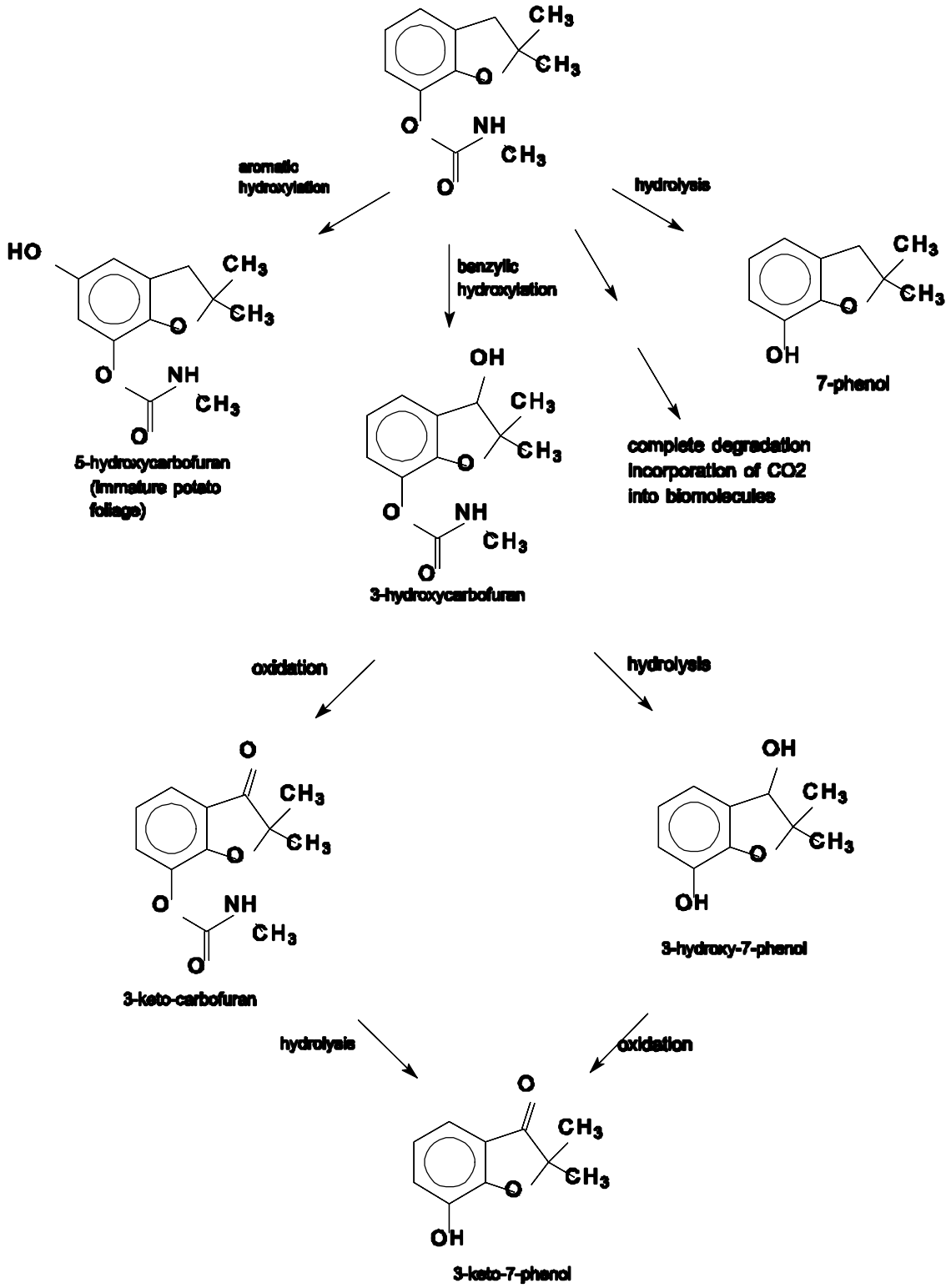
Compound	Forage		Silage	
	% of TRR	mg/kg as carbofuran	% of TRR	mg/kg as carbofuran
carbofuran	14	0.11	0.18	<0.001
carbofuran aglycone	2.4	0.019	2.1	0.003
3-keto-carbofuran	1.6	0.013	-	-
3-keto-carbofuran aglycone	0.28	0.003	0.91	0.001
3-hydroxy-carbofuran	13	0.11	1.3	0.002
3-hydroxy-carbofuran aglycone	9.7	0.078	7.9	0.011
7-phenol	0.47	0.004	0.088	<0.001

Compound	Forage		Silage	
	% of TRR	mg/kg as carbofuran	% of TRR	mg/kg as carbofuran
7-phenol aglycone	7.5	0.060	2.8	<0.001
3-keto-7-phenol	4.8	0.039	1.4	0.002
3-keto-7-phenol aglycone	5.6	0.045	2.4	0.003
3-hydroxy-7-phenol	2.4	0.020	0.88	0.001
3-hydroxy-7-phenol aglycone	3.6	0.029	2.3	0.003
Total	65	0.53	22	0.026

The major components of the radiolabelled residue identified in the forage were carbofuran and 3-hydroxy-carbofuran, free and conjugated and in the silage 3-hydroxy-carbofuran, free and conjugated. The amount of radioactivity that could not extracted with solvent or released by mild acid hydrolysis increased with the PHI, suggesting incorporation of the radiolabel into plant constituents.

The metabolites in the three crops (maize, potatoes and soya beans) are similar and are consistent with metabolism by hydroxylation and oxidation at C-3 and hydrolysis of the carbamate linkage (C-7). Aromatic hydroxylation was seen only in immature potato foliage. The proposed metabolic pathways are shown in Figure 3.

Figure 3. Proposed metabolic pathways of carbofuran in plants.



Environmental fate in soil

The rate and degree of the aerobic degradation of [^{14}C]carbofuran and its metabolites in acid and alkaline soils were determined in a study conducted in accordance with US EPA Guidelines (Saxena *et al*., 1994c). An acidic sandy loam soil (pH 5.7) was collected in Georgia and a portion was made alkaline (pH 7.7) by the addition of lime. The limed soil was incubated for about 2 months at

approximately 25°C until the soil pH and microbial population had reached equilibrium before adding [^{14}C]carbofuran uniformly labelled in the phenyl ring.

The test system consisted of approximately 50 g of oven-dried soil in a 250-ml flask. The soil samples were fortified with [^{14}C]carbofuran at a nominal concentration of 3 mg/kg (equivalent to 6.7 kg ai/ha) and incubated at 25 ± 1°C under aerobic conditions in darkness for 365 days. The apparatus included ethylene glycol to trap organic volatiles and sodium hydroxide to trap CO_2.

Duplicate samples were analysed on days 0, 1, 3, 7, 14, 30, 62, 92, 122, 181, 273 and 365, and a third sample was taken at each interval to measure the pH and microbial population. The solutions in the traps were changed and the soil moisture was adjusted periodically. The samples were analysed immediately after collection: the population of aerobic bacteria and the pH were determined, the radioactivity in the traps was counted by LSC, the soils were extracted and analysed by HPLC and the extracted soil was combusted to measure the ^{14}C. Selected extracts were also analysed by TLC to confirm the identity of [^{14}C]carbofuran. Mass spectrometry was used to confirm the identities of degradation products which accounted for >10% of the TRR. More than 90% of the applied radioactivity was accounted for in all the samples. A summary of the results is given in Tables 14 and 15.

Table 14. Aerobic degradation of carbofuran in acidic soil.

Day	Mean % of applied ^{14}C as							
	carbofuran	3-OH-CF	3-K-7-P	3-K-CF	7-phenol	Volatiles	Soil-bound	Total
0	97.49	0.06	0.16	0.18	0.04	ND	0.40	98.32
1	96.10	0.19	0.31	0.31	ND	0.01	2.91	99.82
3	95.06	0.13	ND	0.29	ND	0.03	4.64	100.15
7	92.68	0.32	0.03	0.70	ND	0.05	5.94	99.71
14	88.89	0.15	0.13	2.10	ND	0.09	8.35	99.71
30	84.30	0.56	0.02	2.08	0.02	0.16	10.96	98.10
62	82.72	ND	ND	2.60	ND	0.29	13.01	98.62
92	74.98	0.56	ND	6.36	ND	0.55	15.00	97.45
122	69.86	ND	ND	7.13	ND	0.94	20.89	98.81
181	58.29	ND	ND	12.41	ND	2.52	24.59	97.80
273	53.85	0.55	ND	11.41	ND	3.98	29.28	99.05
365	43.58	0.63	1.91	11.14	0.33	4.96	35.41	97.95

Table 15. Aerobic degradation of carbofuran in alkaline soil.

Day	Mean % or applied ^{14}C as							
	carbofuran	3-OH-	3-K-7-P	3-K-CF	7-phenol	Volatile	Soil-bound	Total
0	96.63	0.36	0.11	0.12	ND	ND	0.52	97.73
1	93.22	0.18	0.33	0.09	0.03	0.01	3.23	97.08
3	91.73	0.10	0.16	0.05	ND	0.02	7.18	99.23
7	87.73	0.79	0.37	0.07	0.28	0.11	9.98	99.32
14	83.00	0.92	0.77	0.13	0.05	0.25	12.98	98.08
30	77.39	0.33	0.20	0.02	ND	0.61	18.00	96.54
62	66.53	0.14	0.12	0.17	ND	1.67	27.48	96.11
92	59.65	ND	ND	ND	0.59	3.18	29.60	93.01
122	25.07	1.32	0.84	0.31	0.32	8.31	55.62	91.78
181	27.14	1.32	0.14	0.22	0.38	10.97	55.95	96.10
273	23.27	0.36	0.24	ND	1.08	14.11	59.23	98.27
365	20.96	0.56	0.26	0.14	0.36	16.60	57.83	96.71

3-0H-CF: 3-hydroxy-carbofuran
3-K-7-P: 3-keto-7-phenol
3-K-CF: 3-keto-carbofuran

The pH of the acidic soil samples showed no significant change during the study and ranged from 5.2 to 5.8. The pH of the alkaline samples remained between 7.4 and 8.0 in most samples but was 7.0 on day 181 and 6.6 on day 273. The microbial population remained viable and stable during the one-year period in both soils.

The only major degradation product (>10% of the applied radioactivity) in the acidic soil extracts was 3-keto-carbofuran, which reached a maximum of 12.41% of the applied radioactivity by day 181 and then decreased to 11.14% by day 365. The structure of 3-keto-carbofuran was confirmed by mass spectrometry and the structure of carbofuran was confirmed by two-dimensional TLC. Radioactivity from the alkaline soil in the NaOH traps was confirmed to be due to $^{14}CO_2$ by barium chloride precipitation. No degradation products exceeding 10% of the applied radioactivity were detected in the alkaline soil extracts. The other major products of degradation were soil-bound residues in both soils. A maximum of 35.41% (on day 365) and 59.23% (on day 273) of the applied radioactivity was incorporated in bound residues in the extracted acidic and alkaline soils respectively. Fractionation of the bound residues into humic acid, fulvic acid and humin indicated the presence of radioactivity in all three fractions.

The [^{14}C]carbofuran decreased from 97.49% at day 0 to 43.58% on day 365 in the acidic and from 96.63% on day 0 to 20.96% on day 365 in the alkaline samples. The half-life of [^{14}C]carbofuran in the test system calculated according to a first-order rate constant was 321 days and 149 days in the acidic and alkaline soils respectively.

The photodegradation of [^{14}C]carbofuran labelled in the phenyl ring, was studied in accordance with US EPA Guidelines under natural sunlight on a sandy loam soil at a field application rate of 1.7 kg ai/ha at approximately 22°C (McGovern and Shepler, 1989). The soil was sieved (2 mm) and sterilized before treatment. The control soil samples were covered to prevent exposure to light. All samples were placed in temperature-controlled chambers. Ethylene glycol and 10% NaOH were used to trap volatile organic compounds and CO_2 respectively, and air was drawn through both the irradiated and control chambers into separate sets of traps. Duplicate irradiated and control samples were analysed at 0, 3, 8, 15, 22 and 30 days after treatment.

The soil samples were extracted with methanol/water and the extracts assayed by LSC and analysed by HPLC. The remaining soil was combusted and assayed by LSC. More than 90% of the ^{14}C was recovered from all the samples. The ^{14}C from carbofuran decreased to 77% of the applied activity by day 30. The degradation products were the phenol, 3-hydroxy-carbofuran, the 3-hydroxy-7-phenol and CO_2, each <10% of the applied ^{14}C. Carbofuran was also found to be degraded to the 7-phenol in the dark, showing that the showing that 7-phenol is not (all) photochemically derived. The calculated photolysis half-life of carbofuran was 78 days and the half-life of carbofuran in the dark was 720 days.

Three terrestrial field dissipation studies were in accordance with the US Environmental Agency Pesticides Assessment Guidelines in vineyards in California. In all three Furadan 4F was incorporated into plots of soil at the maximum use rate of 11.2 kg ai/ha. Triplicate soil cores were taken from treated and control plots before and immediately after application, and then at intervals for about a year. The cores were to a depth of 120 cm for the first 14 days and 240 cm thereafter. Each core was divided into 15 cm sections which were composited in groups of three to provide

triplicate samples at each 15 cm depth, which were analysed for carbofuran, 3-hydroxy-carbofuran and 3-keto-carbofuran.

The first study was in Napa in 1987 (Daly and Tanner, 1988). The treated plot was approximately 30 x 30 m (11 rows of 10 vines) and the control plot was 72 m from the treated plot. The soil cores were taken before and immediately after incorporation and on days 3, 7, 14, 40, 90, 120, 150, 180, 304, 335 and 360. The soil was classified as a loam with the following characteristics.

pH	6.6
% Sand	41
% Silt	13
% Clay	46
CEC (meq/l)	28.7
Bulk density (g/cc)	1.4

The analytical limit of detection was 0.01 mg/kg and the limit of quantification 0.05 mg/kg. More than 77% of the residue in the soil was carbofuran. Residues of 3-keto-carbofuran and 3-hydroxy-carbofuran increased in the 0-15 cm soils for 30 days then decreased to <0.05 mg/kg by 108 days. The average total carbamate residue in the top 15 cm ranged from 3.16 to 6.66 mg/kg during the first 30 days. Quantifiable residues were not detected below the 105-120 cm. depths at any time except on day 30 at a level of 0.73 mg/kg at 120-135 cm. After 181 days the residues were below the limit of quantification at all depths. The first-order half-life calculated from the 0-15 cm depth was 43 days. A summary of the results is given in Table 16.

Table 16. Carbamate residues in soil dissipation study (Napa, 1987-88).

Mean total carbamate residue, mg/kg											
Depth,	Days after application										
inches	0	5	7	14	30	108	119	150	181	282	388
0-6	3.16	4.65	4.07	6.66	4.45	0.55	0.26	0.15	0.14	(0.02)	(0.04)
6-12	0.29	0.34	0.14	0.05	0.12	0.28	0.10	(0.04)	(0.03)	ND	ND
12-18	0.13	0.07	0.11	(0.02)	ND	0.36	0.19	0.05	(0.02)	ND	ND
18-24	0.09	0.06	0.07	(0.01)	(0.02)	0.11	0.25	0.05	0.05	(0.01)	ND
24-30	0.11	0.08	0.06	(0.02)	ND	0.08	0.16	(0.02)	0.09	(0.01)	ND
30-36	0.08	0.07	0.06	(0.01)	ND	(0.04)	0.10	(0.02)	0.06	(0.01)	ND
36-42	0.07	0.09	(0.04)	ND	(0.01)	(0.02)	0.06	(0.01)	(0.02)	(0.01)	ND
42-48	0.10	0.08	0.06	(0.03)	(0.04)	(0.01)	(0.01)	ND	(0.03)	ND	ND
48-54	NS	NS	ND	NS	0.73	ND	ND	ND	(0.04)	ND	ND
54-60	NS	NS	NS	NS	ND	ND	ND	ND	(0.03)	ND	ND
60-66	NS	NS	NS	NS	ND	ND	ND	ND	(0.03)	ND	ND
66-72	NS	NS	NS	NS	ND	ND	ND	ND	ND	ND	ND
72-78	NS	NS	NS	NS	(0.01)	ND	ND	ND	ND	ND	ND
78-84	NS	NS	NS	NS	(0.02)	ND	ND	ND	ND	ND	ND
84-90	NS	NS	NS	NS	ND	ND	ND	ND	ND	ND	ND
90-96	NS	NS	NS	NS	ND	ND	ND	(0.02)	ND	ND	ND

NS: no soil core sample was taken ND: undetectable (<0.01 mg/kg)
Values in parenthesis are estimated, below the limit of quantification (0.05 mg/kg) but above the limit of detection (0.01 mg/kg)

A second study was in Farmersville in 1988-1989 (Herbert, 1989). The treated plot was 36 x 24 m (10 rows of 10 vines) and the control plot was 72 m from the treated plot. Core samples were taken before and immediately after incorporation and on days 3, 7, 14, 40, 90, 120, 150, 180, 304, 335 and 360. The soil was classified as a loam with characteristics shown in Table 17.

Table 17. Soil characteristics, Farmersville dissipation study.

{PRIVATE }Soil depth (inches)	0-12	12-24	24-36	36-48	48-60	60-72	72-84	84-96
PH	7.49	7.55	7.54	7.51	7.55	7.48	7.99	8.01
% Sand	51.4	66.8	69.8	70.8	70.8	68.6	70.6	71.6
% Silt	32.0	18.4	13.4	12.4	11.4	18.2	19.2	17.2
% Clay	16.6	14.8	16.8	16.8	17.8	13.2	10.2	11.2
CEC (meq/l)	10.1	5.1	5.5	5.4	5.5	7.7	8	12.4
% organic matter	1.04	0.34	0.07	0.04	0.06	0.20	0.09	0.10
Bulk density (g/cc)	1.32	1.37	1.40	1.35	1.38	1.47	1.49	1.56

The limit of detection of the analyses was 0.02 and the limit of quantification 0.05 mg/kg. More than 80% of the total residue in the soil was carbofuran. The average total carbamate residue in the 0-15 cm depth ranged from 4.41 to 6.07 mg/kg during he first 14 days, with minimal leaching. The dissipation of residues in this layer in 360 days was significant. Quantifiable residues were not found below 45 cm at any time except on day 40 at a level of 0.08 mg/kg at 120-135 cm and on day 304 at 0.25 mg/kg at 105-120 cm. The first-order half-life calculated from the 0-15 cm soil depth was 23 days. The results are given in Table 18.

Table 18. Carbamate residues in soil dissipation study (Farmersville, 1988-89).

{PRIVATE } Depth	Mean total carbamate residue, mg/kg											
	Days after application											
Inches	0	3	7	14	40	90	120	150	180	304	335	360
0-6	5.91	6.07	5.74	4.41	0.92	0.18	(0.02)	0.05	ND	ND	(0.03)	(0.03)
6-12	0.48	0.86	0.81	1.07	1.13	(0.02)	ND	(0.04)	ND	ND	ND	ND
12-18	ND	ND	ND	ND	0.51	(0.02)	ND	0.05	ND	ND	ND	ND
18-24	ND	ND	ND	ND	(0.02)	ND	ND	(0.03)	ND	ND	ND	ND
24-30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
30-36	ND	ND	ND	ND	ND	ND	ND	(0.02)	ND	0.09	ND	ND
36-42	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.26	(0.02)	ND
42-48	ND	ND	ND	ND	ND	(0.02)	ND	ND	ND	0.25	ND	ND
48-54	NS	NS	NS	NS	0.08	ND	ND	(0.04)	ND	(0.02)	ND	ND
54-60	NS	NS	NS	NS	(0.04)	ND	ND	ND	ND	ND	ND	ND
60-66	NS	NS	NS	NS	ND	ND	ND	ND	ND	ND	ND	ND
66-72	NS	NS	NS	NS	ND	ND	ND	ND	ND	ND	ND	ND
72-78	NS	NS	NS	NS	ND	ND	ND	ND	ND	ND	(0.04)	ND
78-84	NS	NS	NS	NS	ND	ND	ND	ND	ND	ND	ND	ND
84-90	NS	NS	NS	NS	ND	ND	ND	ND	ND	ND	ND	ND
90-96	NS	NS	NS	NS	ND	ND	ND	ND	ND	ND	ND	ND

NS: no soil core sample was taken
ND: undetectable (<0.02 mg/kg)
Values in parenthesis are estimated, below the limit of quantification (0.05 mg/kg) but above the limit of detection (0.02 mg/kg)

A third study was in conducted in Porterville 1988-1989 (Leppert, 1989). The treated plot was 36 x 24 m (10 rows of 10 vines) and the control plot was 48 m from the treated plot. Core samples were taken before application and on days 0, 3, 7, 14, 50, 61, 90, 120, 150, 180, 307 and 387. The soil was classified as a sandy loam. Its characteristics are shown in Table 19.

Table 19. Soil characteristics, Porterville dissipation study.

Soil Depth (inches)	0-4"	4-8"	8-12"	12-24"	24-36"	36-48"	48-60"	60-72"	72-84"	84-96"
pH	7.27	7.36	7.51	7.52	7.51	7.45	7.41	6.94	6.81	7.24
% sand	64.6	68.8	71.6	75.6	76.2	77.2	66.2	24.2	28.0	32.0

% silt	22.4	18.2	16.4	12.2	12.8	10.8	17.8	47.8	40.0	31.0
% clay	13.0	13.0	12.0	12.2	11.0	12.0	16.0	28.0	32.0	37.0
CEC (meq/l)	4.1	4.0	4.1	5.0	4.4	4.3	6.0	17.3	15.8	17.6
% organic matter	0.38	0.26	0.13	0.2	0.13	0.1	0.2	0.42	0.37	0.15
Bulk density (g/cc)	0.92	0.95	1.05	1.35	1.3	1.32	1.29	1.25	1.32	1.38

The limit of detection was 0.02 mg/kg and the limit of quantification 0.05 mg/kg. More than 80% of the total residue in the soil was carbofuran. The levels of 3-hydroxy-carbofuran did not increase, and it was undetectable by day 50. Residues of 3-keto-carbofuran increased for 14 days in the 15 cm layer, then decreased to undetectable levels by 50 days. The average total carbamate residue in the top 0-15 cm ranged from 4.05 to 5.19 mg/kg during the first 14 days, with minimal leaching. During this period there was little or no rainfall. At the next sampling, day 50, the residues had almost disappeared from the top 15 cm layer with movement of low levels into the lower depths. By 61 days, corresponding to the start of rainfall and irrigation, the residues had permeated the soil strata from 0-240 cm. The levels found, however, were much lower than the original 0-15 cm residues suggesting that various factors such as soil microbial activity, pH and sorption reduced the movement, particularly of oxidized carbamates, through the soil. Quantifiable residues were not found below the 150 cm depth at any time except on days 61 and 150 when residues were found at low levels (0.19 mg/kg) in the 225-240 cm layer. No residues were detectable at any depth after 150 days. A half-life of 13 days was calculated from all of the 0-15 cm residues.

A summary of the results is shown in Table 20.

Table 20. Carbamate residues in soil dissipation study (Porterville, 1988-89).

Mean total carbamate residue, mg/kg													
Depth	Days after application												
Inches	-1	0	3	7	14	50	61	90	120	150	180	307	387
0-6	ND	5.19	4.36	4.17	4.05	(0.04)	0.06	ND	ND	0.07	ND	ND	ND
6-12	ND	0.75	1.01	0.94	1.40	0.05	ND	ND	ND	0.08	ND	ND	ND
12-18	ND	ND	ND	ND	0.08	0.08	ND	ND	ND	0.06	ND	ND	ND
18-24	ND	ND	ND	ND	ND	0.09	0.05	ND	ND	0.05	ND	ND	ND
24-30	ND	ND	ND	ND	ND	0.08	0.11	ND	ND	(0.04)	ND	ND	ND
30-36	ND	ND	ND	ND	ND	(0.4)	0.15	ND	ND	0.05	ND	ND	ND
36-42	ND	ND	ND	ND	0.11	0.08	0.48	0.07	ND	ND	ND	ND	ND
42-48	ND	ND	ND	ND	0.71	0.12	0.64	0.08	ND	ND	ND	ND	ND
48-54[1]	ND	NS	NS	NS	NS	(0.04)	0.24	0.07	ND	0.14	ND	ND	ND
54-60	ND	NS	NS	NS	NS	(0.02)	0.22	0.09	ND	0.17	ND	ND	ND
60-66	ND	NS	NS	NS	NS	ND	0.20	(0.02)	ND	0.32	ND	ND	ND
66-72	ND	NS	NS	NS	NS	ND	0.21	ND	ND	0.28	ND	ND	ND
72-78	ND	NS	NS	NS	NS	ND	0.19	(0.02)	ND	0.18	ND	ND	ND
78-84	ND	NS	NS	NS	NS	(0.04)	0.21	(0.04)	ND	0.21	ND	ND	ND
84-90	ND	NS	NS	NS	NS	0.05	0.06	(0.02)	ND	0.19	ND	ND	ND
90-96	ND	NS	NS	NS	NS	(0.02)	0.19	(0.04)	ND	0.17	ND	ND	ND

NS: no soil core sample was taken.
ND: undetectable (<0.02 mg/kg)
Values in parenthesis are estimated, below the limit of quantification (0.05 mg/kg) but above the limit of detection (0.02 mg/kg)
[1]The sampling equipment may have caused contamination of the cores below 120 cm. The equipment could take only a 120 cm core, so to take a deeper core the probe had to be reinserted into the same hole after the 0-120 cm core had been taken. In some cases, leaf debris and root fragments were found where there should have been none

Adsorption/desorption was studied in a 0.01 M $CaCl_2$ solution with [^{14}C]carbofuran labelled in the phenyl ring (Leppert, 1989). The nominal test concentrations were 0, 0.5, 1.5 and 10 mg/kg. The samples were maintained in an environmentally-controlled chamber at approximately 25°C. Two soils, a silt loam and a sandy loam, were used. Concentrations of carbofuran were estimated in the

aqueous phase by LSC and in the soils by combustion followed by LSC after the desorption phase. Adsorption and desorption constants were determined for the silt loam, but desorption could not be accurately determined for the sandy loam owing to the small amount of test material adsorbed during the adsorption phase. The mass balances for the silt loam and sandy loam were 106% and 104% respectively. The average K_{oc} of 24.7 indicates that carbofuran has the potential to be mobile in the two soils tested. The results are summarized below.

	% organic		Adsorption		Desorption	
Soil type	carbon	pH	K_d	K_{oc}	K_d	K_{oc}
Silt loam	1.2%	7.1	0.246	20.5	0.243	20.3
Sandy loam	0.4%	6.5	0.115	28.9		

Column leaching study was conducted to determine the mobility of [*phenyl*-^{14}C]carbofuran and its degradation products in four agricultural soils (Saxena *et al*., 1994). The soils were a sandy loam from Georgia (GA), a clay loam and a loam from Ohio, and a sandy loam from California (CA). The CA sandy loam had an organic matter content of less than 1%. The characteristics of the soils are tabulated below.

Source	Type [USDA]	pH	% OM	CEC meq/ 100g	Sand, %	Silt, %	Clay, %	Bulk density, g/cm^3
Georgia	sandy loam	5.7	1.2	4.3	73	16	11	1.42
Ohio	clay loam	5.8	4.6	24.7	23	38	39	1.13
California	sandy loam	6.8	0.6	5.0	65	28	7	1.39
Ohio	loam	7.6	1.5	13.9	29	46	25	1.12

OM: organic matter
CEC: cation exchange capacity

A preliminary study was conducted to determine the rate of degradation of [^{14}C]carbofuran on each soil type, and hence the sampling intervals and the length of time for the aerobic aging-phase in the definitive study (one half-life or 30 days, whichever was shorter). The four soils were fortified with [^{14}C]carbofuran at a concentration of 3.2 mg/kg and incubated at 25 ± 1°C under aerobic conditions for 15 days. The soil moisture was maintained at approximately 75% of field capacity throughout the preliminary study. Duplicate soil samples were collected on days 0, 5, 10 and 15. The samples from days 5 and 10 were frozen upon collection. The samples from days 0 and 15 were extracted immediately after collection and the extracts analysed by HPLC. Since less than 20% of the carbofuran in the two sandy loams and the clay loam was degraded by day 15, the sampling points for these soils in the definitive study were days 0, 15, 22 and 30. In the loam soil approximately 41% of the carbofuran was degraded by day 15 so the samples from days 5 and 10 were extracted and analysed to determine the half-life of carbofuran, which was calculated to be 21.9 days. Samples in the definitive study were therefore taken on days 0, 9, 15, 20 and 23.

In the major study each of the four soils was fortified with [*phenyl*-^{14}C]carbofuran at a concentration of 3.2 mg/kg (equivalent to 6.7 kg ai/ha, which represents the highest single application for row crops) and incubated aerobically at 25 ± 1°C. The soil moisture was maintained at approximately 75% field capacity throughout the study. $^{14}CO_2$ and other volatile products were trapped and quantified. Duplicate soil samples were extracted immediately upon collection and analysed by HPLC.

The mean recoveries of the applied radioactivity and the half-life of carbofuran in each soil are shown in Table 21.

Table 21. Recoveries of ^{14}C and half-life of carbofuran in four soils.

Soil	^{14}C recovered, %				Half-life, days
	Extracted	Volatile	Bound	Total	
GA sandy loam	87.6	0.1	9.9	97.6	90.8
clay loam	73.1	2.7	22.0	97.8	53.0
CA sandy loam	86.2	2.4	9.2	97.8	99.9
loam	49.4	4.1	42.4	95.9	21.9

The proportion of the recovered radioactivity associated with each of the compounds determined by HPLC is shown in Table 22.

Table 22. Distribution of recovered ^{14}C.

Soil	^{14}C, % of recovered and mg/kg as carbofuran		
	Carbofuran, %	3-OH-carbofuran (% mg/kg)	3-keto-carbofuran (%, mg/kg)
GA sandy loam, day 30	81.6	2.1 (0.07)	1.6 (0.05)
Clay loam, day 30	67.1	0.2 (<0.01)	2.8 (0.09)
CA sandy loam, day 30	79.1	0.8 (0.03)	3.4 (0.11)
Loam, day 23	46.1	0.1 (<0.01)	0.1 (<0.01)

The remaining radioactivity was distributed among soil-bound residues and $^{14}CO_2$. The identification of 3-ket°Carbofuran was confirmed by LC-MS.

The remaining soil samples of each soil from the definitive study were combined and used as the aged soil in the leaching study. The soils were packed in columns to a height of 30 cm and the aged soil containing the [^{14}C]carbofuran residues at a nominal concentration of 6.7 kg ai/ha was applied to each column. The columns were maintained at approximately 25 ± 1°C and leached with 50 column cm of 0.01 N $CaCl_2$ at an approximate rate of 1.5 cm per hour. Four fractions of leachate (approximately 12.5 cm or 600 ml each) were collected from each column. The leachates and the soils in the columns were assayed for radioactivity. The proportion of the applied radioactivity in the leachates and soil sections were as follows.

% of radioactivity applied to column

	Georgia sandy loam	Clay loam	California sandy loam	Loam
Aged soil layer	42.5	31.1	13.7	49.2
Total in six soil sections	9.4	26.5	2.8	9.0
Total in leachates	53.4	40.9	78.2	33.2
Mass balance	105.3	98.5	94.7	91.4
K_d	0.73	*	0.25	*

*less than 50% of the applied radioactivity was in the leachate

Leachate fractions that contained >10% of the applied ^{14}C were analysed by HPLC. The identity of carbofuran was also confirmed by two-dimensional TLC. More than 94% of the ^{14}C from all four soils was due to [^{14}C]carbofuran. Minor components (less than 1% of the applied radioactivity) detected in the leachates and/or aged layer sections included 3-keto-carbofuran (0.8%) the 7-phenol (0.2% in GA sandy loam, 0.2% in clay loam, 0.5% in loam), the 3-keto-7-phenol (0.2%) and 3-keto-carbofuran (91.8% in CA sandy loam).

The proportion of leached radioactivity was greatest in the CA sandy loam, followed by GA sandy loam, clay loam and loam. The results indicate that carbofuran and its degradation products have the potential to be mobile in all four soils under the "worst-case" conditions of applying 50 column cm of water.

Environmental fate in water/sediment systems

Cook (1974) studied the hydrolysis of [*phenyl*^{14}C]carbofuran in aqueous solutions buffered to pH 5, 7 and 9 at a concentration of 2 mg/l. At room temperature (28°C), carbofuran was hydrolytically stable over the 28-day test period at pH 5 and was slowly hydrolysed at pH 7 with a calculated half-life of 26 days. At pH 9 only 20% of the carbofuran remained after 1 day at 26°C and the half-life was 12 hours. At 5°C the half-life was 1.5 days. The hydrolysis product was the 7-phenol.

Degradation in water/sediment systems

In a study in accordance with US EPA Guidelines (Saxena *et al.*, 1994b) the rate and degree of the anaerobic aquatic degradation of [^{14}C]carbofuran was determined in acidic pond water plus sediment systems (approximate pH 5.4) consisting of approximately 82 g wet sediment, equivalent to 50 g oven-dried sediment and 100 ml of pond water in sealed bottles. Test systems were prepared and incubated under anaerobic conditions in the dark at approximately 25°C for at least 30 days (pre-anaerobic incubation) before adding [^{14}C]carbofuran uniformly labelled in the phenyl ring at a nominal concentration of approximately 3 mg/kg (equivalent to 6.7 kg ai/ha) and incubating at 25°C under anaerobic conditions for 12 months in the dark. The test systems were incubated in a “static” anaerobic apparatus that permitted the trapping of organic volatiles and $^{14}CO_2$. Duplicate samples of sediment plus water were collected immediately after dosing (day 0) and after 1, 3, 7, 14, 31, 60, 98, 122, 183, 273 and 365 days. The samples were flushed with nitrogen to collect organic volatiles, $^{14}CO_2$ and [^{14}C]methane and the dissolved oxygen content, pH and redox potential of the water samples were determined. The populations of aerobic and anaerobic microbes were also measured. The sediment and water were extracted and analysed by HPLC. The identities of compounds that accounted for more than 10% of the applied radioactivity were confirmed by liquid chromatography-

mass spectrometry (LC-MS). The extracted sediment was combusted to determine the amount of bound radioactivity.

More than >90% of the ^{14}C was accounted for in all the samples. Volatile radioactivity was negligible and reached a maximum of 0.5% by day 273. [^{14}C]carbofuran decreased from 96.2% at day 0 to 24.9% by day 365. One major product, the 7-phenol, reached a maximum of 53.7% by day 365, when the maximum level of 20.4% of the applied radioactivity was observed. Fractionation of the bound residues into humic acid, fulvic acid and humin indicated the presence of radioactivity in all three fractions. The observed redox potential and dissolved oxygen values indicated that anaerobic conditions were maintained. A summary of the results is given in Table 23.

Table 23. Anaerobic degradation in a water/sediment system.

Day	% of ^{14}C as					
	carbofuran	3-keto-7-phenol	7-phenol	Trapped volatiles	Bound residues	Total
0	96.2	ND	0.4	NA	0.3	96.8
1	91.1	ND	0.4	0.1	0.8	92.3
3	90.9	0.1	0.5	0.1	1.1	92.6
7	90.4	0.3	0.9	0.3	3.0	94.8
14	85.9	0.1	0.8	0.2	7.8	94.7
31	75.9	0.4	9.6	0.2	11.8	97.8
60	64.7	0.4	17.9	0.3	11.7	94.9
98	55.5	ND	26.6	0.3	14.3	96.7
122	47.0	ND	30.9	0.3	16.1	94.3
183	41.9	ND	39.3	0.3	16.7	98.2
273	34.0	ND	45.3	0.5	18.8	98.6
365	24.9	ND	53.7	0.2	20.4	99.1

The average half-life of [^{14}C]carbofuran in the test system, assuming first order kinetics, was approximately 189 days.

The rate and degree of aerobic degradation of [^{14}C]carbofuran uniformly labelled in the phenyl ring was determined in an acidic pond water/sediment system (approximate pH 5.4) by Saxena and Marengo (1994). Each vessel contained 50 g of pond sediment and 100 ml of pond water fortified with [^{14}C]carbofuran at a concentration of 3.05 mg/kg (equivalent to 6.7 kg ai/ha which represents the highest single application for row crops) and incubated at 25 ± 1°C under aerobic conditions for 30 days in darkness. The vessels were connected in pairs to a set of traps (ethylene glycol for organic volatiles, sodium hydroxide for CO_2) and CO_2-scrubbed humidified air was bubbled through the overlying water of the first vessel of each pair into the water of the second and then into the traps.

Pairs of sediment/water vessels were taken on days 0, 1, 3, 7, 10, 20 and 30 and the contents analysed immediately upon collection. The population of aerobic bacteria, pH, dissolved oxygen content and redox potential of the test system were determined, the radioactivity in the traps was counted by LSC, that in the sediment and water was extracted and the extracts analysed by HPLC and the extracted sediment was combusted to determine bound ^{14}C. Selected extracts were also analysed by TLC to confirm the products detected by HPLC. The identities of compounds accounting for more than 10% of the applied radioactivity were confirmed by LC-MS.

The recovery of applied radioactivity from individual samples was >90% at all times. The distribution of the radioactivity at 0 and 30 days was as follows.

Mean % of applied radioactivity

Day	Water layer	Sediment extract	Extracted sediment	Traps	Total
0	71.07	24.81	3.92	0	99.8
30	15.29	45.86	32.78	1.87	95.8

The first sample in each pair remained acidic throughout the 30-day study with a pH of about 5, and the second sample remained acidic on days 0-10. A shift in the pH of the overlying water in the second vessel of the pair to about 8 was observed at days 20 and 30. A significant difference between the two samples in the degradation of carbofuran was caused by the shift in pH (carbofuran is known to be hydrolysed rapidly at an alkaline pH to the 7-phenol).

The 7-phenol was a major product in the two alkaline samples on days 20 and 30 (23.74 and 17.30% of the applied radioactivity respectively, compared with 0.61 and 1.89% in the first vessels). The other major degradation products were soil-bound residues which accumulated to an average of 32.67% by day 30. Fractionation of the 30-day sediments into humic acid, fulvic acid and humin indicated the presence of radioactivity in all three fractions. Carbofuran decreased to an average of 39.65% by day 30. Minor amounts (<1%) of 3-hydroxy-carbofuran, the 3-keto-7-phenol and 3-keto-carbofuran were detected in all the samples. No unidentified compounds were detected.

An additional study was conducted to determine whether the original arrangements of the vessels in pairs caused the second samples to become alkaline and hence the differences on days 20 and 30. Each vessel, with the same contents as before, was now connected to its own set of traps. Duplicate vessels were collected on days 0, 10, 20 and 30. The contents of the day 0 and day 30 vessels were analysed immediately upon collection as before. The pH, dissolved oxygen and redox potential were measured at days 10 and 20 and the samples were then stored frozen without further analysis.

The mass balance was >90% of the applied radioactivity in the day 0 and day 30 samples. A slight increase in the pH of the overlying water with time was observed in the individual vessels. The pH of the individual water samples at day 0 ranged from 5.23 to 5.50 and a maximum pH of 6.44 was observed in any individual sample during incubation. The 7-phenol was detected at a maximum level of 2.88% and a mean of 2.84% by day 30. No unidentified compounds were detected, the analyses of the duplicate samples at each time interval agreed, and the water samples remained acidic throughout the study. Evidently the connection of the vessels in sequence in the main study caused the pH change to alkaline and the differences between "replicates" on days 20 and 30.

The half-life of carbofuran was calculated by linear regression to be approximately 41 days.

Aqueous photolysis was studied with [^{14}C]carbofuran labelled in the phenyl ring at a concentration of 20 mg/l in a sterile buffer solution at pH 5 (McGovern and Shepler, 1989a). The samples were exposed to natural sunlight in a water bath at approximately 25°C together with control samples wrapped in aluminum foil. Duplicate irradiated and control samples were analysed 0, 3, 6, 12, 20 and 31 days after treatment. Ethylene glycol and 10% NaOH were used to trap volatile organic compounds and CO_2 respectively. Air was drawn through both the irradiated and control sample tubes into separate sets of traps. All samples were analysed directly by LSC and HPLC. The average recovery of ^{14}C from all samples was 97.1%. The 7-phenol and CO_2 were the only degradation products observed. The 7-phenol reached a maximum of 3.7% and CO_2 a maximum of 0.3% of the applied ^{14}C in the irradiated samples. The extrapolated half-life for photolysis was 1200 days, implying a half-life of 450 days in summer daylight conditions. Carbofuran was also found to be

degraded slowly in the dark to the 7-phenol and CO_2 with a half-life of 2100 days, showing that the 7-phenol is not photochemically derived wholly from photolysis.

An aquatic field dissipation study was conducted in the USA in Louisiana and California (Novak, 1987a,b) to determine the distribution of carbofuran and its metabolites in soil, water and rice. Each site consisted of two 20 x 30 m rice plots, one control and one treated, surrounded by a levee. At the Louisiana site, the rice plots were flooded to a depth of 10 cm when the rice plants had reached this height, and the depth of the flood water was maintained between 8 and 21 cm until the rice was mature. The Louisiana plot was treated with Furadan 3G at 0.67 kg ai/ha 19 days after permanent flooding of the planted rice. The California plot was treated with Furadan 5G at 0.56 kg ai/ha immediately after sowing the rice seed before flooding. The plots were flooded to a depth of 20 cm and maintained at 15 to 20 cm. At both sites, an additional plot was planted with crops and irrigated with water from the treated rice plot.

At the Louisiana site, soil core samples were taken from the treated plot to a depth of 64 cm before and 24 hours after application, and then at days 3, 7, 14, 21, 30, 60 and 120. At the California site, soil samples were taken at the same intervals and also at days 162, 196 and 225. The soil cores were taken in the treated plot to a depth of 64 cm during the unflooded phase and 16 cm during the flooded phase. The soil cores were divided and analysed in 8 cm sections. Water samples were taken just before and 8 hours after application at both sites and on days 7, 14, 21, 30, 60 and 99 in Louisiana and 3, 7, 9, 12, 14, 16, 19, 21 and 27 in California. Four 1-l water samples were taken at each sampling at each site.

The soil samples were analysed for carbofuran and 3-hydroxy-carbofuran (Schreier, 1987). The stated limits of determination and detection were 0.1 and 0.02 mg/kg. The analyses showed only low levels (≤0.09 mg/kg) of carbofuran at both sites, and 3-hydroxy-carbofuran was not detected in any of the samples. The maximum residue of carbofuran at the California site, 0.09 mg/kg, occurred 7 days after treatment in the 0-8 cm section. Only the 1-60 days sample at the California site were analysed: both analytes were undetectable to a depth of 16 cm at 60 days. The maximum residue of carbofuran at the Louisiana site was 0.04 mg/kg in the 0-8 cm section after 3 days. Only the 1-21 day samples were analysed because both compounds were undetectable to a depth of 16 cm in the 7-21 day samples. The results are shown in Table 24.

Table 24. Aquatic field dissipation: carbofuran and 3-hydroxy-carbofuran in soil and sample.

Residue, mg/kg		
Site/Interval/Depth	carbofuran	3-hydroxy carbofuran
Louisiana		
Day 1 0-8 cm	(0.04)	ND
Day 1 8-16 cm	ND	ND
Day 3 0-8 cm	(0.04)	ND
Day 3 8-16 cm	ND	ND
Day 7 0-8 cm	ND	ND
Day 7 8-16 cm	ND	ND
Day 14 0-8 cm	ND	ND
Day 14 8-16 cm	ND	ND
Day 21 0-8 cm	ND	ND
Day 21 8-16 cm	ND	ND
California		
Day 1 0-8 cm	(0.05)	ND
Day 1 8-16 cm	ND	ND
Day 3 0-8 cm	(0.06)	ND
Day 3 8-16 cm	ND	ND

Residue, mg/kg		
Site/Interval/Depth	carbofuran	3-hydroxy carbofuran
Day 7 0-8 cm	(0.09)	ND
Day 7 8-16 cm	ND	ND
Day 14 0-8 cm	(0.07)	ND
Day 14 8-16 cm	ND	ND
Day 21 0-8	0.05	ND
Day 21 8-16 cm	ND	ND
Day 30 0-8 cm	(0.07)	ND
Day 30 8-16 cm	ND	ND
Day 60 0.8 cm	ND	ND

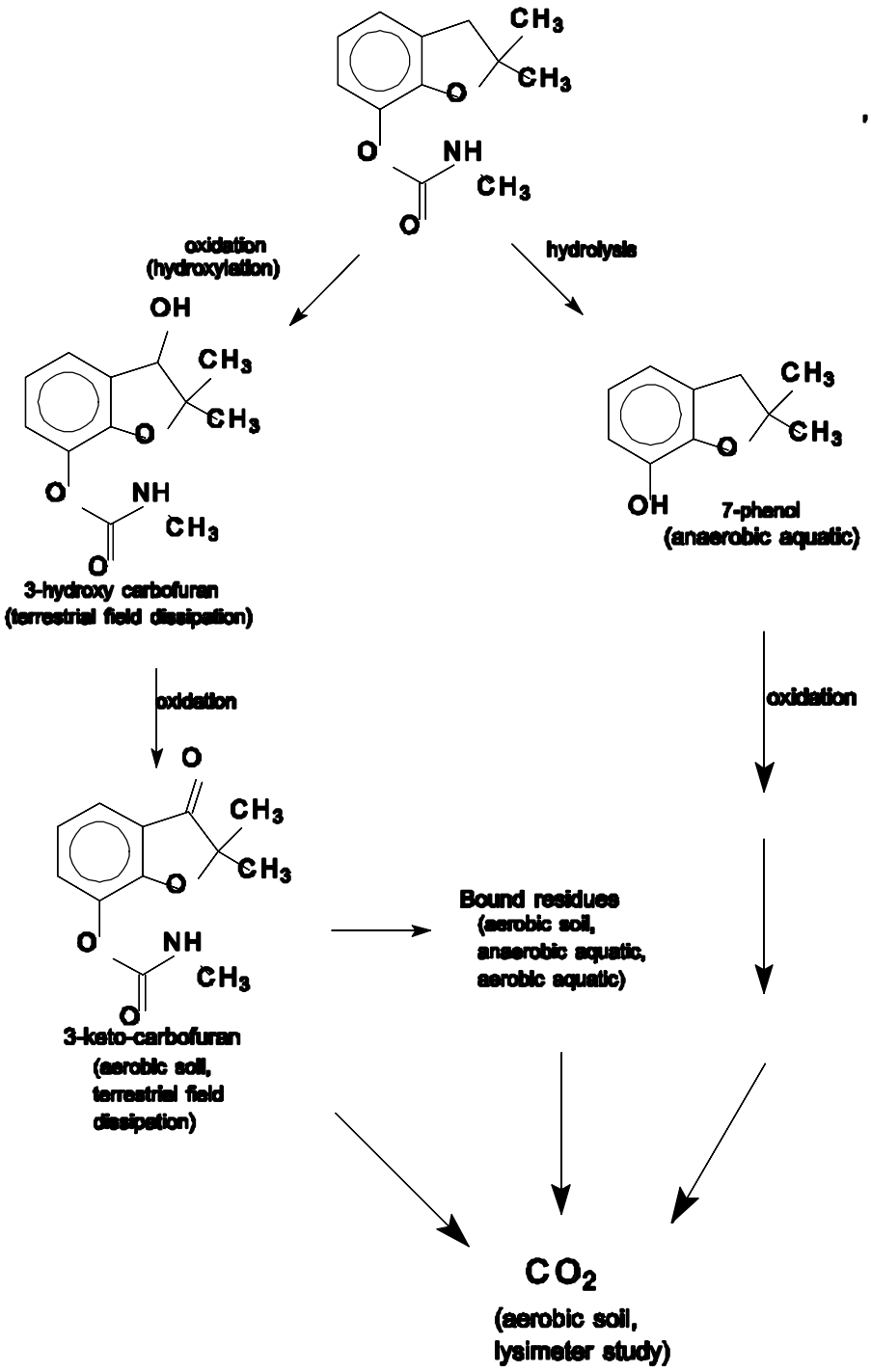
ND: undetectable (<0.02 mg/kg)
Results in parenthesis are estimated levels below the limit of determination (0.1mg/kg) but above the limit of detection (0.02 mg/kg)

Rice paddy water samples were also analysed for carbofuran and 3-hydroxy-carbofuran (Beauchamp, 1987). The limit of determination was 2.5 µk/kg and the limit of detection 1 µk/kg. The water from the Louisiana site contained a mean maximum level of carbofuran of 417 µk/kg after 8 hours, which decreased to 3 µk/kg after 30 days. 3-Hydroxy-carbofuran was detected only in one sample, at 1.0 µk/kg on day 7. The water from the California site contained a mean maximum level of carbofuran of 33 µk/kg after 8 hours which became undetectable by day 27. The half-life of carbofuran in rice paddy water was <10 days at both sites.

Carbofuran dissipated rapidly in soil and water after application to rice plots. Carbofuran was the only residue found in the soil and was undetectable by day 60. In the water, residues of carbofuran were ≤3 mg/kg by day 30 and no residues of 3-hydroxy-carbofuran were found except for a level of 1 µk/kg on day 7. No residues of carbofuran or 3-hydroxy-carbofuran were found in the rice grain or straw.

Proposed degradation pathways of carbofuran in soil and water/sediment systems are shown in Figure 4.

Figure 4. Degradation pathways of carbofuran in soil and water/sediment systems.



Rotational Crops

In a confined crop rotation study [*phenyl*-^{14}C]carbofuran was applied directly to a silt loam soil at an application rate of 3.4 kg ai/ha, based on a 76 cm row space. Wheat, soya beans and sugar beet were seeded into the treated soil 4 and 12 months after treatment and grown to maturity. Wheat forage, straw and grain, soya bean silage, stems, pods and beans and sugar beet tops and roots from both plantings were assayed separately for ^{14}C. Table 25 shows that residues above 0.01 carbofuran equivalents were found in all the samples from both plantings.

Table 25. Total radioactive residues in mature rotational crops, as carbofuran.

Crop	Sample	^{14}C, mg/kg as carbofuran	
		4 months	12 months
Wheat	Forage	-	1.40
	Straw	54.0	0.30
	Grain	0.60	0.04
Soya bean	Silage	16.0	0.50
	Stem	18.0	0.70
	Pod	5.0	0.10
	Bean	1.0	0.08
Sugar beet	Top	0.40	0.05
	Root	0.20	0.05

Subsamples of each plant part were extracted with methanol/water (1:2) and separated into non-polar and polar fractions which were concentrated and analysed separately to determine the nature of the residues. Conjugated metabolites were hydrolysed with 0.25 N hydrochloric acid. Metabolites were identified by TLC, with co-chromatography with reference standards.

The phenolic metabolites (3-hydroxy-7-phenol, the 3-keto-7-phenol and 7-phenol) were the principal degradation products found in the plants. The carbamates (carbofuran, 3-hydroxy-carbofuran and 3-keto-carbofuran) constituted a small proportion of the total radioactive residue; none of them individually exceeded 10% of the TRR in any of the crops sown at 4 or 12 months.

A field rotational crop study was conducted with [*phenyl*-^{14}C]carbofuran applied to the soil at rates of 1.1, 3.4 and 6.7 kg ai/ha. Ten months after treatment, sorghum, soya beans, sugar beet, lettuce, cabbage and wheat were planted in the field and grown to maturity. No ^{14}C was detectable in mature sorghum, soya beans, sugar beet, wheat grain or lettuce from any of the three application rates. Low levels of the total residue (0.01 mg/kg as carbofuran) were observed in mature cabbage harvested from the 3.4 and 6.7 kg ai/ha treatments. Wheat straw and soya bean stems harvested from the 6.7 kg ai/ha treatment contained 0.21 mg/kg and 0.63 mg/kg respectively, but residues were not detectable at the two lower treatment rates. Residues in the immature crops from the 1.1 and 3.4 kg ai/ha treatments harvested 30 and 58 days after planting were generally below the detection limit. Detectable levels of radiocarbon (0.017-0.084 mg/kg as carbofuran) were found in immature crops from the 6.7 kg ai/ha treatment. Low levels of carbofuran (maximum 0.02 mg/kg) remained in the soil ten months after application.

The results indicate that under normal field use conditions the potential for accumulation of carbofuran into ten-month rotational crops is minimal at application rates of 1.1-6.7 kg ai/ha. The residues from all treatment rates were below the limit of detection in all the edible commodities except cabbage where they were at the limit of detection at the two higher treatment rates.

METHODS OF RESIDUE ANALYSIS

Analytical methods

Schreier (1989) provided a GLC method for the determination of carbofuran, 3-hydroxy-carbofuran and 3-keto-carbofuran in green and dry alfalfa, field corn silage and grain, oranges, peanut nutmeat and hulls, potato tubers, sorghum, sugar beet roots and tops and cow milk and muscle. The weighed sample was macerated, hydrolysed by refluxing for one hour with 0.25 N HCL and filtered. The filtrate was partitioned with dichloromethane, transferred to hexane and cleaned up on a Florisil column. Ethyl acetate was used to elute the compounds of interest. The final solutions were analysed on a 10 or 12 m methyl silicone capillary column, either 0.53 mm with direct injection and a nitrogen-phosphorus detector (NPD) or 0.2 mm with splitless injection and a mass-selective detector (MSD). Calibration was with external standards. Chromatograms and raw data were provided for fortified control samples to demonstrate the limits of determination given in Table 26. Limits of determination were also claimed for milk and muscle (0.5 mg/kg for each analyte) and several plant crops, but no chromatograms or data were submitted.

Table 26. Limits of determination of carbofuran and carbamate metabolites by the method of Schreier (1989).

{PRIVATE }Sample	Detector	Limit of determination, mg/kg		
		Carbofuran	3-hydroxy-carbofuran	3-keto-carbofuran
Maize silage	MSD	1.0	3.0	1.0
Peanut kernels	MSD	0.5	0.5	0.5
Peanut hulls	MSD	2.0	2.0	2.0
Potato	MSD	0.5	0.5	0.5
Sorghum	NPD	1.0	1.0	1.0
Sugar beet tops	NPD	1.0	1.0	1.0

Modifications to the Schreier method, e.g. by Brutschy (1984) included ethoxylation of 3-hydroxy-carbofuran and a procedure for the isolation and determination of the phenol metabolites. For the determination of phenol metabolites the hydrolysed and filtered sample was partitioned with methylene chloride/diethyl ether (3:3), butylated hydroxytoluene (1 ml of 10 mg/kg in methylene chloride) and ethanol were added to the extract and the solution was concentrated to remove methylene chloride, acidified, and refluxed for 45 minutes. It was then partitioned into methylene chloride, which was concentrated, cleaned up on a silica gel solid-phase extraction column conditioned with methanol/water (1:1) and eluted with methylene chloride. The eluate was concentrated and analysed by GLC on a cross-linked dimethyl silicone capillary column operated in the splitless mode. Detection was mass-selective with monitoring of the molecular ions of the 7-phenol, 3-keto-7-phenol and 3-ethoxy-7-phenol ($164^{+\cdot}$, $178^{+\cdot}$ and $208^{+\cdot}$). Calibration was with external standards.

Mollhoff (1975a) described a method for the determination of carbofuran, 3-hydroxy-carbofuran and 3-hydroxy-carbofuran glycoside in plants and soil. Plant samples (cereal grains, potatoes) were macerated with methanol and the macerate filtered, concentrated and extracted with chloroform. The residual aqueous fraction was hydrolysed with acid to convert any glycoside conjugate to 3-hydroxy-carbofuran aglycone and extracted with chloroform. Soil samples were macerated with a mixture of methanol, water and hydrochloric acid and extracted with chloroform. Analyses were by GLC on a packed column (Ucon LB 550 X with 0.5% KOH on Chromosorb G AW DMCS). A limit of determination of 0.1 mg/kg claimed and recoveries were reported for several

crops, but no data were provided. Recoveries of the conjugate were generally unacceptable at or below 0.6 mg/kg. Several acceptable recoveries of the conjugates were reported at 1.0 mg/kg.

Leppert *et al*. (1983) reported a GLC method for the determination of carbosulfan and carbofuran residues in soil, plants and water. Crops with a high water content, e.g. green alfalfa or citrus, were blended with hexane and 2-propanol (2:1), diluted with water and the hexane fraction retained. Soil and crops with a low water content, e.g. hay and straw were blended with methanol and pH 8 buffer and the filtered solution was extracted with methylene chloride. Oily samples, e.g. citrus oil, were diluted with hexane and extracted with acetonitrile. Water samples were extracted with methylene chloride after salting. Various column clean-up procedures were used, including gel permeation, Darco-Attaclay, aluminum oxide and Florisil. After the Darco-Attaclay fractionation, the ethyl acetate eluate was concentrated and treated with ethanol and concentrated HCl to ethoxylate 3-hydroxy-carbofuran. The final extracts were analysed on a packed column of Chromosorb W-HP with nitrogen-selective detection. A limit of determination of 0.1 mg/kg carbofuran was reported for citrus fruit.

Smith (1991) reported a method for the determination of parts-per-billion levels of carbofuran in water. Samples were concentrated on a C-18 solid-phase extraction column, eluted with acidified methanol and analysed with by HPLC on a cyclohexyl column with a UV detector (220 nm). The mobile-phase was a water/acetonitrile gradient. Adequate resolution and sensitivity were demonstrated for a rice-water sample fortified with carbofuran at 11 µk/kg .

Barros (1995) described a multi-residue method for the determination of carbosulfan and its metabolites in or on oranges. In addition to carbosulfan, the method determines carbofuran, 3-keto-carbofuran, and the 3-hydroxy-carbofuran, 3-keto-7-phenol, 7-phenol and 3-hydroxy-7-phenol.

To determine the carbamates, macerated oranges were hydrolysed with 0.25 N HCl under reflux, the mixture was filtered and an aliquot of the filtrate was loaded onto a C-18 solid-phase extraction cartridge conditioned with methanol and 0.25 N HCl. The compounds of interest were eluted with 1% methanol in methylene chloride and passed through an aminopropyl solid-phase cartridge. The final residue was re-dissolved in acetonitrile and analysed by reverse-phase HPLC (C-18) with a post-column reactor and fluorescence detector. The demonstrated limit of determination was 0.03 for each analyte.

To determine the phenols, a separate aliquot of the original filtrate was loaded onto a C-18 cartridge and the dried cartridge was eluted with 5% ethanol in methylene chloride. The phenols were derivatized with pentafluorobenzyl bromide and the 3-hydroxy-7-phenol derivative was ethylated. A final ethanol solution of the analytes was analysed by gas chromatography with a mass-selective detector. The demonstrated limit of determination was 0.03 mg/kg for each analyte.

Geno (1991) reported validation of the Barros method for maize silage. The independent laboratory validation was in accordance with US EPA PR Notice 88-5, 40 CFR Part 160. Control maize silage was fortified with 0.05 or 0.25 mg/kg each of carbofuran, the 3-keto-carbofuran and 3-hydroxy-carbofuran. Adequate recoveries were demonstrated for all three compounds (83-102%, 95-102% and 96-108% respectively). The method was not validated for the phenol metabolites.

Blass and Philipowski (1992) reported a method for the determination of methylcarbamate residues, including carbofuran, by HPLC with post-column reaction. Samples with little or no fat were extracted with methylene chloride/water and fatty samples with acetonitrile saturated with hexane. The latter extract was washed with hexane, concentrated and extracted with methylene chloride. The final organic extracts were cleaned up on an "Extrelut" cartridge. Aqueous extracts

were prepared for the determination of 3-hydroxy- carbofuran. The analytes were separated on a Spherisorb RP 18 column and the eluted methylcarbamates converted in a two-stage reactor to (1-hydroxyethylthio)-2-methylisoindole and the indole measured with a fluorimeter (excitation 340 nm, emission 455 nm). The limit of determination is approximately 0.04 mg/kg for carbofuran and 3-hydroxy-carbofuran. The recoveries given in Table 27 were reported for various samples fortified with carbofuran and 3-hydroxy-carbofuran. Sample chromatograms were provided from barley grain, wheat straw, sugar beet foliage and lettuce.

Table 27. Recoveries of carbofuran and 3-hydroxy-carbofuran by the method of Blass and Philipowski (HPLC with post-column derivatization).

{PRIVATE }Sample	Fortification, mg/kg	Recovery, %	
		Carbofuran	3-hydroxy-carbofuran
Apple	0.04; 1.0	84; 100	
Beet foliage	0.04; 1.0	76; 85	73; 84
Carrot	0.04; 1.0	94; 94	79; 87
Cherry	0.04; 1.0	90; 85	
Maize grain	0.04; 0.1; 1.0	99; 99; 97	100; 98; 95
Lettuce	0.04; 1.0	92; 95	88; 89
Melon	0.04; 1.0	92; 84	78; 75
Pepper	0.04; 1.0	86; 91	78; 80
Potato	0.04; 1.0	79; 97	78; 80
Rice	0.04; 1.0	91; 91	90; 90
Soya beans	0.04; 1.0	96; 91	101; 98
Wheat straw	0.10; 1.0	102; 95	
Sunflower seed	0.04; 0.1; 1.0	92; 94; 97	86; 78; 94
Barley grain	0.04; 1.0	100; 96	
Asparagus	0.04; 1.0	104; 92	80; 84
Bulb onion	0.04; 1.0	82; 90	74; 83
Tomato	0.04; 1.0	94; 93	86; 76
Sugar beet foliage	0.04; 1.0	85; 91	78; 85
Sugar beet root	0.04; 1.0	90; 90	80; 83

The sponsors claim that the Blass method is the official enforcement screening method for use in Europe (see the official multi-residue methods of The Netherlands, below).

A multi-residue method is published in the US Food and Drug Administration (FDA) Pesticide Analytical Manual (PAM) for determining total residues of carbofuran in food for the enforcement of tolerances.

Chen (1995a) described a method for the determination of carbosulfan and its metabolites, including carbofuran and the metabolites of Figure 1, in ruminant commodities. Milk and tissues are extracted with acetone. The acetone extract is centrifuged and cleaned up by a combination of liquid-liquid extraction, solid-phase extraction and/or gel permeation chromatography. Carbofuran and carbamate metabolites are determined by HPLC with a post-column reactor and fluorescence detector. The phenolic metabolites are extracted and analysed by a similar procedure to that of Barros (GC-MS). Limits of determination of 0.025-0.50 mg/kg were demonstrated for carbofuran and the metabolites in milk and tissues. The recoveries from fortified controls are shown in Table 28.

Table 28. Recovery of carbofuran and its carbamate and phenol metabolites from milk and ruminant tissues by the method of Chen (1995a).

Sample	Fortificn., mg/kg	No. of samples	Recovery, %					
			carbofuran	3-K-CF	3-OH-CF	7-phenol	3-K-7-P	3-OH-7-P
Milk	0.025	19	93 ± 13	92 ± 11	84 ± 13			
Milk	0.025	18				88 ± 12	99 ± 11	104 ± 15
Muscle	0.050	4	88 ± 14	97 ± 17	94 ± 14			
Muscle	0.050	2				70	100	80
Kidney	0.050	2	88	102	72			
Kidney	0.050	2				78	103	91
Kidney	0.50	2				87	114	85
Fat	0.050	3	76 ± 1.5	89 ± 9.2	71 ± 8.6			

Abbreviated compound names: see Figure 1, p.

The Netherlands submitted official multi-residue methods for the determination of carbofuran and 3-hydroxy-carbofuran (The Netherlands, 1997). Fruits, vegetables and potatoes were chopped, homogenized and extracted with acetone/methylene chloride/petroleum ether (1:1:1). Nuts, cereals, oil seeds, tropical seeds and dried fruits were extracted with acetone/methylene chloride (1:1). The extracts were analysed without clean-up, by gas chromatography with electron capture or ion trap detection. The limits of determination were stated to be in the range of 0.01-0.05 mg/kg, with recoveries of >80%. A second, HPLC method, consisted in extraction with acetone, partitioning into methylene chloride/petroleum ether and clean-up on a solid-phase extraction cartridge if necessary. The final extract was analysed on a reverse-phase HPLC column, with post-column hydrolysis and derivatization of the resulting amine with *o*-phthaldialdehyde. Detection was by fluorescence at 340 and 455 nm. The stated limit of determination was 0.005 mg/kg. The HPLC method is essentially that of Blass and Philipowski (1992).

The Netherlands also reported two methods for the determination of residues in field trial samples (The Netherlands, 1997). The GLC method is that of Mollhoff (1975a). A limit of determination of 0.1 mg/kg was reported for carbofuran, metabolites and conjugates. This method was used for strawberries, red cabbage, onions, leek, celery, celeriac, cauliflower, carrots and Brussels sprouts. The second method consisted in extraction with methylene chloride, concentration, clean-up on alumina and determination of carbofuran and 3-hydroxy-carbofuran of a reverse-phase HPLC column with a UV detector (220 nm). The limits of determination were 0.02 mg/kg for carbofuran and 0.01 mg/kg for 3-hydroxy-carbofuran. Recoveries were reported to be 95 ± 6% (n = 6) for carbofuran at fortifications of 0.06-1.1 mg/kg and 85 ± 7% (n = ?) for 3-hydroxy-carbofuran at fortifications of 0.05-0.50 mg/kg.

Stability of pesticide residues in stored analytical samples

Storage stability studies were reported for green and dry alfalfa, maize grain and silage, oranges, peanut kernels and hulls, potatoes, sorghum stalks, sugar beet tops and roots and cow milk and muscle (Schreier, 1989b). Fortified control samples were stored at -18°C for about 2 years. Samples were analysed after intervals of 9-11 and 24-26 months by the method of Schreier (1989a). The results are shown in Table 29. CLICK HERE to continue