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Effect of commercial processing procedures on carbofuran residues in soybean oil

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Dry seeds of soybean from ^{14}C-carbofuran-treated plants contained about 1% of the originally applied radioactivity. Extraction of the seeds with hexane and methanol left 50% of the ^{14}C in the cake. Analysis of residues showed the presence of free products in the oil and conjugated metabolites in the methanol extract. The free substances were identified as carbofuran and its phenol. The percentage of the latter increased during the successive refining processes. The refined oil had only 16% of the radioactivity originally present. The methanol extract contained four glucosides, mostly those of 3-hydroxy carbofuran. Refining soybean oil fortified with ^{14}C-carbofuran reduced the residue in the oil by 78%. Most of the residue remaining was carbofuran.

INTRODUCTION

Carbofuran is a broad spectrum long residual insecticide and nematicide. It is a potent anti-cholinesterase agent (Vettorazzi, 1979) effective by contact, stomach and systematic action. At recommended doses, it is nonphytotoxic and is compatible with most other insecticides (Kuhr and Dorough, 1982). Carbofuran is used in the protection of several crops and is effective against a large number of insect pests including corn borer, sugar cane borer, alfalfa weevil, pea aphid and thrips (Kuhr and Dorough, 1982). The metabolism of carbofuran has been extensively investigated in mammals, insects plants and soil (Kuhr and Dorough, 1982).

The present investigation aims to determine carbofuran residues in soybean oil before and after refining the oil gained from carbofuran-treated soybean plants. In parallel, the effect of the refining processes on the level and nature of ^{14}C-residues in soybean oil fortified with ^{14}C-carbofuran was also studied.

MATERIALS AND METHODS

The radiochemicals

Labelled carbofuran (3-^{14}C) was purchased from Izinta Isotope Trading Enterprise of the Institute of Isotopes, Budapest, Hungary. The insecticide was purified by thin layer chromatography on silica gel to remove carbofuran phenol using benzene–ether (3:1). About 85% of pure ^{14}C-carbofuran could be recovered by this method. The insecticide had a specific activity of 687.5 MBq mmol^{-1} and a radiometric purity of 98%. The ^{14}C-insecticide was diluted with non-labelled carbofuran to obtain a preparation with a specific activity of 0.592 MBq mg^{-1}.

Synthesis of possible metabolites

Carbofuran phenol, 3-hydroxy carbofuran and 3-keto carbofuran were prepared according to known procedures (Metcalf *et al.*, 1968).

Field experiment

Sound whole seeds of soybean (var. Catler 71) were cultivated by the end of March in an isolated and controlled field area. Plants were irrigated and fertilized as in practice. Labelled carbofuran was applied in a spray form to the plants shortly after blooming. Healthy leaves of the plants were treated twice (10 days apart) with a dose equivalent to 67.5 μg plant^{-1} each time. At maturity pods were collected and dried seeds used for preparation of oil and cake and determination of radioactivity.

Dry seeds were crushed and extracted with hexane for 12 h using a Soxhlet apparatus. After evaporation of

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hexane under reduced pressure, radioactivity in the soybean oil was measured. The residue remaining after hexane extraction was further extracted with methanol.

Fortification of soybean oil with ^{14}C-carbofuran

Crude soybean oil (100 g) was fortified with ^{14}C-carbofuran at a concentration of 7.5 ppm to study the effects of refining on the residue.

Table 1. Distribution of ^{14}C-carbofuran residues in 100 g soybean seeds

Fraction	Weight (g)	^{14}C-residues (μg)	%
Soybean seeds	100	4.0	100
Hexane extract (containing oil)	12–15	0.4	10
Methanol extract	5–6	0.8	20
Cake	78–80	2.0	50
Total recovery	98	3.2	80

Data are means of 2 replicates.

Commercial processing procedures

Alkali refining

Crude soybean oil was vigorously stirred with 2 N sodium hydroxide solution for 20 min at 27°C. The amount of alkali exceeded that calculated for the acid value of the oil by 20%. After settlement, the mixture was centrifuged to separate the soap and then washed several times with hot water. This process removed the fatty acids from the oil which was then counted for radioactivity.

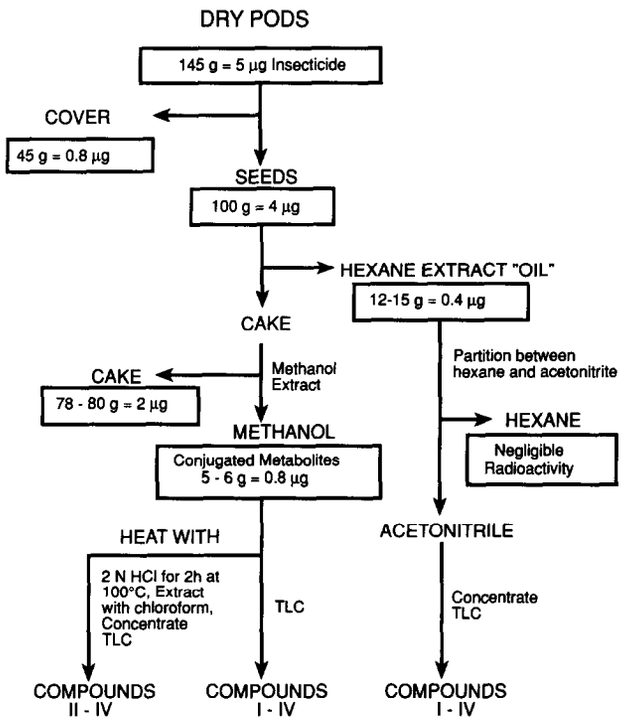
Bleaching

The neutralized oil was heated and agitated on an oil bath at 80–100°C for 10 min, treated with a factory grade Fuller's earth 'Tonsil' and then further stirred for 10 min at the same temperature. The bleached oil was filtrated and analysed for ^{14}C-activity.

Table 2. Conjugated carbofuran metabolites extracted from soybean cake with methanol

Compound	%	R_f		
		Systems 1–3	System 4	System 5
1. 3-Glucoside	48	0.00	0.11	0.14
2. 3-Keto-7-glucoside	26	0.00	0.25	0.28
3. 3-Hydroxy-7-glucoside	23	0.00	0.40	0.45
4. 7-Glucoside	3	0.00	0.50	0.64

Total radioactive products in methanol = 100%. System 1: benzene–ether (3:1). System 2: hexane–ether (1:3) saturated with water. System 3: hexane–ethyl acetate (1:1) saturated with water. System 4: ethyl acetate–*n*-propanol–water (5:3:2). System 5: *n*-butanol–ethanol–water (10:2:3).



Scheme 1. Analysis procedure for residues in oil obtained from soybean plants treated with ^{14}C-carbofuran.

Winterization

The clear dry oil was winterized at 5°C for 3 days and the high saturated glycerides, which separated, were removed by filtration.

Deodorization

This process was simulated by heating the oil to 200–220°C while passing steam under reduced pressure for 4 h.

Table 3. Effect of commercial processing procedures on ^{14}C-carbofuran residues in soybean oil

Compound	Retained ^{14}C-residues			
	In fortified		In oil with aged residues	
	%	ppm	%	ppm
	100	7.5	100	0.024
Alkali refining	80	6.0	75	0.018
Bleaching	60	4.5	54	0.013
Winterization	36	2.7	33	0.008
Deodorization	22	1.7	16	0.004

Data are means of two replicates.

Table 4. ^{14}C-carbofuran and its metabolites in soybean oil after subjecting it to commercial processing procedures

Substance	R_f			Relative percentage				
	System 1	System 2	System 3	Crude oil	A	B	C	D
Carbofuran phenol	0.77	0.80	0.83	25	30	45	65	75
3-Ketocarbofuran phenol	0.56	0.63	0.66	10	15	20	25	15
Carbofuran	0.51	0.54	0.59	60	40	25	10	5
3-Hydoxycarbofuran	0.39	0.40	0.42	5	15	10	5	5

System 1: benzene–ether (3:1). System 2: hexane–ether (1:3) saturated with water. System 3: hexane–ethyl acetate (1:1) saturated with water. Residue in oil = 100%. A, alkali treatment; B, bleaching; C, winterization; D, deodorization.

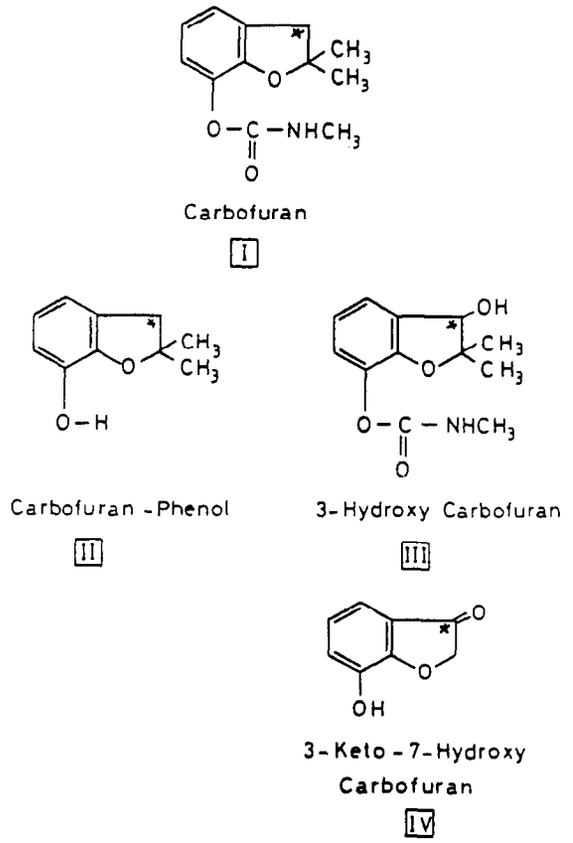


Fig. 1. Main degradation products of ^{14}C-carbofuran in soybean oil.

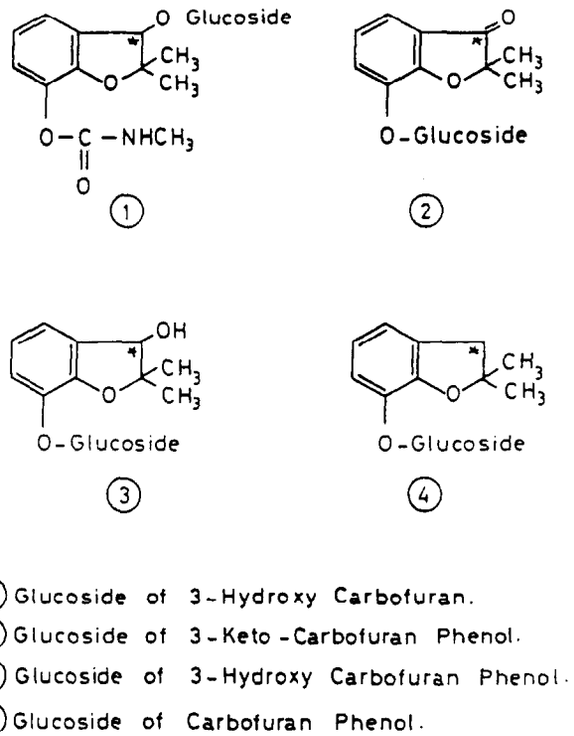


Fig. 2. Conjugated metabolites of ^{14}C-carbofuran in soybeans.

Isolation and characterization of radioactive residues

Oil obtained from an individual refining process was partitioned between acetonitrile and hexane (Scheme 1). After concentration of acetonitrile, the residues were characterized by TLC in different solvent systems. ^{14}C-residues in the methanol extract were also analysed by TLC. Spots were made visible by spraying the plate with vanillin (2.5% ethanolic solution)–sulfuric acid reagent (1:5). Carbofuran and its metabolites gave pink-purple spots upon heating the plates at 110°C for 5 min; conjugated metabolites gave greyish spots under the same conditions (Mostafa *et al.*, 1992).

Radiometric measurements

The radioactivity in solutions was measured by direct liquid scintillation counting. Radioactivity in solids or oil was determined by combustion in a Harvey Biological Oxidizer (Model OX-600), followed by liquid scintillation counting.

RESULTS AND DISCUSSION

^{14}C-residues in seeds and oil

The foliar application led to appearance of ^{14}C-activity in the pods and in dry seeds. The total radioactive residues in pods amounted to 1.3% of applied dose. Only 80% of the radioactivity in pods was associated with dry seeds. The distribution of radioactive residues in oil and cake is shown in Table 1. Hexane contained the least amount of radioactivity, while the cake contained 50% of the original residue in seeds. The major residues in crude oil were identified as carbofuran and its phenol (Fig. 1).

The residues in the methanol extract constituted about 20% of the total residues in seeds. These were hydrophilic and insoluble in organic non-polar solvents (Table 2); presumably a mixture of conjugated metabolites (glucosides; Fig. 2). No attempt was made to separate the individual glucosides. The products were liberated by heating the mixture with 2 N HCl, extracted with chloroform and identified by TLC. Analysis showed the presence of 3-hydroxycarbofuran as the major constituent and carbofuran phenol as a minor product (Fig. 1).

Effects of refining processes

Commercial processing procedures led to a gradual decrease in the total amount of residues in oils with aged residues and in oils fortified with ^{14}C-carbofuran (Table 3).

The refined oil lost 84% of its aged residues and 78% of its fortified residues. The metabolites of carbofuran and their percentages in the oil with aged residues following the individual processing procedures are shown in Table 4. Carbofuran phenol was the main metabolite and its percentage increased progressively during refining of the oil and accounted for about 75% of the residue. Residues remaining in fortified processed oil consisted mainly of the parent compound together with a small amount of carbofuran phenol (< 2%), probably formed via hydrolysis of the parent compound during alkali treatment.

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