Alteration of Carbosulfan [2,3-Dihydro-2,2-dimethyl-7-benzofuranyl (Di-*n*-butylaminosulfenyl)methylcarbamate] in the Rat Stomach

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The alteration of carbosulfan [2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-*n*-butylaminosulfenyl)-methylcarbamate] in the rat stomach at short intervals after oral administration was examined. Carbosulfan was more stable in the stomach than expected from previous results on acid-catalyzed degradation studies but was slowly converted into a variety of products. Major alteration products were carbofuran, biscarbofuran *N,N'*-disulfide, and a compound of unknown structure. In addition, five polysulfide derivatives of carbosulfan or biscarbofuran *N,N'*-disulfide were detected as minor products. In dilute aqueous hydrochloric acid, carbosulfan was converted mainly into carbofuran. The lower mammalian toxicity of carbosulfan relative to carbofuran is attributed to the unexpectedly high stability of carbosulfan in the rat stomach and to the conversion of carbosulfan into the less toxic polysulfide derivatives.

The experimental insecticide carbosulfan [Marshal, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-*n*-butylaminosulfenyl)methylcarbamate], the dibutylaminosulfenyl derivative of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) is 5–10-fold less toxic to the rat than is carbofuran. In an earlier study, carbosulfan was found to be unstable in acidic water–methanol buffer, breaking down rapidly to carbofuran as the principal degradation product (Umetsu et al., 1980). Subsequent studies (Umetsu et al., 1981a,b; Umetsu and Fukuto, 1982) showed that carbosulfan also was unstable in acidic organic solvents, e.g., acetonitrile–acetic acid, but in this case a wide variety of alteration products were observed, the major products being carbosulfan polysulfides, biscarbofuran polysulfides, and carbofuran. All of the polysulfide derivatives were substantially less toxic to the white mouse than carbofuran.

The increased mammalian safety of the sulfenylated derivatives of toxic methylcarbamate esters has been attributed to the delay factor provided by the *N*-sulfenyl moiety; i.e., derivatization allows the compound to be metabolized to nontoxic products before intoxication can take place (Fukuto and Fahmy, 1981). However, because of the rapid conversion of carbosulfan to carbofuran in acidic buffer, the basis for the lower mammalian toxicity of carbosulfan was not clear owing to its expected rapid conversion into the highly toxic carbofuran in the acidic environment of the animal's stomach. However, the possibility remained that carbosulfan was transformed in the stomach into the various polysulfide derivatives observed in acidic organic solvents and that the formation of these alteration products was, in part, responsible for the improved mammalian toxicity of carbosulfan. Therefore, the alteration of carbosulfan in the rat stomach at short time intervals after oral treatment was examined in an effort to determine the basis for the lower mammalian toxicity of carbosulfan compared to carbofuran.

MATERIALS AND METHODS

Chemicals. [*carbonyl*-^{14}C]Carbosulfan (specific activity 25.2 mCi/mmol), biscarbofuran sulfide (CFSCF), (dibutylaminosulfonyl)carbofuran (carbosulfan sulfone), and the various carbosulfan and biscarbofuran polysulfides were available from earlier studies (Umetsu et al., 1979, 1980, 1981a,b). Structures and TLC properties of each compound are given in Table I.

Other chemicals were analytical reagent grade and redistilled solvents were used throughout this investigation.

Toxicological Evaluation. Mammalian toxicity of carbosulfan and carbofuran was determined by oral administration to female Swiss white mice and Sprague-Dawley derived rats purchased from Simonsen Laboratories, Gilroy, CA. Corn oil and propylene glycol were used as carriers.

Alteration of Carbosulfan in the Rat Stomach. The fate of [^{14}C]carbosulfan in the stomach was examined in three female rats, weight range 137.6–148.0 g. Each rat was fasted for 15–17 h prior to oral administration of 30 mg/kg labeled carbosulfan (1.25–2.63 μCi) contained in 0.2 mL of propylene glycol. Rats were sacrificed at 15, 35, and 80 min following treatment, the stomach from each rat was removed immediately, and the contents were transferred to a smaller beaker with the aid of 5 mL of distilled water and 25 mL of pH 7.5 0.1 M phosphate buffer. The pH of the stomach contents was determined prior to washing with the phosphate buffer. The time required to remove the stomach and collect the stomach contents was 5 min. The stomach was washed with 10 mL of ether which was combined with the aqueous phase, an additional 30 mL of ether was added, and the mixture was agitated vigorously for 3 min. Centrifugation at 3000 rpm for 10 min gave two phases which were separated. The ether extraction was repeated 3 times, and the extracts were combined (designated as the *ether-soluble* fraction). After being dried over anhydrous sodium sulfate, aliquots of the ether solution were concentrated and analyzed for radioactivity and by TLC. The residual aqueous phase (designated as the *water-soluble* fraction) also was analyzed for radioactivity.

Alteration of Carbosulfan in Dilute Hydrochloric Acid. Samples of carbosulfan (12.0 mg; 0.514 μCi) dissolved in 0.8 mL of propylene glycol were combined with 8.0 mL of 0.01 N (pH 2.0) or 0.001 N (pH 2.95) hydrochloric acid, and the mixtures were gently shaken at 40 °C. Samples were removed at intervals ranging from 20 min to 24 h and extracted with ether. The ether and aqueous phases were analyzed as described for the stomach contents.

Analyses. Precoated silica gel GHLF plates (0.25-mm thickness; Analtech, Inc.) and silica gel $KC_{18}F$ reversed-phase plates (0.2-mm thickness; Whatman, Inc.) were used for analytical TLC. Location of spots on the plates was

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0021-8561/82/1430-0555$01.25/0

Table I. Thin-Layer Chromatographic Properties of Possible Alteration Products of Carbosulfan

compound	abbreviation	R_f value for TLC system[a] A	B	C	D
carbofuran		0.45	0.22	0.73	0.71
(dibutylaminosulfonyl)carbofuran	carbosulfan sulfone	0.76	0.54	0.61	0.47
biscarbofuran N,N'-sulfide	CFSCF[b]	0.75	0.40	0.63	0.51
biscarbofuran N,N'-disulfide	CFS_2CF	0.77	0.43	0.63	0.47
biscarbofuran N,N'-trisulfide	CFS_3CF		0.39	0.63	0.44
biscarbofuran N,N'-tetrasulfide	CFS_4CF		0.39	0.59	0.39
biscarbofuran N,N'-pentasulfide	CFS_5CF		0.39	0.56	0.33
biscarbofuran N,N'-hexasulfide	CFS_6CF		0.39	0.52	0.28
(dibutylaminosulfenyl)carbofuran	carbosulfan	0.79	0.58	0.51	0.31
carbosulfan disulfide	CFS_2NBu_2	0.81	0.62	0.48	0.26
carbosulfan trisulfide	CFS_3NBu_2	0.81	0.62	0.42	0.20
carbosulfan tetrasulfide	CFS_4NBu_2		0.62	0.37	0.15
carbosulfan pentasulfide	CFS_5NBu_2		0.62	0.33	0.11
carbosulfan hexasulfide	CFS_6NBu_2		0.62	0.27	0.08

[a] TLC and solvent system: (A) silica gel TLC, dichloromethane–acetonitrile (9:1); (B) silica gel TLC, hexane–ether (1:1); (C) reversed-phase silica gel TLC, acetonitrile; (D) reversed-phase silica gel TLC, acetonitrile–water (9:1). [b] CF is the abbreviation for the carbofuran moiety which is bonded to sulfur at the carbamyl nitrogen atom.

Table II. Toxicity of Carbosulfan to Rats and Mice Using Corn Oil and Propylene Glycol as Carriers

compound	LD_{50} for rat, mg/kg corn oil	propylene glycol	LD_{50} for mouse, mg/kg corn oil	propylene glycol
carbosulfan	87 (63-106)[a]	51 (30-81)[a]	129 (107-148)[a]	74 (59-124)[a]
carbofuran	11.9 (9.4-14.4)[b]	6.4[b]	19 (16-24)[a]	11 (10-12)[a]

[a] 95% confidence limit. [b] Data from U.S. Environmental Protection Agency, Office of Pesticide Programs (1976).

by iodine vapor or by ultraviolet detection. The location of radioactive spots was accomplished by means of a Berthold (Varian–Aerograph) thin-layer radioscanner (Model LB 2723) equipped with a dot printer and confirmed by autoradiography using Kodak X-ray film (BB-5) exposed for 7–15 days.

Radioactivity was quantitated with a Beckman Model LS-230 liquid scintillation counter by using 10 mL of a scintillation cocktail consisting of 6 g of PPO, 0.2 g of POPOP, 333 mL of Triton X-100, and 666 mL of toluene. Radioactivity in each spot on TLC plates was determined by scraping the spot from the plate and placing the silica gel in counting vials with scintillation cocktail.

RESULTS

Toxicity of Carbosulfan. Data for the rat and mouse acute oral toxicities of carbosulfan administered in corn oil or propylene glycol are presented in Table II. In all cases where comparison may be made, carbosulfan and carbofuran were significantly more toxic to mice and rats when propylene glycol was used as the carrier. However, in either carrier carbofuran was substantially more toxic to mice and rats (6–7-fold) than was carbosulfan.

Alteration of Carbosulfan in the Rat Stomach. The fate of [^{14}C]carbosulfan (propylene glycol carrier) in the stomach of rats sacrificed 15, 35, and 80 min following oral administration at 30 mg/kg was determined. Data summarizing the distribution, identity, and relative amounts of alteration products are presented in Table III. The individual compounds were identified by two-dimensional thin-layer cochromatography with silica gel (first solvent, 9:1 dichloromethane–acetonitrile; second, 1:1 hexane–ether) and silica gel KC_{18} reversed-phase plates (first solvent, acetonitrile; second, 9:1 acetonitrile–water). Recovery of administered radioactivity diminished with time, from 75.7% at 15 min to 44.6% at 80 min, and most of the recovered radioactivity was extractable into ether (96.2–97.8%).

In spite of the high acidity of the fluid in the rat stomach (pH 2.5–3.8), carbosulfan was far more stable in the stomach than expected. Previous work (Umetsu et al., 1980) showed carbosulfan to be highly unstable in pH 3.0 methanol–citrate–phosphate buffer (40% methanol), 90% being converted into carbofuran within 10 min. However, about 50% of the radioactivity recovered from the stomach 80 min following treatment was in the form of carbosulfan. Major alteration products at all time intervals were carbofuran, CFS_2CF, and an unknown (III). CFS_2CF was contaminated with a trace amount of CFS_3CF owing to the difficulty in attaining clean separation of these compounds by TLC. Other alteration products observed in minor amounts were CFS_4CF, CFS_5CF, CFS_2NBu_2, and CFS_3NBu_2. Except for unknown III, all alteration products were previously observed in earlier studies involving the acid-catalyzed alteration of carbosulfan in organic solvents (Umetsu et al., 1981a,b; Umetsu and Fukuto, 1982). Unknown III did not cochromatograph with any

Table III. Products Obtained from the Alteration of [*carbonyl*-^{14}C]Carbosulfan in the Rat Stomach

product	% of recovered radioact at indicated time interval 15 min	35 min	80 min
total recovery	75.7	55.1	44.6
ether soluble			
unknown I	0.3	0.4	0.3
carbofuran	14.6 (1.93)[a]	20.3 (1.95)	15.8 (1.23)
unknown II	0	0.8	1.1
unknown III	6.5	8.2	9.0
CFS_2CF[b]	11.1	17.0	18.4
CFS_4CF	0.7	0.9	1.0
CFS_5CF	0.5	1.2	0.7
carbosulfan	60.0 (13.6)	46.8 (7.7)	49.5 (6.6)
CFS_2NBu_2	0.9	0.8	1.0
CFS_3NBu_2	0.5	0.1	0.2
unknown IV[c]	1.1	0.8	0.8
subtotal	96.2	97.3	97.8
water soluble	3.8	2.5	2.3
total	100.0	99.8	100.1

[a] Parenthetical values represent actual amount of carbofuran or carbosulfan in milligrams per kilograms of rat. [b] Contained small amounts of CFS_3CF. [c] Consisted of several components.

Table IV. Alteration of [*carbonyl*-^{14}C]Carbosulfan in 0.001 N (pH 2.95) and 0.01 N (pH 2.0) Hydrochloric Acid

	% of total carbosulfan applied				
	0.001 N HCl				0.01 N HCl,
product	20 min	1.5 h	4 h	24 h	1.5 h
total recovery	97.4	96.3	95.0	96.6	96.3
ether soluble					
unknown	3.0	1.5	2.6	1.7	2.1
carbofuran	12.1	31.0	45.8	49.3	38.7
CFS_nCF ($n \geq 2$)	0.7	1.2	2.0	2.7	1.4
carbosulfan	82.2	60.3	41.7	40.6	51.9
CFS_2NBu_2	1.3	2.2	2.6	1.8	0.7
CFS_3NBu_2	0.5	0.4	0.6	0.7	0.3
CFS_4NBu_2		0.4	0.5	0.5	0.3
CFS_5NBu_2		0.2	0.3	0.4	0.3
CFS_6NBu_2			0.2	0.4	0.2
subtotal	99.8	97.2	96.3	98.1	95.9
water soluble	0.2	2.7	3.9	2.0	4.0
total	100.0	99.9	100.0	100.1	99.9

of the standards listed in Table I, but its TLC characteristics were very close to that of CFSCF.

Alteration of Carbosulfan in Dilute Hydrochloric Acid. Since the acidity of the mammalian stomach is caused by secretion of hydrochloric acid, the alteration of carbosulfan in dilute aqueous hydrochloric acid was examined. [^{14}C]Carbosulfan dissolved in propylene glycol was added to 0.001 N (pH 2.95) or 0.01 N (pH 2) aqueous hydrochloric acid, and alteration products were determined at intervals up to 24 h at 40 °C.

Data summarizing the different products which were identified are given in Table IV. Most of the radioactivity (95.9–99.8%) was extractable as ether-soluble material at each time interval. As was the case in the rat stomach studies, carbosulfan was relatively stable in dilute hydrochloric acid. More than 40% of the recovered radioactivity was in the form of carbosulfan after 24 h in 0.001 N hydrochloric acid. However, in contrast to results from the stomach study, carbofuran was the principal alteration product in hydrochloric acid. CFS_2CF was present in only trace amounts, and unknown III was not observed at all. Small amounts of the polysulfides of carbosulfan (CFS_nNBu_2, n = 2–6) were observed.

DISCUSSION

Results on the behavior of carbosulfan in the rat stomach following oral administration at 30 mg/kg are in line with the lower mammalian toxicity of carbosulfan compared to that of carbofuran. The various reactions leading to the different alteration products of carbosulfan in the rat stomach are indicated in Figure 1. The lower toxicity of carbosulfan may be explained by (1) greater-than-expected stability of carbosulfan and (2) conversion of carbosulfan to less toxic polysulfide derivatives.

Carbosulfan was surprisingly stable in the acidic environment of the stomach, and relatively low levels of carbofuran were observed at each of the three time periods when analyses were made. The actual amounts of carbofuran present were 1.93 mg/kg at 15 min, 1.95 mg/kg at 35 min, and 1.23 mg/kg at 80 min. Thus, the level of carbofuran in the stomach at each interval was substantially lower than the LD_{50} value for carbofuran (6.4 mg/kg in propylene glycol carrier).

The formation of the biscarbofuran and carbosulfan polysulfides in the rat stomach is also significant from a toxicological standpoint. Next to carbofuran, CFS_2CF was the most abundant alteration product and actually was present in a larger amount at the 80-min period. Earlier

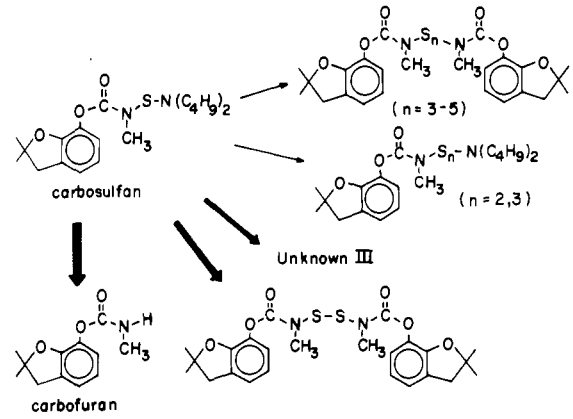

Figure 1. Alteration pathways for carbosulfan in the rat stomach.

work (Umetsu et al., 1981b) showed that CFS_2CF was less toxic (and less insecticidal) to mice (LD_{50} > 100 mg/kg) than carbosulfan. Therefore, conversion of carbosulfan to CFS_2CF should contribute to the lower mammalian toxicity of carbosulfan.

The structure of the third major alteration product of carbosulfan in the rat stomach was not determined (unknown III). The TLC behavior of this compound was similar to that of CFSCF, but KC_{18} reversed-phase two-dimensional cochromatographic analysis showed that they were not the same. The toxicity of this compound to mammals is not known. The other alteration products observed in minor amounts, i.e., CFS_2NBu_2, CFS_3NBu_2, CFS_4CF, and CFS_5CF, were previously shown to be much less toxic than carbofuran to mammals (Umetsu et al., 1981b).

The examination of the alteration of carbosulfan in aqueous hydrochloric acid of pH similar to that of the rat stomach showed that other factors besides acidity are involved in the breakdown of carbosulfan in the stomach. For example, CFS_2CF and unknown III, each major alteration products of carbosulfan in the rat stomach, were not detected in dilute hydrochloric acid or only in trace amounts. However, it should be pointed out that CFS_2CF was a major alteration product when carbosulfan was allowed to stand in 9:1 acetonitrile–1 N hydrochloric acid (Umetsu and Fukuto, 1982).

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Received for review July 27, 1981. Accepted February 8, 1982. This investigation was supported in part by federal funds from the Environmental Protection Agency under Grant R804345. The contents do not necessarily reflect the views and policies of the Environmental Protection Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.