Distribution and Excretion of Dicamba by Rats as Determined by Radiotracer Technique

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The herbicide, dicamba, following administration of C^{14}-labeled compound to rats by esophageal intubation, incorporation in the food, and subcutaneous injection, was in all instances excreted rapidly via the urine. Following subcutaneous injection, a small proportion was excreted via the feces. The urinary excretion was of chemically unchanged dicamba, approximately one fifth of which was conjugated with glucuronic acid. Dynamic balance in the tissues of rats with dietary dicamba appeared to be reached in 2 weeks. The highest levels were in the aqueous rather than in the fatty tissues, and were approximately in proportion to dosage at the levels of 1000 p.p.m. or less. Early dynamic balance and rapid clearance from the tissues of dicamba given by intubation showed an absence of storage in the ordinary use of the term.

The compound 2-methoxy-3,6-dichlorobenzoic acid has valuable potential as a herbicide (Faivre-Dupaigne and Rognon, 1964; Huey *et al.*, 1964). The compound as produced for commercial use is known as technical dicamba. The authors have investigated the toxicology of this material further by the determination of the extent of its absorption from the alimentary tract, the rates and routes of its elimination, the chemical form in which it is excreted with the character of its conjugation, and the quantitative distribution of the compound in selected, representative tissues.

PROCEDURE

A radioactive form of 2-methoxy-3,6-dichlorobenzoic acid, labeled with C^{14} in the carboxyl position (minimum purity 98%, New England Nuclear Co.), mixed with technical dicamba (the mixture is designated herein as dicamba-C^{14}) was administered to Charles River, CD strain, albino rats by three modes—esophageal intubation of one dose in peanut oil, inclusion in the diet, and subcutaneous injection of solutions in peanut oil. All animals were housed individually in stainless steel metabolism cages equipped with funnels designed for the separate collection of feces and urine. The radioactivity of specimens of excreta and of tissues was measured by liquid scintillation counting in a Packard Model 314X liquid scintillation spectrometer. Samples were prepared by the suspension of urine, alkaline digests of tissues, or neutralized acidic digests of feces on Cab-O-Sil, in a modified dioxane–PPO–POPOP scintillation medium, according to Tye and Engel (1965). The efficiency of this system is approximately 45%.

Esophageal Intubation, Group I. Each member of two subgroups of rats, aged 6 months (each subgroup containing eight females and four males), was given one oral dose in peanut oil at two levels—0.1 and 0.93 gram of dicamba per kg. of body weight. The female rats were killed at 1, 3, 5, 7, 9, 24, 48, and 72 hours, respectively, after the administration of the dose, while the male rats were killed, correspondingly, after 1, 5, 9, and 72 hours. Pertinent quantitative data are given in Table I.

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Each level of dosage was given to eight female rats in order to have homogeneous populations sufficiently large to define the relationships, in time, of the absorption from the intestine, the clearance from representative tissues, and the excretion of the dicamba or its metabolites. Four males were expected to be sufficient to establish the general temporal relationship of males to females; and since the feeding experiments were to provide additional information concerning the responses of both sexes, a number of males as large as the number of females was deemed unnecessary.

The solution of dicamba in peanut oil from which to derive each level of dosage was prepared by dissolving dicamba-C^{14} and technical dicamba in acetone, evaporating the solvent with nitrogen, and grinding the dried residue with peanut oil in a mortar. The resulting solutions or slurries were administered to the rats by means of a syringe with a specially modified, long, blunt needle.

The rats that were to be killed more than 9 hours after the administration of a dose were given Purina Laboratory Chow *ad libitum*. Water was given *ad libitum* to all rats.

The rats were anesthetized with Nembutal, and blood was taken via cardiac puncture. The liver, kidneys, gastroenteric tract, and remaining carcass were saved and refrigerated for later analysis. Samples of urine and feces were frozen immediately after their collection.

Subcutaneous Injection, Group S. One male and one female (Charles River, CD strain, 7 months old) were injected subcutaneously in the region of the hip with dicamba-C^{14} in peanut oil, composed of 98% of technical

Table I. Quantitative Data, Dosages of Dicamba-C^{14} in Peanut Oil Administered to Rats, Group I

Av. Dosage, G. of Dicamba-C^{14} Per Kg. Per Rat	Sex	Av. Weight of Rats, G.	Vol. of Dose, Ml.	Dicamba Concn., Mg./Ml.	
				Technical	Radiotracer[a]
0.1	F	280	3.0	9.6	0.20
	M	451	4.8	9.6	0.20
0.93	F	280	3.0	87	0.25
	M	454	4.8	87	0.25

[a] Specific activity, 8.5 μc. per mg.

dicamba and 2% of concentrated radiotracer. Food and water were given *ad libitum*. Urine and feces were collected and analyzed periodically for their content of C^{14}. The rats were killed at 72 hours after being injected. The pertinent data concerning dosages are assembled in Table II.

Inclusion in Diet, Group F. Five subgroups of rats (aged 6 months) were fed on diets containing dicamba-C^{14} in respective concentrations of 10, 100, 1000, 10,000, and 20,000 p.p.m. incorporated by thorough grinding of powdered, dry compound with portions of feed, followed by appropriate dilution and mixing. All of the dicamba-C^{14}, at the level of 10 p.p.m., was concentrated radiotracer (specific activity 8.5 μc. per mg). In each of the remaining diets, 15 p.p.m. were concentrated radiotracer and the remainder was technical dicamba, the radiotracer and diluent having been mixed in solution and recovered by evaporation of the solvent. Each subgroup contained five males and five females. The food containing the highest concentration (20,000 p.p.m.) was apparently unpalatable and was not well taken by this group of animals. This level of concentration was adopted following a brief pilot study of three female rats fed on diets containing dicamba at levels of 30,000, 40,000, and 50,000 p.p.m. The three rats ate 19, 14, and 9%, respectively, of average normal quantities of food, and all lost approximately 20% of their weight in 6 days. Therefore, 20,000 p.p.m. was believed to be the maximum concentration at which adequate nutrition and health could be maintained for an extended period.

The rats were fed on a diet of Purina Laboratory Meal moistened with 55% of water to prevent the spread of radioactive dust. Drinking water was supplied *ad libitum*. The rats were housed in metabolism cages equipped with tunnel feeders to minimize the spread of food and excreta. Food was given according to the median level of consumption of each sex as found, during 6 days of *ad libitum* feeding prior to the administration of dicamba-C^{14}, to be 20 grams of dry feed for males and 17 grams for females.

The specific activity of the four highest concentrations was 0.122 μc. per gram of food. Analyses of aliquots of the food at each level yielded 95 to 100% recovery of C^{14}.

Carefully weighed portions of food were given to each rat on each day, and any food remaining in the feeder was weighed and discarded daily. The quantity of dicamba-C^{14} consumed by each rat was determined, thereby, with adequate accuracy. All urine and feces were collected daily and were frozen immediately.

One rat of each sex from each of the subgroups was killed after 1, 3, 6, 13, and 24 days of feeding. The rats were anesthetized with Nembutal, and blood was taken by cardiac puncture. The liver, kidney, gastroenteric tract, and the remaining carcass were saved and refrigerated. Portions of the abdominal fat were saved from the rats killed on the 24th day. Most of these tissues, along with the excreta, were analyzed for their content of radiotracer.

Determination of Chemical Identity of Radiotracer in Urine. A sample of 456 grams of urine, composited from

Table II. Dosages of Dicamba-C^{14} in Peanut Oil Injected Subcutaneously into Rats, Group S

	Male	Female
Weight of rat, g.	420	345
Dicamba-C^{14} in peanut oil, %	1.05	1.05
Specific activity, μc./ml.	1.68	1.68
Volume of oil given, ml.	4.5	3.7
Equivalent dose of dicamba, g./kg.	0.1	0.1

Table III. Distribution of Radiotracer Administered Orally to Rats of Group I[a]

Time, Hours	Sex	Dose, G./Kg.	Site				
			Feces	Urine	G.e. tract	Blood	Other
1	F	0.1	0.0	8.1	86.5	1.8	5.0
1	M	0.1	0.0	0.4	90.5	2.3	4.4
3	F	0.1	0.3	38.0	56.3	1.4	2.9
5	F	0.1	0.2	49.7	43.2	1.1	7.3
5	M	0.1	0.0	49.0	36.3	0.7	6.1
7	F	0.1	0.0	70.8	23.2	0.3	3.2
9	F	0.1	0.0	81.6	13.9	1.2	7.0
9	M	0.1	0.0	70.3	18.4	0.4	7.6
24	F	0.1	0.2	93.2	0.6	0.1	0.5
48	F	0.1	0.9	92.3	0.1	0.0	0.5
72	F	0.1	1.0	94.5	0.0	0.0	0.0
72	M	0.1	1.1	92.8	0.0	0.0	0.5
1	F	0.93	0.0	3.7	89.7	1.6	2.4
1	M	0.93	0.0	0.0	84.0	1.0	8.1
3	F	0.93	0.0	15.1	83.0	1.1	3.9
5	F	0.93	0.0	26.7	61.8	2.1	9.4
5	M	0.93	0.0	10.3	77.5	1.2	8.8
7	F	0.93	0.3	46.5	37.5	2.1	11.6
9	F	0.93	0.1	47.5	32.0	1.1	8.0
9	M	0.93	0.1	45.4	38.0	1.9	8.9
24	F	0.93	0.3	92.0	12.5	0.3	1.5
48	F	0.93	2.4	87.5	0.4	0.1	1.4
72	F	0.93	0.8	99.1	0.3	0.0	2.1
72	M	0.93	0.8	97.7	0.1	0.0	0.2

[a] Percentage of total dose.

that collected on 13 days scattered throughout the 24-day period of the experiment, from the male rats (Group F) on the diet containing dicamba-C^{14} in the concentration of 10,000 p.p.m. was acidified with 35 ml. of 1.0*N* HCl (producing pH 5) and extracted repetitively with ethyl ether. The combined extract, totaling 1.5 liters, was evaporated to dryness with a stream of N_2 at temperatures under 40° C. The partially crystalline residue weighed 1.680 grams. The urine was further extracted, repetitively, with ethyl acetate (200-ml. total), and the levels of radioactivity of the raffinate and of the extracts were determined. The material extracted by ether was recrystallized from toluene, and the levels of radioactivity of the crystals and of the mother liquor were determined. The identity of radiotracer in the mother liquor and in the ethyl acetate was investigated by the addition of measured portions (with known radioactivity) of each of these solutions, to solutions in cyclohexane containing weighed portions of authentic, pure dicamba such that the compound represented by the radiotracer was quantitatively negligible. The resulting solutions were partially evaporated. Upon cooling, crystals of dicamba were formed. These crystals were washed with cyclohexane, dried, and the specific activity was determined. Depending upon the degree to which other compounds were not included in the crystals, the observed calculated specific activity indicated the fraction of radioactivity attributable to unchanged dicamba.

Whether dicamba is excreted in a conjugated state by rats was investigated by measuring etherial sulfate and glucuronic acid.

Table IV. Dicamba in Urine and Feces of Rats, Group S, Injected Subcutaneously with Dicamba-C^{14}

Time, Hours	Percentage of Dose			
	In Urine		In Feces	
	Male	Female	Male	Female
5	70.00	80.00	...	...
11	19.20	9.15	...	...
24	6.55	8.95	0.90	1.20
32	0.43	0.46	...	...
48	0.49	1.04	0.15	0.90
55	0.12	0.08	...	...
72	0.24	0.33	0.11	0.20
Total	97.03	100.01	1.16	2.30

Table V. Excreted Radiotracer from Rats, Group F, at Various Dietary Levels[a]

Dietary Level, P.P.M.	Male			Female		
	Urine[b]	Feces[c]	Total	Urine[b]	Feces[c]	Total
10	102	5	107	103	2	105
100	94	7	101	96	7	103
1,000	95	4	99	96	3	99
10,000	98	3	101	97	2	99
20,000	98	3	101	95	5	100

[a] Percentage of quantity ingested.
[b] Each value is average of determinations on 10 separate samples, representing 13 days scattered throughout experimental period.
[c] Average of determinations: 2 composites, 11 days.

RESULTS AND DISCUSSION

A general delineation of the physiologic disposition of dicamba-C^{14} by rats was obtained from group I (Table III). Whether administered to rats by esophageal intubation or by subcutaneous injection, the greater part of the dicamba-C^{14} was excreted in the urine within 24 hours (Tables III and IV). In group F the rate of urinary excretion had reached 96% of the rate of dietary intake during the second period of 24 hours (Table V). The proportion of radiotracer appearing in the urine does not appear to be a function of dosage within the range administered.

Elimination of radiotracer in the feces of males or fe-

Table VI. Accumulation of Radiotracer in Tissues Relative to Dosage at Levels from 10 to 20,000 P.P.M., Group F

Tissue	Days	P.P.M. in Tissue/P.P.M. in Food × 10^4									
		10[a]		100		1000		10,000		20,000	
		Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Liver	1	0	7	8	5	2	5	7	9	8	7
	3	4	4	4	9	8	18	6	24	5	22
	6	8	13	8	13	7	16	9	5	14	24
	13	13	9	13	19	9	30	55	29	23	43
	24	17	14	7	13	9	14	29	27	15	16
Kidney	1	...	55	55	7	6	138	12	16	16	28
	3	7	23	8	36	8	10	10	53	9	34
	6	29	68	19	62	12	65	21	3	24	36
	13	14	39	51	89	13	99	43	85	21	83
	24	45	78	7	19	7	17	47	48	52	27
Whole blood	1	13	16	7	9	7	9	10	4	15	7
	3	18	10	13	18	4	27	12	23	15	7
	6	20	20	10	10	15	15	11	11	15	15
	13	20	26	16	20	8	38	16	63	25	55
	24	15	36	5	5	9	17	20	30	16	18
Muscle	13	4	4	1	6	3	12	11	21	15	28
	24	1	6	2	5	2	3	10	7	18	12
Fat	24	4	4	5	12	2	3	2	7	8	11

[a] 10 p.p.m. dicamba-C^{14} in food.

Table VII. Distribution of Radiotracer in Various Fractions of Urine

Site	Radiotracer C.p.m.	%
Crystals	15,100,000	65
Mother liquor of crystals	6,700,000	29
Extract 2 (ethyl acetate)	1,150,000	5
Remaining in urine	160,000	0.7
Total	23,110,000	99.7

males of group F was at the rate of about 4% of the ingested amount and this percentage apparently was independent of the dietary level. Appearance of the radiotracer in the feces of group S shows that some dicamba-C^{14} is excreted by this route, but comparison of data from the various groups shows that perhaps half of that which appeared in the feces of group F represented tracer that was never absorbed.

Modest concentrations appeared in the tissues within one hour after esophageal administration of dicamba-C^{14}, but, with the exception of the alimentary tract and urine, the portion within the body never exceeded 15% of the dose (Table III). The concentrations in the tissues had begun to fall within 9 hours. Thus, the compound was excreted (Table III) about as rapidly as it was absorbed, and little or none of it was detectable within the tissues after 2 or 3 days. The accumulation in selected tissues (group F), resulting from absorption from the dietary intake during a period of 24 days, is shown in Table VI. Equilibration appears to have occurred early in that period. There were no marked differences between the concentrations that appeared in the kidney, liver, and blood, and the concentrations were in approximate proportion to the levels of dosage.

Male rats of group F, at the dietary level of 10,000 p.p.m. of dicamba-C^{14} excreted the compound unchanged. Almost all of the radiotracer was extracted from the urine (Table VII).

The crystals obtained from the urinary extracts were substantially pure dicamba, melting at 113.5° to 114.5° C. (Fisher-Johns block), and having an ultraviolet spectrum indistinguishable from that of the compound obtained by twice recrystallizing technical dicamba from toluene (m.p., 114° to 115° C.).

The total radioactivity of the fractions was equivalent to 23,110,000 c.p.m. (Table VII), corresponding to 1.72 grams of technical dicamba and corresponding to 1.46 grams of the pure compound. Thus, the portion of the compound recovered in the crystals was 67% by weight, in excellent agreement with the 65.5% of the total radiotracer contained therein.

Inclusion of radioactivity from the mother liquor and the extract in ethyl acetate, in otherwise nonradioactive dicamba, yielded crystals with 93% of the expected radioactivity showing that most or all of the radiotracer in these fractions had been chemically unchanged. Ultraviolet spectra of all of the radioactive fractions showed no evidence of hydrolysis to 3,6-dichlorosalicylic acid. Thus, over all, little or none of the radiotracer in the urine appeared to have undergone chemical alteration, with the possible exception of reversible conjugation.

Conjugation with glucuronic acid apparently occurred to the extent of about one eighth at the level of dosage of 10,000 p.p.m. and one fifth at the level of 1000 p.p.m., on the basis of spectrophotometric data. There was no evidence of conjugation with sulfate.

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