Fate in Rats of the Radiocarbon from Ten Variously Labeled Methyl- and Dimethylcarbamate-C^{14} Insecticide Chemicals and Their Hydrolysis Products

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The radiocarbon content of expired CO_2, excrements, and certain tissues was determined, for the most part, 2 days after intraperitoneal administration to male white rats of 19 carbon-14–labeled materials, as follows: eight methylcarbamates-carbonyl-C^{14}; two dimethylcarbamates-carbonyl-C^{14}; three methyl-C^{14}-carbamates; two methylcarbamates labeled in a ring or a ring substituent; four hydrolysis products. Expired $C^{14}O_2$ accounted for 25 to 77% of the radioactivity administered as carbonyl-C^{14}-labeled carbamates. Such methylcarbamates without a para-substituent in the phenyl ring gave rise to more radioactivity in the urine and less in the tissues than those with a substituent in this position. The radioactivity-distribution pattern was specific and distinctive for each methyl- and dimethylcarbamate, for each hydrolysis product, and for each of the sites of radiocarbon in the molecule.

In certain studies on the detoxication in mammals of carbaryl (1-naphthyl methylcarbamate), Baygon (2-isopropoxyphenyl methylcarbamate), and Zectran (4-dimethylamino-3,5-xylyl methylcarbamate), it was assumed or established that the carbamate ester group was initially hydrolyzed and that the phenolic products were subsequently degraded and/or conjugated (*2*, *5*, *10*, *36*, *37*). Previous studies have shown that carbaryl is not stored in the animal body (*9*, *17*, *23*, *28*) and is so rapidly metabolized that trace amounts of carbaryl appear in the milk of treated, lactating animals (*3*, *11*, *12*, *15*, *28*, *36*).

Urinalysis has resulted in tentative identification of the following products derived from the methyl- and dimethylcarbamates used in this study: 1-naphthyl *N*-hydroxymethylcarbamate, 4-hydroxy-1-naphthyl methylcarbamate, 5-hydroxy-1-naphthyl methylcarbamate, 5,6 - dihydro - 5,6 - dihydroxy - 1 - naphthyl methylcarbamate, 1-hydroxy-5,6 - dihydro - 5,6 - dihydroxynaphthalene, 1-naphthol, and at least 11 other unidentified metabolites from carbaryl in rabbits (*22*); 1-naphthyl sulfate from carbaryl in cows (*36*), free and conjugated 1-naphthol from carbaryl in humans and guinea pigs (*2*, *36*), and free and conjugated 1-naphthol, possibly as a glucuronide, in rats (*5*); a conjugate of 2-isopropoxyphenol, presumed to be a glucuronide, from Baygon and 2-isopropoxyphenol in humans (*10*); free and conjugated 4-dimethylamino-3,5-xylenol, conjugated 2,6-dimethylhydroquinone, and unconjugated, unidentified acidic and nonacidic materials from Zectran in dogs (the conjugates probably consisted of both glucuronides and ethereal sulfates) (*37*); and two unconjugated, unidentified carbamate-metabolites from dimetilan (2-dimethylcarbamoyl-3-methyl-5-pyrazolyl dimethylcarbamate) in rats (*40*).

Metabolism of 1-naphthol and methylamine, hydrolysis products of carbaryl, has also been studied in mammals. 1-Naphthyl sulfate, 2-hydroxy-1-naphthyl sulfate, 1-naphthyl glucuronic acid, *N*-acetyl-*S*-(1-naphthyl)-cysteine, and 1,2-dihydro-2-hydroxy-1-naphthyl glucuronic acid are formed from 1-naphthol in rats (*33*). Methylamine is metabolized in mice and other mammals to yield formaldehyde, formic acid, and carbon dioxide (*30*, *35*).

The detoxication rate of methylcarbamate insecticides can be reduced by certain inhibitors. Piperonyl butoxide, α - [2 - (2 - butoxyethoxy)ethoxy] - 4,5 - methylenedioxy-2-propyltoluene, is an effective synergist for the insecticidal activity of Baygon (*25*) and greatly increases the persistence of Baygon in houseflies, *Musca domestica* L. (*32*). SKF-525A, β-diethylaminoethyl diphenylpropylacetate hydrochloride, reduces the rate of carisoprodal, *N*-isopropyl - 2 - methyl - 2 - *n* - propyl - 1,3 - propanediol dicarbamate, metabolism in rats (*19*). Both of these inhibitors are effective in reducing the rate of in vitro metabolism of carbaryl by rat liver microsomes (*22*).

Metabolism of many unsubstituted carbamates, used as drugs, also involves hydrolysis, hydroxylation, and conjugation mechanisms (*4*, *13*, *14*, *24*, *27*, *31*, *34*, *38*, *39*). When rapid and near complete in vivo hydrolysis occurs with a carbamate, the labeled carbamate and the corresponding labeled hydrolysis product yield essentially the same ultimate distribution for the radiocarbon. This has been clearly shown with ethyl-1-C^{14} carbamate, ethyl carbamate-carbonyl-C^{14}, ethanol-1-C^{14}, and sodium bicarbonate-C^{14} in mice (*31*).

Excretory products in urine from the carbamate-carbonyl-C^{14} compounds examined in this study should include phenols or pyrazolones and their conjugates approximately equivalent to that portion of the ester that is hydrolyzed (the amount of $C^{14}O_2$ from the carbonyl label). However, if a high proportion of the radiocarbon from carbamate-carbonyl-C^{14} compounds is excreted in the urine, this indicates the importance of nonhydrolytic detoxication mechanisms which form relatively stable carbamate metabolites, which may be largely associated with conjugates of hydroxylated derivatives. Many such conjugates of unsubstituted carbamates and their hydroxylated derivatives have been demonstrated in the urine of animals treated with these drugs, but no conjugates of hydroxylated derivatives of methyl- and dimethylcarbamates have been identified as yet. The carbamate ester grouping appears to be relatively resistant to in vivo hydrolysis, in many cases, and the balance of the various detoxication reactions is dependent on the organism involved and particularly on the nature of the groupings present in the molecule.

Therefore, it appeared desirable to compare the detoxication and elimination mechanisms for several methyl- and dimethylcarbamate insecticides, and fragments that might be liberated on their hydrolysis, under a standard set of conditions in a mammal. Accordingly, the following labeled compounds were administered to rats to determine their fate:

Chemical Name	Common or Trade Name	Compound No.
1-Naphthyl methylcarbamate-carbonyl-C^{14}	Carbaryl	I
2-Isopropoxyphenyl methylcarbamate-carbonyl-C^{14}	Baygon	II
3-Isopropylphenyl methylcarbamate-carbonyl-C^{14}	UC-10854	III
3,5-Diisopropylphenyl methylcarbamate-carbonyl-C^{14}	HRS-1422	IV
2-Chloro-4,5-xylyl methylcarbamate-carbonyl-C^{14}	Banol	V
4-Methylthio-3,5-xylyl methylcarbamate-carbonyl-C^{14}	Mesurol	VI
4-Dimethylamino-3,5-xylyl methylcarbamate-carbonyl-C^{14}	Zectran	VII
4-Dimethylamino-3-cresyl methylcarbamate-carbonyl-C^{14}	Matacil	VIII
1-Naphthyl methyl-C^{14}-carbamate	Carbaryl	IX
2-Isopropoxyphenyl methyl-C^{14}-carbamate	Baygon	X
2-Chloro-4,5-xylyl methyl-C^{14}-carbamate	Banol	XI
1-Naphthyl-1-C^{14} methylcarbamate	Carbaryl	XII
2-Isoprop-(1,3-C^{14})-oxyphenyl methylcarbamate	Baygon	XIII
1-Isopropyl-3-methyl-5-pyrazolyl dimethylcarbamate-carbonyl-C^{14}	Isolan	XIV
2-Dimethylcarbamoyl-3-methyl-5-pyrazolyl dimethylcarbamate-carbonyl-C^{14}	Dimetilan	XV
Sodium carbonate-C^{14}	...	XVI
Methyl-C^{14}-amine hydrochloride	...	XVII
1-Naphthol-1-C^{14}	...	XVIII
2-Isoprop-(1,3-C^{14})-oxyphenol	...	XIX

All of these compounds were administered intraperitoneally to male rats, each of which was kept individually in a metabolism cage for 48 hours. The radioactive contents of the expired carbon dioxide excrements, and certain tissues were determined. In a separate series of experiments, variously labeled samples of carbaryl and its labeled hydrolysis products were used and similar determinations of radioactivity were made only 4 hours after treatment. In a supplemental study of 48 hours' duration, carbonyl-labeled carbaryl (Compound I) was administered orally to male and female rats to determine differences between the sexes in the elimination and tissue distribution of radioactivity. Supplemental studies were also made with carbonyl-labeled Baygon (Compound II) using male rats to determine the effect, if any, of route of administration and possible metabolic inhibitors on $C^{14}O_2$ expiration.

Materials and Apparatus

Radiolabeled Compounds. The 19 C^{14}-labeled compounds listed in the tabulation above were used. Compounds I to X, inclusive, were prepared via methyl isocyanate-C^{14} according to a procedure previously described (*21*). Following preparation of methyl isocyanate-C^{14}, the respective substituted phenol was introduced into the other compartment of the break-seal reaction tube without triethylamine, and the phenol and isocyanate were made to react at 100° C. for 2 hours (*7*, *21*). Purification on a Florisil column (*21*) and recrystallization from hexane-ether mixture yielded labeled materials with infrared spectra and melting points identical with those of the respective known, nonlabeled compounds. The radiochemical purity for each of these compounds was greater than 99% as determined by thin-layer chromatography on Silica Gel G with ether-hexane (4 to 1) (*11*). The yields varied from 65 to 90%.

Other labeled carbamates were obtained from the following sources: compound XI from A. J. Lemin, The Upjohn Co., Kalamazoo, Mich.; compound XII from N. C. Leeling, Michigan State University, East Lansing, Mich.; compound XIII from J. W. Wright, World Health Organization, Geneva, Switzerland, as prepared by the Radiochemical Center, Amersham, England; compounds XIV and XV from D. P. Ryskiewich, Geigy Research Laboratories, Yonkers, N. Y. Compound XIX was obtained by alkaline hydrolysis of compound XIII and chromatographic purification on a Florisil column. Each of these compounds (XI to XV and XIX) were of greater than 98% purity, based on thin-layer chromatography and radioautography (*11*, *40*). Compounds XVI, XVII, and XVIII, and sodium acetate-1-C^{14} were obtained from the Nuclear-Chicago Corp., Des Plaines, Ill.

All labeled compounds were adjusted with their respective pure, nonlabeled compounds to a specific activity of 1.0 mc. per mmole prior to use.

Chemicals and Test Materials. Pure nonlabeled phenols and their corresponding methylcarbamates were obtained from sources previously cited (*21*) with the exception of the following compounds in the nonlabeled form: IV and its phenol component (Hooker Chemical Corp., Niagara Falls, N. Y.); V and its phenol component (Upjohn Co.); VIII and its phenol component (Chemagro Corp., Kansas City, Mo.). The scintillation mixture used in the radioactive counting contained 0.55% of 2,5-diphenyloxazole in 2-methoxyethanol–toluene mixture (1 to 2, both analytical reagent grade) (*16*). The carbon dioxide absorbent was a 2 to 1 mixture of 2-methoxyethanol and monoethanolamine (purified, Fisher Scientific Co., Fair Lawn, N. J.) (*16*). The source of the piperonyl butoxide was the U. S. Industrial Chemicals Co., New York, N. Y., and the SKF-525A came from the Smith, Kline, and French Co., Philadelphia, Pa.

White rats of the Sprague-Dawley strain, weighing 160 to 170 grams, were used. Those used for the oral experiments, the inhibitor study, and the intraperitoneal injections of compounds I, IX, XII, XVI, XVII, and XVIII were obtained from the Rolfsmeyer Farms, Madison, Wis., and those for the rest of the studies from the Berkeley Pacific Laboratories, Berkeley, Calif. The food for the rats was Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.).

Apparatus. The radioactivity was counted on a Packard Tri-Carb Scintillation spectrometer (Model 3324). Intraperitoneal administrations were made with a 0.25-ml. tuberculin syringe with a 22-gage hypodermic needle. The 2.5-liter glass metabolism unit designed for quantitative collection of expired $C^{14}O_2$, volatile bases, urine, and feces was a modification of one described by Roth *et al.* (*29*) and included an acid trap prior to the carbon dioxide absorption columns; additional columns for removing water and carbon dioxide from the influent air were omitted. One-liter glass flasks described by Kalberer and Rutschmann (*18*) were used for burning dried samples of tissues and feces.

Methods

Test Conditions. The labeled compounds were administered intraperitoneally in the abdominal region of male rats while the animals were under light ether anesthesia, using a uniform, separate dose of 7.5 μmoles per kg. Each rat received 0.25 ml. of a solution of the compound. The vehicle for administration was 2-methoxyethanol for all but two compounds: 0.01*N* sodium hydroxide was used for sodium carbonate-C^{14} (compound XVI), and water for methyl-C^{14}-amine hydrochloride (compound XVII). Immediately before and after each injection, duplicate 5- and 10-μl. aliquots were placed in scintillation vials and the radioactivity in each vial was determined by scintillation counting. In the case of isolan (compound XIV), the uniform dose was lethal; therefore, a dose of 1.9 μmoles per kg. was used immediately following the injection of 10 mg. of atropine sulfate in aqueous solution. Even with this reduced dose and pretreatment with atropine, symptoms of poisoning appeared for a short duration after treatment with isolan. Toxic symptoms also occurred with 3-isopropylphenyl methylcarbamate (compound III), which was administered without atropine, but not with any of the other compounds studied, where atropine was not used. During the course of the experiments, the rats were provided with food and water *ad libitum*.

Immediately after injection of the labeled compound, the rat was placed in the individual metabolism chamber. By means of suction, ordinary laboratory air was drawn into the chamber (at a rate of approximately 0.4 to 0.6 liter per minute) and the air effluent was passed through a bubbler containing 100 ml. of 5% hydrochloric acid and, finally, through two carbon dioxide absorption towers connected in series or in parallel. For time intervals of up to 5 hours, the towers were connected in series and each tower contained 21 ml. of the carbon dioxide absorbent; for intervals of 5 to 9 hours, the towers were connected in parallel and each contained 45 ml. of the absorbent.

In each trial, air was passed through the chamber for periods up to 48 hours, and the carbon dioxide absorbent was replaced 11 or 12 times during this period. The used absorbent was held at −20° C. in the dark up to 48 hours prior to warming to room temperature and scintillation counting. Urine was removed from the metabolism unit every 12 or 24 hours by carefully washing down the surface in contact with the urine with distilled water to make to a final volume of 30 to 70 ml. Two to three drops of aqueous formaldehyde were added to each urine sample prior to frozen storage at −20° C. Feces were collected after 24 and 48 hours and the samples were combined for combustion analysis. The hydrochloric acid bubbler was not changed during the entire experiment and its contents were discarded except in a few cases when its contents were radioassayed. In such exceptional cases, the acid solution in the bubbler was replaced every 12 hours, a 10-ml. aliquot was transferred to a scintillation vial containing 10 mg. of nonradioactive methylamine hydrochloride, and the resulting solution was evaporated to dryness. The methylamine hydrochloride residue was dissolved in 3 ml. of a 2 to 1 mixture of 2-methoxyethanol and monoethanolamine in preparation for radioactive counting.

The rats were sacrificed 4 or 48 hours after treatment. Immediately after sacrificing, a blood sample was withdrawn from the heart and transferred to a tube containing a trace of sodium heparin. This tube was centrifuged at 2° C. to separate the plasma and corpuscles and each component phase was separately kept in frozen storage. The following tissues were removed as completely as possible from each rat and weighed: bone (both femurs), brain, fat (subcutaneous layer from the lumbar region), heart, kidneys, liver, lungs, muscle (femoral), spleen, and testes. After these tissues and organs were removed, the residual rat carcass was chilled, ground with a meat grinder, and thoroughly mixed and a 10-gram portion was taken and frozen. The tissue samples were also held frozen separately at −20° C. until analyzed.

The samples were allowed to thaw under room conditions. Fat and bone samples were cut into small pieces and the other samples were reduced to a pulp by cutting. The resulting material was weighed, dried over phosphorus pentoxide in a vacuum desiccator for 96 hours or more, and reweighed to calculate the percentage dry weight. The fat samples were reduced to fine pieces by cutting and the other samples were finely ground with a mortar and pestle. All dried samples were stored in small screw-cap vials at room temperature until used.

Combustion of Tissue and Feces Samples. Samples (dry basis) of tissue and feces (50-mg.) were burned in oxygen using the procedure described by Kalberer and Rutschmann (*18*), with a few modifications. Six milliliters of a 2 to 1 mixture of 2-methoxyethanol–monoethanolamine were placed in the lower chamber of the combustion flask and this chamber was immersed in a container filled with powdered dry ice. When the combustion was complete and the flask had cooled to room temperature, the dry ice was removed, and the liquid in the lower chamber was allowed to warm up to room temperature. The flask was then shaken and the liquid was swirled over the surface of the flask bulb, then allowed to drain into the lower chamber of the flask. This operation was repeated six times over a 15-minute period. A 3.0-ml. portion of the liquid was transferred to a scintillation vial for radioactive counting.

Determination of Radioactivity and Proof of Efficiency. For radioactivity measurements, samples were transferred to scintillation vials, and the contents of each vial were adjusted to consist of the equivalent of 15 ml. of scintillation mixture and 3 ml. of a 2 to 1 mixture of 2-methoxyethanol and monoethanolamine. For counting of samples of $C^{14}O_2$ from combustion or expiration, this involved the direct addition of 3 ml. of carbon dioxide absorbent to 15 ml. of scintillation mixture. Frozen samples of urine were thawed and duplicate 0.1-ml. aliquots from the clear supernatant of each sample were directly radioassayed. The efficiency of the counting was 54 to 56% in all cases. The counts of the 5- and 10-μl. aliquots of the solution of the labeled compound, taken before and after injection into the rat, were averaged to calculate the total radioactivity administered to the rat. In the analysis of the combustion products, radioassays on duplicate samples were made, and the results were averaged and calculated in terms of micromoles per kilogram of fresh weight, based on the total carbon-14 measured (without regard to the chemical nature of the labeled compounds present).

The efficiency of the scintillation counting was unaltered by carbon dioxide absorbed in the 2-methoxyethanol-monoethanolamine mixture, even to

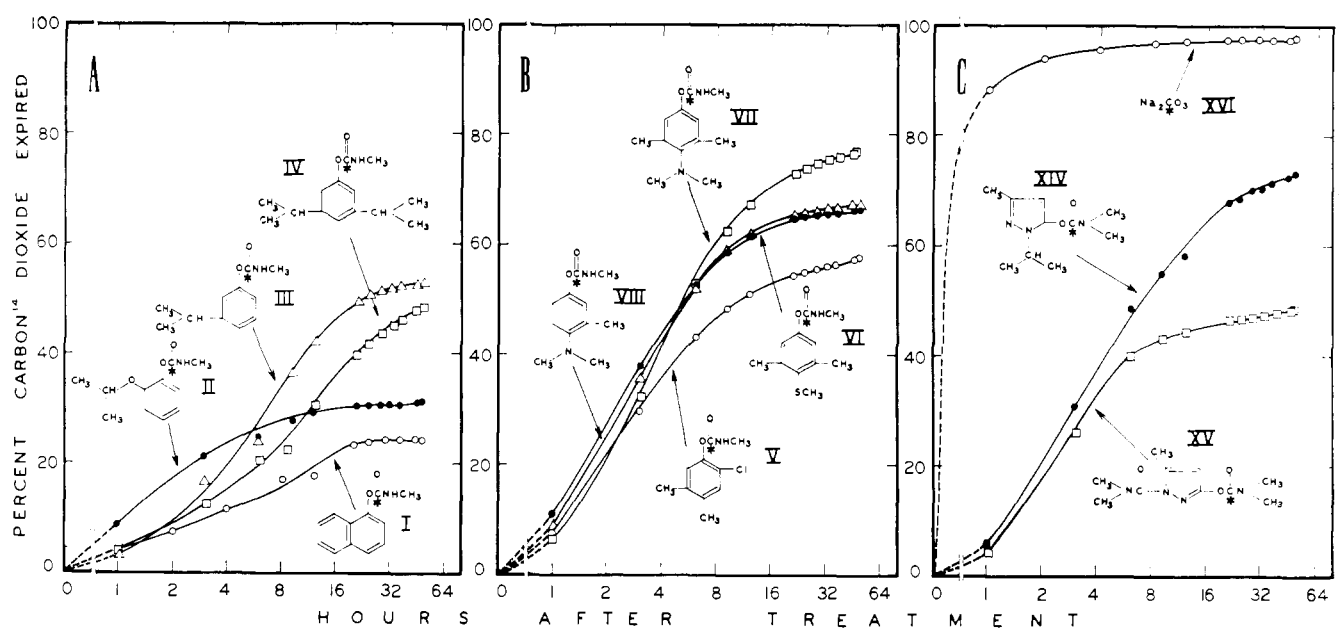

Figure 1. Carbon-14 dioxide expired from male rats treated intraperitoneally with 7.5 μmoles per kg. of carbonyl-C^{14}–labeled methylcarbamate insecticide chemicals

A. Monomethylcarbamates lacking ring substituent para to carbamate group
B. Monomethylcarbamates containing ring substituent para to carbamate group
C. Dimethylcarbamates and sodium carbonate

Roman numeral designations refer to compounds as numbered in text and tables. Results are average of two replicate experiments, with duplicate analyses on each sample

the point of saturation, as long as the solution remained clear; this is in agreement with previous experience (*16*). This was true for carbonate derived from expired air in the metabolism cages and for that resulting from combustion of the tissues. No quenching resulted from addition of up to 10 mg. of nonlabeled methylamine hydrochloride to the scintillation vial. Aliquots of 0.1 ml. from the urine samples resulted in no quenching of the scintillations.

In regard to the trapping of the expired carbon dioxide in the columns containing the 2-methoxyethanol-monoethanolamine mixture, absorption of $C^{14}O_2$ was nearly complete in the first column; over 99% of the recovered counts always appeared there. No loss of radioactivity occurred during the drying of the methylamine hydrochloride. Methyl-C^{14}-amine liberated within the metabolism cage by adding sodium hydroxide to methyl-C^{14}-amine hydrochloride, yielded 45% of the radioactivity in the hydrochloric acid trap and no radioactivity in the column containing the 2-methoxyethanol–monoethanolamine mixture. After 48 hours, the acid trap contained only 0.8% of the administered radioactivity from rats treated with methyl-C^{14}-amine hydrochloride. None was detected in tests with five other compounds.

Proof of the efficiency of the metabolism unit in quantitative trapping of the expired $C^{14}O_2$ was provided not only by the studies on carbamates and their hydrolysis products but also by a separate study with sodium acetate-1-C^{14} conducted by the same procedure, using a dose of 4.8 μmoles per kg. At different time intervals (in hours) after treatment, the respective cumulative percentage recoveries of $C^{14}O_2$ were: 0.5–34, 1–59, 2–77, 4–86, 8–89, 12–92, 24–93, 96–98. In the metabolism unit, some cross contamination of the feces by urine probably occurred, and the low level of radioactivity noted in the feces in all possibility was partially the result from such cross contamination. The degree of urine and feces separation was considered adequate for the present studies, where the primary objective was to account for the total amount of carbon-14 expired as $C^{14}O_2$ or excreted from the body as compared with that remaining in the animal.

The efficiency of the combustion technique was proved by burning known amounts of C^{14}-labeled compounds under various conditions. Recoveries of 95 to 100% of the total radioactivity resulted from combustion of sodium acetate-1-C^{14} on filter paper, and toluene-C^{14} on filter paper, powdered cellulose, purified protein, and dried, powdered samples of bone, liver, and feces from normal, untreated rats. Most of the dried tissue samples left almost no residue after combustion. Liver and, particularly, bone left a residue after combustion; these residues were reburned but no additional radioactivity was liberated, indicating that the residues were not of a carbonaceous nature. No correction was made for radioactivity losses, if any, which may have occurred in preparation and vacuum drying of the tissue samples over phosphorus pentoxide prior to combustion.

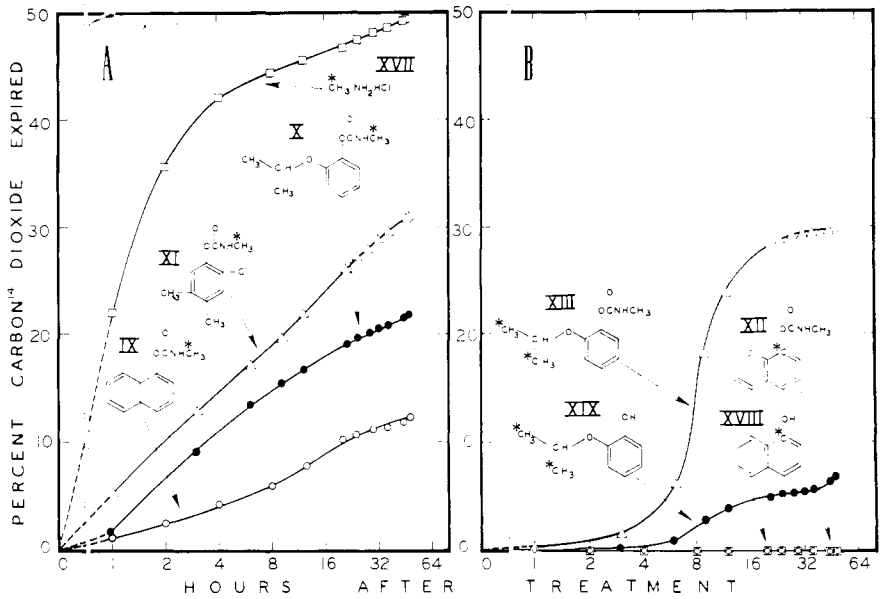

Figure 2. Carbon-14 dioxide expired from male rats treated intraperitoneally with 7.5 μmoles per kg. of C^{14}-labeled methylcarbamate insecticide chemicals and their hydrolysis products

A. Monomethyl-C^{14}-carbamates and methyl-C^{14}-amine hydrochloride
B. Monomethylcarbamates with carbon-14 in their ring or ring substituents and phenols formed on their hydrolysis
Roman numeral designations refer to compounds as numbered in the text and tables. Results are average of two replicate experiments, with duplicate analyses on each sample

Results and Discussion

Figures 1 and 2 show the rates of expiration of $C^{14}O_2$ from rats treated intraperitoneally with C^{14}-labeled methyl- and dimethylcarbamates and certain of their C^{14}-labeled hydrolysis products. Table I gives the recovery of radiocarbon in expired carbon dioxide, urine, feces, and the body of rats so treated. In Table II is shown the distribution of radiocarbon in a variety of tissues from the same rats, 48 hours after treatment. Table III gives the fate and distribution of radiocarbon in rats 4 hours after intraperitoneal injection of carbaryl-containing carbon-14 in the carbonyl, *N*-methyl, and ring position and of certain of its C^{14}-labeled hydrolysis products. Unless specifically noted otherwise, all results tabulated are the average of two replicate experiments with duplicate analyses on each sample.

Fate of Radiocarbon from Carbonyl-C^{14}-Labeled Methylcarbamates and Sodium Carbonate-C^{14} in Rats. Carbonyl-C^{14}-labeled methylcarbamates all yielded $C^{14}O_2$ when injected into male rats (Table I, Figure 1). It is assumed that the expired radioactivity originated from hydrolysis of the administered carbamate, or its metabolites, to yield labeled carbonate which was subsequently expired as $C^{14}O_2$. Sodium carbonate-C^{14} was rapidly and almost quantitatively eliminated as $C^{14}O_2$ (Figure 1, *C*), with little radioactivity appearing in the urine or feces or remaining in the body (Table I). Thus, the rate-limiting step in the elimination of $C^{14}O_2$ from the methylcarbamates-carbonyl-C^{14} (compounds I to VIII, XIV, and XV) was probably the hydrolysis of the ester group (or, less likely, the decomposition of the carbamic acid or salt intermediates, as discussed later) rather than the subsequent expiration of $C^{14}O_2$ from the liberated carbonate.

Radioactivity remaining in the body after 48 hours accounted for 2.1% of that administered for 2-isopropoxyphenyl methylcarbamate, 4.0% for 3-isopropylphenyl methylcarbamate, and 8.5 to 11.9% for the other carbonyl-C^{14} compounds. The radioactivity in the feces accounted for 2.5% or less of the administered dose with all these methylcarbamates except in the case of the 3-isopropylphenyl, the 3,5-diisopropylphenyl, and the 4-dimethylamino-3-cresyl methylcarbamates. From 86 to 98% of the administered radioactivity appeared in the urine and $C^{14}O_2$ (Table I) except with 2-chloro-4,5-xylyl methylcarbamate, where this value was only 74%. The substituents on the ring had a great influence on whether the metabolites were excreted in the urine, or were hydrolyzed and expired as $C^{14}O_2$. This difference was not as great between dimethylcarbamates (compounds XIV and

Table I. Fate of Carbon-14 in Male Rats 48 Hours after Intraperitoneal Administration of Methylcarbamate-C^{14} Insecticide Chemicals and Their Hydrolysis Products

Compound and Labeled Position	Administered Carbon-14 Recovered, %					
		Urine				
	Expired $C^{14}O_2$	0–24 hr.	24–48 hr.	Feces	Body	Total
METHYLCARBAMATE-CARBONYL-C^{14} OF:						
I. 1-Naphthol	24.5	62.1	2.4	2.1	9.5	100.6
II. 2-Isopropoxyphenol	31.2	59.8	0.7	1.2	2.1	95.0
III. 3-Isopropylphenol	52.7	43.3	1.8	5.6	4.0	107.4
IV. 3,5-Diisopropylphenol	48.7	32.4	4.7	3.0	8.5	97.3
V. 2-Chloro-4,5-xylenol	57.8	14.4	1.6	1.8	9.4	85.0
VI. 4-Methylthio-3,5-xylenol	66.1	20.1	2.2	2.5	8.9	99.8
VII. 4-Dimethylamino-3,5-xylenol	76.7	11.3	1.1	2.5	11.6	103.2
VIII. 4-Dimethylamino-3-cresol	67.2	22.9	1.7	3.8	8.8	104.4
METHYL-C^{14}-CARBAMATE OF:						
IX. 1-Naphthol	12.3	54.6	3.4	3.9	12.7	86.9
X. 2-Isopropoxyphenol	21.7	67.8	2.6	2.1	10.0	104.2
XI. 2-Chloro-4,5-xylenol	31.2	42.7	6.1	2.6	19.4	102.0
METHYLCARBAMATE OF:						
XII. 1-Naphthol-1-C^{14}	0.2	74.2	2.3	8.9	6.7	92.3
XIII. 2-Isoprop-(1,3-C^{14})-oxyphenol	29.9	71.3	0.9	0.7	3.5	106.3
DIMETHYLCARBAMATE-CARBONYL-C^{14} OF:						
XIV. 1-Isopropyl-3-methyl-5-pyrazolone	73.5	23.0	1.9	1.6	11.9	111.9
XV. 2-Dimethylcarbamoyl-3-methyl-5-pyrazolone	48.7	47.3	2.2	0.8	8.5	107.5
HYDROLYSIS PRODUCTS:						
XVI. Sodium carbonate-C^{14}	97.7	2.6	<0.1	0.4	0.8	101.5
XVII. Methyl-C^{14}-amine hydrochloride	49.5	11.8	1.5	0.6	22.4	85.8
XVIII. 1-Naphthol-1-C^{14}	<0.1	86.4	3.3	1.4	7.5	98.6
XIX. 2-Isoprop-(1,3-C^{14})-oxyphenol	6.8	76.1	1.4	3.9	4.0	92.2

Dose was 7.5 μmoles per kg. for all treatments, except for compound XIV which was 1.9 μmoles per kg. (administered with atropine sulfate). Generally results are average from two replicate experiments, with duplicate analyses on each sample; however, results other than $C^{14}O_2$ for compounds XII and XVIII and other than $C^{14}O_2$ and feces radioactivity for compounds I, IX, XVI, and XVII are each from one rat only. Sprague-Dawley white rats, weighing 160 to 170 grams, were used.

XV) and monomethylcarbamates (compounds I to VIII) as it was within the monomethylcarbamates with varying ring substituents. One interpretation of the results from the monomethylcarbamates is that a high proportion of the dose is hydrolyzed and eliminated as $C^{14}O_2$ for compounds with a para-substituent in the ring (compounds V to VIII) and that hydrolysis of the original compound or metabolites is less readily effected with a consequent higher proportion of radioactivity appearing in the urine when no para-substituent is present (compounds I to IV). However, it is not clear that the effect can be attributed solely to the position of the substituent, as its nature could also have an effect. A higher proportion of hydrolysis and elimination as $C^{14}O_2$ occurred with isolan (compound XIV) than with dimetilan (compound XV).

Radioactive materials remaining in various tissues 48 hours after administration of the carbonyl-C^{14}-labeled methylcarbamates also varied greatly with the nature of the administered compound (Table II). Reincorporation of liberated carbonate ion probably had relatively little effect on the tissue residue values found for the methylcarbamates, because sodium carbonate-C^{14} yielded much lower values for tissue retention than did any of the carbonyl-C^{14}-labeled carbamates. With only few exceptions, residual radioactivity in the tissues was higher with the methylcarbamates containing a para-substituent (compounds V to VIII) than those without this para-substituent (compounds I to IV). The dimethylcarbamate levels fell between those for the two types of monomethylcarbamates. These generalizations do not hold for the residues in bone, brain, and fat. Generally, Zectran (compound VII) yielded the highest and Baygon (compound II) the lowest persisting tissue residues (Table II); this was in agreement with the analyses on the whole animal after 48 hours (Table I). Each of the carbamates yielded much higher radioactivity in the blood corpuscles than in the plasma. The ratio of radioactive materials in the corpuscles relative to that in the plasma was much higher for the dimethylcarbamates (compounds XIV and XV) and the methylcarbamates with a para-substituent on the phenyl group (compounds V to VIII) than for the methylcarbamates lacking a para-substituent on the phenyl group (compounds I to IV). Persisting radioactivity in the spleen, liver, and kidney also varied with the type of carbamate. Levels in the liver were higher than in the spleen or kidney with the dimethylcarbamates (compounds XIV and XV) and with the methylcarbamates lacking a para-ring-substituent (compounds I to IV); radioactivity in the spleen was higher than in the liver or kidney with the methylcarbamates containing a para-ring-substituent (compounds V to VIII).

Fate of Radiocarbon from Methyl-C^{14}-carbamates and Methyl-C^{14}-amine. Results with methyl-C^{14}-carbamates are more difficult to evaluate than with methylcarbamates-carbonyl-C^{14} because administered sodium carbonate-C^{14} is almost completely expired as $C^{14}O_2$ while methyl-C^{14}-amine yields intermediate metabolites that result in persisting radioactivity in the animal (Table I, Figure 2, *A*). Only half of the administered dose of methyl-C^{14}-amine was expired as $C^{14}O_2$ and only a trace (0.8%) was recovered that might represent expired methyl-C^{14}-amine (as such). One eighth of the radioactive dose was excreted in the urine and feces, and one quarter was still present in the animal after 48 hours. The methyl-C^{14}-carbamates yielded 50 to 70% of the amount of expired $C^{14}O_2$ found with the carbonyl-C^{14}-labeled methylcarbamates. Excretion of radioactive metabolites in the urine was much higher for the methyl-C^{14}- than for the carbonyl-C^{14}-label with Banol (compounds V and XI) but this difference was less evident or was not present with Baygon (compounds II and X) and carbaryl (compounds I and IX). In each case, the methyl-C^{14}-label yielded higher radioactivity in the feces than did the carbonyl-C^{14}-label. Persisting radioactivity in the animal was higher with the methyl-C^{14}-label but it is not clear whether the chemical form remaining resulted from metabolism of the methyl-C^{14} group while still a part of the carbamate ester or while a part of methylamine or hydroxymethylamine (after hydrolysis of the ester).

Tissue radioactivity at 48 hours after treatment was much higher with methyl-C^{14}-amine than with sodium carbonate-C^{14} (Table II). Methyl-C^{14}-Banol (compound XI) yielded higher radioactivity than methyl-C^{14}-carbaryl (compound IX) or -Baygon (compound X) in all tissues except brain and fat. This same relationship appeared with the carbonyl-C^{14}-labeled compounds, with the exception of the bone and brain. Carbonyl-C^{14}- and methyl-C^{14}-labels yielded similar levels of radioactivity in the fat with carbaryl and Banol, but Baygon left high residues in fat only in the case of the methyl-C^{14}-labeled material; however, methyl-C^{14}-amine also yielded high

Table II. Distribution of Carbon-14 in Various Tissues of Male Rats 48 Hours after Intraperitoneal Administration of Methylcarbamate-C^{14} Insecticide Chemicals and Their Hydrolysis Products

Compound and Labeled Position	μmoles per Kilogram of Fresh Tissue Based on Total Carbon-14											
	Blood											
	Corpuscles	Plasma	Bone	Brain	Fat	Heart	Kidney	Liver	Lungs	Muscle	Spleen	Testes
METHYLCARBAMATE-CARBONYL-C^{14} of:												
I. 1-Naphthol	0.38	0.16	0.26	0.33	0.16	0.39	0.47	0.63	0.32	0.18	0.33	
II. 2-Isopropoxyphenol	0.69	0.17	1.27	0.64	0.03	0.26	0.27	0.48	0.25	0.08	0.35	0.07
III. 3-Isopropylphenol	0.42	0.18	0.32	0.44	0.29	0.32	0.44	0.93	0.56	0.16	0.66	0.13
IV. 3,5-Diisopropylphenol	0.40	0.14	0.28	0.44	0.41	0.35	0.66	0.76	0.28	0.15	0.39	0.12
V. 2-Chloro-4,5-xylenol	2.42	0.26	0.53	0.23	0.72	0.99	1.00	1.02	0.59	0.46	1.13	0.28
VI. 4-Methylthio-3,5-xylenol	2.75	0.65	0.47	0.58	0.49	1.19	1.00	1.32	0.95	0.54	1.68	0.34
VII. 4-Dimethylamino-3,5-xylenol	3.70	0.44	0.93	1.23	0.34	1.52	1.54	2.62	1.02	0.57	4.78	0.42
VIII. 4-Dimethylamino-3-cresol	3.08	0.35	0.57	0.52	0.25	1.00	0.99	1.34	0.91	0.51	1.51	0.30
METHYL-C^{14}-CARBAMATE OF:												
IX. 1-Naphthol	0.51	0.22	0.47	0.67	0.19	1.23	1.37	1.78	0.97	0.49	0.97	
X. 2-Isopropoxyphenol	0.50	0.43	0.44	0.43	0.95	0.95	1.31	1.62	0.85	0.55	0.91	0.55
XI. 2-Chloro-4,5-xylenol	1.40	0.83	0.95	0.65	0.71	1.80	2.06	2.52	1.88	1.22	2.22	1.05
METHYLCARBAMATE OF:												
XII. 1-Naphthol-1-C^{14}	0.02	0.02	0.19	0.03	0.18	0.06	0.17	0.09	0.05	0.03	0.26	0.09
XIII. 2-Isoprop-(1,3-C^{14})-oxyphenol	0.13	0.18	0.20	0.12	0.50	0.32	0.33	1.55	1.01	0.14	0.58	0.15
DIMETHYLCARBAMATE-CARBONYL-C^{14} OF:												
XIV. 1-Isopropyl-3-methyl-5-pyrazolone	1.14	0.17	0.41	0.34	0.13	0.51	0.71	0.81	0.28	0.23	0.58	0.23
XV. 2-Dimethylcarbamoyl-3-methyl-5-pyrazolone	1.50	0.22	0.35	0.30	0.53	0.71	0.60	1.05	0.39	0.30	0.60	0.21
HYDROLYSIS PRODUCTS:												
XVI. Sodium carbonate-C^{14}	0.02	0.06	0.17	0.03	0.08	0.05	0.10	0.02	0.09	0.04	0.12	
XVII. Methyl-C^{14}-amine hydrochloride	0.57	0.58	2.14	0.57	0.73	1.47	2.68	2.20	2.46	1.22	3.78	
XVIII. 1-Naphthol-1-C^{14}	<0.01	0.06	0.03	0.01	0.04	0.01	0.05	0.14	0.02	0.06	0.13	
XIX. 2-Isoprop-(1,3-C^{14})-oxyphenol	0.11	0.24	2.81	2.35	6.30	0.37	0.62	0.84	0.07	0.10	1.71	0.07

Dose and other considerations same as in Table I, analyses on same animals. Results of analyses from bone for compounds I, IX, XVI, XVII, and XVIII are each from one rat only. Results for Compound XIV are expressed as if the administered dose were 7.5 μmoles per kg. in accordance with the other compounds, although only 1.9 μmoles per kg. were actually administered; this expression facilitates direct comparison of the results for this compound with the other materials, if it is assumed that doses of 1.9 and 7.5 μmoles per kg. are distributed similarly and metabolized; actual values for μmoles per kg. of fresh tissue for compound XIV can be obtained by multiplying the tabulated values by 0.254.

radioactivity levels in the fat. In only a few cases was the tissue radioactivity lower with the methyl-C^{14}-labeled compounds than with the carbonyl-C^{14}-labeled compounds, and these included the corpuscles, bone, and brain with Baygon, and corpuscles with Banol (Table II). Tissues retaining high levels of radioactivity from methyl-C^{14}-amine or the methyl-C^{14}-carbamates were generally the liver, kidney, spleen, heart, and lungs.

Fate of Radiocarbon from 1-Naphthol-1-C^{14} and 2-Isoprop-(1,3-C^{14})-oxyphenol and Their Methylcarbamates. 1-Naphthol-1-C^{14} and 1-naphthyl-1-C^{14} methylcarbamate (compounds XVIII and XII, respectively) yielded almost no $C^{14}O_2$ (Table I, Figure 2, *B*). Urine contained more radioactivity from administration of naphthol (90%) than from the methylcarbamate (77%), but the feces contained more with the carbamate (8.9%) than with naphthol (1.4%). Although approximately 7% of the radioactivity remained in the animal after 48 hours, only low levels were evident in the tissues analyzed (Table II). A comparison of the residual carbon-14 from the studies with the three labeling positions clearly shows that the carbamate ester did not remain intact and that the naphthol portion was largely eliminated. Appreciable levels of the unhydrolyzed compound or its unhydrolyzed metabolites could have been present only in the bone, fat, kidney, and spleen, although the results do not necessarily indicate that compounds with the carbamate ester group intact were present even in these tissues.

2-Isoprop-(1,3-C^{14})-oxyphenol and its methylcarbamate (compounds XIX and XIII, respectively) were not distributed and eliminated in a similar way (Table I, Figure 2, *B*). The phenol yielded 7% $C^{14}O_2$ while the carbamate yielded 30% $C^{14}O_2$. In each case, approximately 75% of the administered dose appeared in the urine and approximately 4% remained in the animal. In the 48-hour period, the feces contained more radioactivity from the phenol (3.9%) than from the carbamate (0.7%). Tissue radioactivity varied considerably between the labeled isopropoxyphenol and its methylcarbamate (Table II). The phenol yielded much higher radio-

Table III. Fate and Distribution of Carbon^{14} in Tissues of Male Rats 4 Hours after Administration of Carbaryl-C^{14} and Its Hydrolysis Products

Source of Carbon-14	Dry Weight, %	μmoles per Kilogram of Fresh Tissue Based on Total Carbon-14					
		Carbaryl			Hydrolysis Products		
		Carbonyl-C^{14}	Methyl-C^{14}	Naphthyl-1-C^{14}	Sodium carbonate-C^{14}	Methyl-C^{14}-amine	1-Naphthol 1-C^{14}
Blood							
Corpuscles		1.67	1.62	0.63	0.09	0.50	0.24
Plasma		1.06	1.27	2.38	0.15	1.22	4.48
Bone	60.1	0.92	1.97	0.31	0.66	4.55	0.58
Brain	23.1	0.99	1.02	0.06	0.09	0.99	0.13
Fat	80.7	0.67	0.74	1.27	0.17	1.57	1.22
Heart	24.4	1.20	2.16	0.37	0.12	2.19	1.09
Kidney	25.7	3.11	5.80	2.04	0.26	4.48	1.83
Liver	30.8	4.56	5.60	1.96	0.74	4.23	1.31
Lungs	21.1	0.99	2.32	0.39	0.10	5.05	0.69
Muscle	26.7	0.62	1.19	0.17	0.07	2.00	1.54
Spleen	29.3	1.38	3.09	0.62	0.26	14.60	0.53
Expired $C^{14}O_2$		11.5	4.2	<0.01	95.6	42.2	<0.01
Urine		33.1	40.4	60.3	1.9	12.7	82.9
Feces	42.8	0.2	0.2	<0.01	0.2	0.5	0.03
Body	32.1	41.9	50.2	44.4	2.6	42.1	16.4
Total		86.7	95.0	104.7	100.3	97.5	99.3

Dose and other considerations same as in Table I. Dry weights are average results from rats. Results for bone, urine, feces, and body are each from one rat only.

activity at 48 hours than the carbamate for the bone, brain, fat, and spleen, whereas the reverse was true for the liver and lungs. The elimination of the radiocarbon of the isopropoxy-labeled Baygon from the animal more closely approximated that of the carbonyl-labeled or the methyl-labeled Baygon. Tissue distribution differed greatly with the three labeled samples of Baygon, and only the liver and lung retained high levels of radioactivity 48 hours after administration of the isopropoxy-labeled material.

Fate of Radiocarbon after 4 Hours from Carbon-14-Labeled Carbaryl and Its Hydrolysis Products. In a separate series of experiments, the fate of carbaryl labeled in the methyl, carbonyl, or naphthyl position and the labeled hydrolysis product of each of these materials (methyl-C^{14}-amine, sodium carbonate-C^{14}, and 1-naphthol-1-C^{14}) was determined only 4 hours after intraperitoneal injection (Table III). About 12% of the carbaryl was hydrolyzed by this time, based on $C^{14}O_2$ expiration from the carbonyl-labeled compound. Radioactivity, 42 to 50%, was still retained in the body at 4 hours with each of the labeling positions for carbaryl. The extent of elimination as $C^{14}O_2$ or of excretion of radioactivity in the urine and feces was as anticipated, based on the data from the 48-hour studies. Only about four-fifths of the radiocarbon from the naphthyl-labeled carbaryl, two-thirds from methyl-labeled carbaryl, and one-half from carbonyl-labeled carbaryl that was excreted in the urine during the 48-hour period were eliminated via this route in 4 hours. However, excretion in the urine of the radioactivity from the three labeled hydrolysis products was nearly complete in the first 4 hours. Possibly naphthol derivatives formed on rapid hydrolysis of a portion of the carbaryl dose are excreted proportionately faster than the metabolites with the carbamate group intact, which must also be excreted to explain the radioactivity in the urine from carbonyl-labeled carbaryl.

Radiocarbon found in the tissues after 4 and 48 hours was generally greater with methyl-C^{14}-amine and methyl-C^{14}-labeled carbaryl than with the other labeled materials. Exceptions appeared with the plasma and fat at 4 hours, where the naphthyl-C^{14}-labeled carbaryl yielded higher radioactivity than carbaryl labeled in the two other positions. Radioactivity in the brain, corpuscles, and many other tissues was higher with carbonyl- and methyl-labeled carbaryl than with naphthyl-labeled carbaryl, at both 4 and 48 hours. This might indicate the presence of methylcarbamoyl proteins, although the experimental data are not adequate to clarify this point. After both 4 and 48 hours, methyl-C^{14}-labeled carbaryl yielded a higher radiocarbon level in the liver and kidney than in the spleen, while methyl-C^{14}-amine yielded a high level of radiocarbon in the spleen.

Variation in Carbon-14 Dioxide Expiration from Carbonyl-C^{14}-Labeled Carbaryl and Baygon as Influenced by Route of Administration, Sex, and Possible Metabolic Inhibitors. Supplemental tests were made with carbonyl-C^{14}-labeled samples of carbaryl (compound I) and Baygon (compound II) to determine the influence of administration route, sex, and possible metabolic inhibitors on the rate and amount of $C^{14}O_2$ expiration.

In the supplemental oral administration experiments with carbonyl-C^{14}-labeled carbaryl, the following distribution of the radioactivity was found, after 48 hours:

Source of Radioactivity	Recovery of Radioactivity	
	Male	Female
$C^{14}O_2$, %	26.0	26.0
Body, %	3.4	6.5
Urine, %	64.0	72.0
Feces, %	4.0	4.0
Tissues, μmoles per kg.		
Cecum	0.17	0.60
Esophagus	0.05	0.05
Large intestine	0.20	0.30
Small intestine	0.06	0.08
Kidney	0.06	0.07
Liver	0.11	0.12
Spleen	0.05	0.08
Stomach	0.07	0.14

There was no marked difference with sex in regard to the metabolism of carbonyl-C^{14}-labeled carbaryl following oral treatment of rats. The residual radioactivity in the tissues after oral treatment differed between male and female rats only in the cecum, large intestine, and stomach; it was less than that after intraperitoneal treatment, where comparable tissues were analyzed. The expired $C^{14}O_2$ with either sex (26%) was similar to that obtained with the male rats treated intraperitoneally (Table I). Half of this amount of $C^{14}O_2$ (13%) was expired in approximately 4 hours by the male and 6 hours by the female rat following oral administration.

Details of the procedure and results with carbonyl-C^{14}-labeled Baygon have been reported by Krishna (*20*). The $C^{14}O_2$-expiration rate and amount were the same for male rats treated either intraperitoneally or orally by stomach tube; intraperitoneal administration of SKF 525-A at 50 mg. per kg. simultaneously with the Baygon, or either 1.5 or 3 hours before Baygon injection, had little, if any, effect on the $C^{14}O_2$ expiration; intraperitoneal administration of piperonyl butoxide at 1 ml. per kg. simultaneously with the Baygon appeared to have some effect in increasing the amount of $C^{14}O_2$ expiration. Additional studies on the effect of carbamate synergists and/or inhibitors on the metabolism of carbamates by mammals appear warranted.

Conclusions

Despite the lack of chemical characterization of the metabolites, other than $C^{14}O_2$, this study shows the fate in rats of the radiocarbon from 10 methyl- and dimethylcarbamate insecticide chemicals and the results make possible a partial interpretation of the nature of the metabolites. Generally, the data obtained confirm the ease of biodegradation of methyl- and dimethylcarbamate insecticides (*6*). However, certain uncharacterized fragments derived from the methylcarbamate moiety as a result of metabolism of these insecticides remain in the body for at least 48 hours. These fragments include those arising from the *N*-methyl group of the carbamate ester and from the carbonyl group of substituted-phenyl methylcarbamates with para-substituents (the same substituted-phenyl methylcarbamates which were the most completely hydrolyzed). Certain of the results suggest that "transmethylcarbamoylation" occurs in vivo. If this is the case, certain of the persisting fragments may be methylcarbamoyl proteins (*6*).

Each methyl- and dimethylcarbamate, each of the sites of the radiocarbon in the molecule, and each probable hydrolysis product yielded a different balance of the radiocarbon which was expired as $C^{14}O_2$, excreted in urine or feces, or remained in the tissues. Therefore, hydrolysis was not the only and, in some cases, not even the major detoxication mechanism involved for the methyl- and dimethylcarbamates studied. Baygon labeled in the isopropyl group (compound XIII) yielded about the same amount of $C^{14}O_2$ as Baygon labeled in the carbonyl group (compound II), and much more than isoprop-C^{14}-oxyphenol (compound XIX). In this case, ether cleavage probably preceded ester cleavage for a portion of the dose. The proportion of the injected dose of the carbonyl-C^{14}-labeled carbamates that was hydrolyzed by the male rats ranged between one-fourth for carbaryl (compound I) and one-third for Baygon (compound II) to three-fourths for isolan (compound XIV) and Zectran (compound VII). The remaining portion of the dose was, in part, retained in the body, but most of it was excreted.

The relative amounts of $C^{14}O_2$ from the different carbonyl-labeled carbamates, following administration to rats, does not appear to be related to their rate of hydrolysis in aqueous alkali (*7*, *8*), or to their stability on incubation with rat liver enzyme systems, where metabolism is dependent on a reduced nicotinamide adenine dinucleotide and several carbamate metabolites are formed from each compound (*26*).

The previous discussion has been based on the assumption that the following hydrolytic intermediates are very unstable under biological conditions: $CH_3NHCOOH$, $(CH_3)_2NCOOH$, and their *N*-hydroxymethyl derivatives, if present. The fate of these intermediates was not independently examined by directly administering them to rats. Further study will be necessary to establish whether the rate-limiting step in formation of $C^{14}O_2$ from the carbonyl- and methyl-labeled carbamates is the hydrolysis of the carbamate grouping or the subsequent degradation of the intermediates.

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INSECT METABOLISM

Metabolism of 3-Hydroxy-*N*-methyl-*cis*-crotonamide Dimethyl Phosphate (Azodrin) by Insects and Rats

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AZODRIN (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate) is a substituted vinyl-phosphate insecticide that has shown unusual effectiveness against certain lepidopterous insect pests in field trials with cotton plants (*3*). A water-soluble compound, Azodrin can function either as a contact or systemic insecticide.

Recent investigations (*1*, *6*) demonstrated that, in certain biological systems, significant quantities of a closely related compound, Bidrin (3-hydroxy-*N*,*N*-dimethyl-*cis*-crotonamide dimethyl phosphate), were converted to Azodrin by oxidative demethylation via a relatively stable *N*-methylol intermediate. Evidence indicated that further oxidation changed Azodrin to its *N*-methylol derivative, which in turn was converted in minor quantities to the unsubstituted amide derivative (*6*). Metabolic detoxification of both Bidrin and Azodrin was primarily by hydrolysis of vinyl-phosphate and methyl-phosphate bonds, and both insecticides were converted in plants to apparent conjugates that were poten-