SYMPOSIUM ON METABOLISM

Introductory remarks by Louis Lykken, University of California, Berkeley, and the address made by John E. Casida were published in the September/October issue of the Journal of Agricultural and Food Chemistry. Mr. Casida received the International Award for Research in Pesticide Chemistry at the Joint CIC-ACS Meeting in Toronto.

Metabolism of Insecticidal Methylcarbamates in Animals

H. Wyman Dorough

The life of methylcarbamate insecticides in the animal body is very short. From 70 to 80% of an administered carbamate dose is usually eliminated, primarily in the urine, by 24 hr. The compounds are metabolized by hydrolytic and oxidative mechanisms and the resulting metabolites are excreted largely as sulfate and/or glucuronide conjugates. Some carbamates are reported to be conjugated directly, forming *N*-glucuronides, and then excreted. Hydrolysis of the carbamic acid ester results in detoxication of the insecticide. Carbamate metabolites formed by oxidation may be more or less toxic than the original compound. The toxicological properties of the conjugate metabolites have not been critically evaluated. This paper reviews recent findings on carbamate metabolism in animals, with particular emphasis on their metabolism by dairy cows and their residues in the milk.

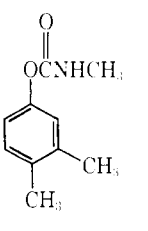
The toxicological significance of most foreign compounds entering the animal body is dependent, in part, on the manner in which they are metabolized. Some chemicals, such as strong bases, strong acids, and highly chlorinated hydrocarbons, are highly resistant to metabolism (Williams, 1963). These compounds, if toxic, would tend to retain their toxic action until excreted from the body. Usually, a chemical entering the body does undergo some type of transformation. The new chemical may be more toxic, less toxic, or equal in toxicity to the original compound. All three of these possibilities are known to occur as the result of metabolic transformation of certain carbamate insecticides by animal systems.

The carbamate insecticides are esters of carbamic acid and those discussed herein are monomethyl derivatives of such esters. The first insecticidal carbamates synthesized were dimethyl carbamates (Gysin, 1952; Wiesmann, 1951; Wiesmann *et al.*, 1951) but these did not have adequate insecticidal activity, and emphasis on the development of carbamate insecticides was placed on the more effective methylcarbamates (Casida, 1963; Fukuto, 1961; Kolbezen *et al.*, 1954). The structures and chemical and common names of some of these compounds are given below.

Although each of the compounds is a monomethyl carbamate, these three materials demonstrate that the carbamate insecticides are highly variable in their chemical makeup. Additional evidence as to the variability of the chemistry of this group may be seen by observing the list of organic insecticides compiled by Kenaga and Allison (1969). These authors list 21 carbamate insecticides, 18 of which are monomethyl derivatives of carbamic acid. Those listed represent but a small fraction of the thousands of carbamate chemicals which have been tested for their insecticidal activity during the past 20 years. Interest in these compounds as insecticides is greater than ever, as evidenced by their continued synthesis and evaluation (Fukuto *et al.*, 1969; Gilbert *et al.*, 1968; Kilsheimer *et al.*, 1969; Mahfouz *et al.*, 1969; Nikles, 1969).

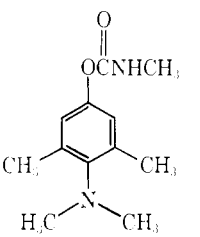
The insecticidal action of the carbamates is attributed to their ability to inhibit the nerve enzyme cholinesterase. While most authorities agree on this point, there has been some debate on whether the enzyme is inhibited primarily by carbamylation or by a complex formation reaction between carbamates and the cholinesterase (Casida *et al.*, 1960; Casida, 1963;

Meobal



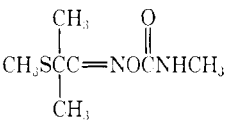
3,4-xylyl *N*-methylcarbamate

Zectran



4-dimethyl-3,5-xylyl *N*-methylcarbamate

Aldicarb



2-methyl-2-(methylthio)-propionaldehyde *O*-(methylcarbamoyl)-oxime

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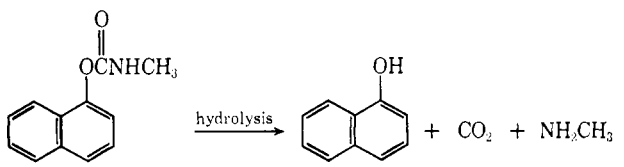
Kolbezen *et al.*, 1954; Metcalf and Fukuto, 1965; O'Brien, 1965; O'Brien *et al.*, 1966; Wilson *et al.*, 1960, 1961). In addition to being potent anticholinesterase agents, the carbamate insecticides also inhibit aliphatic esterase activity (Baron *et al.*, 1966; Plapp and Bigley, 1961).

Prior to the mid 1960's, little was known concerning the metabolism of insecticidal carbamates. As pointed out by Casida (1963), it was assumed that hydrolysis of the ester linkage was the predominant metabolic pathway. Consequently, a great deal of emphasis was placed on determining the importance of hydrolysis on the mechanism of action and efficacy of the carbamates (Carpenter *et al.*, 1961; Casida and Augustinsson, 1959; Casida *et al.*, 1960; Kolbezen *et al.*, 1954). Data from these and other studies indicated that the metabolism of carbamate insecticides involved the hydrolysis of the carbamic acid ester as exemplified below with carbaryl.



1-naphthyl *N*-methylcarbamate carbaryl

1-naphthol

Although much important information resulted from these studies, the techniques employed were of such a nature that only hydrolytic products could be detected. Thus, there was no convincing evidence that nonhydrolytic mechanisms might be important in the metabolism of the carbamates. It is not surprising, then, that residue methods for the detection of carbamate insecticides were based on the colorimetric quantitation of the phenolic moiety of the hydrolyzed compound (Dawson *et al.*, 1964; Miskus *et al.*, 1959).

In 1960, preliminary evidence was presented to support the hypothesis that pathways other than hydrolysis were involved in the metabolism of carbamate compounds (Hodgson and Casida, 1960). It was reported that many dimethylcarbamates, including several insecticides, were metabolized by a rat liver microsome system, requiring $NADPH_2$ and oxygen, to formaldehyde-yielding derivatives which in some cases appeared to be anticholinesterase agents. The nature of the metabolites and the system in which they were formed strongly suggested that oxidative pathways were evident in the metabolism of these carbamates. This view was supported by additional findings presented in a detailed report (Hodgson and Casida, 1961).

It is probably safe to say that our knowledge of the metabolism of carbamates would be only slightly improved over that of 1960 had it not been for the development and refinement of two techniques now considered basic for any metabolism study. Most important was the synthesis of carbon-14-labeled carbamates, followed very closely by the use of thin-layer chromatography. These techniques provided, for the first time, the basic requirements for conducting fate studies of the carbamate insecticides, namely, adequate sensitivity and resolution of metabolic products. As stated before, these are now standard laboratory procedures and there is no need to dwell on them at this point. The use of radiotracer techniques for studying the metabolism of insecticide chemicals has recently been reviewed by Casida (1969).

The first carbamate insecticide to be studied in detail using radiotracer techniques was carbaryl. In this case, the carbamate was radiolabeled at three different sites on the molecule with carbon-14. One preparation had carbon 1 of the naphthyl ring radioactive (Skraba and Young, 1959), another batch of carbaryl was labeled on the carbonyl carbon, and the final preparation was labeled on the *N*-methyl carbon (Krishna *et al.*, 1962). With this arrangement, each labeled preparation of carbaryl could be subjected to identical experimental parameters and the presence or absence of each labeled carbon on a particular metabolite determined. Subsequent studies using these techniques showed that the metabolism of carbaryl in rats, rabbits, and goats could involve hydrolysis, hydroxylation of the ring and of the *N*-methyl group, and conjugation (Dorough *et al.*, 1963; Dorough and Casida, 1964; Leeling and Casida, 1966).

Metabolism involving hydroxylation and conjugation mechanisms is not restricted to the carbamate insecticide carbaryl. One *in vivo* study of the fate of 19 carbamates in rats led the authors to conclude that hydrolysis was not the only pathway operating in the metabolism of carbamates and, in fact, was not always the major pathway (Krishna and Casida, 1966). An examination of the metabolism of 33 carbamate compounds by a rat liver microsome system revealed that at least one carbamate metabolite was produced from each compound (Oonnithan and Casida, 1968). Reactions which resulted in carbamate metabolites, some of which inhibited plasma-cholinesterase more than the parent material, were reported as follows: *N*-demethylation, conversion of carbamate *N*-methyl to *N*-hydroxymethyl groups, conversion of ring *N*-methyl to *N*-formamide, aromatic ring hydroxylation, formation of a dihydrodihydroxy derivative, *O*-dealkylation, alkyl hydroxylation of an aralkyl substituent, and sulfoxidation. Little more needs to be said to convince one of the complexity of the metabolism of carbamate insecticides.

The *in vivo* metabolism of carbaryl and several other methylcarbamates have been investigated in detail and their metabolic pathways in various animal species proposed. A summary of these data are presented.

1-NAPHTHYL METHYLCARBAMATE (CARBARYL)

Studies of the metabolic fate of carbaryl have been made in rats (Hassan *et al.*, 1966; Knaak *et al.*, 1965; Krishna and Casida, 1966), in the guinea pig, man, monkey, pig, sheep, and dog (Knaak *et al.*, 1965; Knaak and Sullivan, 1967; Knaak *et al.*, 1968) in chickens (Paulson *et al.*, 1970), and in dairy cows.

In rats, 25 to 30% of a carbaryl-C^{14} dose was hydrolyzed and 70 to 80% of the administered radioactivity excreted in the urine by 24 hr. The hydrolytic and carbamate metabolites were excreted primarily as sulfate and/or glucuronide conjugates. The metabolism of carbaryl was similar in all species except the dog, which did not liberate 1-naphthol or hydroxylate this carbamate. Based on the reports cited above, a metabolic pathway for carbaryl in animals may be proposed (Figure 1). Knaak *et al.* (1965) reported that a metabolite suspected of being 1-naphthyl methylimidocarbonate δ-glucuronide was a principal metabolic product of carbaryl in animals. However, further evaluation of the identity of this metabolite showed it to be 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide (Sullivan *et al.*, 1970). A minimum of 7% of a carbaryl dose to rats was excreted as this conjugate. The structures of the nonconjugated metabolites and the carbaryl-contributing portion of the conjugates have been identified based on comparative analysis of the unknowns with authentic standards prepared by the various investigators listed above, the manufacturer of carbaryl (Bartley, 1970), and by scientists in Casida's labora-

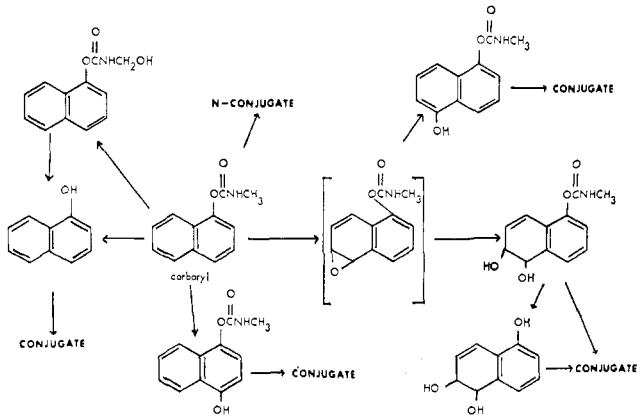


Figure 1. Proposed metabolic pathway for carbaryl in animals

tory (Balba and Casida, 1968; Balba *et al.*, 1968). In addition to the metabolites shown in Figure 1, Paulson *et al.* (1970) identified a metabolite from chickens as the 5,6-dihydroxy derivative of carbaryl. Though the pathway of carbaryl metabolism is indeed complex as now presented, several other metabolites remain unknown at the present time (Dorough, 1970).

As with other insecticidal carbamates, the least defined area in carbaryl metabolism involves the conjugated metabolites. Many metabolites suspected of being conjugates have not received even tentative identification, and few of those tentatively identified have been confirmed. Based on the current search of the literature and on recent reviews of carbamate metabolism (Ernst, 1967; Fukuto and Metcalf, 1969; Lykken and Casida, 1969), it appears that no detailed evaluation of the toxicology of any conjugate metabolite has been reported. The recently reported techniques for isolating intact conjugates of carbaryl metabolites by gel filtration should greatly facilitate investigations of this type (Paulson *et al.*, 1970).

The toxicological importance of the hydrolytic and oxidative metabolites of carbaryl is indicated by the data presented in Table I. Hydrolysis is obviously a detoxication reaction while oxidation may result in metabolites which are still biologically active. The 5-hydroxy derivative of carbaryl was more toxic to rats than the parent compound when considered on an acute oral basis. Its chronic toxicity, however, was much less than carbaryl, indicating a very rapid detoxication and/or excretion from the animal. Although these data would not appear to be of great significance in the overall toxic effect of carbaryl, they do serve to demonstrate that the toxicity of oxidative metabolites of carbamates is unpredictable. Therefore, when residues of such metabolites are formed, it is necessary that they be critically evaluated before the parent compound is deemed safe for pest control use.

Table I. Toxicity of Carbaryl and Some of Its Metabolites to Rats[a]

Compound	Acute oral LD_{50}	7-Day feeding LD_{50}	I_{50}[b]
Carbaryl	430	125–250	5×10^{-8}
4-Hydroxycarbaryl	1190	>1000	4×10^{-7}
5-Hydroxycarbaryl	297	>1000	4.6×10^{-8}
N-Hydroxymethyl carbaryl	5360	250–500	1.4×10^{-5}
1-Naphthol	2590	500–1000	1×10^{-3}

[a] Carpenter and Weil (1970). [b] Enzyme source, bovine AChE.

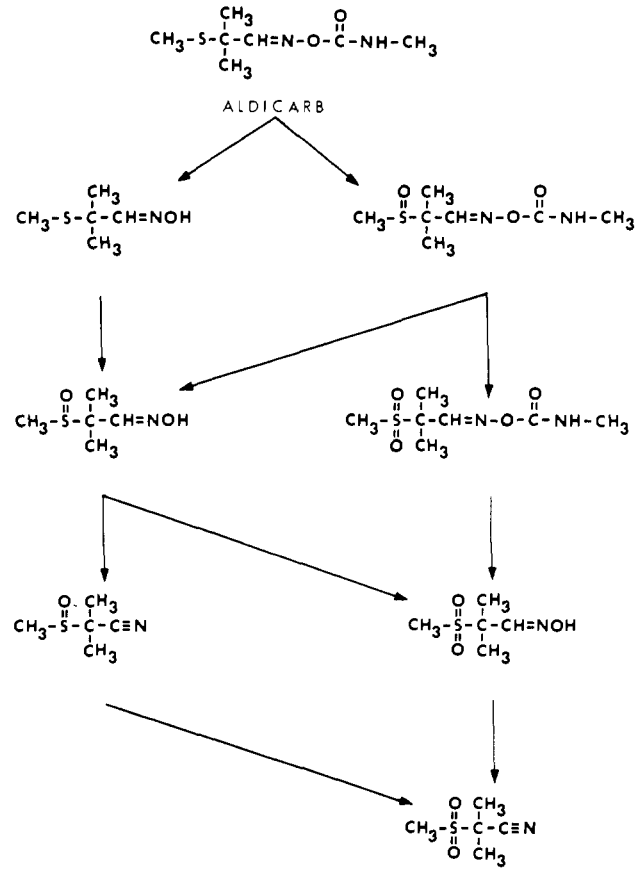


Figure 2. Proposed metabolic pathway for aldicarb in animals

2-METHYL-2-(METHYLTHIO)PROPIONALDEHYDE *O*-(METHYLCARBAMOYL) OXIME (ALDICARB)

When administered orally to rats, aldicarb was metabolized and excreted from the body very rapidly (Andrawes *et al.*, 1967; Knaak *et al.*, 1966). Over 80% of the dose was eliminated in the urine and about 4% in the feces within 24 hr after treatment. Based on the recovery of $C^{14}O_2$ from animals dosed with carbonyl-C^{14}-aldicarb, approximately 61% of the dose was hydrolyzed by 24 hr. The metabolites formed by the animals are shown in Figure 2. Although a variety of metabolites were formed, the most significant ones biologically appear to be the sulfoxide and sulfone derivatives of the carbamate. Both metabolites are more potent *in vitro* anticholinesterase agents than aldicarb (Metcalf *et al.*, 1966). In the rat, aldicarb and aldicarb sulfoxide were almost equal in their anticholinesterase activity following an oral dose of 0.33 mg per kg (Knaak *et al.*, 1966). By 6 hr, the cholinesterase activity of the rat brain and red blood cells had returned to pretreatment levels. Quantitatively, the aldicarb sulfoxide was of much greater importance than the aldicarb sulfone in the metabolism of this carbamate in rats. According to Andrawes *et al.* (1967), about 20% of the dose was excreted in the first-day urine as the sulfoxide, while less than 1% was present in the urine as the sulfone. Knaak *et al.* (1966) showed 40% of the dose in the urine as the sulfoxide and none as the sulfone. The remaining metabolites were primarily oximes and their degradation products.

The *N*-hydroxymethyl derivative of aldicarb was not reported as a metabolite in rats. However, this type of reaction was suspected in laying hens where small amounts of a material tentatively identified as the *N*-methylol form of aldicarb sulfone was found in the excreta (Hicks *et al.*, 1970). Re-

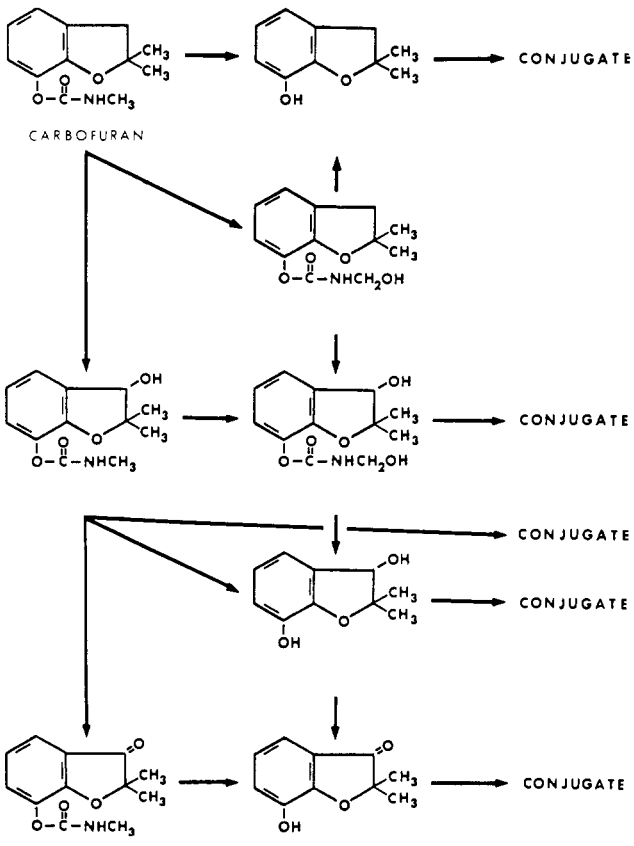


Figure 3. Proposed metabolic pathway for carbofuran in animals

cent findings by these workers suggest that this metabolite was also formed by cows. A metabolite in cow's milk and urine designated as unknown 3 by Dorough *et al.* (1970) could not be separated by tlc techniques from an authentic sample of hydroxymethyl aldicarb sulfone. Similarly, metabolites from the cow which were designated as unknowns 1 and 3a could not be separated from samples of 2-methyl-2-(methylsulfinyl)propanol and 2-methyl-2-(methylsulfonyl)-propanol, respectively. It is possible that some of the unknown aldicarb metabolites from rats could be these same materials.

2-3-DIHYDRO-2,2-DIMETHYL-7-BENZOFURANYL METHYLCARBAMATE (CARBOFURAN)

Carbofuran was metabolized by hydrolytic and oxidative mechanisms in the rat (Dorough, 1968). By 24 hr after treatment, 72% of the dose was eliminated in the urine, 2% in the feces, and about 43% of the administered dose was hydrolyzed. A metabolic pathway proposed for carbofuran based on this study is shown in Figure 3. Over 95% of the material excreted in the urine was in the form of conjugated metabolites. The major metabolite was conjugated 3-keto-carbofuran phenol while conjugated 3-hydroxycarbofuran was the predominant carbamate metabolite. Both metabolites also were present in the free form. Other metabolites shown in Figure 3 were present in lesser quantities and, with the exception of *N*-hydroxymethyl- and -3-ketocarbofuran, all were detected as free and conjugated metabolites. The two aforementioned products were not detected as conjugates.

The biological activity of the carbamate metabolites of carbofuran as estimated by inhibition of housefly-head cholinesterase was less than the parent compound but by no means could they be considered inactive. The molar I_{50} values for the isolated metabolites were reported as follows: carbofuran, 3.3×10^{-8}; 3-hydroxycarbofuran, 2.1×10^{-7}; 3-ketocarbofuran, 3.3×10^{-7}; and 3-hydroxy-*N*-hydroxymethylcarbofuran, 1.4×10^{-6} (Dorough, 1968). Metcalf *et al.* (1968) reported lower anticholinesterase activity for these metabolites, molar I_{50}'s of 10^{-6} to 10^{-5}, about the same magnitude they reported for the highly active carbamate, aldicarb, and its sulfoxide and sulfone derivatives (Metcalf *et al.*, 1966). These workers reported that *N*-hydroxymethylcarbofuran and 3-ketocarbofuran were about equal in their anticholinesterase activity.

A report on the metabolism of carbofuran in white mice (Metcalf *et al.*, 1968) showed that these animals metabolized and excreted the carbamate in a manner very similar to that reported for rats. The major pathways were identical in both species.

3,4-DIMETHYLPHENYL METHYLCARBAMATE (MEOBAL)

A thorough study of the metabolism of this insecticide showed it to be rapidly eliminated from the body when administered orally to rats (Miyamoto *et al.*, 1969). Ninety percent of the dose was eliminated in the urine and 5% in feces by 24 hr after treatment. As with the other carbamates discussed thus far, there was hydrolysis at the ester linkage, oxidation to form the *N*-hydroxymethyl derivative, and the formation of various carbamate and noncarbamate conjugates (Figure 4). These workers were successful in obtaining direct evidence for oxidation of alkyl side chains of substituted *N*-methylcarbamates. Although such a reaction was postulated earlier (Oonnithan and Casida, 1968), the identity of

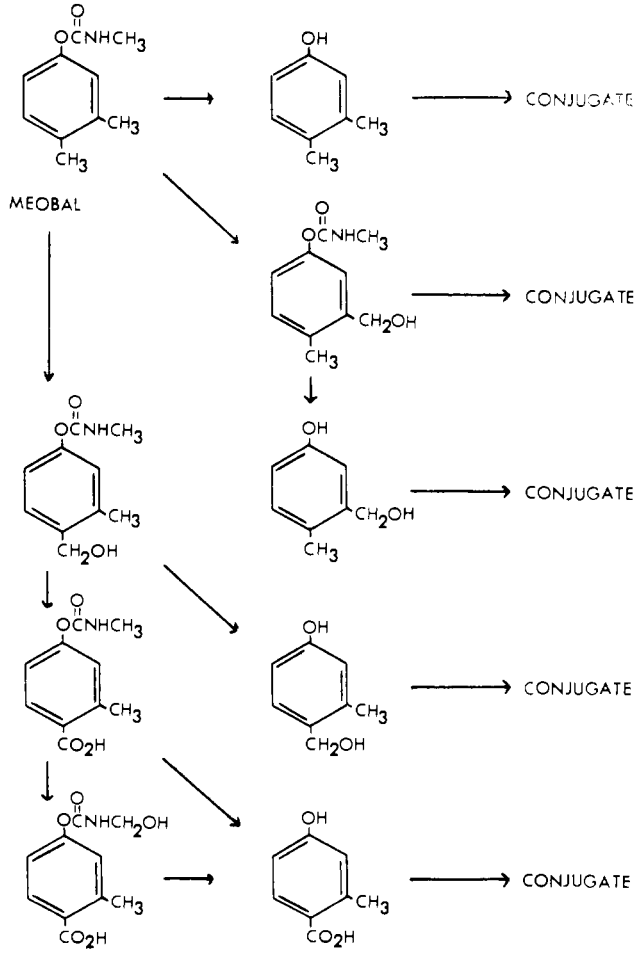
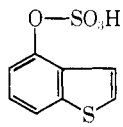


Figure 4. Proposed metabolic pathway for Meobal in animals

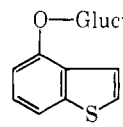
such a metabolite had not been confirmed. Both 3-methyl-4-hydroxymethylphenyl and 3-hydroxymethyl-4-methylphenyl *N*-methylcarbamate were formed in the rats and then further oxidized to the carboxy analogs (Miyamoto *et al.*, 1969). It appeared that direct hydrolysis of the original carbamate was a minor pathway, since major metabolic products were identified as 3-methyl-4-carboxyphenyl *N*-methylcarbamate, its *N*-hydroxymethyl analog, and its component phenol. The ring-hydroxymethyl derivatives and the phenols were conjugated mainly as glucuronides. *N*-Glucuronic acid conjugation did not occur. Biological activity of the carbamate metabolites was not presented.

4-BENZOTHIENYL METHYLCARBAMATE (MOBAM)

Twenty-four hours after rats were dosed orally (2 mg per kg) with radioactive Mobam, 87% of the radioactivity had been excreted in the urine and 8% of the feces (Robbins *et al.*, 1969). Excretion was slightly lower when the dose was increased to 13 mg per kg. The major metabolites in the urine were 4-benzothienyl sulfate and 4-benzothienyl glucuronide.



4-benzothienyl sulfate

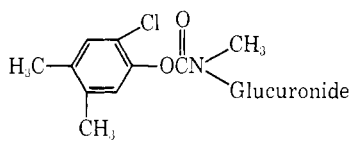


4-benzothienyl glucuronide

These metabolites accounted for 83 and 87% of the excreted radioactivity from rats dosed at 2.0 and 13.0 mg per kg, respectively. No carbamate metabolites were reported. However, the authors reported the presence of a number of unidentified metabolites and acknowledged that carbamate compounds could account for some of the unknowns. Even so, the nature of the identified metabolites made it clear that hydrolysis was the major metabolic pathway for Mobam in rats.

6-CHLORO-3,4-DIMETHYLPHENYL *N*-METHYLCARBAMATE (BANOL)

It was suggested that direct conjugation of a uronic acid moiety to Banol was a principal pathway of metabolism for this carbamate in rats (Baron and Doherty, 1967). By 48 hr, about 18% of an oral carbonyl-C-^{14}-Banol dose to rats was excreted in the urine as a metabolite thought to have the following structure.



Banol-*N*-glucuronide

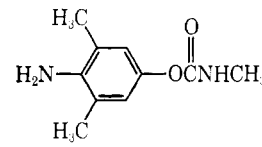
The identification of the metabolite was based on indirect evidence that showed the material was not hydrolyzed by glucuronidase, did not yield formaldehyde upon degradation, was completely alkaline degradable, and was partially acid labile. Although these characteristics of the metabolites were reportedly consistent with other *N*-glucuronic acid conjugates, final acceptance of the proposed structure must await synthesis of this compound and a comparative study with the isolated material.

The overall fate of Banol in rats was not too unlike that for the other carbamates mentioned above. Hydrolysis of the oral dose was rather rapid, 50% by 24 hr, over 90% was excreted in the 0–24 hr urine, and about 6% of the dose was excreted in the feces by 48 hr. About 35% of the administered dose was present as acidic urinary metabolites containing the intact carbamate skeletal structure C—O—C(O)NHCH$_3$. Oxidation of the ring or methyl groups on the ring was not ruled out as possible neutral metabolites or as conjugate metabolites.

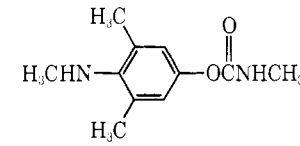
4-DIMETHYLAMINO-3,5-XYLYL METHYLCARBAMATE (ZECTRAN)

An investigation designed to identify the metabolites of Zectran in dog urine showed that hydrolysis and subsequent conjugation of the phenolic derivatives were the predominant metabolic reactions (Williams *et al.*, 1964). No unchanged Zectran was found in the urine and the only conjugated metabolite identified was 4-dimethylamino-3,5-xylenol. Sulfate and glucuronide conjugates of this phenol accounted for 86% of the urine metabolites.

Although the metabolism of Zectran in dogs appeared to be quite simple, *in vitro* studies have shown that its metabolism could be very complex. Rat liver microsomes metabolized Zectran to nine nonconjugated products, four of which were identified as carbamate materials (Oonnithan and Casida, 1968). Two of the metabolites, 4-amino-3,5-xylyl methylcarbamate and 4-methylamino-3,5-xylyl *N*-methylcarbamate, shown below, were more active anticholinesterase agents than the parent compound and were more toxic to mice (Abdel-



4-amino-3,5-xylyl *N*-methylcarbamate



4-methylamino-3,5-xylyl *N*-methylcarbamate

Wahab and Casida, 1967). The practical significance of these metabolites has yet to be defined. However, since their formation is possible biochemically, it is imperative that their existence be considered when evaluating Zectran and other similar insecticidal compounds.

A recent study of the fate of carbonyl-C^{14}-Zectran in mice showed that orally dosed animals hydrolyzed 75% of the carbamate in 48 hr (Miskus *et al.*, 1969). Though individual metabolites were not identified, the data demonstrated that hydrolytic detoxication of Zectran in mice was a major pathway. These data were part of an investigation to determine the effect of metabolic pathway on the toxicity of *N*-acetyl Zectran and were not intended as a detailed report on Zectran metabolism.

N-Acetyl Zectran (4-dimethylamino-3,5-xylyl *N*-acetyl-*N*-methylcarbamate) was first reported in 1965 (Fraser *et al.*, 1965) and has been of interest because of its selectivity. *N*-acetylation of several aryl-*N*-methylcarbamates decreased their anticholinesterase activity and reduced mammalian toxicity (Lewis, 1967; Miskus *et al.*, 1968; Reay and Lewis, 1966). With Zectran, the decreased toxicity did not extend to the spruce budworm larvae (Miskus *et al.*, 1968). This selectivity was shown to result from differences in which the carbamate was metabolized in mice and in budworm larvae (Miskus *et al.*, 1969). Like Zectran, the *N*-acetyl analog was detoxified in mice by hydrolysis of the carbamic acid ester. Approximately 72% hydrolysis occurred within 48 hr; 72% of the dose was excreted in the urine and about 1% in the

feces. Spruce budworm larvae metabolized *N*-acetyl Zectran to two toxic metabolites, Zectran and 4-methylamino-3,5-xylyl *N*-methylcarbamate.

3,4,5- AND 2,3,5-TRIMETHYLPHENYL METHYLCARBAMATE (LANDRIN)

Landrin is an insecticide containing approximately 75% of 3,4,5-trimethylphenyl methylcarbamate and 18% of the 2,3,5-isomer. Mice treated orally with 40 μg of the insecticide hydrolyzed approximately 35% of the dose within 48 hr (Slade and Casida, 1970). An equivalent amount of the dose was excreted in the urine as nonhydrolytic products. Although only a small portion of the metabolites in the urine was tentatively identified, glusulase treatment reportedly liberated ring-hydroxymethyl-dimethylphenyl methylcarbamates. The data indicated that these compounds were present as conjugates and probably as β-D-glucuronides. The *N*-hydroxymethyl derivatives of the carbamates were not detected in the living mice but were reported as metabolites in enzyme systems of mouse liver microsomes. Other metabolites were suspected of being conjugates of acids resulting from oxidation of ring-methyl groups. Since carbonyl-C^{14} insecticide was used, the products from hydrolysis of the carbamate groupings were not considered in this study.

METABOLISM IN DAIRY COWS

Based on the detection of carbaryl by the coupling reaction of *p*-nitrobenzene-diazonium fluoborate with 1-naphthol following alkaline hydrolysis of the carbamate, several workers reported that neither carbaryl nor 1-naphthol was present in the milk of treated cows (Claborn *et al.*, 1963; Gyrisco *et al.*, 1960; Whitehurst *et al.*, 1963). Although the sensitivity of the method was good, approximately 0.01 ppm, it was obvious that this colorimetric method of detection was very limited insofar as the types of metabolites that could be detected. Therefore, measurement of total carbaryl equivalents in the milk and other products as well had to be considered using a technique capable of detecting metabolites of varied chemical nature.

Treatment of a lactating goat with carbonyl-C^{14}-carbaryl demonstrated that carbamate metabolites were excreted in the milk, but that only traces of the parent compound were present (Dorough and Casida, 1964). Most of the residues were of a water-soluble nature, suggesting that they were conjugated carbamate metabolites. Several nonconjugated materials also were isolated, the major one being tentatively identified as 3,4-dihydro-3,4-dihydroxy-1-naphthyl *N*-methylcarbamate. The compound was later identified as the 5,6-dihydro-5,6-dihydroxy derivative of carbaryl (Leeling and Casida, 1966). This metabolite was insensitive to the colorimetric residue method cited above. Since carbonyl-labeled material was used to treat the goat, the fate of the naphthyl ring after hydrolysis could not be determined. However, this initial study did establish that nonhydrolytic pathways were important in the metabolism of carbaryl by lactating animals. Recently, these findings were confirmed in cows treated with carbonyl-C^{14}-carbaryl (Baron *et al.*, 1968, 1969).

The inability to detect major hydrolytic products is not the only disadvantage of using carbonyl-C^{14}-labeled carbamates in metabolic fate studies. Carbonyl-C^{14}-carbaryl and carbofuran, when administered to cows, were shown to result in the formation of radioactive naturally occurring products (Baron, 1968; Dorough and Ivie, 1968a,b). Carbon-14-labeled carbon dioxide formed by the hydrolysis of the carbamate was the apparent precursor of these radioactive components. Radioactive lactose was the principal metabolite in the milk of cows treated with carbonyl-C^{14}-labeled carbaryl and carbofuran. Because of the difficulty in interpreting results obtained with carbonyl-C^{14} carbamates, only those studies using compounds labeled at sites other than the carbamate moiety are considered in the following discussion.

Table II. Fate of Single Oral Doses of Carbamate Insecticides in Dairy Cows

Insecticide	Dose mg/kg	% of dose by 24 hr after treatment[e] Urine	Feces	Milk
Carbaryl[a]	0.3	68(0.002)	11.0(2.4)	0.25
Aldicarb[b]	0.1	83	0.6(0.2)	1.20
Carbofuran[c]	1.0	90	0.5	0.16(0.01)
Mobam[d]	1.9	87	12.0	1.0

[a] Dorough (1967). [b] Dorough and Ivie (1968). [c] Ivie and Dorough (1968). [d] Robbins *et al.* (1970). [e] The percent of the dose as the parent carbamate, if detected, is shown in parentheses.

Data in Table II show the fate of four *N*-methylcarbamates in dairy cows when administered as a single oral dose. These data show that cows metabolized and excreted the carbamates very rapidly, as did rats and other species discussed earlier. As with the other animals, the urine was the major route of elimination of the carbamates by cows. Up to 90% of the doses were excreted by this route within 24 hr after treatment. Only about 0.5% of the dose was excreted in the feces of cows treated with aldicarb or carbofuran after 24 hr, whereas approximately 12% of the carbaryl and Mobam doses were excreted in the first-24 hr feces. Elimination of the four carbamates by these two routes continued after the first 24 hr, although it proceeded at a slower rate.

Milk collected during the first 24 hr after treatment contained from 0.16% of the dose, as in the case of carbofuran, to 1.2% for aldicarb (Table II). Although rapid excretion of the carbamates in the urine undoubtedly contributed to the low residues in the milk, no definite correlation between quantities excreted in the urine or feces and that amount in the milk is apparent when all four carbamates are considered. For example, about 90% of the carbofuran and Mobam doses were excreted in the urine, but the percentage of the carbofuran dose excreted in the milk was only 1/6 that of Mobam. Similarly, excretion of the carbaryl dose in the milk constituted a lesser percentage of the dose than was found to occur with other carbamates whose total excretion far exceeded that of carbaryl. Thus, the level of total carbamate insecticide equivalents in the milk of treated cows is not a function of rapidity of excretion but must be dependent upon the nature of the individual carbamates.

Although estimates of the levels of pesticides equivalents in the milk or other products are interesting from a metabolic fate standpoint, they are of little value in establishing the toxicological importance of these residues. For this, something of their chemical nature must be known. With the carbamates and dairy cows, it is clear that only small fractions of the administered doses are excreted as the parent material (Table II). While traces of carbofuran were detected in the milk, none of the other carbamates *per se* were reported as components in the milk. Small amounts of carbaryl were in the urine and feces, and some aldicarb was detected in the feces. Since 80% or more of all the carbamates were excreted from the cows by 24 hr after treatment and little, if any, as the parent compound, it is apparent that these carbamate insecticides (Table II) are almost completely metabolized within 1 day.

RESIDUES IN MILK

The chemical nature of the metabolites located in the milk of cows treated with the carbamates listed in Table II are summarized below. Detailed analyses of the urine and feces are not considered, because these results largely just confirm the metabolic pathways already described for rats and other animals.

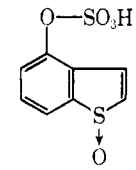
Carbaryl. Carbaryl-naphthyl-C^{14} administered orally to a cow at 0.25 mg per kg resulted in maximum residues in the milk of 0.06 ppm carbaryl equivalents (Dorough, 1967). Maximum residues in the milk when the cow was treated with 3.05 mg per kg of the carbamate was 0.95 ppm. Less than 6% of these residues were detected when colorimetric methods of analysis were employed. The predominant nonconjugated metabolite was the 5,6-dihydro-dihydroxy-carbaryl, which accounted for about 30% of the total residues in milk collected 6 hr after treatment. Other metabolites in the milk were almost entirely in the aqueous portion of the milk extract or in the milk solids. An experiment currently in progress has yielded preliminary data suggesting that these latter products were, at least in part, sulfate and glucuronide conjugates of 1-naphthol and of certain unidentified hydrolytic and carbamate metabolites (Dorough, 1970). This same study showed that the levels of residues in milk remained the same, approximately 0.2% of the daily dose, when cows were fed carbaryl-naphthol-C^{14} for 14 consecutive days.

Aldicarb. The nature of aldicarb metabolites was the same when cows were given a single oral dose of the carbamate, or when it was given daily over a 14-day period (Dorough and Ivie, 1968; Dorough *et al.*, 1970). In the 14-day study, milk from cows fed 0.12, 0.6, and 1.2 ppm aldicarb-C^{14} in the diet contained average concentrations of aldicarb-C^{14} equivalents of 1.4, 5.7, and 13.3 ppb, respectively. There was no evidence of continued buildup of residues as a result of continued exposure of the animals to aldicarb.

At least 50% of the radiolabeled metabolites was as known hydrolytic products; the major one, nitrile sulfone, accounted for 30% of the total residues in the milk. Aldicarb sulfone constituted from 15 to 19% of the residues, while the only other carbamate metabolite identified as a component of the milk, aldicarb sulfoxide, accounted for about 4%. Radioactive materials remaining in the milk solids after extraction made up 15 to 20% of the residues in the milk.

Carbofuran. Maximum radioactive residues, 0.26 ppm carbofuran equivalents, were detected in milk collected 8 hr after a cow had received a 1.0 mg per kg single oral dose of the ring-C^{14} carbamate (Ivie and Dorough, 1968). Considering all milk collected for 12 hr after treatment, 34% of the residues was carbamate metabolites, 56% was hydrolytic products, and 10% was as unidentified materials. The major product, 45% of total residues, was conjugated 3-keto-carbofuran phenol. Approximately 30% of the residues was 3-hydroxycarbofuran. Over 95% of this material was present in the free, nonconjugated form. Other carbamate metabolites were tentatively identified as 3-hydroxy-*N*-hydroxymethyl-carbofuran (2.5%) and 3-ketocarbofuran (1.3%). Carbofuran, *per se*, accounted for 0.6% of the total residues in the milk.

Mobam. Radioassay of samples collected at 8, 16, and 24 hr after a cow was given an oral dose, 1.9 mg per kg, of ring-C^{14} Mobam showed that milk contained 1.3, 0.7, and 0.1 ppm residues at each sampling time, respectively (Robbins *et al.*, 1970). Analysis of the 8-hr sample showed that 98% of the residues consisted of 4-benzothienyl sulfate 1-oxide,



4-benzothienyl sulfate 1-oxide

1% as 4-benzothienyl sulfate and the remaining as three unknown materials. Unlike carbaryl, carbofuran, and aldicarb, treatment of a cow with Mobam apparently did not result in carbamate metabolites in the milk. Furthermore, residues in milk of the Mobam-treated cow were quantitatively removed from the milk by simple methanol extraction, a situation quite unlike that encountered with the other carbamates. It was also noted by the authors (Robbins *et al.*, 1970) that the metabolism of Mobam in cows was different than in the rat. One of the major metabolites in rats, 4-benzothienyl glucuronide, was not found in the urine or milk of the cow.

CONCLUSIONS

Although a limited number of carbamate insecticides have been subjected to critical evaluations of their metabolic fate in animals, there does appear to be a general metabolic pattern which applies to this group of compounds. However, it should be remembered that simple hydrolysis of the carbamic acid ester was once assumed to be the only significant pathway in carbamate metabolism. Data are now available which show this not to be true. It will be a wise investigator who, although he uses the current information, will always approach the study of carbamate metabolism on an individual-compound basis and not be too influenced by what the carbamate is "supposed" to do in a given animal system.

Using the data which are now available, the following generalizations may be made concerning the metabolism of methylcarbamate insecticides in animals:

(1) They are rapidly metabolized by animals, with the majority of metabolic transformation occurring within 12 hr after oral administration.

(2) Excretion of the metabolites occurs very rapidly. Usually, an equivalent of 80% of the dose is eliminated in the urine and from 0.5 to 15% in the feces by 24 hr after treatment.

(3) Metabolism of the consumed carbamate is virtually complete. Little, if any, of the parent compound is present in animal excreta.

(4) Only small amounts of carbamates and their metabolites are excreted in the milk of dairy animals. Usually only 0.1% to 1% or an oral dose is excreted in the milk by 24 hr. Furthermore, the continued feeding of carbamate insecticides does not cause a continuing increase of residues in the milk, nor is the nature of the residues changed significantly.

(5) Although hydrolysis is not the only pathway involved in the metabolism of carbamates, it is by far the predominant pathway for some compounds, and must be considered an important pathway in the metabolism of all *N*-methylcarbamates studied thus far in the animal.

(6) While hydrolysis of the carbamic acid ester is an obvious detoxication reaction, oxidative metabolism of carbamates may yield products which are less toxic, just as toxic, or more toxic than the parent carbamate. Metabolites of different carbamates resulting from similar types of oxidation, such as the formation of *N*-hydroxymethyl carbamates, possess varied biological activity, depending on the individual compound involved.

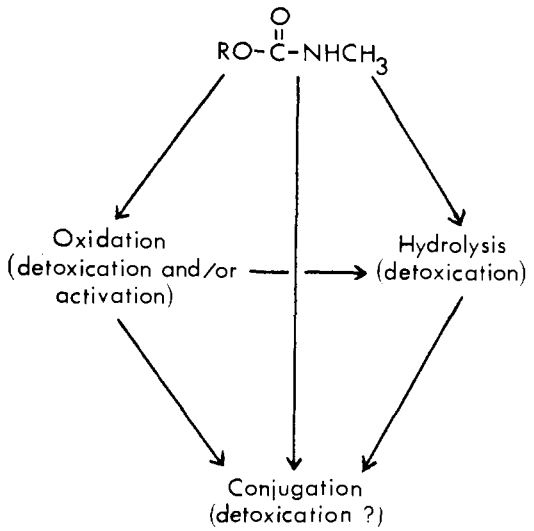


Figure 5. General metabolic scheme for carbamate insecticides in animals

(7) The majority of an oral carbamate dose is eliminated from the animal body as sulfate and/or glucuronide conjugates. Conjugation may involve the parent carbamate directly and/or its hydrolytic and oxidative metabolites.

A general metabolic scheme for carbamate insecticides in animals is shown in Figure 5. Though simple in appearance, it represents a major breakthrough insofar as carbamate metabolism is concerned. With continued study, its full implications in the action, safety, and future of carbamate insecticides will evolve.

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