products were formed by dechlorination of the double bond, but no cage compound was obtained. In the case of CIPC, addition of 2% acetone to the photolysis solution not only increased the rate of CIPC disappearance ca. 30-fold, but also gave rise to a second extractable photolysis product, identified as IBQ, in addition to 3-HOIPC.

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A New Degradation Product of the Insecticide Mexacarbate Found in Fresh Water

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The carbamate insecticide mexacarbate (4-dimethylamino-3,5-xylyl methylcarbamate) is degraded in plants and animals by oxidation of the dimethylamino group and by hydrolysis of the carbamate moiety. In water solutions of different pH values, essentially the same degradation products were found: 4-methylamino-3,5-xylyl methylcarbamate, 4-amino-3,5-xylyl methylcarbamate, 4-methylformamido-3,5-xylyl methylcarbamate, 4-formamido-3,5-xylyl methylcarbamate, and 4-dimethylamino-3,5-xylyl hydroxymethylcarbamate. Additional products identified included 4-dimethylaminoxylenol and 2-hydroxy-3,5-dimethyl-*p*-benzoquinone, a new degradation product. Bioassay of this new degradation product on sixth stage western spruce budworm (*Choristoneura occidentalis* Freeman) larvae indicated little or no toxicity at the rate of 0.1 mg/g body weight by topical application and 0.05 mg/g body weight by injection.

Mexacarbate has been used widely to control the western spruce budworm (*Choristoneura occidentalis* Freeman) in forest environments. Therefore, it is important that as many of the degradation products as possible found in water be identified so their toxicity to insects and other animals can be determined.

This paper reports the isolation and identification of some products formed from mexacarbate exposed to aquatic conditions and the toxic effects of a hydrolytic product on the western spruce budworm.

Schmiege et al. (1970) reported on the toxic effects of some of the oxidation products of mexacarbate of this highly destructive forest pest. Other studies have shown that mexacarbate is degraded by various substrates to several oxidative products. All of these products have the ring and carbamate moiety intact (Abdel-Wahab et al., 1966; Oonnithan and Casida, 1966; Abdel-Wahab and Casida, 1967; Tsukamoto and Casida, 1967; Roberts et al., 1969).

During a study of the effects of forest soil and water microorganisms on mexacarbate, Dickerson (1975) found that a pink color developed in the control flasks containing only water and mexacarbate. This observation was made several times. Colored products in the form of azo compounds have been identified by others working with anilide herbicides (Bartha and Pramer, 1967; Bartha et al., 1968; Bartha, 1968, 1969; Briggs and Ogilive, 1971). Our literature search did not reveal similar information dealing with carbamate insecticides. However, several workers have reported on the degradation of mexacarbate in aqueous environments (Knaak, 1971; Eichelberger and Lichtenberg, 1971) and the effects of mexacarbate on microbial activity in forest soil (Bollen et al., 1970) and in water and soil (Benezet and Matsumura, 1974; Dickerson, 1975).

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METHODS AND MATERIALS

Production of Degradation Products. We prepared 100 mL of 0.2% (w/v) 99.6% pure mexacarbate in tap and distilled water in solutions with differing pH values—4.5, 6.5, 7.5, and 9.5.

The buffer solution contained 2.2 mM potassium phosphate, 7.6 mM ammonium sulfate, 0.4 mM magnesium sulfate, and 1.9 mM potassium nitrate. These solutions were left exposed to air and laboratory fluorescent lighting or sunlight. At various time intervals, 10-mL aliquots were removed and a preliminary analysis was made by thin-layer chromatography (TLC) for degradation products. The water used in these studies was not sterilized because Dickerson (1975) compared the effects of sterilized and nonsterilized water and found that degradation occurred in both conditions. Development of the colored product was monitored by reading absorption at 513 mμ in a B & L Spectronic 20 spectrophotometer.

In an attempt to understand the mechanism involved in the various pathways of degradation of mexacarbate,

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Table I. Chemicals Examined for Color Production[a]

Compd no.	Common name	
1	Mexacarbate	4-Dimethylamino-3,5-xylyl *N*-methylcarbamate
2	DMAX	4-Dimethylamino-3,5-xylenol
3	Dimethyl quinone	3,5-Dimethyl-*p*-benzoquinone
4		3,5-Dimethyl-*p*-benzo-hydroxyquinone
5	AX	4-Amino-3,5-dimethylxylenol
6		4-Dimethylamino-2,6-dimethylxylenol
7		3,5-Dimethylphenol
8		2,6-Dimethylphenol
9		3,3′,5,5′-Tetramethyldi-phenoquinone
10		3,5-Dimethyl-*p*-hydroxy-benzo oxime
11		2,6-Dimethyl-*p*-benzo-hydroxyquinone
12	Matacil	4-Dimethylamino-3-toluoyl *N*-methylcarbamate
13	Mesurol	4-Methylthio-3,5-xylyl *N*-methylcarbamate
14	Landrin	3,4,5-Trimethylphenyl *N*-methylcarbamate, 75% 2,3,5-Trimethylphenyl *N*-methylcarbamate, 18%
15	SD-9077	4-Methoxy-3,5-xylyl *N*-methylcarbamate
16	4-Hydroxy mexacarbate	4-Hydroxy-3,5-xylyl *N*-methylcarbamate
17	Matacil toluenol	4-Dimethylamino-3-toluenol

[a] Compounds 4, 5, 9, 10, and 11 were synthesized by Dr. Melvin Look. The remaining compounds are commercially available.

we examined five closely related insecticides—Landrin, Mesurol, Matacil, SD-9077, and compound 16—and several suspected intermediates (Table I).

Extraction. Products were isolated from the water by extracting twice with hexane. The hexane was reduced to near dryness by rotary evaporation under reduced pressure. The remaining volume was spotted on silica gel G (0.3 mm) TLC plates and chromatographed for subsequent isolation and identification.

The colored product was then isolated from the remaining aqueous solution by adjusting the pH to 4. This process rearranged the quinone structure from the ortho (pink) to the para (yellow) positions, the latter being much more soluble in organic solvents. This solution was extracted twice with chloroform, and the chloroform fractions were combined and reduced to dryness by rotary evaporation under reduced pressure. The colored product was then isolated by sublimation at 200 mmHg and 50 °C.

Spectral Analyses. Infrared analyses of the colored product were obtained from a KBr disk on a Perkin-Elmer Model 137 Infracord Spectrophotometer. Ultraviolet analyses were obtained from ethanol and chloroform solutions from a Unicam SP 800 UV-visible spectrophotometer. Mass spectra (DuPont "CEC" 21-110) and nuclear magnetic resonance (Varian HA-100) spectral data were also obtained to confirm identification.

Thin-Layer Chromatography (TLC). TLC on 0.35-mm silica gel G in solvent A (chloroform–benzene–acetic acid, 65:45:15 v/v) was used to separate and aid identification of the colored product. The products extracted by hexane were separated by TLC with solvent B (ether–hexane–ethanol, 77:20:3 v/v) (Abdel-Wahab and Casida, 1967). Areas containing the degradation products were scrapped into vials, and the silica gel was washed with acetone or methylene chloride and the extract was analyzed by GLC. Two chromogenic reagents were used to detect degradation products on TLC plates: (1) 0.2% ninhydrin in 95% ethanol for identification of amino-containing compounds and (2) ferric chloride–potassium ferrocyanide–glacial acetic acid for identification of oxidation products.

Gas–Liquid Chromatography (GLC). GLC analysis was done on a Varian Model 2700 gas chromatograph equipped with an alkali flame ionization detector with a rubidium sulfate tip on a 92 cm × 2 mm i.d. glass column packed with 7% (w/w) QF-1 on 80–100 mesh Chromosorb W. Operating current was set at 16×10^{-12} amps/mV. Operating parameters were: column temperature, 100–175 °C; injector temperature, 210 °C; detector temperature, 190 °C; nitrogen carrier gas flow rate, 20 mL/min; air, 235 mL/min; hydrogen, 35 mL/min.

Synthesis. Several of the compounds (Table I) were not commercially available and had to be synthesized. Compound **4** (3,5-dimethyl-*p*-benzohydroquinone) was made by sodium hydrosulfite reduction of the corresponding commercially available quinone. Compound **5** (4-amino-3,5-xylenol) was synthesized by hydrogenation of 3,5-dimethyl-*p*-hydroxybenzo oxime. Compound **9** (3,3′,5,5′-tetramethyldiphenoquinone) was synthesized according to the procedures described by Auwers (1905). The 2-nitroso-5-hydroxy-1,3-dimethylbenzene (compound **10**) was synthesized by nitrosation of the 3,5-xylenol according to procedures described by Fischer (1901). Compound **11**, 2-hydroxy-3,5-dimethyl-*p*-benzoquinone, was prepared by the Thiele (1898) reaction with acetic anhydride and sulfuric acid catalyst on 3,5-dimethyl-*p*-benzoquinone, followed by base hydrolysis and ferricyanide oxidation of the intermediate trihydroxydimethylbenzene. The 2-hydroxy-3,5-dimethyl-*p*-benzoquinone was then isolated by sublimation at 200 mmHg and 50 °C.

Bioassay. The insecticidal activity of the colored product was determined on the sixth stage larvae of the western spruce budworm reared in our laboratory on an artificial diet described by Lyon et al. (1972). The compound was dissolved in acetone and applied topically in 1-μL quantities with a calibrated syringe and ISCO Model M microapplicator to the dorsum of the prothorax. Sixty insects were topically treated with six different concentrations ranging from 0.5 to 100 μg/g body weight. Forty insects were injected with seven different concentrations ranging from 0.5 to 50 μg/g body weight. Mortality was tallied at the third and seventh days after treatment.

RESULTS

Mexacarbate decomposition products identified included 4-methylamino-3,5-xylyl methylcarbamate (MA), 4-amino-3,5-xylyl methylcarbamate (A), 4-methylformamido-3,5-xylyl methylcarbamate (MF), 4-formamido-3,5-xylyl methylcarbamate (F), 4-dimethylamino-3,5-xylyl hydroxymethylcarbamate (ZOH), and 4-dimethylamino-3,5-xylenol (DMAX). The MA, A, and DMAX were confirmed by GLC analysis and cochromatography on TLC plates (Table III). The MF, F, and ZOH were confirmed only by cochromatography. Table II lists the products identified on TLC by the ninhydrin reaction and the ferric chloride–ferricyanide reaction.

A previously unreported decomposition product of mexacarbate, 2-hydroxy-3,5-dimethyl-*p*-benzoquinone (DMHQ), was also isolated from the aqueous reaction mixture. This product was identified by GLC and TLC where it chromatographed exactly coincident with known standards (Table III).

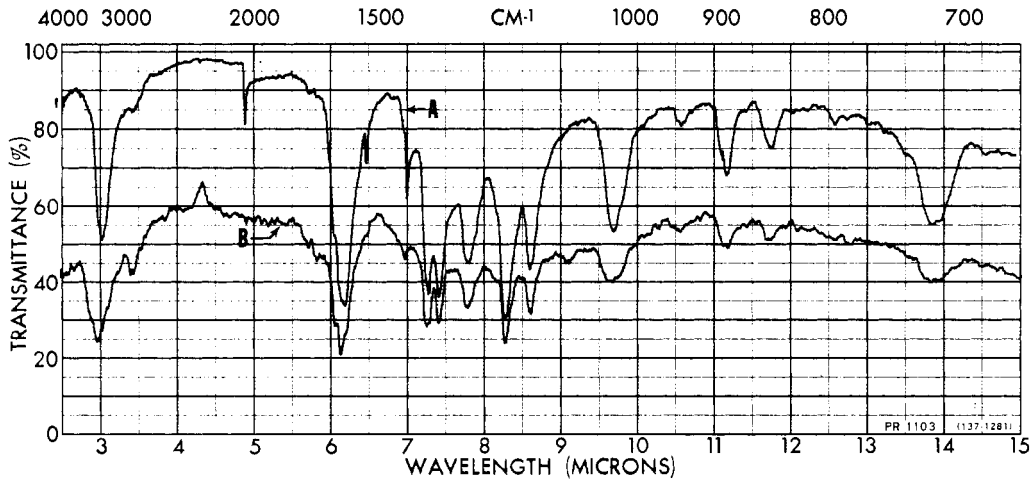


Figure 1. Infrared spectra of synthesized (A) and sample (B) of 2-hydroxy-3,5-dimethyl-*p*-benzoquinone.

Table II. Compounds Identified on TLC[a] Plates by the Ninhydrin and the Ferric Chloride-Ferricyanide-Acetic Acid Reactions

Compd no.	R_f, ninhydrin	Identification[b]	R_f, Ferric chloride-ferricyanide
1	0.94	I	0.96
2	0.88	DMAX	0.88
3	0.78[c]	DMHQ	0.80
4	0.72	Mexacarbate	0.75
5		II	0.67
6	0.58	III	
7	0.44	ZOH	0.48
8	0.40	MA	0.41
9	0.30	A	0.32
10	0.24	MF	0.27
11	0.15	IV	0.18
12		F	0.09
13	0.04	V	0.04

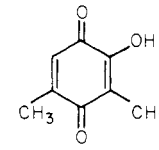
[a] TLC plates developed in solvent B. [b] Roman numerals indicate unidentified compounds. [c] The 2-hydroxy-3,5-dimethyl-*p*-hydroxyquinone was not identified by the ninhydrin reaction, but was observed on TLC plates as a purple spot before spraying.

Table III. R_f Values and Retention Times of Degradation Products of Mexacarbate Identified by TLC and GLC

Compd	R_f values solvent A	R_f values solvent B	Retention time, min Unknown	Retention time, min Standard	Col. temp, °C
A		0.32	5.5	5.5	175
MA		0.41	3.85	3.8	175
Mexacarbate	0.081	0.75	2.3	2.4	165
DMHQ	0.72	0.80[a]	2.55	2.6	100
DMAX	0.023	0.88	3.8	3.8	100

[a] R_f for acid form. R_f for base form is 0.36.

In addition, we confirmed the identity of the DMHQ by several spectral methods. The IR (Figure 1) and UV spectra of the DMHQ and of the synthesized material were identical. The NMR spectra and the melting point of the monoclinic crystals, 103 °C, of the DMHQ agreed with that reported by Sadtler (1971) (Table IV). The mass spectrum indicated a molecular weight of 152 and an elemental composition of $C_8H_8O_3$, based on high-resolution mass measurement (calcd for $C_8H_8O_3$, 152.0472; found, 152.0476). Consecutive losses of carbon monoxide from the molecular ion yielded peaks at *m/e* 124 and 96, as expected for benzoquinone (Bowie et al., 1966). The occurrence of an ion of composition $C_4H_7O_2$, *m/e* 83, ostensibly of structure $O{=}C{=}C(CH_3)C{=}O^+$, indicated a 2-methyl-3-hydroxy substitution of the benzoquinone ring (Bowie et al., 1966). We found no direct mass spectral evidence for the position of the remaining methyl group. The spectrum of the product and of the synthetic material matched within experimental error. Table IV summarizes the information obtained from NMR, UV, and mass spectra analyses. All the above information indicated the following structural formula:



We found that the formation of DMHQ would occur in both tap and distilled water over a pH range of 4 to 9.5. At pH values of 9.0 or greater, formation occurred in less than an hour. At pH 4.5 or lower, it required several days. The reaction rate depends on concentration and is optimum at pH of 9.5. Flasks held in the dark produced the DMHQ as rapidly as those held in the light, and oxygen bubbled through an aqueous solution of mexacarbate did not increase the rate of its formation.

Toxicology. Bioassay of DMHQ on western spruce budworm indicated that it does not cause mortality either by injection or topical treatment at doses as high as 100

Table IV. Physical Data for Mexacarbate Degradation Product, 2-Hydroxy-3,5-dimenthyl-*p*-benzoquinone

	NMR signals[a] CH_3	CH_3	H	OH	Mass spectra (intensity) M^+	*m/e*	*m/e*	*m/e*	Mp, °C	UV, EtOH max, nm Acid	Base
Product	1.97 (s)	2.09 (d, 1.5 Hz)	6.55 (q, 1.7 Hz)	6.89 (br)	152	124	96	83	103	261 207	224 267
Synthesized					152	124	96	83	103	261 206	223 267
Literature[b]	1.96	2.09	6.52	7.06	152				103	263 ($CHCl_3$) 402	

[a] Run in $CDCl_3$ ca. 71 °C. [b] Sadtler NMR Spectral Data (1971), No. 10756M; Morton (1965).

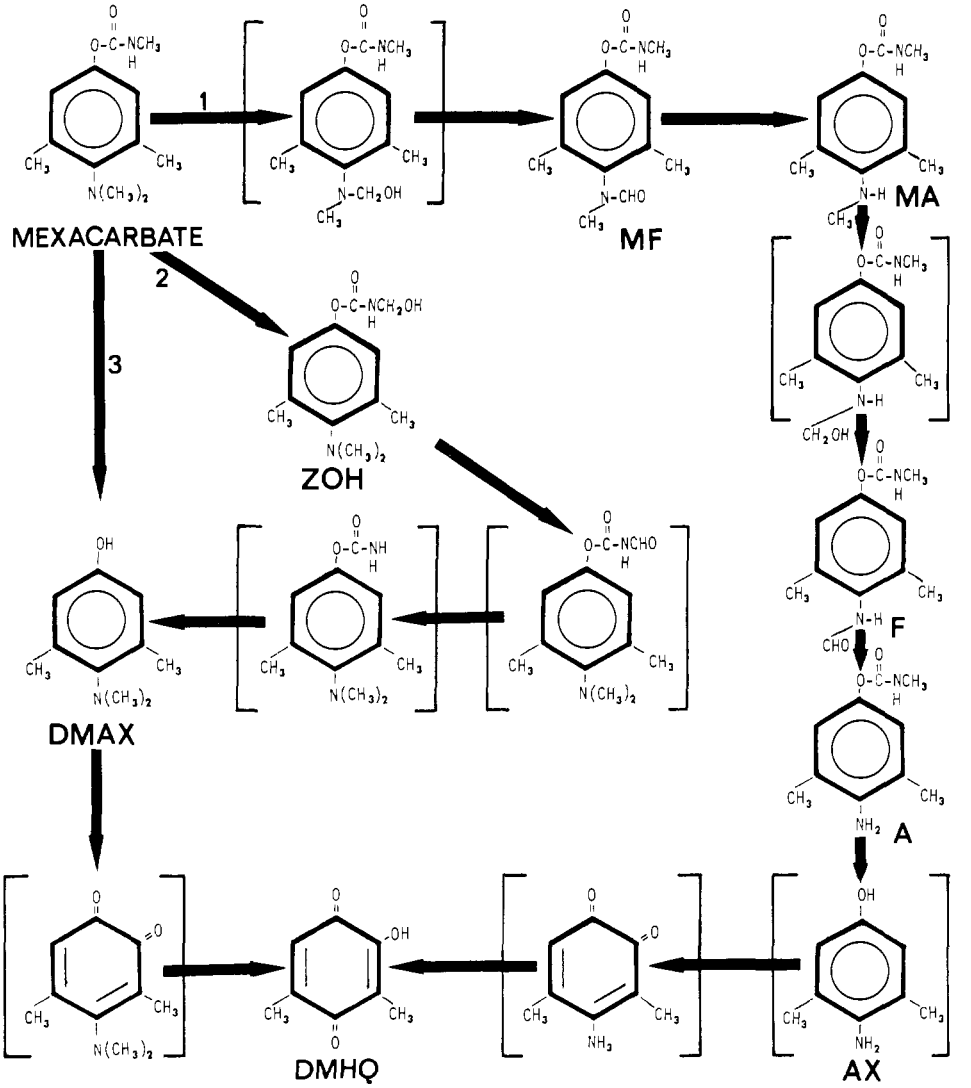


Figure 2. Postulated sequence of reactions for the degradation of mexacarbate in water.

μg/g body weight. By comparison, the LD_{90} for mexacarbate on this insect, as determined by topical application, is about 0.3 μg/g body weight.

DISCUSSION

As Meikle (1973) points out, there have been a variety of different products formed as a result of mexacarbate decomposition in plants, animals, and soil, and these products are, for the most part, common to each of these biological systems. We found most of these same products, with the exception of conjugates. These compounds arise as a result of oxidative demethylation, deamination, hydroxylation, and hydrolysis.

Two of the products of mexacarbate, 3,5-dimethylaminoxylenol and 2-hydroxy-3,5-dimethyl-*p*-benzoquinone, were formed ultimately after hydrolysis of the carbamate moiety. Williams et al. (1964a, b) found that mexacarbate degrades to a similar quinone (3,5-dimethyl-*p*-benzoquinone) in broccoli and the hydroquinone analogue occurred as a metabolite in dog urine. However, Wheeler and Strother (1971) reported no products of mexacarbate with the carbamate moiety removed in whole blood taken from dogs, humans, and rats. It is possible that some oxidation and subsequent hydrolysis occurred in these plants and animals, producing the metabolites present in the aqueous portion, perhaps including the quinone products reported by Williams et al. (1964a, b) and the DMHQ reported here.

Knaak et al. (1970) concluded that mexacarbate in water would degrade to the DMAX and the oxime. Eichelberger and Lichtenberg (1971) indicated that mexacarbate and the suspected decomposition product, DMAX, were not detected after mexacarbate disappeared in aqueous solutions and further that mexacarbate had completely disappeared in 2 weeks. An analysis of their methods leads us to conclude that it is unlikely they would have identified DMHQ, because their system of detection was selective for the xylenol or the aromatic amine. It is quite likely, however, that the DMHQ was present.

Our results are similar to those published by Eichelberger and Lichtenberg (1971). While the DMAX was produced after decomposition of mexacarbate, it seemed to be transient, as our analysis indicated, at most, only barely detectable amounts of this compound present at any one time. Consequently, after formation of the DMAX, there apparently was rapid conversion to the DMHQ. Our analysis for the oxime products of mexacarbate was negative.

Ring hydroxylation of methylcarbamate insecticides is not unusual (Dorough and Casida, 1964; Williams et al., 1964; Kuhr and Casida, 1967; Tsukamoto and Casida, 1967; Oonnithan and Casida, 1968; Knaak et al., 1970; Knaak, 1971; Paulson et al., 1972). In addition, hydroxylation of the carbamate moiety (ZOH) has been reported (Abdul-Wahab and Casida, 1967; Roberts et al., 1969). Most of these studies were conducted on animal tissues or cell

fractions, consequently, an enzyme system was probably involved in causing this hydroxylation. The ZOH and the DMHQ reported here were hydroxylated in water alone and in a wide range of pH (4.0–9.5). Therefore, it seemed to us that very mild conditions were involved to cause this hydroxylation.

In an attempt to understand the mechanism involved in the production of the DMHQ, we found that a colored product (quinone?) formed in water with compounds **1**, **2**, **5**, **7**, **8**, **11**, and **12** (Table I). Initially, we thought that the symmetry of the xylyl derivatives and an oxidizable group at the para position on the ring system were necessary for production of the product. However, since Matacil was the only insecticide, besides mexacarbate, that produced a colored product, it is obvious that a group that is easily oxidized is necessary in the para position, and the symmetry of xylyl compounds is not requisite. The substituent groups in the para position on the "xylyl" insecticides Mesurol and SD-9077 did not contribute to color production.

We have postulated a sequence of reactions for the degradation of mexacarbate in water (Figure 2). It has three pathways: Pathway 1 has been postulated through and including compound A by others (Abdel-Wahab and Casida, 1967; Roberts et al., 1969; Meikle, 1973). The fact that we produced the DMHQ from the amino xylenol (AX) (compound **5**, Table I) indicated that this reaction would continue to the DMHQ as we have postulated in pathway 1. Pathway 2 was postulated through DMAX by Meikle (1973). We concluded that pathway 3 was present because we identified the DMAX as a degradation product and produced the DMHQ from DMAX as a starting material. We feel that this pathway is not mediated by light as we found that the rate of formation of the DMHQ was the same in either dark or light conditions. Photooxidation followed by hydrolysis was probably involved in producing the products in the other two pathways.

In this study we used saturated solutions of mexacarbate in water. These concentrations would be much higher than would be found in any field spray formulation, so the chance of finding these products in the forest aquatic environment after a spray operation would be highly unlikely.

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