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Extraction of Aminocarb and a Metabolite from Whole Fish and Derivatization for Electron-Capture Gas Chromatography

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A method is presented for the detection and measurement of residues of aminocarb and one metabolite in whole fish using electron-capture gas chromatography. The procedure, a combination of unrelated extraction and derivatization procedures, had a mean recovery for aminocarb of approximately 90% and a mean recovery for the metabolite MAM [4-(methylamino)-3-methylphenyl *N*-methylcarbamate] of approximately 88%. Detection limits for aminocarb and MAM were 0.02 and 0.3 μg/g, respectively. The extraction process was straightfoward. The derivatization process was rapid, requiring only 30 min at room temperature. Derivatized residues were stable for at least 7 days if stored under appropriate conditions.

A limited number of methods for gas chromatographic analysis of carbamate pesticides and their metabolites in biological tissue samples are described in the literature (Stanley and Delphia, 1981; Szeto and Sundaram, 1980; Sundaram and Szeto, 1979; Sundaram et al., 1976; Wong and Fisher, 1975; Lau and Marxmiller, 1970). Some use nitrogen-specific detectors that permit rapid, direct assessment of these residues, but problems can arise with the inherent instability of some carbamate molecules when exposed to thermal stress (Seiber, 1972). Those employing an electron-capture detector require derivatization (reacted with trifluoroacetic anhydride, heptafluorobutyric anhydride, etc.) of the carbamate molecule to permit detection. Derivatized carbamates are more stable under heat stress (Seiber, 1972) and offer a high degree of sensitivity due to the addition of several electron-capturing components (halogens) to the molecule. However, derivatization generally requires time-consuming procedures for cleanup or for the derivatization reaction itself (Stanley and Delphia, 1981; Sundaram et al., 1976; Wong and Fisher, 1975; Lau and Marxmiller, 1970). The purpose of this paper is to describe a method used in this laboratory to evaluate aminocarb [4-(dimethylamino)-3-methylphenyl *N*-methylcarbamate] and one of its metabolites [4-(methylamino)-3-methylphenyl *N*-methylcarbamate] (MAM) in whole fish, using electron-capture gas chromatography. It combined separate, unrelated extraction and derivatization procedures found in the literature. These methods were successfully combined and provided a comparatively simple and quick analysis.

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EXPERIMENTAL SECTION

Apparatus. A Hewlett-Packard Model 5713A gas chromatograph fitted with a ^{63}Ni electron-capture detector was used. Operating conditions: injection port, 200 °C; oven, 175 °C; detector, 250 °C; carrier gas, 5% methane/95% argon; carrier gas flow rate, 50 mL/min. The column was glass, 1.8-m length, 2-mm inside diameter, packed with 3% OV-17 on Chromsorb W (HP) (Chromatographic Specialties Ltd.).

Reagents. A 0.1 M solution of trimethylamine (TMA) in benzene was used as a catalyst for the derivatization reaction. This TMA solution was prepared by adding cooled (0 °C) trimethylamine (Kodak Inc.) to cooled, tared benzene to produce a solution of 1 M. Of this solution 10 mL was diluted to 100 mL with benzene in a 100-mL volumetric flask to produce a solution of 0.1 M.

Procedure. *Extraction.* Brown bullhead (*Ictalurus nebulosus* Lesueur) of approximately 5 g were extracted according to the procedure. In a Sorval blender, fish were extracted according to Szeto and Sundaram (1980). Cleanup of the extract was achieved by extracting the aqueous phase of the procedure (phosphoric acid solution) three times with 25 mL of hexane, as done by Sundaram and Szeto (1979), and not by filtration. The extraction and cleanup were then completed according to Szeto and Sundaram (1980).

Derivatization. Fish extracts were derivatized according to the procedure of Lawrence (1976). To the residue in the vial was added 15 μL of heptafluorobutyric anhydride (HFBA) (Kodak Inc.) and 1.0 mL of the 0.1 M TMA solution. The vial was capped tightly, swirled, and allowed

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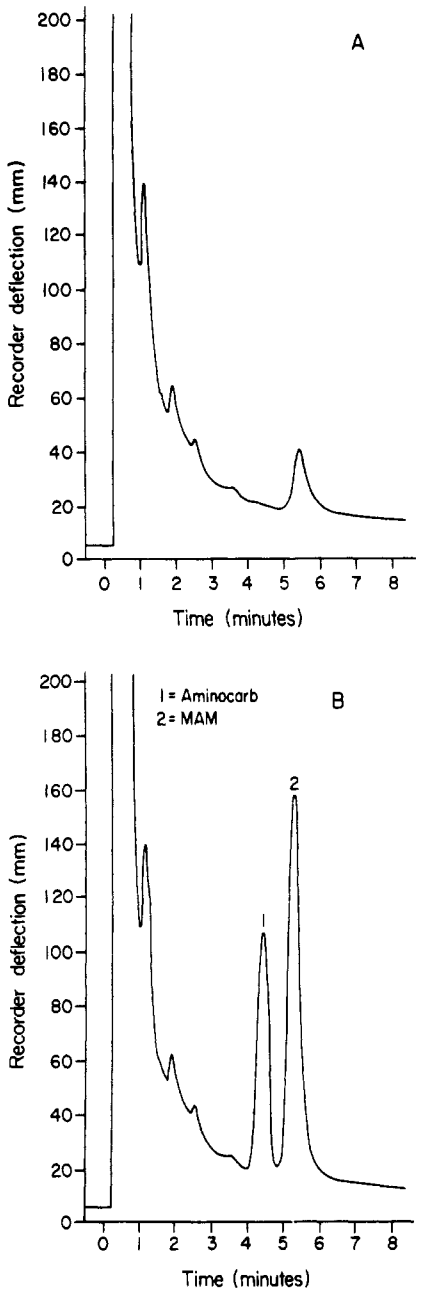

Figure 1. Gas chromatogram of a derivatized extract of a nonspiked fish (A) and a fish spiked at 1.0 μg/g with aminocarb and MAM (B).

to stand at room temperature for 30 min. Hexane (4.0 mL) and distilled water (10 mL) were then added, after which the vial was capped and shaken vigorously for 30 s. The vial was centrifuged for 5 min at about 3000 rpm to separate the phases, and a 5-μL aliquot of the organic phase was injected for analysis.

RESULTS AND DISCUSSION

Derivatization reaction time was determined by reacting 2.41 μg of aminocarb with 15 μL of HFBA and 1.0 mL of the 0.1 M TMA solution. Reagents were permitted to react for 0, 5, 10, 15, 30, 60, and 90 min. The derivatization reaction was found to be complete after 10 min; however, 30 min was permitted for all subsequent derivatizations.

To examine the influence of catalyst quantity on derivatization, 2.41 μg of aminocarb was reacted with 15 μL of HFBA for 30 min in the presence of 0, 0.1, 0.2, 0.4, 0.8, and 1.2 mL of 0.1 M TMA solution. A minimum of 0.2 mL of the TMA solution was required for complete reaction, so to ensure an excess of catalyst, 1.0 mL of TMA solution was used.

Fifteen microliters of HFBA was determined to be an adequate quantity of derivatizing agent by reacting 241.0 μg of aminocarb, in the presence of 1.0 mL of TMA solution, with 15 and 100 μL of HFBA for 30 min. No difference was observed.

The stability of HFB derivatives was assessed by storing derivatized standards under different conditions for up to 7 days. Conditions: 21, 0, and −20 °C; organic phase left in contact with the water phase, organic phase removed and dried with anhydrous sodium sulfate. Complete stability for up to 7 days was only observed with the HFB derivatives stored at −20 °C on anhydrous sodium sulfate.

The mean recovery for aminocarb from five fish spiked at 1.0 and 0.1 μg/g was 89.35 ± 0.95% (±1 SD) and 92.9 ± 8.4%, respectively. For MAM, mean recovery was 88.0 ± 1.4% and 87.9 ± 4.8% from five fish spiked at 5.0 and 0.5 μg/g, respectively. The retention times for the HFB–aminocarb and HFB–MAM were 4.0 and 4.8 min, respectively (Figure 1B). A contaminant peak evident after 5 min (Figure 1A) resulted in a lower limit of detectability for MAM of 0.3 μg/g of fish compared to the limit for aminocarb of 0.02 μg/g. This interference could be resolved with alternative liquid and/or solid phases in the GC column, although this was not attempted here.

Extraction and cleanup of aminocarb and MAM residues were simple and straightforward. Derivatization was simple and rapid with no additional heating, as was used by Sundaram et al. (1976) and Seiber (1972), nor was prolonged reaction time needed, as used by Wong and Fisher (1975). There was no problem encountered in terms of moisture inhibiting the derivatization, as implied by Stanley and Delphia (1981), and the HFB derivatives were stable for at least 7 days, contrary to Sundaram et al. (1976) and Stanley and Delphia (1981), provided they were kept at −20 °C on anhydrous sodium sulfate. The reagents were stable for more than 6 months provided that the containers were kept tightly closed and anhydrous sodium sulfate was added to the flask. It was concluded that this procedure was suitable for the EC gas chromatographic analysis of fish tissue containing aminocarb residues that possess the carbamic acid moiety.

Registry No. MAM, 10233-96-2; aminocarb, 2032-59-9.

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