gates **2A** and **3A** are not present in the bile or urine of ip-treated rats and mice.

The metabolites in bile from [*acid*-^{14}C]tetramethrin are identical with those from [^{14}C]chrysanthemic acid (i.e., three polar compounds that are probably conjugates: R_f 0.33, 0.37, and 0.43; BAW), despite large differences in the rates of biliary excretion (71% in 2 h for chrysanthemic acid and 6% in 2 h and 51% in 24 h with **1A**). A portion of these biliary conjugates probably undergoes enterohepatic circulation and cleavage prior to urinary excretion since oral (Elliott et al., 1972) and ip (current study) administration of [^{14}C]chrysanthemic acid yields 50–66% urinary radiocarbon in 24 h.

Speculation on Possible Neuropharmacological Significance of Thiol Adducts of Tetramethrin. Tetramethrin differs from other commercial pyrethroids in its uniquely high potency (3×10^{-13} M) and transient action on cockroach cercal sensory nerves (Gammon et al., 1981) and in readily adding sulfur nucleophiles. These phenomena may be related if tetramethrin undergoes reversible coupling with a critical thiol in the pyrethroid receptor site.

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Analysis of Formaldehyde in Shrimp by High-Pressure Liquid Chromatography

A method is described for the analysis of formaldehyde in shrimp. The procedure involves converting formaldehyde to its 2,4-dinitrophenylhydrazone which is analyzed by high-pressure liquid chromatography. Characteristics of the method include an estimated detection limit of 0.05 mg of formaldehyde/kg of shrimp, an average recovery of 72.3% at the 10 mg/kg level, and a total analysis time of 2 h. The results of formaldehyde analyses of fresh shrimp obtained from typical commercial outlets, and also of shrimp maintained live in a laboratory aquarium, are reported.

Recent reports of the health hazards of formaldehyde (Loomis, 1979) have stimulated an interest in methods for its analysis both in biological systems and in the environment (Janos et al., 1980; Jordan, 1980). It has long been accepted that formaldehyde develops post-mortem in marine fish and crustaceae (Sundsvold et al., 1969), probably from enzymatic reduction of trimethylamine oxide (Amano and Yamada, 1964). Thus, the analysis of seafood for formaldehyde is of particular importance.

Shrimp represent a major seafood resource. Many of the methods previously employed for the determination of formaldehyde in shrimp involve the use of specific reagents to generate colored complexes which are analyzed photometrically (Hansel and Wurziger, 1968; Sundsvold et al., 1971a; Flores and Crawford, 1973). The direct application of colorimetric methods to analyze formaldehyde in shrimp homogenates may be limited by interferences from other organic compounds. In addition, the drastic conditions used to form the complexes may result in the production of formaldehyde from trimethylamine oxide or from other amino compounds which occur naturally in shrimp (Sundsvold et al., 1971b). Isolation of formaldehyde from shrimp homogenates by distillation effectively eliminates these drawbacks, but obtaining reproducible results using this procedure appears to require considerable technique (Sundsvold et al., 1971a).

A traditional approach to the determination of low molecular weight aldehydes and ketones in foods has been to isolate them as 2,4-dinitrophenylhydrazones [for example, see Halvarson (1972)]. The mixture of 2,4-dinitrophenylhydrazones is either analyzed directly by thin-layer chromatography or converted to a mixture of the free carbonyl compounds which is analyzed by gas chromatography. Recently the separation of low molecular weight 2,4-dinitrophenylhydrazones by high-pressure liquid chromatography (HPLC) was described (Selim, 1977).

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Using this work as a basis, we have developed the procedure reported here for the analysis of formaldehyde in shrimp.

EXPERIMENTAL SECTION

Reagents and Standards. Toluene (Burdick and Jackson; "distilled in glass") was obtained free from carbonyl compounds by a procedure previously used to purify benzene (Parsons, 1966). 2,4-Dinitrophenylhydrazine (Aldrich Chemical Co.), recrystallized twice from methanol, had mp 197 °C dec, lit. mp 198 °C dec. Trichloroacetic acid (AR, Fisher Scientific Co.) was used without further purification.

The 2,4-dinitrophenylhydrazine reagent was prepared by dissolving 50.0 g of trichloroacetic acid in 100 mL of distilled water, adding 1.0 g of 2,4-dinitrophenylhydrazine, and, after most of the solid had dissolved (about 30 min), diluting to 1 L with distilled water. For removal of impurities, the reagent was stirred overnight with 300 mL of toluene, after which the aqueous phase was separated and filtered through a Whatman No. 1 paper.

Formaldehyde 2,4-dinitrophenylhydrazone (DNP), prepared as described by Vogel (1966) and recrystallized 3 times from ethanol, had mp 166 °C, lit. mp 166 °C. Acetone DNP prepared similarly had mp 128 °C, lit. mp 128 °C.

Extraction Procedure. One shrimp tail (10.0–20.0 g) was blotted dry with a paper towel, weighed, and homogenized for 2 min with 100 mL of 2,4-dinitrophenylhydrazine reagent. The homogenate was transferred into a 250-mL Erlenmeyer, and about 30 mL of toluene was added, followed by exactly 2 mL of a solution of 150 mg of acetone DNP in 1 L of toluene. The mixture was stirred magnetically for 1 h and then filtered through cheesecloth into a 250-mL separatory funnel. After filtration ceased, the cheesecloth was pressed with a spatula to expel residual toluene. The toluene layer was separated, dried with sodium sulfate, filtered through a Whatman No. 1 paper, and concentrated to about 2 mL by using a rotary evaporator.

HPLC Analysis. All HPLC analyses were conducted with a system composed of a Valco UHP-7K injector, a Tracor 995 pump, a Tracor 970A variable-wavelength detector set at 348 nm and 0.04 AUFS, a Hewlett-Packard 3380A recorder–integrator operated at attenuation ×32, an Altex 15 cm × 4.6 mm i.d. column packed with 5-μm Ultrasphere-ODS, and a Rheodyne cartridge-type guard column packed with 10-μm ODS. A sample size of 2 μL was used, with a mobile phase of methanol–water (2:1 v/v) and a flow rate of 1.1 mL/min.

Standards were prepared containing 150 mg/L acetone DNP and 35, 70, or 140 mg/L formaldehyde DNP in toluene. These are equivalent to 1, 2, or 4 mg of formaldehyde/kg of shrimp, respectively, based on a 10-g sample. Detector response to the standards was linear.

Triplicate injections were made for each extract, and average area counts were computed for the formaldehyde DNP and the acetone DNP peaks. Relative standard deviations from the mean peak areas were <5% in all cases. The formaldehyde DNP peak area was corrected for contributions from the reagent by subtracting the area counts obtained from analysis of a "blank", which was included in each set of analyses. Formaldehyde contents in milligrams per kilogram were calculated by comparing these data with those obtained for the closest standard.

$$\text{mg/kg formaldehyde in shrimp} = (\text{formaldehyde equivalents of standard in mg/kg}) \times [(\text{g of shrimp})/10](A/B)(C/D)$$

A = peak area of formaldehyde DNP in sample; B = peak area of formaldehyde DNP in standard; C = peak area of acetone DNP in standard; D = peak area of acetone DNP in sample.

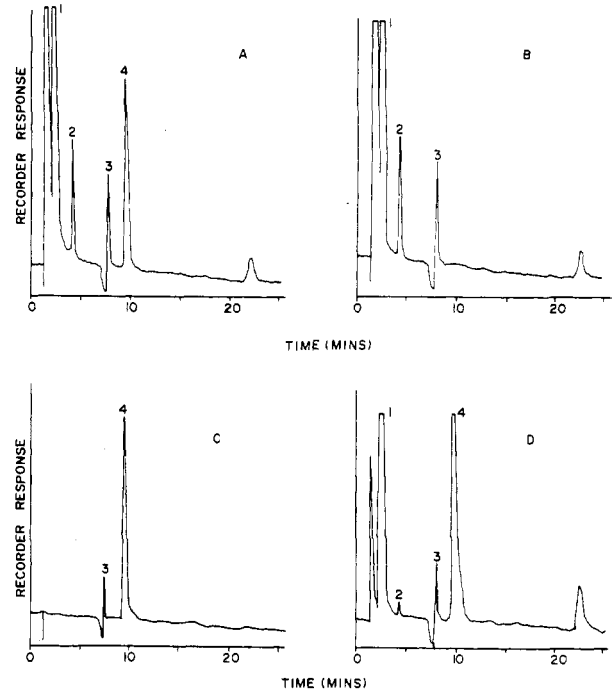

Figure 1. Liquid chromatograms of (A) shrimp extract with internal standard, (B) shrimp extract without internal standard, (C) internal standard in toluene, and (D) extract from blank analysis. Peaks were assigned as follows: (1) 2,4-dinitrophenylhydrazine; (2) formaldehyde DNP; (3) toluene; (4) acetone DNP.

Ultraviolet (UV) Spectroscopy. UV spectra were determined with a Cary 219 spectrophotometer equipped with 1-cm quartz cells. A range of 0.01 AUFS was used, and the scan rate was 0.5 nm/s. Samples were dissolved in toluene.

RESULTS AND DISCUSSION

Prior to using the present method for the routine analysis of formaldehyde in shrimp, it was necessary to establish its validity. Thus, data were obtained relating to (1) the assignment of the formaldehyde DNP peak in chromatograms of shrimp extracts, (2) interferences from the reagent and the internal standard, (3) the stability of trimethylamine oxide and related compounds under the extraction conditions, (4) the average percent recovery of added formaldehyde from shrimp homogenates, and (5) the detection limit of the method.

A liquid chromatogram obtained during an analysis of shrimp containing approximately 1 mg/kg formaldehyde is shown in Figure 1A. Peaks were assigned on the basis of retention times determined for authentic compounds. Additional support for the assignment of peak 2 to formaldehyde DNP was derived from preparative HPLC and ultraviolet spectroscopy. Identical ultraviolet spectra with maxima at 348 nm were obtained for toluene solutions of pure formaldehyde DNP and of collected material corresponding to peak 2 of a shrimp extract. An attempt to obtain further confirmatory evidence using GC–MS was unsuccessful, as the quantity of material isolated by preparative HPLC proved to be insufficient for analysis.

The suitability of acetone DNP as the internal standard was established by analyzing shrimp without the addition of the internal standard and by determining the purity of the internal standard by HPLC. The relevant chromatograms are shown in parts B and C of Figure 1, respectively.

Table I. Formaldehyde Content (Milligrams per Kilogram) of Shrimp

sample		formaldehyde[a]
market 1		0.39
market 2		1.44
market 3		1.33
market 4		0.59
live shrimp:[b]	0[c]	0.99
	1	1.12
	2	1.80
	6	2.15

[a] Averages of duplicate determinations, not corrected for extraction efficiency. [b] Species *Penaeus setiferus*. [c] Time in days since death.

A comparison of the chromatograms in parts A and B of Figure 1 confirmed that no peaks from the shrimp extract coincided with that of the internal standard. The chromatogram in Figure 1C established that the internal standard contained no impurities with the same retention as formaldehyde DNP.

Interferences from the reagent were evaluated by conducting analyses without the addition of shrimp (i.e., analyses of blanks). A typical chromatogram from analysis of a blank is shown in Figure 1D. It will be noted that peak 4, due to the internal standard, is off scale, as the chromatogram was recorded at higher recorder–integrator sensitivity (×16) than that used for shrimp analyses. At this attenuation, a small peak was present at the retention of formaldehyde DNP. Efforts to trace its origin were not successful. Small corrections were made to formaldehyde DNP peak areas during shrimp analyses to account for the blank. The correction necessary at a level of 1 mg/kg formaldehyde in shrimp was approximately 1% of the uncorrected peak area.

The stabilities of trimethylamine oxide, trimethylamine, dimethylamine, and methylamine, all of which occur naturally in shrimp, were checked under the extraction conditions. It was found that none of the four compounds was converted to formaldehyde under these conditions to a significant degree, and therefore they would not be expected to interfere with the analysis of formaldehyde in shrimp.

The average recovery of formaldehyde added to shrimp homogenate was 72.3% based on four analyses at the 10 mg/kg level. The percent recovery was not improved by using an extraction time longer than 1 h. This observation was consistent with the results of model experiments which showed that in the absence of shrimp, formation of formaldehyde DNP was >95% complete in 30 min.

The direct determination of a detection limit for the present method did not prove to be feasible as all the shrimp samples obtained contained measurable levels of formaldehyde. However, HPLC analyses of standards suggested that a detection limit of 0.05 mg/kg should be attainable.

On the basis of the data discussed above, it was concluded that the method reported here is valid for the determination of formaldehyde in shrimp.

Some typical results obtained during analyses of fresh shrimp from local commercial outlets are given in Table I. The values are comparable with those reported for similar analyses using colorimetric procedures. Formaldehyde levels are known to increase in shrimp during post-mortem storage (Flores and Crawford, 1973). Thus, differences in the data given in Table I may reflect variations in freshness between the four commercial samples.

Also included in Table I are results of analyses of ocean shrimp kept live in the laboratory. These data show that formaldehyde was present in the shrimp immediately after death, and the level slowly increased during refrigeration of the dead shrimp at 5 °C. The question of whether formaldehyde is present at significant levels in living shrimp has previously been speculated upon (Flores and Crawford, 1973). The results reported here suggest that, for shrimp maintained live in a laboratory aquarium, formaldehyde is either present in the living tissue or its development is very rapid after death.

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