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Received for review January 7, 1983. Accepted April 7, 1983. Reference to a company and/or product named by the U.S. Department of Agriculture is only for purposes of information and does not imply approval or recommendatioon of the product to the exclusion of others which may also be suitable.

Dynamic Heated Headspace Analyses of Volatile Organic Compounds Present in Fish Tissue Samples

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An analytical procedure was developed for the determination of selected volatile organic compounds in fish and shellfish tissue samples. The method employs a dynamic heated headspace isolation technique with solvent desorption of an activated carbon adsorbent media. Separation and identification, using capillary column gas chromatography with a flame ionization detector, allowed the determination of recovery values for all the compounds investigated in this study. The method was evaluated to quantitate the tissue burdens of estuarine organisms, which are reported herein.

Federal regulations, such as the Water Pollution Control Act Amendments of 1973 (*Fed. Regist.*, 1973), require the quantitation of organic chemicals in domestic and industrial wastewaters. The U.S. Environmental Protection Agency (EPA) has undertaken major studies to determine the concentration of organic pollutants in discharge water under the National Pollutant Discharge Elimination System (NPDES) permit program. Some states, such as New Jersey, have extended the scope of the effort to include the determination of fish and shellfish tissue burdens of volatile organic compounds. This action was initiated because of the commerical importance of the fishing industry in the State.

A survey of the literature showed that the acceptable Federal method (*Fed. Regist.*, 1979) for the determination of volatile organic compounds in water and wastewater was that developed by Bellar and Lichtenberg (1974). This procedure is based on a dynamic headspace isolation technique with adsorption of volatile components on a multiple adsorbent trap. Thermal desorption and carrier gas backflushing of the adsorbent trap is followed by gas chromatography for the separation and quantitation of the volatile organic compounds.

Another dynamic headspace procedure developed at Cook College, Rutgers University (Sabatino, 1981), replaces the thermal desorption and carrier as backflushing of the adsorbent trap by solvent desorption of an activated carbon trapping media (White et al., 1970) with carbon disulfide. The Rutgers method is the basis of the two techniques evaluated in this study. The advantages of the procedures evaluated are a lower cost purging apparatus, reduction of the attended time for the analysis, and the use of capillary column gas chromatography for high-resolution and analyte identification. In phase II of this study a self-contained tissue grinder/purging apparatus is evaluated.

The volatile organic compounds listed in Table I were investigated in this study. In general, the aromatic hydrocarbons studied exhibit greater water solubility than the halogenated aliphatic compounds, but both groups of compounds are less than 2% soluble in water (Ogata and Ogura, 1976). The compounds evaluated are commonly observed as contaminants in surface waters and discharge waters because of their widespread industrial use.

EXPERIMENTAL SECTION

Materials. All organic compounds and solvents used as standards were ACS-certified grade obtained from J. T. Baker and Fisher Chemical Co. Most of these compounds are flammable and/or cancer-suspect agents, and the appropriate handling procedures should be observed (Walters, 1979). Gas chromatography supplies were obtained from Supelco, Inc. (Bellefonte, PA). Glassware used in phase I was obtained from Wheaton Scientific, Vineland, NJ, from designs supplied by T. Sabatino. Glassware used in Phase II was fabricated by R. Shipmann from designs by T. Sabatino.

Procedures. *Phase I.* This procedure used a 100-mL glass purging vessel as shown in Figure 1. Ten grams of knife-cut tissue sample was placed into the vessel, which was secured in a water bath held at 50 °C. A National Institute of Occupational Safety and Health (NIOSH) (100/50 mg) type activated carbon tube (White et al., 1970) was attached to the glass exit arm of the apparatus with a Teflon bushing and a Viton (registered trademark of Du Pont) O ring. The helium purge gas was connected to a 227-mm Pasteur pipet by using 1 mm i.d. Teflon tubing, a cylindrical silicon through hole septum, and a 26 gauge syringe needle. The tissue was fortified with 100 μL of a

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0021-8561/83/1431-1057$01.50/0

Table I. Volatile Organic Compounds and Percent Recoveries

compounds	molecular weight	density, g/mL	% recoveries phase I	% recoveries phase II
carbon disulfide	76	1.26	solvent	solvent
methanol	32	0.79	–[a]	solvent
chloroform	119	1.48	65 (51-84)[b]	24 (24-27)
bromochloromethane	129	1.99	65 (51-84)	–
1,1,1-trichloroethane	133	1.34	48 (48)	32 (17-33)
1,2-dichloroethane	99	1.24	53 (48-70)	18 (2-20)
benzene	78	0.879	45 (34-60)	33 (19-34)
carbon tetrachloride	154	1.59	45 (34-60)	32 (17-33)
trichloroethylene	99	1.34	61 (43-70)	53 (53-65)
1,2-dichloropropane	113	1.16	61 (43-70)	43 (22-44)
dibromomethane	174	2.49	71 (57-97)	–
bromodichloromethane	164	1.49	59 (52-59)	28 (28-45)
toluene	92	0.867	52 (38-99)	43 (22-44)
1,1,2-trichloroethane	133	1.34	81 (59-97)	42 (26-43)
dibromochloromethane	208	2.45	73 (73)	32 (29-32)
tetrachloroethylene	168	1.54	40 (39-57)	24 (24-27)
chlorobenzene	113	1.11	54 (37-54)	41 (17-44)
ethylbenzene	106	0.867	45 (34-49)	36 (14-36)
m-xylene	106	0.864	45 (29-45)	37 (14-39)
p-xylene	106	0.861	45 (29-45)	42 (14-43)
o-xylene	106	0.88	44 (42-44)	22 (21-25)
bromoform	253	2.89	70 (70-79)	25 (2-27)
styrene	104	1.55	39 (33-39)	–
1,1,2,2-tetrachloroethane	168	1.59	47 (46-55)	–
1,3-dichlorobenzene	147	1.29	48 (48)	–
1,2-dichlorobenzene	147	1.31	39 (36-51)	–
1-chloro-3-ethoxypropane	122	–	–	8.7 (9)
1-chloro-2-ethoxyethane	108	–	–	33 (33)
1,1-dichloroethane	99	1.18	–	32 (17-33)

[a] Compound not spiked. [b] Range of percent recoveries.

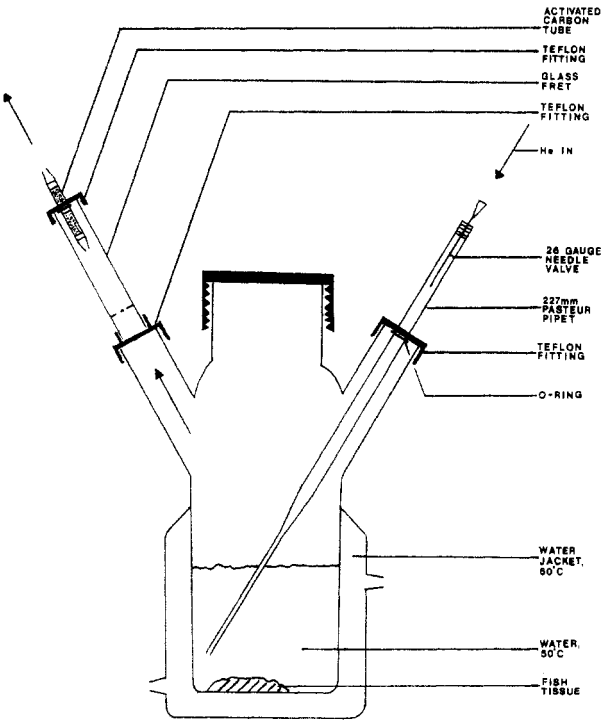

Figure 1. One hundred milliliter purge and trap vessel; developed by Thomas Sabatino, Rutgers University (Wheaton Scientific).

standard solution (10 ppm of each compound in methanol). In addition, 5 g of anhydrous sodium sulfate and 90 mL of organic-free water were placed into the purge vessel. The fortification was allowed to age for 0.5 h prior to extraction.

Helium purge gas was initiated at a flow of 100 mL/min for a 2-h interval with the sample vessel held at 50 °C in a 60 °C water bath. These conditions were monitored very closely. The carbon tube was removed and the carbon adsorbent placed into a 2-mL serum vial. A 1.9-mL volume of carbon disulfide was added to the vial to desorb the volatile organic compounds from the carbon adsorbent. The serum vial containing both the carbon disulfide and activated carbon was sealed with a Teflon-lined septum and stored at 0 °C prior to analysis. So that it could be cleaned, the purge vessel was disassembled and washed with hot tap water. The apparatus, once reassembled, was heated to 50 °C and purged for 0.5 h with a helium flow of 100 mL/min. The apparatus was then ready for another sample extraction.

Sample extracts were separated and quantitated by gas chromatography, using a Hewlett-Packard 5840A gas chromatograph equipped with a Supelco SE-54 (60 m × 0.25 mm i.d.) WCOT glass capillary column. The gas chromatograph was operated in the splitless injection mode using 1.5 atm of hydrogen head pressure (90 cm/s) and temperature programmed from an initial temperature of 50 °C, held for 10 min to a final temperature of 70 °C at 8 °C/min and held at 70 °C for 15 min. A flame ionization detector was used and set at a temperature of 300 °C with 40 mL/min nitrogen makeup gas. An external standard method of calculation was used to quantitate the results. Both external standard solutions and fortification solutions were prepared daily from a stock solution of the organic compounds (at an 8 g/L concentration).

Phase II. The glass grinder/purging apparatus used in this phase of the study is shown in Figure 2. The apparatus was placed into an Erlenmeyer flask containing water, which was heated to 80 °C by using a hot plate. Two grams of tissue and 1 g of anhydrous sodium sulfate (a grinding aid) were placed into the lower portion of the apparatus. The short, purge gas inlet arm of the vessel was plugged with a solid length of 6-mm o.d. with an O ring and a Teflon bushing glass rod during the grinding operation. An activated carbon trap was secured with an O ring and a Teflon bushing to the longer arm. The tissue sample was fortified with 100 μL of an eight-ppm solution

Table II. Sample Results[a] (Concentrations in ppm)

	1,2-dichloroethane	benzene, carbon tetrachloride	*m*-xylene, *p*-xylene	1,2-dichlorobenzene
site A				
Tylosurus marinus	0.73	0.69	–[b]	–
Menidia menidia	1.09	0.69	0.1	0.3
Modiolus demissus	–	0.45	0.1	–
site B				
Paleomonetes pugio	0.59	0.68	0.2	0.33
Modiolus demissus	–	0.58	0.14	–
site C				
Fundulus sp.	3.2	1.03	–	–
Menidia menidia	–	0.99	0.18	–

[a] Sample size equals 2. [b] Not determined.

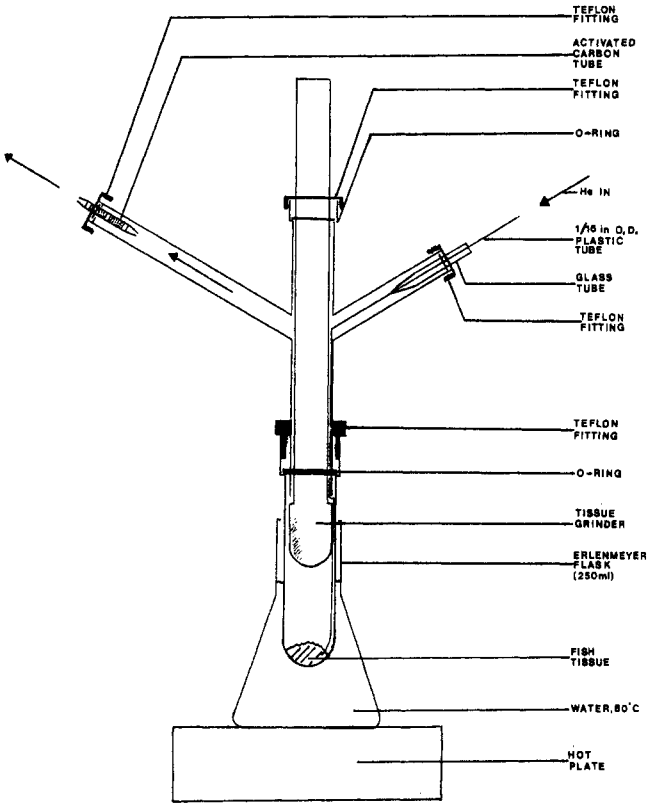

Figure 2. Self-contained grinding purge and trap vessel; developed by Thomas Sabatino, Rutgers University (Ace Scientific).

0.5 h prior to grinding. The tissue was ground into fine particles by using the self-contained grinder. The grinding rod was then lifted to the upper portion of the apparatus, where it was sealed into place with an O ring and a Teflon bushing. A 1 mm i.d. Teflon helium inlet line was placed at the bottom of the grinding apparatus and held in place with a glass seal, an O ring, and a Teflon bushing. The helium purge gas was initiated at 100 mL/min for a period of 1 h. After the purging cycle, the carbon adsorbent was treated in the same manner as phase I.

Phase II chromatographic conditions differ from phase I conditions. The separation of components was accomplished on a 60 m × 0.25 mm i.d. glass capillary column, WCOT, coated with SP-1000. The head pressure was held at 0.75 atm of hydrogen (50 cm/s), and the temperature program was held at an initial temperature of 50 °C for 10 min and increased to a final temperature of 170 °C at 5 °C/min. The final temperature was held for 10 min. The detector and makeup gas parameters were the same as those used in phase I. Quantitation was carried out in the same manner as in phase I.

RESULTS

The recovery values obtained for the compounds used in this study are listed in Table I. In phase I, all fortified compounds were recovered (24 out of 24 compounds); the average recovery values ranged from 39% for styrene to 81% for 1,1,2-trichloroethane for seven replicate determinations. In phase II, all fortified compounds were again recovered by the procedure; however, the average recovery values differed. The recovery rates ranged from a low of 8.7% for 1-chloro-3-ethoxypropane to 53% for trichloroethylene.

The results obtained from the analyses of estuarine fish and shellfish tissue samples are listed in Table II. These values were obtained by using the Phase I procedure. Due to the limited number of samples, a statistical evaluation of the results was not calculated. Many of the compounds found by the procedure were near the minimum detectable limit for the method (0.1 ppm of benzene for 10-g sample size).

DISCUSSION

The recovery data obtained during this study indicate the procedure outlined under phase I, in general, gave better results than the procedure outlined under phase II. In both procedures, compounds exhibiting higher water solubility yielded higher recovery values. This difficulty may be overcome by adjusting purging parameters such as purge temperature (Neff et al., 1976). The precision calculated for the compounds studied ranged from ±5 to ±10% at the fortification levels employed.

From Table II, different species of fish and shellfish apparently exhibit different affinities for accumulating the volatile organic compounds, although differences were not statistically determined due to the small sample size. At site A, both *Tylosurus marinus* (garfish) and *Menidia menidia* (silverside) accumulated 1,2-dichloroethane but to a different concentration, while *Modiolus demissus* (ribbed mussel) did not accumulate the compound to a detectable level. *M. menidia* and *M. demissus* accumulated 0.1 ppm of both *m*- and *p*-xylene at site A; however, *T. marinus* did not exhibit detectable levels of these compounds. At site B, *Paleomonetes pugio* (grass shrimp) and *M. demissus* accumulated benzene and carbon tetrachloride to approximately the same degree. These results also occurred at site C between *Fundulus* spp. (killifish) and *M. menidia*. The differences noted may be due to the organism's tissue lipid content (Phillips, 1978).

Excessive foaming was encountered in the procedure described in phase I. The foam would at times reach the carbon adsorbent trap, introducing interference to the subsequent GC determination (Rose and Colby, 1979). Attempts were made to reduce the foaming problem by the introduction of an antifoaming agent. Silicon antifoam surfactants were employed; the resulting foam was greatly reduced, but extraneous chromatographic components and peak masking were encountered in trial runs. These results

were obtained by using sodium sulfate as the antifoaming agent.

The inherent volatility of the organic compounds in this study made it necessary to prepare the tissue sample for purging without volatilization of these materials. The appartus described in phase II allows the sample preparation to be carried out in a closed system; however, a more detailed evaluation of the operational variables for the apparatus described in phases I and II would be necessary prior to their use as valid analytical tools.

Registry No. Carbon disulfide, 75-15-0; methanol, 67-56-1; chloroform, 67-66-3; bromochloromethane, 74-97-5; 1,1,1-trichloroethane, 71-55-6; 1,2-dichloroethane, 107-06-2; benzene, 71-43-2; carbon tetrachloride, 56-23-5; trichloroethylene, 79-01-6; 1,2-dichloropropane, 78-87-5; dibromomethane, 74-95-3; bromodichloromethane, 75-27-4; toluene, 108-88-3; 1,1,2-trichloroethane, 79-00-5; dibromochloromethane, 124-48-1; tetrachloroethylene, 127-18-4; chlorobenzene, 108-90-7; ethylbenzene, 100-41-4; *m*-xylene, 108-38-3; *p*-xylene, 106-42-3; *o*-xylene, 95-47-6; bromoform, 75-25-2; styrene, 100-42-5; 1,1,2,2-tetrachloroethane, 79-34-5; 1,3-dichlorobenzene, 541-73-1; 1,2-dichlorobenzene, 95-50-1; 1-chloro-3-ethoxypropane, 36865-38-0; 1-chloro-2-ethoxyethane, 628-34-2; 1,1-dichloroethane, 75-34-3.

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Received for review October 30, 1981. Revised manuscript received September 3, 1982. Accepted June 6, 1983. New Jersey Agricultural Experiment Station, Publication No. D-07523-1-83, supported by State funds.

Degradation of the Tri-*n*-butyltin Species in Water

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Some physical and chemical characteristics of the tri-*n*-butyltin moiety from bis(tri-*n*-butyltin) oxide (TBTO) are reported that indicate that it may be moderately persistent in water. The tri-*n*-butyltin species dissolved in water neither volatilizes nor loses butyl groups over a period of at least 2 months in the dark at 20 °C; in sunlight, however, it undergoes slow ($t_{1/2}$ > 89 days) photolytic decomposition, at least partially by stepwise debutylation to inorganic tin. At 20 °C, log K_{ow} of the tributyltin species is 3.2 at pH 6; for the parent TBTO at 20 °C, the aqueous solubility is 0.7–7 mg/L at pH 5–7, and the vapor pressure is estimated to be 6.4×10^{-7} mmHg.

Organotin compounds are used in three major ways, viz., as thermal stabilizers for poly(vinyl chloride), as catalysts in the production of polyurethane foams, and as biocides (Zuckerman et al., 1978). The increasing annual usage of organotin compounds raises the possibility of environmental pollution. Organotin compounds are a chemical class about which more information is sought under Canada's Environmental Contaminants Act (Canada Department of Environment and Department of National Health and Welfare, 1979) regarding toxicology and environmental fate. We chose to determine the aquatic fate of bis(tri-*n*-butyltin) oxide (TBTO) and have recently reported the occurrence of butyltin species in Ontario lakes and rivers (Maguire et al., 1982). Concurrent with our field studies in an attempt to estimate the aquatic persistence of TBTO by determining the relative importance of a variety of routes of degradation and dissipation. This article deals with (i) basic properties such as aqueous solubility, vapor pressure, and octanol–water partition coefficient and (ii) aqueous stability and volatilization from, and photolysis in, water.

The structure of TBTO in water deserves comment at this point, and a conclusion may be drawn from several indirect lines of evidence. First, it appears that TBTO dissolved in water yields the same species as do other Bu_3SnX (Bu = *n*-butyl) compounds. Support for this contention comes from (i) observations that the thin layer (Fish et al., 1976; Kimmel et al., 1977) and high-performance liquid (Jewett and Brinckman, 1981) chromatographic behavior of Bu_3SnX compounds (X = F, Cl, Br, OAc, and $OSnBu_3$) is independent of the nature of X, probably because of anion exchange on chromatography in acidic solvents, (ii) the observation of Fish et al. (1976) that the nature of the metabolites of Bu_3SnX (X = Cl, OAc, and $OSnBu_3$) produced by rat liver microsomal monooxygenase at pH 7.4 is independent of X, and (iii) observations that the variation of X within any particular series of R_3SnX compounds usually has little effect on the biological activity [e.g., Davies and Smith (1980)]. By analogy with the more soluble lower trialkyltin compounds (Tobias, 1966, 1978), therefore, the dissolution of TBTO in pure water likely produces the hydrated Bu_3Sn^+ ion, which behaves as a simple monoprotic acid [in 44% ethanol the pK_a is 6.58 (Janssen and Luijten, 1963)]. For brevity, the tri-*n*-butyltin, di-*n*-butyltin, and n-butyltin species are referred to in this article as though they existed only in cationic form (e.g., Bu_3Sn^+), since we were more interested in debutylation reactions than in cation hydrolysis, largely because in general the toxicity of butyltin compounds decreases with decreasing number of butyl groups (Davies and Smith, 1980). It is recognized that for Bu_3Sn^+ dissolved in water, phenomena such as partitioning into or-

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0021-8561/83/1431-1060$01.50/0