88-7900249

DU PONT

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898
CENTRAL RESEARCH & DEVELOPMENT DEPARTMENT

HASKELL LABORATORY
FOR
TOXICOLOGY AND INDUSTRIAL MEDICINE

8EHQ-1279-0249
Follow Up
8EHQ-1078-0249

December 3, 1979

12/10/79

Document Control Officer
Chemical Information Division
Office of Toxic Substances (WH-557)
Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Gentlemen:

EPA DOCUMENT CONTROL
NO. 8EHQ-1078-0249

This is in response to Mr. Joseph J. Merenda's letter dated November 1, 1979 regarding our October 6, 1978 submission of biocentration data for commercial Motor Fuel Antiknock Compounds containing tetraethyl lead (TEL) and tetramethyl lead (TML).

As noted in our October 6, 1978 letter, we provided the bioaccumulation information to the Agency in accordance with our policy of advising appropriate government agencies of toxicity and environmental test developments that may be of potential significance. It was and still is our view that bioaccumulation of TEL or TML could occur only in the unlikely event of a transportation catastrophe involving bulk quantities and therefore the "potential for widespread exposure and any nontrivial adverse effect" does not exist and that accordingly a submission under Section 8(e) of TSCA was not required.

Mr. Merenda's letter expresses the Agency's preliminary conclusion that the information does meet the criteria for submission under TSCA Section 8(e). He also provided a copy of a status report on our submission prepared by EPA's Office of Toxic Substances (OTS), and requested specified additional information said to be needed to answer several remaining technical questions.

The following is provided in response to Mr. Merenda's letter:

(1) the requested additional information;

(2) a discussion of the information before the Agency and the conclusions properly drawn therefrom; and

8

(3) our views on the criteria for submission under TSCA Section 8(e).

Requested Additional Information

Mr. Merenda's letter requested (1) a full copy of the final report of our TEL and TML bioaccumulation study; (2) a description of any toxic effects observed in the test oysters; and (3) any available data on the stability of TEL in aquatic systems.

Attached hereto as Appendix A is the full copy of the final report "Bioaccumulation and Depuration of Lead by Eastern Oysters (Crassostrea virginica) Exposed to a Tetraethyl Lead Motor Fuel Antiknock Compound" dated April 1978.* The report contains a full description of the test protocols and a discussion of the toxic effects observed (i.e., a shell deposition study performed as a preliminary test for the final study design and effects on growth during the study).

Attached hereto as Appendix B are the following reports on the stability of TEL in aquatic systems and the toxicity of trialkyl lead, the primary degradation product of tetraalkyl lead.

Robinson, J. W., E. L. Kissel and I. A. L. Rhodes. 1979. "Studies of Interactions Between Tetraethyl Lead and Sea Water Using GC-AA." J. Environ. Sci. Health, A14(2):65-85.

Maddock, B. G., and D. Taylor. 1977. "The Acute Toxicity and Bioaccumulation of Some Lead Alkyl Compounds in Marine Animals." presented at the International Experts Discussion Meeting on: Lead-Occurrence, Fate and Pollution in the Marine Environment. Rovinj, Yugoslavia, October 18-22, 1977.

Towers, W., and A. Whittingham. "The Solubility and Breakdown of Lead Alkyls in Natural Water-Part II." The Associated Octel Company Limited, Research and Engineering Department. Unpublished report, 21 pp.

Mr. Merenda's November 1, 1979 letter focuses upon the fact that TEL bioaccumulation is above the 5,000 level stated in EPA's March 16, 1979 "Statement of Interpretation and Enforcement Policy" and concludes that "given the widespread use of leaded gasoline as a fuel for boats, cars, etc., the potential for widespread exposure certainly exists". The letter does not take issue with our statements regarding these materials in bulk form or with the data for TML.

---

*Also attached is a full copy of the comparable report on TML.

Our view is that the Agency's conclusion that potential for widespread exposure to TEL bioaccumulation exists due to its use in leaded gasoline does not properly recognize key properties of TEL such as its solubility in gasoline and water, vapor pressure, density and stability, and the properties of gasoline such as solubility in water, density, vapor pressure and its film forming ability. These properties, as discussed below, bear upon the significance of our reported data (which were derived in a laboratory simulation designed to create bioaccumulation conditions) in evaluating the potential for actual bioaccumulation exposure. It is noted that certain aspects of this issue are recognized by the Agency staff on page 2 of the status report, as follows:

> "A significant question is whether TEL is stable in the environment. If not, the true hazards of this material might be better indicated by studies on its environmental byproducts."

We believe that the following analysis of the data now before the Agency will help answer this question and will amply demonstrate there is no reasonable potential for widespread exposure and bioaccumulation of TEL due to the use of leaded gasoline.

All the ingredients of Motor Fuel Antiknock Compounds are completely miscible in hydrocarbons while they have a very low solubility in water. Therefore, any TEL in gasoline spilled on water should remain in the gasoline rather than mixing with the water or going into solution in the water. The gasoline would, of course, remain on the surface of the water where the TEL content would be subject to oxidation due to exposure to air, and photo-degradation due to exposure to sunlight. Additionally, some portion of the TEL content would be expected to evaporate along with the gasoline. The combined effect of these mechanisms is expected to result in removal of an appreciable amount of TEL from the system, although it is not possible to determine the precise amount. However, even if no TEL were removed and the total TEL content of an average gasoline (dosage of 3.1 g. TEL/gal.) dissolved in water, the result would be a water layer 3.3 inches thick having a TEL concentration of 10 ppb.

Although TEL is highly soluble in gasoline, it is poorly soluble in water and when it dissolves will rapidly decompose to triethyl lead. For example, Robinson, et al. (1979), provided herein in Appendix B, shows TEL solubility limits of approximately 10 ppm in seawater in experimental systems designed to enhance TEL dissolution and retention. A considerably lower limit, 0.12 ppm, was derived by earlier workers (Towers, et al., 1974 unpublished, also in Appendix B). However, in the case of leaded gasoline on water, these limits could not be approached because of the limited availability of TEL due to its relatively low concentration in the gasoline. Once dissolved, TEL rapidly degrades to the trialkyl lead

0 0 0 4

salt. Robinson,et al. (1979) indicate that the conversion from tetra to trialkyl lead was nearly 95% complete within 2-3 hours. Other investigators have also reported rapid dealkylation of TEL in seawater (Maddock, et al., 1977, also in Appendix B). Indeed the degradation of TEL is so rapid that Maddock, et al. (1977) elected to study the bioconcentration of trialkyl lead rather than TEL, reasoning that "...In the context of accumulation, the tetraalkyl lead compounds are of only minor interest since they have only a transitory existence in seawater..."

TEL's primary dealkylation product, triethyl lead, has substantially lesser toxicity and bioconcentrative potential than TEL. With a solubility limit of > 2 g/100 ml, triethyl lead is considerably more soluble in water than TEL (Maddock, et al., 1977). In addition, triethyl lead is less toxic than TEL in aquatic species. Maddock, et al. (1977) have shown that the 96-hour LC50 for triethyl lead is greater than that for TEL by 290, 11 and 7 times in shrimp, mussels and fish, respectively. Most importantly, the bioconcentration factor for triethyl lead is substantially less than that for TEL. Maddock, et al. (1977) have reported triethyl lead bioconcentration factors of 70 and 13 in mussels (*Mytilus edulis*) and fish (*Limanda limanda*), respectively, exposed at 10% of the 96-hour LC50.

It is our view therefore that, based upon the above described properties of TEL, there is no reasonable potential for widespread exposure and bioaccumulation of TEL.

Criteria for Submission Under TSCA Section 8(e)

Mr. Merenda's letter cited the March 16, 1978, "Statements of Interpretation and Enforcement Policy" 43 Fed. Reg. 11110 in assessing our October 6, 1978 letter with regard to criteria for submission under TSCA Section 8(e). Our foregoing comments seek to relate the information to the EPA statement and are believed to be sufficient to demonstrate that these criteria are not met. Although Du Pont considers the EPA statement to be a valuable guide, we believe that the legal obligation to report under Section 8(e) is self-executing and is established solely by the statutory provisions *per se*.

We hope that the above information and comments will be of assistance to OTS in completing its evaluation of our submission. If additional information is necessary or desirable, please contact me at Haskell Laboratory (302) 366-5285.

Sincerely,

Charles F. Reinhardt

Charles F. Reinhardt, M.D.
Director

CFR/jch
Attachments

APPENDIX A

HLO- 0220-78
MR- 2132-001

88-78 00249

Bioaccumulation and depuration of lead by eastern oysters (Crassostrea virginica) exposed to a tetraethyl lead motor fuel antiknock compound

PAGE 004

H# 9506 - LEAD, TETRAETHYL (MOTOR ANTIKNOCK

Submitted to

E. I. du Pont de Nemours & Company

Haskell Laboratory for Toxicology

and Industrial Medicine

Newark, Delaware

Project Number C56-500

Report Number BP-78-4-026

EG&G, Bionomics
Marine Research Laboratory
Route 6, Box 1002
Pensacola, Florida 32507
April 1978

45

Studies were conducted with eastern oysters (Crassostrea virginica) and DuPont TEL Motor Antiknock-Red-01, a tetraethyl lead motor fuel antiknock compound (TEL-MFAC), to evaluate the rate of bioaccumulation and depuration of lead (Pb). In order to determine the appropriate concentrations for the bioaccumulation and depuration test, the acute effect of TEL-MFAC on new shell growth of oysters was evaluated.

0050

MATERIALS AND METHODS

Test material

The TEL-MFAC, which contained 0.6247 grams (g) ~~of Pb per~~ milliliter (mℓ), was received in a metal bottle sealed inside a metal shipping can. The TEL-MFAC was a clear, red liquid. Because of known mammalian toxicity, safe handling procedures provided by E. I. du Pont de Nemours & Company were carefully followed.

Stock solutions of TEL-MFAC for the acute shell deposition test were prepared with reagent grade absolute ethanol as the solvent/carrier; those for the bioaccumulation test were prepared with reagent grade acetone. For the acute test, one set of stock solutions was sufficient for the 96-hour exposure. For the bioaccumulation study, new stock solutions were prepared at least weekly. All stock solutions were kept in capped, amber glass bottles.

It should be noted that concentrations are expressed in this paper as micrograms (μg) of Pb per liter (ℓ) of seawater (μg Pb/ℓ) or micrograms of Pb per g of tissue (μg Pb/g), parts per billion (ppb) or parts per million (ppm), respectively.

The concentrations of Pb in seawater were determined as tetraalkyl Pb in both the shell deposition test and the bioaccumulation test; concentrations of Pb in tissue were determined as total lead.

Test animals

Oysters used in the shell deposition test were 2-4 centimeters (cm), umbo to distal valve edge, and were selected from groups of oysters that had been collected from Big Lagoon, an estuary adjacent to Bionomics Marine Research Laboratory (BMRL). The oysters were held in tanks of flowing, natural seawater of ambient salinity and temperature ~~for a~~ minimum of two weeks before testing.

Oysters for the bioaccumulation and depuration study were collected from Mississippi Sound near Ocean Springs, Mississippi, and were 5-8 cm, umbo to distal valve edge. These oysters were held at BMRL in flowing, natural seawater of ambient salinity and temperature for a minimum of one week before testing.

All oysters appeared to be in good condition at the initiation of the tests.

Test conditions

A. Shell deposition test

Methods for the 96-hour, continuous-flow test were those of Butler et al. (1960) and Butler (1965). Sixty individual oysters of similar size were selected, cleaned of attached organisms and ground by hand on a fine-grit grinding wheel to remove approximately 2-5 millimeters (mm) of peripheral shell. Six groups of 10 oysters each were impartially

separated and a group was placed in each test container. The oysters were removed from the test containers after 96 hours of continuous exposure and the new shell growth of each oyster was measured to the nearest 0.1 mm by using a binocular dissecting scope equipped with a micrometer. The mean shell growth of control oysters, less the mean growth of oysters from each concentration, divided by the control growth and multiplied by 100 gave the percentage reduction of shell growth for oysters from each concentration relative to the control.

The test was conducted in a glass system (except for short lengths of Tygon® tubing connections) that was covered, where feasible, to reduce volatilization of the TEL-MFAC. For each of the five test concentrations and the control, the exposure apparatus (Figure 1) was an individual system consisting of three basic components: (1) The mixing chamber, a 4-ℓ vacuum flask, was located above the other components. It received 30 ℓ/hour continuous flow of natural, unfiltered seawater via a calibrated glass siphon which led to a glass funnel fitted through a neoprene stopper that capped the flask. An appropriate volume of TML-MFAC stock solution or control ethanol was metered into the mixing chamber by a syringe pump. The delivery line from the syringe passed through the funnel and into the mixing chamber. An electric stirrer was mounted above the flask; its stirring shaft extended through the neoprene stopper and into the mixing chamber. The rate of stirring was regulated by a rheostat. (2) A 6-ℓ rectangular glass settling tank was

positioned between the mixing chamber and exposure aquarium to allow unsolubilized particles of TEL-MFAC to settle before entering the exposure chamber. The settling tank was divided in half by a glass partition. Test solution flowed by gravity from the mixing chamber through a short length of Tygon® tubing into one end of the settling tank, passed over the partition into the other end and exited through a standpipe into the exposure aquarium. (3) An 8-ℓ glass aquarium containing 10 test oysters received the seawater/TEL-MFAC mixture from the settling tank. Water flowed from the exposure aquarium through a standpipe to a drain pipe and into the BMRL waste treatment system. The volume of the entire exposure system was 18 ℓ and approximately 99% replacement occurred every 3 hours (Sprague, 1969).

The nominal test concentrations were 1, 10, 56, 100 and 200 μg Pb/ℓ. The control received as great a volume of ethanol (1.3 mℓ per hour) as did any treatment but no Pb. To confirm concentrations of Pb in seawater, water samples were taken from each aquarium daily and analyzed at BMRL for tetraalkyl lead. A complete description of methodology for water sample analyses is contained in Appendix A.

Oysters could obtain plankton and other particulate matter from the unfiltered seawater in which they were tested; there was no supplemental feeding.

The test was conducted from 11-15 August 1976.

B. Bioaccumulation and depuration test

Two separate groups of 80 oysters each were continuously

PAGE 0053

exposed to TEL-MFAC for 84 days in flowing, natural seawater. The nominal test concentrations were 0.1 and 1.0 μg Pb/ℓ; the control received as great a volume of acetone (0.74 mℓ per hour) as did the test concentrations but no TEL-MFAC.

For each concentration and the control, the exposure apparatus (Figure 2) consisted of two basic components, a 4-ℓ rectangular glass mixing chamber and a 60-ℓ fiberglass resin-coated plywood exposure aquarium. No settling tank was used because of the low concentrations being tested. The mixing chambers, placed above the exposure aquaria, received 160 ℓ/hour of seawater of ambient salinity and temperature via calibrated glass siphons. A syringe pump metered appropriate volumes of TEL-MFAC stock solution or acetone into the mixing chambers. An electric stirrer was mounted above each mixing chamber and the stirring shaft extended into the chamber. Incoming seawater and the stock solutions were mixed in the chambers, then flowed by gravity through short lengths of Tygon® tubing into the exposure aquaria which contained the oysters. The volume of the system was 64 ℓ and approximately 99% replacement occurred every 2 hours (Sprague, op. cit.).

Absolute ethyl alcohol was used as the solvent/carrier for TEL-MFAC during days 1-3 of the bioaccumulation study. However, the ethanol apparently induced an extremely heavy deposit of a slime-like growth in all mixing and exposure tanks. Acetone was substituted for ethanol as the solvent/carrier on day 4 and the problem ceased.

During the exposure, test water was analyzed for tetra-alkyl Pb and oyster tissue was analyzed for total Pb. Water

SD

samples were taken weekly. Three oysters were removed from the control and each test concentration on days 3, 7, 10, 14, 21, 28, 42, 56, 70 and 84 of exposure, shucked and the meats (whole-body) combined in tared containers and weighed. Twenty milliliters of iodine monochloride solution were added to each container to fix the Pb in the oysters for later chemical analyses by atomic absorption spectrophotometry. Complete descriptions of methodology for water and tissue analyses are contained in Appendixes A and B.

After 84 days of exposure, the remaining oysters were placed in flowing seawater to which no TEL-MFAC was added to determine the rate of depuration of Pb. Oysters from the control and each test concentration were sampled for chemical analysis of total Pb in tissue on days 3, 7, 10, 14 and 28 of the depuration period.

Oysters could obtain plankton and other particulate matter from the unfiltered seawater in which they were tested; there was no supplemental feeding.

The growth of oysters during the 84-day exposure was monitored by comparing the average in-water weights (Andrews, 1961) of oysters from each test concentration and the control on day 0 and day 84. The in-water weights were determined by placing groups of 4 oysters on a tray that was suspended from the weighing hook of a top-loading balance. The tray and oysters were immersed in seawater.

The test was conducted from 24 September 1976 - 14 January 1977.

Statistical analyses

Differences between the concentrations of Pb in exposed oysters as compared to the background concentrations found in control oysters were determined with "Student's t-test and were considered to be statistically significant at the 99% confidence level ($P<0.01$) (Sokal, 1973).

RESULTS AND DISCUSSION

A. Analyses of tetraalkyl Pb in seawater

During the 96-hour shell deposition test, the mean measured concentrations of tetraalkyl Pb, based on analyses of seawater from each exposure tank, were 1.0, 5.2, 36, 72 and 131 µg Pb/ℓ (Table 1). The measured concentrations were used to estimate the 96-hour EC50 and, hereafter in this report, test concentrations during the acute shell deposition test are given as the mean measured concentrations of tetraalkyl Pb in seawater.

The mean measured concentration of tetraalkyl Pb in seawater in the 1.0 µg Pb/ℓ nominal concentration during the bioaccumulation study was 0.8 µg Pb/ℓ (Table 2). Concentrations of Pb were not measured in the 0.1 µg Pb/ℓ nominal concentration because of the limit of detection of the analytical method based on the water sample volume. Hereafter in this report, test concentrations during the bioaccumulation study are given as the mean measured concentration (0.8 µg Pb/ℓ) for the higher concentration and as the nominal 0.1 µg Pb/ℓ for the unmeasured, lower concentration.

It should be noted that methods for the analysis of tetraalkyl Pb in seawater during the bioaccumulation study were slightly modified from the methods outlined in Appendix A. Because of a malfunction of the Beckman Model B Spectrophotometer, analyses after day 5 of exposure were made by using a Bausch and Lomb Spectronic 20 spectrophotometer. Changing instruments also necessitated transferring the dithizone layer by pipet from the modified 200-mℓ absorption cell to a conventional cuvette for measuring the absorbance. The transferred dithizone layer became cloudy as it cooled; therefore, the cuvettes were placed in a warm water bath to prevent the liquid from becoming cloudy before the absorbance was determined.

B. Shell deposition test

The estimated 96-hour EC50 for oysters exposed to TEL-MFAC in continuously flowing, natural seawater was >1.0<5.2 µg Pb/ℓ. The percentage reduction of new shell growth of the exposed oysters as compared to the control oysters was from 43% in the 1.0 µg Pb/ℓ test concentration to 100% in 131 µg Pb/ℓ (Table 3).

The new shell growth of the control oysters (mean 0.7 mm) was not as great as we have observed in other tests. However, the conditions at the time of testing were not optimal. Oysters along the northcentral coast of the Gulf of Mexico appear to grow best in the early spring and late fall. This test was conducted during the summer when growth was likely inhibited by high water temperature (28-30 degrees Celsius [°C]).

Despite the relatively low rate of shell growth, the acute test served as a valid means of selecting appropriate test concentrations of TEL-MFAC for the bioaccumulation study.

C. Bioaccumulation and depuration study

Concentrations of total Pb in tissues of oysters exposed to a nominal concentration of 0.1 μg Pb/ℓ were significantly greater than the concentration of total Pb in control oysters after three days of exposure and throughout the remainder of the 84-day exposure (Table 4). Total Pb in oyster tissue was apparently in equilibrium with the ambient water concentration by day 28, and this equilibrium was maintained throughout at least day 70 of the study. A line of best fit was constructed (Figure 3) by using a Hewlett-Packard 9815A computer and 9817A plotter to fit the day of exposure/tissue concentration data to a 2nd degree polynominal regression equation.

A bioconcentration factor of 17,600X was calculated by dividing the nominal concentration of tetraalkyl Pb in seawater (0.1 μg Pb/ℓ) into the equilibrium concentration of total Pb above control concentration (2.25 μg Pb/g - 0.49 μg Pb/g = 1.76 μg Pb/g ÷ 0.1 μg Pb/ℓ), the value of 2.25 μg Pb/g being obtained from the line of best fit on day 49 (Figure 3).

Oysters in the higher concentration also accumulated significantly greater concentrations of total Pb than was present in control oysters (Table 4). It appeared that an equilibrium between tissue and water was not reached but was being approached (Figure 4). A maximum observed bio-

concentration factor of 18,138X was calculated by dividing the mean measured concentration of tetraalkyl Pb in seawater into the maximum tissue concentration of total Pb above control concentration (15 μg Pb/g - 0.49 μg Pb/g = 14.51 μg Pb/g ÷ 0.8 μg Pb/ℓ). This concentration factor was very similar to the concentration factor observed in the lower concentration, again suggesting that equilibrium was being approached.

Oysters rapidly depurated the Pb which they had accumulated. After 7 days of depuration in seawater to which no TEL-MFAC was added, oysters previously exposed to 0.1 μg Pb/ℓ had depurated 90% of the Pb they had accumulated and concentrations of total Pb were near control oyster tissue concentrations. (A concentration of 29 μg Pb/g was reported on day 3 of depuration from what was assuredly a contaminated sample.) Oysters previously exposed to 0.8 μg Pb/ℓ had depurated 90% of the accumulated Pb after 10 days and concentrations in oysters were near control oyster tissue concentrations after 14 days of depuration.

Although some oysters died in all treatments, mortality was not excessive. Of the initial population of 80 per treatment, 27 oysters were sampled and the number which died was as follows: control, 10; 0.1 μg Pb/ℓ, 7; and 0.8 μg Pb/ℓ, 15.

There was a slight effect on growth of exposed oysters as compared to growth of control oysters. The in-water weights of oysters from the lower concentration and the

control increased slightly during the 84 days of exposure, but the weight of the oysters in the higher concentration did not increase. The control oysters increased by 9%; the 0.1 μg Pb/ℓ-exposed oysters, 5%; and the 0.8 μg Pb/ℓ-exposed oysters, 0% (Table 5).

Salinity was 30-32 parts per thousand (°/oo) during the shell deposition test and 21-33 °/oo during the bioaccumulation and depuration study; temperature was 28-30°C and 10-28°C, respectively (Table 6).

SUMMARY

Shell growth of oysters exposed to TEL-MFAC in seawater was reduced by more than 50% in concentrations of tetraalkyl Pb ≥5.2 μg Pb/ℓ during an acute exposure. The estimated 96-hour EC50, based on mean measured concentrations of tetraalkyl Pb in seawater, was >1.0<5.2 μg Pb/ℓ.

Oysters exposed to TEL-MFAC in seawater for 84 days accumulated significantly greater ($P<0.01$) concentrations of Pb than were present in control oysters. In the lower concentration (0.1 μg Pb/ℓ of tetraalkyl Pb), an apparent equilibrium between Pb in tissue and Pb in water was reached by day 28 and a concentration factor of 17,600X was calculated. Oysters exposed to the higher concentration (0.8 μg Pb/ℓ of tetraalkyl Pb) also accumulated significant ($P<0.01$) amounts of Pb above control concentrations. Although equilibrium between Pb in tissue and water was not apparently reached in this concentration, a concentration factor of 18,138X suggested that equilibrium was approached.

36

Oysters rapidly depurated the Pb which they had accumulated. The concentration of Pb in oysters from both exposed groups was near control oyster tissue concentrations after 7-14 days of depuration in seawater to which no TEL-MFAC was added.

REFERENCES

Andrews, J.D. 1961. Measurement of shell growth in oysters. Proc. Natn. Shellfish. Assoc., 52: 1-11.

Butler, P.A., A.J. Wilson, Jr., and A.J. Rick. 1960. Effect of pesticides on oysters. Proc. Natn. Shellfish. Assoc., 51: 23-32.

_____. 1965. Reaction of some estuarine mollusks to environmental factors. U.S. Dept. of Health, Education, and Welfare, Public Health Service Publication No. 99-WP-25. p. 92-104.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press, London. 333 p.

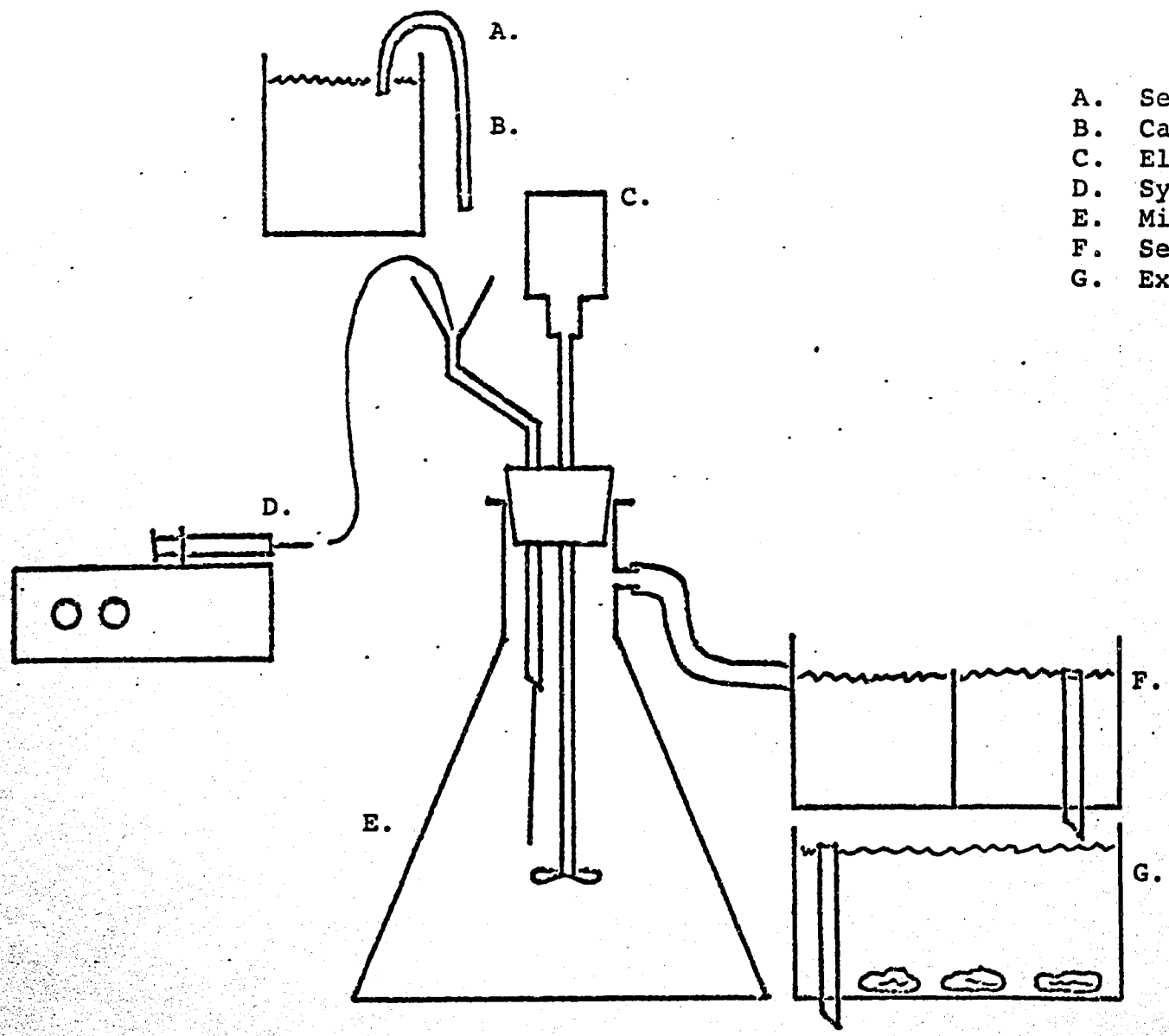
Sokal, R.R. and F.J. Rohlf. 1973. Introduction to Biostatistics. W.H. Freeman and Company, San Fransicso. 368 p

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Research, 3(11): 793-821.

U.S. Environmental Protection Agency. 1976. Bioassay procedures for the ocean disposal permit program. EPA-600/9-76-010. 96 p.

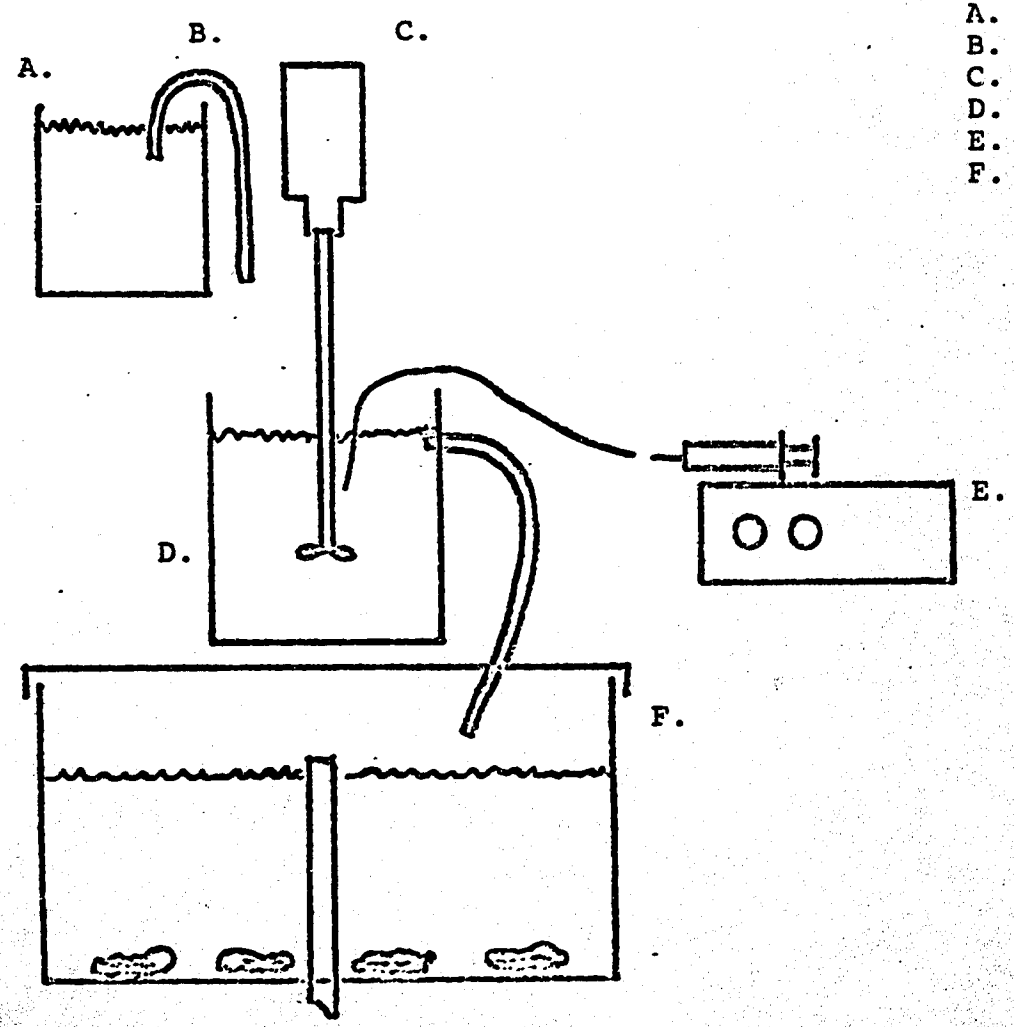
_____. 1974. Methods for chemical analysis of water and wastes. EPA-625/6-74-003. 298 p.

FIGURE 1. Diagram of the exposure system used during a 96-hour shell deposition test with eastern oysters (*Crassostrea virginica*) and a tetraethyl lead motor fuel antiknock compound.



A. Seawater headbox
B. Calibrated glass siphon
C. Electric stirrer
D. Syringe pump
E. Mixing chamber
F. Settling tank
G. Exposure aquarium

FIGURE 2. Diagram of the exposure system used during a bioaccumulation and depuration study with eastern oysters (Crassostrea virginica) and a tetraethyl lead motor fuel antiknock compound.



A. Seawater headbox
B. Calibrated glass siphon
C. Electric mixer
D. Mixing chamber
E. Syringe pump
F. Exposure aquarium

TABLE 1. Measured concentrations of tetraalkyl lead (Pb) in seawater during a 96-hour shell deposition test with eastern oysters (*Crassostrea virginica*) and a tetraethyl lead motor fuel antiknock compound in flowing, natural seawater. Salinity was 30-32 °/oo and temperature, 28-30°C.

Nominal concentration of total alkyl Pb (μg Pb/ℓ)	Measured concentration of tetraalkyl Pb (μg Pb/ℓ)					
	24 h	48 h	72 h	96 h	$\bar{X}$	SD[a]
Control	ND[b]	ND	ND	ND	ND	---
1.0	1.1	-[c]	-	0.8	1.0	±0.2
10	-[c]	3.1	7.2	-	5.2	±2.9
56	43	29	26	44	36	±9.3
100	66	90	58	75	72	±14
200	130	142	138	115	131	±12

[a]Standard deviation.

[b]Not detectable; <0.05μg Pb/ℓ.

[c]No sample.

TABLE 2. Measured concentrations of tetraalkyl lead in seawater during a bioaccumulation study in which eastern oysters (*Crassostrea virginica*) were exposed to a tetraethyl lead motor fuel antiknock compound in flowing, natural seawater for 84 days.

Concentrations of tetraalkyl Pb (μg Pb/ℓ)					
Nominal	Mean	SD	Percent of nominal	Range	Number of samples
Control	ND[a]	---	---	0.0-0.2	14
0.1	-[b]	-	-	-	-
1.0	0.8	±0.3	80	0.2-1.3	14

[a]Not detectable, <0.05 μg Pb/ℓ.

[b]Analyses were not conducted on water samples from this concentration because of the limit of detection of the analytical method.

TABLE 3. Effects of tetraethyl lead motor fuel antiknock compound on shell deposition of eastern oysters (*Crassostrea virginica*) exposed for 96 hours in flowing, natural seawater. Salinity was 30-32 ‰ and temperature, 28-30°C. Shell deposition is the mean value of shell measurements of ten oysters.

Mean measured concentration of tetraalkyl Pb (μg Pb/ℓ)	Shell deposition, mm (Mean and std. dev.)	Reduction[a] (%)
Control	0.7 ± 0.6	---
1.0	0.4 ± 0.2	43
5.2	0.1 ± 0.2	85
36	0.1 ± 0.1	85
72	0.1 ± 0.1	85
131	0.0 ---	100

$$^{a}\% \text{ reduction} = \frac{\text{Shell deposition of control oysters minus shell deposition of exposed oysters}}{\text{Shell deposition of control oysters}} \times 100$$

TABLE 4. Measured concentrations of total lead (Pb) in whole-body tissue of eastern oysters (*Crassostrea virginica*) exposed to a tetraethyl lead motor fuel antiknock compound (TEL-MFAC) in a bioaccumulation and depuration study in flowing, natural seawater. Salinity was 21-33 °/oo and temperature, 10-28°C. Each value represents analysis of a pooled sample of 3 oysters. Whole-body concentrations are listed under the appropriate exposure to TEL-MFAC (control, 0.1 μg Pb/ℓ and 0.8 μg Pb/ℓ).

		Tissue concentration (μg Pb/g, wet weight)		
		(Control)	(0.1)	(0.8)
Exposure	3	0.57	0.63	2.29
	7	0.53	1.46	4.64
	10	0.55	0.86	4.16
	14	0.52	0.92	4.80
	28	0.70	1.60	7.80
	42	0.50	1.60	9.20
	56	0.44	2.80	10.30
	70	0.42	2.50	7.20
	84	0.37	1.00	15.00
Depuration	3	0.39	29.0[a]	0.62
	7	≤0.30	≤0.20	2.60
	10	≤0.20	≤0.20	1.30
	14	≤0.20	≤0.30	0.41
	21	≤0.25	≤0.25	≤0.30
	28	≤0.30	≤0.30	≤0.30

[a]Contaminated sample.

Figure 3. Accumulation of lead (Pb) by eastern oysters (*Crassostrea virginica*) exposed to a tetraethyl lead motor fuel antiknock compound (TEL-MFAC) in flowing, natural seawater. Tissue concentrations are given as total lead in whole-body, wet-weight oysters. The nominal concentration of tetraalkyl Pb in seawater was 0.1 µg Pb/ℓ. The line of best fit was constructed from a 2nd degree polynominal regression equation on a Hewlett-Packard 9815A computer.

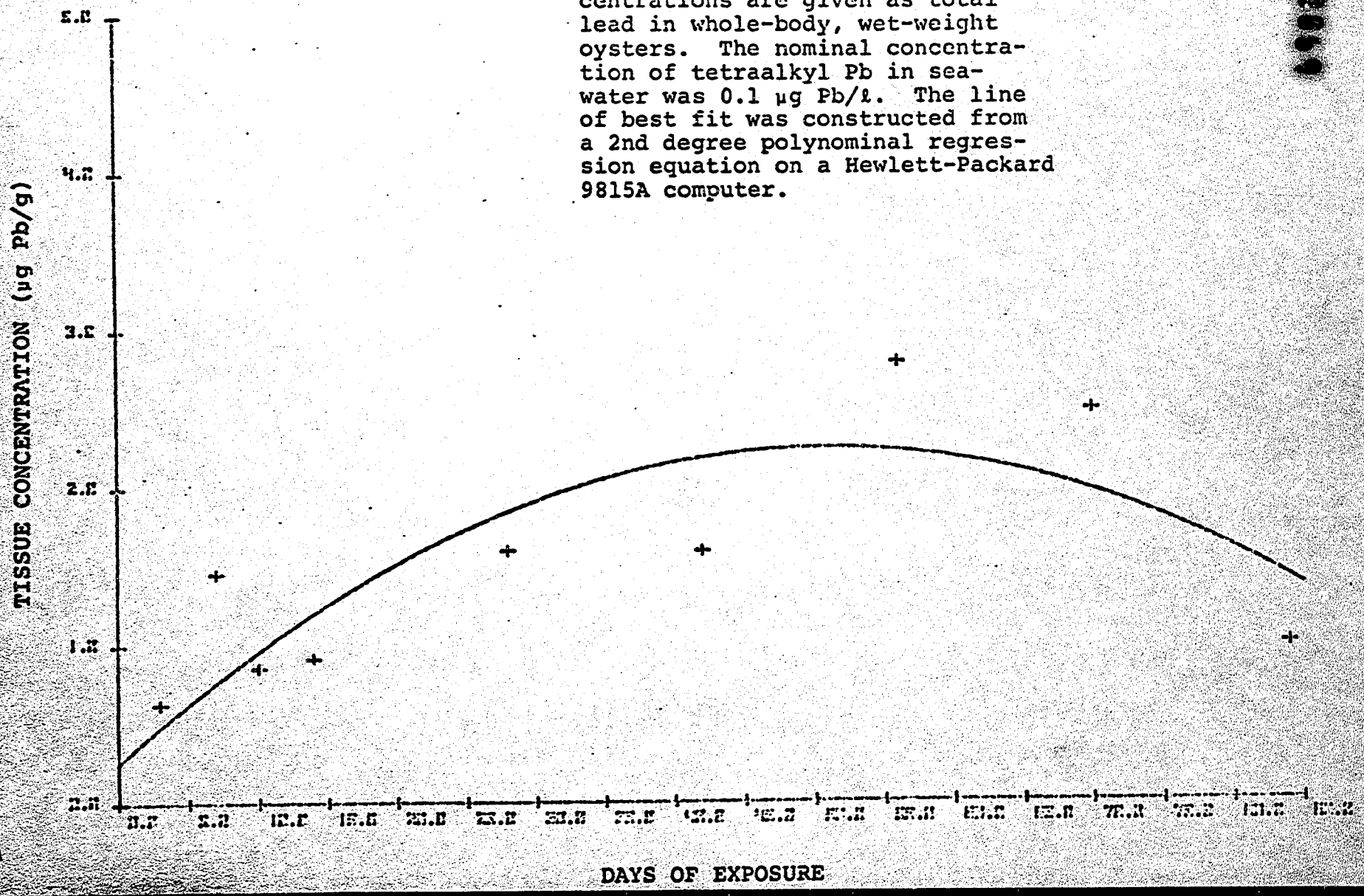


Figure 4. Accumulation of lead (Pb) by eastern oysters (Crassostrea virginica) exposed to a tetraethyl lead motor fuel antiknock compound (TEL-MFAC) in flowing, natural seawater. Tissue concentrations are given as total lead in whole-body, wet-weight oysters. The mean measured concentration of tetraalkyl Pb in seawater was 0.8 µg Pb/ℓ. The line of best fit was constructed from a 2nd degree polynominal regression equation on a Hewlett-Packard 9815A computer.

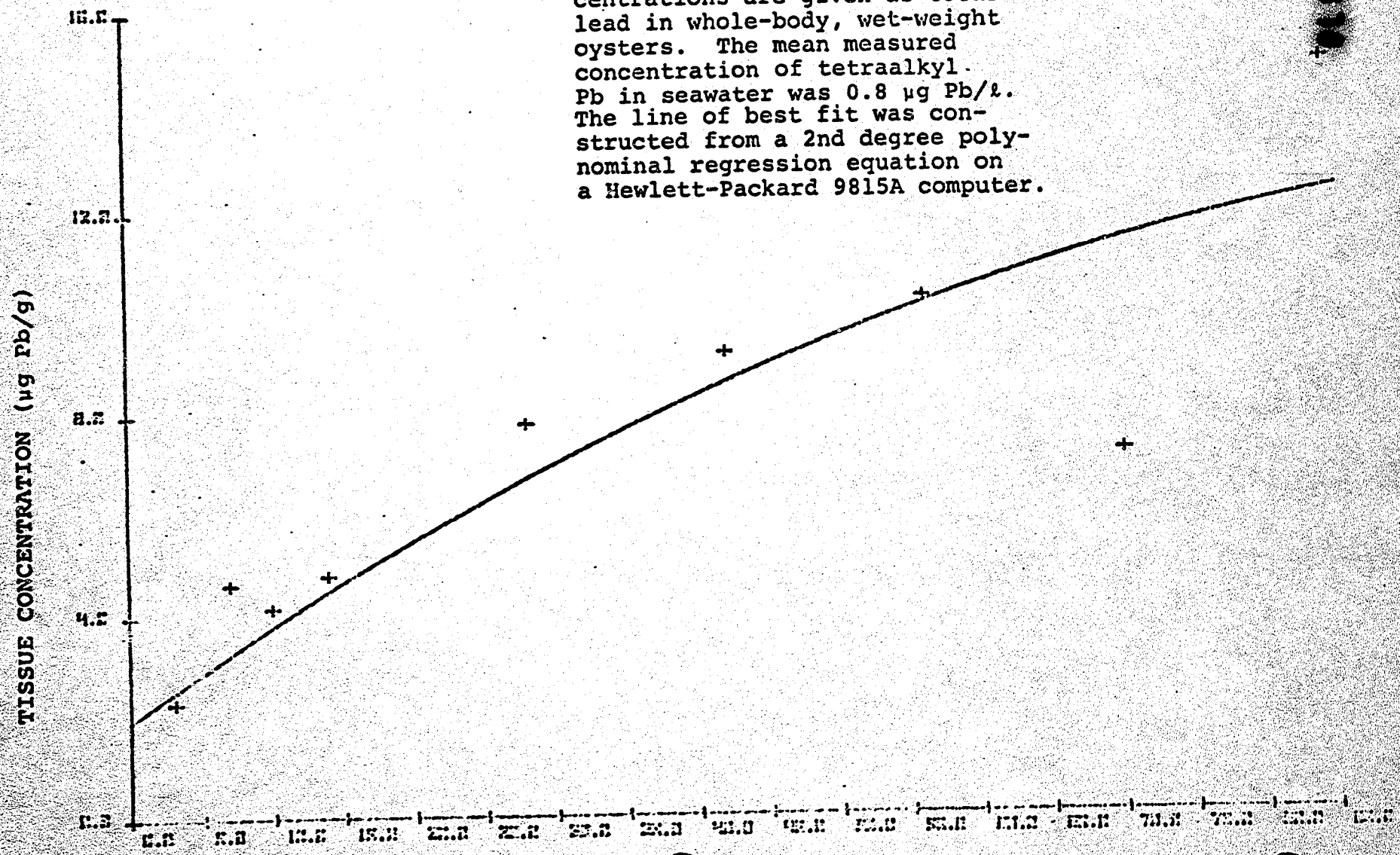


TABLE 5. Weights of eastern oysters (*Crassostrea virginica*) during a bioaccumulation study. The oysters were weighed in water at the initiation of the study and after 84 days of exposure to a tetraethyl lead motor fuel antiknock compound in flowing, natural seawater. Salinity was 21-33 °/oo and temperature, 11-23°C. The in-water weight of groups of 4 oysters was determined, except as noted, and are listed under the appropriate exposure treatment (control, 0.1 μg Pb/ℓ and 0.8 μg Pb/ℓ).

Group	Initial weight (g)			Day 84 weight (g)		
	(Control)	(0.1)	(0.8)	(Control)	(0.1)	(0.8)
1	193	151	156	160	194	151
2	159	168	178	173	171	162
3	146	108	139	159	139	159
4	189	178	199	172	154	141
5	158	150	182	187	187	116
6	206	167	157	215	155	166
7	155	136	110	183	119	158
8	136	149	126	184	146	142
9	177	130	128	169	159	165
10	185	126	135	225	136	92[b]
11	152	168	131	134[a]	190	-[c]
12	134	143	120	-[c]	56[b]	-[c]
13	144	126	148			
14	163	177	120			
15	185	180	128			
16	186	123	146			
17	178	130	198			
18	161	166	170			
19	165	143	214			
20	151	123	141			
Average per oyster	42	37	38	46	39	38
Percentage increase	---	---	---	9	5	0

[a]Group of three oysters.

[b]Group of two oysters.

[c]No oysters remained.

TABLE 6. Daily measurements of the salinity and temperature of flowing, natural seawater in which a bioaccumulation and depuration study was conducted with tetraethyl lead motor fuel antiknock compound and eastern oysters (*Crassostrea virginica*).

Date	Test day	Salinity (°/oo)	Temperature (°C)
24 Sep 76	0	28	26
25 Sep 76	1	28	27
26 Sep 76	2	28	27
27 Sep 76	3	27	27
28 Sep 76	4	25	28
29 Sep 76	5	27	28
30 Sep 76	6	31	27
1 Oct 76	7	28	27
2 Oct 76	8	28	26
3 Oct 76	9	26	27
4 Oct 76	10	28	26
5 Oct 76	11	29	27
6 Oct 76	12	30	25
7 Oct 76	13	29	25
8 Oct 76	14	30	24
9 Oct 76	15	26	21
10 Oct 76	16	27	23
11 Oct 76	17	25	22
12 Oct 76	18	29	22
13 Oct 76	19	26	22
14 Oct 76	20	29	23
15 Oct 76	21	28	22
16 Oct 76	22	29	23
17 Oct 76	23	30	23
18 Oct 76	24	30	20
19 Oct 76	25	30	21
20 Oct 76	26	29	21
21 Oct 76	27	29	19
22 Oct 76	28	27	20
23 Oct 76	29	28	20
24 Oct 76	30	30	21
25 Oct 76	31	30	21
26 Oct 76	32	30	20
27 Oct 76	33	30	19
28 Oct 76	34	30	19
29 Oct 76	35	30	18
30 Oct 76	36	32	19
31 Oct 76	37	31	18

(continued)

TABLE 6, continued.

Date	Test day	Salinity (°/oo)	Temperature (°C)
1 Nov 76	38	28	17
2 Nov 76	39	28	17
3 Nov 76	40	28	17
4 Nov 76	41	26	17
5 Nov 76	42	27	17
6 Nov 76	43	27	16
7 Nov 76	44	28	16
8 Nov 76	45	30	16
9 Nov 76	46	30	16
10 Nov 76	47	29	17
11 Nov 76	48	31	18
12 Nov 76	49	32	18
13 Nov 76	50	30	17
14 Nov 76	51	32	17
15 Nov 76	52	33	17
16 Nov 76	53	33	16
17 Nov 76	54	32	15
18 Nov 76	55	30	15
19 Nov 76	56	30	16
20 Nov 76	57	30	15
21 Nov 76	58	30	15
22 Nov 76	59	28	14
23 Nov 76	60	26	14
24 Nov 76	61	28	14
25 Nov 76	62	28	15
26 Nov 76	63	30	17
27 Nov 76	64	32	18
28 Nov 76	65	31	17
28 Nov 76	66	25	14
30 Nov 76	67	24	11
1 Dec 76	68	22	11
2 Dec 76	69	23	13
3 Dec 76	70	25	13
4 Dec 76	71	24	14
5 Dec 76	72	28	13
6 Dec 76	73	30	14
7 Dec 76	74	28	14
8 Dec 76	75	24	12

(continued)

TABLE 6, continued.

Date	Test day	Salinity (°/oo)	Temperature (°C)
9 Dec 76	76	23	12
10 Dec 76	77	28	14
11 Dec 76	78	31	13
12 Dec 76	79	31	14
13 Dec 76	80	27	14
14 Dec 76	81	22	13
15 Dec 76	82	22	14
16 Dec 76	83	21	15
17 Dec 76	84	24	13
	Depuration		
18 Dec 76	1	22	15
19 Dec 76	2	25	15
20 Dec 76	3	28	15
21 Dec 76	4	29	13
22 Dec 76	5	28	14
23 Dec 76	6	25	13
24 Dec 76	7	26	13
25 Dec 76	8	25	14
26 Dec 76	9	25	14
27 Dec 76	10	24	14
28 Dec 76	11	24	13
29 Dec 76	12	24	13
30 Dec 76	13	25	13
31 Dec 76	14	26	14
1 Jan 77	15	27	14
2 Jan 77	15	27	13
3 Jan 77	17	28	12
4 Jan 77	18	31	11
5 Jan 77	19	31	14
6 Jan 77	20	31	14
7 Jan 77	21	29	13
8 Jan 77	22	30	13
9 Jan 77	23	29	13
10 Jan 77	24	30	13
11 Jan 77	25	25	11
12 Jan 77	26	25	10
13 Jan 77	27	25	10
14 Jan 77	28	27	13

APPENDIX A

Determination of Tetraalkyl Lead in Seawater

Method in brief

Tetraalkyl lead compounds are first extracted from water with n-heptane and then extracted from n-heptane with an aqueous iodine monochloride solution. The lead content of the iodine monochloride solution is measured by a simplified colorimetric dithizone method.

Reagent

1. n-Heptane: Pure grade, 99 Mol% minimum. Phillips Petroleum Company, Bartlesville, Oklahoma 74004.
2. Reducing solution: Dissolve 80 g of dibasic ammonium citrate, 40 g of potassium cyanide, 400 g of anhydrous sodium sulfite, 15 g of disodium ethylenediaminetetraacetate in 2500-mℓ of water. Add 2500 mℓ of ammonium hydroxide (28% NH_3), mix well to dissolve all reagents and store in a sealed bottle.
3. Iodine monochloride solution (approximately 0.1 m): Mix 500 mℓ of potassium iodide solution (250 g potassium iodide diluted to 1 ℓ with water) with 500 mℓ of concentrated hydrochloric acid (37%). Slowly add 84 g of reagent grade potassium iodate with constant stirring until all the free iodine has redissolved to give a clear orange-red solution. Dilute to 10 ℓ with distilled water.

   Note: Iodine monochloride and iodine will react with ammonium ions under certain conditions to yield nitrogen triiodide. It is important to always have an excess of reducing agent (sodium sulfite) present when either of the above halogens are mixed with ammonium hydroxide solutions.

4. Dithizone solutions: Dissolve 40 mg of diphenylthiocarbazone (dithizone) in 1 ℓ of reagent grade chloroform. Mix well and store in a brown bottle in the absence of direct sunlight.

Apparatus

1. Modified absorption cell (Figure 1). Purchased from E. Leitz Inc., Rock Leigh Industrial Park, Rock Leigh, NJ 07647, catalog number 94626.
2. Beckman Model "B" Spectrophotometer with modified cell compartment and modified cell compartment cover. Note: Modified instrument loaned to Bionomics Marine Research Laboratory for lead bioaccumulation studies.

   Note: The modified absorption cell fits directly into a Leitz Spectrophotometer without need for modifying this instrument.
3. Separatory funnels (cylindrical and graduated). 250 mℓ, 500 mℓ and 2,000 mℓ sizes depending on sample size to be analyzed.
4. Graduated cylinders. 25 mℓ and 50 mℓ sizes.
5. Pipet. 5.0 mℓ size.
6. Syringe. 10 mℓ, 30 mℓ and 50 mℓ sizes. B&D all glass syringes with number 17 needles.

Standardization

A precise calibration factor (F) may be obtained by adding known quantities of Pb (as tetraalkyl lead) to water samples as follows:

Add known quantities of Pb with a syringe well beneath the surface of water samples contained in separatory funnels. Analyze

the lead in these known samples by the procedure described below. Calculate the calibration factor as follows:

$$\text{Calibration factor (F)} = \frac{\mu g \text{ of Pb in known sample}}{(\text{absorbance of known sample} - \text{absorbance of blank})}$$

Procedure (1.0 - 5,000 ppb tetraalkyl lead)

1. Siphon the seawater sample (see Table 1 for sample size) directly into a graduated cylindrical separatory funnel containing n-heptane (see Table 1 for volume). Protect the sample from sunlight and immediately analyze it to avoid possible decomposition of tetraalkyl lead.
2. Shake the mixture vigorously for 2 minutes. The tetraalkyl lead will extract into the n-heptane solution.
3. After the two layers have separated, drain the aqueous solution into a graduated cylinder (100 mℓ to 2,000 mℓ depending on sample size). Discard the water after recording its volume.

   Note: If the sample volume was measured in step "1" above, it need not be measured here.
4. Add 100 mℓ of distilled water to the original separatory funnel and shake the n-heptane and water mixture for 1 minute. After the two layers have separated, drain and discard the water layer.
5. Add iodine monochloride solution (either 20 mℓ or 40 mℓ, see Table 1) to the n-heptane present in the original separatory funnel. Shake it vigorously for approximately 2 minutes. Immediately add 50 mℓ of distilled water, shake approximately 1 minute and allow the two layers to separate. At this point all the tetraalkyl lead has been extracted by the iodine monochloride solution.

6. Drain the iodine monochloride solution into a modified absorption cell (Figure 1).
7. Add 30 mℓ of reducing solution to the modified absorption cell. Mix well by shaking the stoppered cell to eliminate all black particles.
8. Add 5.0 mℓ of dithizone solution to the modified absorption cell, shake it vigorously for 30 seconds, and allow the two layers to separate.
9. Insert the modified absorbance cell into the spectrophotometer and record the absorbance of the lower layer at 485 nanometers (nm) wavelength.
10. Analyze a blank using all reagents and distilled water, but use no seawater sample.

Calculations

$$\text{ppm of Pb} = \frac{(\text{absorbance of sample} - \text{absorbance of blank}) \times F}{\text{volume of seawater, m}\ell}$$

Note: The calibration factor (F) should be approximately 18.5 when using 5.0 mℓ of dithizone solution.

Comments

1. If an analysis is made and the color of the lead alkyl dithizonate is too dense to read, the analyst should add an additional 5.0 mℓ of dithizone solution to both the sample and blank and then repeat the measurements.

   The Pb content of the diluted sample was calculated by multiplying the answer according to No. 10 above by the total mℓ of dithizone solution used ÷ 5.0 mℓ of dithizone used for obtaining the factor (F). The addition of more

dithizone solution simply dilutes the color.

2. The above procedure measures tetraalkyl lead in water when the concentration of tetraalkyl lead decomposition products does not exceed 20-40% of the total quantity of lead present.

   If high concentrations of decomposition products are present (80-99% of the total lead present), additional extractions of n-heptane with water are needed to remove all the decomposition products from the n-heptane.

3. The analytical method described above measures only tetravalent lead.

4. Upon cooling, a slight turbidity may form in the n-heptane phase prior to spectrophotometric measurement. Shaking the stoppered cell for 2-3 seconds will remove the excess water (turbidity) from the lead dithizonate solution.

5. If a modified absorption cell (Figure 1) and spectrophotometer with a modified cell compartment are unavailable, perform the color development test in a 250-mℓ Squibb-type separatory funnel and drain the chloroform-lead dithizonate solution through cotton inserted in the stem of the separatory funnel directly into a conventional 10-mm absorption cell. Measure the absorbance of the lead dithizonate solution in the 10-mm cell by conventional means.

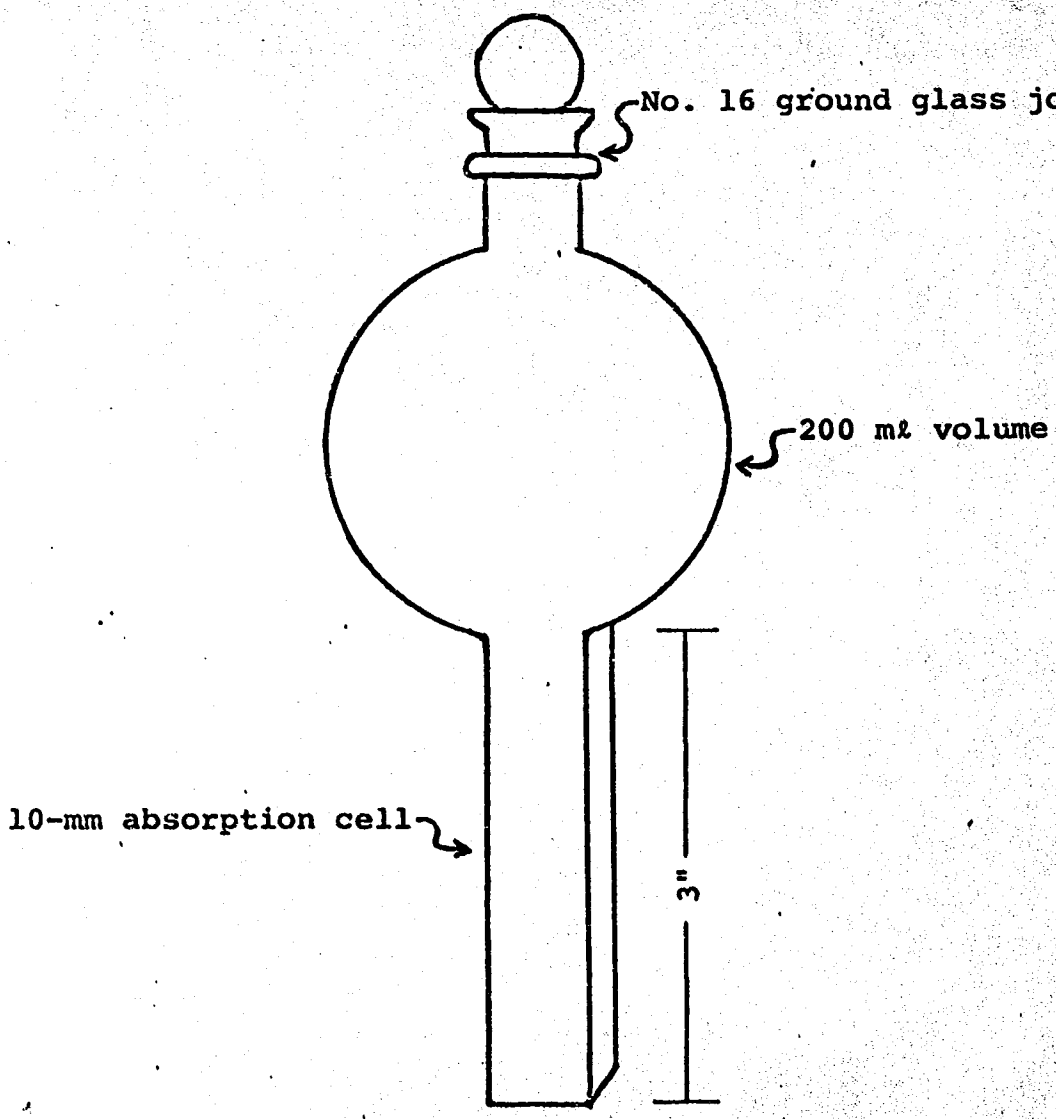
   The first 2-3 mℓ of dithizone solution passing through the cotton must be discarded to minimize contamination. Therefore, a minimum of 10 mℓ of dithizone solution must be used rather than the 5.0 mℓ specified in the method.

TABLE 1. Selection of sample volume, glassware and quantity of reagents for the separation, decomposition and analysis of tetraalkyl lead compounds in water.

Tetraalkyl lead present (ppb)	Graduated cylindrical sep. funnel	n-heptane (mℓ)	Sample volume (mℓ)	ICl solution (mℓ)
5,000	250-mℓ	50	5	20
1,000	250-mℓ	50	20	20
100	500-mℓ	50	200	20
10	2,000-mℓ	100	1,800	40
1	2,000-mℓ	100	1,800	40

Note: When analyzing seawater containing 500-5,000 ppb of tetraalkyl lead, use an all glass syringe to collect and to accurately measure small samples. Inject them immediately into the iodine monochloride solution at the sampling site.

FIGURE 1. Modified absorption cell



APPENDIX B

Chemical Procedure for the Analysis of Total Lead in Oyster Tissue

Equipment and reagents

1. Technicon BD-40 block digester with automatic temperature programmer.
2. Perkin-Elmer Model 305-A atomic absorption spectrophotometer (AAS) with a Boling three-slot burner.
3. Intensitron lead hollow cathode lamp.
4. Deuterium arc background correction module.
5. Perkin-Elmer Model 56 strip chart recorder.
6. Acid digest mixture: two parts of 30% hydrogen peroxide (Fisher Catalog No. 74879, lot 755296) and one part of 70% perchloric acid (Mallinkrodt Catalog No. 2766, lot ABC).
7. Nitric acid (Baker Catalog No. 5-9603, lot 524040).
8. Glass-distilled, deionized water.
9. Iodine monochloride (0.1 M) supplied by Ethyl Corporation.

Digestion procedure

Oyster tissue (3 oysters/sample) were received in polyethylene bottles containing 20 mℓ of iodine monochloride (ICl) solution. Fifteen mℓ of acid digest were added to the tissue in the polyethylene bottle, mixed and the contents were transferred to a 250-mℓ beaker. The polyethylene bottle was rinsed with an additional 25 mℓ of acid digest and the rinse was added to the beaker. The mixture stood at room temperature for one hour and was then placed on the hot plate and heated to 70°C. Heating was continued until digestion was near completion, as evidenced by a nearly clear solution and cessation of frothing (about 8 hours). The temperature was

then increased to 120°C for about 4 hours to destroy any residual peroxides and until a clear solution resulted.

The digests were cooled to room temperature, transferred to volumetric flasks and diluted to volume with distilled, deionized water. Background or control oyster digests were diluted to 25 mℓ and all other samples were diluted to 50 mℓ. The digests were analyzed for lead by AAS.

Analytical procedure

The following instrumental conditions were used throughout the work:

Wavelength: 283.3 nm was used for increased sensitivity for control, background and depuration samples; 217.0 nm was used for all other samples.

Bandpass: 0.7 nm (slit #4) Deuterium arc background correction.

Flame: Lean, acetylene/air.

Nebulizer flow rate: 5.5 mℓ/minute.

Standard lead solutions were prepared by dilution of a 1,000 mg/ℓ lead nitrate stock solution (U.S. Environmental Protection Agency, 1974) to 0.1, 0.5 and 1.0 μg Pb/mℓ of solution. All standards contained sufficient acid digest to match the acid content of digested oyster samples. The solutions were aspirated directly into the flame and the resultant recorder response was labeled and subsequently measured to the nearest mm. Lead standards were analyzed at the beginning and end of each series of samples and a curve of peak height in mm versus concentration in μg/mℓ was constructed. The peak heights of samples were measured and the corrections of lead in the acid digests were interpolated from the graph.

The minimum detectable concentration of lead in the acid digest volume (25 mℓ) was 0.1 ppm. Therefore, since 2.5 μg of lead were detected and a maximum of 10 g of oyster tissue was present in the 25 mℓ digest volume, the minimum detectable concentration of lead in oyster tissue was 0.25 mg/kilogram (kg).

Calculations

The concentration of lead in oysters (wet-weight basis) was calculated as follows:

$$\text{mg Pb/kg tissue} = \frac{(\mu g/m\ell \text{ Pb in acid digest})\ (\text{Volume acid digest in } m\ell)}{\text{Wet weight of oyster in g}}$$

Percentage recovery of TML from oyster tissue

Several oyster tissue samples were analyzed according to the above procedure to determine control oyster tissue lead concentration (background) and the percentage recovery of TEL. Control oysters and oysters spiked with 5.0 mg TEL (as Pb)/kg were prepared and shipped from BMRL in Pensacola, Florida, to the analytical laboratory in Wareham, Massachusetts. Therefore, the recovery of TEL from these spiked samples was subject to potential loss (if any) during shipment and handling.

The results of the recovery study were as follows:

Sample	Wet weight (g)	Concentration added	TEL found (μg Pb/g)
P2079	4.86	0	<0.57
P2080	7.00	0	<0.36
P2081	3.59	0	<0.70
P2082	6.07	0	<0.41
P2083	4.14	0	<0.60
P2076	9.6	5.0	4.79
P2077	10.0	5.0	4.89
P2078	13.1	5.0	4.72

The mean recovery (and standard deviation) of 5.0 mg

TEL/kg tissue was 96±2%. Therefore, the reported results were not corrected for the percentage recovery of the analytical method.

SUBMITTED BY:

EG&G, Bionomics
Marine Research Laboratory
Route 6, Box 1002
Pensacola, Florida 32507

PREPARED BY:

Tom Heitmuller

Tom Heitmuller
Biologist

APPROVED BY:

Rod Parrish

Rod Parrish
Director, Marine
Research Laboratory

APPENDIX A

HR- 2132-001

88-7800249

Bioaccumulation and depuration of lead by eastern oysters (Crassostrea virginica) exposed to a tetramethyl lead motor fuel antiknock compound

HR 0014

H# 9509 LEAD, TETRAMETHYL (MOTOR ANTIKNOCK)

Toxicity Test Report

Submitted to

E. I. du Pont de Nemours & Company

Haskell Laboratory

Newark, Delaware

EG&G, Bionomics
Marine Research Laboratory
Route 6, Box 1002
Pensacola, Florida 32507
September 1977

12

Studies were conducted with eastern oysters (Crassostrea virginica) and a tetramethyl lead motor fuel antiknock compound (TML-MFAC) to evaluate the rate of bioaccumulation and depuration of lead (Pb). In order to determine the appropriate concentrations for the bioaccumulation and depuration test, the acute effect of TML-MFAC on new shell growth of oysters was evaluated.

MATERIALS AND METHODS

Test material

The TML-MFAC, which contained 0.6235 grams (g) of Pb per milliliter (mℓ) of fluid, was received in a metal bottle sealed inside a metal shipping can. The fluid was a clear, red liquid. Because of known mammalian toxicity, safe handling procedures provided by E. I. du Pont de Nemours & Company were carefully followed.

Stock solutions of TML-MFAC for both the acute shell deposition and bioaccumulation tests were prepared with reagent grade acetone as the solvent/carrier. For the acute test, one set of stock solutions was sufficient for the 96-hour exposure. For the bioaccumulation study, new stock solutions were prepared at least weekly. All stock solutions were kept in amber glass bottles.

It should be noted that concentrations are expressed in this paper as micrograms (μg) of Pb per liter (ℓ) of seawater (μg Pb/ℓ) or micrograms of Pb per g of tissue (μg Pb/g), parts per billion (ppb) or parts per million (ppm), respectively.

The concentrations of Pb in seawater were determined as total alkyl Pb in the shell deposition test and as tetraalkyl Pb in the bioaccumulation test; concentrations of Pb in tissue were determined as total lead.

Test animals

Oysters used in the shell deposition test were 2-4 centimeters (cm), umbo to distal valve edge, and were selected from groups of oysters that had been collected from Big Lagoon, an estuary adjacent to Bionomics Marine Research Laboratory (BMRL). The oysters were held in tanks of flowing, natural seawater of ambient salinity and temperature for a minimum of two weeks before testing.

Oysters for the bioaccumulation and depuration study were collected from the Mississippi Sound near Ocean Springs, Mississippi, and were 5-8 cm, umbo to distal valve edge. These oysters were held at BMRL in flowing, natural seawater of ambient salinity and temperature for a minimum of one week before testing.

All oysters appeared to be in good condition at the initiation of the tests.

Test conditions

A. Shell deposition test

Methods for the 96-hour continuous-flow test were those of Butler et al. (1960) and Butler (1965). Fifty individual oysters of similar size were selected, cleaned of attached organisms and ground by hand on a fine-grit grinding wheel to remove approximately 2-5 millimeters (mm) of peripheral shell. Five groups of 10 oysters each were impartially separated and a group was placed in each test container. The oysters were removed from the test

containers after 96 hours of continuous exposure and the new shell growth of each oyster was measured to the nearest 0.1 mm by using a binocular dissecting scope equipped with a micrometer. The mean shell growth of control oysters less the mean growth of oysters from each concentration divided by the control growth and multiplied by 100 gave the percentage reduction in shell growth for oysters from each concentration relative to the control.

The test was conducted in a glass system (except for short lengths of Tygon® tubing connections) that was covered, where feasible, to reduce volatilization of the TML-MFAC. For each of the four test concentrations and the control, the exposure apparatus (Figure 1) was an individual system consisting of three basic components: (1) The mixing chamber, a 4-ℓ vacuum flask, was located above the other components. It received 30 ℓ/hour continuous flow of natural, unfiltered seawater via a calibrated glass siphon which led to a glass funnel fitted through a neoprene stopper that capped the flask. An appropriate volume of TML-MFAC stock solution or control acetone was metered into the mixing chamber by a syringe pump. The delivery line from the syringe passed through the funnel and into the mixing chamber. An electric stirrer was mounted above the flask; its stirring shaft extended through the neoprene stopper and into the mixing chamber. The rate of stirring was regulated by a rheostat. (2) A 6-ℓ rectangular glass settling tank was positioned between the mixing chamber and exposure aquarium to allow unsolubilized particles of TML-MFAC to settle out before entering the exposure chamber. The settling tank was divided in half by a glass

partition. Test solution flowed by gravity from the mixing chamber through a short length of Tygon® tubing into one end of the settling tank, passed over the partition into the other end and exited through a standpipe into the exposure aquarium. (3) An 8-ℓ glass aquarium, containing 10 test oysters, received the seawater/TML-MFAC mixture from the settling tank. Water flowed from the exposure aquarium through a standpipe to a drain pipe and into the BMRL waste treatment system. The volume of the entire system was 18 ℓ and approximately 99% replacement occurred every 3 hours (Sprague, 1969).

The nominal test concentrations were 50, 100, 500 and 1,000 μg Pb/ℓ. Control oysters received as great a volume of acetone (1.3 mℓ per hour) as did any treatment but no Pb. To confirm concentrations of Pb in seawater, water samples were taken from each exposure aquarium daily and analyzed at BMRL for alkyl lead. A complete description of methodology for water sample analyses is contained in Appendix A.

Oysters could obtain plankton and other particulate matter from the unfiltered seawater in which they were tested; there was no supplemental feeding.

The test was conducted from 12-16 June 1976.

B. Bioaccumulation and depuration test

Two separate groups of 80 oysters each were continuously exposed to TML-MFAC for 56 days in flowing, natural seawater. The nominal test concentrations were 0.9 and 9.0 μg Pb/ℓ; a group of control oysters received as great a volume of acetone (0.74 mℓ per hour) ad did any treatment but no TML-MFAC.

The exposure system (Figure 2) for each test group consisted of two basic components, a 4-ℓ rectangular glass mixing chamber and a 60-ℓ fiberglass resin-coated plywood exposure aquarium. No settling tank was used because of the low concentrations being tested. The mixing chambers, placed above the exposure aquaria, received 160 ℓ/hour of seawater at ambient salinity and temperature via calibrated glass siphons. A syringe pump metered appropriate volumes of TML-MFAC stock solution or acetone into the mixing chambers. An electric stirrer was mounted above each mixing chamber and the stirring shaft extended into the chamber. Incoming seawater and the stock solutions were mixed in the chambers, then flowed by gravity through short lengths of Tygon® tubing into the exposure aquaria which contained the oysters. The volume of the system was 64 ℓ and 99% replacement occurred every 2 hours (Sprague, op. cit.).

Absolute ethyl alcohol was used as the solvent/carrier for TML-MFAC during days 1-3 of the bioaccumulation study. However, the ethanol apparently induced an extremely heavy deposit of a slime-like growth in all mixing and exposure tanks. Acetone was substituted for ethanol as the solvent/carrier on day 4 and the problem ceased.

During the exposure, test water was analyzed for alkyl Pb and oyster tissue was analyzed for total Pb. Water samples were taken weekly. Three oysters were removed from the control and each test concentration on days 3, 7, 10, 14, 21, 28, 42 and 56 of exposure, shucked and the meats (whole-body) combined in tared containers and weighed. Twenty milliliters of iodine monochloride solution were added to each container to fix the Pb in the oysters

for later chamical analyses by atomic absorption spectrophotometry. Complete descriptions of methodology for water and tissue analyses are contained in Appendixes A and B.

After 56 days of exposure, the remaining oysters were placed in flowing seawater to which no TML-MFAC was added to determine the rate of depuration of Pb. Oysters from the control and each test concentration were sampled for chemical analysis of total Pb in tissue on days 3, 7, 10, 14 and 28 of the depuration period.

Oysters could obtain plankton and other particulate matter from the unfiltered seawater in which they were tested; there was no supplemental feeding.

The growth of oysters during the 56-day exposure was monitored by comparing the average in-water weights (Andrews, 1961) of oysters from each test concentration and control on day 0 and day 56. The in-water weights were determined by placing groups of 4 oysters on a tray that was suspended from the weighing hook of a top-loading balance. The tray and oysters were immersed in seawater.

The test was conducted from 24 September-17 December 1976.

Statistical analyses

Based on the results of the 96-hour oyster shell deposition test, a 96-hour EC50 (the concentration of TML-MFAC effective in reducing shell growth of exposed oysters by 50% as compared to growth of control oysters) was calculated. Test concentrations were converted to logarithms and corresponding percentage reductions of shell depositions were converted to probits (Finney, 1971). The 96-hour EC50 was then calculated by linear regression.

PAGE 0020

Differences between the concentrations of Pb in exposed oysters as compared to the background concentrations found in control oysters were determined with "students" t-test and were considered to be statistically significant at $P<0.05$ (Sokal, 1973).

RESULTS AND DISCUSSION

A. Analyses of alkyl Pb in seawater

During the 96-hour shell deposition test, the mean measured concentrations of total alkyl Pb, based on daily analyses of seawater from each exposure tank, were 47, 133, 641 and 1,277 µg Pb/ℓ (Table 1). The measured concentrations were used to calculate the 96-hour EC50 and, hereafter in this report, test concentrations during the acute shell deposition test are given as the mean measured concentrations of total alkyl Pb.

The mean measured concentrations of tetraalkyl Pb in seawater in the exposure aquaria during the bioaccumulation study were 0.7 and 6.8 µg Pb/ℓ (Table 2). Hereafter in this report, test concentrations during the bioaccumulation study are given as the mean measured concentrations of tetraalkyl Pb.

It should be noted that methods for the analysis of tetraalkyl Pb in seawater during the bioaccumulation study were slightly modified from the methods outlined in Appendix A. Because of a malfunction of the Beckman Model B Spectrophotometer, analyses after day 5 of exposure were made by using a Bausch and Lomb Spectronic 20 spectrophotometer. Changing instruments also necessitated transferring the dithizone layer by pipet from the modified 200-mℓ absorption cell to a conventional cuvette for

measuring the absorbance. The transferred dithizone layer became cloudy as it cooled; therefore, the cuvettes were placed in a warm water bath to prevent the liquid from becoming cloudy before the absorbance was determined.

B. Shell deposition test

The calculated 96-hour EC50 for oysters exposed to TML-MFAC in continuously flowing, natural seawater was 98 µg Pb/ℓ with 95% confidence limits of 19-509 µg Pb/ℓ. The percentage reduction of new shell growth of the exposed oysters as compared to the control oysters was from 25% in the 47 µg Pb/ℓ test concentration to 100% in 1,227 µg Pb/ℓ (Table 3).

The new shell growth of the control oysters (mean 1.2 mm) was not as great as we have observed in other tests. However, the conditions at the time of testing were not optimal. Oysters along the northcentral coast of the Gulf of Mexico appear to grow best in the early spring and late fall. This test was conducted during the summer when growth was likely inhibited by high water temperature (26-28 degrees Celsius, °C).

Despite the relatively low rate of shell growth, the acute test served as a valid means of selecting appropriate test concentrations of TML-MFAC for the bioaccumulation study.

c. Bioaccumulation and depuration study

Concentrations of total Pb in tissues of oysters exposed to 0.7 µg Pb/ℓ were very near background for all samples taken through day 28 of the exposure. On day 42, the concentration of Pb in tissue was 0.08 µg Pb/g above control concentration and on day 56 the residue was 0.33 µg Pb/g above background (Table 4). However, comparison of data over the entire 56-day exposure showed no significant difference ($P<0.05$) between the control concentrations and the

concentrations of Pb found in the oysters exposed to 0.7 μg Pb/ℓ.

Oysters accumulated measurable amounts of Pb from the higher test concentration of TML-MFAC in seawater. After 3 and 7 days of exposure, oysters from the 6.8 μg Pb/ℓ test concentration had accumulated 0.29 and 0.31 μg Pb/g above control concentrations, respectively. After 10 days of exposure, they had accumulated 0.74 μg Pb/g of Pb above the control concentration. Further sampling on days 14, 28, 42 and 56 indicated that a plateau had been reached by day 10 of exposure (Table 4). The mean residue of Pb in tissues of oysters sampled from the 6.8 μg Pb/ℓ test concentration on days 10, 14, 28, 42 and 56 of exposure was 0.57 μg Pb/g above control concentration. Comparison of the data over the entire 56-day exposure showed a statistically significant difference ($P<0.05$) between the concentrations of Pb in the control oysters and the concentrations of Pb measured in tissues of the oysters exposed to 6.8 μg Pb/ℓ. A bioconcentration factor of 84 was calculated by dividing the mean measured concentration of tetraalkyl Pb in seawater (6.8 μg Pb/ℓ) during the 56-day exposure into the mean concentration of Pb above control concentrations (0.57 μg Pb/g) found in oysters during the plateau interval.

Oysters rapidly depurated the Pb which they had accumulated. Concentrations of total Pb in oysters from both exposure concentrations were near background concentrations after 3 days of depuration in seawater to which no TML-MFAC was added. On day 3, Pb concentrations in oysters previously exposed to 0.7 and 6.8 μg Pb/ℓ of tetraalkyl Pb were 0.44 and 0.48 μg Pb/ℓ, respectively (Table 4).

There was no meaningful effect on growth of exposed oysters as compared to growth of control oysters. The in-water weights of oysters from both test groups and the control group increased slightly during the 56 days of exposure. The control oysters increased by 5%; the 0.7 μg Pb/ℓ-exposed oysters, 5%; and the 6.8 μg Pb/ℓ-exposed oysters, 3% (Table 5).

Salinity was 18-27 parts per thousand (°/oo) during the shell deposition test and 21-33 °/oo during the bioaccumulation and depuration study; temperature was 26-28°C and 11-28°C, respectively (Table 6).

SUMMARY

Shell growth of oysters exposed to TML-MFAC in seawater was reduced by more than 50% in concentrations of total alkyl Pb ≥133 μg Pb/ℓ during an acute exposure. The calculated 96-hour EC50, based on mean measured concentrations of total alkyl Pb in seawater, was 98 μg Pb/ℓ with 95% confidence limits of 19-509 μg Pb/ℓ.

Oysters exposed to TML-MFAC in seawater for 56 days accumulated no statistically significant concentrations of Pb above background concentrations in the lower concentration (0.7 μg Pb/ℓ of tetraalkyl Pb), while oysters exposed to the higher concentration (6.8 μg Pb/ℓ of tetraalkyl Pb) accumulated statistically significant amounts of Pb above background concentrations. A plateau was reached after 10 days of exposure; the calculated bioconcentration factor was 84.

Oysters rapidly depurated the Pb which they had accumulated. The concentration of Pb in oysters from both exposed groups was near the background concentration after 3 days of depuration in seawater to which no TML-MFAC was added.

REFERENCES

Andrews, J.D. 1961. Measurement of shell growth in oysters. Proc. Natn. Shellfish. Assoc. 52:1-11.

Butler, P.A., A.J. Wilson, Jr. and A.J. Rick. 1960. Effect of pesticides on oysters. Proc. Natn. Shellfish. Assoc. 51:23-32.

Butler, P.A. 1965. Reaction of some estuarine mollusks to environmental factors. U.S. Dept. of Health, Education, and Welfare, Public Health Service Publication No. 99-WP-25. p. 92-104.

Finney, D.J. 1971. Probit Analysis. Cambridge University Press, London. 333 p.

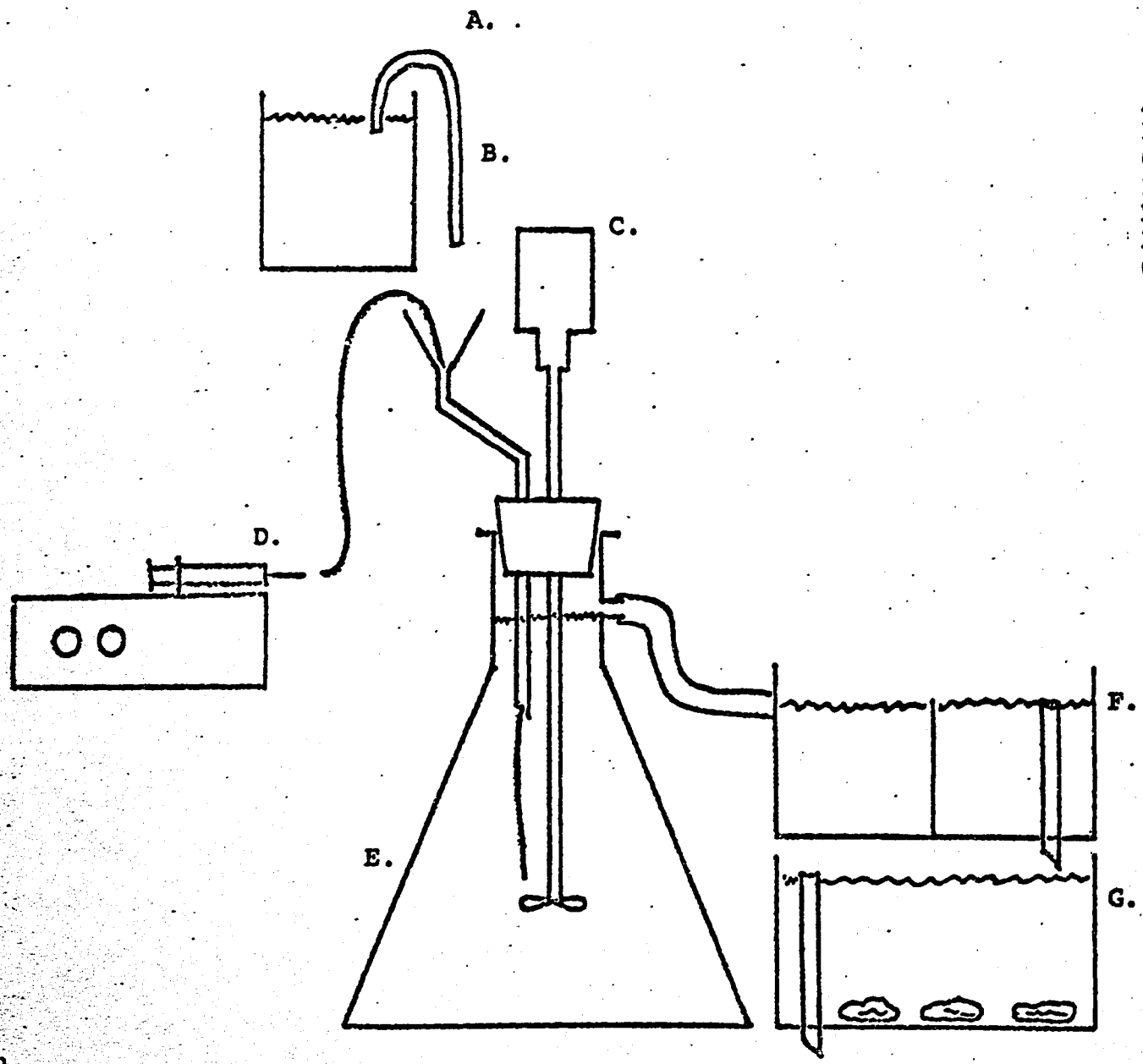
Sokal, R.R. and F.J. Rohlf. 1973. Introduction to Biostatistics. W.H. Freeman and Company, San Francisco. 368 p.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Research 3(11): 793-821.

U.S. Environmental Protection Agency. 1976. Bioassay procedures for the ocean disposal permit program. EPA-600/9-76-010, 96 p.

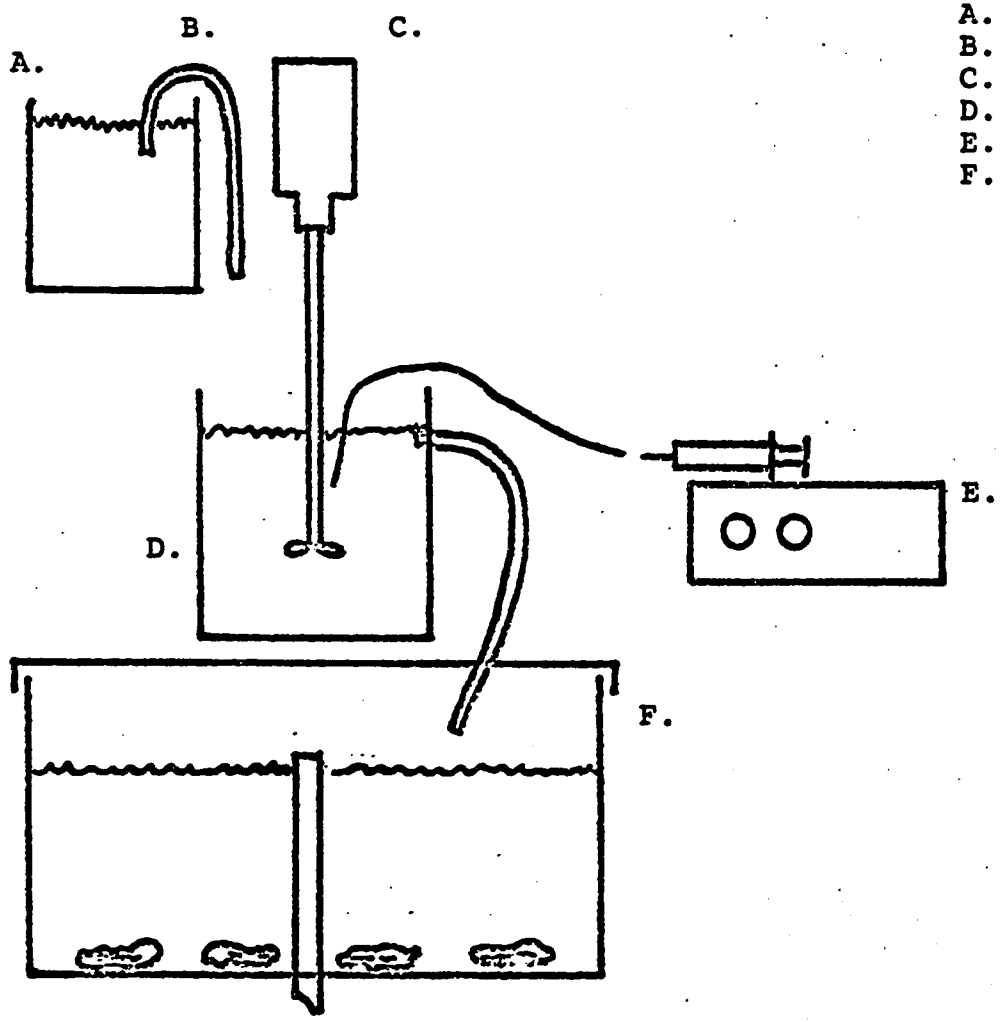
_____. 1974. Methods for chemical analysis of water and wastes. EPA-625/6-74-003. 298 p.

FIGURE 1. Diagram of the exposure system used during a 96-hour shell deposition test with eastern oysters (*Crassostrea virginica*) and a tetramethyl lead motor fuel antiknock compound.



A. Seawater headbox
B. Calibrated glass siphon
C. Electric stirrer
D. Syringe pump
E. Mixing chamber
F. Settling tank
G. Exposure aquarium

FIGURE 2. Diagram of the exposure system used during a bioaccumulation and depuration study with eastern oysters (Crassostrea virginica) and a tetramethyl lead motor fuel antiknock compound.



A. Seawater headbox
B. Calibrated glass siphon
C. Electric mixer
D. Mixing chamber
E. Syringe pump
F. Exposure aquarium

TABLE 1. Measured concentrations of total alkyl lead (Pb) in seawater during a 96-hour shell deposition test with eastern oysters (*Crassostrea virginica*) and a tetramethyl lead motor fuel antiknock compound in flowing, natural seawater. Salinity was 18-27 ‰ and temperature, 26-28°C.

Nominal concentration of total alkyl Pb (µg Pb/ℓ)	Measured concentration of total alkyl Pb (µg Pb/ℓ)				$\bar{X}$	SD[a]
	24 h	48 h	72 h	96 h		
Control	ND[b]	ND	ND	ND	ND	-
50	49	35	48	57	47	±9
100	129	108	117	179	133	±32
500	648	655	712	552	641	±66
1,000	2,200	1,573	655	481	1,227	±806
500 (heptane-extracted)	-[c]	-	-	416		

[a]Standard deviation.

[b]Not detectable; <0.05 µg Pb/ℓ.

[c]To confirm the alkyl Pb as tetraalkyl Pb, one water sample was extracted with n-heptane and then analyzed for tetraalkyl Pb.

TABLE 2. Measured concentrations of tetraalkyl lead in seawater during a bioaccumulation study in which eastern oysters (*Crassostrea virginica*) were exposed to a tetramethyl lead motor fuel antiknock compound in flowing, natural seawater for 56 days.

Concentrations of tetraalkyl Pb (μg Pb/ℓ)					
Nominal	Mean	SD	Percent of nominal	Range	Number of samples
Control	ND[a]	-	-	0.0-0.2	8
0.9	0.7	±0.3	78	0.4-1.2	8
9.0	6.8	±0.6	76	5.8-7.5	8

[a]Not detectable, <0.05 μg Pb/ℓ.

PAGE 0030

TABLE 3. Effects of a tetramethyl lead motor fuel antiknock compound on shell deposition of eastern oysters (*Crassostrea virginica*) exposed for 96 hours in flowing, natural seawater. Salinity was 18-27 °/oo and temperature, 26-28°C. Shell deposition is the mean value of shell measurements of ten oysters.

Mean measured concentration of tetraalkyl Pb (µg Pb/ℓ)	Shell deposition, mm (Mean and std. dev.)	Reduction[a] (%)
Control	1.2 ± 0.4	---
47	0.9 ± 0.3	25
133	0.4 ± 0.5	67
641	0.2 ± 0.3	83
1,227	0.0	100

$$^{a}\% \text{ reduction} = \frac{\text{Shell deposition of control oysters minus shell deposition of exposed oysters}}{\text{Shell deposition of control oysters}} \times 100$$

PAGE 0031

TABLE 4. Measured concentrations of total lead (Pb) in whole-body tissue of eastern oysters (*Crassostrea virginica*) exposed to a tetramethyl lead motor fuel antiknock compound (TML-MFAC) in a bioaccumulation and depuration study in flowing, natural seawater. Salinity was 21-33 °/oo and temperature, 11-28°C. Each value represents analysis of a pooled sample of 3 oysters. Whole-body concentrations are listed under the appropriate exposure to TML-MFAC (control, 0.7 µg Pb/ℓ and 6.8 µg Pb/ℓ).

		Tissue concentration (µg Pb/g, wet weight)		
		(Control)	(0.7)	(6.8)
Exposure	3	0.57	0.58	0.86
	7	0.53	0.60	0.84
	10	0.55	0.59	1.29
	14	0.52	0.46	1.00
	28	0.70	0.65	1.20
	42	0.50	0.58	0.98
	56	0.41	0.77	1.10
Depuration	3	–[a]	0.44	0.48
	7	–[a]	0.68	0.56
	10	–[a]	0.53	0.57
	14	0.42	0.68	0.45
	21	–[a]	0.43	0.56
	28	0.37	0.37	0.56

[a]No sample taken.

NE 0032

TABLE 5. Weights of eastern oysters (*Crassostrea virginica*) during a bioaccumulation study. The oysters were weighed in water at the initiation of the study and after 56 days of exposure to a tetramethyl lead motor fuel antiknock compound in flowing, natural seawater. Salinity was 25-33 °/oo and temperature, 15-28°C. The in-water weight of groups of 4 oysters was determined, except as noted, and are listed under the appropriate exposure treatment (control, 0.7 µg Pb/ℓ and 6.8 µg Pb/ℓ).

Group	Initial weight (g)			Day 56 weight (g)		
	(Control)	(0.7)	(6.8)	(Control)	(0.7)	(6.8)
1	193	174	143	176	201	118
2	159	157	137	189	162	168
3	146	144	140	147	138	141
4	189	132	131	181	132	112
5	158	154	143	193	178	147
6	206	184	134	207	161	112
7	155	143	169	174	206	129
8	136	115	133	176	135	149
9	177	105	152	186	157	188
10	185	143	161	161	149	150
11	152	142	109	163	77[a]	133
12	134	174	121	83[b]	-[c]	173
13	144	166	113			
14	163	145	150			
15	185	178	142			
16	186	163	150			
17	178	213	158			
18	161	181	111			
19	165	195	111			
20	151	150	161			
Average per oyster	42	39	35	44	41	36
Percentage increase	-	-	-	5	5	3

[a]Group of three oysters.

[b]Group of two oysters.

[c]No oysters remained.

TABLE 6. Daily measurements of the salinity and temperature of flowing, natural seawater in which a bioaccumulation-depuration study was conducted with tetramethyl lead motor fuel antiknock compound and eastern oysters (*Crassostrea virginica*).

Date	Test day	Salinity (°/oo)	Temperature (°C)
24 Sep 76	0	28	26
25 Sep 76	1	28	27
26 Sep 76	2	28	27
27 Sep 76	3	27	27
28 Sep 76	4	25	28
29 Sep 76	5	27	28
30 Sep 76	6	31	27
1 Oct 76	7	28	27
2 Oct 76	8	28	26
3 Oct 76	9	26	27
4 Oct 76	10	28	26
5 Oct 76	11	29	27
6 Oct 76	12	30	25
7 Oct 76	13	29	25
8 Oct 76	14	30	24
9 Oct 76	15	26	21
10 Oct 76	16	27	23
11 Oct 76	17	25	22
12 Oct 76	18	29	22
13 Oct 76	19	26	22
14 Oct 76	20	29	23
15 Oct 76	21	28	22
16 Oct 76	22	29	23
17 Oct 76	23	30	23
18 Oct 76	24	30	20
19 Oct 76	25	30	21
20 Oct 76	26	29	21
21 Oct 76	27	29	19
22 Oct 76	28	27	20
23 Oct 76	29	28	20
24 Oct 76	30	30	21
25 Oct 76	31	30	21
26 Oct 76	32	30	20
27 Oct 76	33	30	19
28 Oct 76	34	30	19
29 Oct 76	35	30	18
30 Oct 76	36	32	19
31 Oct 76	37	31	18

(continued)

TABLE 6. Continued.

Date	Test day	Salinity (°/oo)	Temperature (°C)
1 Nov 76	38	28	17
2 Nov 76	39	28	17
3 Nov 76	40	28	17
4 Nov 76	41	26	17
5 Nov 76	42	27	17
6 Nov 76	43	27	16
7 Nov 76	44	28	16
8 Nov 76	45	30	16
9 Nov 76	46	30	16
10 Nov 76	47	29	17
11 Nov 76	48	31	18
12 Nov 76	49	32	18
13 Nov 76	50	30	17
14 Nov 76	51	32	17
15 Nov 76	52	33	17
16 Nov 76	53	33	16
17 Nov 76	54	32	15
18 Nov 76	55	30	15
19 Nov 76	56	30	16
	Depuration		
20 Nov 76	1	30	15
21 Nov 76	2	30	15
22 Nov 76	3	28	14
23 Nov 76	4	26	14
24 Nov 76	5	28	14
25 Nov 76	6	28	15
26 Nov 76	7	30	17
27 Nov 76	8	32	18
28 Nov 76	9	31	17
29 Nov 76	10	25	14
30 Nov 76	11	24	11
1 Dec 76	12	22	11
2 Dec 76	13	23	13
3 Dec 76	14	25	13
4 Dec 76	15	24	14
5 Dec 76	16	28	13
6 Dec 76	17	30	14
7 Dec 76	18	28	14
8 Dec 76	19	24	12

(continued)

TABLE 6. Continued.

Date	Test day	Salinity (°/oo)	Temperature (°C)
9 Dec 76	20	25	12
10 Dec 76	21	28	14
11 Dec 76	22	31	13
12 Dec 76	23	31	14
13 Dec 76	24	27	14
14 Dec 76	25	22	13
15 Dec 76	26	22	14
16 Dec 76	27	21	15
17 Dec 76	28	24	13

APPENDIX A

Determination of Tetraalkyl Lead in Seawater

Method in brief

Tetraalkyl lead compounds are first extracted from water with n-heptane and then extracted from n-heptane with an aqueous iodine monochloride solution. The lead content of the iodine monochloride solution is measured by a simplified colorimetric dithizone method.

Reagent

1. n-Heptane: Pure grade, 99 Mol% minimum. Phillips Petroleum Company, Bartles-Nille, Oklahoma 74004.
2. Reducing solution: Dissolve 80 g of dibasic ammonium citrate, 40 g of potassium cyanide, 400 g of anhydrous sodium sulfate, 15 g of disodium ethylenediaminetetraacetate in 2500-mℓ of water. Add 2500 mℓ of ammonium hydroxide (28% NH_3), mix well to dissolve all reagents and store in a sealed bottle.
3. Iodine monochloride solution (approximately 0.1 m): Mix 500 mℓ of potassium iodide solution (250 g potassium iodide diluted to 1 ℓ with water) with 500 mℓ of concentrated hydrochloric acid (37%). Slowly add 84 g of reagent grade potassium iodate with constant stirring until all the free iodine has redissolved to give a clear orange-red solution. Dilute to 10 ℓ with distilled water.

   Note: Iodine monochloride and iodine will react with ammonium ions under certain conditions to yield nitrogen triiodide. It is important to always have an excess of reducing agent (sodium sulfite) present when either of the above halogens are mixed with ammonium hydroxide solutions.

4. Dithizone solutions: Dissolve 40 mg of diphenylthiocarbazone (dithizone) in 1 ℓ of reagent grade chloroform. Mix well and store in a brown bottle in the absence of direct sunlight.

Apparatus

1. Modified absorption cell (Figure 1). Purchased from E. Leitz Inc., Rock Leigh Industrial Park, Rock Leigh, NJ 07647, catalog number 94626.
2. Beckman Model "B" Spectrophotometer with modified cell compartment and modified cell compartment cover. Note: Modified instrument loaned to Bionomics Marine Research Laboratory for lead bioaccumulation studies.

   Note: The modified absorption cell fits directly into a Leitz Spectrophotometer without need for modifying this instrument.
3. Separatory funnels (cylindrical and graduated). 250 mℓ, 500 mℓ and 2,000 mℓ sizes depending on sample size to be analyzed.
4. Graduated cylinders. 25 mℓ and 50 mℓ sizes.
5. Pipet. 5.0 mℓ size.
6. Syringe. 10 mℓ, 30 mℓ and 50 mℓ sizes. B&D all glass syringes with number 17 needles.

Standardization

A precise calibration factor (F) may be obtained by adding known quantities of Pb (as tetraalkyl lead) to water samples as follows:

Add known quantities of Pb with a syringe well beneath the surface of water samples contained in separatory funnels. Analyze

the lead in these known samples by the procedure described below. Calculate the calibration factor as follows:

$$\text{Calibration factor (F)} = \frac{\mu g \text{ of Pb in known sample}}{(\text{absorbance of known sample} - \text{absorbance of blank})}$$

Procedure (1.0 - 5,000 ppb tetraalkyl lead)

1. Siphon the seawater sample (see Table 1 for sample size) directly into a graduated cylindrical separatory funnel containing n-heptane (see Table 1 for volume). Protect the sample from sunlight and immediately analyze it to avoid possible decomposition of tetraalkyl lead.
2. Shake the mixture vigorously for 2 minutes. The tetraalkyl lead will extract into the n-heptane solution.
3. After the two layers have separated, drain the aqueous solution into a graduated cylinder (100 mℓ to 2,000 mℓ depending on sample size). Discard the water after recording its volume.

   Note: If the sample volume was measured in step "1" above, it need not be measured here.
4. Add 100 mℓ of distilled water to the original separatory funnel and shake the n-heptane and water mixture for 1 minute. After the two layers have separated, drain and discard the water layer.
5. Add iodine monochloride solution (either 20 mℓ or 40 mℓ, see Table 1) to the n-heptane present in the original separatory funnel. Shake it vigorously for approximately 2 minutes. Immediately add 50 mℓ of distilled water, shake approximately 1 minute and allow the two layers to separate. At this point all the tetraalkyl lead has been extracted by the iodine monochloride solution.

6. Drain the iodine monochloride solution into a modified absorption cell (Figure 1).
7. Add 30 mℓ of reducing solution to the modified absorption cell. Mix well by shaking the stoppered cell to eliminate all black particles.
8. Add 5.0 mℓ of dithizone solution to the modified absorption cell, shake it vigorously for 30 seconds, and allow the two layers to separate.
9. Insert the modified absorbance cell into the spectrophotometer and record the absorbance of the lower layer at 485 nanometers (nm) wavelength.
10. Analyze a blank using all reagents and distilled water, but use no seawater sample.

Calculations

$$\text{ppm of Pb} = \frac{(\text{absorbance of sample} - \text{absorbance of blank}) \times F}{\text{volume of seawater, m}\ell}$$

Note: The calibration factor (F) should be approximately 18.5 when using 5.0 mℓ of dithizone solution.

Comments

1. If an analysis is made and the color of the lead alkyl dithizonate is too dense to read, the analyst should add an additional 5.0 mℓ of dithizone solution to both the sample and blank and then repeat the measurements.

   The Pb content of the diluted sample was calculated by multiplying the answer according to No. 10 above by the total mℓ of dithizone solution used ÷ 5.0 mℓ of dithizone used for obtaining the factor (F). The addition of more

dithizone solution simply dilutes the color.

2. The above procedure measures tetraalkyl lead in water when the concentration of tetraalkyl lead decomposition products does not exceed 20-40% of the total quantity of lead present.

   If high concentrations of decomposition products are present (80-99% of the total lead present), additional extractions of n-heptane with water are needed to remove all the decomposition products from the n-heptane.

3. The analytical method described above measures only tetravalent lead.

4. Upon cooling, a slight turbidity may form in the n-heptane phase prior to spectrophotometric measurement. Shaking the stoppered cell for 2-3 seconds will remove the excess water (turbidity) from the lead dithizonate solution.

5. If a modified absorption cell (Figure 1) and spectrophotometer with a modified cell compartment are unavailable, perform the color development test in a 250-mℓ Squibb-type separatory funnel and drain the chloroform-lead dithizonate solution through cotton inserted in the stem of the separatory funnel directly into a conventional 10-mm absorption cell. Measure the absorbance of the lead dithizonate solution in the 10-mm cell by conventional means.

   The first 2-3 mℓ of dithizone solution passing through the cotton must be discarded to minimize contamination. Therefore, a minimum of 10 mℓ of dithizone solution must be used rather than the 5.0 mℓ specified in the method.

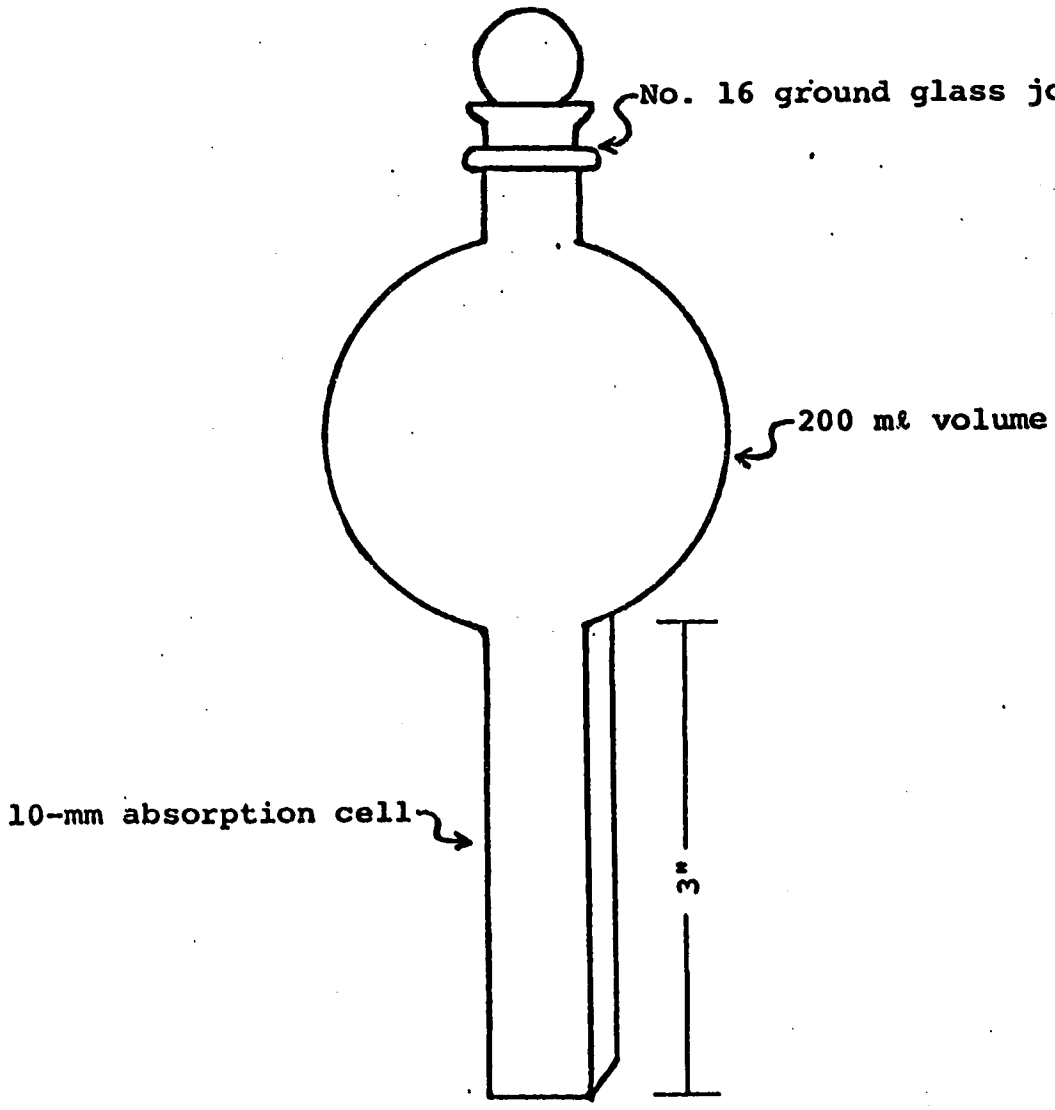
PAGE 0041

TABLE 1. Selection of sample volume, glassware and quantity of reagents for the separation, decomposition and analysis of tetraalkyl lead compounds in water.

Tetraalkyl lead present (ppb)	Graduated cylindrical sep. funnel	n-heptane (mℓ)	Sample volume (mℓ)	ICl solution (mℓ)
5,000	250-mℓ	50	5	20
1,000	250-mℓ	50	20	20
100	500-mℓ	50	200	20
10	2,000-mℓ	100	1,800	40
1	2,000-mℓ	100	1,800	40

Note: When analyzing seawater containing 500-5,000 ppb of tetraalkyl lead, use an all glass syringe to collect and to accurately measure small samples. Inject them immediately into the iodine monochloride solution at the sampling site.

FIGURE 1. Modified absorption cell



PAGE 0043

APPENDIX B

Chemical Procedure for the Analysis of Total Lead in Oyster Tissue

Equipment and reagents

1. Technicon BD-40 block digester with automatic temperature programmer.
2. Perkin-Elmer Model 305-A atomic absorption spectrophotometer (AAS) with a Boling three-slot burner.
3. Intensitron lead hollow cathode lamp.
4. Deuterium arc background correction module.
5. Perkin-Elmer Model 56 strip chart recorder.
6. Acid digest mixture: two parts of 30% hydrogen peroxide (Fisher Catalog No. 74879, lot 755296) and one part of 70% perchloric acid (Mallinkrodt Catalog No. 2766, lot ABC).
7. Nitric acid (Baker Catalog No. 5-9603, lot 524040).
8. Glass-distilled, deionized water.
9. Iodine monochloride (0.1 M) supplied by Ethyl Corporation.

Digestion procedure

Oyster tissue (3 oysters/sample) were received in polyethylene bottles containing 20 mℓ of iodine monochloride (ICl) solution. Fifteen mℓ of acid digest were added to the tissue in the polyethylene bottle, mixed and the contents were transferred to a 250-mℓ beaker. The polyethylene bottle was rinsed with an additional 25 mℓ of acid digest and the rinse was added to the beaker. The mixture stood at room temperature for one hour and was then placed on the hot plate and heated to 70°C. Heating was continued until digestion was near completion, as evidenced by a nearly clear solution and cessation of frothing (about 8 hours). The temperature was

then increased to 120°C for about 4 hours to destroy any residual peroxides and until a clear solution resulted.

The digests were cooled to room temperature, transferred to volumetric flasks and diluted to volume with distilled, deionized water. Background or control oyster digests were diluted to 25 mℓ and all other samples were diluted to 50 mℓ. The digests were analyzed for lead by AAS.

Analytical procedure

The following instrumental conditions were used throughout the work:

Wavelength: 283.3 nm was used for increased sensitivity for control, background and depuration samples; 217.0 nm was used for all other samples.

Bandpass: 0.7 nm (slit #4) Deuterium arc background correction.

Flame: Lean, acetylene/air.

Nebulizer flow rate: 5.5 mℓ/minute.

Standard lead solutions were prepared by dilution of a 1,000 mg/ℓ lead nitrate stock solution (U.S. Environmental Protection Agency, 1974) to 0.1, 0.5 and 1.0 μg Pb/mℓ of solution. All standards contained sufficient acid digest to match the acid content of digested oyster samples. The solutions were aspirated directly into the flame and the resultant recorder response was labeled and subsequently measured to the nearest mm. Lead standards were analyzed at the beginning and end of each series of samples and a curve of peak height in mm versus concentration in μg/mℓ was constructed. The peak heights of samples were measured and the corrections of lead in the acid digests were interpolated from the graph.

The minimum detectable concentration of lead in the acid digest volume (25 mℓ) was 0.1 ppm. Therefore, since 2.5 µg of lead were detected and a maximum of 10 g of oyster tissue was present in the 25 mℓ digest volume, the minimum detectable concentration of lead in oyster tissue was 0.25 mg/kilogram (kg).

Calculations

The concentration of lead in oysters (wet-weight basis) was calculated as follows:

$$\text{mg Pb/kg tissue} = \frac{(\mu g/m\ell \text{ Pb in acid digest})\ (\text{Volume acid digest in } m\ell)}{\text{Wet weight of oyster in g}}$$

Percentage recovery of TML from oyster tissue

Several oyster tissue samples were analyzed according to the above procedure to determine background lead and the percentage recovery of TML. Control oysters and oysters spiked with 5.0 mg TML (as Pb)/kg were prepared and shipped from BMRL in Pensacola, Florida, to the analytical laboratory in Wareham, Massachusetts. Therefore, the recovery of TML from these spiked samples was subject to potential loss (if any) during shipment and handling.

The results of the recovery study were as follows:

Sample	Wet weight (g)	Concentration added	TML found (µg Pb/g)
P2079	4.86	0	<0.57
P2080	7.00	0	<0.36
P2081	3.59	0	<0.70
P2082	6.07	0	<0.41
P2083	4.14	0	<0.60
P2073	7.2	5.0	5.1[a]

(continued)

Sample	Wet weight (g)	Concentration added	TML found (µg Pb/g)
P2074	7.6	5.0	4.7
P2075	11.0	5.0	4.2

[a]The mean recovery (and standard deviation) of 5.0 mg TML/kg tissue was 94±9.0%. Therefore, the reported results were not corrected for the percentage recovery of the analytical method.

SUBMITTED BY:

EG&G, Bionomics
Marine Research Laboratory
Route 6, Box 1002
Pensacola, Florida 32507

PREPARED BY:

Tom Heitmuller

Tom Heitmuller
Biologist

APPROVED BY:

Rod Parrish

Rod Parrish
Director, Marine
Research Laboratory

APPENDIX B

REFERENCE FILE No. RA 6486

88-7800249 Copy

COPY No. 9

THE ASSOCIATED OCTEL COMPANY LIMITED

RESEARCH & ENGINEERING DEPARTMENT

PART II.

PAGE 0 1 0 9

THE SOLUBILITY AND BREAKDOWN OF LEAD ALKYLS IN NATURAL WATER.

(a) The behaviour of Antiknock Compounds using the Jones-Kaplan Equilibrator.
(b) Open tank experiments on Antiknock Compounds.

AUTHORS. W. TOWERS
A. WHITTINGHAM

APPROVED BY: J.R. GROVE

PASSED FOR CIRCULATION: S.F. GROVER

BEST PAGE AVAILABLE

CIRCULATION:

1. Technical Manager
2. Production Manager
3. Works General Manager
4. Manager, Environmental Affairs
5. Commercial Manager
6. Manager, R. & E.
7. Manager - Planned
8. A. Whittingham, W. Towers
9-11. Information Department
11 Assistant to Managing Director

PHOTO COPY

ELLESMERE PORT
OCTOBER 1974

103

CONTENTS

		Page
1.	SUMMARY AND CONCLUSIONS	1
2.	INTRODUCTION	3
3.	EXPERIMENTAL AND RESULTS	4
	3.1 Studies using the Jones-Kaplan Equilibrator	4
	3.2 Studies using open tank experiments	5
4.	DISCUSSION OF RESULTS	12
	4.1 Jones-Kaplan Equilibrator experiments	12
	4.2 Open tank experiments	13
	4.2.1. TEL-B/distilled water/with and without sand	14
	4.2.2. TEL-B/sea water/sand	14
	4.2.3. TML-CB/distilled water	16
	4.2.4. TML-CB/sea water	17
5.	COMPARISON OF THE BEHAVIOUR OF TEL AND TML ANTI-KNOCK COMPOUNDS IN AQUEOUS SYSTEMS	21
6.	ACKNOWLEDGEMENTS	21
7.	REFERENCES	21

PAGE 0 1 1 0

PHOTO COPY

BEST PAGE AVAILABLE

1. SUMMARY AND CONCLUSIONS

A study of the behaviour of both TEL and TML Antiknock Compounds contacted with water under Jones-Kapton conditions i.e. closed system, was undertaken. However, due to emulsion formation in the aqueous phase, the experiments were curtailed and no meaningful evaluation of the effect of scavengers and inerts on the solubilities of the various lead alkyl species could be made.

A study of the behaviour of Antiknock Compounds in contact with water was made using an open tank method. In this, a large glass tank, shielded from the light, was filled with water and a layer of Compound placed on the floor of the tank. The aqueous layer was mechanically stirred and monitored for organo lead species over a period of approximately thirty days. After this time the Compound layer was removed and the decay of the organo lead species present in the aqueous phase followed over a further thirty to sixty days. It was found that:

1. (a) the steady state Et_4Pb concentration in distilled water in contact with TEL-B was 0.16 ppm Pb.

1. (b) the breakdown pattern over the contact period showed that Et_3Pb^+ was slowly being formed and there was evidence for Et_2Pb^{2+} formation after approximately ten days.

2. (a) the steady state Et_4Pb concentration in sea water in contact with TEL-B was 0.12 ppm Pb.

2. (b) the breakdown pattern over the contact period showed that Et_3Pb^+ was slowly being formed and again there was evidence for the very slow formation of Et_2Pb^{++}.

2. (c) the decay of the soluble lead species over fifty eight days showed that:

(1) In the dark the Et_4Pb concentration remained unaltered, the Et_3Pb^+ concentration decayed by ~ 30% but there was no real evidence for Et_2Pb^{2+} or Pb^{2+} formation.

(2) In diffuse light, the Et_4Pb concentration remained unaltered, the Et_3Pb^+ concentration decayed by ~ 25%, the Et_2Pb^{2+} concentration remained very low but the Pb^{2+} concentration showed a measurable increase.

It was found that, whereas the total soluble lead in the dark system was reduced by 30%, in the light the reduction was less than 5%.

PAGE 0 1 1

BEST PAGE AVAILABLE
PHOTO COPY

3. (a) the steady state Me_4Pb concentration in distilled water in contact with TML-CB was of the order of 20 ppm Pb.

3. (b) the breakdown pattern over the contact period showed that Me_3Pb^+ was slowly formed but the Me_2Pb^{2+} and Pb^{2+} concentrations were barely detectable.

3. (c) the decay of the soluble lead species over fifty seven days showed that, both in diffuse laboratory light and in the dark, the Me_4Pb concentration rapidly dropped to $\leq$ 0.1 ppm Pb, the Me_3Pb^+ concentration increased slightly, but the Me_2Pb^{2+} and Pb^{2+} concentration remained below 0.05 ppm Pb and showed no signs of increasing.

4. (a) the steady state Me_4Pb concentration in sea water in contact with TML-CB, was of the order of 7 ppm Pb.

4. (b) the breakdown pattern over the contact period again demonstrated that Me_3Pb^+ was formed as the major breakdown species and further degradation to Me_2Pb^{2+} and Pb^{2+} was not observed.

4. (c) the decay of the soluble lead species over sixty days was similar in both light and dark systems. The Me_4Pb concentration very rapidly fell to $\leq$ 0.1 ppm Pb, the Me_3Pb^+ concentration decayed by ~ 5% to Me_2Pb^{2+} and to a lesser degree Pb^{2+}, the total soluble lead concentration in solution remaining essentially unaltered.

Thus the overall behaviour of TEL-B and TML-CB in aqueous systems may be summarised as follows:-

For TEL-B, the concentration of Et_4Pb in the aqueous phase is always less than the solubility concentration of pure TEL [1] and this is due to evaporative loss of Et_4Pb from the surface. The tetraethyl lead species slowly degrades to triethyl lead and the rate of breakdown is slightly enhanced in sea water compared to distilled water. Further breakdown to diethyl lead and inorganic lead is even slower but there is evidence to suggest that, in the presence of diffuse light, more inorganic lead is formed.

For TML-CB, the concentration of Me_4Pb in the aqueous phase is of the same order as the solubility figures quoted for TML/toluene in sea and distilled water [1]. Again, evaporative loss of Me_4Pb from the aqueous surface is important such that, once the source of Me_4Pb is removed, the concentration of the tetramethyl lead species rapidly falls to less than 0.1 ppm Pb. The major breakdown product is Me_3Pb^+ and its formation is greatly enhanced in the presence of chloride ion. Further degradation to Me_2Pb^{2+} and Pb^{2+} is barely detectable in distilled water and very slow in sea water. Diffuse light had no noticeable effect on the breakdown pattern.

PAGE 0 1 1 2

PHOTO COPY

BEST PAGE AVAILABLE

INTRODUCTION

Part I[1] of this study into the behaviour of lead alkyls in aqueous systems concentrated on the determination of the solubilities of TEL and TML in pure and sea water. From the results it was clear that factors such as the salinity of the aqueous phase, the presence of air and organic liquids (such as toluene in TML/toluene experiments) affected not only the equilibrium solubility of the R_4Pb species but also the rate of breakdown of the R_4Pb species to R_3Pb^+, R_2Pb^{2+} and Pb^{2+} species.

Part I was considered a necessary preliminary to the more pertinent study of the behaviour of Antiknock Compounds under aqueous conditions, since these are the materials transported around the world. The first part of this report deals with the behaviour of Antiknock Compounds under Jones-Kaplan conditions i.e. closed system and the results are contrasted with those obtained in Part I.

The second part of this report deals with the behaviour of Antiknock Compounds in aqueous systems exposed to the atmosphere i.e. open system.

PHOTO COPY

BEST PAGE AVAILABLE

3. EXPERIMENTAL AND RESULTS

3.1 Studies using the Jones-Kaplan Equilibrator.

Experimental.

closed system

The apparatus and general method are identical to those used in Part I[1].

A series of runs were carried out at varying contact times, ranging from one to twenty four hours. At the completion of each run, the water layer was removed and analysed immediately for R_4Pb, R_3Pb^+, R_2Pb^{2+} and Pb^{2+} species.

The systems studied were TEL-B, TEL-CB and TML-CB in distilled water under air at 20°C (all results quoted as ppm Pb).

Results

TEL-B/distilled water System.

Time.	Et_4Pb	Et_3Pb^+	Et_2Pb^{2+}	Pb^{2+}	Total.	Comments.
4 hours.	0.3	10.6	0.1	Nil	11.0	
18 hours.	4.8	14.4	0.2	Nil	19.4	* Cloudy aqueous phase.
24 hours.	..... Not measured.....				66.5	* Very heavy cloudiness

TEL-CB/distilled water System.

Time.	Et_4Pb	Et_3Pb^+	Et_2Pb^{2+}	Pb^{2+}	Total.	Comments.
4 hours.	0.62	0.54	Nil	0.02	1.18	
18 hours.	36.2	16.6	0.2	Nil	53.0	* Very cloudy aqueous phase.
24 hours.	7.7	4.0	0.2	Nil	11.9	* Very heavy cloudiness. Appearance like colloid.

TML-CB/distilled water System.

Time.	Me_4Pb	Me_3Pb^+	Me_2Pb^{2+}	Pb^{2+}	Total.	Comments.
4 hours.	5.9	0.3	Nil	Nil	6.2	
18 hours.	12.5	6.8	Nil	Nil	19.3	
24 hours.	.....Not measured.....				49.0	* Very cloudy, [illegible] not [illegible]

PHOTO COPY BEST PAGE AVAILABLE

PAGE 0114

* Investigation of the cloudiness produced in the aqueous phase.

TEL-B and distilled water were equilibrated for 24 hours in the usual way under a nitrogen atmosphere. At the end of this period the aqueous phase was cloudy. The experiment was continued for a further 3 days at which time the aqueous phase had cleared but an insoluble lower phase had separated out.

This lower phase was separated, dried and analysed by GLC. It was found to be a mixture of TEL and dibromoethane.

PAGE 0115

3.2 Studies using open tank experiments

Not using 5 l aqueous solution or in closed system

The apparatus consisted of an open cylindrical glass tank (30 cm diameter) holding twenty litres of water. Approximately 80 cm^3 of Antiknock Compound, ammonia extracted to remove water soluble material, were required to cover the bottom of the tank to a depth of 3 mm. The aqueous phase was agitated with a stirrer rotating at about 80 rpm and this was sufficient to give a gentle flowing action of the water over the Compound layer. Evaporative losses and volumes removed during sampling were replaced by an automatic constant head device.

The first experiment, performed on TEL-B, was done in the presence of a layer of sand to discover whether or not the breakdown of Compound was affected by sand.

The results are tabulated on the following page (quoted as ppm Pb).

BEST PAGE AVAILABLE

PHOTO COPY

Table 1. TEL-B, distilled water, 20°C, shielded from the light, sand present.

Time.	Et_4Pb	Et_3Pb^+	Et_2Pb^{2+}	Pb^{2+}	Total Pb.
1 hour.	0.04	1.80	Nil	0.02	1.86
2 hours.	0.08	1.85	Nil	0.01	1.94
4 hours.	0.02	2.05	Nil	0.04	2.11
6 hours.	0.06	2.04	Nil	0.03	2.13
24 hours.	0.10	2.08	0.02	0.03	2.23
2 days.	0.12	2.17	Nil	0.01	2.30
3 days.	0.08	2.21	0.03	0.05	2.37
7 days.	0.12	2.33	0.09	0.01	2.55
10 days.	0.18	2.44	0.12	0.02	2.76
15 days.	0.14	3.88	0.18	Nil	4.20
20 days.	0.12	4.73	0.15	0.03	5.03
24 days.	0.16	5.55	0.19	Nil	5.90
30 days.	0.22	7.43	0.21	Nil	7.86

Table 2. TEL-B, distilled water, shielded from the light, 20°C, no sand present.

Time.	Et_4Pb	Et_3Pb^+	Et_2Pb	Pb^{2+}	Total Pb.
1 hour.	0.06	1.50	0.05	0.04	1.65
2 hours.	0.07	1.75	0.04	0.02	1.88
4 hours.	0.07	1.82	0.02	0.05	1.96
6 hours.	0.09	1.88	0.02	0.04	2.03
25 hours.	0.18	2.15	0.03	0.02	2.38
2 days.	0.18	2.31	0.01	0.02	2.52
3 days.	0.24	2.34	0.01	Nil	2.59
7 days.	0.20	2.69	0.01	Nil	2.90
10 days.	0.14	2.66	Nil	Nil	2.80
15 days.	0.16	2.79	0.01	Nil	2.96

PHOTO COPY

BEST PAGE AVAILABLE

Table 3. TEL-B, sea water, 20°C, shielded from the light, no sand present.

Time.	Et_4Pb	Et_3Pb^+	Et_2Pb^{2+}	Pb^{2+}	Total Pb.
1 hour.	0.16	1.77	0.09	0.01	2.03
2 hours.	0.08	1.88	0.14	0.01	2.11
4 hours.	0.07	2.00	0.14	0.02	2.23
6 hours.	0.17	1.98	0.06	0.11	2.32
24 hours.	0.14	2.10	0.02	0.02	2.28
2 days.	0.12	2.47	0.23	0.01	2.83
6 days.	0.12	3.02	0.32	0.19	3.65
12 days.	0.10	3.79	0.14	0.03	4.07
15 days.	0.10	4.26	0.22	0.04	4.62
20 days.	0.12	4.39	0.22	0.02	4.75
26 days.	0.28	4.88	0.20	0.01	5.37

Table 4. TML-CE, distilled water, 20°C, shielded from the light, no sand present.

Time.	Me_4Pb	Me_3Pb^+	Me_2Pb^{2+}	Pb^{2+}	Total Pb.
1 hour.	0.58	0.20	0.04	0.04	0.86
2 hours.	1.16	0.36	0.02	0.03	1.57
4 hours.	2.66	0.40	Nil	0.03	3.09
6 hours.	3.68	0.84	Nil	0.01	4.53
1 day.	5.32	0.92	Nil	0.02	6.26
2 days.	11.97	1.25	0.02	0.01	13.25
4 days.	11.98	3.65	Nil	0.01	15.64
7 days.	16.98	6.52	Nil	0.03	23.53
14 days.	18.50	10.00	Nil	0.06	28.56
21 days.	18.98	11.32	Nil	0.02	30.32
30 days.	19.96	11.72	0.02	0.08	31.78

BEST PAGE AVAILABLE

PHOTO COPY

PAGE 0117

Table 5. TEL-C3, sea water, shielded from light, no sand present.

Time.	Me_4Pb	Me_3Pb^+	Me_2Pb^{2+}	Pb^{2+}	Total Pb.
1 hour.	0.41	0.13	Nil	Nil	0.54
2 hours.	0.80	0.23	Nil	Nil	1.03
4 hours.	1.75	0.38	Nil	0.01	2.14
6 hours.	1.97	0.50	0.02	Nil	2.49
1 day.	6.72	2.09	0.02	0.01	8.84
2 days.	5.69	4.48	Nil	Nil	10.17
3 days.	6.02	6.54	0.03	Nil	12.59
7 days.	7.13	12.90	Nil	0.02	20.05
14 days.	7.57	20.96	Nil	Nil	28.53
21 days.	8.17	29.15	0.02	0.01	37.35
34 days.	6.50	39.50	Nil	0.01	46.01

Table 6. Decay of sea water sample after contact with TEL-B for 26 days (see Table 3) shielded from light.

Time.	Et_4Pb	Et_3Pb^+	Et_2Pb^{2+}	Pb^{2+}	Total Pb.
1 day.	0.18	4.70	Nil	0.01	4.89
2 days.	0.22	4.70	Nil	0.03	4.95
3 days.	Nil	4.28	Nil	0.03	4.31
7 days.	0.24	3.64	Nil	0.07	3.95
11 days.	0.16	3.66	Nil	0.06	3.88
22 days.	0.02	3.82	Nil	Not determined.	3.84
31 days.	0.04	4.14	0.06	0.09	4.33
44 days.	0.16	3.82	0.38	0.14	4.50
58 days.	0.10	3.20	0.06	0.10	3.46

PAGE 0118

PHOTO COPY

BEST PAGE AVAILABLE

Table 7. Decay of sea water sample after contact with TML-B for 26 days (see Table 3) not shielded from light.

Time.	Et_4Pb	Et_3Pb^+	Et_2Pb^{2+}	Pb^{2+}	Total Pb.
1 day.	0.10	4.24	0.36	0.07	4.77
2 days.	0.14	4.74	0.14	0.15	5.17
3 days.	0.26	4.84	Nil	0.12	5.22
7 days.	0.04	4.76	Nil	0.23	5.03
11 days.	Nil	4.60	Nil	0.38	4.98
22 days.	0.10	4.16	Nil	0.53	4.79
31 days.	0.18	3.82	0.08	0.73	4.81
44 days.	0.16	3.60	0.08	0.90	4.74
58 days.	0.16	3.54	0.01	0.92	4.63

Table 8. Decay of distilled water sample after contact with TML-CB for 30 days (see Table 4) shielded from light.

Time.	Me_4Pb	Me_3Pb^+	Me_2Pb^{2+}	Pb^{2+}	Total Pb.
1 day.	0.18	12.82	0.02	0.02	13.04
2 days.	0.02	12.88	0.02	0.05	12.97
6 days.	0.08	12.36	0.05	0.01	12.50
13 days.	0.05	12.08	0.02	Nil	12.15
20 days.	0.01	12.37	0.03	0.13	12.54
28 days.	0.18	13.48	0.06	0.02	13.74
35 days.	0.07	14.53	0.04	0.01	14.65
41 days.	0.09	14.00	0.03	0.02	14.14
57 days.	0.01	14.18	0.02	0.04	14.25

PAGE 0119

BEST PAGE AVAILABLE

PHOTO COPY

Table 9. Decay of distilled water sample after contact with TML-CB for 30 days (see Table 4) not shielded from light.

Time.	Me_4Pb	Me_3Pb^+	Me_2Pb^{2+}	Pb^{2+}	Total Pb.
1 day.	0.29	12.56	Not determined.	0.04	12.89
2 days.	0.10	12.92	0.05	0.03	13.10
6 days.	0.06	13.20	0.02	0.02	13.30
13 days.	0.05	13.31	0.04	0.02	13.42
20 days.	Not determined.	13.27	0.03	0.09	13.39
28 days.	0.07	12.77	0.06	0.03	12.93
35 days.	0.07	13.43	0.04	0.01	13.55
41 days.	0.03	13.67	0.06	0.04	13.80
57 days.	Not determined.	13.56	0.12	0.04	13.72

Table 10. Decay of sea water sample after contact with TML-CB for 34 days (see Table 5) shielded from light.

Time.	Me_4Pb	Me_3Pb^+	Me_2Pb^{2+}	Pb^{2+}	Total Pb.
1 day.	0.1	39.6	Nil	0.03	39.73
4 days.	Not determined.	39.0	0.1	Nil	39.10
7 days.	0.2	39.4	0.02	0.02	39.64
22 days.	Not determined.	38.6	0.30	0.09	38.99
30 days.	Not determined.	38.9	0.76	0.07	39.73
38 days.	0.05	37.6	0.96	0.11	38.72
46 days.	0.01	37.1	1.11	0.14	38.35
53 days.	0.01	36.3	1.17	0.14	37.62
60 days.	0.01	37.5	1.53	0.16	39.20

PAGE 0120

BEST PAGE AVAILABLE

PHOTOCOPY

Table 11. Decay of sea water sample after contact with Me_4Pb for 54 days (see Table 5) not shielded.

Time.	Me_4Pb	Me_3Pb^+	Me_2Pb^{2+}	Pb^{2+}	Total Pb.
1 day.	0.1	37.2	0.02	0.01	37.33
4 days.	0.05	39.0	0.03	Nil	39.13
7 days.	0.1	37.7	Nil	0.02	37.82
22 days.	0.02	36.4	0.40	0.04	36.86
30 days.	0.01	37.9	0.86	0.13	38.90
38 days.	0.06	36.8	0.98	0.15	37.99
46 days.	0.01	36.1	1.02	0.19	37.32
53 days.	0.01	35.7	1.06	0.22	36.99
60 days.	0.01	36.0	1.46	0.24	37.70

PAGE 0121

BEST PAGE AVAILABLE

PHOTO COPY

4. DISCUSSION OF RESULTS

4.1 In Part 1[1] of this study, TML, TEL and TEL/toluene were contacted with water, using the Jones-Kaplan Equilibrator, and the equilibrium concentrations of the various R_4Pb species in the aqueous phase were determined. Certain effects were noted i.e. the effect of chloride ion, presence of toluene in TEL/toluene, on these R_4Pb solubilities and, in addition, information was obtained on the nature and stability of the breakdown products formed in the aqueous phase.

The studies, again using the Jones-Kaplan Equilibrator, were extended to the commercial Antiknock Compounds TEL-B, TEL-CB and TML-CB. Based on the previous work it was predicted that the non-lead containing components of Antiknock Compound i.e. dibromoethane, dichloroethane and various hydrocarbons, would lower the R_4Pb concentrations in the aqueous phase and, since breakdown products were formed from the R_4Pb species in the aqueous phase, would also lower the concentrations of the R_3Pb^+, R_2Pb^{2+} and Pb^{2+} species. Unfortunately the technique broke down since cloudiness and ultimately phase separation occurred in the aqueous phase quite early on in the experiments.

For the TEL-B/distilled water system the lower insoluble phase was analysed by GLC and shown to be a mixture of dibromoethane and TEL.

From available data it can be calculated that the partial pressure of dibromoethane and TEL above TEL-B maintained at 20°C are 4.2 and 0.13 mm Hg respectively, i.e. in the ratio of 30 : 1. However, the aqueous solubilities of these two compounds at 20°C are 4000 ppm and 0.8 ppm i.e. in the ratio of 5000 :1.

Thus, in the Jones-Kaplan Equilibrator, despite the higher concentration of dibromoethane in the vapour phase, the aqueous phase will be saturated with TEL before it becomes saturated with dibromoethane. At this point the TEL/water system is at equilibrium but absorption of dibromoethane vapour continues.

The aqueous phase must be considered as a ternary system i.e. TEL, dibromoethane and distilled water. If one assumes that the solubility of TEL will be lowered by the presence of dibromoethane (this is generally true for two completely miscible liquids which are partially miscible with a third liquid) then it is to be expected that phase separation will occur until equilibrium is re-established.

PAGE 0122

BEST PAGE AVAILABLE

PHOTO COPY

Experimental results (tabulated in Section 3.1) show that phase separation was always observed in the Jone-Kaplan system whenever Antiknock Compounds were being studied and support the argument that the aqueous solubility of R_4Pb is lowered by the presence of other organic compounds.

If one attempts to draw comparisons between the systems containing Antiknock Compounds and those containing the lead alkyls before the onset of cloudiness i.e. at four hours contact, then for TEL-B/distilled water/air atmosphere the Et_4Pb concentration was 0.3 ppm Pb compared to 0.3 ppm Pb for TEL/distilled water/air atmosphere. Under identical conditions for TEL-CB the Et_4Pb concentration was 5.9 ppm Pb at four hours and for TML-CB, the Me_4Pb concentration was 5.9 ppm Pb (compared to 12.0 ppm for TML/toluene/distilled water/air atmosphere). There is no apparent trend since, from the results, the R_4Pb concentration for TML-CB is depressed, for TEL-B the same and for TEL-CB enhanced.

PAGE 0123

4.2 The open tank experiments, however, gave no such problems of cloudiness or emulsion formation and the results can be analysed with a greater degree of confidence. These experiments were designed to give conditions which approximate more closely to those conditions likely to be met in practice.

At the outset it was felt necessary to see whether sand had any effect on the breakdown of the lead alkyl species present in the system. The results given in Tables 1 and 2 (for TEL-B in contact with distilled water with, and without, sand present) showed that sand had no measurable effect. It seemed unlikely that sand would absorb the breakdown products but to confirm this, the sand layer was extracted with solvent at the end of the experiment. Analyses for lead in both the solvent extract and residual sand gave very little evidence for adsorption of brakdown products. As a result, all further experiments were performed in the absence of sand.

The systems are considered in turn and where applicable, the results will be compared to those obtained in Part 1[1].

PHOTO COPY

BEST PAGE AVAILABLE

4.2.1 System TEL-B/distilled water/with and without sand.

The aqueous phase in contact with TEL-B showed a maximum concentration of TEL of ~ 0.24 ppm Pb though the average concentration was generally about 0.16 ppm Pb. This compares to the solubility figure of 0.8 ppm Pb determined for TEL in distilled water (1).

The major breakdown product was Et_3Pb^+ and the concentration of this species gradually increased with time. For both experiments (with and without sand) the concentration of Et_3Pb^+ after one hour of 1.8 ppm Pb and 1.5 ppm Pb respectively, suggested and initial high rate of breakdown. This phenomenon had also been observed in all the Jones-Kaplan i.e. closed system, experiments.

The Et_3Pb^+ concentration reached 7.4 ppm Pb at thirty days whilst the Et_2Pb^{2+} and Pb^{2+} concentrations remained low though the Et_2Pb^{2+} concentration was showing an upward trend from seven days onward (from 0.04 ppm Pb at seven days to 0.21 ppm Pb at thirty days).

The breakdown pattern of Et_4Pb can only be compared with the closed system up to twenty four hours and it is seen that, for this period, the pattern is very much like that recorded for TEL/distilled water/air atmosphere though the concentrations are lower throughout. This reinforces the view that Et_3Pb^+ only comes from breakdown of Et_4Pb in the aqueous phase and it follows that, if the concentration of Et_4Pb is lower, the Et_3Pb^+ concentration will be correspondingly reduced.

4.2.2 System TEL-B/sea water/sand.

The aqueous phase in contact with TEL-B showed a maximum concentration of 0.28 ppm Pb though the average concentration was nearer 0.12 ppm Pb. This compares to the solubility figure of approximately 2.4 pp. Pb for TEL in sea water (1).

The major species in solution was Et_3Pb^+ and the concentration of this species rose with time to reach 4.88 ppm Pb at twenty six days. This is not like the closed system experiments where the Et_4Pb species predominated *

PAGE 0 1 24

BEST PAGE AVAILABLE
PHOTO COPY

and very little breakdown to Et_3Pb^+ occurred. In the open tank the 'normal' breakdown pattern is observed i.e. Et_3Pb^+ predominating, a low, but rising Et_2Pb^+ concentration and a very low Pb^{2+} concentration.

To summarise the behaviour of TEL-B in contact with both distilled and sea water it is seen that:

(1) The Et_4Pb concentration in the aqueous phase is much lower than the equilibrium concentrations recorded for the closed systems. This is attributed to evaporative losses of Et_4Pb from the surface of the aqueous phase.

(2) The Et_3Pb^+ species are the major species present in the aqueous phase. The salinity of the system has a slight effect on the breakdown pattern though, in both cases, the Et_3Pb^+ concentrations are initially high and rise only slowly with time.

(3) The Et_2Pb^{2+} concentrations are low but show a gradual increase with time but are never more than 10% of the Et_3Pb^+ concentration.

(4) The Pb^{2+} concentration is always low.

The most striking feature of the behaviour of lead alkyls in aqueous systems is the apparent stability of the trialkyl lead species, R_3Pb^+. To determine whether this species was resistant to further degradation or whether some type of equilibrium was established with the R_4Pb species which depressed R_2Pb^{2+} formation, decay experiments were performed on the aqueous phase removed from the TEL-B/seawater system after twenty six days contact. The sample was divided into two parts and the decay pattern monitored over a fifty eight day period. One sample was shielded from diffuse laboratory light to see whether this had any effect. The results are given in Tables 6 and 7. The results showed:

(1) The Et_4Pb concentration remained constant throughout the 58 decay period at between 0.1 and 0.16 ppm Pb although some scatter beyond this range was noted.

This is rather puzzling since, with oxygen present, one would have expected the Et_4Pb concentration to have decayed completely. One explanation is that Et_3Pb^+ in the presence of chloride ion might establish an equilibrium thus:

$$Et_3Pb^+ + Cl^- \rightleftharpoons Et_3PbCl \overset{Cl^-}{\rightleftharpoons} Et_3PbCl_2^- \overset{Cl^-}{\rightleftharpoons} Et_3PbCl_3^{2-}$$

and that the analytical method is determining the neutral species Et_3PbCl not Et_4Pb. Such an equilibrium

Control Et_4Pb levels an artifact of system

BEST PAGE AVAILABLE

BEST PAGE AVAILABLE PHOTO COPY

PAGE 0125

is known to exist [2]. Further experiments using aqueous systems containing variable amounts of chloride ion might resolve this matter. However the "apparent" Et_4Pb concentration is only of the order of 0.1 to 0.15 ppm Pb and no certainty can be placed upon this figure except to say that there is some evidence for a neutral organo lead species present at low concentration.

(2) Diffuse laboratory light has a slight effect on the system in that more Pb^{2+} was formed in the light system.

(3) The Et_3Pb^+ concentration decreased by about 1 ppm Pb over the fifty eight days.

(4) Generally there was a slow decay in the total dissolved lead concentration in both the light and dark experiments of the order of 1 ppm Pb. The scatter in these results is difficult to account for but the downward trend is clear. Since all the dissolved species are below their solubility levels (pH of solution ~ 4) this loss of lead may be due to:

(a) deposition on the walls of the vessel.

(b) evaporated loss from the surface.

The lead loss is due to a reduction in the Et_3Pb^+ concentration. If, as previously suggested, an equilibrium is established in the presence of chloride ion, then it is possible that the neutral species Et_3PbCl will have a vapour pressure above the surface of the liquid and this is lost to the vapour phase to restore "equilibrium" and this accounts for the slight lead loss from the system. Analysis of the atmosphere above the tank might verify this hypothesis.

4.2.3 System TML-CB/distilled water.

The aqueous phase in contact with TML-CB showed a rising concentration of Me_4Pb throughout the experiment, though the rise after fourteen days was only 1.5 ppm Pb over the next sixteen days. This suggests an equilibrium concentration of approximately 19 - 20 ppm Pb. The solubility of TML/toluene in distilled water, from the Jones-Kaplan experiments, was found to be of the order of 15 ppm Pb. This higher figure in the open tank experiments was rather unexpected since it was assumed that evaporative losses from the surface would have given rise to a much lower concentration of Me_4Pb in the aqueous phase. There was no evidence for entrainment of TML-CB which, in any case, would have given a scatter to the

PAGE 0 1 2 b

PHOTO COPY

BEST PAGE AVAILABLE

results. No scatter was observed. However it is clearly seen that it takes a much longer time to reach an equilibrium Me_4Pb concentration in an open tank compared to a closed system i.e. time to reach 15 ppm Pb was six days compared to six hours.

The breakdown pattern showed that the Me_4Pb species was slowly degraded to Me_3Pb^+ which was essentially stable, very little of the Me_2Pb^{2+} and Pb^{2+} species were formed up to thirty days.

At the completion of the thirty day period, the aqueous phase was divided into two parts and, in a manner identical to that reported for TEL-B, the decay of the soluble lead species was monitored over fifty seven days. The results are given in Tables 8 and 9.

The most obvious fact was the rapid loss of Me_4Pb from solution, from an initial 20 ppm Pb to 0.2 ppm Pb at one day and to less than 0.1 ppm Pb after two days. The Me_3Pb^+ concentration remained essentially unaltered and, in fact, rose slightly by approximately 1 ppm Pb over the fifty seven day period. This rise in concentration is most curious and an explanation is not immediately apparent. The concentrations of Me_2Pb^{2+} and Pb^{2+} remained at very low levels throughout and showed no signs of rising. Thus one comes to the conclusion that, in distilled water, Me_3Pb^+ is a stable species and does not readily undergo either oxidative or hydrolytic breakdown.

4.2.4 System TML-CB/sea water.

The aqueous phase in contact with TML-CB showed a concentration of 6.7 ppm Pb at one day which remained, with some small variations, at this level until the experiment was terminated at thirty four days. This compares to a solubility figure of approximately 9 ppm Pb for TML/toluene in sea water i.e. a slight reduction which was to be expected. The breakdown pattern showed a rising Me_3Pb^+ concentration but very little evidence for the formation of Me_2Pb^{2+} and Pb^{2+}. The major species in solution was Me_4Pb until day three after which Me_3Pb^+ predominated.

If the behaviour of TML-CB in sea water and distilled water are compared, the total soluble lead figures are similar but the species in solution are such that, in distilled water, Me_4Pb appears more stable and is the major species whereas, in sea water, Me_3Pb^+ is the major species.

PAGE 0127

PHOTO COPY

BEST PAGE AVAILABLE

If one assumes that breakdown is due to the presence of oxygen then the two systems should behave in a similar manner unless the dissolved oxygen content is affected by the salinity of sea water. There is no evidence to support this. Other possibilities that can be considered are as follows:-

(a) Does Cl^- ion enhance the breakdown of Me_4Pb i.e.
$Me_4Pb + Cl^- \longrightarrow Me_3PbCl + R^-$

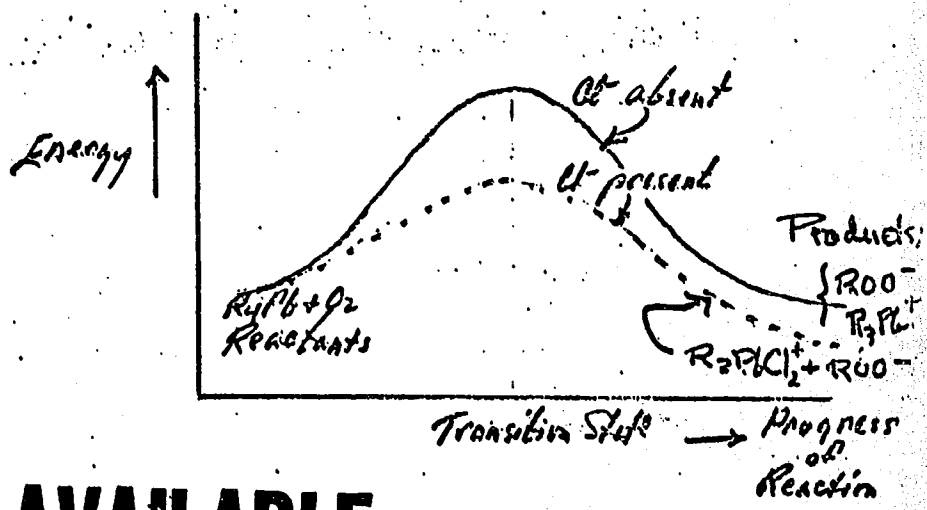
This seems unlikely since R_4Pb compounds are noted for their resistance to nucleophilic attack.

(b) Does Cl^- ion complex with the neutral Me_4Pb to give Me_4PbCl^- which is not extracted with hydrocarbons during the analysis for Me_4Pb. The decay experiments, described later, argue against this.

(c) If one refers back to the behaviour of TEL-CB in sea and distilled water, a slight effect of enhanced formation of Et_3Pb^+ was noted in sea water. Since Cl^- ion can stabilise the Et_3Pb^+ species in solution by co-ordination i.e.

$$Et_3Pb^+ \overset{Cl^-}{\rightleftharpoons} Et_3PbCl \overset{Cl^-}{\rightleftharpoons} Et_3PbCl_2^- \overset{Cl^-}{\rightleftharpoons} Et_3PbCl_3^{2-}$$

it might be inferred that Cl^- would also stabilise the transition state which occurs when the Et group is being cleaved i.e. lowers the activation energy for cleavage reaction.



PAGE 0128

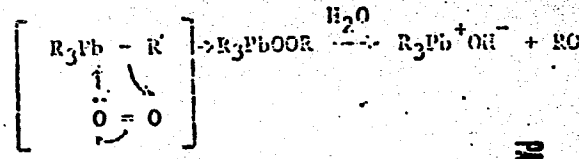
BEST PAGE AVAILABLE

PHOTO COPY

122

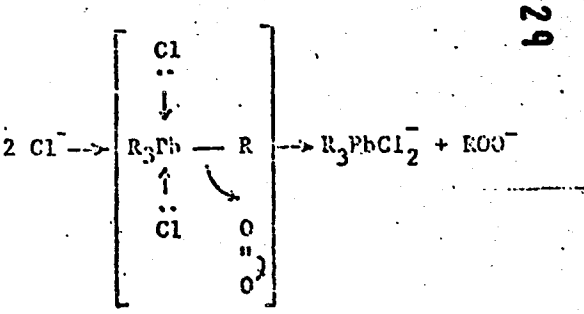
Possible transition states might be:

(1) Cl^- absent



transition state

(2) Cl^- present



transition state

In (2) it is postulated that Cl^- co-ordinates with the central lead atom weakening the lead-carbon bond and making the α carbon atom more susceptible to electrophilic attack by oxygen. This mechanism may explain the enhanced breakdown of Me_4Pb in sea water compared to distilled water.

At the completion of the contact experiment, the aqueous phase was separated from TML-CB, divided into two equal portions and the decay pattern observed for both light and dark systems.

The two systems behaved in a similar manner and the pattern observed was as follows:-

(a) rapid loss of Me_4Pb species

(b) a slowly reducing Me_3Pb^+ concentration (approximately 5% reduction over sixty days).

PHOTO COPY

BEST PAGE AVAILABLE

In connection with the distribution of Me_4Pb and Me_3Pb^+ species in solution, it was thought possible that part of the identified "Me_3Pb^+" might have been Me_4PbCl^- in equilibrium with Me_4Pb. If this had been the case then removal of Me_4Pb by surface loss would have lowered the identified "Me_3Pb^+" concentration i.e.

$$\uparrow Me_4Pb + Cl \rightleftharpoons Me_4PbCl^-$$

The equilibrium would have been moved to the left. This was not observed which suggested that Me_3Pb^+ was in fact the true species present in solution and not the complexed form of Me_4Pb.

However even this argument can be questioned. In the presence of excess chloride ion the equilibrium might lie completely to the right and the measured Me_4Pb is, in fact the solubility of Me_4Pb in salt solution i.e.

$$Me_4Pb \begin{pmatrix}\text{Compound}\\ \text{phase}\end{pmatrix} \rightleftharpoons Me_4Pb \begin{pmatrix}\text{aqueous}\\ \text{phase}\end{pmatrix} \rightleftharpoons Me_4Pb \begin{pmatrix}\text{gaseous}\\ \text{phase}\end{pmatrix}$$

$$\begin{array}{ccc} & O_2 \text{ steady state concentration} & \\ \swarrow & & \uparrow \\ Me_3Pb^+ & & \downarrow Cl^- \text{ (slow step)} \\ & & Me_4PbCl^- \\ & & \text{(slowly rising concentration)} \end{array}$$

The results obtained do not differentiate between Cl^- complexing with Me_4Pb or catalysing the oxidative cleavage of Me_4Pb to Me_3Pb^+. It is possible that experiments performed with variable Cl^- concentrations would help to resolve this point.

(c) The concentrations of Me_2Pb^{2+} and Pb^{2+} were initially very low (at the limit of detection) and showed a slight tendancy to rise with time i.e. measurable after seven days, suggesting that they are slowly formed in aqueous systems.

In general, the total lead levels, after the initial drop due to evaporative loss of Me_4Pb, remained fairly constant over the period of the experiments, though the Me_3Pb^+ concentration was slowly being replaced by Me_2Pb^{2+} and Pb^{2+}.

PAGE 0130

PHOTO COPY

BEST PAGE AVAILABLE

124

COMPARISON OF THE BEHAVIOUR OF TEL AND TML AGITATION CONDITIONS IN AQUEOUS SYSTEMS

The experimental results show that the breakdown products R_3Pb^+, R_2Pb^{2+} and Pb^{2+} are produced from the R_4Pb species present in the aqueous phase and not from decomposition reactions in the bulk compound phase. Thus it should be possible to compare the relative breakdown rates for the various systems using the relationship $k = R/R_4Pb$ where R_4Pb is equal to the "steady state" concentration of R_4Pb in the aqueous phase and R is equal to the total amount of breakdown products produced per day during steady state conditions.

The results are given in the following table:-

SYSTEM	R_4Pb concentration (steady state) *	Breakdown products produced *	Period considered (days)	R	$k = R/R_4Pb$
TEL-B/distilled water/ sand.	0.12	0.45	1-10=9	0.05	0.42
TEL-B/distilled water.	0.18	0.46	1-10=9	0.05	0.28
TEL-B/sea water.	0.11	1.25	2-12=10	0.125	1.14
TML-CB/distilled water.	19.15	1.76	14-30=16	0.11	0.005
TML-CB/sea water.	7.34	26.59	7-34=27	0.98	0.13

* Figures quoted as ppm Pb.

The general trend is clearly seen in that enhanced breakdown of R_4Pb occurs in sea water both for TEL and TML systems. In all systems TEL breaks down more readily than TML although, due to the higher concentrations of TML in the aqueous phases, the absolute formation of methyl lead species is greater.

6. ACKNOWLEDGEMENTS

The authors wish to acknowledge the fortitude and patience of Mr. D.J. Hodges and others in the Analytical Section for carrying out the analyses quoted in this report.

7. REFERENCES

(1) Part 1 - Studies using the Jones-Kaplan Equilibrator to determine the solubilities of TEL and TML in natural waters - F.G. Noden, W.Towers, and A. Whittingham. Report No. RA 6320. (March 1974).

(2) R. Barbieri et al., Ric. Sci., 33, 975, (1963).

PAGE 0131

PHOTO COPY

BEST PAGE AVAILABLE

125

appendix B

83-7800249

PRESENTED AT THE INTERNATIONAL EXPERTS DISCUSSION MEETING ON:

LEAD - OCCURRENCE, FATE AND POLLUTION IN THE MARINE ENVIRONMENT

ROVINJ, YUGOSLAVIA - OCTOBER 18-22, 1977.

PAGE 0133

THE ACUTE TOXICITY AND BIOACCUMULATION OF SOME LEAD ALKYL COMPOUNDS IN MARINE ANIMALS

Maddock B G
Taylor D

Imperial Chemical Industries Ltd.,
Brixham Laboratory
Devonshire
United Kingdom

PHOTO COPY

BEST PAGE AVAILABLE

126

INTRODUCTION

Alkyl lead compounds are used as additives to petrol as a convenient and economic method of increasing the octane rating of fuels for use in high compression internal combustion engines.

The United Kingdom is the largest European producer of these materials, some 11% (40,000 tonnes) of the total amount of lead used in the United Kingdom is converted into these additives, and approximately 80% of the UK production is exported by sea (Anon, 1974).

In the last few years, increasing concern has been expressed about the possible harm to aquatic life that might be caused by the accidental release of dangerous materials carried in cargo ships due to shipwreck or collision. An IMCO Working Party is preparing regulations that should minimise the risk of environmental damage caused by such accidents, but in order to do this effectively, adequate data is required about the effects of the various compounds on aquatic life.

In addition to the possibility of accidental releases of man-made lead alkyls, it has recently been shown (Jarvie et al, 1975; Schmidt and Huber, 1976; Wong et al, 1975) that the possibility exists, in the aquatic environment, for the transformation of inorganic lead compounds into alkylated forms by chemical or bacterial mechanisms. Furthermore, Sirota and Uthe (1977) have demonstrated that tetra alkyl lead compounds can be detected is some Canadian fish products.

In terms of its acute toxicity to aquatic life, lead is one of the least studied of the heavy metals, and most of the data that has been reported concerns the effects of inorganic lead compounds. Prior to 1975, when the present study began, the only work reported in the literature, concerning the toxicity of alkyl lead compounds to fish was the paper by Turnbull et al (1954) who reported that the 24 hr LC_{50} of Bluegill Sunfish

PAGE 0134

BEST PAGE AVAILABLE PHOTO COPY

(Lepomis macrochirus) exposed to tetra ethyl lead was 2.0 mg/l. He also quoted the figure of 0.2 mg/l as a "safe level", but without producing evidence to support that conclusion.

This data has been quoted extensively and used as the basis for assessment of the risk caused by spillages of tetra ethyl lead (Anon, 1968b). In addition to this study, there had been two other investigations of the effects of tetra ethyl lead on lower forms of aquatic life. Kozyura et al (1961) had studied its effects on two species of blue-green algae; Cladophera sp. and Scenedesmus sp. and Siegal et al (1971) had compared its toxicity with a range of organo-mercurials to a flat worm. Since 1975, two further papers have been published. (Krupinska (1976) has studied the effects of tetra ethyl lead on two species of Bryophytes whilst Silverberg et al (1977) have investigated the effect of bacterially produced tetra methyl lead on various species of green algae.

The results of all these studies, with the exception of Silverberg et al, must be interpreted with caution since the high volatility and degradability of these materials may have been underestimated. The object of the present study was to measure the acute toxicity of a range of alkyl lead compounds and to gain information on the potential bioaccumulation of these materials in marine animals.

It should be noted that throughout this paper, concentrations of alkyl lead compounds in water are quoted as mg/l total lead and all tissue concentrations refer to mg/Kg (dry Weight) unless stated otherwise.

PHOTO COPY

BEST PAGE AVAILABLE

MATERIALS AND METHODS

Alkyl Lead Compounds

The most important organic lead compounds in terms of scale of manufacture and use, are the lead alkyl derivatives. A wide range of these materials are manufactured but the most significant are the tetra alkyl lead compounds; Tetra methyl lead (TML) and tetra ethyl lead (TEL). These two materials are unstable in seawater and undergo progressive dealkylation eventually forming inorganic lead. (Grove, 1977). The initial step in this sequence proceeds very rapidly to produce the trialkyl lead chloride, although subsequent steps in the sequence are considerably slower. Thus the acutely toxic effects of a release of a tetra alkyl lead compound into the marine environment will initially be due to the parent compound, but within a few hours the toxic effects of the trialkyl lead compounds will become of considerable significance and in the longer term the effects of the di-substituted materials may be important. Consequently we have studied both the tetra alkyl lead compounds and the primary and secondary dealkylation products. In addition, since the mammalian toxicity of TML and TEL are significantly different (Magistretti et al, 1963), we have investigated both the methyl and ethyl derivatives of these materials.

In the context of accumulation, the tetra alkyl lead compounds are of only minor interest since they have only a transitory existence in seawater. Thus, although some information has been obtained on the accumulation of these materials during short term exposures to high concentrations, the majority of the work has been concerned with the accumulation of the trialkyl lead compounds during long term exposures.

The test materials were supplied by the Ellesmere Port Research Laboratories of the Associated Octel Company Limited and with the exception of the tetra methyl lead, all materials had a purity of 98%. Tetra methyl lead was tested as an anti knock

PAGE 0136

BEST PAGE AVAILABLE

PHOTO COPY

formulation (TML-CB) containing dibromo and dichloroethane in addition to the lead alkyl. Throughout the investigations, the safe handling techniques recommended for these materials (Anon, 1968 a) were followed.

PAGE 0137

Analytical Techniques

Water samples were analysed for lead alkyl compounds using the technique of Noden (1977). The total amount of the tetra, tri and di-alkyl lead components is obtained by reducing the tetra and tri alkyl lead to dialkyl lead by treatment with iodine monochloride, and then measuring the total dialkyl lead content spectrophotometrically as a complex with 4(2-Pyridylazo) resorcinol (PAR) in the presence of 1.2.-Diamino-cyclohexane-NNN'N'-tetra acetic acid (CDTA) which masks inorganic lead, calcium, magnesium and other metal salts. The amount of the tri and dialkyl lead compounds is then measured in a similar manner on another portion of the solution which has been extracted with hexane to remove the tetra alkyl lead. Finally the dialkyl lead component is measured on a further portion of the extracted solution using the PAR complex. Trialkyl lead does not form a complex with PAR. The precision of this technique is ca. $\pm$ 5% at a detection limit of 0.05 mg/l. Periodic measurements of total lead were also carried out using atomic absorption techniques. During studies with the tetra alkyl lead compounds a lower detection limit (0.005 mg/l) was required and this was achieved by extracting the tetra alkyl lead from solution with hexane, converting to inorganic lead with bromine in carbon tetrachloride, followed by determination of the lead using atomic absorption spectrophotometry with deuterium arc background correction.

During the investigation of the formulated lead alkyl (TML-CB), measurements were made of the dibromoethane and dichloroethane content of the test solutions. The method adopted was a GLC technique involving direct injection of aqueous samples.

PHOTO COPY BEST PAGE AVAILABLE

Dissected tissue samples (0.1g - 10g) were ashed at 100°C in Aristar grade concentrated nitric acid for several hours prior to measurement of lead by atomic absorption spectrophotometry using background correction. The precision of the technique was ± 10% at a detection limit of 0.5 mg/Kg. The method has been used to analyse two standard biological reference materials, Bowen Standard Kale (Bowen, 1967) and Monoco Oyster (Fukai, 1976). In each case the method produced a value within 25% of the accredited value.

PAGE 0 13

Experimental Animals

Five species, representing four phyla were selected for investigation (Table 1). With the exception of the algae, the species are of commercial importance and could provide a pathway for the transfer of lead alkyls from seawater to man. In addition, all the species are commonly found in estuarine and coastal areas where there is a greater risk of a serious spillage of lead alkyl compounds than in deeper oceanic waters. All the test animals were obtained from areas free from the effects of industrial and domestic effluent discharges, and, at the commencement of the exposure tests, contained lead concentrations equivalent to those quoted for uncontaminated animals (Bowen, 1966). All animals were maintained in flowing seawater at the laboratory for a minimum of seven days prior to their exposure to test materials.

BEST PAGE AVAILABLE

PHOTO COPY

PAGE 0139

BIOASSAY TECHNIQUES

Acute Toxicity Experiments

The techniques used are conveniently subdivided into three sections dealing with the tetra alkyl compounds, the degradation products and the algal assay procedure.

(a) Tetra Alkyl Lead Compounds

The tetra alkyl lead compounds are volatile and relatively insoluble in seawater, hence they are readily lost from solution to the atmosphere. Secondly, they rapidly undergo dealkylation in seawater to produce the trialkyl lead derivatives (Grove, 1977). Consequently it was necessary to design a test system that would permit the exposure of aquatic animals to constant levels of the materials for periods of up to 100 hours. The system used is shown in Figures 1 and 2. In view of the high mammalian toxicity and high volatility of the tetra alkyl lead compounds, the test equipment was constructed in a self contained and vented enclosure, and the waste discharged into a sealed drainage system. Seawater (salinity 34.9^{o}/oo) was pumped directly from the sea at Brixham and supplied at a constant temperature (15°C) to the system. At the design flow rate of 100 ml/min, the 90% replacement time, was 7.5 hours and, with the exception of small pieces of silicone rubber in the peristaltic pumps, those parts of the system which were in contact with the toxicant were made of either glass or PTFE. This system ensured minimum contact between the test solution and the atmosphere and hence minimal losses due to volatilisation.

Stock solutions were prepared by gently agitating 22 litres volumes of water with 10g of the lead alkyl for four hours, allowing the excess to settle for two hours and then pumping the top 20 litres into a sealed stock vessel. This procedure enabled stock solutions of seawater saturated with lead alkyls to be produced without the use of solvents. The stock solutions were used to supply five test vessels simultaneously,

BEST PAGE AVAILABLE PHOTO COPY

and the total rate of utilisation of the stock solution was maintained, with the aid of a bleed line to waste, at a constant rate of 1.2 l/hr. This ensured that the stock solutions were always replaced before decomposition of the lead alkyl became significa Since the dealkylation of the tetra alkyl lead compounds is promoted by light, the sto vessels were painted black and the complete test system isolated from direct sunlight. Using these procedures it was possible to maintain concentrations of the tetra alkyl lead compounds in the test vessels with a variation of ± 10% over the range 0.01 mg/l to 0.7 mg/l.

The halogenated ethanes present in the TML formulation are very soluble in seawater by comparison to TML, and although the ratio of TML to halogenated ethane in the original formulation was 1:0.4, in a saturated solution this ratio can become 1:1000. In our experiments, it was not possible to maintain the 1:0.4 ratio, but by using peristaltic pump tubing which was more permeable to the halogenated ethanes than to the lead alkyls, it was possible to achieve ratios of 1:10 for dichloroethane and 1:4 for dibromoethane. The toxicological significance of the presence of these materials, and the dealkylation products in the test vessels is discussed in a subsequent section.

The test procedure was as follows:

Ten individuals of the appropriate species were transferred from the laboratory holding tanks to each of the five test vessels and maintained in flowing seawater for 24 hours. The injector pump was then started, and preselected amounts of the stock solution were fed continuously into four of the vessels. The fifth vessel acting as a control. This procedure results in a gradual increase in the concentration of the toxicant from zero to its set value over the first 3-4 hours of exposure, but allows the animals to acclimatise to the actual test conditions.

PHOTO COPY
BEST PAGE AVAILABLE

The test vessels were regularly monitored for the deaths of the individual animals, and the time of death recorded. Water samples were taken from the stock solution and the test vessel overflow lines twice daily and analysed for the tetra alkyl component and its degradation products and where appropriate for the halogenated ethanes. In addition the temperature, pH and dissolved oxygen content of the water in each vessel was measured daily.

The test exposure terminated after 96 hours. The test animals were fed prior to but not during the exposures.

(b) Tri and Di Alkyl Lead Compounds

The methyl and ethyl derivatives of the tri and di alkyl lead chlorides are all soluble in water at>2g/100 ml. (Calingaert et al, 1948). They are not readily lost from solution by volatilisation, adsorption or dealkylation, the latter process proceeding only slowly in seawater. Consequently, a much simpler bioassay procedure was used for these materials.

A series of 15 litre volumes of seawater containing a range of concentrations of the appropriate material were prepared in 30 litre glass tanks. Each tank was fitted with an aeration system supplying a flow of 300 ml/min of air, covered with a loose fitting lid and maintained at 15°C in a constant temperature room. Experimental animals, in groups of ten, were transferred to the test vessels from the laboratory holding tanks and observed at regular intervals over a period of 96 hours. Dead animals were removed, deep frozen for subsequent analysis, and the time of death recorded. The levels of the toxicant, its dealkylation product and the pH, temperature and dissolved oxygen levels were measured daily and the test solutions changed at 24 hour intervals.

This procedure permitted concentrations of tri and di alkyl lead chlorides to be

BEST PAGE AVAILABLE PHOTO COPY

maintained to an accuracy of ± 5% over the range 0.1 - 420 mg/l. The level of the dealkylation products ie. dialkyl lead dichloride was always less than the detection limit of the method used ie. 0.05 mg/l.

(c) Algal Assay

The procedure used to study the effects of these materials on algal productivity was based on the technique developed by Johnstone (1974) from the organic productivity assessment technique of Steeman-Nielsen (1952). A standardised quantity of algae, in this case Phaeodactylum tricornutum was incubated for 6 hours, under controlled conditions, in the presence of a range of concentrations of the toxicant and a standard amount of C^{14} labelled carbonate. The accumulation of C^{14} by the control cultures, after correction for adsorption indicates the "normal" activity of the algae which can be compared to the activity of the algae exposed to the toxicant.

Accumulation Experiments

Two accumulation experiments have been undertaken involving the exposure of mussels (Mytilus edulis) and Dabs (Limanda limanda) to seawater (salinity 34.9°/oo) containing either trimethyl or triethyl lead chloride. Since these materials are soluble and relatively stable in seawater, the relatively simple experimental system, shown diagrammatically in Figure 3, was used.

In the experiment with mussels, constant temperature seawater (14°C) was supplied at a rate of 200 ml/min. to exposure tanks consisting of 35 litre all glass aquaria, fitted with glass lids. This produced a 95% replacement time of 6 hours. The dabs were exposed in substantially larger (ie. 300 litre) glass fibre tanks and these received a flow of 900 ml/min. of constant temperature seawater (12°C) producing

PAGE 0 1 4 2

PHOTO COPY

BEST PAGE AVAILABLE

a 95% replacement time of 13 hours. In order to maintain a homogenous toxicant distribution, it was necessary to equip each of these large tanks with a low volume aeration system. All the apparatus which was in contact with the toxicant was made of glass, fibre glass or PTFE with the exception of peristaltic pump tubing.

A standard procedure was used. An appropriate number of animals (60) mussels, 50 dabs) were transferred from the laboratory holding tanks to each of four test tanks (two for triethyl and two for trimethyl exposure) and a control tank. These animals were then maintained in flowing seawater for at least 48 hours prior to the commencement of the experiment. At the start of the exposure period, the peristaltic pumps were started and then a suitable volume of stock solution was added to each tank, with gentle agitation, to produce the appropriate test concentration. During the first few days of the exposure period, the trialkyl and dialkyl lead content of the test solutions was monitored at three hourly intervals, subsequently the frequency was reduced to daily measurements of trialkyl lead with occasional checks on the dialkyl lead content. The level of this latter material in the test vessels was always <0.05 mg/l. The temperature of the test tank water was recorded continuously and the pH, dissolved oxygen content and the flow rates of seawater and stock solution were measured daily. At the end of the exposure period, the peristaltic pumps supplying the toxicant were stopped and 95% of the test tank water replaced immediately with clean seawater at the same temperature.

The mussels were able to obtain sufficient food from the seawater supply used. However, the fish were fed three times per week on a diet of ox liver. At each feed, the total amount of food given was equivalent to 1% of the total weight of the experimental animals.

PAGE 0143

BEST PAGE AVAILABLE
PHOTO COPY

Animals were removed from the tanks at regular intervals. On each occasion three individuals were selected at random, dissected and the tissues analysed immediately for total lead content. Four tissues from the mussel (gill, digestive gland, gonad and foot) and two tissues from dabs (liver and flesh) were examined.

PAGE 0144

In any long term experimental work with animals, aimed at detecting chronic or sub-lethal effects of pollutants, it is necessary to know if the experimental animals are under excessive stress. Bayne (1975) has defined stress in this context as "A measurable alteration of a physiological (or behavioural, biochemical or psychological) steady state which is induced by an environmental change and which renders the individual (or the population or the community) more vulnerable to further environmental change". Several indices have been proposed for describing this physiological condition and an estimation of one of these indices gives an easily determined general guide to the metabolic state of the experimental animals and allows an estimate to be made of the stress induced in the control population by the test system itself in the absence of any toxic materials.

In this work we have used the following condition factors:-

$$\text{Condition Factor}_{\text{Mussel}} = \frac{\text{Flesh Weight}}{\text{Total Body Weight}} \times 100$$

$$\text{Condition Factor}_{\text{Dab}} = \frac{\text{Liver Weight}}{\text{Total Body Weight}} \times 100$$

The factor for mussels has been used previously by several workers e.g. Bayne and Thompson, 1970; Roberts, 1972. The factor for dabs was suggested by Thompson, 1976.

BEST PAGE AVAILABLE

PHOTO COPY

RESULTS AND DISCUSSION

Acute Toxicity

The acute toxicity of four alkyl lead compounds (tetra methyl lead, tetra ethyl lead, trimethyl lead chloride and triethyl lead chloride) have been measured to three marine species (mussel, shrimp and plaice) and the effect of these materials on the photosynthetic activity of Phaeodactylum tricornutum has been established. In addition the toxicity of dimethyl and diethyl lead dichlorides have been measured to plaice.

Results

The results, as 96 hour LC_{50} values are presented in Table 2 and diagrammatically in Figure 4. All the 96 hour LC_{50} values have been calculated after the method proposed by Doudoroff et al, (1951) and in addition, where appropriate data was available, by the method of Litchfield and Wilcoxon (1949). Since the values calculated using the Doudoroff technique fell within the 95% confidence limits of the other method, only the Doudoroff results are presented.

It can be clearly seen from the results that in general the ethyl derivatives are more toxic than the corresponding methyl derivatives and that the toxicity decreases with decreasing degree of alkylation; the dialkyl lead dichlorides having a similar order of toxicity to inorganic lead. There are two exceptions to this order, mussels are more sensitive to trimethyl than triethyl lead chloride, although the difference in toxicity is so slight that it is probably justifiable to consider them of similar order of toxicity. The other exception is that plaice seem to be rather resistant to the effects of tetra ethyl lead.

BEST PAGE AVAILABLE
PHOTO COPY

The results of the algal exposures are shown in greater detail in Figure 5, from which it can be seen that the nature of the alkyl group has much more effect than the degree of alkylation.

Several other materials were present in the test vessels during part of the study. In the investigations of the toxicity of the tetra alkyl compounds, trialkyl lead chlorides were inevitably present as breakdown products. However, in all but one instance (where mussels were exposed to tetra ethyl lead), the concentration of trialkyl lead chloride present in the test solution was always well below the level that had been shown to cause no deaths in that species over 96 hours. In the study of the tetramethyl lead formulation, dibromoethane (EDB) and dichloroethane (EDC) were also present in the test solutions. The concentration of EDC in the test solutions was always < 5 mg/l and Taylor (1974) has shown that levels of 60 mg/l and 25 mg/l had no apparent effect on dabs (Limanda limanda) and prawns (Palaemon serratus) respectively in 96 hour exposures. The only data in the literature on EDB is the report by Davis and Hardcastle (1959) that the 48 hour LC_{50} to Bluegill sunfish (Lepomis macrochirus) in freshwater was 18 mg/l. Compared to our test concentrations in seawater of < 2 mg/l.

We conclude that, although synergistic and antagonistic effects cannot be ruled out, the contaminants measured in the test vessels did not materially affect the recorded toxicity of the lead alkyl compounds with the possible exception of mussels exposed to tetra ethyl lead, where the presence of triethyl lead chloride may have had a minor effect.

BEST PAGE AVAILABLE

PHOTO COPY

Discussion

The toxic effects of inorganic lead compounds on aquatic animals are largely governed by the chemical form in which the lead is present. In freshwater the dominant controlling factor has been shown to be the hardness of the water, (Pickering and Henderson, 1966). Lead has a low solubility of 0.5 mg/l in soft water and an even lower solubility (0.003 mg/l) in hard water, (Anon, 1972a) although considerably higher concentrations of suspended and colloidal lead may remain in the water. In hard water, the lead will be mainly present as carbonate salts and the amount of "free ionic" lead ie. uncomplexed, which is thought to be the most toxic species, will be relatively small compared to the situation is soft water. This has been confirmed by Davies et al (1976), who were able to monitor electrochemically the levels of "free ionic" lead in their test solutions in addition to che total lead concentration. They reported that on the basis of the "free ionic" lead concentration there was no difference in the toxicity in hard and soft waters, whereas in terms of total lead concentration the toxicity differed by a factor of 100.

As in the case with many materials, juveniles are more sensitive than adult animals. McKim et al (1975) state that the 96 hour LC_{50} for juvenile rainbow trout (Salmo gairdneri) is 0.14 mg/l, ie. 10% of the value recorded for adults by Brown (1968).

There have been few studies of the acute toxicity of lead to marine animals, primarily because of the difficulty of maintaining solutions of lead in seawater at concentrations large enough to cause toxic effects. The most reliable data available at the present time is that of Portmann and Wilson (1971) who report 48 hour LC_{50} values for the Pink Shrimp (Pandalus montagui) and the cockle (Cardium edule) of 375 and 500 mg/l respectively.

PHOTO COPY

BEST PAGE AVAILABLE

The acute toxicity of inorganic lead to aquatic animals can be summarised as follows: In soft freshwater, the acute toxicity, as indicated by the 96 hour LC_{50} value fall between 1-10 mg/l. In hard freshwater or in saline waters the acute toxicity falls between 300-500 mg/l.

No data has been published relating to the aquatic toxicity of the trialkyl or dialkyl lead compounds and in general, the data published on the toxicity of the tetra alkyl lead compounds is not directly comparable with the present study. The 24 hour LC_{50} value of 2.0 mg/l quoted by Turnbull et al (1954) for the Bluegill sunfish (Lepomis macrochirus) in freshwater may be an underestimate when compared to data reported in the present study for marine fish (ie. 0.2 mg/l) which would be expected to be less sensitive. This may be due to losses of the toxin from the test environment, by volatilisation and degradation, in previous studies.

The reduction in algal photosynthesis was studied by Silverberg et al (1977) with a method similar to that used in the present study. They report a 4 hour EC_{50} value of $<$0.3 mg/l for the freshwater alga Ankistrodesmus folcatus compared to the 6 hour EC_{50} of 1.3 mg/l for the marine alga Phaeodactylum tricornutum determined in the present study.

The toxicity of inorganic lead compounds to aquatic animals was originally attributed to coagulation film anoxia (Westfall, 1945). This phenomenon, which was first reported for lead by Carpenter in 1949, involves the formation, by the action of a toxicant, of a veil like film of coagulated mucus on the body surface of the fish. If this film affects the gill tissue, the fish suffers acute respiratory distress and dies from lack of oxygen. This type of mucus development was not detected in the present study. More recently, other workers (Jackim, 1970) have indicated that direct action of the metal on enzyme systems may be responsible for their acute effects, and such responses may be involved in the case of the alkyl lead compounds.

PHOTO COPY

141

BEST PAGE AVAILABLE

In mammals, the toxicity of tetraethyl lead is several times greater than the toxicity of tetramethyl lead. This has been demonstrated in studies with rats (Magistretti et al 1963; Schepers, 1964) and in man (De Treville et al, 1962). However, the toxicity is not simply dependant on the size of the alkyl group since Cremer and Callaway (1961) have shown that tetraproply lead is less toxic than tetraethyl lead. In the present study a similar order has been established for marine animals ie. tetra ethyl lead is more toxic than tetramethyl lead. However, in mammals, the trialkyl lead compounds are more toxic than the tetra alkyl lead compounds and the presently accepted view (Cremer, 1959) is that the tetra alkyl lead compounds are rapidly broken down in the liver of mammals to produce trialkyl lead derivatives which are then responsible for the toxic effects, primarily to the central nervous system. This study, however, appears to show the reverse situation in marine animals since the trialkyl compounds are shown to be considerably less toxic than the tetra alkyl compounds.

This is probably due to the fact that the hydrophobic tetra alkyl lead compounds can be readily absorbed into the aquatic animal where they may exert their toxic action after dealkylation as suggested by Cremer (1959), whereas the more hydrophilic and ionic trialkyl lead chlorides have more difficulty in penetrating the gill membranes.

It is desirable, for monitoring and control purposes, to obtain an estimate of the "safe" concentration of any contaminant. "Safe" is defined here to mean that concentration of the contaminant which is not expected to cause any acutely toxic response in the animal. The "safe" concentration can be obtained from the 96 hour LC_{50} value using an appropriate "application factor". This factor, which may range from 0.1-0.001, is intended to allow for the existence of more sensitive species than those studied and to compensate for the difference between the 96 hour

PAGE 0149

PHOTO COPY BEST PAGE AVAILABLE

LC_{50} value and the incipient LC_{50} or lethal threshold value which is the concentration just lethal to 50% of the animals after indefinite exposure.

A more satisfactory procedure is to obtain the incipient LC_{50} value from a plot of the concentration against the median time of death of the animal (Median Survival Period - MSP curve) and apply an appropriate safety factor to this value. This procedure, which is applied below, eliminates errors caused by the variable slope of the MSP curve. Insufficient data is available from this study to allow the complete median survival period curve to be plotted, since in some of the experiments, the maximum exposure level that could satisfactorily be obtained, did not cause sufficient mortality within 96 hours. However, sufficient data is available to produce points on the curve representing 24, 48 and 96 hour LC_{50} values. (Figure 6). This data shows that in most cases, a threshold value had not become evident after 96 hours. This is in agreement with the work of Cairns (1957) who reported similar findings with tetra ethyl lead. However, the data can be used to predict threshold values for plaice of 0.04 mg/l for TML-CB and 1.0 mg/l for triethyl lead chloride. This information, coupled with a reasonable application factor of 0.1 (Sprague, 1971) produces estimated safe levels of 0.004 mg/l for TML-CB and 0.1 mg/l for triethyl lead chloride. This latter figure will also apply to trimethyl lead chloride, since although no threshold value can be predicted from the data, its toxicity is far less than the triethyl derivative. Similarly since the toxicity of TEL is similar to that of TML-CB and ca. 70-80x that of the trialkyl compounds a "safe" level in the region of 0.001 mg/l for both the tetra alkyl compounds seems reasonable. Support for these levels is provided by the data obtained during the accumulation experiments, which are discussed in the next section. In these studies, marine animals were maintained for periods in excess of 30 days without apparent ill effect, in concentrations of tri-alkyl lead chlorides ranging from 0.1-2.0 mg/l.

PAGE 0 1 50

PHOTO COPY

BEST PAGE AVAILABLE

Thus in the light of existing evidence we can propose tentative "safe" levels for the avoidance of acutely toxic effects in the marine environment of 0.001 mg/l for tetra alkyl lead compounds and 0.1 mg/l for trialkyl lead compounds. Since the tetra alkyl lead compounds are rapidly degraded to the trialkyl lead derivative, it is the latter figure which is of most importance.

PAGE 0151

Bioaccumulation

There are two principal routes by which aquatic animals may accumulate a contaminant, via food or via water. Exposure of animals to contaminants in food is usually used to assess the significance of food chain transfer and is usually considered to be secondary to accumulation direct from the water. At this stage in the investigation, the water route was considered to be of primary importance, since if no significant accumulation occurred by this route, the possibility of food chain transfer and magnification would be relatively small.

The tetra alkyl lead compounds have only a short lifetime in seawater and therefore they are unlikely to be accumulated over long periods by marine life. Thus, although some information has been obtained on the accumulation of lead from these materials during short-term exposures to high concentrations, most of the experimental work discussed is concerned with the accumulation of lead from trialkyl lead compounds during long term exposures.

BEST PAGE AVAILABLE

PHOTO COPY

Results

(a) Short Term Exposures

The animals, which had been exposed for 96 hours to a wide range of concentrations of four alkyl lead compounds during the acute toxicity investigations, were subsequently analysed for their lead content. The data obtained from the experiment with shrimps (Crangon crangon) is shown in Figure 7.

The results obtained must however, be treated with caution since at exposure concentrations in excess of the 96 hour LC_{50} value a significant proportion of the animals would have diedbefore the end of the test period. The behaviour of these animals under high stress, such as immediately prior to death, may have caused anomalies in the rate of accumulation of the material being investigated, and, in addition, since it was not practicable to remove dead animals from the test system the analytical data obtained for these species may be unrepresentative due to decomposition. The data obtained is of little use for predicting the results of long term exposures, but can be used to indicate the likely concentrations of lead in the tissues of animals exposed to high levels of lead alkyls in the immediate vicinity of a spillage.

The results of these short term exposures are summarised in Table 3. The relevant tissue levels, as far as hazards to fish consumers are concerned is that produced by exposure to the 96 hour LC_{50} value since higher exposure levels will lead to substantial mortalities in the exposed population. As can be seen the concentration factors (see Table 3) measured were all relatively small with the tetra alkyl compounds showing a greater tendency to accumulate.

(b) Long Term Exposures

Two species, the common mussel (Mytilus edulis) and the dab (Limanda limanda) both of

BEST PAGE AVAILABLE

PHOTO COPY

which are of commerical importance and provide a pathway for the transfer of lead alkyls to man were chosen for investigation. The test conditions were selected to represent typical seawater and the exposure levels set at approximately 10% of the 96 hour LC_{50} values.

PAGE 0153

1. Mussel Experiment

The results of this experiment are summarised in Table 4. The shape of the accumulation curves was similar for both compounds and both concentrations tested and a typical example is shown in Figure 8.

After an initial rapid accumulation of lead, during which intake was greater than loss, the mussels reached an equilibrium. The time taken to reach this plateau varied from 5-9 days but was apparently independent of the concentration of alkyl lead. No further accumulation took place although the exposures were continued for up to 35 days. After termination of the exposure the accumulated lead was rapidly lost with a half life of ca. 3 days.

At equilibrium, the average tissue level of lead was directly proportional to the exposure concentration with a concentration factor of ca. 70x. The maximum lead concentration, 68 mg/Kg, was measured in the mussels that had been exposed to 0.1 mg/l of trimethyl lead chloride (20% of the 96 hour LC_{50} value) and represented an order of magnitude increase in lead concentration compared to the control population.

The distribution of lead within the mussels could be represented as follows:

GILL > DIGESTIVE GLAND > FOOT > GONAD

The condition factor of the control population indicated no significant signs of stress and, this is confirmed by the low cumulative mortality of ~ 5% observed over the 60 day experimental period. There was some evidence of a decline in

BEST PAGE AVAILABLE PHOTO COPY

condition in the exposed animals for the first ten days of the experiment but this w within the natural variation.

2. Dab Experiment

A summary of the data is shown in Table 5 and the accumulation curves are shown in Figures 9 and 10. In this animal, two tissues were analysed, muscle since it constitutes the major part of the animal and is eaten by human beings and the liver sin this is both a storage organ and a major part of the animals detoxification system.

In contrast to the study with mussels, the increase in tissue concentration of lead, with one exception, was linear with respect to time, and there was no evidence for equilibrium being reached even though the exposure was continued for 41 days. The exception is illustrated in Figure 9 where, in the liver of fish exposed to trimethy lead chloride, an equilibrium was reached after ca. 20 days. The reason for this is not known.

The rate of accumulation of lead into the fish tissues was very low. After 41 days, the concentration factors were 2x for trimethyl and 12x for triethyl lead chloride. liver and muscle tissues accumulated similar levels of lead with the average concent reaching a maximum 30 mg/Kg.

In contrast to the mussel experiment, the rate of loss of lead from the fish, after termination of the exposure, was very slow with half lives in excess of 41 days. Th low rate of loss was expected, since the linear accumulation curves had implied that the rate of loss was lower than the rate of accumulation. Since the accumulated lea levels in liver and muscle were very similar it seems unlikely that the liver is involved in any major detoxification mechanism in the animal, although the observati that the liver concentrations tended to increase after the exposure had terminated

PHOTO COPY BEST PAGE AVAILABL

(Fig. 10) implies that some translocation of lead to the liver may take place.

During the course of the experiment, there was a gradual but minor decline in condition of all the experimental animals. It is interesting to note that the mean deterioration in condition of the fish exposed to triethyl lead chloride was identical to that of the control population (0.004%/day) whereas the corresponding figure for fish exposed to trimethyl lead chloride was 0.008%/day.

PAGE 01 55

Several workers have studied the accumulation of inorganic lead compounds by members of the Mytilus family. (Coombs, 1975; Majori and Petronio, 1973; Schulz-Baldes 1972, 1974). Majori and Petronio, using a Mediterranean species Mytilus galloprovincialis reported concentration factors of 200-300x at exposure levels of 0.05 - 0.10 mg/l. However, most other workers have found the accumulation of inorganic lead to be substantially greater in mussels than these authors have reported.

The most extensive study has been made by Schulz-Baldes (1972, 1974) with Mytilus edulis and a direct comparison of his results with the present study is presented in Table 6. The major difference between inorganic lead, as reported by Schulz-Baldes, and trialkyl lead, as reported here, is that for inorganic lead there is no evidence of a steady state being achieved. Thus, although the tissue levels recorded by both groups after 7 days exposure are similar, after 35 days exposure the mussels exposed to trialkyl lead chloride have not accumulated any more lead (Concentration Factor (Dry wt 400)whereas the mussels exposed to inorganic lead have maintained their initial high rate of accumulation to reach a substantially higher level (Concentration Factor (Dry wt) = 6000). These results are shown diagrammatically in Fig. 11.

These high concentration factors for inorganic lead have been confirmed by Coombs (1975) who reported values of 3,000 in tissues of Mytilus edulis exposed to 0.1 mg/l inorganic lead in seawater.

PHOTO COPY

BEST PAGE AVAILABLE

This difference between inorganic and trialkyl lead compounds appears to be due to the existence of a rapid excretion process for trialkyl lead in this animal. In the present study the half life of the lead accumulated from trialkyl lead solutions was ca. 3 days. However, Schulz-Baldes quotes a half life for inorganic lead of > 35 days and it has been reported (Kauranen and Jarvenpaa, 1972) that the half life of lead in mussels could be as long as 300 days. At the present time the form which lead is present in those animals that have been exposed to trialkyl lead chlorides is not known, but it would appear from these results that it is not present simply as inorganic lead.

Some similarities do exist between the accumulation of lead from inorganic and trialkyl lead compounds. In both cases the tissue residue levels at a fixed time are directly proportional to the exposure concentration (Fig. 11). Also, the tissue distribution of lead within the mussel appears to be unaffected by the type of lead compound to which the animal is exposed. Coombs and Schulz-Baldes both found the same ranking order for the tissue distribution as was found in this study.

No data has been found on the accumulation of inorganic lead in marine fish that can be contrasted with the present study. Some work has been reported with freshwater fish (Berg, 1973; Merlini and Pozzi, 1977a, b) but comparisons must be made with care. Merlini and Pozzi (1977) showed that the accumulation of inorganic lead by the sunfish (Lepomis gibbosus) was directly related to the species of lead present in the water and they quote concentration factors of 400-900x after 27 day exposures (compared to 2-10x in the present study). They also report in contrast to this study that the liver tissue contained from 20-30x more lead than the muscle tissue.

It is interesting to compare the data obtained in the present study with that produced in the case of mercury accumulation. Several workers have studied the accumulation of inorganic mercury into fish (Macleod and Pessah, 1973; McKone, 1971) and shellfish

PHOTO COPY

BEST PAGE AVAILABLE

149

(Kopfler, 1974; Unlu et al, 1970) and the generally agreed concentration factor is ca. 20-60x. However, experiments by Burkitt (1974) and Kopfler (1974) indicate that the concentration factor for alkyl mercury compounds in fish and shellfish is approximately an order of magnitude greater at ca. 500-800x. Thus alkyl mercury compounds have higher concentration factors in fish and shellfish than inorganic mercury compounds whereas the reverse situation applies in the case of lead.

PAGE 0151

BEST PAGE AVAILABLE

PHOTO COPY

CONCLUSIONS

The aim of the present study was to provide data which could be used to assess the risks caused by an accidental release of lead alkyl compounds in the marine environment.

PAGE 0158

It has been demonstrated that the toxicity of the alkyl lead compounds is directly related to the degree of alkylation and to a lesser extent to the nature of the alkyl group. The tetra alkyl lead compounds are the most toxic with 96 hour LC_{50} values of approximately 0.1 mg/l, but these rapidly degrade in seawater to the trialkyl lead chlorides which have 96 hour LC_{50} values in the range of 1-10 mg/l. Tentative "safe" levels for these two groups of compounds in the marine environment have been calculated as 0.001 mg/l and 0.1 mg/l, and these levels would not be expected to cause any acutely toxic effects.

The accumulation of lead by marine animals is important both because of the possible effect on the animal itself and due to the possible effect that the accumulated levels might have on predatory organisms, one of which is man. There is, at the present time, some considerable dispute with regard to the toxicological significance of lead residues in food and in man (Anon, 1972b), however the Joint FAO/WHO Expert Committee on Food Additives (1972) have recommended a provisional tolerable weekly intake of 3 mg lead which is equivalent to 430 ug/day.

In the United Kingdom, the lead content of food for human consumption is regulated by either the Food and Drugs Act or the Lead in Food Regulations. These regulations have recently been reviewed by the Food Additives and Contaminants Committee of the Ministry of Agriculture Fisheries and Food (Anon, 1975) and they have recommended in the light of all the available evidence that in future there should be a statutory lead limit of 2.0 mg/Kg for fish and 5.0 mg/Kg for shellfish (equivalent to 14.0 and 35.0 mg/Kg on a dry weight basis).

BEST PAGE AVAILABLE

PHOTO COPY

It has long been known that lead is present in marine animals (Vinogradov, 1953), although due to the inaccuracies of early analytical methods, data prior to 1970 must be treated with caution. The distribution of lead in molluscs and particularly mussels obtained from the environment has recently been reviewed by a number of authors (Alexander and Young, 1976; Chow et al, 1976; MAFF 1971, 1975; Nickless et al, 1972; Topping, 1973A) and their results are shown in Table 7(a) alongside data from the present study. The corresponding data for fish is presented in Table 7(b), although in this case the data is not as extensive and does not always refer to the dab, but to other similar species such as the plaice (Pleuronectes platessa) and flounder (Pleuronectes flesus).

PAGE 159

The results of the present study indicate that the long term exposure of mussels and dabs to concentrations of trialkyl lead chlorides equivalent to 10% of the 96 hour LC_{50} value produce lead residues equivalent to twice that permitted for human consumption, although the form of the accumulated lead is not known. The production of significantly higher residue levels would require exposure to higher concentrations of the lead alkyl which would cause significant mortality. For example, Table 7 indicates that residue levels of 7x the permitted maximum could be achieved by exposure of mussels to tetra methyl lead for 96 hours but under these conditions 50% of the exposed population would die.

Thus, the major environmental impact of a release of tetra alkyl lead compounds into the marine environment is more likely to be due to acutely toxic effects than those of bioaccumulation.

PHOTO COPY

BEST PAGE AVAILABLE

ACKNOWLEDGEMENT

The authors would like to express their thanks to the Associated Octel Company for the provision of materials and considerable technical assistance, Mr J E Caunter and Mr E Gillings of the Brixham Laboratory for assistance with the experimental work and to Dr L Carter of the same establishment for his helpful and constructive criticism.

PAGES 160

PHOTO COPY

BEST PAGE AVAILABLE

Fig 1. DOSING SYSTEM

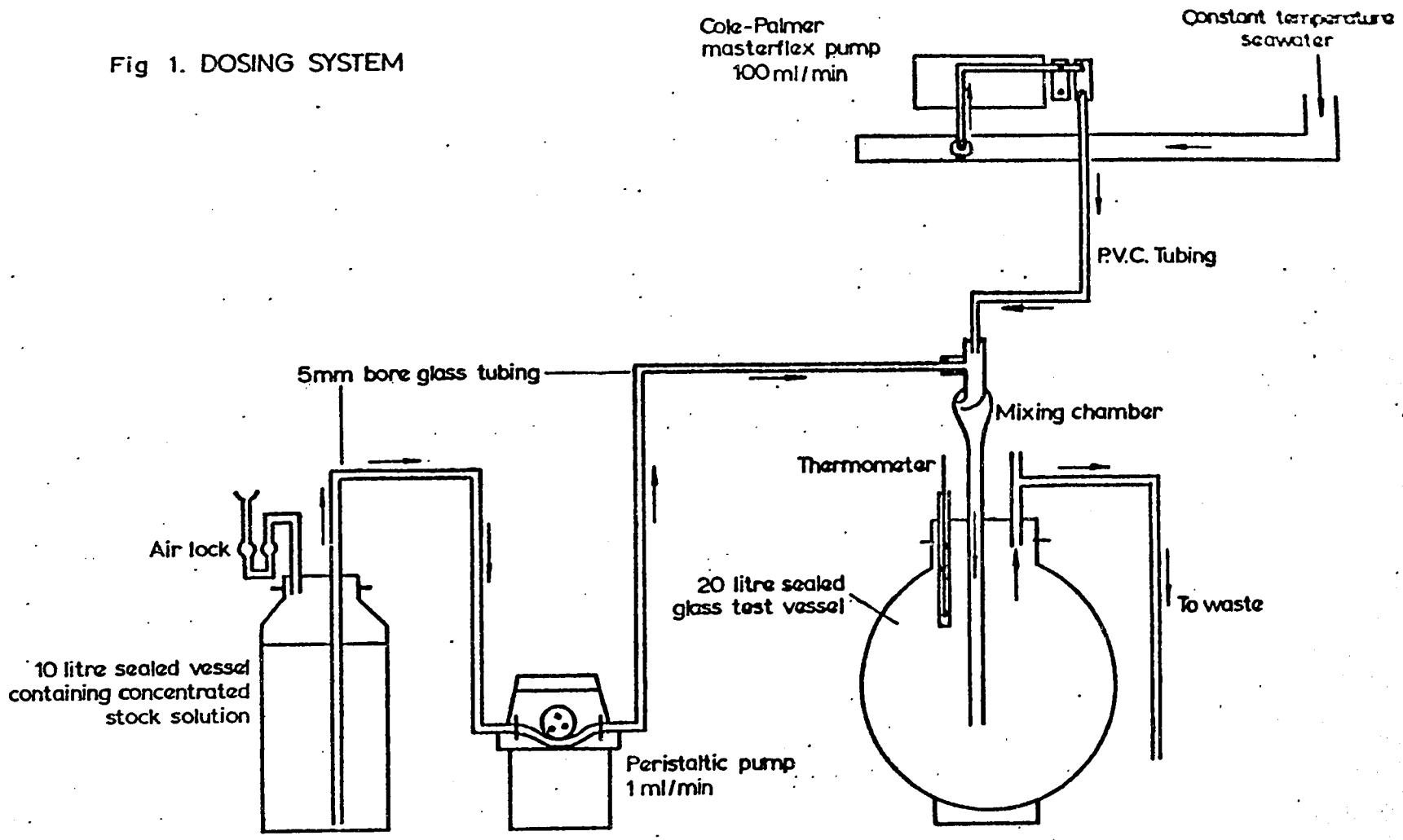


PHOTO COPY BEST PAGE AVAILABLE

151

BEST PAGE AVAILABLE

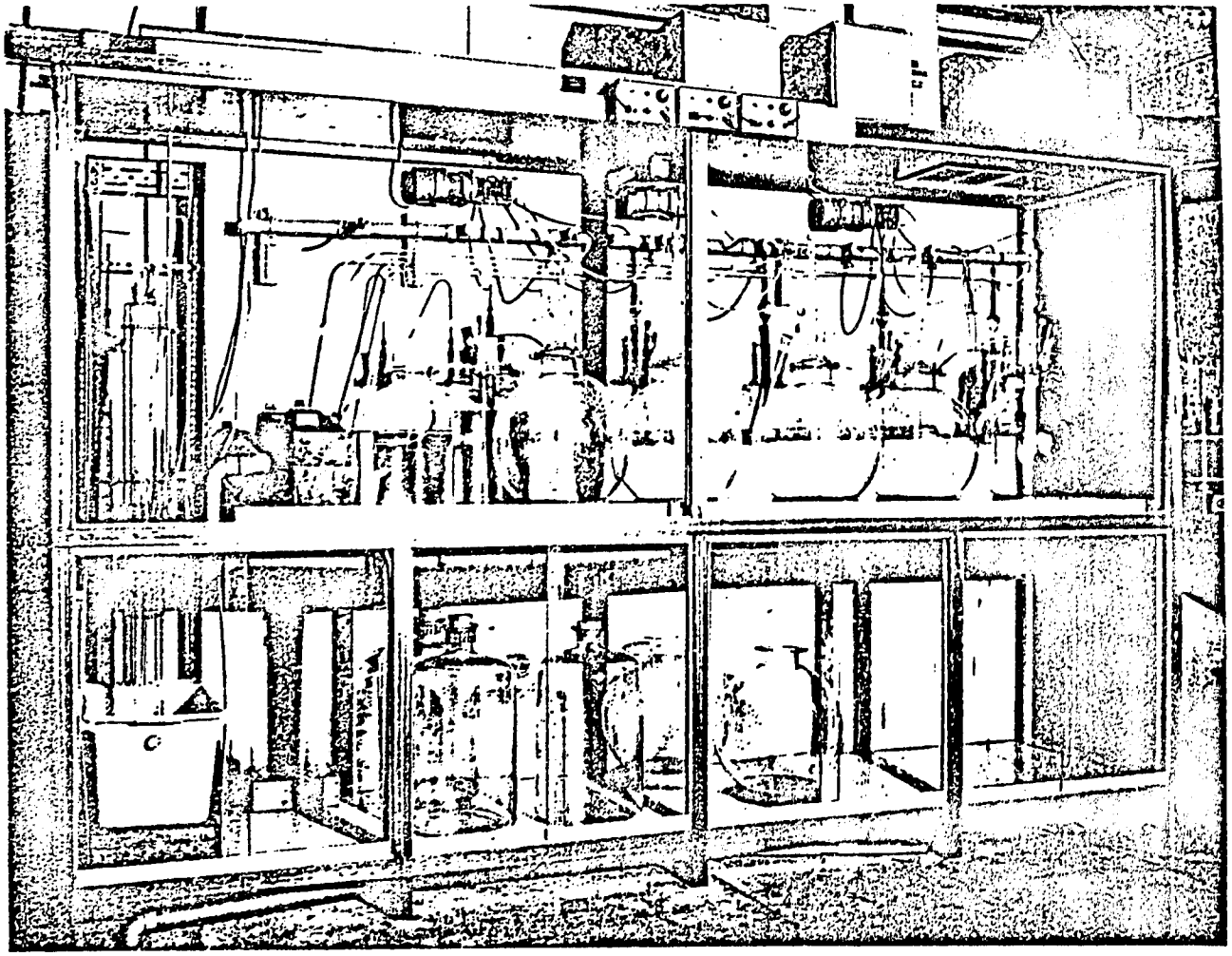


Fig. 2 - Bioassay System

PHOTO COPY

Fig 3. TEST APPARATUS FOR MUSSEL ACCUMULATION

PAGE 0163

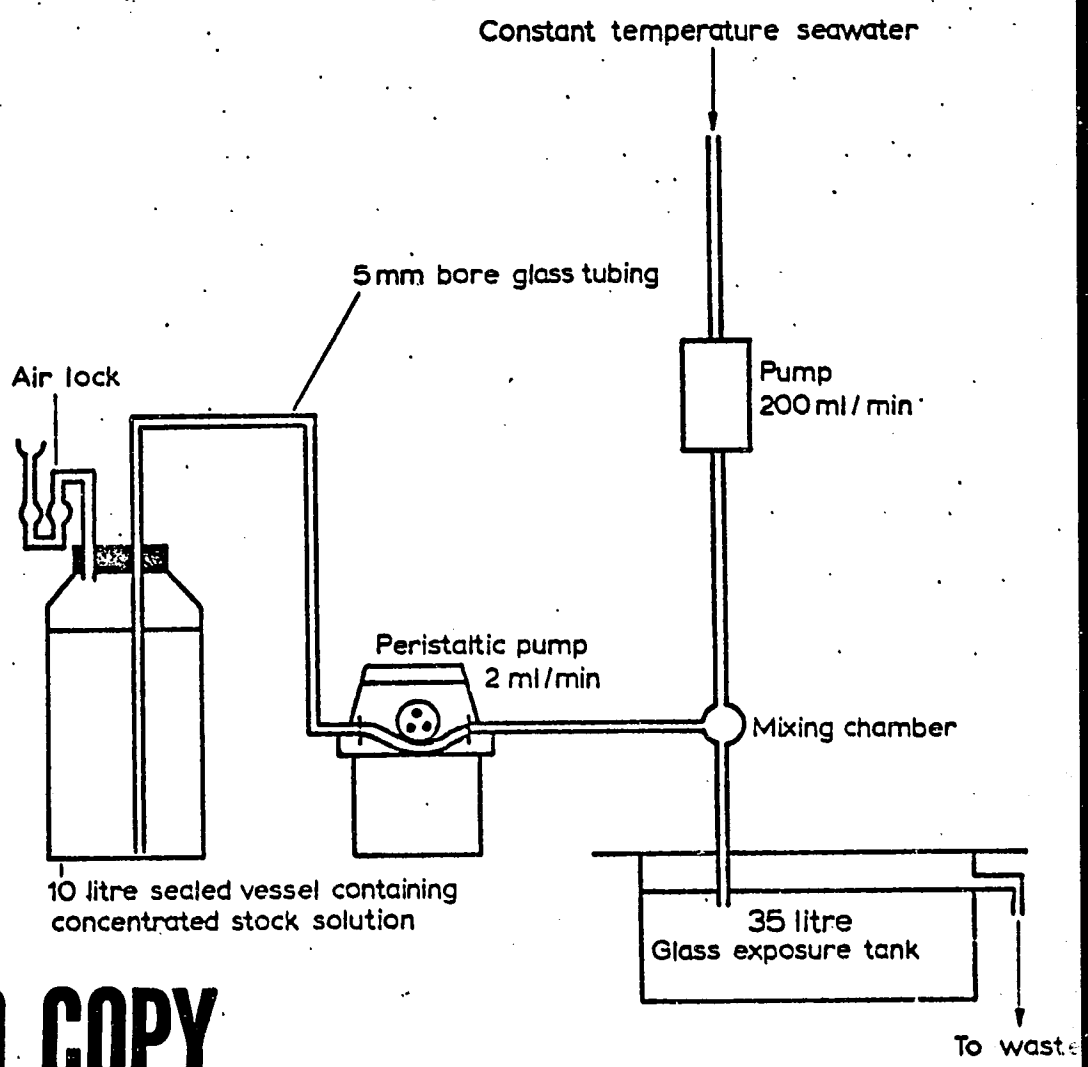


PHOTO COPY

BEST PAGE AVAILABLE

Fig 4. TOXICITY OF LEAD COMPOUNDS TO MARINE ANIMALS

PAGE 0164

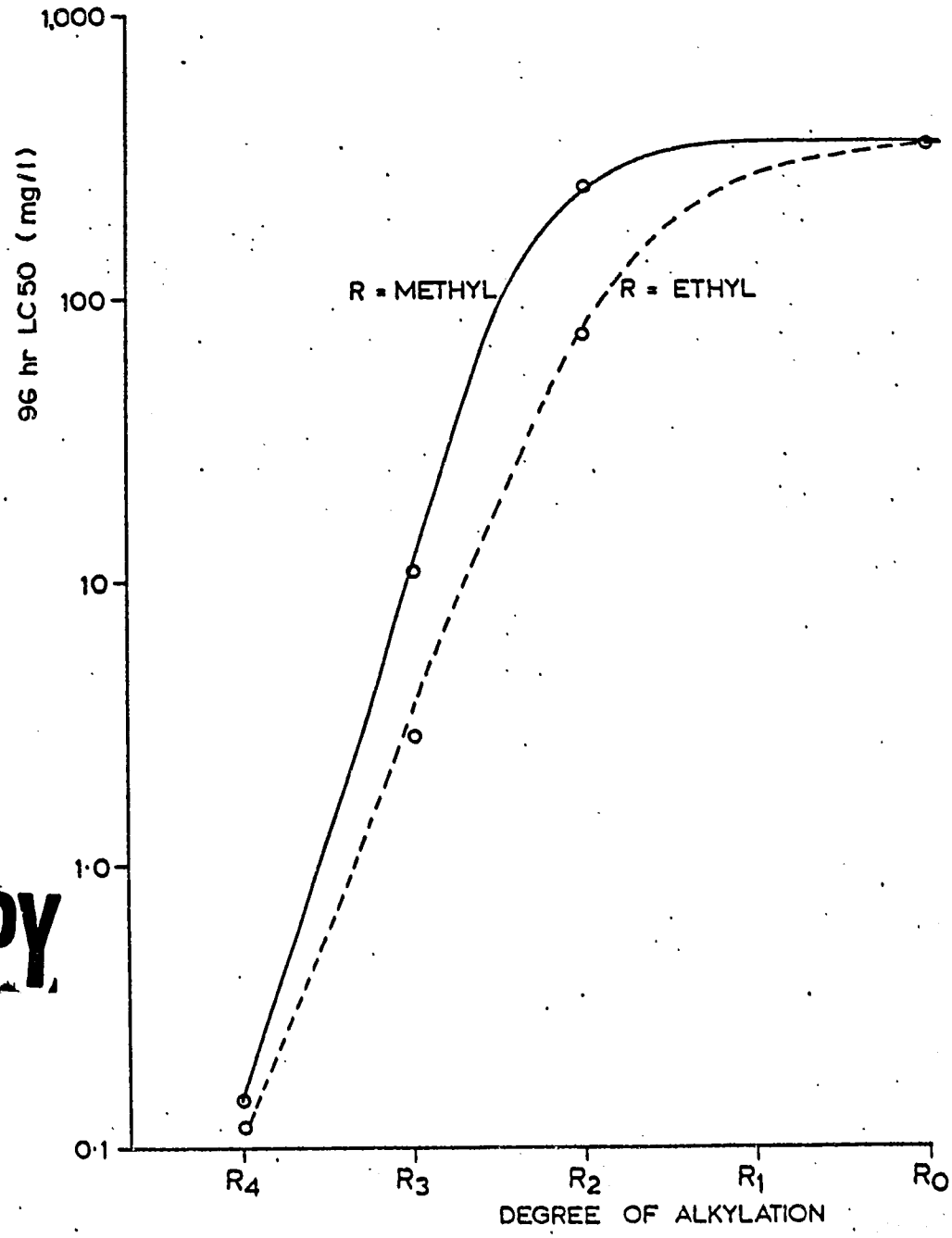
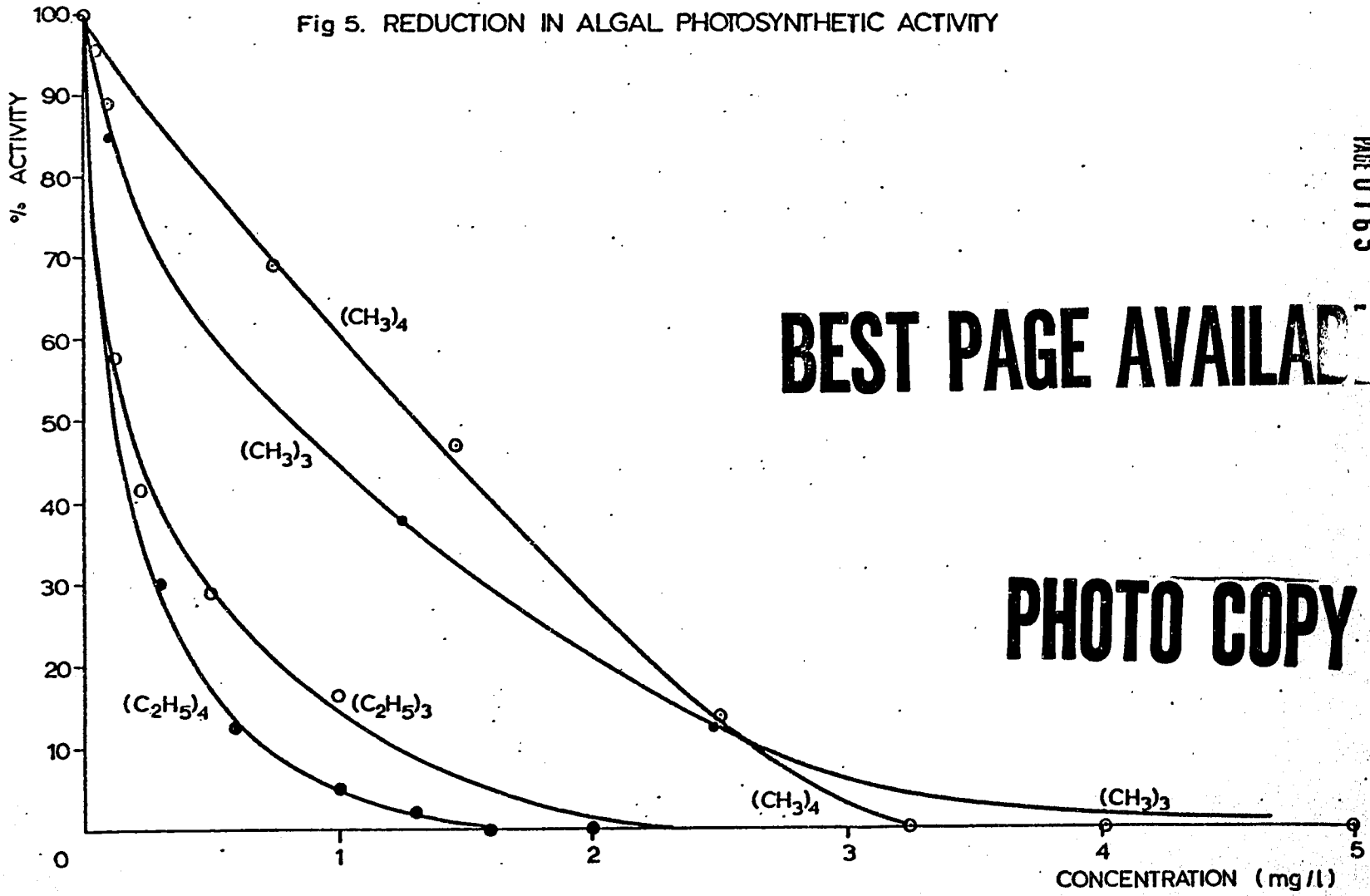


PHOTO COPY

BEST PAGE AVAILABLE

Fig 5. REDUCTION IN ALGAL PHOTOSYNTHETIC ACTIVITY



BEST PAGE AVAILABLE

PHOTO COPY

159

Fig 6 (A) MEDIAN SURVIVAL PERIOD PLOT — T.M.L.- C.B.

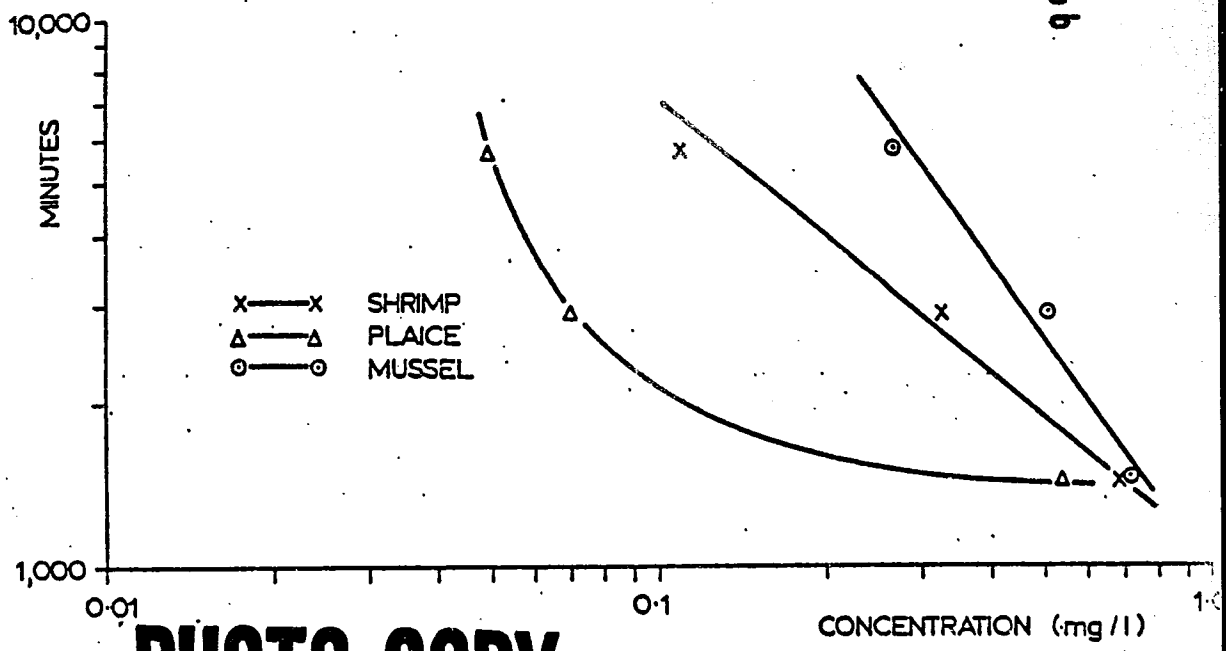
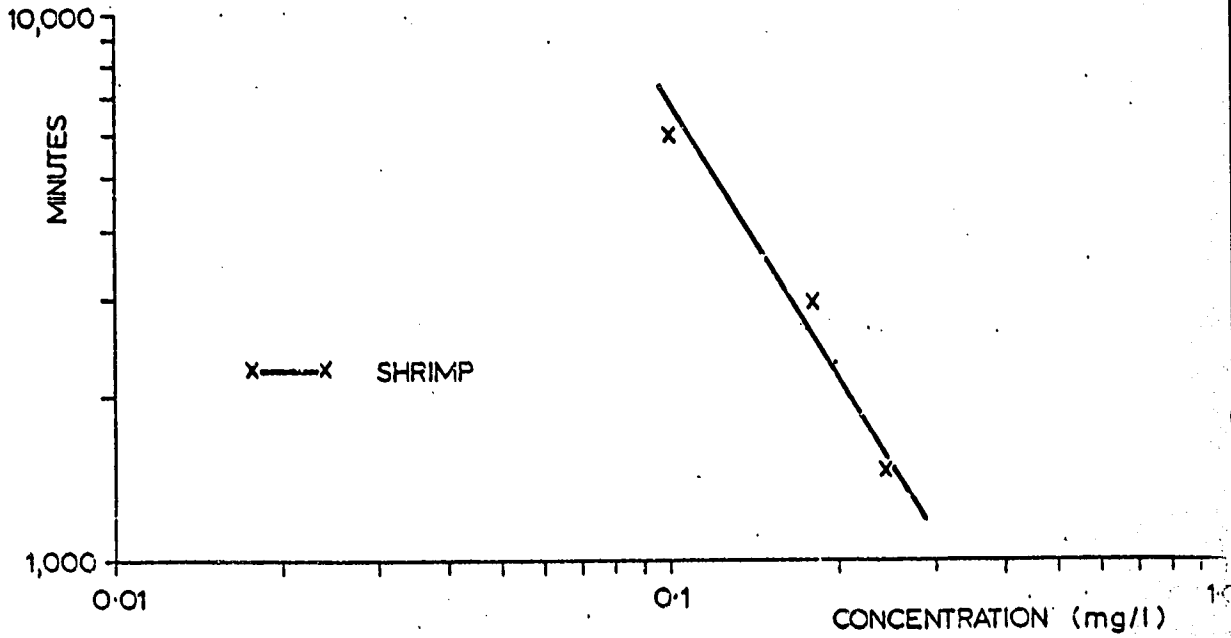


PHOTO COPY

Fig 6 (B) MEDIAN SURVIVAL PERIOD PLOT — T.E.L.



BEST PAGE AVAILABLE

Fig 6 (C) MEDIAN SURVIVAL PERIOD PLOT — TRIMETHYL LEAD CHLORIDE

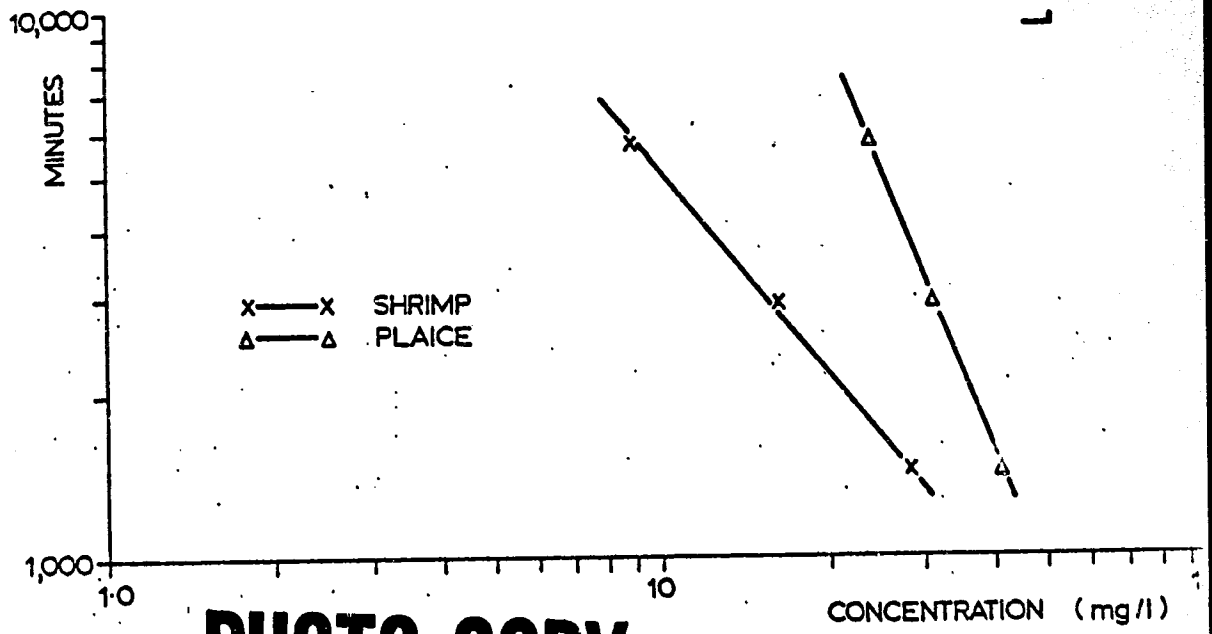
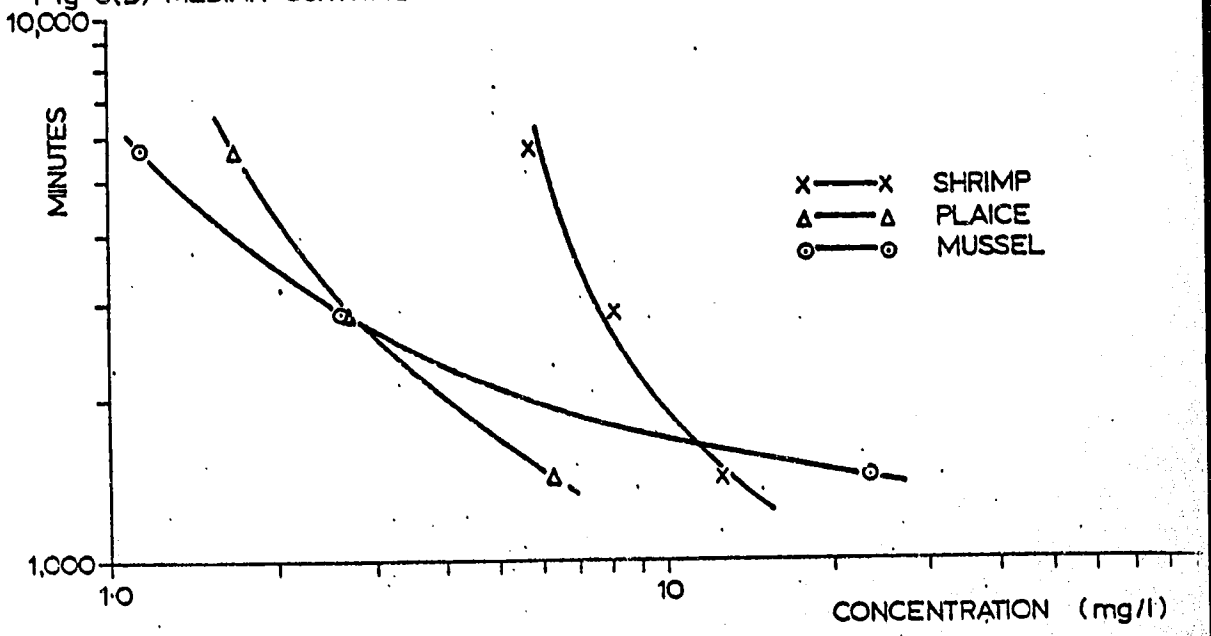


PHOTO COPY

Fig 6(D) MEDIAN SURVIVAL PERIOD PLOT — TRIETHYL LEAD CHLORIDE



BEST PAGE AVAILABLE

Fig 7. ACCUMULATION OF LEAD ALKYLS BY SHRIMPS (CRANGON CRANGON) DURING 96 hr EXPOSURES

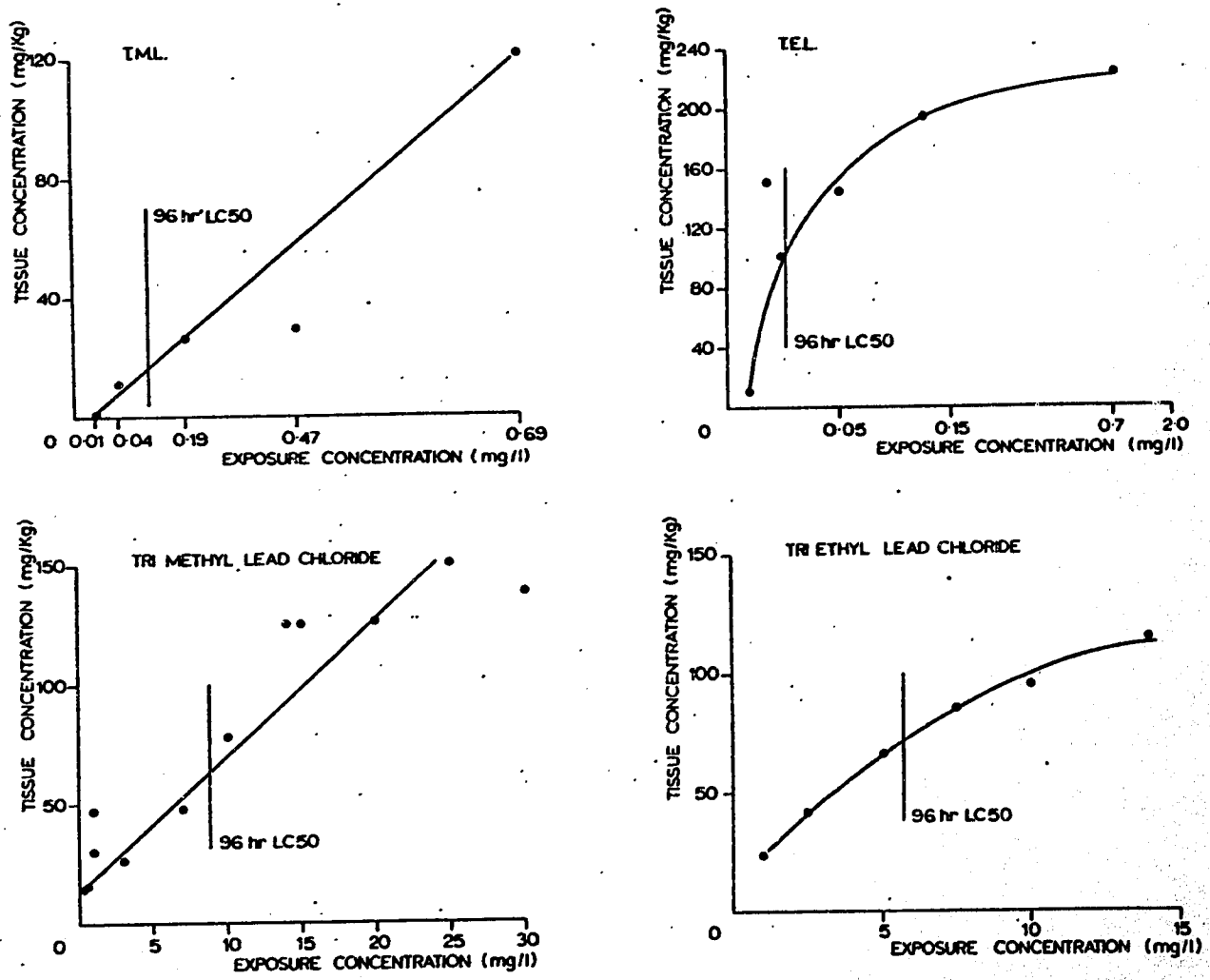
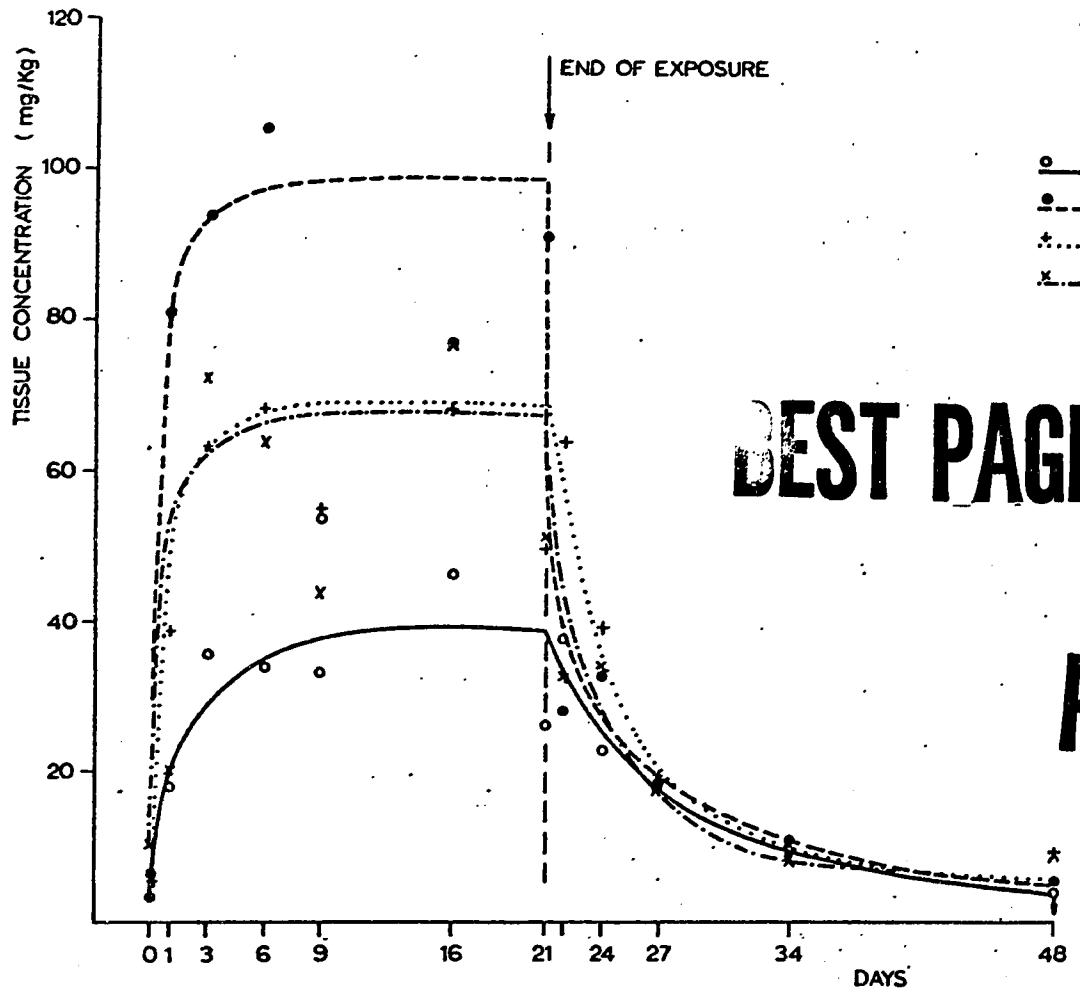


PHOTO COPY
BEST PAGE AVAILABLE

Fig 8. ACCUMULATION OF LEAD BY MUSSELS EXPOSED TO 0·1 mg/l TRI METHYL LEAD CHLORIDE



BEST PAGE AVAILABLE

PHOTO COPY

163

BEST PAGE AVAILABLE

Fig 9. ACCUMULATION OF LEAD BY DABS EXPOSED TO TRI METHYL LEAD CHLORIDE

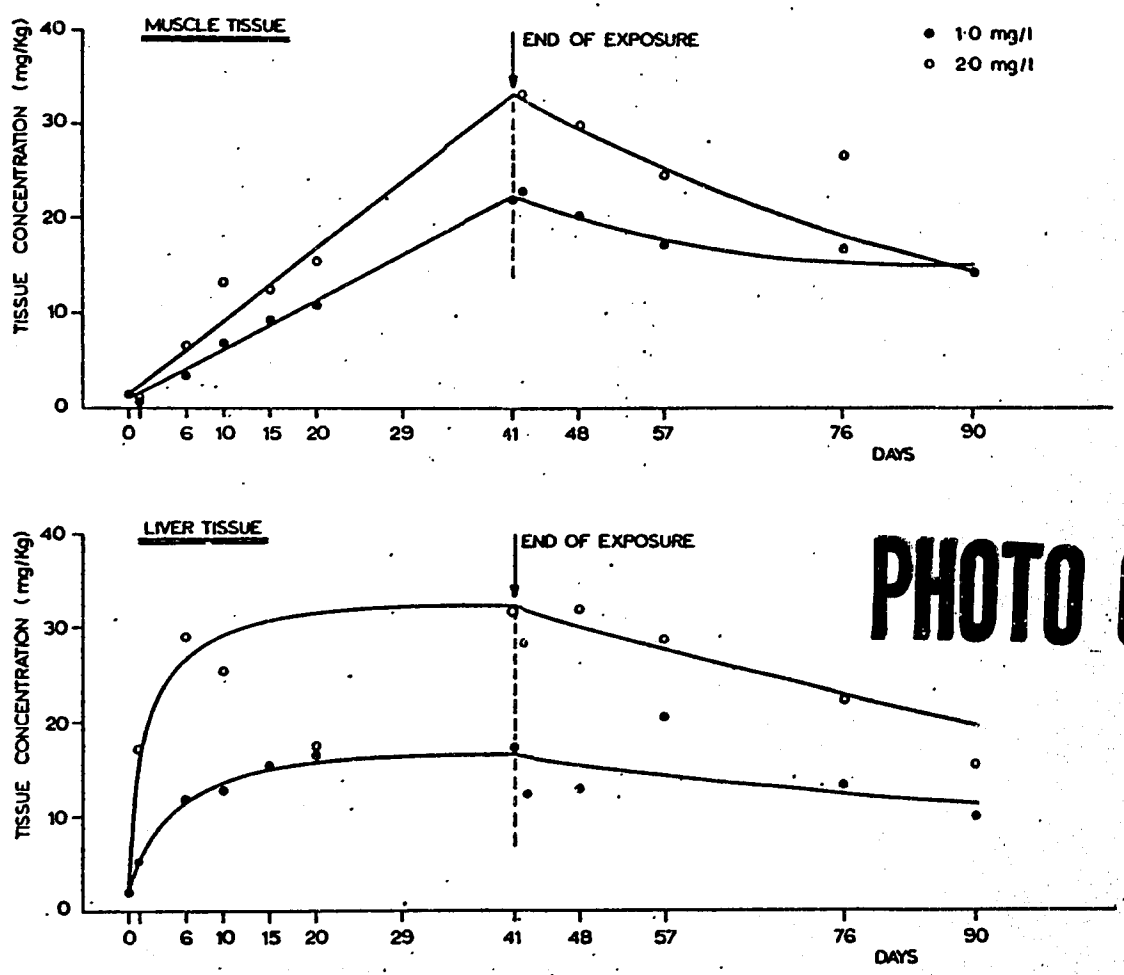
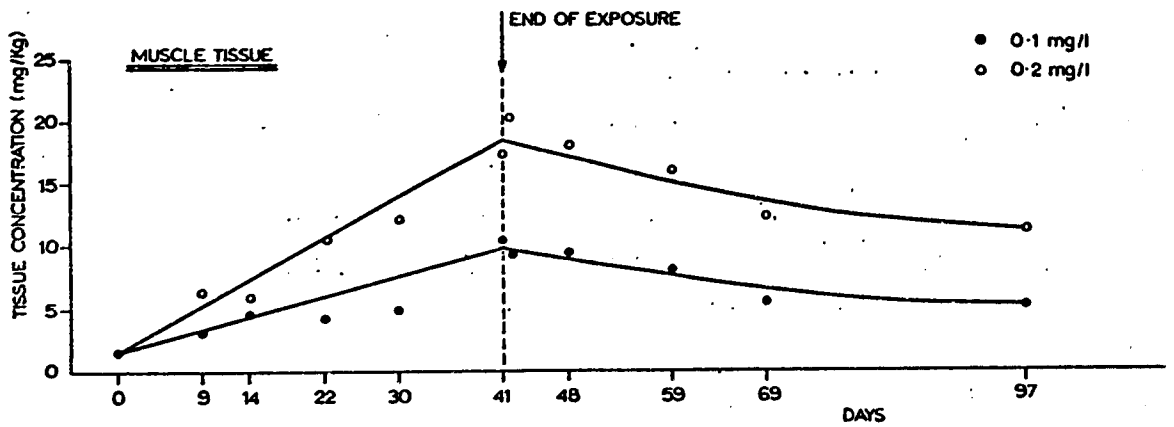


PHOTO COPY

PAGE 0 1 70

BEST PAGE AVAILABLE

Fig 10. ACCUMULATION OF LEAD BY DABS EXPOSED TO TRI ETHYL LEAD CHLORIDE



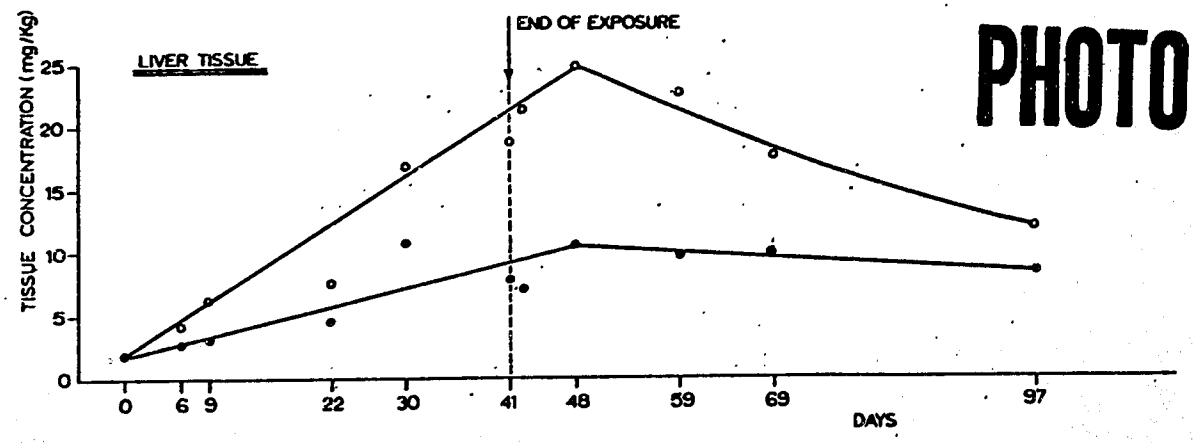
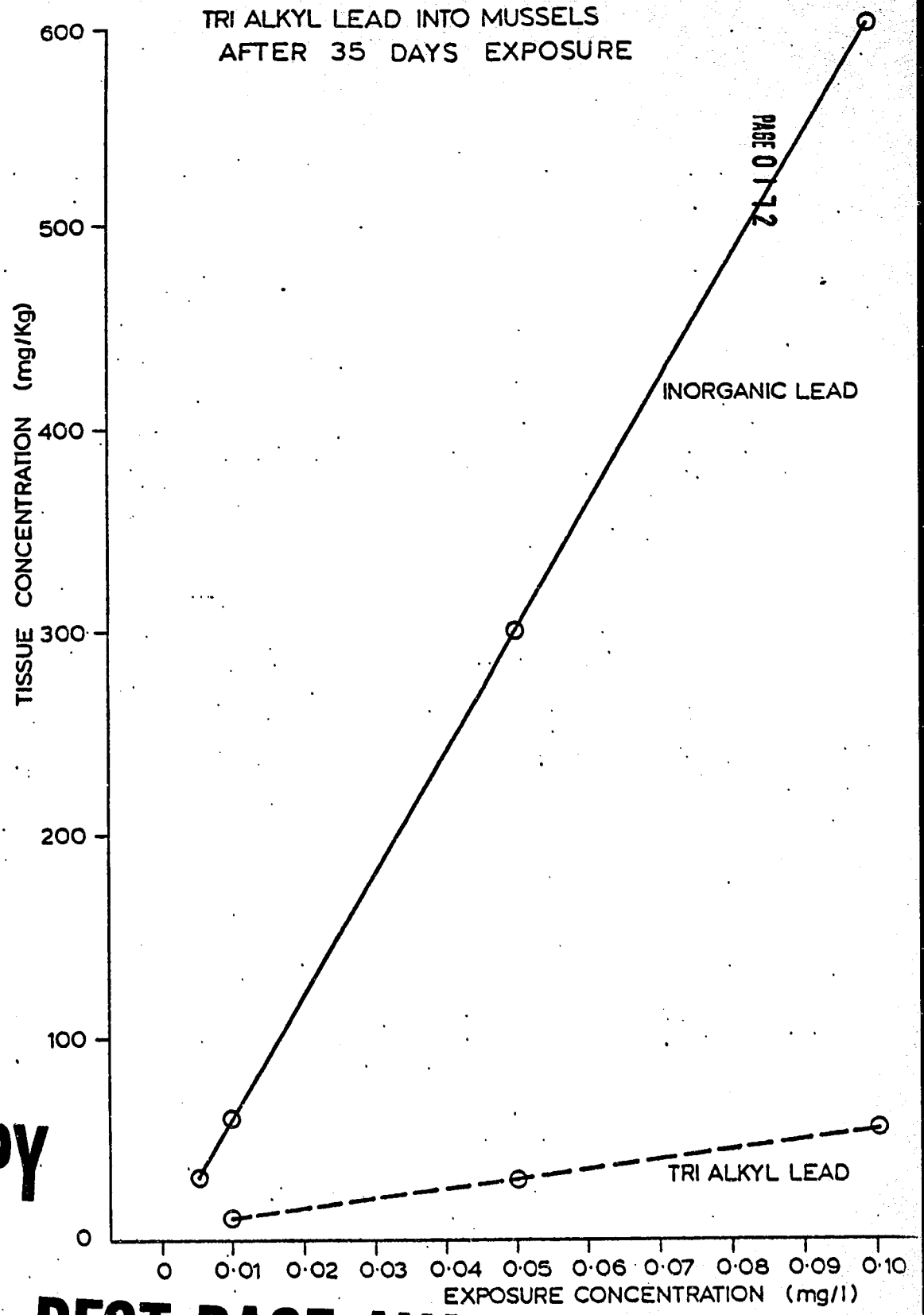


PHOTO COPY

PAGE 0 1 7 1

FIG 11

COMPARISON OF ACCUMULATION OF INORGANIC AND TRI ALKYL LEAD INTO MUSSELS AFTER 35 DAYS EXPOSURE



PAGE 0172

PHOTO COPY

BEST PAGE AVAILABLE

166

TABLE 1

EXPERIMENTAL ANIMALS

Phylum	Species	Mean Length (cm)	Mean Weight (g)	Source	Mode
Algae	Phaeodactylum tricornutum	-	-	Plymouth Collection	Sub-culture on modified Erd-Schrieber (Foyn, 1934)
Mollusc	Mytilus edulis (Common Mussel)	6.4	28.5	Teign Estuary, S. Devon	Hand Collection
Crustacean	Crangon crangon (Brown Shrimp)	4.8	1.1	Tor Bay, S. Devon	Push Net
Fish	Pleuronectes platessa (Plaice)	5.2	2.8	Tor Bay, S. Devon	Otter Trawl
	Limanda limanda (Dab)	18.9	191	Tor Bay, S. Devon	Otter Trawl

BEST PAGE AVAILABLE

PHOTO COPY

PHOTO COPY

TABLE 2

SUMMARY OF 96 Hr. LC_{50} VALUES (mg/l)

Species	Shrimp	Mussel	Plaice	Algae*
1. Tetra Methyl Lead (TML-CB)	0.11	0.27	0.05	1.3
2. Tetra Ethyl Lead (TEL)	0.02	0.10	0.23	0.1
3. Tri Methyl Lead Chloride	8.8	0.5	24.6	0.8
4. Tri Ethyl Lead Chloride	5.8	1.1	1.7	0.1
5. Di Methyl Lead Dichloride			300	
6. Di Ethyl Lead Dichloride			75	
7. Inorganic Lead	(375)[1]	(>500)[1]	(180)[2]	>5.0

* Parameter measured is a reduction in photosynthetic activity (6 hr EC_{50})

Figures in brackets refer to related species - 1. Portman and Wilson (1971)
2. Jackim *et al* (1970)

BEST PAGE AVAILABLE

PAGE 0174

1689

PHOTO COPY

TABLE 3

SUMMARY OF ACCUMULATION DATA FROM 96 HR. EXPOSURES

Compound	Species	Exposure Range (mg/l)	96 Hr LC_{50} (mg/l)	Concentration Factor[1]
1. Tetra Methyl Lead (TML-CB)	Shrimp	0.01 - 0.7	0.11	20x
	Mussel[2]	0.05 - 0.7	0.27	170x
	Plaice	0.02 - 0.7	0.05	60x
2. Tetra Ethyl Lead	Shrimp	0.01 - 0.2	0.02	650x
	Mussel[2]	0.01 - 0.2	0.10	120x
	Plaice	0.02 - 0.2	0.23	130x
3. Tri Methyl Lead Chloride	Shrimp	0.2 - 30	8.8	1x
	Mussel[2]	0.1 - 3.0	0.5	24x
	Plaice	10 - 50	24.6	1x
4. Tri Ethyl Lead Chloride	Shrimp	1.0 - 15.0	5.8	2x
	Mussel[2]	0.1 - 25.0	1.1	10x
	Plaice	1.0 - 10.0	1.7	2x

1. Concentration Factor = $\frac{\text{Concentration in Animal Tissue (mg/Kg. Wet Wt.)}}{\text{Concentration in water (mg/l)}}$ After exposure at 96 hr. LC_{50} Concn.

2. Mussel figures are mean of 4 tissue (Digestive gland, Foot, Gill, Gonad)

BEST PAGE AVAILABLE

BEST PAGE AVAILABLE
PHOTO COPY

TABLE 4

MUSSEL EXPERIMENT - SUMMARY OF ACCUMULATION DATA

		Control	Trimethyl Lead Chloride			Triethyl Lead Chloride		
Exposure								
Exposure Concn. (mg/l)		0	0.01	0.05	0.10	0.01	0.05	0.10
Exposure Time (Days)		35	9	35	21	9	35	21
Time to Equilibrium (Days)		-	-	9	7	-	6	5
Tissue Concn. (mg/Kg Dry Wt.) at Equilibrium	Gill	6	10	39	99	20	52	60
	D. Gland	6	10	32	69	11	33	56
	Gonad	9	15	21	67	10	15	42
	Foot	3	5	19	39	6	18	35
	Mean	6	10	27	68	18	29	48
Concentration Factors								
(a) Tissue (Wet Wt.)/Water		-	60	60	90	90	66	60
(b) Exposed/Control		-	1.6	4.5	11	2.0	4.8	8.0
Depuration								
Half Life (Days)	Gill			2	1		2	-
	D. Gland			2	4		12*	5
	Gonad			4	2		4	2
	Foot			4	6		5	3
	Mean			3	3		4	3
Total Experiment Duration (Days)		62	9	62	48	9	62	48

BEST PAGE AVAILABLE

PHOTO COPY

TABLE 5

DAB EXPERIMENT - SUMMARY OF ACCUMULATION DATA

	Tissue	Control	Trimethyl Lead Chloride		Triethyl Lead Chloride	
Exposure						
Exposure Concn. (mg/l)		-	1.0	2.0	0.1	0.2
Exposure Time (Days)		41	41	41	41	41
Time to Equilibrium (Days)	Liver	-	20	20	N.D.	N.D.
	Muscle	-	N.D.	N.D.	N.D.	N.D.
Tissue Concn. (mg/Kg. Dry Wt.) after 41 days	Liver	1.9	17	32	9	21
	Muscle	1.4	22	33	10	18
	Mean	1.6	20	32	10	20
Concentration Factors @ 41 days						
(a) Tissue (Wet Wt.)/Water		-	2.5	2.0	12	13
(b) Exposed/Control		-	12	20	6	12
Depuration						
Half Life (Days)	Liver		70	62	N.D.	N.D.
	Muscle		N.D.	41	56	N.D.
Total Experiment Duration (Days)		97	90	90	97	97

N.D. = Could not be determined

PHOTO COPY

BEST PAGE AVAILABLE

Table 6

Comparison of the Accumulation of Inorganic and Trialkyl Lead by Mussels

	Exposure Concentration (mg/l)								
	0.01			0.05			0.10		
	Inorganic*	Trimethyl	Triethyl	Inorganic*	Trimethyl	Triethyl	Inorganic*	Trimethyl	Triethyl
Tissue Concentration After Exposure for 7 days (mg/Kg. Dry Wt.)	10	10	9	50	25	29	110	68	48
Tissue Concentration After Exposure for 35 days (mg/Kg.Dry Wt.)	60	10	9	300	27	29	600	68	48
Concentration Factor @ 35 days (Tissue/Water)	6000	400	300	6000	420	460	6000	620	420
Half Life (days)	> 35	-	-	> 35	3	3	> 35	4	3

* Inorganic Data from Schulz-Bades (1974).

Table 7

Distribution of Lead in Marine Animals

(A) Mussels

Author	Concn. Range (mg/Kg. Dry Wt.)	Source
Alexander and Young, 1976	2.4 - 38	California
Chow et. al. 1976	0.2 - 42	"
MAFF. 1971, 1975	<3.5 - 35*	England and Wales
Nickless et. al 1972	1 - 30	Bristol Channel
Topping. 1973 A.	<1.4 - 38*	Scotland
This Study.	70 - 250	Short Term Exposure
	9 - 68	Long Term Exposure
MAFF. Recommended Limit (Anon, 1975)	35	

* Converted from Wet Wt. Data

BEST PAGE AVAILABLE

(B) Flatfish

Author	Species	Concn. Range (mg/Kg. Dry Wt.)	Source
Hardisty et al, 1974	Flounder	14 - 28	Bristol Channel
MAFF 1971, 1975	All Flat Fish	<1.2 - 29*	England and Wales
	All Flat Fish	2.4 - 7.2*	Greenland
Topping, 1973	Plaice	<1.4 - 5.6*	Scotland
This Study	Dabs	25 - 240	Short Term Exposure
	Dabs	10 - 32	Long Term Exposure
MAFF Recommended Limit (Anon, 1975)		14	

* Converted from Wet Wt. Data

PHOTO COPY

PAGE 0 1 79

REFERENCES

Alexander G.V., Young D.R.
Trace Metals in South Californian Mussels
Mar. Poll. Bull 7 (1976) 7-9

Anon
Notes on the handling of lead alkyls in the laboratory
Associated Octel Company Ltd., (1968a)

Anon
Oil and hazardous materials - Emergency procedures in the water environment
U.S. Dept., of Interior (1968b)

Anon
Water quality criteria. A report of the Committee of Water Quality Criteria for the United States Environment Protection Agency
National Acadamy of Science (1972a)

Anon
Lead in the environment and its significance to man
Dept., of Environment Pollution Paper No. 3
HMSO (1974)

Anon
International Symposium on Environmental Health Aspects of Lead (Amsterdam)
Commission of European Communities (1972b)

Anon
Food Additives and Contaminants Committee.
Review of the Lead in Food Regulations 1961
HMSO. (1975) FAC/REP/21

Bayne B.L.
Aspects of physiological condition of Mytilus edulis with special reference to the effects of oxygen tension and salinity
Proc. 9th. European Marine Biological Symposium (Aberdeen) (1975)

Bayne B.L., Thompson R.J.
Some physiological consequences of keeping Mytilus edulis in the laboratory
Helgolander Wiss. Meeresunters 20 (1970) 526-52

Berg A.
Lead in the aquatic environment
Euratom Report (1973) Eur. 5260

Bowen H.J.M.
Trace Elements in Biochemistry
Pub. Academic Press (1966)

Bowen H.J.M.
Comparative analysis of a standard plant material
Analyst 92 (1967) 124-131

Brown V.M.
The calculation of the acute toxicities of mixtures of poisons to Rainbow Trout
Water Research 2 (1968) 723-33

PAGE 0180

HOTO COPY

BEST PAGE AVAILABLE

Burkett R.D.
Influence of temperature on uptake of methyl mercury by Blunt Nose Minnows
Bull. Env. Cont. Toxicol. 12 (1974) 703

Cairns J.
Environment and time in fish toxicity
Ind. Wastes 2 (1957) 1-5

Calingaert G. et al
The decomposition of alkyl lead compounds
J. Am. Chem. Soc. 70 (1948) 3902-6

Carpenter K.E.
The lethal action of soluble metallic salts on fish
J. Exp. Biol. 4 (1927) 378

Chow T.J., Snyder H.G., Snyder C.B.
Mussels as an indicator of lead pollution
Science of the Total Environment 6 (1976) 55-63

Coombs T.L.
Metabolism and mechanism of uptake of heavy metals including lead by estuarine and freshwater invertebrates
EEC. Environmental Research Programme (1975) Contract 086/087-74/7 Env.U.K.

Cremer J.E.
Biochemical studies on the toxicity of tetraethyl lead and other organic lead compounds
Brit. J. Ind. Med. 16 (1959) 191

Cremer J.E., Callaway S.
Further studies on the toxicity of some tetra and trialkyl lead compounds
Brit. J. Ind. Med. 18 (1961) 277

Davies P.H., Goettl J.P., Sinley J.R., Smith N.F.
Acute and chronic toxicity of lead to Rainbow Trout in hard and soft water
Water Research 10 (1976) 199-206

Davis J.T., Hardcastle W.S.
Biological assay of herbicides for fish toxicity
Weeds 7 (1959) 397-404

De Treville R.T.P., Wheeler H.W., Sterling T.
Occupational exposure to organic lead compounds
Arch. Env. Health 5 (1962) 532

Douderoff P., et al
Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish
Sewage and Industrial Wastes 23 (1951) 1380-1397

PAGE 0181

PHOTO COPY

BEST PAGE AVAILABLE

FAO/WHO. Joint Expert Committee on Food Additives (1972)
WHO Tech. Report Series 505

Foyn B.
Lebenszyklus, Cytologie und Sexualitat der Chlorophycae
Arch. Rotistenk 83 (1934) 1-56

Fukai R.
Personal Communication (1976)
IAEA International Laboratory of Marine Radioactivity (Monaco)

Grove J.R.
Investigation into the formation and properties of aqueous solutions of lead alkyls
Proc. Int. Exp. Disc. Lead, Occurrence, Fate and Pollution in the Marine Environment (1977)

Hardisty M.W., Kartar S., Sainsbury M.
Dietary habits and heavy metal concentrations in fish from the Severn Estuary and the Bristol Channel
Mar. Poll. Bull. 5 (1974) 61-3

Jackim E.
Influence of lead and other metals on fish - aminolevulinate dehydrase activity
J. Fish. Res. Bd. Can. 30 (1973) 560-562

Jarvie A.W.P., Markall R.N., Potter H.R.
Chemical Alkylation of Lead
Nature (London) 255 (1975) 217

Johnston R.
Personal Communication
Dept. of Agriculture and Fisheries for Scotland
Marine Laboratory Aberdeen (1974)

Kauranen P., Jarvenpaa T.
Biological Half Times of 210-Po and 210-Pb in some Marine Organisms
Dept. of Radiochemistry. University of Helsink . Annual Report (1972)

Kopfler F.C.
Accumulation of organic and inorganic mercury compounds by the Eastern Oyster Crassostrea virginica
Bull. Env. Cont. Toxicol. 11 (1974) 275

Kozyura A.S., Smirnova A.N., Mirnaya A.P.
Study of the possible decomposition of tetra ethyl lead under natural conditions
Trudy Ob' Edineyii 3 (1961) 55-59

PAGE 0182

BEST PAGE AVAILABLE

PHOTO COPY

Krupinska I.
Influence of lead tetra ethyl on the growth of Funaria hygrometrica L. and Marchantia polymorpha L.
Acta. Soc. Botan. Pol. 45 (1976) 421

Litchfield J.T., Wilcoxon F.
A simplified method of evaluating dose effect experiments
J. Pharm. Exp. Ther. 96 (1949) 99-113

Macleod J.C., Pessah E.
Temperature effects of mercury accumulation toxicity and metabolic rate in Rainbow Trout
J. Fish. Res. Bd. Can. 30 (1973) 485-492

MAFF.
Working Party on the Monitoring of Foodstuffs for Heavy Metals - Second Report.
Survey of Lead in Food
HMSO (1972)

MAFF
Working Party on the Monitoring of Foodstuffs for Heavy Metals - Fifth Report.
Survey of Lead in Food - First Supplementary Report
HMSO (1975)

Magistretti M., Zurio N., Scolla F., Pacillo D.
A comparative study of the toxicity of tetraethyl and tetramethyl lead
Med. Lav. 54 (1963) 486

Majori L., Petronio F.
Accumulation phenomena which takes place in a mussel (Mytilus gallo provincialis) grown in an artificially polluted environment
Ig. Mod. 66 (1973) 79-98

McKim J.M., Benoit D.A., Biesinger K.E., Brungs W.A., Siefert R.E.
Effects of pollution on freshwater fish
J. Wat. Poll. Cont. Fed. 47 (1975) 1711

McKone C.E.
Rapid uptake of mercuric ion by goldfish
Env. Sci. Technol. 5 (1971) 1138

Merlini M., Pozzi G.
Lead and freshwater fish
I. Lead accumulation and water pH
Env. Poll. 12 (1977a) 167-172

Merlini M., Pozzi G.
Lead and freshwater fish
2 Ionic lead accumulation
Env. Poll. 13 (1977b) 119-126

PAGE 0183

BEST PAGE AVAILABLE

PHOTO COPY

177

Nickless G., Stenner R., Terille N.
Distribution of Cadmium, Lead and Zinc in the Bristol Channel
Mar. Poll. Bull. 3 (1972) 188-190

Noden F.G.
The determination of tetra alkyl lead compounds and their degradation products in natural waters
Proc. Int. Exp. Disc. Lead, Occurrence, Fate and Pollution in the Marine Environment (1977)

Pickering Q.H., Henderson C.
The acute toxicity of some heavy metals to different species of warm water fishes
J. Int. Air. Water. Poll. 10 (1966) 453-463

Portmann J.E., Wilson K.W.
The toxicity of 140 substances to Brown Shrimp and other marine animals, MAFF Shellfish Information Leaflet 22.HMSO (1971)

Roberts D.
The assimilation and chronic effects of sub lethal concentrations of endosulfan on condition and spawning in the common mussel (Mytilus edulis)
Mar. Biol. 16 (1972) 119-125

Schepers G.W.H.
Tetraethyl Lead and Tetramethyl Lead
Arch. Env. Health 8 (1964) 277

Schmidt U., Huber F.
Methylation of organo lead and lead (11) compounds to $(CH_3)_4Pb$ by micro-organisms
Nature (London) 259 (1976) 157-8

Schulz-Baldes M.
Toxizitat und. anreicherung von blei bei der miesmuchel Mytilus edulis im laborexperiment
Mar. Biol. 16 (1972) 226-229

Schulz-Baldes M.
Lead uptake from seawater and food, and lead loss in the common mussel Mytilus edulis
Mar. Biol. 25 (1974) 177-193

Siegel S.M. et al
The general and comparative biology of toxic metals and their derivatives : Mercury and Lead
In Mercury in the Western Environment (1971)
Fitz Dahler D.R.

PHOTO COPY

BEST PAGE AVAILABLE

PAGE 0184

Silverberg B.A., Wong P.T.S., Chau Y.K.
Effects of tetramethyl lead on freshwater green algae
Arch. Env. Contam. Toxicol. 5 (1977) 305-13

Sirota G.R., Uthe J.F.
Determination of tetra alkyl lead compounds in biological materials
Anal. Chem. 49 (1977) 823

Sprague J.B.
Measurement of Pollutant Toxicity to Fish Part III
Sub lethal Effects and "Safe" Concentrations
Water Research 5 (1971) 245-266

Steeman-Nielsen E.
The use of radioactive carbon (C^{14}) for the measurement of organic productivity in the sea
J. Du. Conseil. 18 (1952) 117

Taylor D.
Some investigations into the effect of 1.2.Dichloroethane (EDC) on marine life
Brixham Laboratory Report (1974) BL/B/1571

Thompson R.S.
Personal Communication (1976)
I.C.I. Brixham Laboratory

Topping G.
Heavy metals in shellfish from scottish waters
Aquaculture 1 (1973a) 379-384

Topping G.
Heavy metals in fish from scottish waters
Aquaculture 1 (1973b) 373-77

Turnball H., De Mann J.G., Weston R.F.
Toxicity of various refinery materials to freshwater fish
Ind. Eng. Chem. 46 (1954) 324

Unlu M.Y., Heyraud M., Keckes S.
Mercury as a hydrospheric pollutant
1. Accumulation and excretion of $HgCl_2$ in Tapes decussatus
FAO. Poll. Conf. (Rome) (1970) FIR/MP/70.E68

Vinogradov A.P.
The elementary composition of marine organisms
Sears Foundation. Mar. Res. Men. (1953) 647pp

[illegible] B.A.
[illegible] 24 (1945) 283

[illegible] P.T.S., Chau Y.K., Lukon P.L.
[illegible] of lead in the environment
[illegible] (London) 253 (1975) 263

PAGE 0185

PHOTO COPY

BEST PAGE AVAILABLE

Appendix B

88-7800249

J. ENVIRON. SCI. HEALTH, A14(2), 65-85 (1979)

Notice: This material may be protected by copyright law (Title 17 U. S. Code)

STUDIES OF INTERACTIONS BETWEEN TETRAETHYL LEAD AND SEA WATER USING GC-AA

by

J.W. Robinson, E.L. Kiesel and I.A.L. Rhodes
Chemistry Department
Louisiana State University
Baton Rouge, Louisiana 70803

A. INTRODUCTION

It is becoming increasingly clear that the health effects of various heavy metals are dependent, not only on their concentrations, but on their chemical forms. For example, some forms of chromium are known to be carcinogenic while other forms are known to be innocuous. When assessing the projected health effects of various metals, it is therefore important to identify the chemical forms of those metals and if possible, to predict their chemical and physical state in any particular environmental system such as air, water, sea water, etc. Elemental analysis, such as the determination of the total concentration of lead or mercury in the sample provides only part of the information needed to make reliable judgements of the toxicity of the metals. The chemical form has a direct bearing on toxicity. For this reason, speciation studies are of extreme importance.

Even with the increasing use of nonleaded gasoline, tetraethyl lead (TEL) is still of importance as an antiknock agent. Several studies have shown that TEL is toxic. Once introduced into a biological system, TEL is quickly converted by the liver to tri-

Copyright © 1979 by Marcel Dekker, Inc. All Rights Reserved. Neither this work nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

82

ethyl chloro lead ($PbEt_3Cl$) which is very soluble in water (> 2 g/100 ml of water) and known to attack the central nervous system by inhibiting respiration by brain cells. Symptoms produced by dichlorodiethyl lead ($PbEt_2Cl_2$) and inorganic lead salts are completely different from those produced by TEL and $PbEt_3Cl$. Studies involving TEL and $PbEt_3Cl$ with brain slices have shown that $PbEt_3Cl$ is about 100 times more toxic than TEL.[1,2,3] It is therefore of great importance to know whether TEL converts to $PbEt_3Cl$ in a given system at a given time.

Most methods reported in the literature deal with either total lead determination,[4] or the determination of TEL, $PbEt_3Cl$ and diethyldichloro lead by dithizone,[1] the iodine monochloride[5] or the 4-2 pyrydylazo resorcinol (PAR)[6] methods. These methods are time consuming and subject to error. However, GC-AA and GC-MS systems hold the potential of studying the speciation of lead alkyls without prior separation.[7,8,9,10] This paper describes the development and application of such a combination.

In a recent event in the Adriatic Sea, a ship sank with large amounts of TEL as its cargo. Speculations regarding the fate of the TEL and its environmental effects ranged from 'no effect' to 'will destroy the floor of the Mediterranean.' Several important questions that arose include solubility values of TEL in sea water, dissipation of TEL through water and into the atmosphere, possible reaction products of TEL with sea water and their toxicities, etc.

In order to answer these questions, several studies have been undertaken regarding the interactions of TEL with sea water.[11,12] TEL is known to produce $PbEt_3Cl$ in sea water as well as in blood. $PbEt_3Cl$ seems to be stable under experimental conditions.

A GC-AA system previously developed in this lab for the determination of lead alkyls in gasoline[7] permitted the determination of both TEL and $PbEt_3Cl$ directly with no sample preparation of any kind.

PAGE 0008

This present study involves the estimation of short term and long term solubility of TEL in several samples of sea water and the formation and accumulation of $PbEt_3Cl$ with time. A GC-MS system was used for positive identification of $PbEt_3Cl$.

This is a distinct advantage of other methods because it is free from contamination errors or loss from inefficient extraction and provides simultaneous analyses of the lead compounds in the same sample.

Since there are many points of similarity in the chemical composition of sea water and blood, some of the observations made in the TEL sea water studies may be pertinent to the fate of lead alkyls which find their way into the blood stream after inhaling polluted air.

B. EXPERIMENTAL:

1. Equipment:

a. GC-AA System: (See Figure 1)

i. HCL Power Supply
ii. Pressure Gauge for Demountable HCL
iii. Microtek GC-2000-R Gas Chromatograph
iv. Mechanical Chopper
v. Atomic Absorption Detector
vi. Varian - Techtron type M1 (SI-RO-SPEC) Grating Monochomator with Hammatsu Model R-106 Photomultiplier
vii. Hewlet-Packard Model 6515A High Voltage Power Supply
viii. Heathkit Photometric Readout Amplifier Model Ev-703-31
ix. Sargent Model XK Recorder with Variable Range Attachment Power to Atomic Absorption Detector
x. Hollow Cathode Lamp (Designed and built in this lab)
xi. Hamilton 0-10 Microliter Syringe
xii. Precision Sampling Inc. 0-10cc Gas Syringe

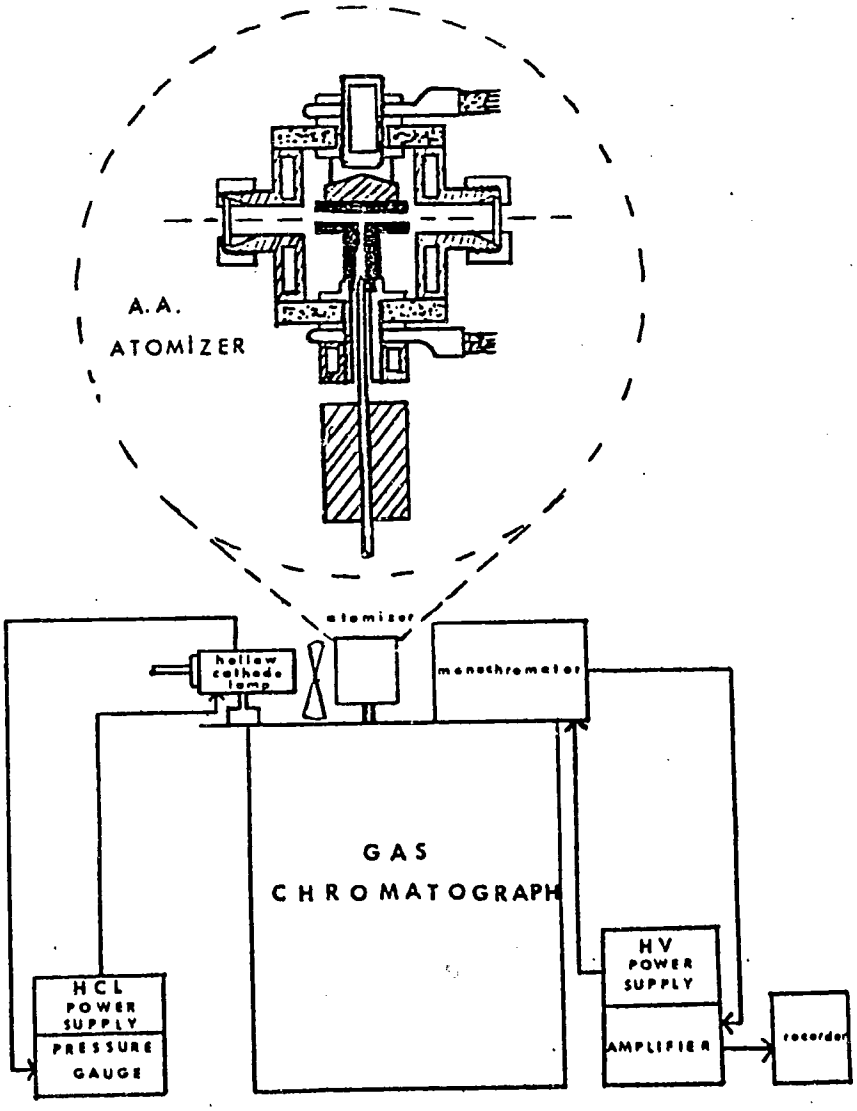


Figure 1: Schematic diagram of GC-AA system with enlarged section of AA atomizer.

85

b. GC Operating Conditions:

i. Column: $^1/_8$" Diameter Teflon Column 18" long. Packed with 20% Ucon Non-Polar on Chromosorb P (Microter Inc.)
ii. Carrier Gas: Argon (~60 cc/min.)
iii. Column Temperature: 140°C
iv. Injection Port Temperature: 150°C
v. Transfer Line Temperature: 140°C

c. AA Operating Conditions:

i. Lamp Current: 35 mA
ii. High Voltage on PM.Tube: 400 V D.C.
iii. Slit Width: 25 μm
iv. Atomizer Temperature: ~1500°C

d. GC-MS System:

i. Hewlett-Packard GC-MS Computer System 5985-A
ii. GC conditions same as in GC-AA

e. Miscellaneous:

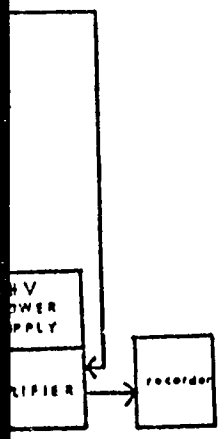
i. 50 ml Erlenmeyer Flasks Equiped with inert valves (Figure 2)
ii. ~ 9 ml contrifuge tubes with tops
iii. E.H. Sargent and Co. centrifuge (bench top)
iv. Orbit Water Bath Shaker
v. Grey Silicone Septums; $^1/_2$ " diameter (Tek Lab)

2. Chemicals:

a. Sea Water: Collected from different places in the Gulf of Mexico
b. TEL
c. Heptane
d. Artificial Sea Water:

. Deoxygenated Deionized Water (1 liter):

. Na^+ as NaCl	.456	mmoles/l
. Mg^{++} as $MgCl_2$, $MgSO_4$	.054	"
. K^+ as KCl	.01	"
. Ca^{++} as $CaCl_2$	.01	"



system with
er.

86

. Cl^- as NaCl, $MgCl_2$, KCl, $CaCl_2$ .543 "
. $SO_4^=$ as $MgSO_4$ .028 "

3. Experimental Procedure - Several studies were undertaken as follows:

I. Vapor Study - A 25 cc sample of sea water was placed in a 50 ml Erlenmeyer flask and 200 µl of TEL were added. The flask was stoppered with a specially designed Teflon valve to prevent air leaks, as shown in Figure 2, and covered with aluminum foil in order to exclude light. 1 ml samples of vapor over the liquid were run in the GC and compared with 1 µl aliquot of standards of dilutions of TEL in heptane.

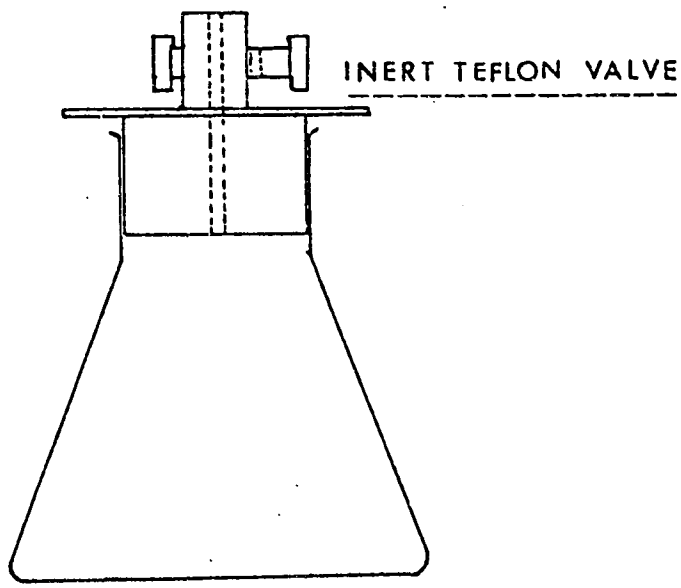


Figure 2: Schematic diagram of reaction vessel with specially designed Teflon valve.

II. Interaction between TEL and Sea Water - Samples composed of artificial and natural sea water were prepared using the setup described in Figure 2. The specially designed valves were again used this time to stopper small bottles holding about 33 ml filled to the top to eliminate any interaction with a vapor phase. To about 33 ml of water, 200 µl of TEL were added. These samples were shaken thoroughly and allowed to settle for at least two months. A typical GC trace of these samples is shown in Figure 3. These samples were periodically checked using the GC-AA system and GC-MS in order to determine the identity of the second unknown peak.

III. Short term solubility of TEL in sea water with no excess of TEL present - Different samples were prepared by mixing 100 ml of sea water with 100 µl of TEL at room temperature. Samples were shaken thoroughly and then allowed to stand until clear. The supernatant solution was then separated from the excess TEL and placed in containers covered with aluminum foil. The solutions were analyzed for TEL concentration by GC-AA using 5 µl samples and compared to TEL standards in heptane.

IV. Short term solubility of TEL in sea water and formation of $PbEt_3Cl$ in presence of an excess of TEL - Samples were prepared by completely filling centrifuge tubes (~9 ml) with different sea waters. A hole was drilled in the plastic top and a silicon septum covered with Teflon was placed between the tube and the top to provide a good seal. The tubes held approximately 9 ml of sea water and about 60 µl of TEL were added to each. All four samples were then shaken for 24 hours in a circular shaker and centrifuged for 5 minutes.

V. Long term solubility of TEL in sea water and formation of $PbEt_3Cl$ in presence of an excess of TEL - Samples were prepared as in IV. They were checked periodically by GC-AA. Samples were manually shaken daily for 10 minutes and centrifuged for 1 minute prior to determination.

DE0093

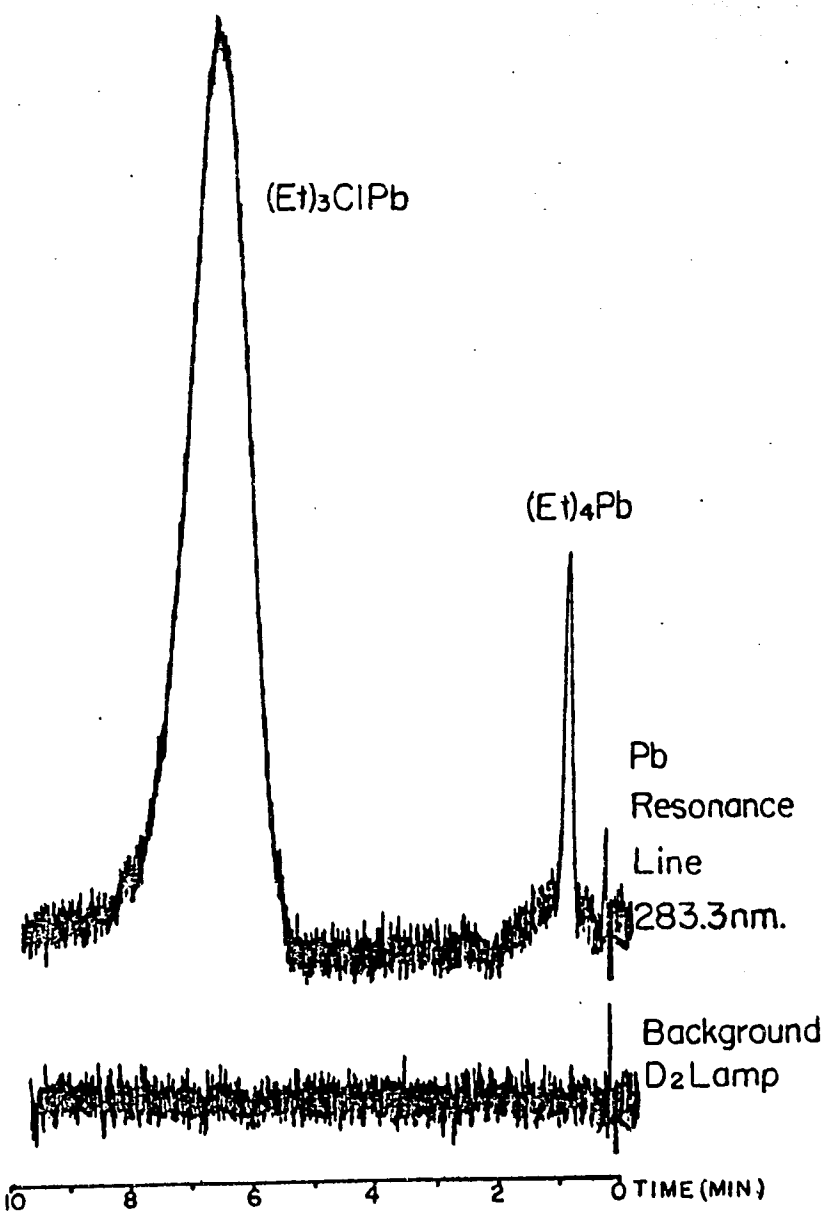


Figure 3: Typical GC-AA trace of a sample of $(Et)_4Pb$ in sea water.

89

VI. Long term solubility of TEL in sea water and formation of $PbEt_3Cl$ in light excluded systems - The samples used in the GC-MS identification of triethylchlorolead (part B) were monitored by GC-AA periodically in order to determine long term effects in stationary, light-tight systems.

VII. Equilibrium between TEL and $PbEt_3Cl$ in sea water with no excess of TEL present - Two samples were prepared by mixing approximately 9 ml of sea water with 60 µl of TEL in centrifuge tubes. The samples were vigorously mixed for 20 minutes and then centrifuged for 15 minutes. After forty days the supernatant sea water was separated from the excess TEL and transferred to clean centrifuge tubes. A third sample was prepared the same way, but the excess TEL was removed after two hours of contact. All samples were analyzed periodically for TEL and $PbEt_3Cl$ concentration. The change in concentration for periods up to twenty-four days was noted.

C. RESULTS AND DISCUSSION:

I. Vapor study - The concentration in the gas phase above the TEL - sea water solution was approximately 430 µg of Pb as TEL per cc of gas. This is an unexpectedly high concentration and indicated that TEL diffused very rapidly through sea water in an open system. In practice this may provide a mechanism for TEL to leave the sea water and enter the atmosphere above within a short time. The fate of the TEL in the atmosphere is not clear, but it is known that TEL is sensitive to radiation from the sun and would probably decompose.

II. Interaction between TEL and sea water - Figure 3 shows the GC-AA trace of a sample of TEL in sea water. Two lead containing compounds are clearly indicated. GC-MS studies of several samples clearly indicate that this second peak was triethylchorolead. Figures 4 and 5 support the identity of this species. Triethylchorolead is a solid at room temperature, very soluble in

Pb
Resonance
Line
283.3nm.

Background
D_2 Lamp

TIME (MIN)

of $(Et)_4Pb$

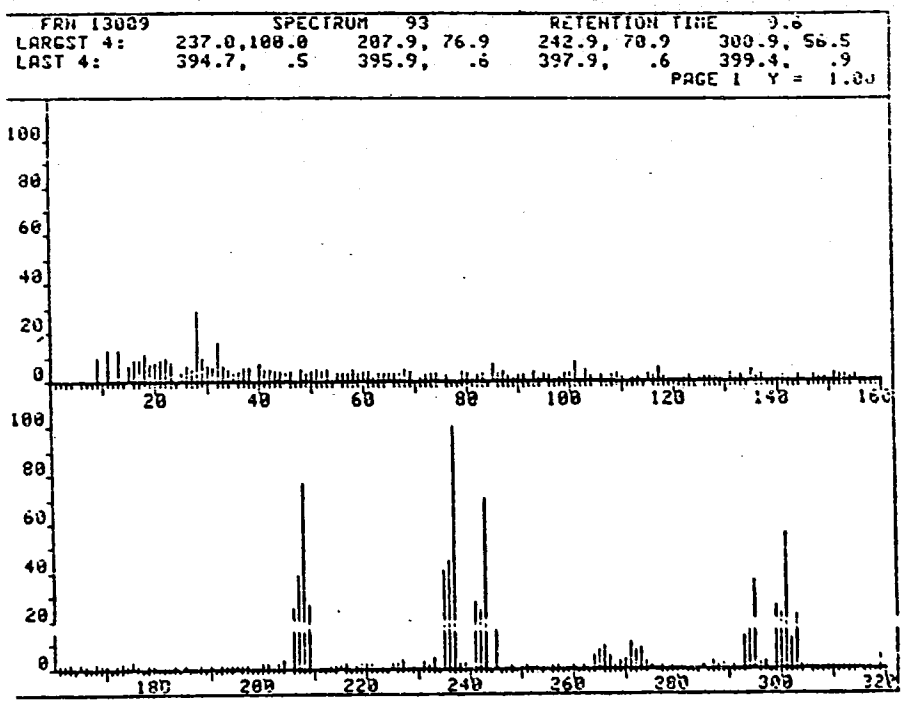
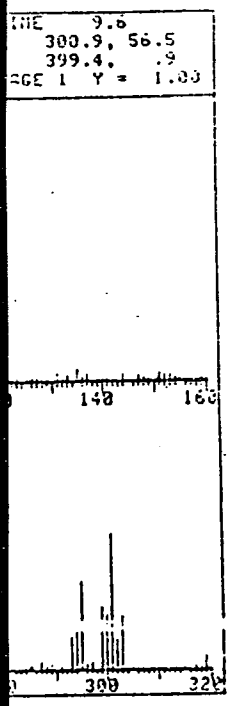


Figure 4: MS of the second peak observed in the GC-AA trace of a sample of $(Et)_4Pb$ in sea water: $(Et)_3ClPb$.

water and is known to be more toxic than TEL. Further, the fact that $PbEt_3Cl$ eluted through the column indicated that it is somewhat volatile at higher temperature and organic in nature, thus supporting the MS identification. The concentration of this species in sea water increased with time.

III. Short term solubility of TEL in sea water with no excess of TEL present - Table I lists results obtained from this experiment. All samples were allowed to stand for two days prior to determination unless otherwise indicated.



erved in the
f $(Et)_4Pb$ in

er, the fact
t it is
in nature,
ration of

ater with
tained from
for two days

MASS	ABUND	
190	.9	
190	1.0	
191	1.3	
192	.8	
194	1.0	
195	.9	
196	1.7	
197	1.1	
199	.4	
200	3.6	
202	.9	
203	2.7	
204	4.2	
206	25.5	Pb_{206}
207	35.2	Pb_{207}
208	78.8	Pb_{208}
209	30.7	
210	1.2	
211	.8	
212	1.6	
213	.6	
214	1.6	
216	.6	
217	3.2	
218	1.8	

MASS	ABUND	
219	2.4	
220	2.2	
221	1.3	
222	1.0	
222	1.2	
223	.8	
224	.6	
225	2.4	
227	1.9	
228	.9	
229	.6	
230	1.1	
231	1.7	
232	1.0	
233	4.7	
234	.8	
235	41.7	
236	46.0	
237	100.0	PbEt
238	2.4	
239	1.0	
241	27.0	
242	25.0	
243	63.3	$PbCl_{35}$
244	9.5	

MASS	ABUND	
245	21.2	$PbCl_{37}$
246	1.1	
247	1.1	
248	.7	
249	.5	
250	.6	
250	.6	
251	.7	
251	1.2	
253	.9	
254	1.0	
254	.7	
255	1.1	
256	.8	
257	1.1	
259	1.6	
260	1.1	
261	.9	
261	.8	
263	1.6	
264	8.1	
265	8.8	
266	10.7	$Pb(Et)_2$
269	2.8	
270	2.9	
271	8.3	

MASS	ABUND	
272	9.0	$PbCl_{35}Et$
273	7.7	
274	4.8	$PbCl_{37}Et$
275	1.4	
275	.9	
276	.9	
277	.9	
278	.8	
279	1.3	
280	.4	
281	.8	
282	.6	
282	1.3	
283	.6	
284	.8	
284	.9	
285	1.1	
286	1.1	
287	2.0	
288	2.0	
289	1.3	
291	1.7	
292	.5	
293	14.7	
294	13.6	
295	32.6	$Pb(Et)_3$

MASS	ABUND	
296	2.1	
297	2.2	
299	24.4	
300	29.6	
301	61.4	$PbCl_{35}(Et)_2$
302	10.3	
303	16.4	$PbCl_{37}(Et)_2$
304	1.4	
305	.4	
306	.5	
308	.4	
309	1.0	
310	.6	
311	.6	
311	.4	
311	.5	
313	1.3	
315	.6	
315	.7	
316	.6	
317	.6	
319	5.4	
320	6.3	
321	9.9	
322	1.1	

MASS	ABUND
323	.5
324	1.0
325	.6
326	.6
327	.4
328	1.4
329	1.4
330	2.5
331	1.5
332	.6
332	.7
333	.9
333	.7
335	.8
336	.4
337	.7
338	.4
339	.6
341	.5
342	.4
343	1.5
344	.6
345	2.7
347	1.3
348	1.0

Figure 5: MS tabulation of Figure 4 & $(Et)_3ClPb$.

92

TABLE I

Sample Source	Concentration of TEL After two days	
1. Artificial Sea Water No. 1 (deoxygenated)	1.3	µg/ml
2. Artificial Sea Water No. 2	1.3	µg/ml
3. Natural Sea Water No. 1 (filtered)	1.2	µg/ml
4. Natural Sea Water No. 2 (filtered)	0.8	µg/ml
5. Deoxygenated Sea Water	3.5	µg/ml
6. Artificial Sea Water No. 1 (after 16 days)	4	µg/ml
7. Artificial Sea Water No. 2 (after 16 days)	3.1	µg/ml
8. Natural Sea Water No. 1 (after 16 days)	2	µg/ml
9. Natural Sea Water No. 2 (after 16 days)	3	µg/ml

These values reported are average values of at least three runs. On several occasions significantly higher data were obtained probably due to inadvertent trapping of pockets of TEL emulsion suspended in the sample analyzed. The values indicate that if excess TEL is removed from the system, the solubility of TEL in water is only a few µg/ml. Errors in this determination are possible. The TEL may exist as a suspension and should be centrifuged prior to analysis. The solubility seemed to increase with time and depended somewhat on the nature of the water used.

IV. Short term solubility study of TEL in sea water and formation of $PbEt_3Cl$ in presence of excess TEL - In the oceans, sea water is in constant motion. It was therefore thought to be of importance to find the solubility of TEL in a constant motion

system when an excess of TEL is present. It was observed that $PbEt_3Cl$ was always formed in the samples. Samples of 3 µl were injected into the GC-AA system and calculated with respect to 3 µl injections of a TEL standard in heptane. Table II shows the results of this study. Triplicate measurements were done.

Boiling and filtration of the sea water was carried out in order to avoid analytical errors caused by bacterial decomposition or foreign bodies. TEL remained at the bottom of the containers in well defined "spheres" and if no air gaps were present, it seemed to dissolve slowly. Formation of $PbEt_3Cl$ was very rapid and it seemed to accumulate. Since all samples were centrifuged prior to analysis, emulsions were reduced and the results were fairly reproducible.

GC studies with TEL in heptane show that $PbEt_3Cl$ was not generated by a reaction of TEL in the GC column, and it was not present as an impurity in TEL but it was formed when TEL was brought in contact with sea water and that its concentration increased with time.

This study also showed that the solubility of TEL in sea water was slightly related to the nature of the sea water used in the analysis. Impurities present in sea water may increase the

TABLE II

	Sample Source	µg TEL/ml Sample	µg $PbEt_3Cl$/ml Sample
1.	Sea Water (boiled and filtered) Panama City, Fla.	11.6	173
2.	Sea Water (boiled and filtered) Panama City, Fla.	16.3	146
3.	Sea Water (boiled and filtered) Destin, Fla.	9.7	185
4.	Sea Water (boiled and filtered) Destin, Fla.	12.0	208

rate of TEL conversion to $PbEt_3Cl$ and thus cause different solubility values for different sea waters. However, overall the reaction rates in sea water were very similar.

V. Long term solubility of TEL in sea water and formation of $PbEt_3Cl$ in the presence of excess of TEL - This study is similar to IV but samples were followed for a period of about two months and shaken daily. Light was not excluded from the TEL - sea water system and TEL seemed to remain at the bottom of the sealed centrifuge tube with no excessive signs of decomposition (some brown spots were observed). Since samples were centrifuged prior to analysis, there was no apparent sign of an emulsion present when sampling took place and the results are due solely to dissolved TEL and $PbEt_3Cl$. Both TEL and $PbEt_3Cl$ concentrations increased with time as can be observed in Figure 6. This study clearly indicated that as long as there was an excess of TEL at the bottom of a constantly moving natural water system the concentration of TEL in sea water increased only slowly with time. But $PbEt_3Cl$, a much more dangerous compound, increased more rapidly.

VI. Long term solubility and stability of TEL and formation of TEL in light excluded systems - Samples used for study B were analyzed periodically for TEL and $PbEt_3Cl$. These were light excluded, undisturbed samples with an excess of TEL at the bottom of the containers. After about 200 days, TEL was still present as a separate phase at the bottom of the container. Concentrations of TEL and $PbEt_3Cl$ with time are shown in Figure 7. These values were somewhat scattered probably due to traces of TEL emulsion. It is clearly indicated that TEL did not convert to less harmful inorganic lead compounds in a short period of time but, in the presence of an excess, its solubility may slowly increase with time with simultaneous formation and accumulation of the much more stable $PbEt_3Cl$. Because there was an excess of TEL in a separate phase, any TEL that converted to $PbEt_3Cl$ was quickly replaced and a dynamic system was established.

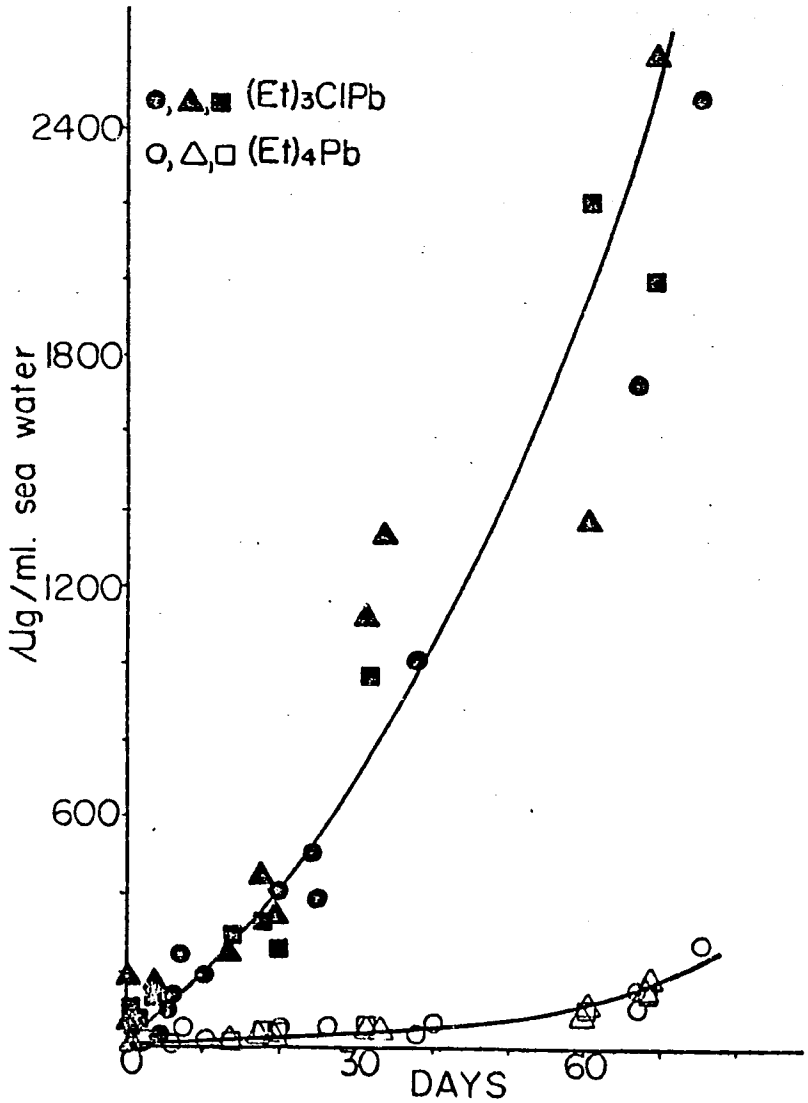


Figure 6: Solubilty of $(Et)_4Pb$ and formation of $(Et)_3ClPb$ in sea water with respect to time.
○, ● sea water, boiled. Panama City, Fla.
△, ▲ sea water, filtered. Panama City, Fla.
□, ■ sea water, filtered. Destin, Fla.

96

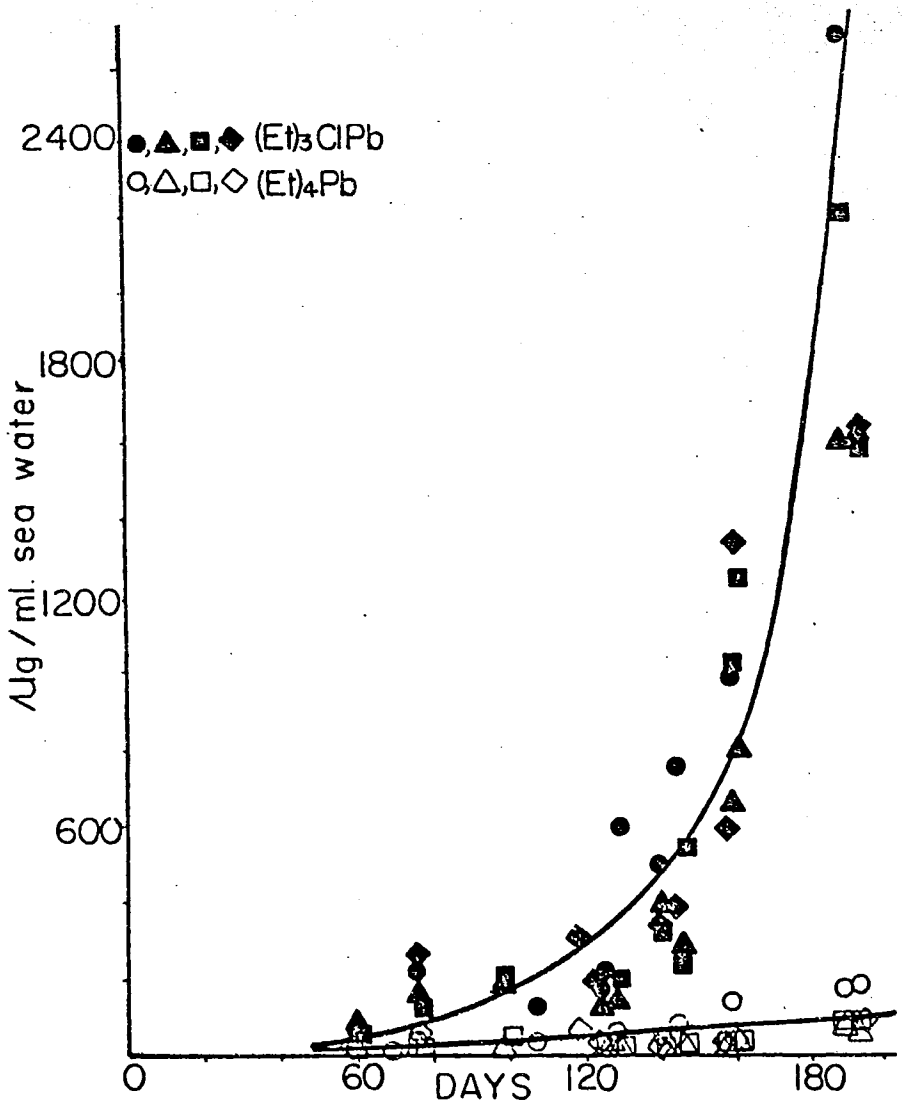
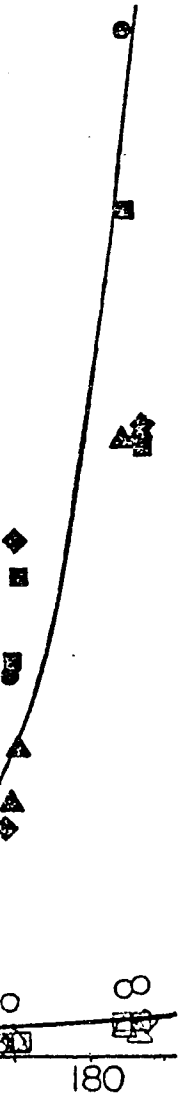


Figure 7: Solubility of $(Et)_4Pb$ and formation of $(Et)_3ClPb$ in sea water with respect to time.
○ ● artificial sea water, deoxygenated.
△ ▲ sea water, filtered.
□ ■ sea water, filtered.
◇ ◆ sea water, deoxygenated.

97



of $(Et)_3ClPb$
e.

VII. Equilibrium between TEL and $PbEt_3Cl$ in sea water with no excess TEL present - Results have demonstrated that TEL, once in contact with sea water, quickly forms $PbEt_3Cl$. It was thought to be of interest to establish (a) if TEL completely converted to $PbEt_3Cl$ and (b) how fast the reaction took place. Since there was no excess of TEL present to replenish any TEL loss due to formation of $PbEt_3Cl$, the disappearance of TEL from solution with time would indicate complete conversion to $PbEt_3Cl$. Figure 8 shows the results obtained.

The time taken to centrifuge off the excess TEL and determine the $PbEt_3Cl$ and TEL was about one hour. The concentration of $PbEt_3Cl$ in the first two samples was about 600 ppm and 900 ppm and the TEL was 20 ppm and 24 ppm. On prolonged standing the TEL concentration remained fairly constant but the $PbEt_3Cl$ seemed to decrease. The results indicated an equilibrium between these two compounds rather then a complete reaction of the TEL. The loss of $PbEt_3Cl$ may have been caused by reaction to $PbEt_2Cl_2$ - which we did not measure, or by adsorption on the containing vessel.

The concentration of $PbEt_3Cl$ in the third sample was considerably lower (320 ppm) perhaps because of the lower exposure time. In contrast to the first two samples, it did not seem to decrease in time. The TEL concentration behaved in the same manner as that observed in samples 1 and 2 again indicating an equilibrium between TEL and $PbEt_3Cl$.

Calingaert[13,14] reported that $PbEt_3Cl$ thermally decomposes by the following mechanisms:

$$3\ PbEt_3Cl \rightleftharpoons 2\ PbEt_4 + PbCl_2 + EtCl \quad 66\%$$

$$2\ PbEt_3Cl \rightleftharpoons Et_4Pb + PbCl_2 + 2\ Et \quad 34\%$$

These mechanisms indicate that even if all of the TEL in solution, temporarily formed $PbEt_3Cl$, TEL would be formed again. The other possible route of decomposition of $PbEt_3Cl$ is to form $PbEt_2Cl_2$. This would indicate a decrease in $PbEt_3Cl$ concentration with time as observed in Figure 8 for samples 1 and 2. Calingaert[13,14] also

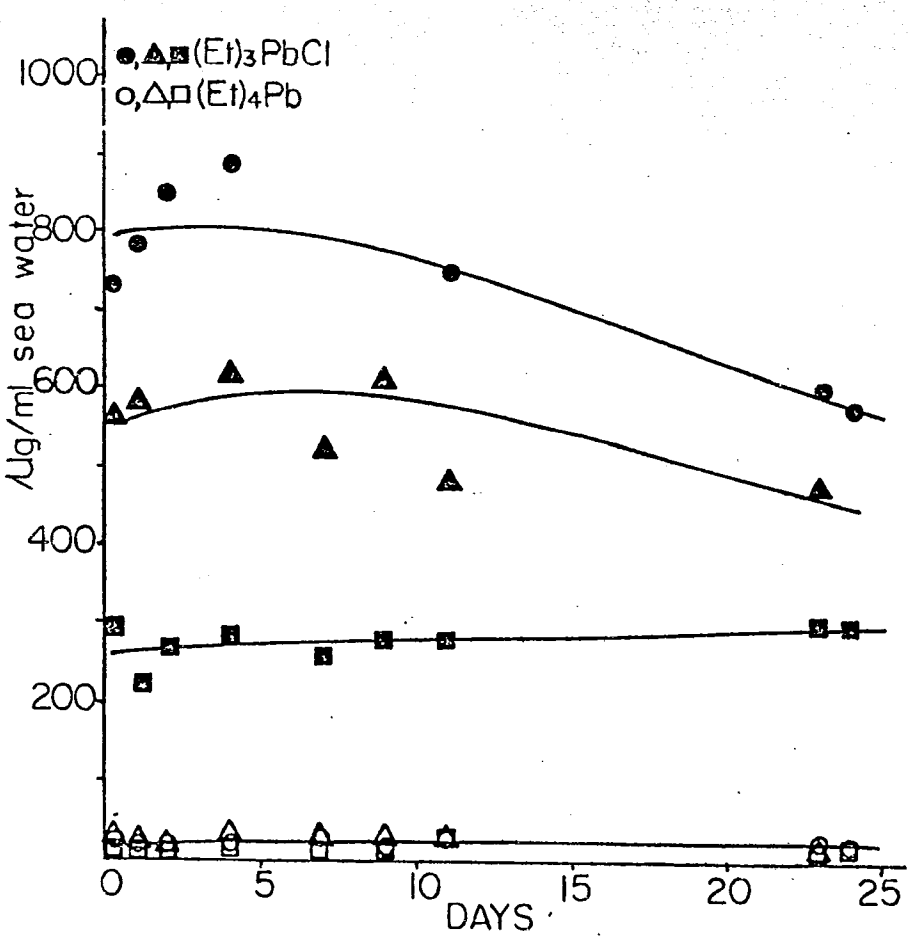


Figure 8: Equilibrium between $(Et)_4Pb$ and $(Et)_3PbCl$ in sea water with respect to time.
○ ● sample 1: sea water, boiled and filtered.
△ ▲ sample 2: sea water, boiled and filtered.
□ ■ sample 3: sea water, boiled and filtered.

reported that $PbEt_2Cl_2$ thermally decomposes by the following mechanisms:

$$2\ PbEt_2Cl_2 \rightleftharpoons PbEt_3Cl + PbCl_2 + EtCl \quad 67\%$$

$$PbEt_2Cl_2 \rightleftharpoons PbCl_2 + C_4H_4 \quad 33\%$$

99

If $PbEt_3Cl$ forms $PbEt_2Cl_2$, the latter in turn may form some $PbEt_3Cl$ again, but this process is very slow. Although $PbEt_2Cl_2$ cannot be determined by the method proposed in this paper, it is certain that all three forms exist in equilibrium. The results obtained for sample 3 indicate basically no change in $PbEt_3Cl$ concentration. The mechanism of decomposition for $PbEt_3Cl$ to $PbEt_2Cl_2$ is probably much slower at lower concentrations that at higher concentrations of $PbEt_3Cl$. Further studies with these equilibrium systems need to be performed in order to clarify these results.

D. CONCLUSION:

The solubility of TEL in sea water was found to be only slightly dependent on the type of water and to increase with time at rates dependent upon the experimental conditions. Results suggest that in practice, TEL lies at the bottom of the sea bed in a separate liquid phase, dissolving into the sea water. Some of the TEL may rapidly migrate to the surface and evaporate into the atmosphere. A large amount of it goes into formation of the very soluble $PbEt_3Cl$ which accumulates steadily. Studies of TEL and $PbEt_3Cl$ in sea water with no excess TEL present indicated an equilibrium between these compounds with the $PbEt_3Cl$ being predominant. There was evidence of further reaction or loss of $PbEt_3Cl$, perhaps to $PbEt_2Cl_2$, but this was not studied.

The method of analyses described here was simple, rapid and allowed direct separation and determination of both TEL and $PbEt_3Cl$, a valuable improvement over existing methods of analysis of these compounds which require time consuming wet chemical procedures. This method has a detection limit of about 1 ug TEL/ml of sample and an uncertainty of $\pm$ 10%. If necessary, the detection limit could be improved an order of magnitude, by optimizing the GC-AA conditions, but under the conditions of these studies, the refinements were not necessary.

The results were informative, but clearly called for a more vigorous study, which we propose to pursue.

References:

1. Cremer, J.E., British Journal of Industrial Medicine, 16, 191-199 (1959).

2. Millar, J.A., Thompson, G.G., Goldberg, A., Barry, P.S.I., Lowe, E.H., British Journal of Industrial Medicine, 29, 317-320 (1972).

3. Maddock, B.G., Taylor, D.,"The Acute Toxicity and Bioaccumulation of Some Lead Alkyl Compounds in Marine Animals," presented at the International Experts Discussion Meeting on: Lead - Occurrence, Fate and Pollution in the Marine Environment. Rovinj, Yugoslavia - October 18-22, 1977.

4. Sirota, G.R., Uthe, J.F., Analytical Chemistry, Vol. 49, No. 6, 823-824 (1977).

5. Moss, R., Browet, E.V., Analyst, 91, p. 428 (1966).

6. Pilloni, P., Plazzogna, G., Analytica Chimica Acta, 35, p. 325 (1966).

7. Robinson, J.W., Vidaurreta, L.E., Wolcott, D.K., Goodbread, J.P., Kiesel, E., Spectroscopy Letters, 8(7), 491-507 (1975).

8. Coker, D.T., Analytical Chemistry, Vol. 47, No. 3, 386-390 (1975).

9. Chav, Y.K., Wong, P.T.S., Saitoh, H., Journal of Chromatographic Science, Vol. 14, 162-164 (1976).

10. Howard, H.E., Ferguson, W.C., Snyder, L.R., Analytical Chemistry, Vol. 32, 1814-1815 (1960).

11. Noden, F.G., "The Determination of tetraalkyllead Compounds and their Degradation Products in Natural Water," presented at the International Experts Discussion Meeting on: Lead - Occurrence, Fate and Pollution in the Marine Environment. Rovinj, Yugoslavia, October 18-22, 1977.

12. Grove, J.R., "Investigations into the Formation and Behavior of Aqueous Solutions of Lead Alkyls," presented at the International

Experts Discussion Meeting on: Lead - Occurrence, Fate and Pollution in the Marine Environment. Rovinj, Yugoslavia, October 18-22, 1977.

13. Calingaert, G., Hymin, S., Dykstra, F.J. and Hess, L., Journal of the American Chemical Society, 70, 3902-3906 (1948).

14. Calingaert, G., Chemical Reviews, 2, 43-83 (1925).