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The determination of tetra-alkyllead and ionic alkyllead compounds in seafood

D S Forsyth* and J R Iyengar
Food Research Division, Bureau of Chemical Safety, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada K1A OL2

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Extraction methodologies were developed for tetra-alkyllead and ionic alkyllead compounds in seafood. Tetra-alkylleads were extracted with n-hexane after the samples had been enzymatically hydrolyzed. The ionic alkylleads were complexed with diphenylthiocarbazone (dithizone) at pH 8 and 9 from enzymatically hydrolyzed samples to optimize recovery. The dithizone extracts were butylated prior to analysis by gas chromatography–atomic absorption spectrometry (GC AA). Instrumental detection limits ranged between 1.6 and 2.3 pg lead. Application to a limited number of seafood samples indicated the possible presence of trace amounts (*ca* 1 ng g^{-1}) of trimethyllead in some samples. No other alkylleads were detected.

Keywords: Tetra-alkyllead, ionic alkyllead, gas chromatography–atomic absorption spectrometry (GC AA), trimethyllead, dithizone, seafood

INTRODUCTION

Concern continues over the health effects of exposure to lead from the environment. Tetra-alkylleads (used as gasoline additives) remain a major source of the environmental lead burden and are considerably more toxic than inorganic lead.[1] Ionic alkylleads result from the metabolic dealkylation,[2–4] hydrolysis[5–6] and photolysis[5,7] of tetra-alkylleads.

Experimental evidence for environmental alkylation of inorganic lead remains controversial,[8] with only some studies reporting positive findings.[9–12] However, some environmental studies do suggest that environmental alkylation of inorganic lead may occur. Forsyth and Marshall[13] determined that methyllead but not ethyllead levels in herring-gull tissues were significantly ($P=0.05$) correlated with lake sediment total lead levels in the Great Lakes region. Hewitt and Harrison[11] found that air masses originating from the North Atlantic had higher alkyllead:total lead ratios than did continental or urban air. Alkylleads have also been reported to occur in some seafoods[14] and freshwater fish.[15]

The purpose of this study was to (a) develop a method for ionic alkyllead and tetra-alkyllead compounds in seafood, and (b) examine several seafood items (edible portion only) to determine if alkyllead enters the diet through this route.

MATERIALS AND METHODS

Instrumentation

A gas chromatograph (GC)–atomic absorption spectrometer (AA) system was assembled with a Varian 3400 gas chromatograph equipped with an autosampler (Dynatech GC311V) interfaced to a Pye–Unicam SP9 atomic absorption spectrometer. The atomization system used was a quartz T-tube furnace.[16]

The GC was fitted with a 2.1 m, 6 mm o.d., 2 mm i.d., glass column packed with 3% OV-73 on Chromosorb WHP 100/120 mesh (3% OV-17 on Chromosorb WHP 80/100 mesh inside the injector). A transfer line between the GC column and quartz T-tube furnace was made from a 1 m section of DB-5 wide-bore (i.d. 0.31 mm) fused silica capillary column (J and W Scientific Inc.). A zero dead volume $\frac{1}{4}$ inch (0.64 cm) to $\frac{1}{16}$ inch (0.16 cm) Swagelok reducing union connected the transfer line to the end of the GC column. The transfer line was enclosed in an insulated,

* Author to whom correspondence should be addressed.

heated $\frac{1}{4}$ inch (0.64 cm) o.d. copper tube. Operating conditions were: carrier gas helium, 30 cm^3 min^{-1}; transfer line temperature, 200°C; injector, 200°C (alkylbutylleads), 175°C (tetra-alkylleads); temperature program (alkylbutylleads), 40°C (1 min hold), linear increase (15°C min^{-1}) to 140°C (no hold), linear increase (10°C min^{-1}) to 170°C (no hold), linear increase (15°C min^{-1}) to 200°C (5 min hold); temperature program (tetra-alkylleads), 35°C (0.5 min hold), linear increase (15°C min^{-1}) to 75°C (no hold), linear increase (20°C min^{-1}) to 200°C (5 min hold). The AA operating conditions were: Photron Super lead lamp current, 8 mA; boost current, 22 mA; wavelength, 217 nm; bandpass, 1 nm; furnace temperature was 900°C with a hydrogen make-up gas flow rate of 50 cm^3 min^{-1}.

Reagents and standards

Alkyllead chlorides (R_3PbCl, R_2PbCl_2, R=CH_3 and CH_3CH_2) and alkyllead butyls (R_3BuPb, R_2Bu_2Pb, R=CH_3 and CH_3CH_2) were prepared as previously described.[13] Solvents were pesticide grade and ACS reagent chemicals were used. Diphenylthiocarbazone (dithizone) was purchased from Fluka Chemical Corp. Enzyme preparations were purchased from Sigma Chemical Co.

The potassium cyanide–sodium sulfite solution (KCN–Na_2SO_3) consisted of 1.6 g KCN and 10 g Na_2SO_3 in 100 cm^3 distilled water. The ammonium phosphate–citrate buffer solution $(NH_4)H_2PO_4$–$(NH_4)_2HC_6H_5O_7$ contained 14.38 g $NH_4H_2PO_4$ and 28.27 g $(NH_4)_2HC_6H_5O_7$ made up to 250 cm^3 with distilled water. The pH was adjusted to 8.0 with concentrated ammonium hydroxide.

Sample preparation

Fish, shrimp and scallop samples were purchased from local supermarkets. The samples were received frozen and were partially thawed before homogenizing with a meat grinder (Moulinex, Model 244). The homogenates were throughly mixed and stored at −20°C until analysis.

Ionic alkyllead methodology

Hydrolysis

Tissue homogenates (5.0 g) were weighed into 50 cm^3 glass screw-cap centrifuge tubes. Buffer (20 cm^3 5% ethanol–0.5 mol dm^{-3} ammonium citrate buffer, pH 8.5) and 50 mg each of lipase (type VII) and protease (type XIV) were added.[13] The samples were then incubated at 37°C for 24 h (shrimp) or 48 h (cod, scallop). The ethanol was used to suppress bacterial growth during incubation.

Extraction

Potassium cyanide–sodium sulfite solution (1 cm^3) was added to the hydrolyzate. The sample pH was adjusted to 8.0 and extracted (rotary-tumbled) once (65 rpm, 5 min) with 0.05% (w/v) dithizone (10 cm^3) in 20% dichloromethane–hexane. Phase separation was hastened by centrifugation at 3000 rpm for 5 min. The organic layer was collected. The hydrolyzate pH was adjusted to 9.0 with 1 mol dm^{-3} sodium hydroxide (NaOH) and then extracted twice more with 10 cm^3 portions of the 0.05% dithizone solution. The pooled dithizone extracts were back-extracted three times (65 rpm, 5 min) with 7 cm^3 of 0.05 mol dm^{-3} nitric acid (HNO_3). The combined acidic extract was neutralized with 1 mol dm^{-3} NaOH, basified with KCN–Na_2SO_3 (1 cm^3) solution and 5 cm^3 of ammonium phosphate–citrate buffer (pH 8.0) and then extracted three times (65 rpm, 5 min) with 5 cm^3 of 0.05% dithizone solution. The pooled dithizone extract was reduced to 1.0 cm^3 in precalibrated tubes under nitrogen at 40°C.

Derivatization

Butylmagnesium chloride (2.0 mol dm^{-3}, 0.5 cm^3, Aldrich Chemical Co.) and tetrahydrofuran (1.0 cm^3) were added to the dithizone extract. The sample tube was then purged with nitrogen, capped, vortexed (10 s) and rotary-tumbled (25 rpm) for 10 min. The sample was then cooled in an ice bath, and prechilled 0.5 mol dm^{-3} HNO_3 (7.5 cm^3) was slowly added to destroy excess Grignard reagent. Iso-octane (0.7 cm^3) was added, the samples tumbled (25 rpm) for 2 min followed by centrifugation (2000 rpm, 5 min). The organic phase was then extracted (25 rpm, 2 min) with 8 cm^3 of distilled water (which was discarded), adjusted to 2.0 cm^3, dried over sodium sulfate and stored in an autoinjector vial.

Tetra-alkyllead methodology

Hydrolysis

The homogenized samples (5.0 g) were weighed into 16 mm × 125 mm screw-cap centrifuge tubes. Buffer (5% ethanol–0.5 mol dm^{-3} ammonium citrate, pH 8.5) and 50 mg each of protease (type XIV) and

lipase (type VII) were added. Hexane (2 cm^3) was layered on top of the sample and further buffer added until the hexane was just below the top of the tube. The sample was capped, slowly inverted and then incubated at 37°C for 24 h (shrimp) or 48 h (cod, scallop). The samples were vortexed briefly several times during hydrolysis to aid dispersion of the enzyme preparation.

Extraction

The samples were cooled for 5–10 min in an ice bath and them extracted (45 rpm) for 10 min. Centrifugation (3000 rpm, 5 min) hastened phase separation. The organic layer was collected and the extraction repeated with an additional 1 cm^3 of hexane. The pooled hexane extract was made up to 3.0 cm^3, dried over sodium sulfate and then stored in an autoinjector vial.

Recovery experiments

Samples of each tested tissue (5.0 g) were spiked (prior to hydrolysis) at two different levels with either a mixture of four ionic alkylleads or tetramethyllead and tetraethyllead. The percentage recovery of each analyte was determined by dividing the mean peak area of the recovered analyte by the mean peak area of either tetraalkyllead or butylated ionic alkyllead (added to a blank hydrolyzate–butyllead extract) diluted to the expected concentration.

Extraction optimization

Ionic alkyllead

Ionic alkylleads (spiking level 3–5 ng g^{-1} as lead) were added to 20 cm^3 distilled water with 5 cm^3 buffer solution (made up to various pH values). The sample was extracted three times with dithizone solution (5 cm^3) for 5 min each time. The pooled organic extract was reduced to 1.0 cm^3 under nitrogen at 40°C and then butylated. Three parameters — buffer, extraction pH and dithizone concentration — were examined in a series of extraction studies. Initial studies indicated that ammonium citrate and ammonium acetate were suitable as buffers. The extraction pH was varied from 8 to 11 and dithizone concentration from 0.01% to 0.05%.

Tetra-alkylleads

Tetraethyllead and tetramethyllead (spiking levels 6 and 25–26 ng g^{-1} as lead) were added to 5.0 g tissue samples. The samples were incubated for 24 h (shrimp) or 48 h (cod, scallop). The initial solvent volume was either 1 or 2 cm^3 n-hexane. The second extraction volume was 1 cm^3. Two extraction times, 5 and 10 min, were tested.

Environmental sample analysis

A 5.0 g sample of each tested tissue was hydrolyzed and extracted under optimized (maximum recovery)

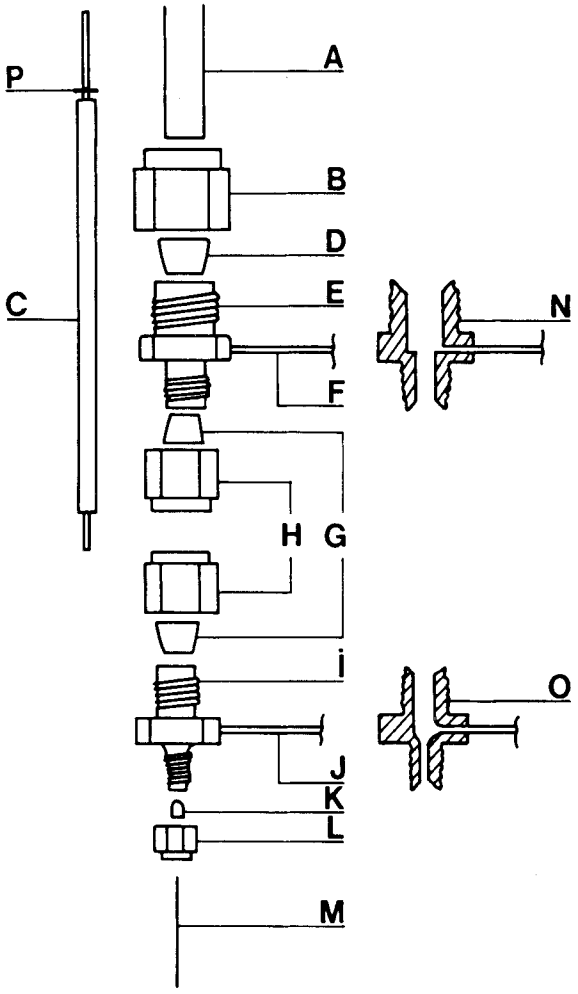


Figure 1 GC AA Interface: A, 6.25 mm o.d. lower tube of quartz T-tube; B, $\frac{1}{4}$ inch (0.64 cm) Swagelok nut; C, ceramic insert; D, $\frac{1}{4}$ inch (0.64 cm) graphite ferrule; E, $\frac{1}{4}$ inch (0.64 cm) to $\frac{1}{8}$ inch (0.32 cm) Swagelok reducing union; F, hydrogen inlet; G, $\frac{1}{8}$ inch (0.32 cm) vespel/graphite ferrules; H, $\frac{1}{8}$ inch (0.32 cm) Swagelok nuts; I, $\frac{1}{8}$ inch (0.32 cm) to $\frac{1}{16}$ inch (0.16 cm) Swagelok reducing union; J, hydrogen/air inlet; K, capillary graphite ferrule; L, $\frac{1}{16}$ inch (0.16 cm) Swagelok nut; M, fused silica capillary column; N, O, longitudinal sections of E and I respectively; P, 3.5 mm × 0.5 mm porcelain disc.

conditions. External standards containing Me_3BuPb, Me_2Bu_2Pb, Et_3BuPb and Et_2Bu_2Pb were used for sample quantitation. Results were corrected for background absorbance occurring at the Me_3BuPb retention time.

RESULTS AND DISCUSSION

Instrumentation

Several modifications have been made to the GC AA system since last reported.[16] A 3.5 mm × 0.5 mm porcelain disc (P, Fig. 1) dissipates heat from the quartz tube of the ceramic insert (C, Fig. 1). A 3.9 mm × 2 mm porcelain disc mounted inside the lower section of the quartz T-tube (at the air–insulation interface) prevents much of the radiant energy (from the heating elements) from reaching the ceramic body of the insert. System response has become more stable with any peak-tailing now associated with chromatographic rather than interface performance. The furnace heating elements are now the open-coiled type for ease of replacement and to eliminate quartz tube recrystallization resulting from contact with hot

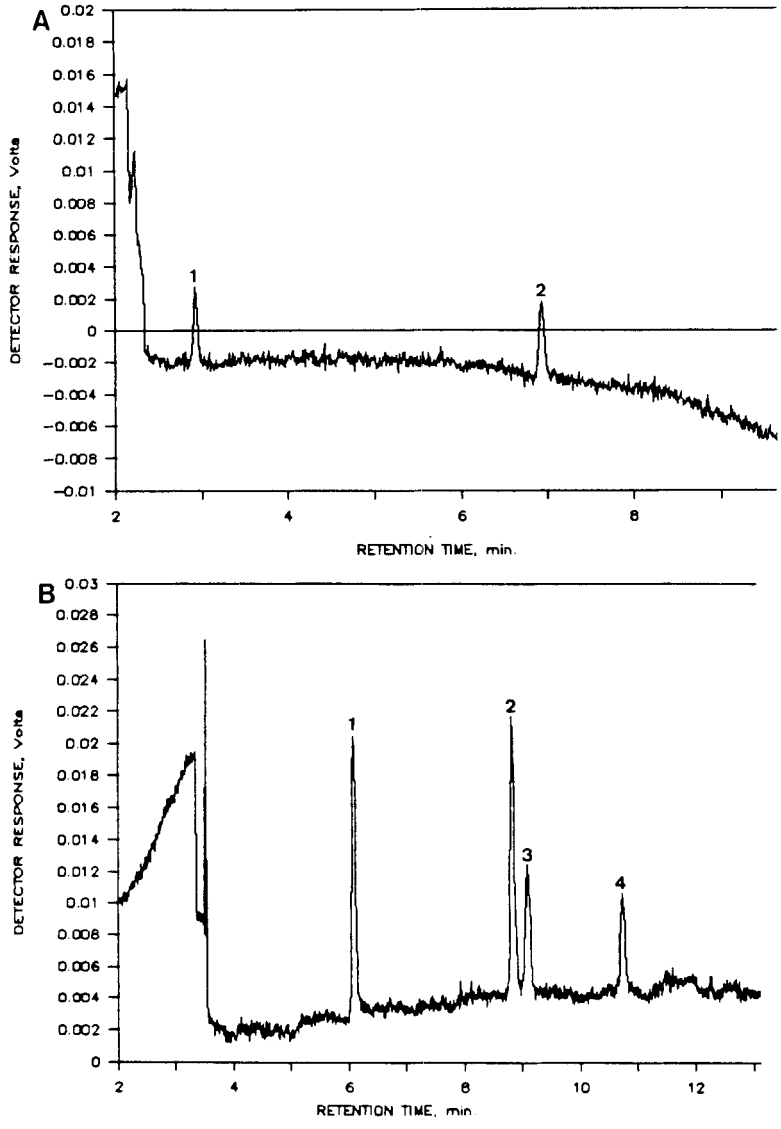


Figure 2 GC AA chromatograms of: A, (1) 6.2 pg (as lead) Me_4Pb and (2) 6.4 pg (as lead) Et_4Pb; B, 13–31 pg (as lead) (1) Me_3BuPb, (2) Me_2Bu_2Pb, (3) Et_3BuPb, and (4) Et_2Bu_2Pb.

alumina-based cementing compounds (previously used to embed the heating elements). The GC AA system can readily detect low picogram quantities of lead as tetra-alkyllead (Fig. 2A) or alkylbutyllead (Fig. 2B) with calculated limits of detection between 1.6 and 2.3 pg lead (Table 1).

Table 1 Limits of detection (LOD)

Compound	Mean[a] N_{p-p}(V)[b]	N_{SD}(V)[c]	Response factor[d]	LOD[e]
Me_3BuPb	0.0005068	0.0002081	1806	2.0
Me_2Bu_2Pb	0.0004676	0.0001856	1588	1.6
Et_3BuPb	0.0004676	0.0001856	1863	1.9
Et_2Bu_2Pb	0.0004839	0.0001829	2233	2.3
Me_4Pb	0.0004770	0.0002779	1240	1.6
Et_4Pb	0.0006135	0.0002967	1280	1.9

[a] Of 20 measurements.
[b] Mean peak to peak baseline noise.
[c] Standard deviation of N_{p-p}.
[d] Inverse of slope from linear regression (pg lead V^{-1}).
[e] (mean N_{p-p} + $3N_{SD}$) * response factor (pg lead).

Optimization and recoveries

Ionic alkylleads

Initial work with seafood samples indicated that extraction pH and dithizone concentration were important parameters to be controlled for optimum recoveries of all four tested analytes. The extraction series using buffered water (Fig. 3) allowed extensive testing of these parameters. Recoveries of triethyllead, dimethyllead and diethyllead dropped as the extraction pH increased above 9 when the dithizone concentration was 0.01% [Fig. 3 (1a, 1b)] or 0.03% [Fig. 3 (2a, 2b)]. When the dithizone concentration was increased to 0.05% only triethyllead recoveries were lowered with increased extraction pH [Fig. 3 (3a, 3b)]. Trimethyllead recoveries were virtually unaffected by extraction pH or dithizone concentration (Fig. 3). None of the analytes appeared to be affected by buffer composition as the recoveries were very similar between the ammonium acetate [Fig. 3 1a–3a)] and ammonium citrate [Fig. 3 (1b–3b)] series. Ammonium citrate did, however, produce hydrolysates with better physical handling characteristics. Triethyllead required both

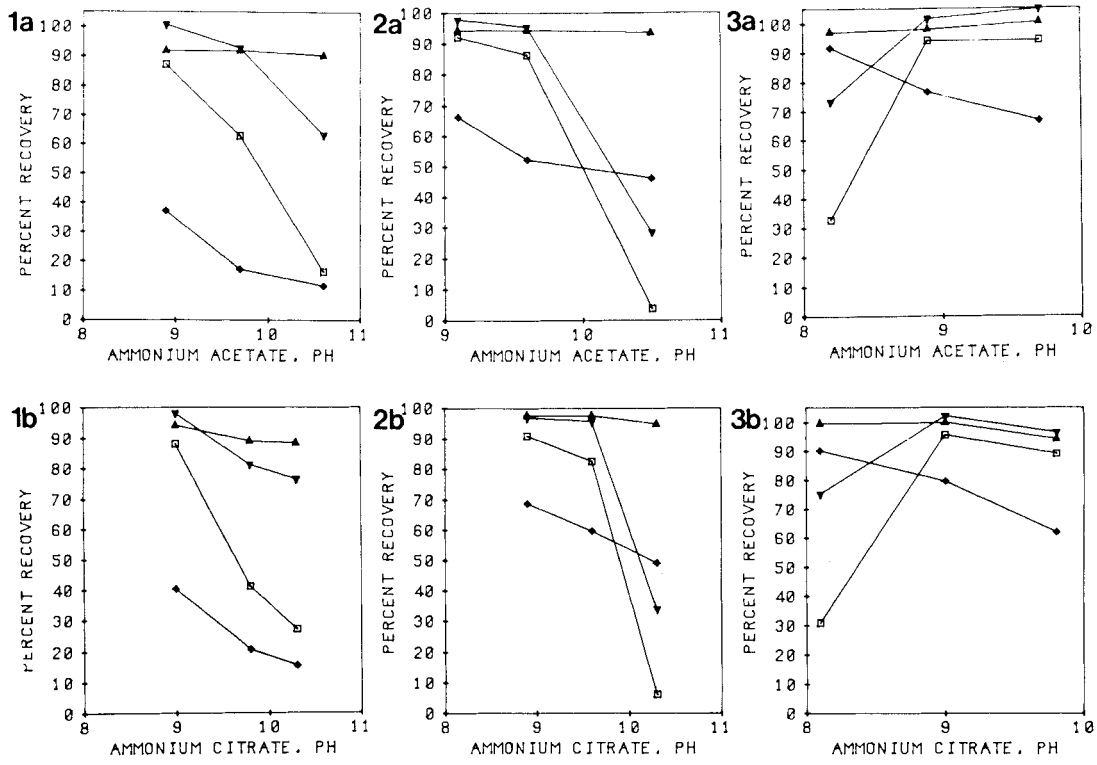


Figure 3 Percentage of recovery of ▲ trimethyllead, □ dimethyllead, ◆ triethyllead, and ▼ diethyllead with (1a, 1b) 0.01% dithizone, (2a, 2b) 0.03% dithizone, and (3a, 3b) 0.05% dithizone.

Table 2 Mean recoveries of ionic alkyllead compounds from seafood

Tissue	N	Spiking level[a](ng g^{-1})	Mean recovery (% ± SD)			
			Analyte			
			Me_3PbCl	Et_3PbCl	Me_2PbCl	Et_2PbCl_2
Cod	4	13–24	103 ± 3	90 ± 2	58 ± 6	76 ± 1
	4	53–95	100 ± 0.1	81 ± 3	66 ± 6	82 ± 3
Shrimp	3	13–24	95 ± 3	82 ± 3	55 ± 5	90 ± 3
	3	53–95	94 ± 0.9	82 ± 2	51 ± 9	76 ± 2
Scallop	3	13–24	97 ± 3	86 ± 0.2	69 ± 11	91 ± 9
	4	53–95	97 ± 0.5	84 ± 1	57 ± 5	78 ± 5

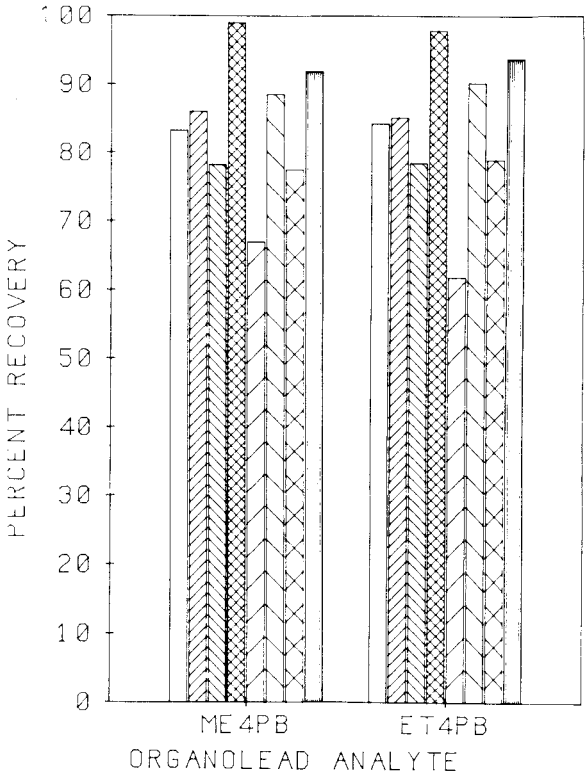
[a] As lead.

high dithizone concentration (0.05%) and low pH (8) to achieve recoveries above 90% [Fig. 3 (3a, 3b)]. However, recoveries of the dialkylleads, particularly dimethyllead, dropped at pH 8 [Fig. 3 (3a, 3b)].

As no single pH value appeared optimal for the tested analytes, pH programming was incorporated into the seafood hydrolyzate extraction methodology; one extraction was done at pH 8, with two additional extractions made at pH 9. As initial work with ammonium phosphate indicated no adverse effects on ionic alkyllead recoveries, ammonium phosphate/citrate was used in the extraction procedure for better buffering capacity at pH 8. This technique gave satisfactory recoveries from cod, scallop and shrimp at both spiking levels (Table 2). Only dimethyllead recoveries were below 75% (ranging between 51 and 69%), but are comparable with[17] or better than most other existing tissue extraction methodologies.[13,18]

Tetra-alkylleads

Tetramethyllead and tetraethyllead behaved similarly under the optimization conditions (Fig. 4). A 2 cm^3 initial extraction volume produced better recoveries than a 1 cm^3 initial volume, regardless of spiking level or extraction time. At both spiking levels, a 10 min extraction time usually gave higher recoveries than a 5 min extraction time whether a 2 or 3 cm^3 total extraction volume was used (Fig. 4). The maximum recoveries of tetramethyllead and tetraethyllead were achieved using a 3 cm^3 total extraction volume with 10 min extraction time (Fig. 4). Under these conditions both analytes were recovered almost quantitatively from cod, scallop and shrimp at both spiking levels (Table 3).



	Spiking level (ng g^{-1}Pb)	Total extraction vol (cm^3)	Extraction time (min)
	6	2	5
	6	3	5
	6	2	10
	6	3	10
	25/26	2	5
	25/26	3	5
	25/26	2	10
	25/26	3	10

Figure 4 Percentage of recovery of Me_4Pb and Et_4Pb under various experimental conditions.

Environmental samples

Three of the consumer seafood samples tested, Boston bluefish *(Pollachius virens)*, haddock *(Melanogrammus aeglefinus)* and ocean perch *(Sebastes marinus)* (Fig. 5, C–E) appeared to contain trace amounts (0.8–1.6 ng g^{-1}) of Me_3Pb^+ (as Me_3BuPb); however, this was not subjected to further confirmation. Shrimp (Fig. 5, B) may contain a trace amount but the reagent blank (Fig. 5, A) makes it difficult to be certain. None of the other peaks in the reagent blank or samples corresponds to organolead standards. None of the samples tested contained tetra-alkyllead.

Table 3 Mean recoveries of tetra-alkyllead compounds from seafood

Tissue	*N*	Spiking level[a](ng g^{-1})	Mean recovery (% ± SD)	
			Analyte	
			Me_4Pb	Et_4Pb
Cod	3	6	90 ± 4	90 ± 5
	3	25–26	96 ± 0.3	99 ± 0.5
Shrimp	3	6	96 ± 2	93 ± 3
	3	25–26	97 ± 2	94 ± 0.5
Scallop	3	6	92 ± 4	88 ± 3
	3	25–26	91 ± 3	87 ± 5

[a] As lead.

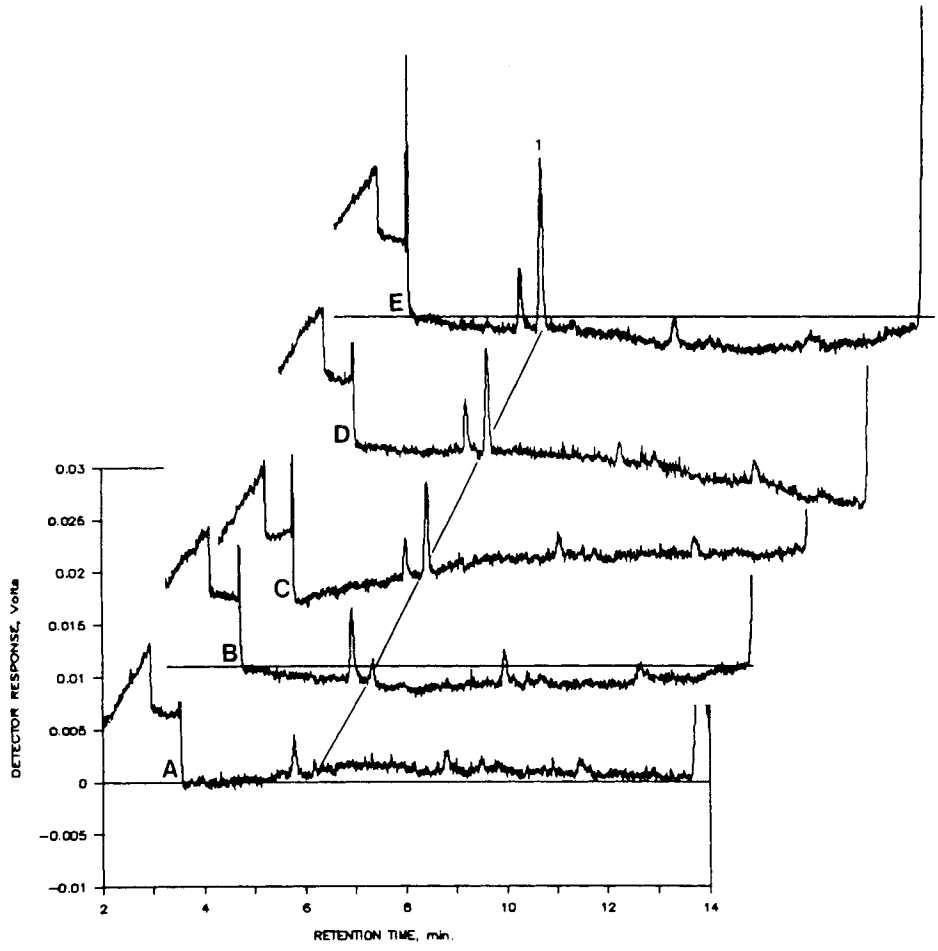


Figure 5 GC AA chromatograms of: A, reagent blank; B, shrimp; C, Boston bluefish fillet [containing 0.8 ng g^{-1} Me_3Pb^+ as (1) Me_3BuPb]; D, haddock fillet [containing 0.8 ng g^{-1} Me_3Pb^+ as (1) Me_3BuPb]; E, ocean perch fillet [containing 1.6 ng g^{-1} Me_3Pb^+ as (1) Me_3BuPb].

Very limited data on organolead levels in seafood from unpolluted sites are available. Sirota and Uthe[14] found 10 ng g^{-1}–4.8 $\mu g\ g^{-1}$ tetra-alkyllead in seafood using less specific extraction methodology and instrumentation. The absence of tetra-alkylleads in the samples examined in the present study may be a result of (a) only edible portions (fillet) being examined; (b) lead level reductions in gasoline since the previous study or (c) limited sample size. The presence of only methyllead in the samples may be caused by the lower stability of ethyllead in the environment[19] or by an environmental source of methyllead.

CONCLUSIONS

Preliminary examination of consumer seafood samples indicates the possible presence of methyllead. Further research is in progress to determine the prevalence and level of alkyllead compounds in the diet.

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