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Ionic alkyl-lead, tetra-alkyl-lead and total lead in fish from the Great Lakes

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Fillets from a variety of fish species collected from Lakes Ontario, Superior and Erie, Canada, were examined for ionic alkyl-lead, tetra-alkyl-lead and total lead compounds. Diphenylthiocarbazone (dithizone)-derivatized extracts were collected at pH 8 and 9 for ionic alkyl-leads from enzymatically hydrolyzed samples. Butylated derivatives were formed prior to analysis by gas chromatography–atomic absorption spectrometry (GC AA). Tetra-alkyl-lead was extracted from the hydrolyzates with hexane.

Most of the fillets contained low (<0.08–2 ng g^{-1}) levels of trimethyl-lead. Several samples contained triethyl-lead or tetraethyl-lead. Dimethyl-lead, diethyl-lead and tetramethyl-lead were detected by GC MS but were below the GC AA method detection limit of 0.06 ng g^{-1}, 0.09 ng g^{-1} and 0.2 ng g^{-1} respectively. Total lead levels were between <1.8 and 96.7 ng g^{-1}.

Keywords: Organoleads, analysis, fish, atomic absorption, mass spectroscopy

INTRODUCTION

Lead has become a ubiquitous environmental contaminant, being detected as inorganic and organic compounds in a variety of food and other matrices.[1–4] Inorganic lead, even at levels previously considered safe, has been linked to learning deficiencies in children.[5,6] Alkyl-leads, although usually present at lower levels than inorganic lead, exhibit greater toxicity; trialkyl-lead salts are 10–100 times more toxic and dialkyl-lead leads up to 10 times more toxic than inorganic lead salts. Acute alkyl-lead poisoning causes hyperactivity, tremors, periodic convulsions and aggressive behavior in rats[7] and humans.[8] Previous Great Lake, Canada, region surveys have sampled freshwater fish primarily from regions near alkyl-lead production facilities.[4] Very few data are available on alkyl-lead levels in edible portions from fish caught for commercial sale from the Great Lakes. The purpose of this study, therefore, was to examine commercial fish species in the Great Lakes for current total and organic lead levels.

MATERIALS AND METHODS

Instrumentation

Details of the gas chromatograph (GC)–atomic absorption spectrometer (AA) system used to quantitate results have been reported previously.[9,10]

The GC column was packed with 3% OV-73 on Chromosorb WHP, mesh 100/120; the 3% OV-17 previously used in the injector port[10] was omitted to simplify periodic repacking of the front portion of the column. Operating conditions were as follows: carrier gas, helium, 30 cm^3 min^{-1}; transfer line temperature, 225 °C; injector temperature (alkylbutyl-lead), 200 °C; injector temperature (tetraalkyl-lead), 175 °C; temperature program (alkylbutyl-lead), 40 °C initially (1 min hold) with a linear increase (15 °C min^{-1}) to 140 °C (no time hold) followed by a linear increase (10 °C min^{-1}) to 170 °C (no time hold) with a final linear increase (15 °C min^{-1}) to 200 °C (5 min hold); temperature program (tetra-alkyl-lead), 35 °C initially (0.5 min hold) with a linear increase (15 °C min^{-1}) to 75 °C (no hold) followed by a linear increase (20 °C min^{-1}) to 200 °C (5 min hold). The AAS operating conditions were: bandpass, 1 nm; wavelength, 217 nm; Photron Super Pb lamp current, 8 mA; boost current, 22 mA; furnace temperature, 900 °C; hydrogen make-up gas flow rate, 50 cm^3 min^{-1}.

Total lead was determined using a Varian Model 775-ABQ atomic absorption spectrometer

equipped with a Perkin–Elmer HGA-400 graphite tube atomizer. A pyrolytic graphite L'vov platform was used in a pyrolytically coated graphite tube. Operating conditions were: bandpass, 1 nm; wavelength 283.3 nm; furnace program, dry at 250–380 °C, ash at 550 °C for 10 s, cool to 40 °C (15 s), and atomize for 6 s at 1800 °C using the gas interrupt mode and a zero second ramp.

A VG Analytical 7070EQ (operated in the conventional sector only) interfaced with a Varian VISTA 6000 GC (on column injection) was used for GC MS confirmation. The system was operated in the electron-impact mode at 30 eV and at a mass resolution of 1000. GC operating conditions were: column, J&W DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film); carrier gas, helium 100 kPa); injector temperature program (alkylbutyl-leads), 80 °C initially (0.5 min hold) with a linear increase (80 °C min^{-1}) to 250 °C; injector temperature program (tetra-alkyl-leads), 100 °C initially (0.5 min hold) with a linear increase (80 °C min^{-1}) to 200 °C; column temperature program (alkylbutyl-leads), 70 °C initially (1.5 min hold) with a linear increase (5 °C min^{-1}) to 85 °C (no hold) followed with a linear increase (15 °C min^{-1}) to 200 °C (no hold) with a final linear increase (20 °C min^{-1}) to 260 °C; column temperature program (tetra-alkyl-leads), 30 °C initially (3 min hold) with a linear increase (5 °C min^{-1})) to 50 °C (no hold) followed by a linear increase (10 °C min^{-1}) to 200 °C.

Ions monitored were *m/z* 293, 294 and 295 for trimethylbutyl-lead (Me_3BuPb); 335, 336 and 337 for dimethyldibutyl-lead (Me_2Bu_2Pb); 321, 322 and 323 for triethylbutyl-lead (Et_3BuPb); 349, 350 and 351 for diethyldibutyl-lead (Et_2Bu_2Pb); 251, 252 and 253 for tetramethyl-lead (Me_4Pb) and 293, 294 and 295 for tetraethyl-lead (Et_4Pb).

Reagents and standards

Pesticide-grade solvents (Caledon Laboratories Ltd, Georgetown, Ontario, Canada) and ACS reagent chemicals were used. Diphenylthiocarbazone and ammonium pyrrolidine dithiocarbamate were purchased from BDH (Toronto, Ontario) and Aldrich Chemical Co. (Milwaukee, Wisconsin, USA) respectively. Lipase (Type VII) and protease (Type XIV) were purchased from Sigma Chemical Co. (St Louis, Missouri, USA). High-purity nitric and perchloric acids were prepared from ACS grade by sub-boiling distillation. The potassium cyanide–sodium sulfite solution contained 1.6 g potassium cyanide and 10 g sodium sulfite made up to 100 cm^3 with 18 MΩ Cm deionized water (Milli-Q Water System, Millipore Corp., USA). The ammonium phosphate–citrate solution contained 14.38 g $NH_4H_2PO_4$ and 28.27 g $(NH_4)_2HC_6H_5O_7$ with distilled water added to a total volume of 250 cm^3. The alkyl-lead standards were prepared as previously described.[10]

Sample preparation

Fish were collected from Lake Ontario, Lake Erie and Lake Superior by inspectors from the Canadian Department of Fisheries and Oceans. Samples were shipped frozen as fillets or gutted whole fish. Portions (100 g) of fillet (minus skin) were partially thawed before homogenizing with a meat grinder (Moulinex, Model 244) and then stored at −20 °C until analysis.

Ionic alkyl-lead method

The procedure has been described previously.[3] Briefly, homogenates were enzymatically hydrolyzed for 24 h using lipase and protease. The resulting hydrolyzates were extracted once at pH 8 and twice at pH 9 with 0.05% (w/v) dithizone made up in 20% methylene chloride/hexane. The pooled dithizone extracts were back-extracted with dilute nitric acid three times. The combined acidic extracts were neutralized with sodium hydroxide, basified with potassium cyanide–sodium sulfite ($KCN–Na_2SO_3$) solution and ammonium phosphate–citrate buffer and then extracted three times with dithizone solution. The pooled dithizone extracts were reduced to 1 cm^3 and derivatized using butylmagnesium chloride.

Samples selected for GC MS confirmation were cleaned up using a purge and trap apparatus described elsewhere.[12] The final extract volume was reduced to 0.5 cm^3 under a gentle stream of nitrogen at 40 °C.

Tetra-alkyl-lead method

The procedure has been reported previously.[3] Homogenates (5 g) were enzymatically hydrolyzed for 48 h with 2 cm^3 hexane present, cooled in ice for 5–10 min and then extracted (rotary-tumbled, 45 rpm) for 10 min. The extraction was repeated with an additional 1 cm^3 of hexane. The pooled extract was made up to 3 cm^3 and dried over sodium sulfate.

Table 1 Mean recoveries of ionic alkyl-lead compounds from freshwater fish

Tissue	N^a	Spiking level[b] ($ng\,g^{-1}$)	Mean recovery (%, ±SD)			
			Me_3PbCl	Et_3PbCl	Me_2PbCl_2	Et_2PbCl_2
Lake trout	4	51–63	99±2	74±1	76±4	98±4
Rainbow trout	3	5–8	94±0.5	76±3	88±8	97±7

[a] N, number of samples. [b] As lead.

Total lead method

The procedure has been reported previously.[11] Samples were analyzed for total lead using a nitric–perchloric acid digestion, neutralized with ammonia, followed by co-precipitation with ammonium pyrrolidine dithiocarbamate with copper and iron as carriers. The only modification was a reduction of the final concentration of ammonium dihydrogen phosphate from 1% to 0.1% to reduce spectral interference.

Recovery experiments

The tested tissues were spiked prior to hydrolysis with a mixture of ionic alkyl-leads (or tetraethyl-lead and tetramethyl-lead) at two different concentration levels (Tables 1, 2). The percentage recovery of each compound was determined by comparing the mean peak area of the recovered compound with the mean peak area of the recovery standards (spiked tissue hydrolyzate extracts) diluted to the expected concentration.

RESULTS AND DISCUSSION

Alkyl-leads

Recoveries of ionic alkyl-leads from freshwater fish generally exceeded 75% (Table 1) at two spiking levels of 5–8 and 51–63 $ng\,g^{-1}$ (as Pb).

Table 2 Selenium in marine plants

N^a	Spiking level[b] ($ng\,g^{-1}$)	Mean recovery (%, ±SD)	
		Me_4Pb	Et_4Pb
8	13	90±4	—
8	16	—	91±4
4	51	—	91±4
4	62	91±1	—

[a] N, number of samples. [b] As lead.

These results were comparable with,[3,13] or better than,[14] those previously reported. Initial work indicated that altering the sample pH from 8 to 9 after the first dithizone extraction (a procedure adopted earlier for seafood analysis[3]) was required. The method detection limits, based on a 5 g sample size, were 0.08 $ng\,g^{-1}$ (Me_3BuPb), 0.06 $ng\,g^{-1}$ (Me_2Bu_2Pb), 0.08 $ng\,g^{-1}$ (Et_3BuPb) and 0.09 $ng\,g^{-1}$ (Et_2Bu_2Pb). Some reagent blanks contained a small interference appearing at the retention time of Me_3BuPb. Me_3BuPb results were, therefore, corrected with the mean reagent blank response obtained from two reagent blanks run with each set of samples. Any environmental sample value falling within the range of the reagent blank values was rejected. The interference was eliminated (Fig. 1,b) by extracting the ammonium citrate buffer once with dithizone solution (25 cm^3), 50-cm^3 portions of methylene chloride (until colourless) and then twice with hexane (50 cm^3). Tetra-alkyl-lead recoveries were 90% or better (Table 2) at both spiking levels of 13–16 and 51–62 $ng\,g^{-1}$ (as Pb). Detection limits for the described method were 0.2 $ng\,g^{-1}$ (Me_4Pb) and 0.1 $ng\,g^{-1}$ (Et_4Pb).

Total lead

Recovery of alkyl-lead and inorganic lead from samples spiked at 37–167 $ng\,g^{-1}$ (Table 3) averaged 104% (84–150%). Detection limits for the analytical runs (3 × standard deviation of seven blanks in a run) averaged 6 ng absolute (3–9 ng). Detection limits for individual samples averaged 3 $ng\,g^{-1}$ (1.4–5.1 $ng\,g^{-1}$).

ENVIRONMENTAL SAMPLES

Trimethyl-lead was found in fish fillet collected from all three Great Lakes and in most (nine of ten) of the fish species examined, with mean values ranging from not detected ($<0.08\,ng\,g^{-1}$) to 0.8 $ng\,g^{-1}$ (Table 4). Individual values did not

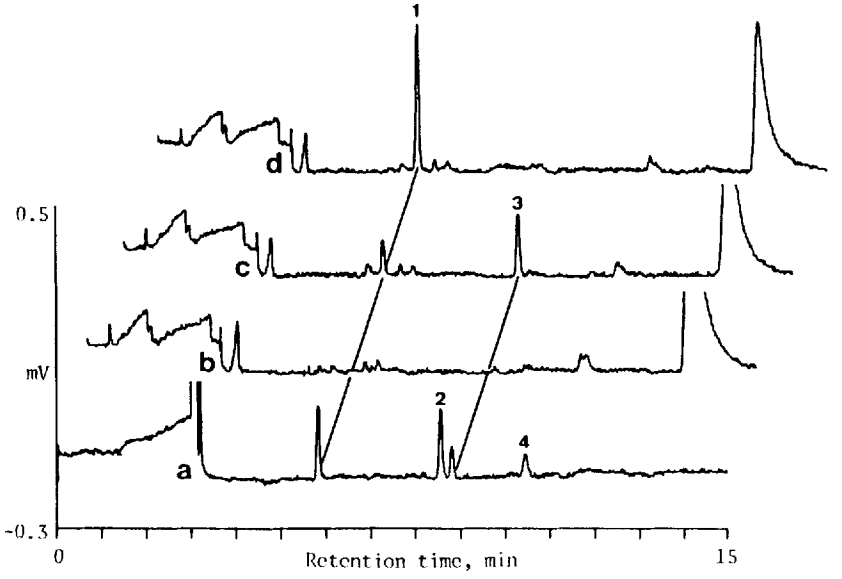

Figure 1 GC AA chromatograms of: a, standard solution; b, reagent blank; c, brown bullhead fillet; and d, trout fillet (Lake Superior) containing 1, Me_3Pb^+ (as Me_3BuPb); 2, Me_2Pb^{2+} (as Me_2Bu_2Pb); 3, Et_3Pb^+ (as Et_3BuPb); and 4, Et_2Pb^{2+} (as Et_2Bu_2Pb).

exceed 2.1 ng g^{-1} (Fig. 1,d). Trimethyl-lead levels did vary among the species examined. American eel (Lake Ontario) contained the highest mean concentration (0.8 ng g^{-1}) whereas herring (Lake Superior, Erie), yellow perch (Lake Erie) and white bass contained less than the detection limit. Trout (Lake Superior), yellow perch (Lake Ontario), white fish (Lake Superior) and menominee contained 0.4 ng g^{-1} Me_3BuPb. Smelt, yellow pickerel, brown bullhead and trout (Lake Erie) contained less, with values ranging between 0.1 and 0.2 ng g^{-1}.

Triethyl-lead was found much less frequently in five (out of 88 samples) than trimethyl-lead although three of the four brown bullhead samples (Lake Ontario) contained a mean value of 0.5 ng g^{-1} (Table 4; Fig. 1,c). White fish (Lake Superior) contained a mean concentration of 0.1 ng g^{-1}. All other samples contained less than the detection limit.

Table 3 Mean recoveries of alkyl-lead and inorganic lead as total lead from fish

Analyte	N^a	Matrix	Spiking level[b] (ng g^{-1})	Mean recovery (%, ± SD)
Me_3PbCl	3	Turbot	64	97 ± 8
Me_2PbCl_2	3	Turbot	77	84 ± 7
Et_3PbCl	3	Turbot	48	150 ± 40
Et_2PbCl_2	3	Turbot	43	115 ± 2
Me_4Pb	3	Turbot	40	90 ± 13
Et_4Pb	3	Turbot	37	99 ± 10
$Pb(NO_3)_2$	4	Lake trout	167	95 ± 3
$Pb(NO_3)_2$	4	Turbot	167	98 ± 3

[a] N, number of samples. [b] As lead.

The presence of ionic alkyl-lead was confirmed in three samples examined by comparison with authentic standards using GC MS. Following derivatization one brown bullhead sample contained all four butylated ionic alkyl-leads (Fig. 2); Me_2Bu_2Pb (m/z 337, $[M—CH_3]^+$) and Et_2Bu_2Pb (m/z 351 $[M—CH_3CH_2]^+$) were present (Fig. 2) although the levels were below the method detection limit using GC AA (0.06–0.09 ng g^{-1}).

Tetraethyl-lead was found in four of the 88 samples analyzed with values ranging between 0.3 and 1.3 ng g^{-1} (Table 4). Two of the samples were confirmed by GC MS. These samples also contained tetramethyl-lead, at levels below the method detection limit (based on GC AA) of 0.2 ng g^{-1}.

Total lead mean values were low, ranging between 2.6 and 28.0 ng g^{-1}. Individual values did not exceed 97 ng g^{-1} (Table 4). Alkyl-lead, when present, constituted 1.6–17% of the mean total lead burden (Table 4). There was not, however, any significant ($P = 0.05$) correlation between total lead and alkyl-lead levels, suggesting independent sources of organic and inorganic lead.

Table 4 Alklyl-lead and total lead levels in fish collected from Lakes Ontario, Erie and Superior

			Analyte concentration, ng g^{-1} wet wt												
			Me_3Pb^+			Et_3Pb^+			Et_4Pb			Total Pb			
Species	Lake	N^a	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Alkyl-lead (%)
Trout	Superior	23	0.4	0.3	<0.08–2.1	<0.08	—	—	<0.1	<0.1	<0.1–1.3	7.4	6.8	<5.6–17.1	5.4
	Erie	3	0.2	0.2	<0.08–0.3	<0.08	—	—	—	—	—	2.8	3.4	<2.2–4.2	7.1
Yellow perch	Erie	9	<0.08	—	<0.08–0.3	<0.08	—	—	—	—	—	8.1	5.8	<5.1–21.7	—
	Ontario	4	0.4	0.4	0.3–0.5	<0.08	—	—	—	—	—	25.0	5.7	<2.8–86.8	1.6
White fish	Superior	6	0.4	0.3	0.2–0.9	0.1	<0.08	<0.08–0.7	—	—	—	18.3	13.3	10.1–39.4	2.7
	Erie	4	0.5	0.5	0.4–0.6	<0.08	—	—	<0.1	<0.1	<0.1–0.3	<2.9	—	<3.2–3.8	—
Herring	Superior	13	<0.08	—	<0.08–0.2	<0.08	—	—	—	—	—	28.0	19.5	7.7–96.7	—
	Erie	2	<0.08	—	—	<0.08	—	—	—	—	—	<3.2	—	<2.6–<3.5	—
Menominee	Superior	9	0.4	0.5	<0.08–0.8	<0.08	—	<0.08–0.2	—	—	—	12.9	8	<5.3–48.3	3.1
Brown bullhead	Ontario	4	0.2	0.2	0.1–0.3	0.5	0.6	<0.08–1.1	—	—	—	6.4	3.8	<4.1–17.0	10.9
American eel	Ontario	4	0.8	0.7	0.3–1.4	<0.08	—	—	—	—	—	4.8	5.0	<4.1–6.0	16.6
Yellow pickerel	Erie	2[b]	0.1	0.1	<0.08–0.3	<0.08	—	—	—	—	—	<5.3	—	<4.7–5.8	—
White bass	Erie	3	<0.08	—	—	<0.08	—	—	—	—	—	2.6	3.1	<1.8–3.3	—
Smelt	Erie	2[b]	0.2	0.2	<0.08–0.4	<0.08	—	—	0.3	0.3	<0.1–0.6	11.2	11.2	7.3–15.0	4.5

[a] N, number of samples.

[b] Yellow pickerel and smelt samples were pools containing three to four and ten fillets each, respectively.

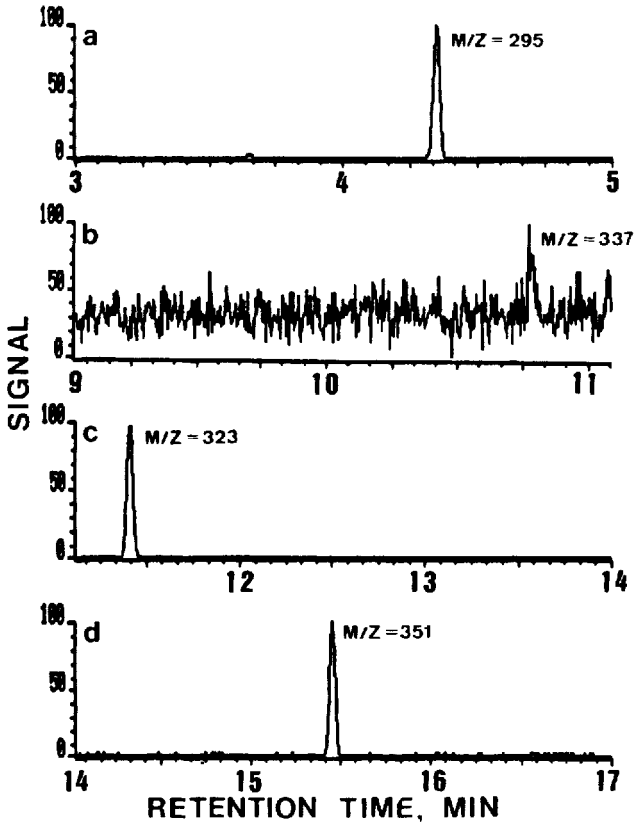

Figure 2 Selected ion monitored GC MS of brown bullhead fillet containing (a) Me_3BuPb; (b) Me_2Bu_2Pb; (c) Et_3BuPb; and (d) Et_2Bu_2Pb.

Most of the data on alkyl-lead levels in fish from the Great Lakes are primarily from regions contaminated by commercial alkyl-lead production.[4] However, some control sites were sampled; alkyl-leads were found in five of 14 fish muscle samples collected in 1983 from the St Lawrence River west of Maitland (upstream of alkyl-lead production). The levels ranged from not detected (less than 8 ng g^{-1}) to 421 ng g^{-1}. The predominant species were tetra- and triethyl-lead. No tetramethyl-lead was found in the samples.

Current levels of alkyl-leads are generally less than previously found[4] as no sample contained more than 2 ng g^{-1}; however, there has been a distinct shift from ethyl-lead to methyl-lead as the predominant alkyl-lead species found in the fish samples; only brown bullhead fillets contained more ethyl-lead than methyl-lead. The recent reduction in ethyl-lead, the only alkyl-lead antiknock additive used in Canadian leaded gasoline, is the most probable reason for the lower ethyl-lead levels found in this study. The methyl-lead could originate from American leaded gasoline (which contains tetraethyl-lead or methylethyl-lead mixtures with tetramethyl-lead) although environmental methylation of inorganic lead remains a possible source.

CONCLUSIONS

The majority of fish fillets collected from Lakes Ontario, Superior and Erie contained low levels of trimethyl-lead. Several fish from Lakes Ontario and Superior contained triethyl-lead. Other ionic alkyl-leads were detected by GC MS but were below the GC AA method detection limit. Tetraethyl-lead was found in only four of the 88 samples analyzed. Total lead levels in the fillets ranged from <1.8 to 96.7 ng g^{-1}.

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