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Received for review August 19, 1987. Accepted December 28, 1987. We gratefully acknowledge the financial support of the U.S. EPA (CR812356-01-0).

Ionic Alkylleads in Salt Marsh Periwinkles (*Littorina irrorata*)

Kannan Krishnan,[†] William D. Marshall,*[,†] and Walter I. Hatch[‡]

Department of Food Science and Agricultural Chemistry, Macdonald College of McGill University, Ste. Anne de Bellevue, Quebec, Canada H9X 1C0, and Division of Natural Sciences and Mathematics, St. Mary's College of Maryland, St. Mary's City, Maryland 20686

■ Salt marsh periwinkles (*Littorina irrorata*), from six sites in Maryland and Virginia, were examined to determine ionic alkyllead concentrations and possible alkyllead sources in lower Chesapeake Bay. Different sources of ethylleads and trimethyllead to this species were demonstrated by statistical comparisons of the concentrations of individual analytes from different sites. These comparisons also indicated (1) that environmentally mediated methylation of Pb^{2+} contributes appreciably to Me_3Pb^+ concentrations in snails and (2) that the relative concentrations of individual analytes were consistent with an environmental methylation of ethyllead salts. Compared to females, males were characterized by significantly higher concentrations of several of the alkyllead analytes. In addition, an unknown lead-containing compound was present in all samples.

Introduction

The potential adverse impacts of the continued release of lead (Pb) into the environment remains an area of considerable concern. The major source of Pb is tetraalkylleads used as gasoline antiknock additives. Tetraalkylleads, which may form a small portion of the total lead emitted from automobiles and from related industries (*1–3*), are volatile but environmentally labile. They are degraded abiotically (*4–6*) and via metabolic processes (*7, 8*) to result, sequentially, in trialkyllead (R_3Pb^+), dialkyllead (R_2Pb^{2+}), and inorganic lead (Pb^{2+}) salts. Ionic alkyllead salts are considered to be more persistent in the environment, and they retain much of the acute toxicity of their tetraalkyl progenitors. Moreover they tend to accumulate, at least temporarily, in soft tissues where they mediate deleterious changes which are distinctly different from classical plumbism. Acute toxicity studies indicate that ionic alkylleads are up to 100 times more toxic to mammals than are inorganic lead salts (*9, 10*). A similar spectrum of activities against representative marine organisms (algae, mollusc, crustacean, and fish) (*11*) and representative microorganisms (*12*) has been reported. Thus, although alkylleads may make only a minor contribution to the total lead burden, they may be anticipated to appreciably alter toxicity.

There is an emerging pattern of low concentrations of tetraalkylleads and alkyllead salts in several environmental compartments: air (*4, 13, 14*); rain, snow, and surface water (*15*); street dusts and urban soils (*16, 17*); freshwater fish (*18, 19*); cod, lobster, and mackerel (*20*); fowl (*21–23*) and

[†] Macdonald College of McGill University.
[‡] St. Mary's College of Maryland.

0013-936X/88/0922-0806$01.50/0

human brains (*24*). The environmental methylation of alkyllead salts, whether biologically (*25, 26*) or chemically (*27, 28*) mediated, is now well established. However, the analogous methylation of inorganic lead cations remains controversial (*29, 30*). Recently reported evidence, based on alkyllead concentrations in herring gulls, favored an environmental methylation of inorganic lead (*23*). The objectives of the current study were to extend previous observations on the origin of individual ionic alkylleads by determining the concentrations of these analytes in a nonavian indicator species which was representative of an aquatic environment. The concentrations of individual alkylleads, in samples from separate geographic locations, were to be compared in an effort to detect separate inputs of these toxicants.

Salt marsh perwinkles (*Littorina irrorata*) are abundant in salt marshes along the Atlantic and Gulf coasts of the United States (*31*). They are commonly associated with the marsh grasses *Spartina alterniflora* and *Juncus roemarianus*, which the periwinkle uses to avoid predators and to escape inundation by rising tides. Periwinkles feed by scraping the surface of the marsh at low tide and the stems of *Spartina* and *Juncus*. As they make their tidal migrations, they ingest detritus particles and associated bacteria and algae (*31*). Tagging experiments have demonstrated that snails are relatively sedentary within small areas of the marsh. Related species, *Littorina littorea* and *Littorina littoralis*, have been used successfully by Bryan et al. (*32*) as biological indicators of heavy metals pollution.

Materials and Methods

Reagents and Standards. Alkyllead chloride (R_3PbCl, R_2PbCl_2; R = CH_3, C_2H_5) and alkyllead butylate (R_3BuPb, R_2Bu_2Pb; R = CH_3, C_2H_5; Bu = C_4H_9) standards were prepared as previously described (*33, 34*). Chromatographic support gases were of prepurified grade, all chemicals were of ACS reagent grade or better, and solvents were distilled in glass grade. The ammoniacal buffer consisted of diammonium citrate (22.6 g), potassium cyanide (4.0 g), and sodium sulfite (24.0 g), which was diluted to 250 mL with distilled water. The pH was adjusted to 10.0 with concentrated aqueous ammonia.

Sample Collection. Collection sites were thought to represent a range of pollution stress with respect to heavy metals (*35–38*). Sites were established at two locations in the Potomac River in southern Maryland (Fisher's Creek, FC; Point Lookout, PL), which were considered representative of relatively pristine conditions. Two locations in the Elizabeth River near Norfolk, VA (Craney Island, CI; Cardinal Point, CP), were thought to be representative of high pollution stress. Specific sites in the latter locale were limited by the relatively few stands of *Spartina* remaining in the area. At each site, collection plots, 8 m wide and extending 5 m into the marsh (across the intertidal zone), were transsected into 0.25-m^2 quadrats. All *L. irrorata* in alternating quadrats were collected and immediately stored on ice. Two other collection plots, considered representative of intermediate pollution stress, were established at Hampton, VA (Hampton North, HN; Hampton South, HS). Collections from these plots were made from alternating 1-m^2 quadrats. All collections were performed between mid-June 1985 and mid-July 1985.

Analytical Methods. (A) Sample Preparation and Enzymatic Hydrolysis. Samples of 30–40 snails, consisting of males or females from the same site, were prepared for analysis by separating the operculum from the foot. The remaining soft tissues were combined in a tissue homogenizer, frozen in an oxymoron, and pulverized, and the resulting powder was thoroughly mixed. Three samples (~2.5 g) in separate 50-mL Nalgene screw-cap centrifuge tubes were combined with 20 mL of 5% ethanol in 0.5 M NaH_2PO_4 buffer (pH 7.5) containing 40 mg of lipase (type VII, Sigma Chemical Co.) and 40 mg of protease (type XIV) and incubated at 37 °C for 24 h.

(B) Extraction. The crude hydrolysate was combined with 5 mL of ammoniacal buffer. The diluted hydrolysate was extracted 3 times with 10 mL of 0.01% diphenylthiocarbazone (dithizone) in hexane. Centrifugation (4400 rpm) hastened phase separation. The pooled organolead dithizonates were concentrated to 1.0 mL in precalibrated tubes (equipped with screw caps and Teflon liners) under a gentle stream of N_2 at 30 °C.

(C) Modified Extraction Procedure. The crude hydrolysate was extracted 3 times with 5 mL of hexane. The organic washes were combined (fraction 1); the aqueous phase was diluted with 5 mL of ammoniacal buffer and reextracted 3 times with 10 mL of 0.01% dithizone in hexane. The combined dithizone extracts (fraction 2) were back-extracted 3 times with 5 mL of 0.01 M HNO_3. The combined acidic washes (fraction 3) were processed following the normal extraction procedure above.

(D) Derivatization. *n*-Butylmagnesium chloride (0.5 mL, 2.27 M in tetrahydrofuran, Alfa Products, Ventron Corp.) was added, under N_2, to the tubes containing organolead dithizonates. The tubes were sealed, vigorously stirred with a vortex mixer for 10 s, magnetically stirred for 10 min at ambient temperature, and then cooled in an ice bath. Excess Grignard reagent was destroyed by the dropwise addition of 1 M HNO_3. The reaction mixture was diluted to 10 mL, shaken for 30 s, and centrifuged for 5 min in a clinical centrifuge (1550 rpm). The aqueous layer was extracted with a further 5 mL of hexane. The two organic washes were combined. The organic extract from the butylation was dried over Na_2SO_4, reduced to 1 mL under a gentle stream of N_2, placed in a sample vial, and capped for immediate analysis.

(E) Sample Analysis. A gas chromatograph (GC)–quartz tube–atomic absorption spectrometer (QT–AAS) system was used for sample quantitation. Operating conditions of this system were as previously described (*34*). Each butylated extract was quantitated twice by comparison with an external standard mixture of Me_3BuPb, Me_2Bu_2Pb, Et_3BuPb, and Et_2Bu_2Pb. Mixed methylethyllead compounds were identified from predicted Kovat's retention indices on the basis of the observed retention times of alkylbutyllead standards. Actual retention times of the methylethylleads were confirmed from transalkylation reaction products. Methylethyllead quantitation was by comparison with a similar organolead analyte for which standards were available. Analyses for total lead were performed as previously described (*17*).

(F) Recoveries. Recoveries of ionic alkylleads from snails were assessed by spiking three samples of female tissue homogenate (from the PL collection site) at a level of 4–5 ng/g (as Pb) with a standard mixture of Me_3PbCl, Me_2PbCl_2, Et_3PbCl, and Et_2PbCl_2. The resulting spiked homogenate was hydrolyzed, extracted, and butylated as above. The percentage recovery was determined by dividing the mean peak area of the recovered butylate by the mean peak area of a butylated spike solution diluted to the expected (assuming 100% recovery) concentration. Recoveries of trimethyllead were corrected for the concentrations of this analyte already present in this tissue homogenate.

Results and Discussion

Within the collection plots, most snails were recovered from the lower portion of the intertidal zone; their numbers

Table I. Mean[a] Percent Recovery[b] (±1 SD) of Ionic Alkyllead Compounds from Snail Homogenate

	analyte			
	Me_3Pb^+	Me_2Pb^{2+}	Et_3Pb^+	Et_2Pb^{2+}
% recovery	105 ± 6	28 ± 5	72 ± 6	47 ± 5

[a] Based on two replicate injections of three separate determinations (i.e., $n = 6$). [b] From tissues spiked at 4–5 ng/g (as Pb) of each alkyllead chloride.

decreased toward the landward edge of the plots. The Elizabeth River samples of *L. irrorata* appeared to be abnormal in several aspects. The numbers of individuals, average sizes, and sex ratios of the other four collection sites fell within the ranges reported in the literature. In contrast, the sizes of the snails from the CP and from the CI sites were greater. These sites were characterized by an almost total absence of juveniles (shell length <15 mm) and small adults (shell length 15–19 mm). The female-to-male ratios were considerably lower at these sites, 1.02 (CI) and 1.11 (CP), relative to that at other sites, 1.32 (HN), 1.42 (HS), 1.57 (PL), and 1.66 (FC). All snails investigated in the present study were selected for similar size (shell length ~22 mm).

Ionic Alkyllead Concentrations in Snails. Recoveries, the averages of three replicate determinations, of individual alkyllead standards added at 4–5 ng/g wet wt (as Pb) to snail homogenate are reported in Table I. Average recoveries of these analytes, although less than quantitative, were comparable to analogous recoveries from chicken liver, kidney, and breast muscle (*23*).

The alkyllead burdens in representative male and female snails from each of the six collection sites are reported in Table II. The values in this table are reported on a dry weight basis (percent dry matter = 26.9) and have been corrected for recoveries (Table I). Ionic alkylleads were ubiquitous in these samples. However, they were present at very low levels (not detected to 8.0 ng/g on an as received basis). Of the seven ionic alkylleads which had been reported previously in environmental samples (R_3Pb^+, R_2Pb^{2+}; R = CH_3, C_2H_5), neither dimethyllead (Me_2Pb^{2+}) nor ethylmethyllead ($EtMePb^{2+}$) was detected in any of the snail samples. Two analytes, trimethyllead (Me_3Pb^+) and an unknown lead-containing species, were observed in all samples. In general, ethyllead salts were present at higher concentrations than mixed ethylmethylleads which were present at higher concentrations than were methyllead salts (i.e., there was a trend to decreasing concentrations from right to left across Table II). Similarly, there was a trend to decreasing burdens from top to bottom of this table.

The unknown lead-containing analyte was particularly interesting. It was observed to cochromatograph with authentic triethylmethyllead under our instrumental conditions. However, if the samples were reanalyzed by a modified extraction procedure, the unknown behaved as an ionic alkyllead salt and not as an uncharged tetraalkyllead. No alkylleads were detected if either fraction 1 or fraction 3 was injected directly into the GC–QT–AAS. Further, this modified procedure did not change the quantities of unknown observed in the final butylated extracts. Thus, the unknown was not present in the sample as a tetraalkyllead. The very low levels of these analytes precluded the possibility of the identification of this unknown by gas chromatography–mass spectrometry. A typical chromatogram of butylated snail extract recorded at 217 nm and again at 283.3 nm (after concentrating the sample approximately twofold) is presented in Figure 1.

The total ionic alkyllead burden (sum of the individual ionic alkyllead concentrations) accounted for less than 1% of the total lead burden in these samples. The fraction of total alkylleads in the total lead burden decreased with decreasing apparent pollution stress of the sites (Table III). Thus, the sum of the concentrations of individual alkyllead salts was greater in samples from Norfolk sites (CI, CP) than from Hampton sites (HN, HS) than from Maryland sites (PL, SM). In contrast, the total lead burden did not appear to be related to the anticipated pollution stress.

Statistical Analyses. An analysis of variance of the individual analyte concentrations with respect to site and to sex (Table IV) indicated significant site–sex interactions for all analytes except Et_3Pb^+ and $EtMe_2Pb^+$. The concentrations of these latter analytes varied significantly both with respect to site and to sex. Males were characterized by higher concentrations of alkyllead salts compared to females. The simple effects of sex were investigated further by an analysis for contrasts (*39*). Except for Me_3Pb^+, each of the analytes varied significantly with respect to sex.

To detect differences in the origins of individual alkyllead species, relationships among the concentrations of the various analytes were studied. A simple correlation of the variables, in which the correlation is the result of both variables varying along with some other primary variable, can be misleading (*40*). Since the alkyllead analyte levels were influenced significantly by site, sex, and

Table II. Ionic Alkyllead Levels (as Alkyllead Butylates) in Soft Tissue of Salt Marsh Periwinkles from Lower Chesapeake Bay

		mean[a] alkyllead[b] concentration[c] ± SD, ng/g dry wt[e]					
source	sex[d]	unknown	Me_3Pb^+	$EtMe_2Pb^+$	Et_2MePb^+	Et_3Pb^+	Et_2Pb^{2+}
Craney Island	M	7.5^{a} ± 0.5	3.6^{b} ± 0.3	6.7^{a} ± 3.1	12.3^{bc} ± 1.0	13.4^{b} ± 1.3	31.8^{a} ± 3.0
	F	5.2^{b} ± 0.6	3.1^{bc} ± 0.3	4.7^{ab} ± 1.0	8.0^{dc} ± 1.3	7.4^{c} ± 0.6	13.2^{b} ± 6.7
Cardinal Point	M	4.6^{bc} ± 0.7	3.0^{bc} ± 0.6	4.0^{ab} ± 1.6	16.0^{a} ± 0.7	18.4^{a} ± 5.7	14.8^{b} ± 2.5
	F	5.9^{b} ± 1.9	5.6^{a} ± 2.1	4.0^{ab} ± 1.6	18.3^{a} ± 4.7	18.3^{a} ± 3.6	13.0^{b} ± 2.9
Hampton South	M	3.4^{cde} ± 0.6	3.1^{bc} ± 1.3	4.3^{ab} ± 1.8[f]	10.3^{dc} ± 3.2[f]	9.0^{c} ± 3.4	13.3^{b} ± 4.0[f]
	F	3.1^{cde} ± 1.0	3.3^{bc} ± 1.3	3.0^{b} ± 0.1	8.9^{dc} ± 0.1	5.7^{c} ± 1.8	ND
Hampton North	M	3.4^{cd} ± 0.7	2.6^{bc} ± 0.8	4.1^{ab} ± 0.7	10.6^{dc} ± 6.1[f]	6.8^{c} ± 3.8	9.7^{bc} ± 4.8[f]
	F	2.8^{de} ± 1.0	3.0^{bc} ± 0.9	2.7^{ab} ± 1.3[f]	ND	6.0^{c} ± 0.6	6.3^{c} ± 3.4[f]
Fisher's Creek	M	3.3^{cde} ± 0.8	3.6^{b} ± 1.0	4.9^{a} ± 1.6[f]	ND	6.1^{c} ± 1.3	13.0^{b} ± 1.4
	F	1.8^{e} ± 0.2	1.7^{c} ± 0.3	ND[g]	7.3^{d} ± 0.7	6.8^{c} ± 0.7	ND
Point Lookout	M	3.5^{cd} ± 0.4	2.1^{bc} ± 0.4	3.5^{b} ± 2.1[f]	8.7^{dc} ± 1.9[f]	6.8^{c} ± 0.7	12.6^{b} ± 1.3
	F	1.8^{e} ± 0.3	3.3^{bc} ± 0.0	ND	ND	ND	ND

[a] Calculated from two replicate injections of three separate determinations (i.e., $n = 6$). [b] No Me_2Pb^{2+} or $EtMePb^{2+}$ was detected in any of the samples. [c] Corrected for mean recoveries. [d] M = male; F = female. [e] Means within a column bearing different superscripts are significantly ($p < 0.05$) different. [f] Detected in only two of the three replicate determinations. [g] None detected (less than 0.7 ng/g dry wt before correction for recovery).

Table III. Total Ionic Alkyllead[a] and Total Lead[b] Concentrations[c] in Salt Marsh Periwinkles

site	males			females		
	total Pb, μg/g	ionic alkyllead, ng/g	percent burden[d]	total Pb, μg/g	ionic alkyllead, ng/g	percent burden[d]
Craney Island	7.91	75.4	0.95	4.79	41.6	0.87
Cardinal Point	15.39	60.9	0.40	10.88	65.1	0.60
Hampton South	14.03	45.5	0.32	10.88	24.2	0.22
Hampton North	11.76	30.4	0.26	9.45	18.5	0.20
Fisher's Creek	14.21	29.4	0.21	10.92	17.6	0.16
Point Lookout	14.96	33.0	0.22	13.77	5.1	0.04

[a] Sum of the individual ionic alkylleads. [b] Mean total lead, μg/g, based on two replicate determinations. [c] Concentrations have been expressed on a dry weight basis. [d] Percent burden = (mean total ionic alkyllead/mean total lead) × 100.

Table IV. Analysis of Variance (ANOVA) of Individual Alkyllead Concentrations in Periwinkles with Respect to Site and to Sex and Simple Effect of Sex Analyzed Further with a Contrast Analysis

analyte	level of significance (p)			
	analysis of variance			contrast analysis
	site	sex	site–sex[a]	(sex)
Me_3Pb^+	0.0213	0.5246	0.0043[c]	0.2523
$EtMe_2Pb^+$	0.0112[b]	0.0086[c]	0.3454	0.0031[c]
Et_2MePb^+	0.0001	0.0237	0.0001[c]	0.0155[b]
Et_3Pb^+	0.0001[c]	0.0302[b]	0.0890	0.0016[c]
Et_2Pb^{2+}	0.0001	0.0002	0.0034[c]	0.0001[c]
unknown	0.0001	0.1218	0.0061[c]	0.0022[c]

[a] Site–sex interaction. [b] Significant at $p < 0.05$. [c] Significant at $p < 0.01$.

Table V. Pearson's Partial Correlation Coefficients for the Burdens[a] of Individual Ionic Alkylleads in Periwinkles

analyte	$EtMe_2Pb^+$	Et_2MePb^+	Et_3Pb^+	Et_2Pb^{2+}	total alkylleads
Me_3Pb^+	0.3410	0.1288	0.3130	0.1408	0.3813
prob > |r|	0.0953	0.5395	0.1276	0.5019	0.0601
$EtMe_2Pb^+$		0.3771	0.3160	0.3377	0.5615
prob > |r|		0.0631	0.1237	0.0987	0.0035[b]
Et_2MePb^+			0.6085	0.7118	0.8664
prob > |r|			0.0013[b]	0.0001[b]	0.0001[b]
Et_3Pb^+				0.5809	0.8137
prob > |r|				0.0023[b]	0.0001[b]
Et_2Pb^{2+}					0.8561
prob > |r|					0.0001[b]

[a] Degrees of freedom = 23. [b] Significant at $p < 0.01$.

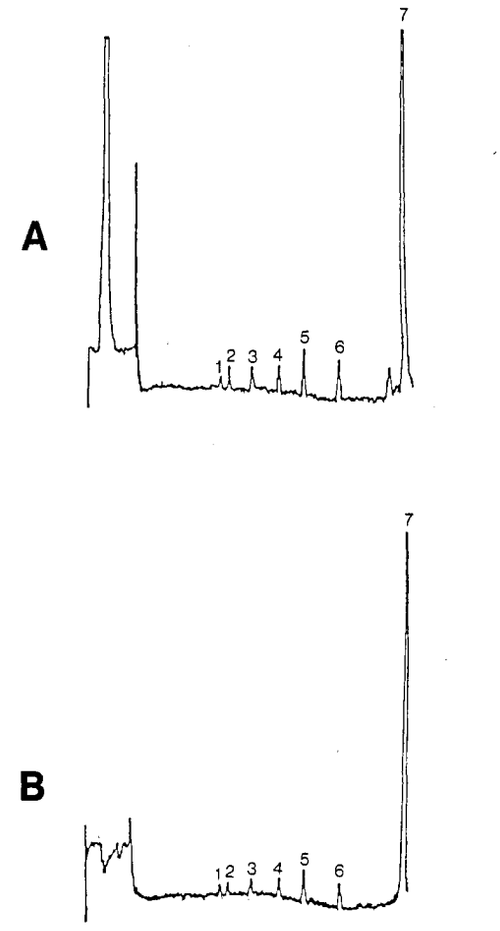

Figure 1. GC–AAS chromatograms of a typical periwinkle sample at (A) 217 nm and at (B) 283.3 nm containing (1) Me_3BuPb, (2) unknown lead-containing peak, (3) $EtMe_2BuPb$, (4) $Et_2MeBuPb$, (5) Et_3BuPb, (6) Et_2Bu_2Pb, and (7) Bu_4Pb. The sample was concentrated approximately twofold prior to analysis at 283.3 nm.

their interaction (Table IV), partial correlations would be useful in determining the relationships (if any) among the analytes. This approach allows one to correlate levels of one analyte with levels of another, if adjustment for the influencing factors are made. With the exception of Me_3Pb^+, the concentration of each of the alkyllead salts was correlated significantly with the total ionic alkyllead level from the same site (Table V). Concentrations of Et_2MePb^+, Et_3Pb^+, and Et_2Pb^{2+} were also correlated significantly ($p < 0.01$) with each other. In contrast, $EtMe_2Pb^+$ and Me_3Pb^+ were not correlated significantly ($p > 0.05$) with any of the other individual alkyllead analytes. Interestingly, as the number of methyl groups on the analyte increased, the levels of correlation with ethyllead (Et_3Pb^+ or Et_2Pb^{2+}) burdens decreased.

The significant correlations between levels of Et_3Pb^+ and Et_2Pb^{2+} were not unexpected and indicate a common origin (from Et_4Pb gasoline additive) for these analytes. Similarly, the significant correlation of levels of ethyllead analytes with concentrations of Et_2MePb^+ indicates that Et_2MePb^+ also results from the dealkylation of gasoline additive. The lack of a significant correlation between the concentrations of Me_3Pb^+ and either of the ethyllead salts suggests a difference in origin of this analyte and the origin of the ethylleads.

Because the initial data set contained several "not detected" values, partial correlation coefficients were also determined in which only data pairs were considered. Thus, if one analyte of the data pair was not detected, the pair was ignored. This explains the varying degrees of freedom in Table VI. Again, concentrations of Et_2Pb^{2+} were correlated significantly with levels of Et_3Pb^+ ($p < 0.05$) and Et_2MePb^+ ($p < 0.01$). In addition, $EtMe_2Pb^+$ concentrations were correlated significantly ($p < 0.05$) with

Table VI. Pearson's Correlation Coefficients between Alkyllead Concentrations in Periwinkle Tissue

analyte	$EtMe_2Pb^+$	Et_2MePb^+	Et_3Pb^+	Et_2Pb^{2+}
Me_3Pb^+	0.1510	0.2856	0.3130	0.0987
prob > |r|	0.5627	0.2835	0.1458	0.7160
df[a]	16	14	21	14
$EtMe_2Pb^+$		0.4967	0.2901	0.4584
prob > |r|		0.0503	0.2282	0.0420[b]
df		14	17	18
Et_2MePb^+			0.5482	0.6459
prob > |r|			0.0649	0.0021[c]
df			10	18
Et_3Pb^+				0.4856
prob > |r|				0.0410[b]
df				16

[a] Degrees of freedom. [b] Significant at $p < 0.05$. [c] Significant at $p < 0.01$.

Table VII. Analysis of Variance and Partial Correlations[a] between Combined Mixed Alkyllead,[b] Combined Ethyllead,[c] and Me_3Pb^+ Concentrations

	level of significance		
analytes	site	sex	site–sex
Me_3Pb^+	0.0401	0.4507	0.0103[d]
Et_2MePb^+ and $EtMe_2Pb^+$	0.0001[e]	0.2819	0.1270
Et_3Pb^+ and Et_2Pb^{2+}	0.0001[e]	0.0029[e]	0.1953

analyte(s)	mixed alkyls[b]	mixed ethyls[c]
Me_3Pb^+	0.2617	0.2653
prob > |r|	0.2064	0.2000
mixed alkyls		0.6398
prob > |r|		0.0006[c]

[a] For 23 degrees of freedom. [b] Sum of Et_2MePb^+ plus $EtMe_2Pb^+$. [c] Sum of Et_3Pb^+ plus Et_2Pb^{2+}. [d] Significant at $p < 0.05$. [e] Significant at $p < 0.01$.

Et_2Pb^{2+} and highly correlated ($p = 0.0503$) with Et_2MePb^+ concentrations. There was also a pronounced lack of correlation between the concentrations of Me_3Pb^+ and the concentrations of any of the other ionic alkylleads.

A third statistical model was also considered. So as to be able to consider all the data for detectable concentrations of the analytes, concentrations of ethylleads (Et_3Pb^+ plus Et_2Pb^{2+}) were summed together for each site and sex and compared with the concentration of pooled mixed alkylleads (Et_2MePb^+ plus $EtMe_2Pb^+$) and with the concentration of Me_3Pb^+. Analysis of the pooled data (Table VII) demonstrated that ethyllead concentrations varied significantly among the sites and were sex dependent, that combined mixed alkyllead concentrations were site dependent but not sex dependent, and that site–sex interactions were significant for Me_3Pb^+. Whereas concentrations of the combined mixed alkylleads were correlated significantly ($p < 0.01$) with concentrations of combined ethylleads, the concentration of Me_3Pb^+ lacked a significant correlation with concentrations of either the mixed alkylleads or the ethylleads.

Finally, a statistically significant ($p = 0.0001$) negative ($r = -0.9664$) correlation was observed between the fraction of trimethyllead in the total ionic alkyllead burden and the fraction of ethyllead salts plus mixed alkylleads in the total alkyllead burden. This negative correlation reflects the significant correlation of individual ethyllead concentrations with the total ionic alkyllead burden, the high correlation of mixed alkylleads (Et_2MePb^+ or $EtMe_2Pb^+$) with the total ionic alkyllead burden, and the lack of variability of Me_3Pb^+ concentrations among the sites.

Conclusions

These statistical analyses indicate separate sources of Me_3Pb^+ and ethylleads to the periwinkles. A considerable portion of Me_3Pb^+ must have come from sources other than directly from demethylation of Me_4Pb gasoline additive. In addition, these results are consistent with a second source of mixed ionic alkyllead salts which would dilute the pool of mixed ionic alkylleads from gasoline additives. An environmentally mediated methylation of ethyllead salts is postulated to account for the reduced levels of correlation. The observation that the concentrations of ethyllead and mixed alkyllead salts in snails were sex dependent was unexpected.

Registry No. Me_3Pb^+, 14570-16-2; Me_2Pb^{2+}, 21774-13-0; Et_3Pb^+, 14570-15-1; Et_2Pb^{2+}, 24952-65-6; $EtMe_2Pb^+$, 103730-90-1; Et_2MePb^+, 105956-70-5.

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Received for review June 25, 1987. Accepted January 20, 1988. It is a pleasure to acknowledge financial support from the Natural Science and Engineering Research Council of Canada.

Chemistry of Individual Aerosol Particles from Chandler, Arizona, an Arid Urban Environment

James R. Anderson, F. John Aggett,[†] Peter R. Buseck,* Mark S. Germani,[‡] and Thomas W. Shattuck[§]

Departments of Chemistry and Geology, Arizona State University, Tempe, Arizona 85287

■ Atmospheric aerosol particles, collected at a site in Chandler, AZ, over a 12-day period in February and March 1982, were chemically analyzed with an automated scanning electron microscope. The fine-particle fraction, with minimum average diameters of 0.4 μm and effective maximum diameters of about 2 μm, contains numerous types of Pb-, Cu-, Zn-, P-, and S-bearing particles, most of probable anthropogenic origin. The concentrations of selected particle types, each with distinct elemental associations, have been tracked over the sampling period, thereby providing information about particle emission and transport. The concentrations of particle types containing one or more metals and S were strongly dependent upon prevailing wind direction; the predominant source was copper smelters located to the southeast of Phoenix. Other particle types probably had local sources, some of which were intermittent. The identification and tracking of chemically distinct types of individual particles are demonstrated to be powerful tools for understanding complex atmospheric aerosol systems.

Introduction

Airborne particles of respirable size, whether of natural or anthropogenic origin, form an important fraction of all atmospheric aerosols. Their importance stems not only from their impact on health and the environment but also from their usefulness as chemical markers of individual pollution sources. Chemical characterization of atmospheric aerosol particles has been largely limited to either bulk samples or small numbers of individual particles. Bulk analysis data have been used in receptor modeling techniques, such as chemical element balances, factor analysis, and target-transformation factor analysis (*1-3*). In many cases the information provided by these techniques can be provided more directly and precisely by the analysis of individual particles. For complex aerosols, a combination of individual particle and bulk methods is optimal, as the individual particle major element data can be combined with trace element data from the bulk samples.

The scanning electron microscope (SEM) with X-ray spectrometers has been an extremely powerful tool for the analysis of particles. A major limitation initially was the difficulty in analyzing submicron particles (*4*). A second limitation was the problem of acquiring the large number of particle analyses needed to characterize an atmospheric aerosol. A solution to the latter problem has been the automation of the SEM and its analytical systems (*5*, *6*).

The advantages of individual particle versus bulk analysis are made clear by studies such as that of Post and Buseck (*7*) of the Phoenix aerosol. They used manual analysis methods but still treated about 8000 particles. They were able to directly determine the chemistry and size distribution of major particle types at each site. Automated methods using an electron microprobe have been used in recent studies to determine the chemistry and size distribution of individual particles in marine atmospheric aerosols and estuarine samples (*8*, *9*).

The objectives of this study were (a) to determine the chemical compositions of selected anthropogenic aerosol particles from a site on the periphery of the Phoenix metropolitan area (the 26th largest U.S. metropolitan area in 1980) and (b) to illustrate the methods employed for automated analysis of particles from an atmospheric aerosol. A series of aerosol samples collected during a 2-week period at a site in Chandler, AZ, 27 km SE of Phoenix, were used (Figure 1). Results from the fine-particle fractions (0.4-~2 μm in diameter) are emphasized here because of their high content of particles of probable anthropogenic origin.

The Chandler aerosol is complex, as it represents a combination of aerosol contributions from the Phoenix urban area, the surrounding desert, and several major copper smelters that lie about 120 km to the southeast. It has been possible to identify a number of distinct anthropogenic particle types and to monitor their concentrations over the collection period.

The general setting of the aerosol of the Phoenix area has been described by Post and Buseck (*7*). In another study, Post and Buseck (*10*) attempted to identify the

[†] Present address: Department of Chemistry, University of Auckland, Auckland, New Zealand.
[‡] Present address: McCrone Associates, Chicago, IL 60616.
[§] Present address: Department of Chemistry, Colby College, Waterville, ME 04901.

0013-936X/88/0922-0811$01.50/0