THE NATURE AND CONCENTRATION OF ORGANOMETALLICS IN NATURAL WATERS

by

HUGH ROBERT POTTER

A thesis presented for the
degree of Doctor of Philosophy
in the University of Aston
in Birmingham.
January 1976.

SUMMARY

Certain compounds containing lead atoms bonded to alkyl groups are of much greater toxicity than their non-alkylated analogues. The little research that has been carried out into the occurrence of such compounds in the environment has almost entirely been concerned with atmospheric concentrations. This dissertation seeks to quantify the amounts of alkyl lead compounds in natural waters.

The major use of alkyl lead compounds is as anti-knock agents in petrol and since they can subsequently be released to the environment through spillage, evaporation, and exhaust emission, this was considered to be the most probable source of alkyl lead compounds in natural systems.

Extraction and estimation procedures for alkyl lead compounds in water and in sediment were developed and spectroscopic studies of pure alkyl lead compounds were made for positive identification.

A widely based geographical survey was carried out to determine the normal concentrations of alkyl lead compounds in natural waters and to locate specific sources. Sampling sites were chosen such that all possible types of waters were sampled in a variety of environments. Alkyl lead compounds were only detected in certain road drainage grids, and concentrations significantly above the limits of detection were observed only in grid sediments from forecourts of garages dispensing petrol.

Since methylation of inorganic lead salts by micro-organisms could also introduce alkylated lead species into the environment, the ability of various biologically active systems to methylate lead salts was studied. In none of these was biological methylation observed despite the known methylation of mercuric salts under similar conditions. An apparent biological methylation of trimethyl lead salts was shown to proceed by a chemical disproportionation.

The dissertation concludes that the concentrations of alkyl lead compounds in natural waters does not constitute any hazard to public health.

ACKNOWLEDGEMENTS

I should like to express my thanks to the following: Dr. A.W.P. Jarvie for her advice and encouragement throughout the course of this work; the Associated Octel Company for the provision of valuable chemicals, and for details of their 'PAR' analytical method; Dr. A.W. Downs for his invaluable suggestions and observations; the staff of the Biological Sciences Department for their help and co-operation; the technical staff of the Chemistry Department for many of the spectra recorded; and the Natural Environmental Research Council who sponsored this project.

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CHAPTER ONE

INTRODUCTION

INTRODUCTION

1.1. DEVELOPMENT AND MECHANISM OF TETRA-ALKYL LEAD COMPOUNDS AS ANTIKNOCK AGENTS.

In their attempt in 1916 to improve the power and economy of the V-12 Liberty aeroengine, Midgeley and Boyd, under the direction of Charles Kettering, began a systematic study of chemical additives for petrol. The specific aim of the project was to prevent combustion 'knock' which was reducing efficiencies of the engine. In their study of over 100 compounds, tetraethyl lead (TEL) was found to be the most effective antidetonant and is still in worldwide use today. Discovered in 1921, it became generally available as an additive to petrol for road vehicles in 1925 and remained the only commercially significant antiknock additive until 1960. At this time tetramethyl lead (TML) and the mixed alkyl leads (Me_3EtPb, Me_2Et_2Pb, and $MeEt_3Pb$) were introduced complementary to the use of TEL[1,2].

In a conventional petrol engine the homogeneous fuel-air mixture in the combustion chamber is subjected to high temperature and pressure as the piston advances. The compressed mixture is then ignited by a spark and the flame theoretically progresses uniformly through the mixture producing a smooth rise in pressure up to the end of compression. However the advancing flame subjects the compressed unburned fuel-air mixture ('end gas') to even higher temperatures and pressures and if the mixture is insufficiently resistant to autoignition causes it to ignite spontaneously in a much more rapid combustion and hence create violent vibrations in the cylinder. This is translated into everyday driving as 'knocking' or 'pinking' of the engine.[114]

It is thought that the extreme conditions created in the end gas lead to decomposition of some of the hydrocarbon molecules and to the formation of hydroperoxides with the oxygen present. It is the subsequent explosion of these hydroperoxides which causes the hammer blow to the pistons instead of a smooth build up of pressure[1,2].

The propagation steps for hydroperoxide formation are:

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$
$$ROO^{\bullet} + HR \longrightarrow ROOH + R^{\bullet}$$

When tetra-alkyl lead (TAL) is present it can decompose the hydroperoxide thus:

$$R_4Pb \longrightarrow Pb^{\bullet} + 4R^{\bullet}$$
$$Pb^{\bullet} + ROO^{\bullet} \longrightarrow PbO^{\bullet} + RO^{\bullet}$$

forming less active products.

Another possible mechanism is:

$$ROO^{\bullet} + R'_4Pb \longrightarrow ROOPbR'_3 + R'^{\bullet}$$

since the rate of reaction of $ROO^{\bullet}$ is much faster at a multivalent metal atom than at a hydrogen centre or alkyl ligand[3].

1.2. EFFECTS ON HEALTH

17 months after the commercial manufacture of TEL began in the U.S.A. in 1923, 139 cases of encephalopathy with 13 deaths had been reported on three separate manufacturing plants. The victims were not only workmen who were ignorant of any danger, but chemists who handled the liquid recklessly, being unaware of the extreme toxicity of the compound. As a result its sale and production were banned for a year until more rigorous data were available on its toxicity. Protective

clothing and breathing apparatus were developed for its bulk handling but it was shown that there was no danger in handling and dispensing petrol containing TEL at filling stations. Nevertheless a dye was added to petrol to indicate that it contained TEL and that it should not be used for cleansing the skin[4]. TAL is absorbed into the body by inhalation of the vapours or by skin absorption[5].

One of the greatest problems concerning the toxicity of TAL is that there are no specific symptoms. Reported symptoms include agitation, excitement, delusions, insomnia, nightmares, impairment of memory, and loss of concentration[4,6]. It is a psychotic poison and the symptoms closely mimic those of conventional psychotic disorders[7]. An example of the difficulty of diagnosis occurred under war conditions in the Middle East. Poor supervision of the cleaning of petrol tanks led to 200 cases of poisoning with 40 deaths. Many of these, because of the symptoms, were not recognised soon enough. They were mistaken for drunkards and lunatics and were sometimes beaten or starved to death instead of being treated properly[4].

Unlike inorganic lead poisoning there is no correlation between the amount of TAL ingested and the blood lead levels[7,8,115]. Thus unless exposure to TAL is known, diagnosis of the cause of poisoning is difficult.

There is no known positive cure for TAL poisoning, EDTA and BAL (2,3 dimercapto-1-propanol) which are used for inorganic lead poisoning being ineffective owing to their inability to complex with the toxic metabolite R_3Pb^+ formed from TAL in the liver[9].

1.3. ACTION IN THE BODY

In rats and rabbits it has been shown that the toxic moiety is the water soluble trialkyl lead cation (R_3Pb^+) formed by dealkylation of the water insoluble TAL. TAL administered to these animals is converted to R_3Pb^+ in vivo[10,11] and by their liver cell microsomes in vitro[9,11]. Once formed, the trialkyl lead species is relatively stable[10,11] and affects the central nervous system by inhibiting the oxidation of glucose[13]. The effects of administering triethyl lead salts is identical to that for TEL[9] but the LD_{50} values for the methyl and propyl derivatives are much greater for the trialkyl than for the tetra-alkyl species. Since methyl and propyl derivatives are dealkylated much more slowly than TEL, the relative toxicity of tetra-alkyl lead compounds is thought to be dependent on the rate of conversion to the trialkyl lead species[13]. Thus the lipid soluble TAL is absorbed through the skin and lungs and converted into the water soluble trialkyl lead cation which is then free to circulate in the body.

In three fatal cases of poisoning by TEL, high concentrations of Et_3Pb^+ were found in the liver, kidney, brains, and pancreas. The values of 2.0 to 22.0 μgg^{-1} were in the same range as the total lead content[14].

Administration of diethyl lead compounds has a different effect from that of tri- or tetraethyl lead, the first named being much less toxic, and comparable in effect to inorganic lead[9]. Like inorganic lead but unlike TEL or Et_3Pb^+, Et_2Pb^{++} can be complexed by BAL. Of these species only inorganic lead is complexed by EDTA[9].

1.4. POTENTIAL SOURCES OF TAL

In 1973, 12,000 tonnes of lead in the form of TAL were used as antiknock additives to petrol in the U.K.[15] Most of this is burnt in internal combustion engines to form inorganic lead salts which are partly retained in the engine and exhaust system but mainly emitted to the atmosphere in a finely dispersed particulate aerosol[16]. However a certain fraction of the 12,000 tonnes escapes unburnt and in view of the much greater toxicity of TAL over inorganic lead, could present a potentially very serious health hazard. This dissertation seeks to quantify further the potential hazard.

Surprisingly few figures have been published on the concentrations of alkyl lead compounds emitted from vehicle exhausts. Wallin[17] quoted that less than 0.03% of the total lead exhausted was TAL and Hirschler and Gilbert[18] that 0.023% of total lead input was emitted as TAL, or approximately 10 μgm^{-3}. The most detailed work is that of Laveskog[19] in which he estimated and identified TAL in air, crank-case emissions, and exhaust gases. For exhaust gases he reported that the majority of TAL was emitted during the first few minutes from a cold start:

Conditions	Concentration of TAL (μgm^{-3})
Cold start, +10 to 20°C.	1000–5000
Idling, warm engine.	50–1000
Driving at constant speed, warm engine.	5–100

Taking an average figure of 50 μgm^{-3} and comparing this with Hirschler and Gilbert's figures gives approximately 0.1% of total lead input exhausted as TAL. On a consumption of 12,000 tonnes p.a. this amounts to 12 tonnes p.a. of lead as TAL exhausted to the atmosphere.

The other important potential source of TAL in the environment is from evaporation of unburnt petrol. This can occur around the carburettor, from crank-case ventilation, from accidental spillage, and from expulsion of vapour during tank filling[20].

Laveskog[19] suggested that 20–80% of the total TAL emission from vehicles came from crank-case emissions which would correspond to a further ca. 12 tonnes p.a. of TAL volatilised into the atmosphere.

The last source mentioned above can also be partly quantified since it has been estimated that 4.7g of petrol vapour escapes per gallon of petrol added to a tank[21], or approximately 0.1%. TEL (B.p. 200°C) tends to concentrate in the heavy ends of average commercial petrol but TML (B.p. 110°C) has a volatility comparable with the higher fractions. The relative volatilities of TEL and TML at 20°C are 1:100[1,5]. Thus, assuming an equal use of TEL and TML and neglecting the use of mixed alkyl leads which is rare in the U.K.[22], this would amount to the vaporisation of approximately 6 tonnes p.a. per transference of petrol. Transference must occur at least three times: from storage tanks to road tankers; from road tankers to underground storage tanks at garages; and from petrol pumps to vehicle tanks, making a total of ca. 18 tonnes p.a. overall.

Adding this to the previous figures we have 42 tonnes of lead as TAL annually discharged into the atmosphere excluding any other sources mentioned above and any TAL that may be volatilised during manufacture. A recent report suggests that the total may be nearer to 140 tonnes[15].

The figures reported for alkyl lead concentrations in air vary greatly, possibly due to differing analytical techniques. Figures range from 0.0 to 3.5 μgm^{-3} and from 0 to 55% of total airborne lead. In general the higher figures relate to sampling sites in the vicinity of petrol filling stations[19,23,24] 113.

Outside the field of antidetonant additives for petrol, commercial applications of organolead compounds are relatively few and small, although several fields are being investigated[2,25,26,27]. These have not been considered in this work as possible sources of environmental pollution.

1.5. POSSIBLE BREAKDOWN PRODUCTS

The breakdown of TAL to trialkyl lead cations in the body, and the toxicity of these ions has been discussed above. In a study of the potential hazards of alkyl lead compounds in the environment it was therefore necessary to consider not only the occurrence of TAL initially released, but also of possible breakdown products. It was thought that the most likely breakdown products would be R_3Pb^+, R_2Pb^{++} and Pb^{++}, RPb^{+++} (R=alkyl) being unstable[28]. Occurrence of Pb^{++} in the environment was outside the scope of this work.

Thus in addition to the detection and determination of TAL in the environment, this dissertation also includes a study of the di- and trialkyl lead salts.

1.6. SYSTEMS STUDIED

The area of study was confined to aqueous systems for two main reasons. Firstly, TAL whether it decomposes or not would be expected to be washed out of the atmosphere during rainfall and enter the drainage systems as is the case for inorganic lead[29,30]. Secondly these drainage systems are eventually channelled into the rivers and these are increasingly being considered as future drinking water supplies. Although total lead analysis is carried out on drinking water, it is conceivable that concentrations of lead within the statutory limits are dangerous owing to a high proportion of lead being in an organic form. It was important therefore to have a method of analysis for the various alkyl lead compounds so that distinction could be made and a survey carried out to ascertain whether or not a potential hazard existed.

1.7. DATA AVAILABLE

Very little data is available on organolead compounds at low concentrations.

There is a considerable volume of literature on the properties of organolead compounds at high concentrations resulting from their mass production as antiknock agents, and in search of related products and of other uses[2,27,31].

There has been an increasing number of papers concerning mercury and organomercury at low concentrations[32] to which lead and its compounds are in some ways analogous. Obvious similarities are that both are 'B' metals and for both elements the alkyl compounds are of greater toxicity than the aryl compounds which are of greater toxicity than the inorganic compounds.

Although these data could be combined to predict the properties of alkyl lead compounds at low concentrations, there was a need for much basic groundwork to be carried out before any research on alkyl lead compounds in the environment was commenced.

1.8. SURVEY

After an initial study of the properties of alkyl lead compounds at low concentrations, extraction techniques were developed to enable estimation of alkyl lead compounds in natural systems. These were then utilised in a survey of the locality to assess any potential health hazard from the use of TAL in petrol.

1.9. METHYLATION OF INORGANIC LEAD

A second potential source of alkyl lead compounds in the environment is methylation of inorganic lead by natural systems. Although methylation of inorganic mercury is well established[32], very few studies have been reported for analogous reactions of lead, despite general similarities of the two elements and their compounds. The methylation of lead would be less likely to occur than that of mercury if the mechanism is one of consecutive monomethylation owing to the instability of the monomethyl lead species[28]. However an investigation of possible chemical and biological methylating systems was carried out.

CHAPTER TWO

PROPERTIES AND ANALYSIS OF ALKYL LEAD COMPOUNDS

2.1. Properties

2.3. Analysis

PROPERTIES AND ANALYSIS OF ALKYL LEAD COMPOUNDS

2.1. PROPERTIES

2.1.1. General

This dissertation is concerned with two distinct groups of organolead compounds. The first group comprises the tetra-alkyl leads: tetraethyl lead (TEL), tetramethyl lead (TML), and the mixed alkyl leads, i.e. methyltriethyl lead ($MeEt_3Pb$), dimethyldiethyl lead (Me_2Et_2Pb), and trimethylethyl lead (Me_3EtPb). Some or all of these compounds are present in all commercially available antiknock fluids and are the only organolead compounds so used[2]. The second group comprises the possible breakdown products of these compounds, i.e. $Me_nEt_{3-n}PbX$ (n=0-3) and $Me_nEt_{2-n}PbX_2$ (n=0-2). Owing to the ionic nature of these compounds in aqueous solution[31], the anion X is of little significance, and in the present work was usually chloride, acetate, or hydroxide.

The properties of TEL and TML have been extensively studied and those of the mixed alkyl lead compounds are generally assumed to lie between those of the two symmetrical compounds. These properties have been reviewed elsewhere[2,28,31,33]. Spectral data have also been independently reviewed[34,35]. Since the alkyl lead salts lack any large scale commercial applications, less data are available on their properties but these have also been reviewed[28,31].

2.1.2. Physical nature and handling

The tetra-alkyl lead compounds are clear, colourless, volatile liquids which are soluble in the common organic solvents but insoluble in water[31]. TEL boils at 200°C and has a vapour pressure of 0.26mmHg at

20°C. TML boils at 110°C and has a vapour pressure of 23.7 mmHg at 20°C. Both are heavy liquids, their densities being 1.650 and 1.995 gml^{-1} respectively[5].

All the tetra-alkyl leads are highly toxic and are absorbed into the body via inhalation and by contact with the skin. They are sufficiently stable to air, hydrolysis, and heat to permit their handling without undue problems or precautions, but their toxicities decree that they must always be handled and stored in an efficient fume cupboard[5]. Owing to their instability in ultra violet light they should be stored in the dark and not handled in direct sunlight. Dilute solutions in benzene or petroleum ether were found to be indefinitely stable when stored in the dark[36].

The alkyl lead salts R_3PbX and R_2PbX_2 are generally white crystalline solids. Where X is the anion of a strong acid the compounds are usually stable to air, water, and gentle heat. When X derives from a weak acid the salts tend to disproportionate[2]. Nevertheless all the salts decompose eventually and for maximum purity should be stored at $\leqslant 0^{\circ}C$ in the dark.

The toxicity of R_2PbX_2 is comparable with that of inorganic lead, but that of R_3PbX is comparable with that of TAL. However handling of R_3PbX does not present the same hazards as that of TAL since these salts are solids and are not easily absorbed through the skin or inhaled. If however the dust of these salts is inhaled it has strong sternatory properties. A number of related compounds were studied for possible offensive uses during the second world war[37].

In our study of detection and determination of organolead compounds in natural waters the solubilities of the salts in different solvents were of considerable importance and the published data[27,38] are summarised in Table 1 along with observations made in the present work.

TABLE 1

Solubilities of alkyl lead salts

	Water	$CHCl_3$	CCl_4	Acetone	Ether	Benzene	Hexane	Alcohol
Et_3PbCl	20	s	50	50	6	800	0.9	50
Et_3PbBr	13		50	50	50	700	9	50
Et_3PbOH	198	s		42.1	1.8	8	0.6	
Et_3PbOAc	60	450		21(r)	2.3	8.6	6	450
$(Et_3Pb)_2CO_3$	8.3		0.017	0.72	0.023	0.016	0.018	17.5
Me_3PbBr	100	275		350	25	50	3	450
Me_3PbOAc	135	13		4	0.3	1	2	100(r)
Et_2PbCl_2	50	i	0.04	22.2	0.03	0.04	0.02	21
Et_2PbBr_2	15		0.02	50	0.09	0.08	0.0	10
$Et_2Pb(OH)_2$	34						0.0	
Et_2PbCO_3	0.21		0.006	0.016	0.01	0.009	0.03	0.013
$PbCl_2$	9.9		0.0	0.003	0.0	0.0	0.0	0.015
$PbBr_2$	8.4		0.0	0.1	0.0	0.0	0.0	0.053

s soluble

i insoluble

r solvent suitable for recrystallisation

2.2. PREPARATION OF ALKYL LEAD SALTS

From a study of the literature it was apparent that certain data were lacking for some of the alkyl lead salts. Apart from being able to provide these data, it was also desirable to have a range of alkyl lead salts available for study during the development of analytical and extractive techniques. Consequently the ethyl lead salts detailed below were prepared by standard procedures. Their infra red and nuclear magnetic resonance spectra were recorded and are discussed in the relevant sections of this chapter where they are compared with the reported data for the methyl lead salts.

Et_3PbCl was prepared according to the method of Heap et al[39] and its identitiy confirmed by comparison of its infra red spectrum with that previously reported[40] and by thin layer chromatography (TLC) against a genuine sample. In the latter method both samples showed a trace of Et_2Pb^{++} which is attributed to decomposition of a small fraction of Et_3PbCl on the TLC adsorbent. Silica is reported to catalyse the decomposition of TAL to tri- and dialkyl lead salts by acetic acid[27].

Et_2PbCl_2 was prepared by two methods, but that of Heap et al[41] gave excessive amounts of inorganic lead as judged by TLC. Identity was confirmed as above.

Et_3PbOH was prepared by the method of Calingaert et al[42] and its identity confirmed by comparison of the infra red spectrum with that previously recorded[43]. As the infra red spectra of Et_3PbOH and $Et_2Pb(OH)_2$ are similar[43], further confirmation of the nature of the cation was obtained by TLC. The sample gave a spot of the characteristic colour of Et_3Pb^+, but at a lower R_f value than Et_3PbCl. In the solvent system used (benzene-acetic acid, 9-1) Et_3PbOH, owing to its basic properties[31], would be converted to Et_3PbOAc. This compound will be more strongly adsorbed onto silica than Et_3PbCl owing to the nature of the anion, and thus will have a smaller R_f value. Et_3PbOAc as prepared below and Et_3PbOH gave identical spots on TLC.

$Et_2Pb(OH)_2$ was prepared by the method of Heap et al[41] and its identity confirmed as for Et_3PbOH.

Et_3PbOAc was prepared according to the method of Heap et al[37]. Its identity was confirmed by its melting point and by TLC, the R_f value being identical with that of Et_3PbOH. The compound was also prepared by neutralisation of Et_3PbOH with acetic acid, thus demonstrating the basic properties of the alkyl lead hydroxides[31].

$Et_2Pb(OAc)_2$. Preparation of this compound was only partly successful owing to the difficulty of its isolation, but the infra red spectrum and TLC were recorded.

Et_2PbI_2. Preparation of this salt was attempted by a method analogous to that of the methyl lead iodides[44,45], but conditions were insufficiently severe. TLC and infra red spectroscopy indicated that a mixture of Et_2Pb^{++} and Et_3Pb^+ was present, but from the nuclear magnetic resonance spectrum it appeared that Et_3PbI was the main product.

2.3. ANALYSIS

2.3.1. General

After isolation from natural systems any suspected organolead compound would require both identification and estimation. These two processes tend to be distinct and separate. Those techniques which give a positive identification of a compound (e.g. infra red and nuclear magnetic resonance spectrosopy) tend to be unsuited to quantitative determinations and those techniques which give a quantitative measure of a compound (e.g. gas liquid and high pressure liquid chromatography, colorimetric techniques) cannot give a positive identification owing to the possibility of unknown species interfering. Thus the analytical techniques studied in this work fall into two categories. The first concerns positive identification of compounds and includes mass spectrometry, infra red and nuclear magnetic resonance spectroscopy, and thin layer chromatography. The second concerns the estimation of alkyl lead compounds and includes a specific extraction technique followed by total lead analysis, gas liquid chromatography, high pressure liquid chromatography, and a colorimetric technique.

2.3.2. Mass Spectrometry

Owing to the volatility of tetra-alkyl lead compounds a considerable volume of literature is available on their mass spectra which has been reviewed elsewhere[34]. Mass spectrometry coupled with gas chromatography has been used to detect and identify trace quantities of TAL in the atmosphere[19]. For both TEL and TML the abundance of ions is in the order $R_3Pb^+ > RPb^+ > Pb^+ > R_2Pb^+ \gg R_4Pb^+$, the parent ion accounting for only 0.5% of the total[46,47]. In the mixed alkyl leads, ethyl groups are lost in preference to methyl groups[48].

The volatility of alkyl lead salts is much lower than that of the tetra-alkyl leads and no mass spectra have been reported. Attempts in the present work to determine spectra of ethyl lead chlorides gave only very weak patterns corresponding to alkyl lead ions, and in all cases those including an iodine or bromine atom were dominant, despite the purity of the ethyl lead chlorides and the absence of bromine or iodine in their preparation. It is thought that the iodides and, to a lesser extent, the bromides are much more volatile than the chlorides and as a consequence trace quantities of these are detected whilst the chloride remains completely undetected.

2.3.3. Infra red spectroscopy

Infra red studies of TML have been numerous[49-53] but those of TEL[40,49,54] and of the mixed alkyl leads[49] less so. For the methyl lead salts, the following have been reported: Me_3PbCl[40,44,55,56], Me_3PbF, Me_3PbBr[40,44], Me_3PbI[44], Me_3PbOAc, $Me_3PbOOCH$, $Me_3PbOOCH_2Cl$[57], Me_2PbCl_2[44,55,56], and Me_2PbBr_2[44]. Of the ethyl lead salts only the following have been reported: Et_3PbCl[40], Et_2PbCl_2[58], Et_3PbOH, and $Et_2Pb(OH)_2$[43]. In addition to the infra red spectra of these ethyl lead compounds, those of Et_3PbOAc and $Et_2Pb(OAc)_2$ have also been recorded in the present work and the principal vibration frequencies are listed in Table 2.

From the spectra of various Me_3PbX compounds it has been deduced that the compounds are ionic, even in the solid state and that the Me_3Pb^+ ion is planar[55,57]. Me_2PbCl_2 is ionic and the Me_2Pb^{++} ion linear[55]. The spectra reported in the present work for ethyl lead compounds confirm that this is also the case for these compounds. The spectra of Et_3PbOAc and $Et_2Pb(OAc)_2$ consist of the superimposition of the spectrum of the acetate ion as in NaOAc or PbOAc and that of Et_3PbCl and Et_2PbCl_2 respectively. Apart from the vibration frequencies of the anions, the spectra of Et_3PbX (X = Cl, OAc, OH) are almost identical, corresponding to vibration frequencies of the Et_3Pb^+ species. Similarly the spectra of Et_2PbCl_2 are almost identical, corresponding to that of Et_2Pb^{++}.

The main contributions to the spectra obviously originate in vibrations of the single CH_3-CH_2-Pb grouping since the spectra of Et_4Pb, Et_3PbX, and Et_2PbX_2 are very similar. Distinctions which could be made between the spectra of Et_3PbX and Et_2PbX_2 compounds

TABLE 2 Principal vibration frequencies (cm^{-1}) of ethyl lead compounds

Et_4Pb	Et_3PbCl	Et_3PbOAc	Et_3PbOH	Et_2PbCl_2	$Et_2Pb(OAc)_2$	$Et_2Pb(OH)_2$	NaOAc	PbOAc	
43	a	a	a	a	a	a	42	a	Ref.
		656		[illegible]	660	[illegible]	647	660	
656 674	680	681	680						
				710	720	700b			
		925					923	931	
930	942	944	945	945	937	942			
957	961	964	960	958	970	965			
		1010			1017		1007	1015	
		1040					1045	1047	
1013	1021	1021	1020						
1153	1150	1152	1151	1136	1162	1160			
1224	1223	1224	1224	1211	1223	1225			
		1337	1340		1340		1339	1340	
1377	1374	1374	1370	1372	1372	1372			
1428	1421	1410b	1410b	1431	1425b	1419	1425	1410	
1463	1453	1455	1455			1460b			
		1570			1575		1582	1540	
2725									
2865	2865	2865	2865	2860w	2850	2870			
2941	2930	2930	2930	2920	2920	2925			
	2950	2950	2950	2970	2960w	2960			
	2980	2980	2980	2985					
			3450b			3500b			
			3620						

a. This work

b. Broad band

w. Weak band

are tabulated in Table 3 and illustrated in Figure 1.

Thus in the event of an unknown alkyl lead salt being isolated from a natural system in sufficient quantity, the identity of the cation could be ascertained by a comparison of its infra red spectrum with the tabulated data. The data were also available for confirmation of the nature of the extracted species after addition of a known compound to the system prior to extraction.

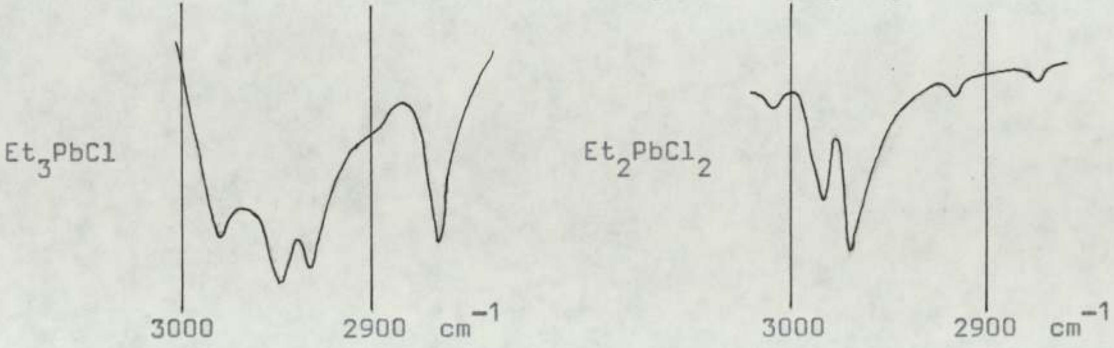
TABLE 3

Differences in IR spectra of Et_3PbX and Et_2PbX_2 (cm^{-1})

	Et_3Pb^+	Et_2Pb^{++}
Strong sharp band	680	710
Medium sharp band	1021	absent
Weak band	1453	absent
Basic pattern for C-H stretch,	2950	2970
decreasing order of intensity.	2930	2985
See also Figure 1.	2865	(3010)
	2980	(2915)
		(2860)

FIGURE 1

Differences in IR spectra of Et_3PbX and Et_2PbX_2



2.3.4. Nuclear magnetic resonance spectroscopy

The nuclear magnetic resonance spectrum of TML consists of a single line at τ 9.2 corresponding to CH_3 protons attached to non-magnetic isotopes of lead, with satellites due to splitting by the magnetic isotope ^{207}Pb ($I=\frac{1}{2}$, natural abundance 21.11%[59]), $J_{Pb-CH_3} = 61$Hz.

The spectrum of TEL consists of an intense central band at τ 8.5 situated symmetrically between two pairs of satellite bands. The central band is caused by ethyl groups attached to non-magnetic isotopes of lead. These do not give the familiar 'ethyl' type spectrum since the chemical shift between the CH_2 and CH_3 is very small (~1Hz at 40MHz[60]). However the difference in spin coupling between ^{207}Pb and the two portions of the ethyl group results in the removal of the degeneracy caused by their chemical shift equivalence and gives the familiar ethyl type spectrum. Thus the central broad band has inner satellites of quartets (J_{Pb-CH_2}=41Hz) and outer satellites of triplets (J_{Pb-CH_3}=125Hz) with coupling between methylene and methyl protons of $J_{CH_2-CH_3}$=8Hz[60,61]. Values for both TML and TEL recorded in the present work agreed with previously reported figures.

The methyl lead salts have relatively simple spectra compared with those of the ethyl lead salts, and the reported data is summarised in Table 4. Of the ethyl lead salts, only the nitrates had been characterised by NMR[62], and these values are tabulated along with those recorded for the ethyl lead salts prepared in the present work (Table 4). The difference in the chemical shifts for the CH_2 and CH_3 group protons are much larger than was the case for TEL and the spectra show the normal 'ethyl' type splitting, the CH_3 triplet being at higher field than the CH_2 quartet. The basic ethyl patterns are complicated by the satellites caused by splitting by the ^{207}Pb isotope. The coupling with CH_3 protons is greater than that with

TABLE 4

NMR data for alkyl lead compounds

Compound	$\tau(CH_3)$	$\tau(CH_2)$	J_{Pb-CH_3}	J_{Pb-CH_2}	Solvent	Ref.
Me_4Pb	9.16		62		80% in toluene	105
Me_3PbH	9.15		66.7		-	115
Me_3PbF	8.67		81		Chloroform	54
Me_3PbCl	8.37		70		Chloroform	54
Me_3PbBr	8.25		68		Chloroform	54
Me_3PbI	8.16		63		Chloroform	54
Me_3PbOH	8.47		76		Chloroform	54
Me_2PbCl_2	-		154.5		DMSO	66
$Me_2Pb(ClO_4)_2$	-		150.0		-	66
Et_4Pb	8.64	8.64	125	41.5	Neat	a
Et_3PbCl	7.98	7.6	177	40	$CDCl_3$	a
Et_3PbI	8.14		180		DMSO	a
Et_3PbOH	8.30	8.0	174	40	$CDCl_3$, D_2O	a
Et_3PbOAc	8.45	8.02	182	40	D_2O	a
Et_3PbNO_3	8.3	7.8	180	40 ∓ 5	D_2O	84
Et_2PbCl_2	7.96	7.18	312	96	DMSO	a
$Et_2Pb(OH)_2$	8.23	7.30	280	70	D_2O	a
$Et_2Pb(NO_3)_2$	8.1	6.8	318	72	D_2O	84

a.Present work

CH_2 protons as was the case for TEL, but the CH_3-CH_2 coupling is similar. The ^{207}Pb-CH_3 splitting is much larger in Et_2PbX_2 than in Et_3PbX which is larger than that of Et_4Pb, as was the case with the analogous methyl compounds.

As with the infra red data discussed above, it has been shown that NMR spectroscopy could be applied to the identification of unknown alkyl lead compounds extracted from natural systems if sufficient were available. In the case of NMR spectroscopy, differentiation between R_2PbX_2 and R_3PbX type compounds would be much more positive due to the large differences in the spectra. Such a technique was used in the identification of the major component of a mixture of Et_2PbI_2 and Et_3PbI after an attempted preparation of Et_2PbI_2. It was also used for identification of the nature of compunds extracted from natural systems, to which a known alkyl lead compound had been added.

2.3.5. Thin layer chromatography

2.3.5.1. Separation as dithizonates

The separation and identification of various metal dithizonates on silica gel plates eluted with non-polar solvents[63] and of methyl mercury dithizonates on alumina plates with petroleum ether/diethyl ether[64] have been reported.

We attempted to adapt these methods to the identification of alkyl lead salts. Aqueous solutions of alkyl lead salts were shaken with excess dithizone in chloroform, and the chloroform solutions were eluted on silica plates with $CCl_4/CHCl_3$. In each case a red spot corresponding to inorganic lead dithizonate was visible immediately above that of unreacted dithizone. The R_f value for inorganic lead dithizonate agreed with that previously reported[63]. No other spots were visible, but spraying of the eluted plates with dithizone solution showed up additional spots whose colours indicated the presence of uncomplexed alkyl lead salts. Since there was excess dithozone present it seems unlikely that R_3Pb^+ salts were extracted uncomplexed into the chloroform solution of dithizone, and it would have been impossible for this to occur for R_2Pb^{++} salts owing to their insolubility in chloroform. Thus we concluded that decomposition of the alkyl lead dithizonate was taking place on the TLC adsorbent, and that this technique would not be suitable for identification of alkyl lead salts.

It was noted however that the uncomplexed Et_3PbCl had run a small distance (R_f ca. 0.1) whilst the other salts (Me_3PbCl, Et_2PbCl_2 and Me_2PbCl_2) remained on the baseline. This observation was used in experiments on the separation of alkyl lead salts described below.

2.5.3.2. Separation as salts

Separation of Ph_4Pb, Ph_3PbCl, and Ph_2PbCl_2 on alumina, and of R_3PbX, R_2PbX_2 (R=Me,Et) on silica plates using benzene[27] or benzene/acetic acid[65,66] as eluant have been reported. In the last separation, plates were developed with a chloroform solution of dithizone which gave a characteristic yellow spot for R_3PbCl and a salmon red spot for R_2PbX_2. Inorganic lead remained on the baseline and developed as a red spot. In the present work it was found that benzene/acetic acid eluant would partially separate methyl and ethyl homologues of the salts, as was previously reported with the use of a mixture of apolar solvent, acetic acid, water and acetylacetonate as eluant[66]. In an attempt to increase the separation between the various mixed methylethyl lead salts, and to improve the lower limit of detection by TLC, various systems were studied using different adsorbants and eluants. The resulting R_f values are reported in Table 5.

TABLE 5 TLC: R_f values for alkyl lead salts

Plate	Eluant	Et_3PbCl	Me_3PbCl	Et_3PbOAc	Et_2PbCl_2	Me_2PbCl_2	$Et_2Pb(OH)_2$	Comments
Silica	30%$CHCl_3/CCl_4$	.1	0	0	0	0	0	
	50% "	.2	.05	.01	0	0	0	
	$CHCl_3$	.3	.1	.05	0	0	0	
	10%toluene/$CHCl_3$	.5	.2	.05	0	0	0	
	10%acetone/$CHCl_3$	.6	.4	.01	0	0	0	Streaky
	Benzene	.1	.05	0	0	0	0	
10%	10%AcOH/acetone				1	1	1	Streaky
	Acetone				.8	.5	0	Streaky
	MEK							Too streaky
	2%AcOH/acetone				.8	.6	.4	
	10%toluene/acetone				.5	.3	0	Streaky
	10%EtOAc/acetone				.6	.3	.1	Streaky
	10%EtOAc/2%AcOH/acetone				.5	.4	.3	
	20%tol/5%AcOH/acetone				.7	.6	.5	
	20%$CHCl_3$/5%AcOH/acetone				.7	.6	.6	
	25%AcOH/toluene	.5	.3	.35	.2	.1	.25	
	25%AcOH/benzene	.5	.3	.35	.2	.1	.25	
	25%AcOH/toluene	.5	.3	.3	.2	.1	.25	Plate dried
	10%AcOH/toluene	.4	.1	.2	.1	.05	.15	
	10%AcOH/$CHCl_3$	.7	.5	.5	.3	.15	.3	
	25%AcOH/$CHCl_3$	.8	.6	.75	.7	.4	.7	
Alumina	25%AcOH/toluene				.8	.8	.8	
	Acetone	1	1	1	.1	.1	.1	
	5%AcOH/toluene	.5	.2	.5	.3	.1	.3	
	2%AcOH/toluene	.4	.15	.4	.3	.1	.3	Me streaky
Cellulose	10%AcOH/benzene	1						
	Benzene	1						
	Pet. ether 60-80°	.7						Too streaky
	15%benzene/P.E.	.7						Too streaky

R_f for Pb^{++} was 0.0 in all cases.

Plates were equilibrated with atmosphere unless otherwise stated.

It should be noted that the use of dithizone as a developer precludes the use of most uv-activated plates since the activating agent is usually a heavy metal salt, with which the dithizone will complex. All adsorbents used in this work were non uv-active materials.

For R_3PbCl the best separation was obtained with elution by 10% toluene in chloroform on silica plates, but R_2PbCl_2 remained on the baseline. For R_2PbCl_2 the best separation was with elution by 2% acetic acid in acetone, but this was not suitable for R_3PbCl. The original 25% acetic acid in benzene[65] or toluene gave a reasonable separation for both R_3PbCl and R_2PbCl_2, but the limits of detection were high. We found that these could be decreased for R_3PbCl in this system by standing the developed plate in a tank of iodine vapour for 15 minutes, removing the iodine adsorbed in a current of air, and redeveloping the plate with dithizone. The resulting spots were all a salmon red colour owing to the conversion of the trialkyl lead salts to dialkyl lead salts. This technique could also be used as a confirmatory test for R_3PbX salts. Other compounds producing a yellow colour with dithizone and with R_f values similar to R_3PbX can be distinguished by their persistant yellow colour after treatment with iodine. Certain impurities in redistilled 4-methyl pentan-2-one (MIBK) were found to have this property.

We found that alumina plates eluted with 5% acetic acid in toluene gave a good separation for both R_3PbCl and R_2PbCl_2 (R=Me,Et) and that the detection limits were at least 10-fold lower than for the previous method (Table 6). Toluene was used rather than benzene owing to its lower toxicity. Both gave identical R_f values.

In the study of TLC systems Et_3PbOAc and $Et_2Pb(OH)_2$ were included for interest and occasionally showed anomalous R_f values. This was probably due to specific interactions with adsorbent and differing

TABLE 6

Limits of detection of alkyl lead salts by TLC

Compound	Limit[a]	Limit[b]	Limit[c]
Et_3PbCl	5	2	0.5
Et_3PbOAc	5	2	0.5
Me_3PbCl	5	3	1
Et_2PbCl_2	2	2	0.5
Me_2PbCl_2	3	3	0.5

[a] Silica plates, 25%AcOH/toluene

[b] Silica plates, 25%AcOH/toluene, after iodine treatment

[c] Alumina plates, 5%AcOH/toluene

All figures given are in μg

TABLE 7

R_f values for alkyl lead salts

Compound	R_f
Et_3PbCl	0.46
Et_3PbOAc	0.46
$Et_2MePbCl$	0.36
$EtMe_2PbCl$	0.26
Me_3PbCl	0.16
Et_2PbCl_2	0.24
$EtMePbCl_2$	0.14
Me_2PbCl_2	0.07

Adsorbent: Alumina

Eluant: 5%AcOH/toluene

solubilities in the eluant compared with the chlorides (see Table 1).

The mixed methylethyl lead salts, $MeEt_2PbCl$, $Me_2EtPbCl$, and $MeEtPbCl_2$ were prepared and run on alumina plates eluted with 5%AcOH in toluene. Each gave a spot with R_f value intermediate to those of the single alkyl lead salts (Table 7).

Preparation of methylethyl lead dichloride was only partially successful due to side reactions. From the TLC it was apparent that not only had $MeEtPbCl_2$ been formed but also the whole range of $Me_nEt_{3-n}PbCl$ (n=0-3). This was undoubtedly due to disproportionation of the dialkyl lead salts which has been previously reported for Me_2PbX_2 in D_2O and DMSO[56]:

$$2Me_2PbX_2 \longrightarrow Me_3PbX + PbX_2 + MeX$$

However by analogy with the trialkyl lead salts it was assumed that the red part of the unresolved spots with R_f values intermediate between that of Me_2PbCl_2 and Et_2PbCl_2 was due to $MeEtPbCl_2$.

Confirmation of the presence of small amounts of TAL in natural extracts was possible by decomposition of the sample in petroleum ether with iodine monochloride followed by extraction into water and evaporation to dryness. The residue was taken up in acetone and eluted by TLC, by which method concentrations of TAL as low as 2 ppm in the petroleum ether could be detected as the Et_2Pb^{++} salt.

2.3.6. Qualitative analysis, general

A recent review of analyses for organolead compounds details all the methods available up to the end of 1972[67]. These methods have been almost exclusively concerned with the analysis of TAL in petrol or the analysis of organolead salts in the absence of unknown interfering species. All these analyses were carried out in known or defined systems where any interfering species could be allowed for in calibration standards. The systems with which the present work is concerned do not fall into these categories, since natural waters and sediments can contain any number of unknown chemical species which may or may not interfere in a particular estimation. It was necessary therefore that any analytical methods used in the subsequent survey should be highly specific for the alkyl lead compound of interest.

Two basic approaches were available. The first was to consider the properties of the alkyl lead compound of interest and to develop a method of extraction of this compound to the exclusion of any other compounds which may have interfered with the final detection and estimation. This approach, described below in section 2.3.7., was the first one to be attempted and was based on the differing solubilities of the alkyl lead and inorganic lead compounds. Since results from this technique proved to be negative, other more specific and sensitive analytical techniques were sought. The three major groups of compounds of interest, R_4Pb, R_3PbX, and R_2PbX_2 have either very different properties (i.e. R_4Pb and the salts) or toxicities (i.e. R_3PbX and R_2PbX_2) and it is necessary therefore to be able to distinguish between the three types of compounds. As a consequence, much of the development of analytical techniques concentrated on one particular group of compounds.

2.3.7. Specific extraction technique

This was based on the differing solubilities of the alkyl lead compounds, particularly R_4Pb and R_3PbX, in organic solvents compared with those of inorganic lead compounds. It was assumed that only alkyl lead compounds would dissolve in organic solvents, and so extraction of natural waters and sediments by organic solvents, followed by concentration and analysis by atomic absorption spectroscopy (AAS) for total lead content would indicate the levels of alkyl lead compunds present originally.

For greatest sensitivity, organic extracts of natural systems had to be concentrated and transferred to a solvent suitable for AAS. Owing to their lack of volatility, alkyl lead salts could be readily concentrated, but tetra-alkyl leads required the development of a suitable concentration techniques, to keep loss due to evaporation to a minimum. Such concentration techniques were developed both with and without decomposition of the TAL.

2.3.7.1. Without decomposition

Concentration of dilute solutions of TEL in methanol was not possible by use of a rotary evaporator owing to the comparable volatilities of the two liquids. Partial concentration was possible from methanol if a fractional distillation was carried out.

Concentration of a dilute solution of TEL in ether was successful by rotary evaporation. Unfortunately diethyl ether is not a suitable solvent for AAS as it causes flare-back, so another organic solvent was sought. 4-methyl pentan-2-one (methyl isobutyl ketone, MIBK) has been used for AAS in the field of metal-complex analysis[68] and we developed a technique for transference of TEL from a low boiling solvent in which the TEL could be concentrated by rotary evaporation, to a small volume of MIBK. This was carried out by addition of a small volume of MIBK to a solution of TEL in organic solvent and removal of the lower boiling solvent by rotary evaporation. The technique was found to be quantitative for acetone and diethyl ether as initial solvents.

To simulate natural extracts of aqueous systems where water would probably still present in the organic extract, the effect of small amounts of water and of drying solutions with sodium and magnesium sulphate were investigated. None of these processes had a significant effect on the final analysis.

2.3.7.2. With decomposition

Solutions of TEL in MIBK and benzene were reacted with thionyl chloride and a trace of pyridine which acts as catalyst for the decomposition[5]. After removal of the solvent, the residue was taken up in nitric acid and analysed by AAS. Recovery was 93% and 98% respectively.

Similar solutions of TEL were exposed to ultra violet light and worked up as above. Recovery from MIBK solution was low probably due to preferential absorption of the light by the solvent. That from benzene was higher (ca. 50%) but not consistent. This may have been due to insufficient exposure to the uv radiation and the low intensity of the far uv produced by the source.

2.3.7.3. Use of AAS with organic solvents

Analysis of organic solutions of alkyl leads were normally carried out in MIBK solution and the instrument calibrated with standard solutions of TEL in MIBK. Previous workers have carried out direct analysis of petrol for lead by dilution with organic solvents[69], but it has been shown that standards must be made up with TAL, rather than inorganic lead salts or complexes, and in the same solvent, due to differing atomisation efficiencies[70,116].

2.3.7.4. Extraction of natural samples

Various sediments were extracted with acetone, methanol, chloroform, benzene, petroleum ether, and water and the extracts worked up appropriately, using the methods developed above for the organic solvents. In no case was any significant concentration of lead detected in the extracts. Consequently more specific and sensitive analytical techniques were studied.

2.3.8. Gas liquid chromatography

Gas liquid chromatography (GLC) is an ideal method for the separation and estimation of tetra-alkyl lead compounds owing to their volatility. Almost all the published work in this field has been concerned with the analysis of petroleum solutions. For this, a selective detector is required since the volatility of TAL is such that the peaks are superimposed upon the hydrocarbon peaks[67]. Such a detector is the electron capture detector (ECD)[71] which with a Ni^{63} source has been used for determination of trace quantities of TAL in fuel oil[72]. This detector responds only to compounds having an electrophilic grouping which can 'capture' free electrons produced by the source. TAL has this property, unlike the hydrocarbons, thus enabling the estimation of the former in the presence of the latter. Such selectivity was also advantageous in the present work since extracts of river sediments contain many components in differing concentrations and the use of an ECD enabled the removal of interference from most of these compounds.

The principal of operation of the ECD is the variation of conductivity of gases in an ionisation chamber by the presence of contamination in the gas. Thus the ECD used in this work comprised an ionisation chamber containing a Ni^{63} source through which passed a stream of pure nitrogen. Beta-ray emenation from the source causes ionisation of the nitrogen with consequent liberation of free electrons. The ionisation chamber is connected to a low voltage potential and a current measuring device. Because of the greater mobility of the electrons over the positive ions, the former migrate to the anode before recombination with the latter can occur, and thus create a 'standing current'.

If an electrophilic compound is now introduced into the chamber, a reaction will occur between this compound and the free electron as follows:

$$e^- + AB \longrightarrow AB^- \qquad \text{Non-dissociative}$$

$$e^- + AB \longrightarrow A^o + B^- \qquad \text{Dissociative}$$

Consequently the highly mobile electrons are replaced by slow moving negative ions. These take longer to migrate to the anode and so have a higher probability of recombination with positive ions before reaching the electrode. A decrease in the standing current is therefore observed.

In general the dissociative reaction requires considerable energy to break the sample-molecule bond, whilst the non-dissociative reaction results in an increase in energy of the sample molecule which has to be dispersed prior to formation of a stable complex. Thus an increase in detector temperature will promote a dissociative reaction and inhibit a non-dissociative reaction. For TAL we found that increased detector temperature led to an increased sensitivity and it was therefore concluded that electron capture occurred via a dissociative reaction. The operating temperature used was the maximum practical (300^oC) which still allowed for an increase to clean the detector (upper operating temperature = 355^oC[72]).

The instrument used in the present work was operated with a pulsed voltage across the ionisation chamber. The duration of the pulse is much smaller than the interval between pulses and gives an increased sensitivity over the constant D.C. mode of operation. During the long intervals between pulses there is no migration of electrons to the anode and the concentration of electrons builds up. Sample entering the detector will therefore have a greater chance of capturing electrons and the quantity of sample needed to produce a given decrease in standing current will be less.

Whilst the high sensitivity and specificity of the detector to electron capturing materials is its chief advantage, it must be remembered that certain common organic solvents are strongly electron capturing and their use must be avoided. Such solvents are alcohol, acetone, diethyl ether, and any chlorinated solvents. Indeed any compounds containing oxygen, sulphur or halogen will be strongly electron capturing and injection of greater than nanogramme quantities will overload or 'saturate' the detector (i.e. remove all the electrons produced), an effect which may last for several hours[71].

Many different column packings have been used in analysis of TAL by GLC[67] but care has to be taken when using an ECD that column 'bleed' does not contaminate the detector. To prevent such contamination columns are 'conditioned' overnight at their maximum working temperature in a flow of carrier gas, but disconnected from the detector. This also removes any high boiling fractions which may have been retained on the column at normal operating temperatures, and which will otherwise plate out onto the Ni^{63} foil and reduce the standing current.

Despite the reported stability of the Ni^{63} detector response over a period of 20 days[72], it was found to be necessary to calibrate the detector at least once per day by injection of TAL standards. This was possibly due to the nature of the samples injected causing [illegible] contamination of the detector. The detector was operated within the limits of linear response and therefore injection of only one standard was necessary for calibration. For convenience this was chosen as close as possible to the sample concentration. Integration of peak areas was carried out electronically.

During experiments involving relatively high concentrations of TAL, it was found advantageous to operate with a non-contaminated detector as this gave a more stable response. However when looking for traces of TAL in natural extracts, operation with a contaminated detector was advantageous. Although the latter conditions tended to give a less stable response to standard injections of TAL, the sensitivity is greatly enhanced (approx.20-fold). This is because contamination of the Ni^{63} foil leads to a decrease in beta-ray emenation and a corresponding decrease in the number of electrons reaching the anode. Thus to produce a certain percentage reduction in the standing current, far less electron capturing material is necessary to produce a given detector response since far less electrons need to be captured. Where possible detection of trace amounts were carried out under these conditions, but for quantitative work, at necessarily higher concentrations, the instrument was operated with a non-contaminated detector.

The detection limit with a contaminated detector was ca. 0.005 ppm and the quantitative detection limit for a clean detector was 0.1ppm in solution (10 μl injection).

Owing to the low levels with which our work was concerned it was found necessary to run separate chromatographs for the detection of TEL and TML. Under conditions giving a retention time for TEL which did not overbroaden the peak (ca. 9 min), the mixed alkyl leads could also be observed, but the signal due to TML was lost in the impurity peaks from the petroleum ether and from natural extracts. Thus TML was estimated separately under conditions giving a retention time of ca. 7 minutes, at which any peaks corresponding to a retention time similar to that of TEL were not visible above the baseline unless present in very high concentrations.

Where samples were suspected of containing any of the mixed alkyl leads, the operating conditions were adjusted to give the optimum retention time for that compound i.e. the longest retention time which did not overbroaden the peak, and its identity confirmed by a mixed injection with an authentic sample.

Routine analyses were carried out using an SE-30 column which gives a boiling point separation of the compounds injected. Confirmatory analyses were carried out on a 1,2,3-tris-(2-cyanoethoxy)-propane (TCEP) column. Attempts to use a polypropylene glycol (PPG) column for further confirmation of suspected TAL in natural extracts proved impractical due to impurities in the redistilled solvents which had retention times corresponding to TEL. Furthermore many of the components of natural extracts had very long retention times on the PPG column and gave large detector responses long after TEL had eluted. Such responses were unacceptable for quantitative analysis of large numbers of samples owing to the long delays necessary between injections. Use of temperature programming was also rejected for similar reasons.

The SE-30 column could be used for analysis of both TEL and TML by altering the conditions, but TML could not be determined on the TCEP column because of its very short retention time. Even when analysing for TEL on the TCEP column the temperature and flow rate had to be maintained as low as possible.

Two compounds which have been reported to interfere with the determination of TAL by GLC[67] are dichloro- and dibromo-ethane, used as scavengers in petrol. In this work these were shown not to interfere. On the SE-30 column the retention times were: TML 6 min; dichloroethane 4 min; dibromoethane 8 min.

2.3.9. High pressure liquid chromatography

TLC had proved to be an excellent qualitative technique for the analysis of alkyl lead salts, but quantitative analysis by TLC is much more difficult. TLC is a basic chromatographic technique and results derived from it usually can be obtained on a large scale by column chromatography. Like TLC this technique has not often in the past been used in quantitative work. The development of high pressure liquid chromatography (HPLC) however has enabled chromatographic separations to be carried out rapidly and quantitatively on small amounts of sample.

The instrument used in this work was a Waters model ALC 202/401 liquid chromatograph fitted with a Waters 6000 solvent delivery system. The latter instrument provided a constant pulseless flow at rate of between 0.1 and 9.9 ml min^{-1} to the chromatograph column after which the solvent passed to detector systems. The pressure on the high pressure side of the column could be up to 6000 psi. Two types of detector were available on the instrument: a refractometer and a uv detector. The uv detector was extremely sensitive for compounds absorbing at the operating wavelength (254nm), the only limitation being that the solvent must not absorb at this wavelength. The trialkyl lead salts absorb at 254nm, but trial runs using the uv detector indicated that decomposition was occuring and uv absorbing products were plating out onto the sides of the cell. This led to an increasing baseline on the detector which was unacceptable for quantitative work.

We attempted to determine the nature of this decomposition by observing the uv spectrum of Et_3PbCl in chloroform. The spectrum had a maximum absorption at 247nm (ϵ=5000) and had ϵ=3000 at 254nm. However the spectrum did not alter with repeated exposure to uv light at 254nm and no plating out was observed.

We concluded that either the alkyl lead salt was decomposing on the column packing to give uv absorbent intermediates which decomposed to give insoluble products in uv light, or that the concentration and intensities used in the uv spectrophotometer were not sufficiently high to induce decomposition of the trialkyl lead salt. For on-scale absorption on the uv spectrophotometer the concentration of Et_3PbCl in chloroform had to be several orders less than that which would have occurred in the detector of the HPLC. Also the light source in this detector is considerably more intense than that in the spectrophotometer since the former has a mercury resonance lamp emitting 90% of the light at 254nm whilst the latter has a deuterium lamp, the wavelength being selected via an optical system. Since the rate of uv decompositions depends on the number of photons absorbed, the conditions of high light intensity and of high sample concentration which occurred in the HPLC detector would be much more likely to lead to uv decomposition of the alkyl lead salt.

It was found that the dialkyl lead salts did not absorb at 254nm, and the uv detector was therefore of no use in detecting these compounds.

The refractometer is a more universal detector, measuring any change in refractive index which occurs in the eluant. Thus any compound which gives rise to a change in the refractive index of the solvent can be detected, but unfortunately the sensitivity is much less than that of the uv detector. The lowest quantitatively detectable amount of Et_3PbCl was 100 μg and the limit of detection only a little lower, thus it was only possible to use the instrument for initial development of extraction techniques for salts on sediments, when relatively large quantities were added.

It is claimed by the manufacturers that the instrument would separate compounds which are separable by TLC, but despite study of various systems with different columns and solvents, separation of Et_3PbCl and Me_3PbCl was not achieved. Neither was a suitable system found for R_2PbX_2 or R_3PbOAc.

2.3.10. Pyridyl-azo-resorcinol

As well as its use as a spray reagent for TLC, dithizone has also been used in a quantitative estimation of alkyl lead salts. Interference from inorganic lead may be calculated from measurements of absorption at different wavelengths[73] or by removal by prior complexing with EDTA[9]. However the dithizonates are unstable to daylight, as is dithizone itself, and it is a relatively unselective reagent. It gives complexes in the yellow-red range with Pb^{++}, Cd^{++}, Fe^{++}, Zn^{++}, Hg^{++}, and Mn^{++} as well as with the organolead salts[63]. A more selective reagent is pyridyl-azo-resorcinal (PAR)[67]. This complexing agent reacts with R_2PbX_2 type molecules to form stable coloured complexes, but forms no coloured complex with R_3PbX[74]. Interference from inorganic lead can be removed by EDTA which does not affect the Et_2Pb^{++}-PAR complex[74]. Thus Et_2Pb^{++} can be estimated spectrophotometrically after complexing with PAR.

This method has been adapted to estimate either Et_2PbX_2 alone, or total Et_3PbX and Et_2PbX_2 after decomposition of Et_3PbX to Et_2PbX_2 by iodine monochloride solution. Interference from other ions including Pb^{++} was removed by 1,2-diaminocyclohexane N,N,N',N'tetra-acetic acid (CDTA)[75].

The only metal which was found to interfere in the determination was iron. This could be removed by coprecipitation with lead by aqueous sodium hydroxide. We found that any iron present in a sample could be readily distinguished from Et_2Pb^{++} by the characteristic absorption peak due to the Fe-PAR complex at 483 nm, at which wavelength absorption is many times that at 515nm, the maximum for Et_2Pb^{++}-PAR (see Figure 2).

We found that the absorbance of Fe^{++} when run under the conditions for trialkyl lead analysis was much greater than when run under

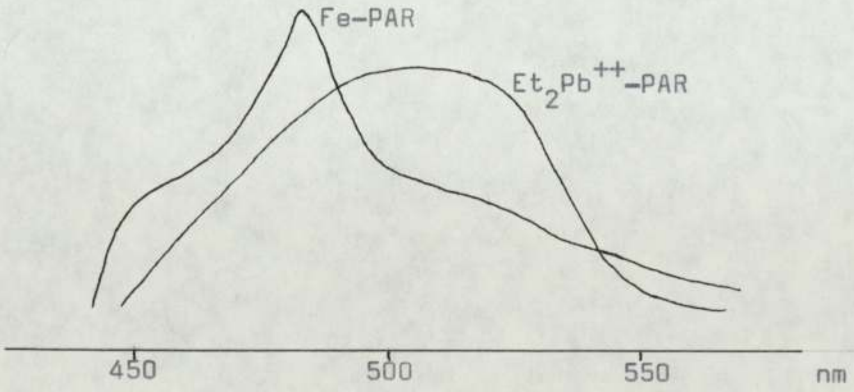
TABLE 8

Interference of iron in PAR method

Sample (5ml)	estimated as	A^{483}	A^{515}
100ppm Fe^{++}	di	0.02	0.00
100ppm Fe^{++}	tri	0.14	0.06
1000ppm Fe^{++}	di	0.49	0.18
1000ppm Fe^{++}	tri	1.43	0.55
100ppm Fe^{+++}	di	0.19	0.08
100ppm Fe^{+++}	tri	0.46	0.18
1000ppm Fe^{+++}	di	1.54	0.58
1000ppm Fe^{+++}	tri	2	2
10ppm Et_2PbCl_2	di	(0.33)	0.39

FIGURE 2

Interference of iron in PAR method



conditions for dialkyl lead analysis, and was the same as that for Fe^{+++} run under conditions for dialkyl lead estimation. We thought initially that the increased absorption was due to oxidation of Fe^{++} to Fe^{+++} by iodine monochloride when run under conditions for trialkyl lead estimation, and that the Fe^{+++}-PAR complex had a greater absorption than the Fe^{++}-PAR complex. However this did not offer a complete explanation since it was also shown that Fe^{+++} when run under conditions for trialkyl lead estimation also gave an enhanced absorption over that observed under conditions for dialkyl lead estimation. The absorption values are given in Table 8.

For samples of alkyl lead salts uncontaminated with iron the limit of detection of this method was 1 μg of Et_2Pb^{++} per 10ml sample, i.e. 0.1 ppm for aqueous solutions.

CHAPTER THREE

EXTRACTION OF ALKYL LEAD COMPOUNDS FROM NATURAL SEDIMENTS

3.1. Extraction of tetra-alkyl lead

3.2. Extraction of alkyl lead salts

EXTRACTION OF ALKYL LEAD COMPOUNDS FROM NATURAL SEDIMENTS

3.1. EXTRACTION OF TETRA-ALKYL LEAD

3.1.1. Vacuum distillation

Small volumes of TEL could be vacuum distilled at low pressures in the absence of sediment, but the efficiency dropped in the presence of sediment. Recovery of TEL depended on the concentration of TEL on the sediment, but was only 25% for ca. 1000ppm(dry weight). Since concentrations in natural systems were expected to be much lower than this, the technique would not have been suitable.

3.1.2. Steam distillation

Steam distillation of large quantities of TEL is used in its purification[31] and this technique seemed to offer an ideal way of separating TAL from the non-volatile alkyl lead salts and other lead compounds on sediments. However attempted steam distillation of small volumes of TAL in the absence of sediment were completely unsuccessful, the majority of TEL remaining in the flask to which it was added, probably as a result of adsorption onto the glass. Further experiments with the presence of sediment were not carried out.

3.1.3. Extraction by petroleum ether

Large volumes of TEL on sediment were extractable by petroleum ether, 40-60°boiling range (P.E.) with 100% recovery.

Smaller amounts of TEL added to various sediments and extracted with P.E. gave 40% recovery on a single extraction and a further 15% on a second extraction. TML and the mixed alkyl leads gave comparable extraction efficiencies. For speed of analysis only a single P.E.

extraction was normally carried out for sample analysis in the survey. Repeated extractions were carried out where TAL was detected. Values reported are the total for two extractions.

3.2. EXTRACTION OF ALKYL LEAD SALTS

3.2.1. 50% acetic acid

It was found that 50% aqueous acetic acid (50%AcOH) would remove Et_3PbCl from sediments. The identity of the extracted product was confirmed by TLC and by comparison of its infra red spectrum with that of Et_3PbCl. No trace of Et_3PbOAc was observed in either of these analyses despite the high concentration of acetic acid used. Presumably Et_3PbCl is not ionised in this system, in contrast to its properties in pure water and solutions of NaOAc. Extraction with benzene of Et_3PbCl in water saturated with NaOAc gives Et_3PbOAc as the only detectable product.

Alkyl lead compounds at various concentrations on sediment were extracted with 50%AcOH and the extracts analysed. The results are given in Table 9.

Although the total weight of extract was comparable to the weight of Et_3PbCl added, quantitative estimation by HPLC showed only 23% recovery, the difference in weight being attributed to the extraction of naturally occurring compounds from the sediments.

TLC of samples of Et_3PbCl extracted from sediment after up to 31 days showed the presence of Pb^{++}, Et_2Pb^{++}, and Et_3PbCl. Extraction of an undosed sample showed no alkyl leads to be present.

Extraction of smaller quantities of added Et_2PbCl_2 showed a recovery of only 6% as estimated by PAR after removal of the acetic acid both by evaporation and by neutralisation with aqueous NaOH.

TABLE 9 Extraction of alkyl lead compounds with 50% aqueous acetic acid

Salt	Weight (mg)	Sediment	Volume (ml)	Extract	Comments
Et_3PbCl	1150	PFC	100	880mg weight	IR identical to original salt.
"	474	PFC	100	430mg weight	After 6d TLC showed Pb^{++},Et_2Pb^{++},Et_3PbCl.
"	513	PFC	100	480 mg weight	After 31d, " " "
"	100	PFC	100	23mg as Et_3PbCl	Estimated by HPLC
TEL	330	PFC	100	310mg weight	After 31d TLC showed Pb^{++},Et_2Pb^{++},Et_3PbOAc.
"	330	PFC	100	-	After 3 months " " "
"	1.6	PFC	70	-	TLC showed Et_2Pb^{++}.
"	330	PFC	100	-	After P.E. extractn, Et_2Pb^{++} only feint.
Et_2PbCl_2	10	LMD	25	6.5% as Et_2Pb^{++}	PAR after evaporation of AcOH.
"	10	LMD	25	6.0% as Et_2Pb^{++}	PAR after neutralisation with NaOH.

Extraction of small quantities of TEL by this method showed only Pb^{++} and Et_2Pb^{++} in the extract. That decomposition of TEL was brought about by acetic acid and not the sediment alone was shown by prior extraction of a large volume of TEL on sediment with P.E. Subsequent extraction with 50%AcOH showed only trace amounts of Et_2Pb^{++}. Had decomposition of TEL to Et_2Pb^{++} been brought about by the sediment alone, large amounts of Et_2Pb^{++} would have been expected in this extract.

Extraction of large volumes of TEL on sediment after one and three months showed Et_3PbOAc, Et_2Pb^{++}, and Pb^{++} by TLC, with only a trace of Et_3PbCl, indicating decomposition of TEL in the presence of sediment to the Et_3Pb^+ species. As was shown above, any undecomposed TEL would react with acetic acid to give Et_2Pb^{++} and Pb^{++} only.

Since only low percentage recoveries were achieved using 50%AcOH, a more efficient extraction technique was sought.

3.2.2. Solvent extraction

3.2.2.1. Benzene/NaCl

It has been reported that Et_3PbCl can be extracted from water into benzene by saturation of the water with sodium chloride[10]. The ionisation of Et_3PbCl

$$Et_3PbCl \rightleftharpoons Et_3Pb^+ + Cl^-$$

in aqueous solution is supressed by the high concentration of chloride ion, thus reducing the solubility of the salt in water and increasing solubility in the non-polar solvent. Et_3PbCl may be back-extracted into water, under which conditions the reverse occurs.

No such technique has been reported for the dialkyl lead salts, probably owing to their greater ionisation and their insolubility in most water immiscible solvents. One solvent in which dialkyl lead salts are soluble is acetone, and this is immiscible with water if the concentration of dissolved salt in the water is sufficiently high. A screening experiment was carried out in which aqueous solutions of Et_3PbCl, Et_2PbCl_2, and $PbCl_2$ were consecutively extracted with P.E. and toluene, then a large amount of $CaCl_2$ added to the water which was subsequently extracted with toluene and acetone. Et_3PbCl was quantitatively extracted from $CaCl_2$ solution into toluene, but neither Et_2PbCl nor $PbCl_2$ were extracted from the aqueous phase.

Subsequent work on the extraction of alkyl lead salts by solvents was carried out on Et_3PbX only, since no solvent system was available for Et_2PbX_2.

The dissociation of Et_3PbCl in aqueous solution was demonstrated by extraction into benzene from water saturated with NaOAc. The only product was Et_3PbOAc.

A similar extraction of Et_3PbOAc from a saturated solution of NaCl gave solely Et_3PbCl.

The efficiency of extraction of Et_3PbCl from NaCl saturated water into benzene was shown to be 84% on a single extraction, and from benzene to water 75%. Consecutive extraction of Et_3PbCl from water saturated with NaCl into benzene, from benzene into water,and back into benzene after saturation of the water with NaCl, gave a 92% recovery for 105 mg of Et_3PbCl originally added, if two extractions were carried out at each stage.

Extraction of Et_3PbCl and Et_3PbOAc from sediment by saturation with NaCl and NaOAc respectively followed by extraction into benzene was shown by TLC to be possible, but the spots corresponding to the alkyl lead salts were obscured by organics which had also been extracted. It was found that these organics were retained in the benzene when this was back-extracted into water. However to concentrate down and to analyse the extract by TLC or HPLC the sample had ideally to be in an organic solvent. The aqueous extract was therefore saturated with NaCl or NaOAc as appropriate and re-extracted into benzene. TLC of this extract showed only Et_3PbCl and Et_3PbOAc respectively.

Quantitative experiments with Et_3PbCl showed a 38% recovery with one initial extraction of NaCl saturated sediment and 10% on the second giving an overall recovery of 48%.

It was thought that the alkyl lead salts might be partly held on the sediment as sulphides, or complexed with larger molecules. In an attempt to break down such compounds and to release free R_3Pb^+ ions, acidification was carried out before saturation with NaCl. The salts were more readily extracted from benzene to water (88% for the first extraction, cf. 63% without acidification) but the overall recovery was similar. These results were thought to be caused by more rapid breakdown of R_3Pb^+ complexes which are in both cases extracted from NaCl saturated sediment into benzene, but are not back-extracted into water. A similar effect was found for the addition of inorganic lead before saturation of the sediment with NaCl (see section 3.2.2.2.).

One possible source of inefficiency was thought to be the extraction of the sediment with benzene. However repitition of the initial extraction with high speed stirring before isolation of the benzene did not give any increase in recovery.

It was found that as the amount of Et_3PbCl added was decreased, so the percentage recovery also decreased. Using a standard volume of sediment of 30 ml (cf. 100 ml above) it was found that more than 50% of Et_3PbCl added at 10 mg or more could be recovered, but reduction in the amount of Et_3PbCl below this value produced decreasing percentage recoveries. At 1 mg Et_3PbCl added, recovery was $<4\%$ (Table 10, first two columns).

TABLE 10

Extraction of Et_3PbCl from sediment by NaCl/benzene

Et_3PbCl added (mg)	%recovery no Pb^{++}	%recovery Pb^{++} added
20	63	76
10	53	74
5	29	72
2	15	55
1	<4	55

Similar inefficiencies in recovery were noted by Westwoo[76] in his work on the extraction of organomercury compounds from certain animal tissues. He found that small amounts of methylmercury salts added to meat could only partly be recovered by his solvent technique, and found no recovery from liver or egg yolks. Addition of inorganic mercury to the system before solvent extraction gave much greater extraction efficiencies in all cases. This was attributed to release of the alkylmercury from complexing sites by the excess of inorganic mercury.

Analogously in the present work it was found that addition of inorganic lead to the sediment before addition of NaCl and extraction into benzene gave much greater percentage recoveries (Table 10).

To enable the estimation of lower amounts of alkyl lead salts, it was decided to change the method of analysis for Et_3Pb^+ from HPLC to PAR since the minimum quantitatively detectable amounts were 100 and 2 μg respectively.

Since the PAR method required that the sample be in aqueous solution, the procedure developed above was stopped before the second benzene extraction, i.e. the aqueous extract of the benzene extract of the sediment was analysed by PAR. As stated previously, most of the organics were retained in the benzene on extraction with water, the extraction of this aqueous solution into benzene being purely for convenience of analysis by HPLC. As a result of the removal of this final extraction stage, recoveries were slightly increased owing to the removal of inefficiencies involved in the extraction.

To the standard volume of sediment was added Et_3PbCl at 1000 μg down to 20 μg and a fall in percentage recovery was once again noted, even after the addition of Pb^{++} to the original sediment. However efficiency was still 45% for 200 μg added dropping to 11% for 20 μg of Et_3PbCl added - the minimum detectable recovery (Table 11). Further increase in the weight of $Pb(NO_3)$ added to the sediment did not improve the recovery, nor did repeated benzene extractions.

TABLE 11

Extraction of Et_3PbCl by NaCl/Pb^{++}/benzene

Et_3PbCl added (μg)	% Recovery (analysis by PAR)
1000	60
500	50
200	45
100	36
50	31
20	11

Me_3PbCl gave an overall recovery of only 29%, attributed to the difference in solubilities of Et_3PbCl and Me_3PbCl in water and benzene. The figures for the analogous bromides may be compared in Table 1.

Recovery of Me_3PbCl from benzene to water was 100% whilst that from water saturated with NaCl to benzene was only 48%. This problem of low extraction efficiencies for Me_3PbCl was overcome in the final extraction technique (section 3.2.3.).

Repeated extraction of Et_3PbCl on sediment showed that only a certain percentage was available for extraction. The first benzene extract removed 45% of the 1000 μg of Et_3PbCl added, leaving 550 μg theoretically available for extraction. However only 150 μg were extracted on the second extraction with benzene (cf. 45% of 550 μg = 250 μg). Thus some kind of permanent adsorption was occurring for small amounts of Et_3PbCl.

3.2.2.2. Action of lead nitrate

Westoo[76] noted that methylmercury was extracted into benzene with or without the addition of Hg^{++}, but that methylmercury was only released from the benzene extract into an aqueous phase if Hg^{++} were present. Thus he deduced that the methylmercury was complexing with organic molecules which were preferentially soluble in benzene. Addition of excess Hg^{++} to the original material, or extraction of the benzene with an aqueous solution of Hg^{++} released the methylmercury from the complex by the preferential adsorption of Hg^{++}.

A similar mechanism was shown to operate in the case of Et_3PbCl on sediment. At levels which gave very low extraction from NaCl saturated sediment without the addition of Pb^{++} it was found that equally good recoveries could be obtained by either adding Pb^{++} to sediment before benzene extraction, or extracting sediment to which no Pb^{++} had been added with benzene, and back-extracting the benzene into an aqueous solution of lead nitrate.

It was also shown that the residual sediment from this latter extraction produced very little extra Et_3PbCl when extracted with benzene after addition of lead nitrate.

Thus it appeared that any extractable Et_3PbCl can be extracted into benzene from sediment saturated with NaCl, but that only if Pb^{++} is available to free the Et_3PbCl is this extractable from benzene into water.

3.2.3. Addition of Pb^{++}/filter

The extraction of trialkyl lead salts into benzene was investigated as this was the only reported method for their isolation from complex matrices. However the method proved to be over-complicated for the analysis of large numbers of samples, in addition to which, the analysis was not applicable to dialkyl lead salts. Consideration was therefore given to simpler techniques.

In the benzene extraction technique developed above, the inorganic lead added to the sediment appeared to be freeing alkyl lead ions from benzene soluble complexing species in the sediment whereupon the NaCl converted these into the chloride and suppressed ionisation, making the Et_3PbCl preferentially soluble in benzene. If this was the case, uncomplexed Et_3Pb^+ was already in solution before saturation with NaCl and benzene extraction. Direct filtration of the sediment plus inorganic lead should therefore have given Et_3Pb^+ in the filtrate. This was found to be the case, recoveries being ca. 90%.

Since no solvent extraction was involved in this technique it could also be applied to diethyl lead salts which gave recoveries of ca. 75%. Me_3PbCl and Me_2PbCl_2 gave recoveries of 90% and 40% respectively.

Extractions with different amounts of lead nitrate added to the initial sediment showed that the optimum amount was 5g per 30 ml of sediment. Study of the effect of pH showed that the trialkyl lead salts were little affected but that the efficiency of extraction increased for Et_2PbCl_2 as the pH decreased to 1. However recovery of Me_2PbCl_2 decreased with decreasing pH, attributed to decomposition. A compromise was made and the sediment was just acidified (i.e. pH 5-6) at which, Me_2PbCl_2 recovery had not dropped significantly, and Et_2PbCl_2 recovery was still 70%.

The effect of the addition of other metals in place of lead was studied but only mercury was found to have a comparable effect. The order of efficiencies was as follows for the divalent ions:

$$Pb \approx Hg > Zn > Fe \approx Sn \approx 0$$

The extraction of Et_2PbCl_2 was carried out from various sediments to check that comparable recoveries could be obtained. These included sediment from a clean and a polluted river, a clean and a polluted canal and from road drains. In all cases extraction was between 60 and 80%. Extraction was also carried out from fine mesh silica from which recovery was 83%. Percentage recovery was greatest where pollution from organics was least.

It has already been shown that detection of Et_3PbCl was possible by TLC after solvent extraction, and it was also shown that,after the extraction above,both Et_2PbCl_2 and Me_2PbCl_2 were detectable by TLC if the extract were concentrated sufficiently.

The decomposition of salts on sediment was estimated by the decrease in recovery with time and the percentage of dialkyl lead salts in the trialkyl lead salts. On immediate extraction there was no increase in the percentage of Et_2PbCl_2in Et_3PbCl, but after Et_3PbCl had been left on sediment for 3 days, the percentage increased from 1 to 5. The total extraction of Et_3PbCl after 3 days dropped from

90 to 70% whilst that for Et_2PbCl_2 dropped from 70 to 7% indicating a much faster decomposition for the latter.

The extraction technique developed in this section was used in all subsequent analyses of sediments for alkyl lead salts and was combined with the solvent extraction of TAL to provide a complete analysis for the samples taken in the survey in Chapter 4.

CHAPTER FOUR

SURVEY

SURVEY

4.1. INTRODUCTION

The total concentration of lead in natural waters is generally low due to adsorption onto sediment[77], unless pH is exceptionally low[78]. Figures quoted for rivers in the Midlands range from 0.01 to 0.08 and, in one case, 0.14 ppm(Pb), averaging 0.03 ppm[79]. The general observation that levels of lead in rain water are twice that in ground water is attributed to this cause[29,30].

Tetra-alkyl lead compounds are insoluble in, and denser than, water[31] and would therefore be expected to accumulate with the sediment. The alkyl lead salts however are generally much more soluble in water than the corresponding inorganic lead salts and will therefore be more likely to remain in solution in the absence of suspended solids. In the presence of suspended solids and sediment in water it has been shown that triphenyl lead acetate is adsorbed onto sediment[80] and experiments carried out in the present work showed that alkyl lead salts at low concentration are totally adsorbed onto a variety of natural sediments. The minimum detectable concentrations of alkyl lead compounds in water are 0.002 ppm for TAL (by extracting the water with one tenth its volume of petroleum ether) and 0.1 ppm for alkyl lead salts (by direct analysis by PAR). Therefore in the presence of sediment, concentrations of alkyl lead compounds are unlikely to exceed these values.

Thus the survey of natural waters was carried out on samples of sediment since this is always present. If high alkyl lead concentrations were found, additional analyses could be carried out on filtered water samples. The concentration of alkyl leads in drinking waters where suspended solids content is very low is discussed in the next chapter.

It was thought that Birmingham was an ideal centre for a survey of the distribution of alkyl lead compounds in natural waters, because of the very high number of petrol engined vehicles in the area and of the variation in character of the area.

Not only is there a high density of traffic on normal commuter roads which involve a range of slow to medium speed driving conditions but there are also the motorways M5, M6, and A38(M) Aston Expressway, all of which normally present conditions for fast driving. It has been shown that the percentage of TAL emitted unburnt from exhausts depends on driving conditions. Figures given by Laveskog[19] indicate that most TAL is emitted in the first few minutes from a cold start and that the amount is less under constant driving conditions than when idling or accelerating.

Within the area surveyed there are large residential housing estates (e.g. Pool Farm and Small Heath) where car engines are subjected to cold start and choked driving conditions and where persons carrying out oil changes may have been tempted to dispose of the used oil into roadside drainage grids. There are also areas of low population (e.g. Clent Hills, Sandwell Valley) which offer sampling sites subjected to less specific pollution sources. Additionally there are areas of heavy industry (e.g. the Black Country) which although not using organolead compounds, may have contributory effects, caused for example by higher levels of inorganic lead in water courses.

Birmingham and much of its surroundings is on a plateau 120-160m above sea level and has therefore relatively few large rivers. Apart from in the east where the Rivers Tame and Cole flow towards the Trent, most of the land drainage is by small streams or canals. Due to the low flow rates in canals and reservoirs these will act as indicators of local sources of pollution. Any alkyl lead compounds which entered such waters would be removed onto suspended solids and precipitated as the upper layer of sediment close to the source of pollution. Streams and rivers on the other hand, with their fast and turbulent flow, could carry such sediments over long distances before depositing them, thus giving a more general indication of the pollution in the area.

A general survey was carried out to determine normal levels of alkyl leads in water, to observe if any overall trends were present dependent on such factors as wind direction, and to locate major sources of pollution and sites where alkyl lead compounds were being concentrated. Using Birmingham as a centre, samples were taken from streams, rivers, canals, and reservoirs within a radius of approximately 10 km, the geographical distribution of sites being as even as possible.

4.2. RESULTS AND DISCUSSION

4.2.1. Analysis of samples

Samples taken in the survey were analysed for TAL and for alkyl lead salts by methods based on those developed in Chapter 3.

Confirmation of the identity of suspected TAL by GLC below levels of 0.02 ppm dry weight (for samples containing 10 g solid, dry weight) proved to be an insuperable difficulty owing to large numbers of other compounds present in solvent extracts of sediments, and to the lack of a more specific method applicable to such small amounts. Above 0.02ppm a quantitative estimation could be made on both SE-30 and TCEP (1,2,3-tris-(2-cyanoethoxy)propane) columns. If the peak corresponding to TAL gave the same concentration value by comparison with standards on both columns, the signal was assumed to be due to TAL.

This method had to be adopted since samples showing no peaks corresponding to TAL on one column showed such peaks on the second column and vice versa. TLC could only be used to confirm the presence of TAL in cases where the concentrations were much higher than average.

The limits of detection of alkyl lead salts was much higher than that of TAL, being approximately 2 ppm (dry weight) for a sample containing 10 g of solid.

TML could not be confirmed by the use of a second column since the retention time was too low on both TCEP and polypropylene glycol columns. However it was only detected in four samples and in these TEL was present in high concentration. Thus the presence of TML was not unexpected owing to the use of both compounds together in petrol (see Table 14).

4.2.2. Description of survey sites

The sites are listed with their grid references in Table 12, and their geographical distribution is shown in Figure 3 along with the major rivers and canals of the area.

Samples 1-15 were taken from lakes, pools, and reservoirs that contain a volume of water in great excess of their daily throughput. Such sites would not only indicate specific sources of alkyl lead pollution close to from where it originated but also, owing to their large surface area, would act as a sink for any alkyl lead compounds washed out of the atmosphere during rainfall and subsequently adsorbed onto suspended solids in the water. These sites are marked 'R' on Figure 3.

Sites 1-2 were close to, but on opposite sides of, the city centre, in old residential areas, and near to main arterial roads. Sites3-4 were in residential areas close to the heavily industrialised

TABLE 12

Survey sites

Sample No.	Site		Grid ref.(SP unless stated
1	Rotten Park Reservoir		046872
2	Ward End Pool		110886
3	Thimble Mill Pool		016871
4	Titford Pools		SO 986878
5	Woodbrooke Pool		038812
6	Witton Lakes		088924
7	Brookvale Park Pool		092915
8	Coldbath Pool		087820
9	Moseley Pool		074834
10	Lifford Reservoir (NW)		058800
11	Lifford Reservoir (NE)		060799
12	Lifford Reservoir (SE)		057797
13	Swan Pool		025920
14	Langley Mill Pool		152968
15	Moxhull Park Pool		180953
16	River Cole:	Scribers Lane	103795
17		Brook Lane	098813
18		Warwick Road	100840
19		Coventry Road	111851
20		Bordesley Green	123868
21		Stechford Lane	128880
22		Packington Avenue	152880
23		Moorend Avenue	183873
24		Old Lichfield Road	197896
25	River Tame:	James Bridge (N of aqueduct)	018952
26		James Bridge (S of aqueduct)	022947
27		Newton Road	025937
28		Hampstead Hill	050923
29		Aldridge Road	070920
30		Lichfield Road/M6	096900

TABLE 12 (Cont'd)

Sample No.	Site	Grid ref.(SP unless stated
31	River Tame: Bromford Bridge	116895
32	Chester Road	139902
33	Water Orton	174914
34	Lichfield Road	187918
35*	Walsall Canal, James Bridge	SO 988977
36	Rushall Canal, Walstead Road	034964
37	Tame Valley Canal: Navigation Lane	016948
38	Holloway Bank	SO 990939
39	Ridgacre Branch Canal: Holloway Bank	SO 995926
40	Ryders Green Road	SO 985918
41	Old Birmingham Canal: Dudley Port	SO 963915
42	Lower City Road	SO 978904
43	Spon Lane	002898
44	New Birmingham Canal: Spon Lane	002898
45	Old Birmingham Canal: Brasshouse Lane	020889
46	Rabone Lane	033889
47*	Dudley Road	050876
48	Birmingham & Fazeley Canal: Bodymoor Heath	198964
49	Lichfield Road	186938
50	Cottage Lane	158924
51	Kingsbury Road	128910
52	Salford/M6	096901
53	Salford/Lichfield Rd	096899
54*	Aston	076881
55*	Grand Union Canal: Saltley Viaduct	094984
56	Sandy Lane	089868
57	Garrison Lane	085860
58	Lincoln Road South	130830
59	Dovehouse Lane	146818
60	Damson Lane	161807
61	Stratford Canal, Kings Norton stop lock	056795
62	Worcester & Birmingham Canal, Pool Farm	053784

* indicates several samples taken from these sites.

TABLE 12 (Cont'd)

Sample No.	Site	Grid ref.(SP unless stated
63	Bristol Road, run off from Edgbaston Pool	057837
64	Bristol Road/Bournville Road, small stream	034813
65	Bournville Road, Griffins Brook	038812
66	Bristol Road, Griffins Brook	032810
67	Pershore Road South, River Rea	049793
68	Lifford Lane, small stream	056796
69	Harborne Road, Chad Brook	044851
70	Harborne Lane, run off from Reservoir	038832
71	Mill Lane, small stream	010827
72	Furnace Hill, River Stour	SO 967845
73	Cradley, River Stour	SO 944853
74	Calthorpe Park, small stream	070846
75	Dog Pool Lane, River Rea	060822
76	Fox Hollies Park, Westley Brook	117820
77	Church Road, small stream	150844
78	Alcester Road South, Chinn Brook	079796
79	Powells Pool, run off from pool	104953
80	Wylde Green Road, Plants Brook	132944
81–101	See Table 16 and Chapter 6 for details	

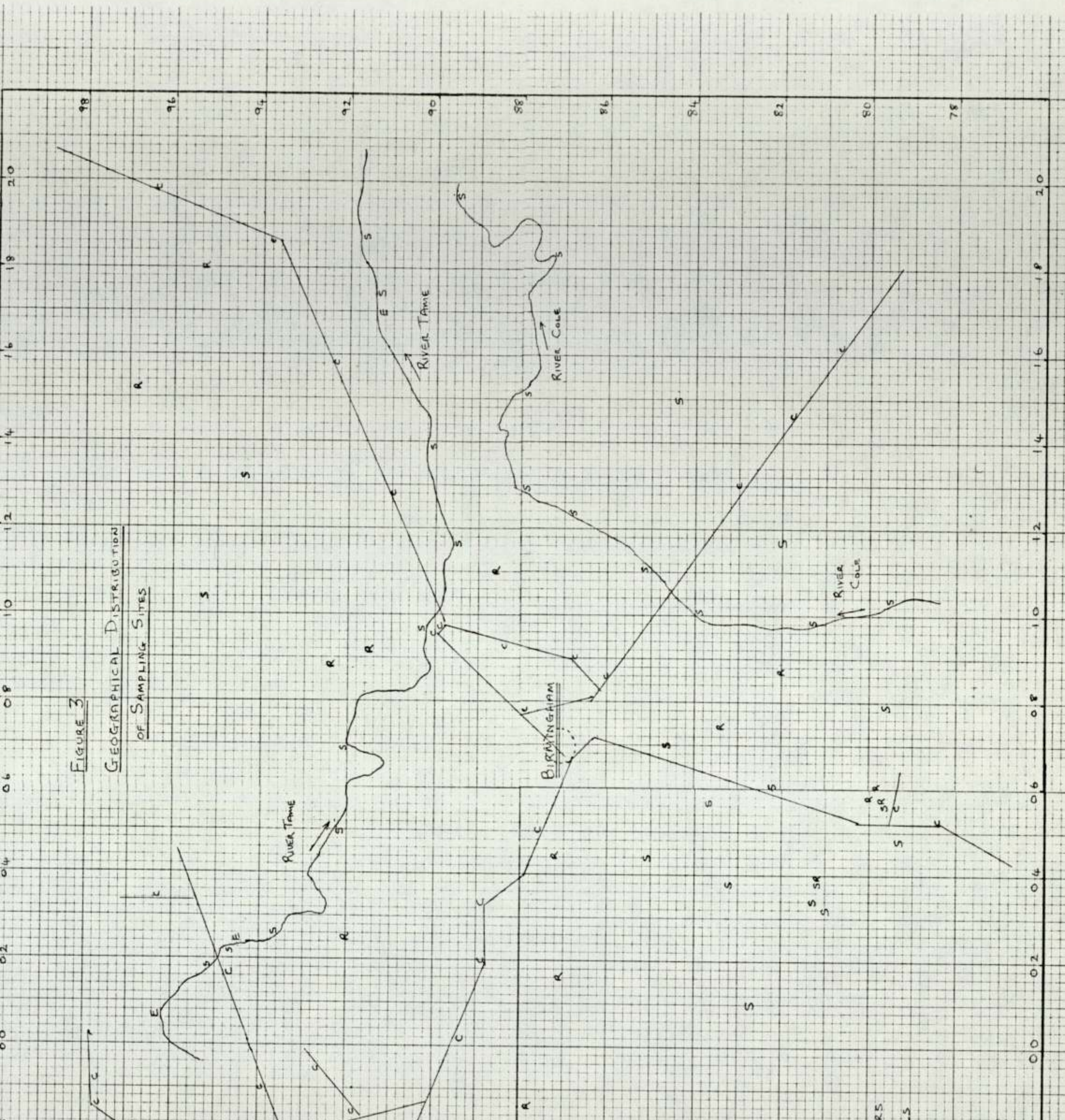


FIGURE 3

GEOGRAPHICAL DISTRIBUTION OF SAMPLING SITES

Black Country town of Smethwick. Sites 5-9 were in residential areas surrounding Birmingham. Samples 10-12 were taken from a polluted reservoir surrounded by heavy chemical industries: site 10 was near the reservoir overflow, and site 12 was near to an inlet. Site 13 was in the Sandwell Valley , an open area of land between the conurbations of Birmingham and the Black Country. Sites 14-15 were in open country to the northeast of Birmingham.

Samples 16-24 were taken from sites 2-4 km apart along the course of the River Cole within the area of the survey. The river runs from the south to the east of Birmingham passing 5 km to the southeast of the city centre. It is a small, generally slow flowing stream, the land immediately adjacent to which has been designated as open space for most of its course through residential suburbs. The course of the river and the sampling points (marked 'S') are shown in Figure 3. All the samples were taken on the downstream side of road bridges in case the roadwater run off made a significant contribution to alkyl lead levels in the immediate vicinity. The road crossings at sites 18-20 were busy commuter roads. Site 16 was immediately downstream of a ford where a minor road crossed the river.

Samples 25-34 were similarly taken from the Tiver Tame. This is a larger, faster river flowing from the northwest to the northeast of Birmingham and passing 5 km to the north of the city centre. Its tributaries rise in the highly industrialised areas of the Black country to the north and west of Birmingham. The section within the survey area to the west of Birmingham flows through open land whilst that to the north and east flows through an industrial area, much of its course being beneath the M6 motorway. A high percentage of the water in the river is treated sewage effluent which enters at Bescot (007962), Ray Hall (023945), and Minworth (170915). These are marked 'E', and the sampling sites marked 'S' on Figure 3. It was thought that the additional biological activity of these non-chlorinated effluents might increase the probability of methylation of inorganic and partially alkylated lead salts occurring in the river. This possibility was investigated further, and is discussed in Chapter 6.

Sites 25-26 were downstream of Bescot, sites 27-34 were downstream of Ray Hall and Bescot, and sites 33-34 were downstream of all three works. Sites 30-32 were almost underneath the M6 motorway which passes overhead and within 400 m of the river for 7 km from 086900 to 160910. Storm water drainage from the motorway enters the river at several points around 096900.

Samples 35-62 were taken from canals within the survey area, the distribution of sites being dependent on the availablity of access to the canal. The sites are marked 'C' on Figure 3, where they are superimposed on a diagrammatic map of the local canal system. Unlike the rivers and streams, the immediate locality of canal sites will be of greater importance owing to the very low flow of water in the canals, and hence the small distances involved in the movement of sediments. Sites 35, 39-47, 52-57 were in heavily industrialised areas, sites 47, 54, 56-57 being within 2 km of the centre of Birmingham. Sites 37-38, 49-51, 58-62 were in residential areas and sites 36 and 48 were in open country. Sites 35, 43-44, 52 were immediately adjacent to motorway crossings, site 44 being immediately below an outfall for motorway storm water drainage. Sites 37-39, 41, 43, 47, 49, 51-56 were close to busy major roads and sites 41,53 were alongside petrol filling stations.

Samples 63-80 were taken from small streams throughout the survey area such that an even geographical distribution of sites was obtained when combined with the previous sampling sites. Many of these streams are interconnected, but it is very difficult to trace their courses since for much of their length they are now culverted. The sites are marked individually as 'S' on the map of Figure 3. In the vicinity of the sampling sites 63-67, 69-71, 74-78 the streams run through residential areas. Sites 68, 71, 72 are close to industrial areas, and sites 79-80 are in open country to the north of Birmingham. Sites 63, 64, 66-67, 78 were sampled close to main commuter roads into Birmingham, and like all sites, were sampled downstream of the road crossing wherever possible.

In addition to these sites around Birmingham where alkyl lead pollution would probably originate in the use of petrol, samples 81-101 taken from rural areas of known high lead burden were also analysed. In such areas it was considered possible that species adapted to an environment of high lead concentration might have developed a detoxification mechanism involving methylation of the lead. This possiblity is discussed at length in Chapter 6 where details of the sites are also listed (Table 16).

4.2.3. Results of geographical survey

In all the samples from sites described above, no alkyl lead compounds of any type were detected within the limits given in section 4.2.1.

As far as was possible we selected the sites to include all possible variations which might affect the concentration of alkyl lead compounds in natural waters. Factors considered were: types of water (e.g. canals, rivers, reservoirs); immediate sources of water (e.g. rainfall, land drainage, road drainage); immediate locality of site (e.g. close to major roads, motorways, garages, sewage outfalls); and general locality (e.g. geographical, urban, rural, industrial, lead mining). If we exclude the possibility of dumping or accidental spillage of large quantities of alkyl lead compounds, our results show that concentrations of alkyl lead compounds are unlikely to exceed our limits of detection of 0.02 and 2 ppm (dry weight) for TAL and alkyl lead salts in sediment respectively, and 0.002 and 0.1 ppm in water in the localities studied.

Excluding the immediate vicinity of a TAL manufacturing plant (of which there is only one in the U.K. - at Ellesmere Port, Cheshire), the variation in factors considered should apply to all areas of the U.K. and we would therefore extrapolate these results to be valid throughout the U.K.

4.2.4. Road drainage grids

Owing to the negative results of the wide-scale survey undertaken above, we decided to sample closer to possible sources of alkyl lead pollution, both to confirm that our analytical techniques were valid on natural samples, and to trace how far, if at all, alkyl lead compounds were being carried into the drainage systems.

During measurements of the concentrations of airborne TAL, Laveskog[19] reported that the passage of a heavily choked car produced values as high as 5000 μgm^{-3} (cf. background of 0-3.5 μgm^{-3}), and that the concentration of TAL was much higher in such cars than in cars running unchoked with a warm engine. Laveskog and other workers[19-21] have reported that the highest general levels of airborne TAL were recorded close to petrol filling stations.

In view of these results we decided to sample road drainage grids near to garages, along busy commuter roads, and in residential areas where cars would be subjected to cold starts and choked running conditions.

TABLE 13

Samples containing alkyl lead compounds

Sample No.	Weight(g) solid in 30 ml	(TEL) SE-30	(TEL) TCEP	Confirm by TLC?	(TML)	(salts)
1	5.2	96	93	yes	5.3	6.1
2	8.6	4.7	5.1	(yes)	0.11	
3	6.4	0.36	0.35			
4	15.3	0.05	0.05			
5	4.8	0.33	0.33			
6	1.1	0.51	0.53			
7	8.0	0.20	0.18			
8	3.5	0.25	0.22			
9	4.5	0.09	0.07			
10	5.2	0.38	0.35			
11	7.3	0.24	0.25			
12	4.3	0.34	0.38			
13	5.4	54	58	yes	1.8	4.0
14	7.2	25	24	yes	0.36	

Sampling sites

1. Garage forecourt, Aston Expressway, adjacent to pumps.
2. " " by entrance.
3. Pool Farm Estate, bottom of Owens Croft.
4. " storm water settling tank.
5. Aston Expressway (N side), downhill from garage (ca. 10m).
6. " " " "
7. " " " (ca. 100m)
8. " (S side), overpass split.
9. " " " (after 14 days)
10. Garrison Lane, downhill from garage (ca. 30m).
11. " " (ca. 50m).
12. Carlton Road, 2nd grid from N (E side).
13. Garage forecourt, Garrison Lane, near pumps.
14. " " near entrance.

49 grids were sampled: 10 in Pool Farm Estate, a modern high density council housing estate; 10 in Small Heath, an area of high density terraced housing built around the turn of the last century; 19 on the A38(M) Aston Expressway; 12 adjacent to garages (6 on the Aston Expressway); and 4 on garage forecourts. Samples which gave positive results are listed in Table 13.

Of these, the only ones which contained alkyl lead compounds in great excess of our limits of detection were those on the two garage forecourts (samples 1-2, 13-14). The presence of TEL in these was confirmed by TLC. The samples also showed the presence of TML and alkyl lead salts. Roadside grids near to garages also showed concentrations of TEL, but only those grids downhill of the garages. (samples 5-7, 10-11) and the concentration decreased with distance from the garage. Those uphill of the garages showed no alkyl lead compounds to be present, indicating that the alkyl leads are transported by rainwater which washes them off the forecourts and into roadside grids. Were the alkyl leads to be washed out of the air around the garages by rainfall, significant concentrations would also be expected uphill of garages.

Grids on the A38(M) Aston Expressway were studied since this road carries both fast and slow moving traffic, depending on the time of day. No alkyl leads were detected in roadside dust, and only one out of the 16 grids sampled (excluding those downhill of the garage mentioned above) had detectable quantities of TEL present. This was attributed to either a local petrol spillage, or a heavily choked car standing for a long period over the grid. Under conditions of rain much of the unburnt petrol and TAL would be washed directly onto the road and hence into the grid. This grid was resampled after 14 days when the concentration of TEL had dropped by 70% presumably due to decomposition. Resampling several months later showed no TEL, due either to complete decomposition or to total removal of the polluted sediment during cleansing operations.

Within residential areas alkyl lead compounds were only detected at two sites, both as TEL, and both of which showed evidence of having had oil poured into them at some time. This was presumed to be waste engine oil dumped by 'do-it-yourself' car mechanics. That such oil contains TEL, and in great excess of TML, was shown in section 4.3. below.

At Pool Farm housing estate the road drainage from all the grids is combined and flows through a solids settling tank before entering the adjacent canal. Only one grid of the 10 sampled contained detectable quantities of TEL, this grid being close to the main solids settling tank. A sample of the sediment in this tank was also analysed and showed detectable quantities of TEL, but lower than that in the single grid. Presumably, some of the sediment containing TEL had been washed from the grid during heavy rain to pass through to the final settling tank. It seemed likely therefore that lesser quantities would also have been washed through to the canal beneath. None was detected however (sample 62, Table 12) probably because further dilution, and decomposition from exposure to light in the shallow edge of the canal, would have decreased the level of TAL below the limits of detection. This series of results demonstrates a possible mechanism for the dilution and removal of alkyl lead compounds settled out on solids in drainage grids.

Apart from these conventional grids, we also sampled motorway storm water drainage tanks, where solids are settled out before the water is discharged to canals or rivers. Sites were at the A38(M)/M6 interchange (098900) and at Spon Lane (010898 and 003899). No alkyl lead compounds were detected in any of these samples.

Only in the samples from garage forecourts (1-2,13-14) could the presence of TEL be confirmed by TLC, and only in these samples was TML detected. Alkyl lead salts (as R_3Pb^+) were only detected in samples 1 and 13, i.e. those containing the highest concentrations of TEL. Insufficient alkyl lead salts were present to determine the nature of the alkyl groups by TLC. Insufficient TML was present for confirmation by TLC, but its presence would be expected when large amounts of TEL are present, owing to their occurrence together in petrol (see Table 14, section 4.3.). No mixed alkyl leads were detected, and none would be expected judging by their absence in any of the petrols and oils analysed in section 4.3. No alkyl lead compounds were detected in samples of filtered water from the grids.

We attributed the predominance of TEL over TML in the grid sediments, despite generally higher levels of TML in petrol (Table 14), to the 100-fold greater volatility of TML over TEL at 20°C[5].

Confirmation of the presence of TAL was attempted by methods other than TLC, and employing the characteristic mass spectra of the alkyl leads, but neither proved suitable. With a mass spectrometer coupled to a GLC operated under conditions similar to those used in the ECD work, TEL in petroleum ether at 1000ppm was not detected. Direct injection of 10 μl at this concentration onto a MS9 mass spectrometer did not give patterns characteristic of TEL even at the highest sensitivity available. Thus analysis of natural extracts which were of considerably lower concentration was not possible.

The results in this section indicate that tetra-alkyl lead compounds do occur in drainage grid sediments, but only close to known or predictable sources. The consistent presence of TEL in grids close to garages, and its isolated occurrence away from garages is supported by data on concentrations of airborne alkyl lead compounds. Such data that is available gives considerably higher figures for sites in proximity to garages than for those adjacent to main roads[19-21]. Accumulation of TEL in grid sediment is much greater than that of TML probably on account of volatility differences. It is apparent that some of the tetra-alkyl lead decomposes to the trialkyl lead salt , as was predicted by our earlier study of the breakdown of TEL on sediment (section 3.2.1.).

The results also demonstrate the validity of our analytical techniques, and show that had any alkyl lead compounds been present above our limits of detection in the other sediments analysed, then they would have been detected.

4.3. PETROL AND OIL

It is reported that the mixed alkyl lead compounds $MeEt_3Pb$, Me_2Et_2Pb and Me_3EtPb are used in petrol today in addition to TEL and TML[2], and a recent survey of 30 petrols showed that 25% contained mixed alkyl leads[81]. Our results, showing as they did the presence of only TEL and TML, were therefore somewhat surprising.

TEL and TML are manufactured in this country in approximately equal quantities, a small proportion of these being mixed and subjected to catalytic redistribution for marketing as mixed alkyl leads. However since over 75% of the total production is exported it is not possible to estimate overall proportions of the different TAL compounds used in this country[22]. Furthermore the actual amounts and proportions added to petrol will vary with the individual batch as the characteristics of the crude oil vary.

We consequently carried out a small survey of the petrols available for sale locally to determine the proportions of the various

TAL compounds that we could expect in samples of sediments. The results are given in Table 14. Out of the 13 petrol samples analysed, all showed only TEL and TML to be present, the latter usually being in excess.

Additionally,used engine oil was analysed, partly to support the theory of oil dumping as a source of TAL in drainage grids, and partly because, if any TAL was present, this would probably be an average of that contained in the most recent batches of petrol used in that car. Again only TEL and TML were detected, the concentrations being given in Table 14.

All these results support our findings of only TEL and TML in the sediments, and as explained previously, the excess of TEL over TML in sediment in contrast to the proportions in petrol, is attributed to the 100-fold greater volatility of TML.

TABLE 14

Concentration of TAL in petrol and oil (ppm)

Sample	Rating	Date(1975)	(TML)	(TEL)	Comments
Esso	**	12.6	400	250	
Ultramar	**	27.6	230	100	
VIP	**	20.6	360	30	
Texaco	**	26.6	380	260	
Texaco	**	16.7	160	310	
Texaco	**	22.7	170	260	
Texaco	**	21.8	190	250	
Texaco	**	11.9	140	200	
Esso	****	1.7	340	165	
Esso	****	21.8	300	30	
Mobil	****	14.7	360	120	
Total	****	17.6	360	80	
Shell	****	15.6	390	350	
Mini van	oil	1.7	trace	47	** fuel
Austin 1100	oil	21.8	7.4	23	****fuel

4.4. CONCLUSION

We initially proposed that the major source of alkyl lead compounds in natural waters would be from its use as an antiknock agent in petrol. The results of our surveys have confirmed this proposition. Alkyl lead compounds were only detected close to places at which petrol is dispensed or burnt, or where used engine oil has been disposed of. The major source of entry of alkyl lead compounds into natural waters is from wash off from garage forecourts, the maximum level detected being 93 ppm(dry weight) in a garage forecourt grid. We have shown that decomposition via R_3Pb^+ and dilution of TAL in drainage grids will occur over a period of time, the latter probably occuring via removal of sediment from the grid settling pot during heavy rainfall and its subsequent mixture with other unpolluted sediments. Roadside grids close to garages had a maximum concentration of alkyl lead compounds of 0.53 ppm and showed a considerable reduction with the distance that rainwater had to travel along the ground before reaching the grid.

In grids distant from garages, alkyl lead compounds were only detected in isolated cases. Two of these were attributed to deliberate dumping of waste engine oil, and one to the accidental spillage of engine oil or petrol, or to the presence of a heavily choked car for a long time in the vicinity of the grid.

In all cases the major compound present was TEL, the occurrence of TML and alkyl lead salts being limited to sites on garage forecourts where levels of TEL were in great excess of the limits of detection of our analytical techniques.

The total absence of the mixed alkyl lead compounds is predictable from the survey of petrol carried out in the locality in which 13 petrols analysed were shown to contain only TEL and TML. The relative absence of TML in sediments is attributed to its 100-fold greater volatility over TEL, and its consequent greater rate of evaporation from spillages.

The lack of detection of alkyl lead salts outside of garage forecourts is partly due to the greater limits of detection involved in their analysis (approximately 100-fold those of TAL). Thus if TAL decomposed to alkyl lead salts, a large accumulation of the latter would have to occur in roadside grids before the concentration reached detectable levels, and we have shown that the salts themselves will decompose on sediment (25% loss in 3 days for Et_3PbCl, 90% for Et_2PbCl_2: see section 3.2.3.). Only on garage forecourts, where TEL concentrations were up to 100 times greater than in roadside grids, were sufficient alkyl lead salts (as R_3Pb^+) present to be detected. It seems unlikely however that significant build up of alkyl lead salts is occurring in roadside grids. Taking the percentage of total alkyl lead compounds occurring in garage forecourt grids from the figures in Table 13, expected maximum levels in roadside grids would be 0.05 ppm for alkyl lead salts.

In larger bodies of water not directly connected to road drainage systems, no alkyl lead compounds were detected at any of the 100 sites sampled. This indicates that there is an adequate trapping and dilution of alkyl lead compounds by settling pots in grids before the water runs into more open bodies of water. It also shows that any other sources, for example wash out of airborne alkyl lead by rainfall, do not contribute sufficiently to the concentration in ground waters to enable detection.

Thus, the results of the surveys indicate that there is no general threat to public health from the presence of alkyl lead compounds in natural waters. Alkyl lead compounds only occur at specific and generally predictable sites and these are such that human contact with the water or sediment is unlikely, even supposing that the levels were dangerous. It is conceivable however that temporarily higher levels will occur from dumping or accidental spillage of petrol or used engine oil.

CHAPTER FIVE

EXTRACTION OF ALKYL LEAD COMPOUNDS FROM WATER

EXTRACTION OF ALKYL LEAD COMPOUNDS FROM WATER

5.1. INTRODUCTION

Despite the fact that low concentrations of alkyl lead compounds are totally adsorbed onto sediment when this is present, it was thought to be desirable to be able to detect very low concentrations of alkyl lead compounds in solution in waters with low suspended solids contents such as drinking waters. Although rigorous standards are applied to drinking waters for the total lead content (W.H.O. maximum permitted level = 0.1 ppm[82]) there is no method available to determine the nature of the lead present. In view of the higher toxicities of some alkyl lead compounds, a level of lead within the accepted limits could present a hazard to health if a large proportion of this was in the form of trialkyl lead salts.

Activated carbon is known to adsorb many trace impurities in water, both organic[83] and inorganic[84], and its properties have been reviewed[85]. Lead by-products from the manufacture of TAL have been reclaimed by carbon[86], and TAL vapours adsorbed from air onto carbon have been estimated by X-ray fluorescence spectroscopy after briquetting of the carbon with stearic acid[87]. It is reported that TEL decomposes on activated carbon since it is not extractable by steam distillation or benzene, but no decomposition products were reported[88].

We investigated the possibility of the removal of low concentrations of alkyl lead compounds from water onto carbon, and subsequently their removal from carbon for estimation.

5.2. TETRA-ALKYL LEAD COMPOUNDS

It was found that TEL, both as a pure liquid and in dilute solutions of organic solvents, was totally adsorbed onto activated carbon. It was not feasible to check the adsorption from water owing to the very low solubility of TAL in water. After adsorption, desorption did not occur significantly (<2%) with organic solvents such as ether, acetone, and chloroform. Extraction of TEL from carbon at low pressures and elevated temperatures was also unsuccessful. This confirms the previously reported unsuccessful attempt to extract TEL from carbon[88], and may imply strong adsorption or decomposition.

With 50% aqueous acetic acid, 18% extraction (as Pb) was achieved. Under similar conditions, 35% of added lead nitrate was extracted, 90% of Et_2PbCl_2, and 80% of Et_3PbCl. TLC showed the extract of TEL to be mainly Et_2Pb^{++} and Pb^{++}. If TEL decomposes to any of the above compounds, extraction of >18% would be expected and the majority of TEL added must therefore be adsorbed very strongly or decomposed to another strongly adsorbed species, possibly a lead oxide.

5.3. ALKYL LEAD SALTS

Dilute aqueous solutions of Et_3PbCl and Et_2PbCl_2 were passed through columns containing activated carbon (50 ml, 30 g). At 16 ppm the salts were initially totally adsorbed , breakthrough occurring at ca. 5 mg of salt adsorbed, after which the effluent solution increased in concentration to ca. 30% of the influent concentration for Et_3PbCl and 50% for Et_2PbCl_2, at which it levelled off. For volumes of up to 1 l of dilute solutions (1-5 ppm) the alkyl lead salts were completely adsorbed and were not eluted by washing the column with water.

Since 50% aqueous acetic acid (50%AcOH) had extracted alkyl lead salts from sediment, we decided to study its suitability for extraction of the salts from carbon. 50% AcOH extracted Et_3PbCl at 80% as estimated on the total lead recovered (64% as alkyl lead salts), and Et_2PbCl_2 at 90% (total lead). Et_2PbCl_2 was also extracted by acetone (14% as estimated on total lead recovered).

For estimation by atomic absorbtion spectroscopy , 50% AcOH was satisfactory but for estimation by PAR to determine the nature of the extracted lead, prior neutralisation had to be carried out. This could be achieved by the use of Na_2CO_3 or NaOH, but care had to be exercised to prevent excessive rises in temperature which were found to lead to the decomposition of the alkyl lead salts. Both reagents however produced excessive heat and a large volume of solution for analysis, and so an extraction with milder solvents was sought. The results of these experiments are given in Table 15.

Et_3PbCl was not extractable in an alkylated form by acetone, benzene, methanol, lead nitrate solution (100 gl^{-1}), or HCl (up to 20%), but could be extracted with a solution of 2% HCl in methanol which removed 26% of Et_3PbCl added. The same eluant removed 30% of added Et_2PbCl_2. The insufficiency of either 2% aqueous HCl or methanol alone to remove the salts indicated that adsorption was possibly of a dual nature, partly due to the ionic character of the salts, and partly due to the presence of organic groups. We noted that Et_3PbCl was eluted partly as Et_2PbCl_2, and attributed this to decomposition by the solvent. Decomposition of R_4Pb to R_3Pb^+ and to R_2Pb^{++} in the presence of HCl and methanol has been previously reported[89], and it is possible that carbon catalyses this reaction.

In an attempt to achieve higher recoveries than that with HCl/methanol, the effect of reduction in concentration of acetic acid

TABLE 15

Extraction of alkyl lead salts from carbon

Salt	Weight (μg)	Eluant	Volume	Fraction 1 (μg)	Fraction 2 (μg)	Total % recovery
Et_3PbCl	9900	2% aq. HCl	250	78	-	0.8
"	9900	10% $Pb(NO_3)_2$	250	187	-	1.9
"	9580	Acetone	250	106	-	1.1
"	9580	Benzene	250	nil	-	-
"	9580	Methanol	250	nil	-	-
"	1145	2%HCl/MeOH	2x200	203	109	26
Et_2PbCl_2	811	"	2x200	185	60	30
Et_3PbCl	1050	5%AcOH	2x200	244	183	40
Et_2PbCl_2	849	"	2x200	448	142	70

from 50% was investigated. We found that reduction to 5% made relatively little difference to the extraction efficiency, giving 70% for Et_2PbCl_2 and 40% for Et_3PbCl. Since only a small amount of NaOH was required for neutralisation, this solvent was used in subsequent extractions.

Using acidic solvents, we found that iron was extracted from the carbon and interfered with determination of alkyl lead salts by PAR. This interference was removed by addition of inorganic lead to the solution before neutralisation. The iron was then coprecipitated with the lead.

The unextractable portion of the alkyl lead salts was presumed to be either decomposed to non-extractable products, or adsorbed on more active sites on the carbon. Et_3PbCl when eluted with 5%AcOH gave an eluant containing both Et_3PbCl and Et_2PbCl_2, the percentage of the latter increasing with elution volume. It is probable therefore that decomposition of Et_3PbCl to Et_2PbCl_2 takes place only in the presence of acetic acid. If this were not the case, Et_3PbCl added to carbon would give some Et_2PbCl_2 in the eluant when eluted with acetone, since acetone will partly elute Et_2PbCl_2. However acetone elutes no alkyl lead salts from Et_3PbCl added to carbon. The fate of the non-extracted portion of the added salts was not investigated further since it was sufficient for the aims of this project to be able to adsorb alkyl lead salts onto carbon and to desorb them still in a partially alkylated state. Determination of relative amounts of R_3PbX and R_2PbX_2 salts could be achieved by extraction with acetone, which removes only R_2PbX_2, before extraction with 5%AcOH.

It was shown by the storage of samples of alkyl lead salts adsorbed onto carbon that they were reasonably stable over a period of several days. Concentrations of salts recoverable fell from 70% to 27% for Et_2PbCl_2 on carbon stored dry in the dark for two weeks and to 20% for the same stored in water in the dark. Values for Et_3PbCl fell from 40% to 24% under both sets of conditions.

It was not feasible in the laboratory to pass large quantities of water containing low concentrations of alkyl lead salts through carbon, as might occur where river or drinking water is continuously passed through activated carbon. An attempt to simulate such conditions was made by passing large volumes of tap water through carbon onto which had been adsorbed small amounts of alkyl lead salts. No removal of the salts by water was observed over and above the reduction in percentage recovery observed in identical samples in a small volume of still water. It was therefore assumed that low concentrations of alkyl lead salts would be removed onto carbon, since this occurs at higher concentrations and there is no indication that the reverse would occur under equilibrium with pure water. A parallel experiment using activated carbon with no added alkyl lead salts showed no alkyl lead compounds to be present in the water, after a throughput of ca. 15,000 l of tap water.

Extraction of carbon through which filtered water from the River Trent (20,000 l) had been passed gave no detectable alkyl lead salts.

Extraction of carbon through which sewage had been passed after treatment with lime, carbon dioxide and sand filter also showed no detectable alkyl lead salts. Extraction of the sand filter by the method used for river sediment likewise gave a negative result. It was shown that treatment of a dilute solution of alkyl lead salts with lime and carbon dioxide gave only a 10% loss of alkyl lead, thus any alkyl lead salts in solution would have been detected.

5.4. CONCLUSION

We have developed a method for the estimation of very low levels of alkyl lead salts occurring in waters of low suspended solids content. Such salts are adsorbed onto granulated activated carbon, and may be subsequently eluted by 5% aqueous acetic acid and estimated. It has not been possible to evaluate this technique under exact field conditions, but a simulation of these conditions gave satisfactory results.

Subsequent analysis of three types of water showed no detectable levels of alkyl lead salts to be present. The waters analysed were: Birmingham drinking water; the River Trent at Colwick (SK 610390); and a treated sewage effluent.

We should not have expected drinking water to contain any alkyl lead salts owing to the lack of opportunity for such compounds to enter the

the water. Neither would water from a river have been expected to contain significant concentrations of alkyl lead salts in solution, since even though the river flow is slow, there is still a considerable concentration of suspended solids.

In sewage however, the relatively high levels of lead concentration and bacterial activity might have been expected to create an ideal environment for the methylation of lead, and we showed that any alkyl lead salts so formed would not be removed by the treatment used before filtration through sand and activated carbon. A second source of alkyl lead compounds might be from road drainage which had entered the foul sewer systems. That no alkyl leads were detected indicates that there is no significant contribution from either of these sources. The absence of biological methylation by sewage was further confirmed, and is discussed in Chapter 6.

CHAPTER SIX

METHYLATION OF LEAD

METHYLATION OF LEAD

6.1. INTRODUCTION

In Minamata, Japan, between 1953 and 1960 at least 111 persons suffered from methylmercury poisoning and 41 died. The source of poisoning took many years to trace, but it was eventually shown that large amounts of inorganic mercury dumped in a river estuary had been bacteriologically converted to methylmercury and concentrated by fish which formed the staple diet of the local population[90].

It seemed desirable to determine whether such methylation might occur for inorganic lead, of which 12,000 tonnes are released annually from internal combustion engines in the U.K. alone[15].

Biological methylation of certain metals and metalloids was recognised many years ago[91] but that of mercury has only recently been discovered[92]. The natural methylation of mercury and its compounds has been reviewed recently[32,93]. It has been shown that there are two mechanisms involved in this methylation. A non-enzymatic route involves methyl cobalamin, a vitamin B_{12} derivative, which readily methylates mercuric salts[94,95] and which is present in many natural species known to methylate mercuric salts. However certain organisms lacking B_{12} will also methylate mercuric salts, and so an enzymatic route has also been proposed[96,97].

The first reported biological methylation of lead was in 1975 by Wong et al, who stated that addition of lead nitrate or trimethyl lead acetate to lake sediment incubated under anaerobic conditions greatly increased TML production. All sediments examined produced TML from trimethyl lead acetate but only certain sediments produced TML from lead nitrate of chloride. No TML production was detected from lead hydroxide, cyanide, oxide, bromide, or palmitate added to sediment[98].

By analogy with the methylation of mercury, a possible mechanism for the reported methylation of lead would be the transfer of a methyl group from methyl cobalamin. In the case of inorganic lead this is unlikely to occur by a simple transfer of one methyl group, since it would involve the formation of the unstable monomethylated species[28] as intermediate. The di- and trialkyl lead compounds however should form the tri- and tetra-alkyl lead compounds respectively if methylation occurs. It has previously been reported that no ^{14}C-TML was formed from Pb^{++} and ^{14}C-methyl cobalamin[20].

We attempted to repeat the work of Wong et al using lake sediments and other natural systems, and also attempted the methylation of inorganic lead and di- and trialkyl lead salts by methyl cobalamin in the hope of establishing the mechanism of methylation.

6.2. ATTEMPTED METHYLATION BY SEDIMENT AND BY CHEMICAL SYSTEMS

We attempted the methylation of various lead and alkyl lead salts using the method that Bertilsson and Neujahr used for the methylation of mercuric salts by methyl coabalamin[94]. During methylation the methyl cobalamin is converted to the hydroxy cobalamin and the resulting changes in the ultra violet spectum can be monitored. As methylation proceeds, the absorption due to methyl cobalamin at 374 nm decreases and that due to hydroxy cobalamin at 350 nm increases.

Bertilsson and Neujahr found that the rate of methylation increased with substrate concentration. A large excess of potential substrate was therefore used in the present work in order to give conditions most favourable to methylation. That the conditions were suitable for methylation to occur was shown by the immediate methylation of $HgCl_2$.

Addition of Me_3PbOAc, Me_3PbCl, Me_2PbCl_2 and $Pb(NO_3)_2$ to methyl cobalamin in aqueous solution produced no change in the uv spectrum of the mixture within 21 days, the spectrum being identical to that of methyl cobalamin in distilled water. Thus methylation does not occur under these conditions and any methylation which occurs in natural systems cannot proceed by this pathway. It must therefore occur either by an enzymatic pathway or via chemical reaction with some other component in the methylating system.

We found that addition of Me_3PbCl and Me_3PbOAc to active lake sediments produced 300 μg TML in the atmosphere above the solution after anaerobic incubation for two weeks. This figure compares favourably with those reported by Wong et al of 125 μg after one week and 642 μg after two weeks[98]. However we detected no TML on incubation of lake sediment with the addition of lead nitrate.

It is known that salts of the type R_3PbX tend to disproportionate according to:

$$2\ R_3PbX \longrightarrow R_2PbX_2 + R_4Pb$$

and the weaker the acid HX, the greater is the tendency to disproportionate[2]. Thus if the anion of Me_3PbOAc or Me_3PbCl were replaced by the anion of a weaker acid, a greater rate of disproportionation would be predicted and a greater rate of production of TML.

We found this to be the case when dilute solutions of trimethyl lead salts were mixed with a solution of sodium sulphide. Me_3PbCl produced 50 μg of TML in the vapour above the solution in two weeks whilst Me_3PbOAc produced 300 μg. Solutions of the alkyl lead salts without added sulphide produced only low background levels of TML. When incubated at the same concentrations in sediment these salts produced 400 and 650 μg TML respectively. Thus it seemed likely that the apparent biological methylation of trimethyl lead salts was due to reaction of the salts with sulphide ion to form $(R_3Pb)_2S$ and the subsequent disproportionation of this compound.

In support of this proposed mechanism the products formed by Et_3PbCl with sediment and with Na_2S were studied. With sediment TEL was detectable after 4 days, the total in the atmosphere above the solution being 0.15 μg. No Et_3MePb was detected. With sulphide the amount of TEL produced after 4 days was 0.8 μg.

Since direct biological methylation would have produced Et_3MePb and not Et_4Pb as was detected, it appears that such methylation does not occur in this case and that some form of disproportionation is again taking place. As in the case for the methyl lead salts, this can be explained by reaction with sulphide.

Sulphide, in the form of hydrogen sulphide, is liberated by many organisms under anaerobic conditions. This would normally react with any metallic ions present to form insoluble sulphides (such as, ferrous sulphide) but, presumably, the trialkyl lead salts compete with these inorganic ions for sulphide. Wong et al[98] stated that no TML was produced after autoclaving of the sediment: in such a system, the amount of free sulphide available will be low because of the reaction of any H_2S present to form insoluble sulphides, and further H_2S will not be made available for the trialkyl lead salts because of lack of biological activity. Thus, if the mechanism proposed here is correct, production of TML from trimethyl lead salts would not be expected to proceed after autoclaving of the sediment[99].

Although the proposed mechanism will explain the production of TML from Me_3PbOAc in natural sediments, it offers no explanation for the reported methylation of inorganic lead[98]. This must involve a highly specific mechanism since lead nitrate and chloride, but not lead bromide hydroxide, cyanide, oxide or palmitate were methylated, and these only by certain lake sediments[98]. Since the monomethylated lead species is unstable and does not exist in a free state[28] any methylation process producing TML from Pb^{++} must substitute at least two methyl groups onto the lead atom before the ion is released into the environment. In an attempt to produce such methylation, and to repeat the work of Wong et al, we studied the various natural systems which are detailed below.

6.3. ATTEMPTED METHYLATION BY SEWAGE

Reports on the methylation of mercuric salts in anaerobic sewage digestors are conflicting. One recent report states that such digestors provide an ideal environment for methylation, being essentially scaled up versions of laboratory systems[100]. Another report however, states that no methylation occurs in such systems possibly owing to inhibition by sulphide[101]. The capacity of aerobic digestors to methylate mercuric salts is not in doubt however[100].

Incubation of sewage under anaerobic conditions produced no TML both with and without the addition of inorganic lead. TML was, however produced from Me_3PbCl, presumably via the mechanism proposed previously. Aerobic digestion of sewage with the addition of TEL produced no Et_3MePb or any other methylated lead species. Under these conditions 75% of the TEL broke down, presumably via Et_3Pb^+ and other more active species, but none of these underwent methylation. No TML was produced from sewage digested aerobically without the addition of TEL, with or without the addition of inorganic lead. 85% of TML added to sewage could be recovered under these conditions, indicating that if sufficient TML were formed from Pb^{++} it would have been detected.

6.4. ATTEMPTED METHYLATION BY LEAD RESISTANT SPECIES

6.4.1. General introduction

The methylation of mercuric salts is considered to be a detoxification mechanism since those species which are most tolerant to Hg^{++} are those which most rapidly produce methylated mercury and subsequently Hg^{o} which is removed from the system by volatalisation[97,102]. It was suggested that if this was the case for lead, than the species most likely to methylate lead would be those that occured in an environment of high lead concentration[103]. The ability of micro-organisms to adapt to withstand toxic environments created by high concentrations of lead has been established[104].

Samples of sediments from areas of known high lead burden were incubated with additional lead nitrate in an attempt to produce detectable amounts of TML. Samples were taken from four main sources: Checkley Sewage Works; Snailbeach Mine; Mid-Wales; and Derbyshire. These sources are described below, the numbers in brackets referring to the specific sites as listed in Table 16.

6.4.2. Checkley Sewage Works

The area serviced by the Checkley Works (SK 036377) situated in the Blythe Valley near Stoke-on-Trent, is mainly residential, but includes several works associated with the pottery industry. These produce effluent high in heavy metal concentrations, particularly in lead and cadmium, and the resultant sewage is therefore relatively high in lead content. Bacteria active in the digestion of this sewage must therefore be able to survive the environment provided by this high lead concentration. Samples were taken from a closed anaerobic digestion tank (2), and from an open tank (1) used as storage for the digested sludge prior to discharge to the drying beds.

TABLE 16 Samples of high inorganic lead content

Sample No.	Grid ref.	pH	Weight(g) solid in 30 ml	$(Pb)H_2O$ (ppm)	(Pb)solid (ppm)
1	SK 036377	8	2.5	0	8000
2	SK 036377	8	2.8	0	7300
3	SJ 377023	7.5	13.5	1.0	4400
4	SJ 377023	6.5	3.5	0.9	4300
5	SJ 375022	5.5	15.5	10.0	50600
6	SN 673808	7	14.7	1.7	7000
7	SN 671805	7	5.3	1.1	6800
8	SN 671805	7	45.2	0.5	490
9	SN 665789	6.5	22.6	0.3	580
10	SN 724736	7	11.6	0.7	2300
11	SN 800744	7	5.9	0	690
12	SN 800744	6	33.0	0.3	530
13	SK 187697	8	10.4	0	1000
14	SK 187697	8	20.3	0	850
15	SK 205661	8	8.3	0	920
16	SK 205661	8	5.1	0	2000
17	SK 232648	8	10.0	0	1700
18	SK 270620	8	9.3	0	2200
19	SK 300572	8	28.2	0	750
20	SK 340532	8	9.2	0	850
21	SK 337532	8	18.9	0	950

Sampling sites

1. Checkley Sewage Works: open digestor.
2. " closed digestor.
3. Snailbeach Mine: lake.
4. " " .
5. " pig effluent.
6. Goginan: bridge.
7. " footpath by stream, sandy.
8. " " organic.
9. R. Rheidol, 4 km below Cwm Rheidol Mine.
10. Below Frongoch Mine, stream.
11. R. Ystwyth, below Cwm Ystwyth Mine: organic.
12. " " sandy.
13. R. Wye, 1 km below sough: side stream.
14. " " main stream.
15. Lathkill Dale: 400m below footbridge.
16. " 100m below footbridge.
17. Alport, below road bridge.
18. Darley Bridge, below road bridge.
19. Cromford, below bridge over Derwent.
20. Ambergate: canal.
21. " river by abstraction point.

6.4.3. Snailbeach Mine

Snailbeach mine (SJ 375023), 12 km SW of Shrewsbury, was Shropshire's most productive lead mine, and was once estimated as being the richest mine per acre in Europe. Lead, zinc, and barium ores are present in a calcite matrix, and lead workings took place in Roman times. More recently it was worked for lead from 1783 to 1911 after which it was worked for barytes until 1955. The only current activity is the removal of fine spar from the exposed spoil heaps for use as pebble dash in the building trade[105].

Samples were taken from a small lake (3-4) once used as a storage reservoir for water pumped from the mine prior to its use for washing the ore, and from an earth lined pit containing effluent from a pigsty (5).

6.4.4. Mid-Wales

Mining of lead, zinc and silver took place extensively in this area from 1820 to 1920, during which time lead containing ores were mobilised and released to the environment in washing processes and in dumping of non-productive measures on spoil tips[106,107].

In the 1930's it was shown that although higher forms of plant and animal life had been virtually eliminated from the River Rheidol area, a relatively wide range of algae were present but in smaller number than in unpolluted streams[106]. Despite cessation of mining activities over 50 years ago, the Rivers Rheidol and Ystwyth still contain moderately high concentrations of lead[108]. This is probably caused by the extensive peat bogs and acid moorland water which drain into the rivers[106]. Such acidity will increase the solubility of lead and thus the leaching action of rivers and streams flowing through the mining waste areas.

A recent survey in the area showed the presence of micro-organisms which had adapted to withstand the toxic environment created by the old lead workings[104].

Samples of sediments were taken from the following sites, the numbers in brackets referring to Table 16.

Goginan (6-8). This mine was one of the largest in the district and was closed in 1922. The River Melindwr flows through the base of the mine past the old waste dumps[106]. Samples were taken from the river 2 km below the mine, and at one site samples of both the sandy and the organic sediment were taken.

Cwm Rheidol (9). The sample was taken from the River Rheidol 4 km downstream of where the river flows past the mine.

Frongoch (10). The sample was taken from a small stream which emanates from the mine. The extensive workings at this mine ceased in 1903 being the last operating mine in the Ystwyth valley[107].

Cwm Ystwyth (11-12). This mine was one of the largest in the valley and closed in 1893[107]. The River Ystwyth runs through the base of the mine and is fed by small tributaries which flow through the mine workings. Samples of both the sandy and the organic sediment were taken from just downstream of the mine.

6.4.5. Derbyshire

In the Peak District of Derbyshire lead occurs mainly as galena, lead sulphide, in the mineral veins of the carboniferous limestone rocks, but occasionally as the oxidised form, cerrussite, lead carbonate. The mineral veins can contain up to 10% galena, averaging 5%, and it is estimated that 2-3 Mtonnes have been mined since working began in Roman times. The associated gange minerals, fluospar, barytes, and calcite form over 90% of the veins and were regarded as waste in the lead mining industry. Nowadays these materials form the main products of mining in the area, lead and other minor ores being regarded as by-products.

Before the seventeenth century, the depth of mining was determined by the water table, which in theory lies at the level of the nearest valley floor. However local irregularities such as impervious layers of rock and other geological complications led to considerable seepage into mines even up to 100 m above normal water table. Where possible this was run off into natural underground cavities or via small tunnels leading to nearby valleys.

By the seventeenth century the larger lead veins had been worked down to the water table and to enable deeper mining to take place, underground channels or 'soughs' were dug. These lowered the local water table by channelling the water away to deeper valleys than those immediately adjacent to the mine, sometimes as far as 5 km away. These 'soughs' were essential to the lead workings and many are still in good condition and carrying water today[109].

Although the solubility of the naturally occurring lead salts is low, especially in the alkaline water resulting from the carboniferous limestone measures, the quantity of water that has flowed through the mineral veins, workings and spoil heaps is large, and considerable amounts of lead must have been carried, both in solution and as suspended solids, into the natural water courses of the area. This is born out by the high lead levels found in the sediments of the rivers and streams sampled (Table 16).

Samples of sediment were taken at the following sites, the numbers in parenthesis referring to the specific sites listed in Table 16.

Magpie Mine (13-14). Built in 1881, the sough from this mine was the last major sough to be built in Derbyshire and still carries a considerable flow of water. The sampling point was about 1 km downstream of where the sough enters the River Wye.

Lathkill Dale (15-16). There are several mines in this area with sough outfalls at 205661, 197661, and 180658. The samples were taken downstream from the lowest outfall.

Alport-by-Youlgreave (17). This area was one of the most intensively worked, tremendous efforts being made to dewater the mines to lower levels by means of many kilometres of soughs. Much of this drainage would have entered the Rivers Lathkill and Bradford and the sample was taken from below their confluence downstream of Alport.

Darley Bridge (18). Many of the mines north of the River Lathkill were drained by the Hillcarr Sough which outfalls into the River Derwent at 258637. The Yatestoop Sough also enters the river above Darley Bridge at 263626 and a small stream entering adjcaent to Darley Bridge carries the Old Mill Close Mine sough water. The sampling point was downstream of these three discharges.

Cromford (19). This sough was the first major one to be undertaken in Derbyshire, work commencing in 1673, the outfall being by the Market Place at 295568. Apart from this there are many mines between Matlock and Cromford, much of the drainage water from which enters the River Derwent. The sampling point was immediately downstream of the Cromford road bridge.

Crich (20-21). The main sough which outfalls into the River Derwent at 331548 and still carries water, once served the dual purpose of removing water pumped from lower levels by a steam engine, and of carrying boats which supplied coal to the engine. Entering the river at almost the same point is the Oxhay Brook which runs directly through the mining area. The river was sampled 2 km downstream of this point as was the canal which also takes local land drainage water[109].

6.4.6. Results and discussion

Before incubation, samples of each sediment were analysed for TAL and alkyl lead salts, but were all found to be negative. Analysis of dried sediments following the method of Hemsley[110] showed that they were all of above average lead concentration. Normal figures for soils in areas devoid of lead bearing ores are 2-100 ppm[111]. The samples studied in the present work ranged from 490-50,600 ppm (Table 16), thus justifying the choice of areas.

The water above the sediments was also analysed for pH and lead concentration and the figures reported in Table 16. In the alkaline waters of the Derbyshire area and in the Checkley sewage sludge, no lead was detected, but in the more acidic samples from Mid-Wales, concentrations of up to 1.7 ppm were recorded. The exceptionally high figure for sample 5 was thought to be due to the highly acidic effluent from a pigsty standing in contact with lead bearing minerals.

In no case was TML produced in detectable quantities upon anaerobic incubation.

Considering the wide variety of samples taken and the known adaptation of micro-organisms to withstand high lead concentrations, it seems unlikely that such adaptation involves detoxification via TML production.

6.5. CONCLUSIONS

As regards the biotransformation of trimethyl lead acetate to TML reported by Wong et al[98], the following results need to be considered. The main non-enzymatic route for the methylation of mercury (i.e. via methyl cobalamin) is not available to trimethyl lead acetate. The production of TML from trimethyl lead acetate in anaerobic sediment is comparable to that from trimethyl lead acetate in aqueous sodium sulphide solution. Micro-organisms in anaerobic sediments are known to produce sulphide ions. Autoclaved sediment which will contain no active micro-organisms will not produce TML from trimethyl lead acetate[98]. TEL, not Et_3MePb is produced from Et_3PbCl in both anaerobic sediment and aqueous sodium sulphide solution. These results indicate that the probable route for the conversion of trimethyl lead acetate to TML is dependent on biological activity only to produce sulphide ions, and not for direct biological methylation. The sulphide ion reacts to produce trimethyl lead sulphide $(Me_3Pb)_2S$ which subsequently disproportionates forming TML as one product.

As regards the reported methylation of inorganic lead by Wong et al[98], we were unable to repeat this experiment. We have shown that the major non-enzymatic route for the methylation of mercuric compounds is not available to lead salts. We have shown that a variety of highly active biological systems will not produce this biotransformation, and that the reported methylation is not a common detoxification mechanism, in contrast to the methylation of mercuric salts.

We can only conclude that by whatever mechanism this biotransformation occurs, it must be highly specific. Since lead nitrate and chloride, but not lead hydroxide, cyanide, oxide, bromide, or palmitate produce TML and since such methylation only occurs in certain sediments, it would appear that a specific organism was present in the sediment used by Wong et al which was absent in all the sediments with which we attempted methylation.

EXPERIMENTAL

1. General

2. Experimental work relating to Chapter 2

3. Experimental work relating to Chapter 3

4. Experimental work relating to Chapter 4

5. Experimental work relating to Chapter 5

6. Experimental work relating to Chapter 6.

EXPERIMENTAL

1. GENERAL

Unless otherwise stated, the following terms, reagents and experimental conditions were used throughout this work.

1.1. Reagents

All reagents were A.R. grade.

Petroleum ether, boiling range 40-60°C is abbreviated to P.E. Where this was used for GLC analyses, it was distilled before use.

Ether unqualified refers to diethyl ether.

Water refers to distilled water.

4-methyl pentan-2-one GPR (Methyl isobutyl ketone, abbreviated to MIBK) was distilled before use.

HNO_3 refers to concentrated nitric acid.

HCl refers to concentrated hydrochloric acid.

Samples of the following compounds were kindly provided by the Associated Octel Company:

Tetraethyl lead (pure)	(Et_4Pb, TEL)
Tetramethyl lead (80% in toluene)	(Me_4Pb, TML)
Mixed alkyl leads (TEL/TML, 50/50, catalitically redistributed)	
Triethyl lead chloride	(Et_3PbCl)
Trimethyl lead chloride	(Me_3PbCl)
Diethyl lead dichloride	(Et_2PbCl_2)
Dimethyl lead dichloride	(Me_2PbCl_2)

1.2. Instrumentation

Atomic absorbtion spectroscopy (AAS) was carried out on a Perkin Elmer PE 303 Spectrophotometer using the 283.3 nm line of a hollow cathode lead lamp, and an air/acetylene flame. Aqueous samples were run against standards of lead nitrate in 1% aqueous HNO_3, and organic samples against standards of TEL in MIBK.

Ultra violet spectra were recorded on a Pye Unicam SP800 spectrophotometer.

Infra red spectra were recorded on a Perkin Elmer PE157G grating spectrometer, as a KBr disc (ca. 1.0%).

Nuclear magnetic resonance spectra were recorded on a Perkin Elmer R-14 (100 MHz) spectrometer at 34°C.

1.3. General techniques

Evaporation of solvents was carried out on a rotary evaporator, the vacuum for which was provided by a water pump.

pH was measured on wide range universal indicator paper (Johnsons).

Sediment specified as LMD refers to Lea Marston Dredgings from a lake (SP 059932) through which water from the River Tame is pumped, and where the majority of suspended solids settle out. PFC refers to Pool Farm Canal sediment, taken from near to a storm water drainage outfall from a municipal housing estate (SP 053784). Approximate total lead concentrations (dry weight) were:LMD, 500 ppm; PFC, 200 ppm. For most experiments 30 ml of sediment was used having a dry weight of solids of 5-10 g.

2. EXPERIMENTAL WORK RELATING TO CHAPTER TWO

2.1. Preparation of alkyl lead salts

All confirmatory TLC work was carried out on silica plates eluted with acetic acid/benzene (1:3). Other conditions are described in section 2.2. below.

2.1.1. Et_3PbCl

To ether (50 ml) saturated with HCl gas, TEL (7.9 ml) was added dropwise. During addition the mixture was shaken and cooled. A white precipitate formed immediately. The mixture was allowed to stand for 20 min at $0^{o}C$ and 30 min at ambient temperature. The white solid Et_3PbCl was filtered off. Yield = 11.0 g (83%). Its IR spectrum (Table 2) was identical to that previously reported[40] and its identity was further confirmed by TLC against a genuine sample. Both showed a second spot corresponding in colour and position to Et_2Pb^{++}. The NMR spectrum was recorded (Table 4).

2.1.2. Et_2PbCl_2

2.1.2.1. TEL (1 ml) in ether (250 ml) was maintained at $-50^{o}C$ whilst HCl gas was bubbled through the solution. The temperature was allowed to increase to $-10^{o}C$ at which point the flow of HCl gas was stopped. As the solution warmed up to ambient temperature, a cloudy white precipitate gradually formed. HCl gas was passed through for a further 5 min during which time a thick white precipitate formed. This was filtered off. The IR spectrum (Table 2) was identical to that previously reported for Et_2PbCl_2[58] and its characteristics on TLC were identical to those of a genuine sample.

2.1.2.2. TEL (6 ml) in toluene (50 ml) was maintained at $90^{o}C$ and HCl gas bubbled through the solution. After 20 min the solution was cloudy, and after 60 min a heavy white precipitate had formed. The precipitate was filtered off after 80 min and washed with boiling toluene. Its identity was confirmed as above. TLC showed the product to be heavily contaminated with inorganic lead, as seen as a crimson spot of R_f 0.0. The NMR spectrum was recorded (Table 4).

2.1.3. Et_3PbOH

Et_3PbCl (1 g) in benzene (15 ml) was shaken with NaOH (5.6 g) in water (8.4 ml) for 20 min. The white solid which formed in the lower layer (cf. Calingaert et al[42]) was separated off, extracted with hot benzene and crystallised by filtering into a flask at 0°C. The Et_3PbOH was filtered off and dried under vacuum over phosphoric oxide for 4h. TLC showed a yellow spot with R_f in between that of Et_3PbCl and Et_2PbCl_2. The IR spectrum (Table 2) was identical to that previously reported[43]. Yield = 0.68 g (72%). The NMR spectrum was recorded (Table 4).

2.1.4. $Et_2Pb(OH)_2$

Et_2PbCl_2 (3 g) in ether (30 ml) was shaken with NaOH (3 g) in water (10 ml). The white suspension disappeared from the ether layer, and the aqueous layer turned cloudy. The mixture was left overnight, the ether decanted, and the solid at the interface filtered off and dried under vacuum over P_4O_{10} for 2 d in the dark. The IR spectrum was identical to that reported for $Et_2Pb(OH)_2$. On TLC, the product gave a salmon red spot of the same R_f value as Et_2PbCl_2. Yield = 1.5 g (56%).

2.1.5. Et_3PbOAc

2.1.5.1. TEL (3.3 ml) in glacial acetic acid (1.0 ml) and a small amount of silica (MFC, 80-200 mesh) were heated under reflux. After 20 min the mixture became a solid white mass which was filtered off after 60 min and recrystallised from benzene. M.p. 158°C (lit.[37],160°C). TLC was identical to Et_3PbOH. The IR spectrum was recorded (Table 2) and the NMR spectrum (Table 4) showed the following integration ratios: $CH_3COO: CH_2: CH_3 = 4:6.5:10$ (Theoretical for $Et_3PbOAc = 3:6:9$).

Yield = 3.9 g (65%).

2.1.5.2. Et_3PbOH (170 mg) in water (1 ml), and glacial acetic acid (1 ml) were mixed and the solvent removed under reduced pressure. The IR spectrum of the product was identical with that of Et_3PbOAc prepared above.

2.1.6. $Et_2Pb(OAc)_2$

2.1.6.1. TEL (3.3 ml) in glacial acetic acid (2.0 ml) and a small amount of silica (MFC, 80-200 mesh) were heated under reflux[27]. After 15 min the mixture became a solid white mass which was filtered off after 90 min. The solid showed two spots on TLC, one corresponding to Et_3PbOAc and the other identical with $Et_2Pb(OH)_2$. After recrystallisation from cyclohexane a small amount of white solid was recovered which showed only the second spot on TLC. The IR spectrum of this compound was recorded (Table 2).

2.1.6.2. $Et_2Pb(OH)_2$ (620 mg) was dissolved in aqueous acetic acid (50%, 1 ml) and the solvent removed under reduced pressure. The residue was extracted with a large volume of hot cyclohexane, and upon removal of the cyclohexane, produced a small amount of white solid which gave an IR spectrum identical to that recorded for $Et_2Pb(OAc)_2$ above.

2.1.7. Et_2PbI_2

TEL (1 ml) in ether (10 ml) was maintained at $-10^{\circ}C$. Iodine (3 g) in ether (10 ml) was added dropwise over 10 min. At first the iodine was rapidly decolorised but later left a permanent colour in the solution. The solution was allowed to warm up to ambient temperature over 1 h, by which time a yellow solid had precipitated. This was filtered off, washed with ether and recrystallised from acetone/ether. TLC showed spots corresponding to both Et_3PbCl and Et_2PbCl_2. The IR and NMR spectra were recorded (Tables 2 and 4).

2.1.8. Me_3PbOAc (aqueous solution)

Aqueous NaOAc (4%) was passed through a column of Amberlite IRA400 anion exchange resin until the eluant gave a chloride negative test with AgOAc. Aqueous Me_3PbCl (300 mgl^{-1}) was passed through the column and the eluant used as a solution of Me_3PbOAc. The solution was chloride negative.

2.2. Thin layer chromatography

2.2.1. General

Silica and alumina refer to MN Kieselgel G and MN Aluminoxid G respectively. These materials were used at a thickness of 0.25 mm on 20 cm glass plates, and were equilibrated with the atmosphere before use.

Cellulose refers to Polygram CEL 300, a 20 cm plastic strip coated with Cellulose MN300 at a thickness of 0.1 mm.

Plates were eluted in a closed atmosphere, saturated with eluant vapour.

Salts were applied from an appropriate solution. Chloroform was found to be suitable for R_3PbX and acetone or water for R_2PbX_2.

With systems giving low R_f values care was necessary not to overload the plates to prevent streaking and consequently false R_f values.

The results of elution of alkyl lead salts under different conditions are given in Table 5.

2.2.2. As dithizonates

Plate coating: silica

Eluant : $CCl_4/CHCl_3$ 100:40

Compounds : Et_2PbCl_2, Et_3PbCl, Me_2PbCl_2, Me_3PbCl, $PbCl_2$

A small amount of each compound (ca. 5 mg) was dissolved in water (0.5 ml) and shaken with a solution of dithizone in chloroform (2% $^{w}/v$, 0.5 ml). After separation, the chloroform solution was run on TLC. After elution all the compounds showed only spots corresponding to inorganic lead dithizonate and to unreacted dithizone. Development of the plate with dithizone gave a salmon spot on the baseline (R_f=0.0) for Me_2PbCl_2 and Et_2PbCl_2, a yellow spot at R_f 0.0 for Me_3PbCl and a yellow spot at R_f 0.1 for Et_3PbCl.

2.2.3. Limits of detection

Solutions were made up of Et_3PbCl, Et_3PbOAc, and Me_3PbCl in chloroform at 1 mgml^{-1} and of Et_2PbCl_2 and Me_2PbCl_2 in water at 1 mgml^{-1}. Measured volumes were applied to TLC plates from a calibrated 10 µl syringe, the solvent allowed to evaporate, and the plates eluted and developed. Plates were studied visually and those for which spots were not observed were placed in iodine vapour for 15 min. On removal from the iodine, the plates were left in a stream of air until all the iodine adsorbed had evaporated (ca. 1 h) when the plates were resprayed with dithizone solution.

Sample volumes of 5, 3, 2, 1, 0.5, and 0.2 µl were applied and the corresponding limits of detection for the various systems are tabulated in Table 6.

2.2.4. Mixed methylethyl lead salts

A mixture of the methyl, ethyl and methyl ethyl lead chlorides, $Et_nMe_{(3-n)}PbCl$ (n=0-3) was prepared according to the method of Calingaert et al[85]. Et_3PbCl (327 mg, 1mM) and Me_3PbCl (285 mg, 1mM) were dissolved in acetone and heated under reflux for 2 h. After cooling and diluting, TLC of the solution showed two yellow spots in addition to, and lying between, those of the starting materials.

Et_2PbCl_2 (359 mg, 1mM) and Me_2PbCl_2 (312 mg, 1mM) were added to acetone (5 ml) and heated under reflux for 2 h. After cooling and diluting, TLC of the solution showed the starting materials with an additional yellow spot between the two. Since a large amount of solid was present undissolved, water was added and the mixture heated again under reflux for 3h. TLC of the resulting mixture showed several spots. Between those of the two starting materials were two unresolved spots, the one of slightly greater R_f being yellow and corresponding to Me_3PbCl. The lower spot was salmon red and was assumed to be due to $EtMePbCl_2$. Other spots present were yellow and corresponded to the various trialkyl lead salts. R_f values for all the salts are given in Table 5.

2.3. Specific extraction technique

2.3.1. Concentration of TAL without decomposition.

2.3.1.1. From methanol by rotary evaporation. The following samples were analysed for (Pb) by AAS:

(1) methanol (200 ml), evaporated down to 16 ml, made up to 200 ml and evaporated down to 17 ml.

(2) TEL (20 μl) added to methanol (200 ml).

(3) (2) evaporated down to 30 ml and diluted to 200 ml with methanol.

(4) Condensed methanol from (3).

(5) (3) evaporated down to 30 ml and diluted to 200 ml with methanol.

(6) Condensed methanol from (5).

Sample (1) had no detectable (Pb). (Pb) in (3) and (4) was equal and half that of (2). The concentration of (5) and (6) were equal and half those of (3) and (4). Thus loss of TEL on rotary evaporation from methanol is ca. 50%.

2.3.1.2. From methanol by fractional distillation. TEL (10 μl) added to methanol was distilled via a 15 cm column packed with glass spirals and collected in 40 ml fractions. Oil bath temperature = 81-83°C, head temp. = 63°C. The first two fractions contained only low (Pb) but the third fraction almost equalled the concentration of the original solution. On dilution to its original volume, the residue from distillation had (Pb) equal to less than half of the original.

2.3.1.3. From ether by rotary evaporation. TEL (10 μl) added to ether (200 ml) was evaporated down to 6 ml. On making the residue up to the original volume, no loss in (Pb) was recorded by AAS. Ether condensed from the evaporation showed only a trace of (Pb).

2.3.1.4. Transference of TEL in ether to MIBK solution. A solution of TEL in ether was made up such that the concentration of (Pb) was 12 ppm. 50 ml were left in equilibrium with distilled water (30 ml) overnight and the layers separated. (Pb) in aqueous layer = zero.

(1) 20 ml of the ethereal layer was transfered directly to MIBK (5 ml) by adding MIBK to ether and removal of latter by rotary evaporation.

(2) 20 ml of the ethereal layer was dried over sodium sulphate before transference to MIBK.

Sample	(Pb)MIBK	(Pb)theoretical
(1)	44	48
(2)	49	48

50 ml of the solution of TEL in ether (12 ppm) was transfered to MIBK by rotary evaporation at various pressures:

Pressure(mmHg)	Temp(°C)	(Pb) MIBK	(Pb) theoretical
4	20	54	60
8	17	55	60
12	18	55	60

Thus ethereal solutions of TEL can be transfered to MIBK with less than 10% loss.

2.3.1.5. Effect of drying MIBK solution of TEL. Solutions of TEL in MIBK (2.1 and 21 ppm as Pb) were equilibrated with water overnight. After separation, the MIBK layer was analysed for (Pb), (1) before drying, (2) after drying with sodium sulphate, and (3) after drying with magnesium sulphate. No significant differences in (Pb) were recorded.

2.3.1.6. Transference of TEL from acetone to MIBK. Solutions of TEL in acetone (50 ml) at various concentrations were added to MIBK (8 ml) and the acetone removed by rotary evaporation at a pressure of ca. 8 mmHg. Solutions were made up to 10 ml with MIBK and the lead concentration determined by AAS.

(Pb) in acetone	(Pb) in MIBK	(Pb) theoretical
2.2	10.6	11.0
1.1	5.0	5.5
0.55	2.1	2.7
0.22	0.9	1.1
0.11	0.6	0.55
0.055	0.3	0.27

2.3.2. Concentration of TAL with decomposition

2.3.2.1. SO_2Cl_2 (1 ml) was added to solutions of TEL in MIBK (50 ml) at various concentrations. The samples were left overnight before the solvent was evaporated off. The solvent removed was condensed and its lead content determined by AAS. The residues were treated with HNO_3 (2.5 ml), made up to 25 ml with water and analysed by AAS. The results are given in the table below.

2.3.2.2. SO_2Cl_2 (1 ml) and pyridine (1 drop) were added to solutions of TEL in benzene (50 ml) at various concentrations. After allowing the sample to stand overnight the solvent was evaporated off and the residue taken up in HNO_3 (1 ml) and made up to 10 ml for AAS.

(Pb)orig	Method 2.3.2.-	(Pb)ppm	(Pb)ppm corrected	(Pb)ppm Theory	%recov	(Pb)ppm condensate
4.25	1	9.0	8.0	8.5	94	0.2
10.5	1	20.5	19.5	21.0	93	1.0
21.0	1	40.1	39.1	42.0	93	0.8
nil	1	1.0	0.0	0.0	–	0.0
1.9	2	10.9	9.5	9.6	99	–
4.8	2	25.5	24.1	24.2	100	–
9.7	2	48.4	47.0	48.4	97	–
4.25	3	2.2	0.5	8.5	6	3.0
10.5	3	2.6	0.9	21.0	4	7.0
21.0	3	7.7	6.0	42.0	14	11.0
nil	3	1.7	0.0	0.0		0.0
1.9	4	5.9	4.9	9.6	51	–
4.8	4	18.5	17.5	24.2	72	–
9.7	4	27.7	26.7	48.4	55	–
nil	4	1.0	0.0	0.0	–	–
nil	2	1.4	0.0	0.0	–	–

2.3.2.3. Solutions of TEL in MIBK were irradiated by ultraviolet light (Phillips TLU20W/12) for 16 h. The solvent was removed and the residue treated with HNO_3 (2.5 ml) and made up to 25 ml with water for lead analysis by AAS. The condensate was also analysed for (Pb).

2.3.2.4. Solutions of TEL in benzene were treated as per 2.3.2.3., the residue being taken up in HNO_3 (1 ml) and made up to 10 ml. The results and percentage recoveries are given in the table above.

2.3.3. Solvent extraction of sediments

Samples of various sediments (50 ml) were extracted in a soxhlet apparatus for 18 h with acetone, chloroform, benzene, petroleum ether, and water. Extracts were worked up by one of the preceding methods and were analysed for (Pb) by AAS. No significant concentrations of lead were found in extracts of any of the following sediments: PFC, Pool Farm drainage settling tank, various sediments from Lea Marston (LMD).

2.4. Gas liquid chromatography

Chromatograph: Pye Model 104 operated at constant temperature

Columns: SE-30: glass, 150 cm: 10% SE-30 on Chromosorb W (60-85 mesh)

TCEP : glass, 300 cm: 10% 1,2,3-tris(2-cyanoethoxy)propane on Chromosorb W (60-85 mesh)

PPG : glass, 150 cm: 10% polypropylene glycol (Phase Separation Ltd.) on Chromosorb W (60-85 mesh)

Carrier gas: Nitrogen purified through molecular seives

Detector: Pye Unicam Electron Capture Detector, Ni^{63} source

Operating temperature: 300°C

Operating mode: pulsed, pulse space = 150 μs

Purge gas: nitrogen purified through molecular seives

Integrator: Kent Chromalog Mark 1.

Conditions: For	column	col.temp. (°C)*	flow rate ($ml\ min^{-1}$)
TEL	SE30	100	60
TML	SE30	64	40
TEL	TCEP	64	40
TEL	PPG	64	40

* With the detector operating at 300°C, 64°C was the minimum attainable working temperature which could be maintained.

Standards: Solutions of TAL in benzene, stored in the dark.

2.5. High pressure liquid chromatography

Instrument: Waters APC202/401 liquid chromatograph with Model 6000 solvent delivery system

Detectors: Ultraviolet and refractometer

Injection: In line injection via septum from high pressure microsyringe

Conditions for estimation of Et_3PbCl:

Solvent: benzene GPR, distilled

Column: 60 cm x 3 mm i.d. stainless steel packed with Corasil II.

Flow rate: 3 mlmin^{-1}; k' = 2.5

Increase in column length or reduction in flow rate on these conditions did not give separation of Et_3PbCl and Me_3PbCl.

Calibration: Measured volumes of a solution of Et_3PbCl in benzene (50.8 mg in 10 ml) were injected from a calibrated microsyringe and the detector response measured by a combined recorder and integrator. The instrument gave a linear response from 0.1 to 2 mg for Et_3PbCl. The response was stable over several weeks, regular checks being made by injection of standard samples.

Exploratory work: A whole range of solvents was used for columns packed with the materials listed below, but suitable retention times were not observed for R_2PbX_2 or Et_3PbOAc.

Packings used:		
	Porasil A Corasil I	normal phase
	C-18 Corasil Phenyl Corasil	reverse phase

2.5.1. Study of uv absorbtion of alkyl lead salts.

A solution of Et_3PbCl in chloroform (60 μgml^{-1}) gave λ_{max} 247 nm (ϵ=5000) with ϵ_{254}=3500. Continuous exposure to uv light at 254 nm (up to 10 min) gave no change in the spectrum.

2.6. Pyridyl-azo-resorcinol (PAR)

2.6.1. Reagents

ICl 1.0M

Potassium Iodide	27.75 g
Concentrated HCl (s.g. 1.18)	111.25 ml
Potassium Iodate	18.75 g
Water	to 250 ml

HCl was added to the KI dissolved in water and cooled to 0°C.

KIO_3 was added to the solution with stirring and the solution allowed to warm up to ambient temperature. The iodine initially liberated redissolved to give a clear orange solution, which was made up to 250 ml in a volumetric flask.

Buffer Solution

Sodium Sulphite, hydrated, $Na_2SO_3.7H_2O$	60 g
Triammonium citrate	46 g
Potassium Cyanide	5 g
Ammonium Hydroxide (s.g. 0.880)	100 ml
Water	to 1000 ml

Sodium sulphite and triammonium citrate were dissolved in water (ca. 600 ml) and transfered to a 1 l volumetric flask. KCN dissolved in water (ca. 50 ml) was added to the solution followed by ammonium hydroxide, and water to volume.

PAR Solution (1.04×10^{-3}M)

Pyridyl-azo-resorcinol, sodium salt (PAR)	0.282 g
Water	to 1000 ml

PAR was dissolved in water and made up to 1 l in a volumetric flask.

CDTA

1,2-diaminocyclohexane-N,N,N',N',tetra-acetic acid (CDTA)	14.56 g
Sodium Hydroxide	4 g
Water	to 200 ml

CDTA was added to a solution of NaOH in water and made up to 200 ml after cooling to ambient temperature.

2.6.2. Method

2.6.2.1. Analysis for (Et_2Pb^{++}). ICl solution (1 ml) was pipetted into a 25 ml volumetric flask and washed from the sides with a small volume of water. Buffer solution (5 ml) was added from a pipette and the solution shaken to dissolve the liberated iodine. A measured volume of sample (1-10 ml) was pipetted into the flask. The total (Et_2Pb^{++}) should not exceed 100 μg. PAR solution (5 ml) was added followed by CDTA solution (1 ml) from pipettes, and the solution made up to volume with water and shaken.

The absorbance of the solution at 515 nm was measured in a 1 cm glass cell and compared with that of a reagent blank comprising the above solutions without the addition of sample.

2.6.2.2. Analysis for (Et_3Pb^+). The concentration of Et_3Pb^+ was determined by the difference in absorbance between the above solution estimated for Et_2Pb^{++}, and a solution estimated for total (Et_3Pb^+ and Et_2Pb^{++}) . Estimation for (Et_3Pb^+ and Et_2Pb^{++}) was carried out by addition of the sample to the ICl solution before addition of the buffer. The mixture of sample and ICl was left 5 min before addition of buffer to allow complete reaction of the Et_3Pb^+ to Et_2Pb^{++}.

2.6.3. Instrumentation

Routine analyses were carried out on a Pye Unicam SP500 spectrophotometer. Confirmatory work on unknown samples was carried out on a Pye Unicam SP800 scanning spectrophotometer between 450 and 600 nm.

2.6.4. Interference by iron

Solutions of $FeSO_4.7H_2O$ were made up in water at concentrations of 100 and 1000ppm (as $FeSO_4.7H_2O$). 5 ml aliquots were substituted for Et_2Pb^{++} samples in the PAR method and their uv spectra recorded between 450 and 600 nm. The absorbance at 483 and 515 nm was noted and is given in Table 8. The experiment was repeated with $FeCl_3.6H_2O$.

3. EXPERIMENTAL WORK RELATING TO CHAPTER THREE

3.1. Extraction of tetra-alkyl lead

3.1.1. Vacuum distillation

TEL with and without sediment was heated in a 250 ml round bottomed flask at 45-50°C, 0.1 mmHg, for 35 min or until the sediment was completely dry. Any volatiles were condensed in a dry ice/acetone trap which was afterwards extracted with benzene and the benzene analysed for TEL by GLC.

Sediment	Volume	TEL(μl) on	TEL(μl) off	%recovery
nil	nil	1	1	100
PFC	50	10	7	70
LMD	70	1	0.25	25

3.1.2. Steam distillation

TEL (1 μl) was steam distilled and 100 ml fractions collected under benzene (10 ml). Each fraction was shaken and the benzene analysed for TEL by GLC.

Fraction 1 = 0.025 μl TEL
Fraction 2 = 0.02 μl TEL
Subsequent fractions= nil

Washing of the condenser with benzene gave no detectable TEL. Washing of the original distillation flask to which TEL had been added showed 0.5 μl TEL remaining.

3.1.3. Solvent extraction

TEL (1 μl) added to sediment (PFC, 50 ml) was shaken with P.E. (50 ml) for 1 min. GLC of the diluted extract showed a recovery of 100%.

10 μl of a solution of TEL in methanol (10 μl in 10 ml) was

added to sediment (LMD, 30 ml) and shaken. The sediment was extracted with P.E.(2x10 ml) and each extract analysed for TEL. The experiment was repeated with TML and the mixed lead alkyls. Volume of TAL added $\approx 10^{-2}$ μl.

TAL	%recovery,1st extract	%recovery,2nd extract
Et_4Pb	40	15
$MeEt_3Pb$	40	14
Me_2Et_2Pb	38	13
Me_3EtPb	36	13
Me_4Pb	41	15

The experiment was repeated for TEL with various sediments. Blank extractions of all the sediments used showed no TAL to be present initially.

Sediment	%recovery,1st extract
LMD	40
PFC	42
Drain sediment	38
Sand (R.Trent)	44
Canal sediment, Smethwick	36

3.2. Extraction of alkyl lead salts

3.2.1. 50% aqueous acetic acid

Sediment, with and without the addition of alkyl lead compounds, was stirred with an equal volume of acetic acid (glacial) overnight. The suspension was filtered and the solvent removed from the filtrate at reduced pressure (ca. 0.1 mmHg) at below 50°C. The residue was extracted with acetone and the acetone evaporated off.

Various experiments were carried out and the extracts studied by IR, TLC, HPLC, and PAR. Results are given in Table 9.

3.2.2. Solvent extraction from water

Et_3PbCl (2.6 mg) in water (100 ml) was extracted with P.E. (50 ml) and the solvent evaporated off from the extract. The residue was taken up in HNO_3 (10 ml) and made up to 100 ml with water for lead analysis by AAS. A small volume of the evaporated aqueous solution was analysed by AAS and the remainder extracted with toluene (50 ml) following the above procedure. To the extracted aqueous solution was added $CaCl_2$ (50 g) and the solution extracted with toluene (2x50 ml).

The experiment was repeated with Et_2PbCl_2 (2.6 mg) and $PbCl_2$ (20 mg), but toluene in the final extraction was replaced by acetone. Figures reported are in ppm (Pb).

Sample	Et_3PbCl	Et_2PbCl_2	$PbCl_2$
Original solution	16.0	15.8	13.3
P.E. extract	nil	nil	nil
Extracted solution	15.8	15.1	13.0
Toluene extract	nil	nil	nil
Extracted solution	15.6	15.1	13.0
$CaCl_2$/toluene extract(1)	13.4	nil	nil
$CaCl_2$/toluene extract(2)	2.0	-	-
$CaCl_2$/acetone extract	-	trace	nil

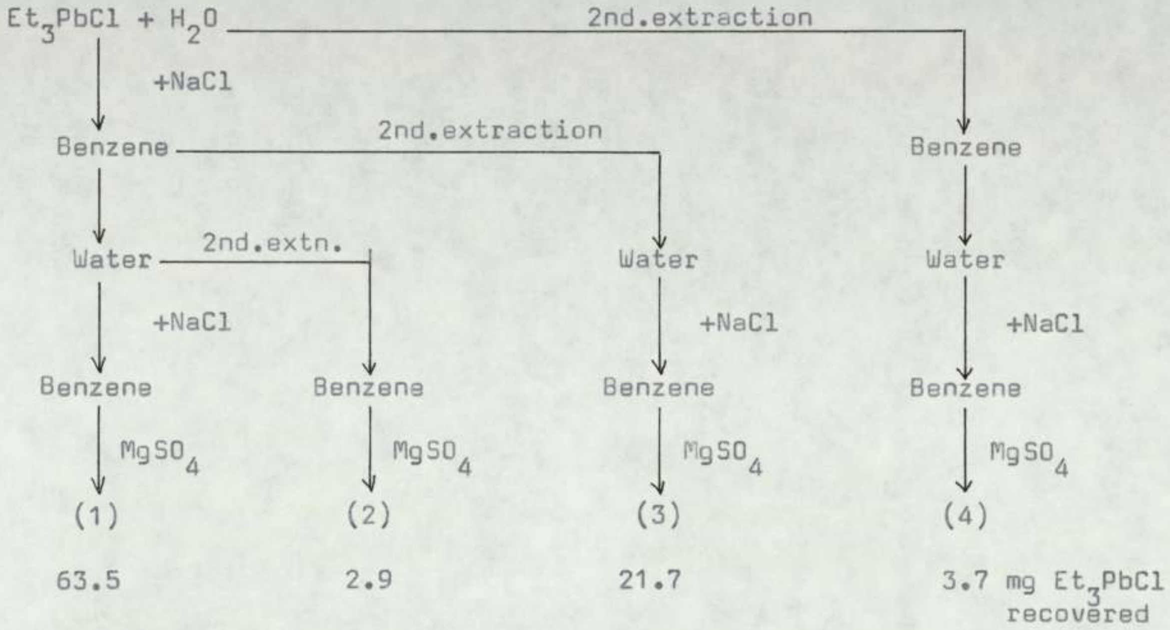
3.2.3. Exchange of anion

Et_3PbCl (100 mg) in water (100 ml) was shaken with NaOAc (10 g) in water (100 ml) and left for 1 h. The mixture was extracted into benzene (10 ml) and analysed by TLC after removal of the bulk of solvent. The product was shown to be Et_3PbOAc. No trace of Et_3PbCl was visible. This was further confirmed by IR spectroscopy.

The experiment was repeated using Et_3PbOAc (90 mg) and NaCl (10 g). The product was exclusively Et_3PbCl. No acetate absorptions were visible in the IR spectrum of the product.

3.2.4. Extraction of Et_3PbCl from water with NaCl/benzene

Et_3PbCl (105 mg) in water (100 ml) was saturated with NaCl and extracted into benzene (100 ml). The benzene was back-extracted into water (100 ml) which was then saturated with NaCl and extracted into benzene for analysis by HPLC after drying over $MgSO_4$. Each extracted sample was re-extracted and worked up following the remainder of the procedure, thus:



$MgSO_4$ from the original extract was taken up in water, saturated with NaCl, extracted into benzene and analysed by HPLC without drying. Et_3PbCl = 5 mg.

Total Et_3PbCl recovered = 96.8 mg (92%)

The experiment was repeated with Et_3PbCl (1 mg) when total recovery was 80%.

3.2.5. Extraction of Et_3PbX from sediment with NaCl/benzene

3.2.5.1. Et_3PbOAc (102 mg) in water (5 ml) was added to sediment (PFC, 5 ml) and shaken. To the suspension was added NaOAc (24 g) before extraction into benzene (100 ml). The bulk of the solvent was removed and the concentrated extract was run on TLC. The experiment was repeated substituting Et_3PbCl (108 mg) for Et_3PbOAc and NaCl (30 g) for NaOAc.

TLC (silica plates: toluene/chloroform, 1:9 eluant) showed spots corresponding to the salts added, obscured by a brown streak due to organics extracted into the benzene.

TLC (silica plates: acetic acid/benzene, 1:3 eluant) showed no spots corresponding to the added salts, due to the presence of excessive organics.

3.2.5.2. 3.2.5.1. was repeated with Et_3PbCl (117 mg) on sediment (PFC, 20 ml), but the benzene extract was back-extracted into water, and the aqueous extract saturated with NaCl and extracted into benzene. TLC showed only Et_3PbCl with both eluants. No organics were visible.

3.2.5.3. Et_3PbCl (100 mg) on sediment (PFC, 100 ml) was extracted as for the aqueous solution of Et_3PbCl in 3.2.4. The final benzene extract was not dried. Et_3PbCl recovered (mg): (1) 27, (2) trace, (3) 11, (4) 10.

Total Et_3PbCl recovered = 48 mg (48%)

Total Et_3PbCl recovered in first benzene extract =

$$27 + 11 + \frac{11}{27}\times 11 = 43 \text{ mg.}$$

Recovery from first benzene extract into first water extract

$$= \frac{27}{43} \times 100 = 63\%$$

3.2.5.4. 3.2.5.3. was repeated with acidification of the sediment to pH2-3 before initial extraction into benzene. Et_3PbCl recovered (mg): (1) 36.6, (2) low, (3) 4.5, (4) 9.4.

Total recovery = 50.5 mg (50%)

Total Et_3PbCl in first benzene extract =

$$36.6 + 4.5 + \frac{4.5}{36.6} \times 4.5 = 41.7 \text{ mg.}$$

Recovery from first benzene extract into first water extract

$$= \frac{36.6}{41.7} \times 100 = 88\%.$$

3.2.5.5. 3.2.5.3. was repeated with the initial extraction carried out under high speed mechanical stirring for 1 h. This extraction was repeated after separation of the first benzene extract. Et_3PbCl recovered (mg): (1) + (3) 37.6, (4) 5.7.

Total recovery = 43.3%.

3.2.5.6. Standard Procedure. Sediment was acidified to pH3-4 with aqueous HCl (20% $^{v}/v$), saturated with NaCl and extracted into benzene (2x50 ml). The benzene was back-extracted into water (2x50 ml). The combined aqueous extracts were saturated with NaCl and extracted into benzene (50 ml). The bulk of the benzene was removed and the concentrated sample analysed by HPLC.

3.2.5.7. Various amounts of Et_3PbCl and Et_3PbOAc were added in a small volume of water to sediment and extracted by the standard procedure, 3.2.5.6. The recoveries are tabulated below.

Salt	Weight(mg)	Sediment	Vol.sed.(ml)	%recov.	Notes
Et_3PbOAc	43	PFC	60	67	
Et_3PbCl	51	PFC	50	57	
"	20	LMD	30	63	
"	15	LMD	30	58	
"	10	LMD	30	53	
"	10	PFC	30	54	
"	5	LMD	30	29	
"	2	LMD	30	15	
"	1	LMD	30	4	
"	100	PFC	100	22	After 2 d.
"	100	PFC	100	48	Immediate extn.

The above recoveries are averages of at least two extractions. Reproducibility of extraction was checked for recoveries of Et_3PbCl (20 mg) added to sediment (LMD, 30 ml). Percentage recoveries were 56, 59, 63, 65, 70, 67, 60.

3.2.5.8. Procedure 3.2.5.6. was modified by addition of lead nitrate (5 g) to the sediment (LMD, 30 ml) before saturation with NaCl. The results are given in Table 10 with figures from 3.2.5.7. for for comparison.

3.2.5.9. Analysis by PAR. Sediment (30 ml) was acidified to pH3-4 with aqueous HCl (20% $^{v}/v$), and lead nitrate added. The solution was saturated with NaCl and extracted with benzene (2x50 ml). The benzene was back-extracted into water (2x10 ml), the aqueous extract made up to 25 ml and analysed by PAR.

3.2.5.10. Variation of Pb^{++} added. Et_3PbCl (1 mg) was added to sediment (LMD, 30 ml) and extracted as in 3.2.5.9. with varying amounts of lead nitrate added.

$Pb(NO_3)_2$ (g)	% Recovery
0	4
2.5	51
5.0	60
10.0	61

3.2.5.11. Various amounts of Et_3PbCl were added to sediment (LMD, 30 ml) and extracted by 3.2.5.9. with the addition of 5 g of lead nitrate. The percentage recoveries are tabulated in Table 11. Addition of 100 μg Et_3PbCl to 100 ml sediment gave a lower recovery of 25%. Increase in the amount of lead nitrate added did not increase these recoveries.

3.2.5.12. Me_3PbCl (1 mg) in water (10 ml) was added to sediment (PFC, 30 ml) and extracted by 3.2.5.9. Recovery = 29% for 5 g $Pb(NO_3)_2$.

Me_3PbCl (1 mg) in water (50 ml) was extracted by 3.2.5.9. with no lead nitrate added. Recovery = 29%.

Me_3PbCl (1 mg) in benzene (50 ml) was extracted into water (25 ml). Recovery = 100%.

3.2.5.13. Efficiency of extraction. Et_3PbCl (1000 μg) was added to sediment (LMD, 30 ml) and acidified to pH3-4 with aqueous HCl (20% $^v/v$). Lead nitrate (5 g) was added and the solution saturated with NaCl. The suspension was extracted with benzene (70 ml) and separated. The benzene was extracted three times with water (40 ml) and each extract analysed for Et_3PbCl by PAR. (Et_3PbCl) = 410, 25, 10 μg. The original suspension was re-extracted with benzene (50 ml), the benzene extracted twice with water (40 ml) and each extract analysed. (Et_3PbCl) = 114, 38 μg.

3.2.5.14. Extraction of Et_3PbCl from benzene into aqueous lead nitrate. Portions of Et_3PbCl (500μg) in benzene (100 ml) were extracted into (a) water (2x20 ml) and (b)aqueous lead nitrate solution (10%, 2x20 ml). After precipitiation of any lead with aqueous NaOH (2M), the solutions were analysed by PAR. Both (a) and (b) gave a 75% recovery of Et_3PbCl.

3.2.5.15. Action of lead. Et_3PbCl in water (200 μg in 20 ml) was added to sediment (LMD, 30 ml) and acidified to pH5-6. The sediment was extracted into benzene (2x50 ml) and the benzene back-extracted into water (2x 25 ml). The water was analysed by PAR. Et_3PbCl = 8 μg (4%).

Aqueous lead nitrate (5 g in 20 ml) was added to the extracted sediment and the sediment extracted with benzene (2x50 ml) and worked up as above. Et_3PbCl = 16.5 μg (8%).

Each benzene extract was re-extracted with aqueous lead nitrate (10%, 2x25 ml), the lead precipitated by aqueous NaOH (2M) and the solutions analysed by PAR.

Et_3PbCl (1st benzene extract) = 91 μg (45%)

Et_3PbCl (2nd benzene extract) = nil

3.2.6. Extraction of alkyl lead salts by addition of Pb^{++} and filtering

3.2.6.1. Basic Procedure. Alkyl lead salts on sediment (LMD, 30 ml) were extracted as follows. The sediment was acidified and its pH measured after evolution of any H_2S had ceased. Aqueous lead nitrate solution was added, the mixture shaken, and allowed to stand for 5 min. The mixture was filtered and the inorganic lead precipitated from the filtrate by addition of aqueous NaOH (2M) until the solution had pH8. The precipitated lead was filtered off, the solution made up to a standard volume and aliquets analysed by PAR. The results are tabulated below, each recovery quoted being the average of at least two duplicates.

3.2.6.2. Et_3PbCl:: pH2:: (Pb^{++}) = 5 g.

Et_3PbCl added(mg)	% Recovery
10	88
5	91
1	90
0.5	90

3.2.6.3. Et_3PbCl = 1 mg:: pH2:: Variation with (Pb^{++}).

(Pb^{++})(g)	% Recovery
0	0
1	71
2	88
5	90

3.2.6.4. Et_3PbCl = 1 mg:: (Pb^{++}) = 5g:: Variation with pH.

pH	% Recovery
8	92
2.5	90
1	92

3.2.6.5. Et_2PbCl_2 = 2 mg:: pH = 2:: Variation with (Pb^{++}).

(Pb^{++})(g)	% Recovery
0	0
2	54
5	75
10	77

3.2.6.6. Et_2PbCl_2 = 2 mg:: (Pb^{++}) = 5 g:: Variation with pH.

pH	% Recovery
8	68
5	70
2.5	73
1	75
1	38

3.2.6.7. Me_3PbCl = 1 mg:: pH3.5:: (Pb^{++}) = 5 g. Recovery = 90%

3.2.6.8. Me_2PbCl_2 = 1.3 mg:: (Pb^{++}) = 5 g:: Variation with pH.

pH	% Recovery
8	43
5.5	39
4.5	33
2.5	34
1.5	27
1	18

3.2.6.9. Me_2PbCl_2 = 1.3 mg:: pH6:: Variation with (Pb^{++}).

(Pb^{++})(g)	% Recovery
0	0
2.5	29
5.0	42
10.0	36

3.2.6.10. Effects of other metals. Et_2PbCl_2 (2 mg) on sediment (LMD, 30 ml) was extracted by 3.2.6.1. with lead nitrate replaced by various divalent metal salts. (M^{++})= 5 g:: pH3.

Salt	% Recovery
$Pb(NO_3)_2$	70
$FeSO_4$	nil
$Zn(OAc)_2$	25
$HgCl_2$	68
$SnCl_2$	nil
None	nil

Extraction of sediment with no added alkyl leads gave nil readings with all these metals.

3.2.6.11. Various sediments. Et_2PbCl_2 (1 mg) was added to various sediments and extracted by 3.2.6.1. pH5.5:: (Pb^{++}) = 5 g.

Sediment	% Recovery
LMD	70
PFC	73
Drain sediment	65
Sand (River Trent)	75
Silica (MFC)	83
Canal sediment, Smethwick	63

3.2.6.12. Identification on TLC. Et_2PbCl_2 (1 mg) and Me_2PbCl_2 (1 mg) were extracted by 3.2.6.1. (pH5:: (Pb^{++}) = 5 g) and the resulting aqueous solution evaporated to dryness. The residue was taken up in acetone and run on TLC. Et_2Pb^{++} showed clearly, but Me_2Pb^{++} showed only feintly.

3.2.6.13. Nature of extracted salt. Et_3PbCl on sediment (LMD, 30 ml) was extracted by 3.2.6.1. (pH3:: (Pb^{++}) = 5 g) and the extract analysed for both Et_3Pb^+ and Et_2Pb^{++} by PAR. The experiment was repeated for Et_2PbCl_2.

Et_3PbCl on (mg)	Et_2PbCl_2 on (mg)	Et_3PbCl off (mg)	Et_2PbCl_2 off
9.9	0.1	8.6	0.1
0.6	8.4	0.8	5.8

3.2.6.14. Decomposition of salts. Et_2PbCl_2 (2 and 10 mg) and Et_3PbCl (2 and 10 mg) on sediment (LMD, 30 ml) were stored in sealed vessels in the light for 3 d before being extracted as per 3.2.6.13., with which the results are compared below.

Salt	Immediate extraction		Extraction after 3 d	
	%Recov.	%di/tri	%Recov.	%di/tri
Et_2PbCl_2	70	-	7	-
Et_3PbCl	90	1	70	5

4. EXPERIMENTAL WORK RELATING TO CHAPTER FOUR

4.1. Analysis of survey samples

Samples were taken from the top few inches of sediment and stored in polypropylene bottles until analysed. 30 ml samples of the sediment were shaken for 1 min with P.E. (10 ml) and centrifuged. The P.E. extract was transfered to a small glass stoppered flask and stored at 0^oC. The extracted sediment was acidified to pH5-6 with dilute HNO_3, allowed to stand until evolution of H_2S had ceased,and lead nitrate added (5 g in 20 ml water) and mixed. After standing for 5 min the sediment was filtered. The solids remaining were dried and weighed. The filtrate was neutralised with aqueous NaOH (2M), filtered and made up to a standard volume (ca.100 ml). 10 ml aliquots of this filtrate were analysed by PAR for Et_3Pb^+. If significant values were recorded, the solution was also analysed for Et_2Pb^{++}. The above operations were carried out on the day of sampling.

Analysis of the P.E. extract for TAL was carried out by GLC using a SE-30 column. Where peaks corresponding to TAL were observed, the sample was rechromatographed under conditions which gave the longest practical retention time. Under these conditions it was also injected with a genuine sample of the TAL in question . In all cases where mixed alkyl leads were apparently present, these were shown not to be genuine by the appearance of a second peak upon mixed injection.

Attempts to confirm the identity of TEL by use of a second column (TCEP) proved difficult for low concentrations of TEL owing to the presence of other electron capturing materials of identical retention times. Only where quantitative measurements could be made was identity shown with any certainty. The use of a third column (PPG) was not suited to natural extracts owing to the long retention times of many other compounds and the presence of impurities in both P.E. and benzene with identical retention time to TEL.

TML was observed only in four samples (all from garage forecourts) and was shown to give only one peak when injected with a genuine sample of TML. The retention time on the TCEP column was not sufficient to allow confirmation.

The presence of tri alkyl lead salts also was found only in the above samples, but the concentration was insufficient to allow confirmation and determination of the alkyl groups present by TLC.

The presence of TEL in the above samples could be confirmed by TLC after treatment of the P.E. extract with ICl and extraction into water.

Sites sampled which gave positive results are listed in Table 13 along with the weight of solid per 30 ml sample and the concentrations of alkyl lead compounds determined. At those sites giving high concentrations of TEL, samples of filtered water from above the sediment was also analysed. 10 ml of the water was extracted with P.E. (1 ml) for analysis by GLC, and 10 ml aliquots were analysed directly by the PAR method. In none of the samples were any alkyl lead compounds detected in the filtered water.

Other sites sampled are listed in Table 12 and displayed on a grid map of the Birmingham area (Figure 3).

Only samples which gave apparent TEL concentrations of comparable magnitude on the two GLC columns have been reported. Those which gave peaks that were too small to quantify have been disregarded. The maximum possible concentration of TEL in such samples is 0.02 ppm (dry weight) for a sample with a dry weight of 10 g.

4.2. Confirmation of TEL

4.2.1. TLC

A sample of sediment was extracted into P.E. (50 ml) and to the separated P.E. was added ICl (0.3 ml). The solution was shaken, allowed to stand for 10 min and extracted into water (10 ml). The water was removed from the aqueous extract and the residue taken up in acetone. After evaporation of the bulk of the acetone, the sample was run by TLC against samples of Et_2PbCl_2 and Me_2PbCl_2. TEL added to TEL-free samples showed the the presence of Et_2Pb^{++} by TLC at concentrations down to 2 ppm in the P.E. extract.

4.2.2. Mass spectroscopy

Direct injection of TEL in P.E. (10 μl, 1000ppm) into a MS9 mass spectrometer gave no patterns characteristic of TEL, even at the highest sensitivity.

4.2.3. GLC/MS

Using a Pye 104 coupled with a Micromass 12B, samples of TEL at 10 and 1000 ppm in P.E. did not show any response for TEL when chromatographed under conditions similar to those used for GLC/ECD.

4.3. Analysis of petrol and oil

Samples of petrol were collected directly from petrol pumps and stored in the dark in plastic topped glass bottles. Samples were diluted 100-fold with P.E. and analysed for TAL by GLC.

Used engine sump oil was also analysed after 10-fold dilution and filtering.

The results are tabulated in Table 14.

5. EXPERIMENTAL WORK RELATING TO CHAPTER FIVE

All work involving carbon was carried out with CAL 12x40 granular activated carbon, supplied by Chemviron. The carbon was washed 5 times with water before use to remove fines. Unless otherwise stated, 50 ml (30 g, dry weight) samples of carbon were used.

5.1. Adsorption and desorption of TEL

5.1.1. Ether

TEL (100 μl, 106 mg as Pb) was added to carbon in water (500 ml) and the mixture stirred for 5 h. The carbon was transfered to a small column and further water (500 ml) passed through. The water was extracted with MIBK (10 ml) and the lead content of the residual water and of the MIBK extract were determined by AAS. Ether was passed through the column and fractions (3x50 ml) collected. These were dried over sodium sulphate and filtered. The ethereal solution was transfered to MIBK and analysed for Pb by AAS.

Sample	μg Pb
MIBK extract of water	10
Residual water	3000
Fraction 1	850
Fraction 2	470
Fraction 3	70

Recovery = 1.3%

Decomposition to water soluble salts = 3%.

5.1.2. Various solvents

TEL (1 μl) was added to carbon in water (500 ml) and stirred. After transference of the carbon to a column, acetone (150 ml), ether (150 ml) and chloroform (150 ml) were passed through and the eluant lead concentration determined by AAS. In no case did the concentration of lead exceed 2% of the initial amount added.

5.1.3. Soxhlet extraction

Portions of TEL (1 µl) on carbon were extracted separately in soxhlet apparatus with acetone, methanol, and chloroform for 24 h. Acetone and methanol extracts were analysed for lead following procedures 2.3.1.6. and 2.3.2.1. respectively. The methanol extract was treated with bromine (2.5 ml), left for 3 h and the methanol evaporated off after removal of excess bromine with sodium thiosulphate. The residue was treated with H_2O_2 and HNO_3 (1 ml) and made up to 20 ml. All the analyses were compared with those for extracts of carbon containing no added TEL and the blamk readings subtracted.

Solvent	TEL (µl) extracted
Acetone	0.008
Methanol	nil
Chloroform	0.006

5.1.4. Reduced pressure

TEL (1 µl) was added to carbon in distilled water (500 ml) and the mixture stirred. After removal of excess water, the carbon was heated to 100°C at 0.2 mmHg pressure and any volatiles trapped in one of two traps maintained at liquid nitrogen temperatures. Any condensate was treated with HNO_3 and diluted for lead analysis by AAS. Only 1% of TEL added was recovered in the traps.

5.1.5. 50% aqueous acetic acid

TEL (200 µl, 209 mg as Pb) on carbon (100 ml, 60 g) was shaken with 50%AcOH (100 ml) for 5 h, filtered, and the solvent evaporated off at 0.1 mmHg and 50°C. The residue was taken up in acetone and estimated for lead by AAS after transference to a water solution. TLC showed the presence of Et_2Pb^{++} only. The acetone insoluble part of the residue was taken up in water and the total lead estimated by AAS. The residue insoluble in both acetone and water was taken up in HNO_3, diluted and analysed by AAS.

AcOH extract	Pb (mg)
Acetone soluble	17.3
Water soluble	1.8
HNO_3 soluble	9.1
Total	28.2

Recovery = 13.5%

5.1.6. 50%AcOH

TEL (50 μl, 53 mg as Pb) on carbon was extracted on a small column with 50%AcOH (4x250 ml). The solvent was removed from each fraction and the residue taken up in HNO_3 for analysis by AAS.

Fraction	Pb (mg)
1	7000
2	1470
3	740
4	350
Total	9560

Recovery = 18%.

5.2. Adsorption and desorption of alkyl lead salts

5.2.1. Total lead analyses

In the following experiments a weighed amount of alkyl lead salt in a known volume of water was passed through carbon on a 20 mm i.d. column, after estimation of the lead content of the solution by AAS. A known volume of water was passed through the column. 250 ml fractions were collected and analysed for lead by AAS. From these figures, the amount of alkyl lead salt adsorbed onto the carbon was calculated.

The column was then eluted with 50%AcOH (1 l) and 250 ml fractions collected. Each fraction was evaporated to dryness, the residue taken up in HNO_3 (10 ml), made up to 100 ml with water and analysed for lead by AAS. A blank run in which no alkyl lead salt was added to the carbon showed no significant lead concentrations at any stage.

5.2.1.1. Et_2PbCl_2 (Pb) = 44240 μg vol = 2.8 l Fractions 1-10

Washing water vol = 1 l Fractions 11-14

Adsorption:

Fraction	(Pb)(μg)
1	trace
2	280
3	630
4	875
5	1225
6	1680
7	1820
8	1820
9	2240
10	2170
11	1505
12	1050
13	770
14	525
Total non-adsorbed	16590

Therefore total adsorbed = 27650 μg.

Elution:

Fraction	(Pb)(μg)
1	20860
2	2830
3	700
4	420
Total desorbed	24810

Recovery = 90%.

5.2.1.2. Et_2PbCl_2 (Pb) = 5880 μg vol = 1 l Fractions 1-4

Washing water vol = 500ml Fractions 5-6

Adsorption:

Fraction	(Pb)(μg)
1	nil
2	trace
3	245
4	245
5	245
6	140
Total non-adsorbed	875

Therefore total adsorbed = 5005 μg.

Elution:	Fraction	(Pb)(μg)
	1	3920
	2	560
	3	98
	4	56
	Total desorbed	4634

Recovery = 93%.

5.2.1.3. Et_3PbCl (Pb) = 58800 μg vol = 4.0 l Fract.1-10(250ml)
11-13(500ml)
Washing water vol = 1.0 l Fract14-15(500ml)

Adsorption:	Fraction	(Pb)(μg)
	1	nil
	2	trace
	3	280
	4	420
	5	525
	6	665
	7	735
	8	1050
	9	1330
	10	1050
	11	2240
	12	2310
	13	2310
	14	1680
	15	1624
	Total non-adsorbed	16219

Therefore total adsorbed = 42581 μg.

Elution:	Fraction	(Pb)(μg)
	1	25900
	2	4500
	3	2590
	4	1050
	Total desorbed	34090

Recovery = 80%.

5.2.1.4. $Pb(NO_3)_2$ (Pb) = 35000 μg vol = 2.5 l Fract.1-10

Washing water vol = 1.0 l Fract11-14

Adsorption:	Fraction	(Pb)(μg)
	1	70
	2	455
	3	630
	4	630
	5	630
	6	665
	7	455
	8	595
	9	630
	10	700
	11	560
	12	455
	13	350
	14	420
	Total non-adsorbed	7245

Therefore total adsorbed = 27755 μg.

Elution:	Fraction	(Pb)(μg)
	1	7280
	2	840
	3	924
	4	602
	Total desorbed	9646

Recovery = 35%.

5.2.1.5. A similar experiment was carried out for Et_2PbCl_2 using acetone as eluant. Fractions were transfered to water for analysis by AAS.

(Pb) = 13020 μg vol = 1.0 l Fract.1-4

Washing water vol = 500ml Fract 5-6

Adsorption:	Fraction	(Pb)(μg)
	1	nil
	2	trace
	3	770
	4	490
	5	385
	6	350
	Total non-adsorbed	1995

Therefore total adsorbed = 11025 μg.

Elution:	Fraction	(Pb)(μg)
	1	980
	2	532
	3	62
	4	nil
	Total desorbed	1574

Recovery = 14%

5.2.2. Analysis by HPLC

5.2.2.1. Et_3PbCl/50%AcOH. Et_3PbCl (50 mg) in water (500 ml) was shaken with carbon for 5 min. The carbon was transfered to a column and water (100 ml) passed through. The total 600 ml of water was saturated with NaCl and extracted into benzene. 50%AcOH (4x250 ml) was passed through the column, the fractions neutralised with sodium carbonate, extracted into benzene, back-extracted into water and, after saturation with NaCl extracted into benzene. Et_3PbCl eluted was determined by HPLC.

Sample	Et_3PbCl (mg)
Extract of orig. soln.	0.5
Fraction 1	28.5
Fraction 2	0.5
Fraction 3	nil
Fraction 4	nil

5.2.2.2. Et_3PbCl/HCl. The above experiment was repeated using Et_3PbCl (63 mg), and HCl as eluant. Fractions were neutralised with NaOH, saturated with NaCl and extracted into benzene for determination by HPLC.

Sample	Et_3PbCl (mg)
Extract of orig. soln.	0.5
Fraction 1 (200ml, 5%HCl)	nil
Fraction 2 (200ml,10%HCl)	nil
Fraction 3 (200ml,20%HCl)	nil

5.2.3. Analysis by PAR

In the following experiments a dilute aqueous solution of alkyl lead salt was passed through carbon in a small column (20 mm i.d.) and the carbon washed with water. Fractions of eluant were analysed by PAR. When the total amount of salt added was 10 mg, over 85% was adsorbed, and when total was 1 mg over 99% was adsorbed. The carbon was eluted with various solvents. Acidic fractions were neutralised with aqueous NaOH (2M) before being analysed for alkyl lead salts by PAR. It was found that with certain solvents iron was eluted from the carbon and subsequently interfered with the PAR determination. Interference was removed by adding lead nitrate (2.5 g in 10 ml water) before neutralisation, when the iron coprecipitated with the lead. Fractions of organic solvents other than acetic acid were added to water and the solvent evaporated off. PAR determinations were carried out after the water had been made up to a standard volume. With all solvents, blank extractions were carried out on carbon with no added alkyl lead salts and the small blank values subtracted from subsequent extractions.

The results are tabulated in Table 15.

5.2.3.1. Stability of Samples. Duplicated samples of carbon onto which was adsorbed (1) Et_3PbCl (2530 μg) and (2) Et_2PbCl_2 (2330 μg) were left under different conditions for 14 d before analysis. One sample was extracted immediately. All extractions were carried out with 5%AcOH (3×200 ml) as above.

Conditions	% Recovery	
	Et_3PbCl	Et_2PbCl_2
Immediate extraction	43	63
Stored in small vol. water, in dark	24	20
Air dried, stored in dark	24	27
Tap water passing through (ca.800mlmin^{-1})	23	19
Tap water passing through, no added salts	0	0

5.2.3.2. Extraction of used carbon. Carbon through which water had been passed was analysed as for 5.2.3.1.

Sample 1 Birmingham tap water	15,000 l	of water passed through
Sample 2 Water from River Trent	20,000 l	"
Sample 3 Treated sewage	unknown vol.	"

In sample 3, sewage had been treated with lime, carbon dioxide, and a prefilter consisting of anthracite, sand, and garnet before passing through the carbon. We showed that treatment of alkyl lead salts in water with lime and carbon dioxide reduced their concentration by 10% (Et_3PbCl) and 8% (Et_2PbCl_2). A sample of the pre-carbon filter was analysed as for sediment and contained no alkyl lead salts.

In all samples, no alkyl lead salts were detected.

5.2.3.3. Treatment of salts with lime and CO_2. Et_3PbCl and Et_2PbCl_2 in water (100 μg in 100 ml) were treated with $Ca(OH)_2$ (10 g) and filtered. Carbon dioxide was passed through until the pH of the solution was 8 and the solution filtered. The filtrate was analysed by PAR and compared with the original solution. Recovery for Et_3PbCl = 90%; for Et_2PbCl_2 = 92%.

6. EXPERIMENTAL WORK RELATING TO CHAPTER SIX

6.1. Methylation by Methyl cobalamin

6.1.1. Methyl cobalamin solution

Methyl cobalamin ($C_{63}H_{91}CoN_3O_{14}P$, Sigma Chemicals Ltd., 7.5 mg) was dissolved in water (25 ml). The solution was stored in the dark, and all experiments involving its use were carried out with the minimum exposure of the solution to daylight.

6.1.2. Methylation of Hg^{++}

Methyl cobalamin solution (1.2 ml) was mixed with an equal volume of aqueous $HgCl_2$ (1.47 gl^{-1}) and the uv spectrum between 300 and 400 nm recorded. The spectrum was compared with that of the methyl cobalamin solution diluted with an equal volume of water. There was an immediate loss of absorption at 374 nm and an increase at 350 nm.

6.1.3. Attempted methylation of alkyl lead and lead salts

6.1.2. was repeated, replacing $HgCl_2$ by $Pb(NO_3)_2$ (5 gl^{-1}), Me_2PbCl_2 (5.3 gl^{-1}), Me_3PbOAc (5 gl^{-1}), and Me_3PbCl (5.4 gl^{-1}). No change in the methyl cobalamin absorptions of these solutions was observed within 21 days. Concentrations of TML in the atmosphere above the solutions was shown to be the same as that above similar solutions with no methyl cobalamin.

6.2. Methylation by natural and by chemical systems

6.2.1. Me_3PbCl and $Pb(NO_3)_2$

Sediment (Langley Mill Pool, 50 ml), nutrient broth (Oxoid CM1, 1 g), glucose (D(+), 0.2 g), and the compounds indicated in the table below were made up to 200 ml with water, and sealed with Subaseals in

250 ml volumetric flasks. The solutions were shaken, and incubated at ambient temperature in the dark. Parallel experiments were carried out in water, with no sediment or nutrients added. The atmosphere above the solutions was periodically sampled with a 100 μl syringe, and analysed for TML by GLC. Figures quoted are the total amount of TML in the atmosphere in μg as TML

	Compounds added (mg)			TML in atmosphere (μg) after			
Matrix	$Pb(NO_3)_2$	Me_3PbCl	Na_2S	1d	13d	17d	32d
Sediment	0	0	0	0	0	0	0
Sediment	15	0	0	0	0	0	0
Sediment	0	15	0	trace	150	320	400
Water	0	17.2	0	0.1	0.1	0.3	0.8
Water	0	17.2	14.5	25	40	50	30

<u>6.2.2</u>. Me_3PbOAc

The above experiment was repeated with Me_3PbOAc

	Compounds added (mg)		TML in atmosphere (μg) after			
Matrix	Me_3PbOAc	Na_2S	5h	2d	7d	14d
Sediment	0	0	0	0	0	0
Sediment	15	0	0	20	150	300
Water	15	0	0	0.5	0.6	0.5
Water	15	15	50	300	300	300

<u>6.2.3</u>. Et_3PbCl

The above experiment was repeated with Et_3PbCl, and the atmosphere above the solution was analysed for Et_3MePb and TEL

	Compounds added (mg)		TEL (μg)		Et_3MePb (μg)	
Matrix	Et_3PbCl	Na_2S	17h	4d	17h	4d
Sediment	0	0	0	0	0	0
Sediment	15	0	0	0.15	0	0
Water	15	0	0	0	0	0
Water	15	15	0.2	0.8	0	0

6.3. Methylation by sewage

6.3.1. Anaerobic

Raw sewage (Coleshill, 500 ml), nutrient broth (Oxoid CM1, 2.5 g), and glucose (D(+), 0.5 g) were incubated with the addition of the compounds listed in the table below. The solutions were stored at ambient temperature in the dark in 1000 ml flasks sealed with Subaseals. The atmosphere above the solutions was sampled at intervals and analysed for TML by GLC.

Cpds. added (mg)		TML in atmosphere (μg) after				
$Pb(NO_3)_2$	Me_3PbCl	1d	11d	13d	17d	32d
0	0	0	0	0	0	0
10	0	0	0	0	0	0
0	10	trace	300	600	400	400

6.3.2. Aerobic

Raw sewage innoculated with activated flock (Coleshill, 400 ml) was placed in a vessel through which air could be drawn via a sinter. After aerating the sewage, the air was drawn through two traps maintained at dry ice/acetone temperature. After 16 h the traps and sewage were extracted with P.E. and analysed for TAL by GLC. None were detected.

Addition of $Pb(NO_3)_2$ (10 mg) to the above system produced no detectable TML.

Addition of TML in methanol (0.5 μl in 100 μl) showed 85% to be recovered in the first cold finger. None was detected in the second nor in the sewage.

Addition of TEL in methanol (0.5 μl in 100 μl) gave 0.1 μl TEL in the first cold finger and none in the second. Extraction of the sewage gave 0.01 μl TEL. No Et_3MePb was detected in the traps or sewage.

6.4. Methylation of Pb^{++} by lead resistant species

Various sediments of high lead concentrations were collected, and the following data recorded in Table 16.

pH of filtered sample .

(Pb) of filtered sample by direct aspiration onto AAS.

Weight of solids per 50 ml sample.

(Pb) of dried sample of solids analysed by the method of Hemsley[110](see 6.5.).

All samples were analysed for TAL and alkyl lead salts and were all found to be negative.

Samples of each sediment (50 ml) were incubated in sealed 250 ml flasks with nutrient broth (Oxoid CM1, 1 g), glucose (D(+), 0.2 g), $Pb(NO_3)_2$ (10 mg) and water to 200 ml. The atmosphere above the samples was analysed periodically for TML by withdrawing up to 100 μl in a syringe and injecting directly onto the GLC. In no case was TML detected above the solutions after 7 or 14 days.

6.5. Total lead analyses

The sediment to be analysed was filtered and the solids dried in an oven at 110°C. 1 g portions in 150 ml tall-form beakers were treated with HNO_3 (2 ml) and aqueous H_2O_2 (50%, 2 ml) and gently heated. The beakers were covered with a watch glass to prevent splashing. Further H_2O_2 was added until all the organic matter had been destroyed (cessation of violent frothing). The solution was cooled, filtered, and made up to 20 ml after the addition of 2.0 ml of a solution of $LaCl_3$ (17.3% $^w/v$). Duplicated samples were run in all cases.

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