BURDENS OF ALKYLLEAD COMPOUNDS IN THE SALTMARSH PERIWINKLE - TOXICITY OF ETHYLLEAD SALTS TO JAPANESE QUAIL

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ALKYLLEADS IN PERIWINKLES AND JAPANESE QUAIL.

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ABSTRACT

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BURDENS OF ALKYLLEAD COMPOUNDS IN THE SALTMARSH PERIWINKLE-TOXICITY OF ETHYLLEAD SALTS TO JAPANESE QUAIL

Saltmarsh periwinkles (Littorina irrorata Say.) collected from six separate sites in lower Chesapeake Bay were analysed for alkyllead levels. Of the six ionic alkylleads quantitated in the tissue, Me_3Pb^+ lacked a significant correlation with the levels of each of the individual alkylleads indicating a different origin from the other alkyllead analytes. The high level of correlation among the ethyl- and methylethyllead analytes was consistent with a methylation of ethyllead salts. In two separate feeding trials, Japanese quail were provided with drinking water amended with Et_3PbCl, Et_2PbCl_2 or $Pb(NO_3)_2$. A rapid dealkylation of tri- to diethyllead and a recirculation of the toxicants among the soft tissues was observed. Suprisingly, a conversion of di- to triethyllead was also observed, though the efficiency of this conversion was low relative to the amount of diethyllead ingested. Even at these levels of intake of ethylleads, mixed alkylleads remained at trace levels, indicating that methylation of ingested ethyllead salts in avian species is not a major metabolic process. Biological methylation of ingested inorganic lead is not a detectable process in quail.

RESUME

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CONCENTRATIONS D'ALKYLPLOMBS CHEZ LA PERVENCHE DES MARAIS SALES- TOXICITE DES SELS D'ETHYLPLOMB CHEZ LA CAILLE JAPONIQUE

Les pervenches des marais salés (Littorina irrorata Say.) provenant de six emplacements différents de la Baie de Chesapeake, ont été analysées pour leur teneur en alkylplomb. Le manque de corrélation significative entre la quantité de Me_3Pb^+ et chacun des autes sels d'alkylplomb indique une différence au niveau de la source de celui-ci. La corrétion des niveaus d'éthyl- et de methylethylplomb s'accorde avec une méthylation des sels d'ethylplomb. Lors de deux essais distincts de toxicité chronique, les cailles japonises ont été approvisionnées en eau contenant Et_3PbCl, Et_2PbCl_2 ou $Pb(NO_3)_2$. Une dealkylation rapide des tri- au diethylplomb et une recirculation des produits toxiques à travers les organes et les muscles ont été observées chez les cailles. Etonnement, la conversion du di- au triethylplomb a aussi été observée bien que l'efficacité de cette conversion était peu élevée relativement à la quantité de diethylplomb ingérée. À l'ingestion de ces concentrations d'ethylplomb, les methylethylplombs sont demeurés à l'état de traces; indiquant que la methylation des sels d'éthylplomb ingérés par les espcées avicoles n'est pas un procédé mebolique majeur. La methylation biologique de plomb inorganique suite à l'ingestion de celui-ci, n'est pas décelé chez la caille.

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1. LITERATURE REVIEW

1.1 Introduction

For the purpose of this dissertation, organoleads are considered to be lead-containing chemicals in which there is at least one covalent bond between a carbon and a lead atom. In these products lead is usually in the tetravalent state. Tetraorganoleads (R_4Pb, where the alkyl substituents are either methyl, ethyl or mixed methylethyl) have been used extensively as gasoline antiknock additives. These additives are thermally labile and are converted to lead oxide, which is formed as a fine mist in advance of the flame front during the combustion process. It is the lead oxide which is responsible for capturing peroxy radicals and the consequent reduction in pre-ignition (Shapiro and Frey, 1968). In the presence of scavengers such as dichloroethane (EDC) and dibromoethane (EDB), lead oxide is converted to the more volatile lead halide (Nickerson, 1954).

Since their introduction in 1923, the production of tetraalkyllead compounds has increased to such an extent that by the mid 1970s only a few other organic compounds were being produced in amounts greater than R_4Pb (Grandjean, 1983). In view of more recent concerns of the adverse health effects of lead, their use has been decreased slowly during recent years.

New automobiles manufactured in North America, are equipped with a catalytic convertor, which is poisoned by leaded gasolines. Whereas some countries have strict legislation to reduce the lead content of gasoline, other countries have not promulgated similar restrictions yet.

It is highly unlikely that the last traces of leadalkyls will be removed from gasolines in the near future, because of (1) the lack of regulations in other parts of the world; (2) the lack of an alternate antiknock agent which would be as economical yet less hazardous; and (3) the slow consumer acceptance of alternate fuels or new formulations. Since recent research has raised the possibility of the alkylation of inorganic forms of lead in the environment, organolead compounds may not be entering the environment exclusively from the leaded gasolines.

1.2 Sources of Alkylleads

1.2.1 Vehicular Sources

Although most of the lead in the exhaust from internal combustion engines is in an inorganic form, at times up to 10 % of emitted lead may remain in an organic form (Laveskog, 1971; Purdue et al., 1973). It has been estimated that up to 1.4 % of the tetraalkyllead formulated into gasolines may enter the atmosphere due to losses during the transport and transfer processes (Huntzicker et

al., 1975). In places where a mixture of tetraethyl- and tetramethyllead has been used as a gasoline additive, higher levels of the latter can be expected due to its higher vapour pressure.

Currently about 55 % of the gasoline consumed in Canada is of the leaded type. By the end of this year (1987) the permissible limit of lead in gasoline will be reduced from 0.77 g/L to 0.29 g/L (Environment Canada, 1985). A reduction in airborne lead in the coming years is to be anticipated. Eisenreich et al. (1986) demonstrated that the atmospheric lead levels decreased correspondingly if the lead level in gasoline was reduced.

1.2.2 Bioalkylation

The term biological methylation (biomethylation) implies the transfer (from another compound), or the addition of an intact methyl group to a chemical compound in question under quasi steady-state conditions (Challenger, 1955). There has not been any direct evidence of the biomethylation of lead in the environment as has been clearly demonstrated for other metals such as tin (Nelson et al., 1973; Huey et al., 1974; Dizikies et al., 1978; Hallas, 1981; Hallas et al., 1982) arsenic (Braman and Foreback, 1973; Cox and Alexander, 1973; Wood, 1975; Shiraki et al., 1981; Rowland and Davis, 1982; Buchet and Lauwerys, 1985) and mercury (Jensen and Jernelov, 1969; Kivimae et al., 1969; Bertillson and Neujahr, 1971; Imura,

1974; Neville and Berlin, 1974; Berman and Bartha, 1986). Somewhat conflicting reports have appeared in recent years, either supporting (Wong et al., 1975; Schmidt and Huber, 1976; Bellenick et al., 1977; Chau and Wong, 1978) or arguing against (Jarvie and Whitmore, 1981; Reisinger et al., 1981; Jarvie et al., 1975, 1983) a biomethylation mechanism in the environment.

Chemical alkylation of divalent lead to tetravalent organolead compounds has been reported (Ahmad et al. 1980; Craig, 1980; Craig and Rapsomanikis, 1985). Ahmad et al. (1980) demonstrated the formation of tetramethyllead in aqueous systems, from lead acetate in the presence of methyl iodide. Since the presence of methyl iodide in natural waters has been reported (Lovelock et al., 1973), this mechanism may explain trace levels of methylleads present in the various environmental compartments.

Wong et al. (1975) and Schmidt and Huber (1976) reported a microbially mediated formation of tetramethyllead from trimethyllead acetate or from lead nitrate under anaerobic conditions, although in case of the latter substrate, the production of Me_4Pb was sporadic. Harrison and Laxen (1978) attributed levels of tetraalkyllead, detected along the coastal regions of England, to the natural sources/formation of tetramethyllead.

Forsyth and Marshall (1986) found that tissue levels of

trimethyllead in Herring gulls, culled from several different colonies located within the Great Lakes, were significantly correlated with the mean inorganic lead (Pb^{+2}) level of the lake. The observation was consistent with a microbially mediated methylation of inorganic lead. Thus, there is considerable indirect evidence for the existence of a non automotive source of methylleads in the environment. However, the mode of formation of these methylleads, whether biologically or chemically mediated, remains controversial.

1.3 Environmental Fate of Alkylleads

Tetraalkyllead compounds which enter the environment as a result of incomplete combustion of leaded gasolines are converted, via a series of sequential dealkylations, into trialkyllead (R_3Pb^+), dialkyllead (R_2Pb^{2+}) and finally into inorganic lead cation (Pb^{2+}). (Stevens et al., 1960; Cremer et al., 1965; Harrison and Laxen, 1978; Roderer, 1980; Jarvie et al., 1981). The ionic alkyllead salts, being water soluble, undergo both dry and wet precipitation processes and may enter the aquatic environment. It is likely that lead compounds are taken up by aquatic organisms and become accumulated through the aquatic food chain. (Mor and Beccaria, 1977; Chau et al., 1979, 1980; Cruz et al., 1980; Wong et al., 1981). Ultimately the remaining lead pool is gradually deposited into sediments with the excreta, detritus or as the particulate-bound lead. (De Jonghe and Adams, 1982).

1.4 Occurrence of Alkylleads

Low levels of ionic alkylleads have been found to occur in several environmental compartments: air (Harrison, 1976; Radziuk et al., 1979; Rohbock et al., 1980; Nielsen et al., 1981; Harrison et al., 1985; Harrison and Radojevic, 1986); sediments (Cruz et al., 1980; Chau et al., 1984, 1985); rain water, snow and surface water (Radojevic and Harrison, 1986a; van Cleuvenberger et al., 1986); street dusts and urban soils (Blais and Marshall, 1986; Radojevic and Harrison, 1986b). The concentrations of organolead compounds in urban air, have been summarized by De Jonghe and Adams (1982) and Forsyth (1985). Fish (Chau et al., 1980; Cruz et al., 1980); fowl (Johnson et al., 1982; Bull et al., 1983; Forsyth and Marshall, 1986) and human brain (Nielsen et al., 1978) have also been found to contain low levels of organolead compounds.

Although alkylleads generally comprise less than one percent of the total lead burdens in most environmental compartments, as much as 38 % of the total lead was in an organic form in fish samples from the St. Clair river (Chau et al. 1985). Four to 84 % of the total lead in human brain was organosoluble (Nielsen et al., 1978). A lack of correlation between the total lead level and the organosoluble fraction has been observed in various environmental compartments (Harrison and Perry, 1977; Nielsen et al., 1978; Blais and Marshall, 1986), but the reason for this observation is not evident.

1.5 Analytical Methodology

The most commonly employed technique for the determination of alkylleads involves a selective extraction procedure, a derivatization sequence and a chromatographic separation followed by metal specific detection. Several researchers have adopted an affordable analytical system involving a gas chromatograph (GC) equipped with a flameless atomic absorption spectrometer (AAS) or quartz T tube furnace as the detector (Estes et al., 1981; Chakraborti et al., 1984; Chau et al., 1984; Forsyth and Marshall, 1985; Radojevic et al., 1986). Whereas the volatile tetraalkyllead compounds can be determined by injecting a suitably preconcentrated sample extract directly into the GC, the ionic alkylleads (R_3Pb^+, R_2Pb^{2+}; R = Me, Et) must be derivatized by alkylation using a Grignard reagent. Thus dialkyllead and trialkyllead compounds are usually butylated to form the corresponding tetraalkyl derivatives prior to analysis by GC-AAS.

Other methods of analysis include capillary column GC with electron capture detection (Forsyth and Marshall, 1983); GC- AAS with electrothermal atomization (Torsi and Palmosano, 1983); or more expensive approaches such as chromatographic separation coupled with plasma emission detection (Reamer et al., 1978; Estes et al., 1981, 1982) or mass spectrometric detection (Nielsen et al., 1981).

1.6 Toxicology

1.6.1 Introduction

Toxicity and health hazards of organolead compounds have been summarized by Grandjean and Nielsen (1979) and Grandjean (1983). The characteristic toxic effects of tetraalkylleads, at least in mammals, are not caused by the tetraalkyl compounds themselves but rather by the trialkyl derivatives, formed by dealkylation which occurs mainly in the liver (Cremer, 1959; Cremer and Callaway, 1961).

1.6.2 Acute Toxicity

The acute LD_{50} values for several organolead compounds are summarized in Table 1 along with values for several inorganic lead compounds which are included for comparison. It can be noted that the relative toxicity of organolead compounds generally is much higher than the inorganic forms. Trialkyls are as toxic as or even more toxic than, their tetraalkyl progenitors. The dialkylleads are less toxic than their tetra- and tri- analogues, but are comparitively more toxic than any of the inorganic forms of lead. Ethyl organoleads are more toxic than the methyl analogues to rats whereas the reverse is true for dogs and mice (Davis et al., 1963; Grandjean and Nielsen, 1979). The lethal dose for man has not been reported.

Tetraethyllead poisoning in mammals is generally associated with the initial onset of the "TEL triad" of

Table 1. The acute LD_{50} values for various lead compounds.

Compound	Animal	Route	LD_{50}	Reference
Et_4Pb	Rat	oral	35.0	Venugopal & Luckey (1978)
	Rat	i.g.	14.2	Schroeder et al. (1972)
	Rat	i.p.	15.4	Cremer (1959, 1961)
	Mouse	i.p.	30.3	Hayakawa (1972)
	Japanese Quail	oral	24.6	Hudson et al. (1984)
	Mallard	oral	107.0	Hudson et al. (1984)
Et_3PbCl	Rat	i.p.	11.2	Cremer (1959)
	Mouse	i.p.	12.8	Hayakawa (1972)
Et_2PbCl_2	Rat	i.g.	120.0	Springman et al. (1963)
$Et_2Pb(OAc)_2$	Mouse	oral	130.0	Jaworski (1978)
Me_4Pb	Rat	i.v.	109.3	Cremer and Callaway (1961)
	Mouse	i.p.	14.3	Hayakawa (1972)
Me_3PbCl	Rat	i.p.	25.0	Cremer and Callaway (1961)
Me_3PbOAc	Rat	i.p.	33.0	Caujolla and Voisin (1966)
$Pb(OAc)_2$	Rat	i.p.	140.0	Venugopal and Luckey (1978)
$Pb_3(AsO_4)_2$	Rat	oral	100.0	"
$PbCl_2$	Guinea pig	oral	2000.0	"
Pb metal	Rat	i.p.	1000.0	"

i.g. (intragastric); i.p. (intraperitonial); i.v. (intravenous).

hypotension, bradycardia and hypothermia, perhaps due to hypothalamic changes (Razsudov, 1976). The acute poisoning with tetra or trialkyllead in rats was characterized by hyperexcitability, tremor, periodic convulsions and aggressive behaviour (Cremer and Callaway, 1961; Springman et al., 1963; Sanders, 1964; Schroeder et al., 1972). No effective treatment exists for acute organolead poisoning (Grandjean and Nielsen, 1979).

1.6.3 Subacute/Chronic Toxicity

The general population is constantly exposed to sub-lethal levels of alkylleads. In man, exposure to a Et_4Pb concentration of 100 mg (as Pb)/m^3, for 1 h may produce illness (Fleming, 1963). A level of 0.075 mg/m^3 of air is considered as the permissible air concentration of Et_4Pb (Ethyl corp., 1962).

Schepers (1964) demonstrated that oral dosing of rats with (1.7 or 170 ug) tetramethyllead or tetraethyllead for a period of 21 weeks produced pathological changes in liver, pancreas, kidney, endocrine glands and the nervous system. However, no treatment-related histological changes appeared in Rhesus monkeys which were given tetramethyllead or tetraethyllead (6 ug (as Pb)/Kg body weight per day) orally for a period of six months (Heywood et al., 1978, 1979).

Although these few studies have identified the hazards associated with the chronic exposure to tetraalkyllead

compounds, analogous studies (either sub-chronic or chronic) with the actual toxic principle (R_3Pb^+) or with its degradation product R_2Pb^{2+} have not been conducted.

1.6.4 Toxicokinetics

1.6.4.1 Absorption

Absorption is of major concern, in cases of tetraalkyllead poisoning because of the lipophilicity of these compounds. The dermal absorption of tetraethyllead increased with increasing application area and exposure time (Kehoe and Thamann, 1931), moreover its distribution throughout the body was faster than for tetramethyllead (Davis et al., 1963). Whereas three daily 30 min dermal exposures (applied to ~ 28 sq. cms) to 10 % Et_4Pb in gasoline caused paralysis and death, absorption from gasoline having concentrations of less than 0.1 % Et_4Pb was not detectible (Kehoe and Thamann, 1931). Grandjean and Nielsen (1979) estimated the efficiency of pulmonary absorption to be about 80 %.

1.6.4.2 Metabolism

The toxic effects of tetraalkylleads appear to be caused by their trialkyl (R_3Pb^+) homologs. Cremer (1959) demonstrated the rapid conversion of tetraethyllead to triethyllead by rat liver, the observed rate being 180 ug Et_4Pb / g wet weight of liver / hour. Triethyllead could be isolated from the livers of rats which had been exposed to tetraethyllead vapours (Stevens et al., 1960).

Bolanowska et al. (1967), Bolanowska (1968), Bolanowska and Wisniewska-Knypl (1971) and Hayakawa (1972) demonstrated the rapid dealkylation of the tetraalkyllead compounds into R_3Pb^+ by either rat or rabbit liver homogenate, and in tetraethyllead-poisoned human beings. They proposed that Et_4Pb was converted to Et_3Pb^+ and then to inorganic lead directly, although their analytical techniques did not permit the analysis of dialkyllead compounds. Sequential dealkylation of tetraalkylleads may proceed to dialkyllead compounds (Casida et al., 1971).

1.6.4.3 Distribution

In contrast to inorganic lead which is known to be excreted or deposited in storage tissues (bones, hair and cartilage) fairly rapidly, alkylleads have been observed mainly in the soft organs of the body. The tissue distribution of Et_3Pb^+ has been studied in rats (Cremer and Callaway, 1961), mice (Hayakawa, 1972) and humans (Bolanowska et al., 1967). The highest levels were observed in liver followed by kidney, brain and blood. In cases of tetraalkyllead poisoning blood lead levels always remained at less than 1 ug Pb/mL and tetramethyllead was retained to a much greater extent than was tetraethyllead. Whereas the half-life of Me_3Pb^+ was 15 days in blood and 40 days in the liver or kidney of rats, the half-life of Et_3Pb^+ was found to be 3-5 days in blood and 15 days in liver or kidney of this species (Hayakawa, 1972).

1.6.5 Toxic Mechanisms

1.6.5.1 Neurotoxic Effects

The brain appears to be the target organ in cases of organolead poisoning. The organolead compounds, being lipophilic are selectively localized in nervous tissue, resulting in the CNS type of toxicity.

Cremer (1962) demonstrated that Et_3Pb^+ at low concentrations inhibited oxygen consumption and output of carbon dioxide by brain slices when labelled ^{14}C-glucose was added as substrate. An increased production of lactic acid, under aerobic conditions, was also observed indicating that this toxicant interferes with the tricarboxylic acid (TCA) cycle at the point where pyruvate normally enters the cycle.

Myelin forming cells were more vulnerable than other cells in the brain of Et_3PbCl intoxicated rats (Konat and Clausen, 1974; Konat et al., 1976). The maturation of the myelin sheath in the forebrain of the subjects seemed to have been retarded appreciably. A high level of correlation between the inhibition values for protein synthesis and for myelin deposition was observed (Konat et al., 1979; Konat and Clausen, 1980)

Tilson et al. (1982) found Et_3PbCl to be a highly neurotoxic agent, which was capable of inducing long lasting alterations in emotional behaviour and reactivity. Triethyllead chloride (1 to 2.5 mg/kg) administered

subcutaneously to adult male rats for 5 days produced hyperexcitability and hyperactivity for 1-2 weeks post-dosing followed by hypoexcitability and hypoactivity 3-4 weeks post-dosing.

It is interesting to note that even the cerebral levels of alkylleads found in North Americans (0.3 to 1.0 x 10^{-6} M) with no history of abnormal exposure can modify the transmission of putative neurotransmitters (eg., dopamine, glutamate) to a considerable extent (Bondy et al., 1979).

1.6.5.2 Biochemical Toxicity

Inhibitory effects of triethyllead cation on serum cholinesterase activity (Galzigna et al., 1969, 1973) and glutathione-S-aryl transferase (Henry and Byington, 1972) have been demonstrated in vitro. Beattie et al. (1972) observed a pronounced reduction in blood aminolevulenic acid dehydrase (ALA-D, the enzyme responsible for the conversion of aminolevulenic acid to porphobilinogen) activity in cases of Et_4Pb poisoning.

Hamilton (1986) reported that dimethyllead dichloride had the greatest inhibitory effect on human erythrocyte ALA-D in vitro. The enzyme was completely inhibited at a concentration of 10^{-4} M and was only 5 % as active in the presence of 5 x 10^{-6} M Me_2PbCl_2 as in the absence of this toxicant. Interestingly, trialkylleads were considered less effective inhibitors in this assay.

1.6.5.3 Cellular Toxicity

Ammitzboll et al. (1978), investigating the use of chicken embryo brain cell cultures in toxicological studies, found that triethyllead chloride inhibited the synthesis of DNA, of sulfatides and the synthesis of those cerebrocides which were devoid of hydroxy fatty acids. The confluent layer of the astroblasts was disrupted in 50 % of the cultures at the tissue culture lethal dose ($TCLD_{50}$ = 1.9 mg/L) and the neurons lost their activity at even lower concentration ($TCED_{50}$ = 0.57 mg/L).

One of the major biochemical effects caused by alkylleads is the uncoupling of oxidative phosphorylation. Bjerrum (1978) demonstrated that both trimethyllead and triethyllead salts could induce an exchange of extracellular chloride with intracellular hydroxyl ions, with triethyllead being ten times more effective than the corresponding trimethyllead salt.

1.6.6 Special Toxicity Studies

Alkyllead compounds cause genetic modifications similar to those caused by the alkyl mercury compounds. Mutagenic effects have been observed at a concentration of about 10^{-7} M, Et_3PbCl being more toxic than Me_3PbCl (Ahlberg et al., 1972; Ramel, 1973). McClain and Becker (1972) and Kennedy et al. (1975) found no teratogenic effects in the progeny of rats or mice which had received Et_4Pb, Me_4Pb or Me_3PbCl, even at levels at which overt

maternal toxicity was observed. Tetraalkylleads are formulated into gasolines along with the lead scavengers, ethylene dibromide (EDB) or ethylene dichloride (EDC), which are known to be carcinogenic (IARC, 1977; NCI, 1978; Nylander, et al., 1978). The combined effects of these toxicants, which have not been investigated, remains an area of concern.

1.7 Alkylleads in Marine Species

1.7.1 Introduction

It has been estimated (Huntzicker et al., 1975) that 20 - 25 % of the lead resulting from the combustion of leaded gasolines, finds its way into the oceans. Whereas the mean total lead concentration in deep water is about 1 ng/L (Patterson and Settle, 1976), it may be more than 100 times this amount near the surface or in polluted waters (Tiravanti and Boari, 1979).

The sinking of an Yugoslavian ship ("Catvat") carrying 325 metric tonnes of alkyllead antiknock formulations, in the Adriatic sea (Tiravanti and Boari, 1979) focused concerns about the possible harmful effects of these compounds to aquatic life.

The accumulation of alkyllead compounds by marine organisms is of concern not only because of their deleterious effects on the organism itself but also becauce of the possible harmful effects that the accumulated levels may have on predators such as man.

Accumulation may be the result of either direct absorption from water and sediments, or ingestion from the food chain.

Speciation of alkylleads in fresh water fish (Botre et al., 1977; Chau et al., 1979, 1980, 1984, 1985), cod, lobster, mackerel and in flounder meal samples (Sirota and Uthe, 1977) has been reported. Alkylleads constituted up to 38 % of total lead burdens in fish samples from the St. Clair river, Ontario (Chau et al., 1985). Tetraalkylleads accounted for about 9.5 - 90 % of the total lead in various marine tissues (Sirota and Uthe, 1977).

1.7.2 Toxicity to Marine Organisms

The estimated concentrations required to kill 50 % of the test marine organisms (LC_{50}) after 96 h exposure to ethyl- and methyllead compounds and inorganic lead are summarized in Table 2. It has been demonstrated, at least for marine organisms, that the toxicity of the alkyllead compounds is directly related to the degree of alkylation (Maddock and Taylor, 1980). Levels of 1 ppb for tetraalkylleads and 100 ppb for trialkylleads have been suggested as "safe" limits - concentrations at which no acute toxic effects would be anticipated (Tiravanti and Boari, 1979).

Toxicity of tetraalkyllead compounds to fish (Turnbull et al., 1954), algae (Kozyura et al., 1961; Silverberg et al., 1977; Marchetti, 1978) and marine animals (Maddock

Table 2. Summary of 96 h LC_{50} values, (Maddock and Taylor, 1980)

Species	Plaice	Shrimp	Mussel	Algae*
Me_4Pb	0.05	0.11	0.27	1.3
Et_4Pb	0.23	0.02	0.10	0.1
Me_3PbCl	24.60	8.80	0.50	0.8
Et_3PbCl	1.70	5.80	1.10	0.1
Me_2PbCl_2			300.00	
Et_2PbCl_2			75.00	
Inorg. Pb	375.00[a]	>500[a]	180.00[b]	>5.0

* Parameter measured was a reduction in photosynthetic activity

a Portman and Wilson (1971)

b Jackim (1973)

and Taylor, 1980) has been investigated. Marchetti (1978) demonstrated that tetraethyllead was more acutely toxic than tetramethyllead to various marine species, and that marine animals were more susceptible to tetraalkylleads than either algae or bacteria.

1.7.3 Bioaccumulation

The accumulation of tetramethyllead by rainbow trout and subsequent depuration were investigated by Wong et al. (1981). The authors observed that the accumulation was limited mainly to fatty tissues, a consequence of the lipophilic nature of the toxicant. Maddock and Taylor (1980) reported that the accumulation in dabs (Limanda limanda) was two fold for trimethyllead chloride and 12 fold for triethyllead chloride.

From this rather limited range of experiments, it has been suggested that the major environmental impact of alkylleads in the marine environment would result from their acute toxicities, rather than their bioaccumulation (Maddock and Taylor, 1980).

1.8 Alkylleads in Avian Species

1.8.1 Introduction

Although most of the tetraalkylleads in gasolines is decomposed during combustion, small amounts of these compounds have been detected in particulates in urban areas (Harrison and Laxen, 1978; Blais and Marshall, 1986). From 22 to 58 % of the lead emitted from motor

vehicles is deposited on the ground or vegetation within the roadside verges (Ward et al., 1975; Little and Wiffen, 1978).

1.8.2 Lead in Avian Tissues

Birds inhabiting roadside verges (areas which constitute about 30.4 million hectares in the U.S.A) (Smith, 1976) may be exposed to lead from motor vehicle exhaust through inhalation or ingestion of contaminated food. Lead concentrations have been measured in the ingesta or the tissues of urban pigeons Columba livia (Tansy and Roth, 1970; Ohi et al., 1974, 1981; Hutton, 1980; Hutton and Goodman, 1980; Johnson et al., 1982), doves (Siegfried et al., 1972) and urban songbirds (Getz et al., 1977; Udevitz et al., 1980; Grue et al., 1984). Tissue levels of wild birds have been summarized by Bagley and Locke (1967).

1.8.3 Alkyllead Burdens in Birds

Johnson et al. (1982) were the first group to report levels of alkylleads in an avian species. Trialkylleads but neither tetraalkyllead nor the dialkyllead were reported in the tissues of pigeons. Concentrations of about 0.3 ppm (in kidney) were observed. Subsequently it was reported (Bull et al., 1983) that alkylleads were the toxicants responsible for the death and the sickness of the 2,400 birds in the Mersey estuary, U.K. in 1983 as well as for mortalities during the years 1980 and 1981.

Alkyllead (unspeciated) concentrations ranged up to 19.42 ppm in the kidney of the dead birds from the Mersey estuary. Much of the lead detected was likely to have been in the trialkyl form.

1.8.4 Speciation of Alkylleads in Avian Tissues

Only four studies (Hutton, 1980; Johnson et al., 1982; Bull et al., 1983; Forsyth and Marshall, 1986) reported the analysis of the avian tissues for alkyllead levels. In the study by Hutton (1980) alkyllead salts were not detected in pigeon tissues, possibly a result of the limit of detection of the analytical methods employed (1 ug of either the tri- or dialkyllead).

Johnson et al. (1982) reported levels of alkylleads in tissues of urban pigeons. Using differential pulse anodic stripping voltammetry, only the nature of the chemical species could be reported as R_3Pb^+ but not the identity of the alkyl substituents.

More recently Bull et al. (1983) reported levels of alkylleads in the tissues of dead and sick birds collected from the Mersey estuary. These workers were only able to measure the total ionic alkyllead levels; they did not identify the individual chemical species.

The only study (Forsyth and Marshall, 1986) that attempted to speciate the alkylleads, found levels of trimethyllead to be high relative to other alkyllead analytes, in the tissues of Herring gulls collected from

the Great Lakes. Alkyllead burdens in tissues (liver, kidney or brain) of mature and immature birds were not significantly different, suggesting that gulls did not bioaccumulate ionic alkylleads. Significant correlations were observed between tissue levels of Me_3Pb^+ and Me_2Pb^{2+} cations and between Et_3Pb^+ and Et_2Pb^{2+} cations which were attributed to sequential dealkylation of the trialkyllead species. However the ethyl- and methyllead burdens were not significantly correlated, indicating independent ethyl and methyllead sources to the gull populations.

1.8.5 Methylleads in Avian Species

The above study also identified a significant correlation between methyllead tissue concentrations of the combined (mature and immature) birds and the lake sediment inorganic lead levels. Trimethyllead cation concentrations in gull egg homogenate were also correlated significantly with mean lake sediment inorganic lead levels as reported by Hodson et al. (1984). Correlations between ethyllead and methyllead tissue levels were not significant; indeed a negative correlation occurred in every case. It is to be noted that Canadian gasolines contain tetraethyllead but not tetramethyllead (Chau et al., 1976; Radziuk et al., 1979; Forsyth, 1985). High levels of trimethyllead detected in the gull tissues, relative to the ethyllead levels, would certainly not be expected. Together with the statistically significant

correlation of concentrations of methyllead salts with the mean lake sediment inorganic lead (Pb^{2+}) levels, it would seem that methylation (possibly microbially mediated) but not ethylation of inorganic lead, is a potential source of methyllead salts in the environment.

1.8.6 Toxicity of Alkylleads to Avian Species

1.8.6.1. Acute Toxicity Studies

The acute oral LD_{50} vaules for tetraethyllead to Mallard ducks and Japanese quail are included in Table 1. Polydypsia, regurgitation, shakiness, hypoactivity, wingdrop, wingspread, ataxia, tremors and anorexia were some of the major signs of intoxication in birds (Hudson et al., 1984).

Osborn et al. (1983), investigating the toxicity of trialkylleads to kestrels, suggested that birds which were burdened with more than 0.5 mg Pb (in the form of trialkyllead) per kg wet weight in liver would be at some risk if they met adverse conditions.

1.8.6.2 Chronic Toxicity Studies

Diehl et al. (1985) reported results of the exposure of laying hens to emission sources of tetraethyllead and tetramethyllead for a period of 6 to 12 months. They found that the tissue total lead burdens decreased with increasing distance from the emission source.

1.8.6.3 Special Studies

Egg shell thinning in raptorial birds has for long been attributed to their exposure to various environmental contaminants. Haegele and Tucker (1974) investigated the effect of tetraethyllead on the egg shell thickness in Japanese quail and Mallard ducks. Whereas a single dose of 6 ppm reduced egg shell thickness significantly ($p < 0.05$) in quail, the same dosage did not produce significant changes in the eggshell thickness in Mallards for up to one week post-dosing.

Forsyth et al. (1985) observed an appreciably greater interaction of the alkyllead salts (R_3PbCl, R_2PbCl_2; R = Me, Et) with the egg yolk fraction than with the equivalent weight of egg white from chicken eggs. In another experiment they demonstrated the rapid transfer of toxicants through the biological membranes within viable American kestrel eggs, to accumulate in the yolk and the developing embryo. Since the chick resorbs the embryo just prior to hatching, these results indicate that the chicks will be burdened with the majority of alkylleads present in the egg.

From this review, it may be concluded that the chronic toxicity of ionic alkyllead compounds to avian species, remains virtually unexplored. The toxicity and metabolism of dialkyllead cations to avian species have not been investigated. There has been neither a metabolic study with sub-lethal doses of triethyllead or diethyllead, nor

a study of the possible biomethylation as a metabolic response to ingested inorganic lead salts by avian species.

1.9 Objectives of this Research Project

The following objectives were defined:

1. To extend previous observations (Forsyth and Marshall, 1986) on the origin of individual ionic alkylleads by investigating the alkyllead burdens in a non-avian indicator species (Saltmarsh Periwinkle) from several separate sites from within an aquatic environment (Chapter 2).

2(a). To assess the possibility of biomethylation, to produce dimethyllead or trimethyllead cations as a metabolic response to ingestion of inorganic lead salts by a suitable avian indicator species (Japanese quail) (Chapter 3).

2(b). To study the accumulation, metabolism and transfer into egg of diethyllead cation, when Japanese quail are administered low chronic doses of diethyllead dichloride under quasi steady-state conditions (Chapter 3).

2(c). To study the accumulation, metabolism and transfer into egg of triethyllead cation, when Japanese quail are administered low chronic doses of triethyllead chloride under quasi steady-state conditions (Chapter 3).

2. BURDENS OF ALKYLLEADS IN THE SALTMARSH PERIWINKLE.

2.1 Introduction

Saltmarsh Periwinkles (Littorina irrorata. Say) are semiaquatic snails associated with the grasses Spartina alterniflora and Juncus roemarianus in the saltmarshes along the Atlantic and Gulf coasts of the U.S.A. (Odum and Smalley,1959; Smalley, 1959). Littorina irrorata feeds by scraping the surface of the marsh at low tide, thus ingesting detritus particles, associated bacteria and microalgae (Smalley, 1959; Alexander, 1979). With rising tides this species mounts the stems of Spartina and Juncus to avoid predation (Bingham, 1972).

The sedentary nature of this species and the intimate association with its habitat suggests that the periwinkle would be a good bioindicator of hazardous materials contamination. Related species, Littorina littorea and L. littoralis, have been successfully used as indicators of heavy metals pollution (Bryan et al., 1983).

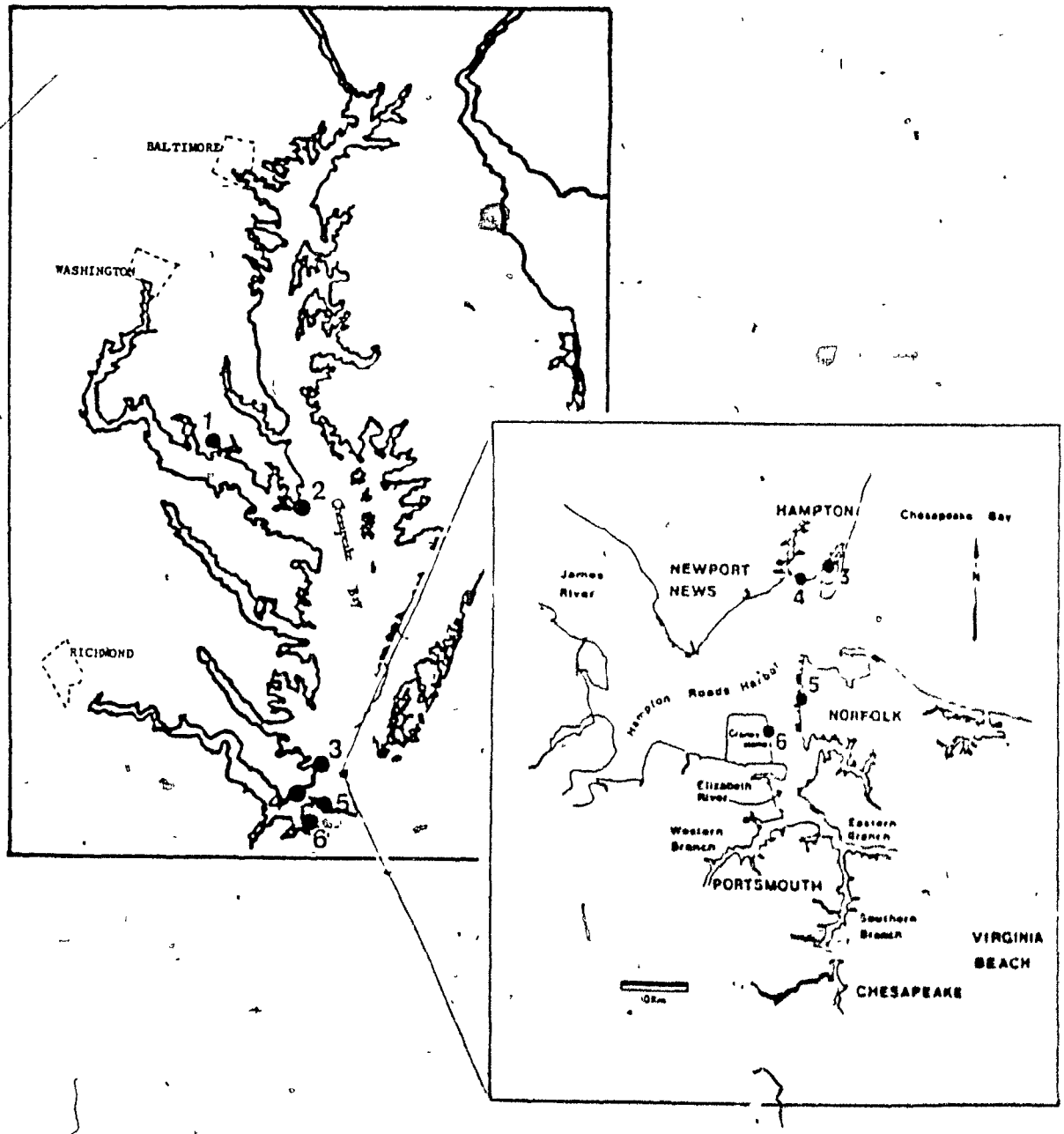
The purpose of this study was to speciate alkylleads in Saltmarsh Periwinkles which had been culled from six separate sites in lower Chesapeake Bay. It was also hoped that this species could be used to corroborate previous observations (Forsyth and Marshall, 1986) regarding methyllead sources in the environment.

2.2 Materials and Methods

2.2.1 Location of Sites

Collection plots for Littorina irrorata were established at two sites in southern Maryland and at four sites in northern Virginia (Figure 1). Sites were chosen to provide different characteristics of pollution stress. The Maryland Sites, Fisher's Creek (FC) and Point Lookout (PL) were located on the St Mary's River and at the mouth of the Potomac River respectively. The former site (FC) is situated near a small college campus surrounded by agricultural lands and the latter site (PL) was also surrounded by farming areas. These two sites were considered to represent relatively pristine conditions. The Elizabeth river was chosen as a location of relatively high pollution stress. Specific sites in this area (CI,CP) were limited by the relatively few stands of Spartina alterniflora remaining in the area. Craney Island (CI) is located between a U.S coast-guard support base and a U.S navy fuel depot, and Cardinal point (CP) is situated in a residential area, in Norfolk, Va. Hampton sites (HS,HN) were located only a short distance from the Elizabeth river sites; however the characteristics of the snail population more closely resembled the population of the southern Maryland sites. These two sites were considered to represent an intermediate pollution stress.

Figure 1. Location of sampling sites in Chesapeake Bay, U.S.A.; 1, Fisher's Creek, (FC); 2, Point Lookout, (PL); 3, Hampton North (HN); 4, Hampton South (HS); 5, Cardinal Point (CP); 6, Craney Island (CI).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WASHINGTON
RICHMOND
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2
3
5
6
HAMPTON
Chesapeake Bay
James River
NEWPORT NEWS
4
Hampton Roads Harbor
NORFOLK
Elizabeth River
Western Branch
Eastern Branch
PORTSMOUTH
Southern Branch
VIRGINIA BEACH
CHESAPEAKE

2.2.2 Sample Collection

At each site, collection plots, 5x8 m^2, were transected into 0.25 m^2 quadrats. Collections were made from alternating quadrats and samples were stored immediately on ice. Collections were performed during June and July 1985. Snails were sexed prior to analyses.

2.2.3 Analyses for Alkylleads

2.2.3.1 Sample Preparation and Enzymatic Hydrolysis

Thirty to forty adult snails from each site, either male or female (shell length 20-25 mm) were pooled after separation from their shells and opercula. Samples were ground in a Virtis tissue homogenizer, frozen in liquid nitrogen, pulverized and the resulting powder was thoroughly mixed. Each sample, ~ 2.5 g, was placed in a 50 mL Nalgene screw cap centrifuge tube, suspended in 20 mL of 5 % ethanol in 0.5 M NaH_2PO_4 containing 40 mg of each of Lipase Type VII and Protease Type XIV (Sigma Chemical Co.) and incubated at 37° C for 24 h.

2.2.3.2 Extraction

2.2.3.2.1 Extraction of tetraalkylleads

The crude hydrolysate was extracted with 5 mL of hexane, and centrifuged to separate the hexane phase. The organic solvent was removed, dried over Na_2SO_4, concentrated to 1 mL under a gentle stream of nitrogen and transferred to capped sample vials for analysis by GC-QT-AAS as described in section 2.2.3.4.

2.2.3.2.2 Extraction of Ionic Alkylleads

The hydrolysate from each sample was combined with 5 mL ammoniacal buffer (consisting of 22.6 g ammonium citrate, 4.0 g KCN and 24.0 g Na_2SO_3 diluted to 250 mL with double distilled water and adjusted to a final pH of 10.0 with concentrated aqueous ammonia) and extracted three times with 5 mL 0.01 % dithizone in hexane. Centrifugation at 4400 rpm hastened phase separation. The hexane extracts were combined and concentrated to 1.0 mL under a gentle stream of nitrogen at ambient temperature.

2.2.3.3 Derivatization

N-butyl magnesium chloride [0.5 mL, 2.27 M in tetrahydrofuran (THF), Alfa Products, Ventron Corp., Denver, Co.] was added, under N_2, to the tubes containing the organolead dithizonates. The tubes were sealed, vortexed for 20 s, and cooled in an ice bath. Excess Grignard reagent was destroyed by the dropwise addition of 1 M HNO_3. The reaction mixture was diluted to 10 mL with water, shaken for 30 s and centrifuged for 5 min in a clinical centrifuge (1550 rpm). The organic phase was removed and the aqueous phase was washed with a further 5 mL hexane. The organic extracts were combined, reduced to 1 mL and dried over Na_2SO_4.

2.2.3.4 Instrumentation

A gas chromatograph - quartz tube - atomic absorption spectrometer (GC-QT-AAS) was used for sample

quantitation. The GC, equipped with an autoinjector, was fitted with a glass column (1.8 m, 6 mm o.d, 2 mm i.d) packed with 10 % OV - 101 on 80-100 mesh Supelcoport. The optimized operating conditions (Forsyth and Marshall, 1985) were: carrier gas, helium, 35 mL/min; hydrogen make up gas, 50 mL/min; injector temperature, 200°C; furnace temperature, 900°C; temperature program, 50°C (1 minute)-raised at 8°C $(min)^{-1}$ to 250°C and held for 1 minute.

2.2.3.5 Quantitation

Each butylated extract was analysed twice; quantitation was performed by comparison of the peak areas with the peak areas of a standard mixture of Me_3BuPb, Me_2Bu_2Pb, Et_3BuPb and Et_2Bu_2Pb. Mixed methylethyllead compounds were identified from the predicted Kovats' retention indices (Kovats, 1965) based on the observed retention times of the alkylbutyllead standards. Actual retention times of the methylethylleads were confirmed by comparison with a standard mixture of transalkylation reaction products. Quantitation of mixed alkylleads ($Et_2MePbBu$ and $EtMe_2PbBu$) was performed by comparison of peak areas with a similar organolead analyte (Et_3Pb^+ and Me_3Pb^+ respectively) for which the standards were available (Forsyth and Marshall, 1986).

2.2.3.6 Recoveries of Ionic Alkyllead from Snail Tissue

Recoveries of ionic alkylleads from the snail tissue were assessed by spiking three samples of female tissue

homogenate (from the Point Lookout site), with a standard mixture of Me_3PbCl, Me_2PbCl_2, Et_3PbCl and Et_2PbCl_2. The resulting spiked homogenate samples were analysed as described in sections 2.2.3.2 to 2.2.3.5. The percent recovery was determined by dividing the mean peak area of the recovered butylate by the mean peak area of a butylated spike solution diluted to the expected concentration (assuming a 100 % recovery). Recoveries of the trimethyllead were corrected for the level of this analyte which was originally present in the samples. The reported burdens of an unknown alkyllead analyte were calculated based on instrumental response for Me_3Pb^+ and the levels have not been corrected for recoveries. A sample calculation is contained in Appendix 1.

2.2.4 Total Lead Analysis

Samples of 1- 1.5 g of the freeze dried tissue pools were thoroughly mixed and digested with HNO_3 (10 mL, 10M) and $HClO_4$ (2 mL, 7M) until clarified. Digests were diluted to volume and analysed by graphite furnace - atomic absorption spectrometry (GF-AAS).

2.3 Results and Discussion

2.3.1 Population Dynamics of Littorina irrorata in the Collection Sites:

Within the collection plots the number of Littorina decreased towards the landwards edge. The snails from the

Elizabeth river sites appeared to be abnormal in many aspects. These sites were characterized by a total absence of juveniles (shell length less than 15 mm) and small adults (shell length 15 - 19 mm). The female to male ratios were considerably lower than other sites: 1.02 (CI), 1.11 (CP) relative to other sites 1.32 (HN), 1.42 (HS), 1.57 (PL) and 1.66 (FC).

2.3.2 Recoveries of Ionic Alkylleads:

The relative recoveries of the spiked lead salts (Table 3) were not quantitative but were comparable to analogous recoveries from avian tissue (Section 3.2.4) and to recoveries reported for avian tissue by Forsyth and Marshall (1986). The recoveries of dimethyllead dichloride was very low, but were in agreement with the previously reported observations by Cremer (1959), Chau et al. (1979), Chau et al. (1984) and Forsyth (1985). These authors concluded that the dimethyllead remained bound to the tissue or existed in the solution as a complex which

Table. 3. Mean Percent recoveries of ionic alkyllead compounds from snail tissue[a] or from deionized distilled water[a,b].

Matrix	Mean[c] analyte percent recovery ± 1 SD			
	Me_3PbCl	Et_3PbCl	Me_2PbCl_2	Et_2PbCl_2
Snail homogenate[a]	105 ± 6	72 ± 6	28 ± 5	47 ± 4
Distilled Water[a]	94 ± 6	86 ± 3	42 ± 8	76 ± 4
Distilled Water[b]	98 ± 4	93 ± 4	84 ± 8	93 ± 6

[a] Spiked at 4-5 ppb (as Pb).
[b] Spiked at 40 - 50 ppb (as Pb) with a mixture of Et_3PbCl, Et_2PbCl_2, Me_3PbCl and Me_2PbCl_2.
c N=3 replicate determinations.

could not be extracted with the solvent. Chau et al. (1984) observed a higher decomposition rate for dimethyllead dichloride than for other alkyllead analytes in aqueous media.

2.3.3 Tetraalklylleads in the Samples:

No tetraalkylleads were detected in any of the samples in this study. Hexane extracts of the crude hydrolysates did not contain any volatile lead compounds. This might be attributed to the loss of the volatile tetraalkyls during the summer sampling times or because of the environmental and/or metabolic dealkylation of these analytes.

2.3.4 Total Ionic Alkyllead Burdens:

The alkyllead burdens in the representative pools of either males or females from each of the six sites is recorded in Table 4 . The values are reported on a dry weight basis (percent dry weight = 25.6) and have been corrected for the percent recoveries, based on the recovery trials (Table 3). Six alkyllead species were quantitated (Me_3Pb^+, $EtMe_2Pb^+$, Et_2MePb^+, Et_3Pb^+, Et_2Pb^{+2} and an unknown lead-containing analyte) in the samples. Whereas, trimethyllead and an unknown lead-containing species were detected in all samples, neither Me_2Pb^{2+} nor $EtMePb^{2+}$ was present in any of the samples. The sum of the ionic alkylleads formed less than 1 % of the total lead

Table 4. Ionic alkyllead levels (as alkylbutylleads) in soft tissue of saltmarsh periwinkles from lower Chesapeake Bay.

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

i. calculated from 2 replicate injections of 3 separate determinations.
ii. no Me_2Pb^{2+} or $EtMePb^{2+}$ was detected in any of the samples.
iii. corrected for mean recoveries.
iv. M = male, F = female
v. means within a column bearing different superscripts are significantly ($p < 0.05$) different.
* detected in only two of the three replicate determinations.
ND none detected (less than 0.7 ng/g dry weight before correction for recovery).

burdens in all the samples. The fraction of total ionic alkyllead in the total lead burdens decreased with decreasing pollution stress of the sites (Table 5). In

Table 5. Total lead[a] and the total ionic alkyllead[b] levels[c] in Saltmarsh Periwinkles

Sex	Males			Females		
Site	Total Lead (ug/g)	Ionic Alkyl lead(ng/g)	Percent Burden[d]	Total Lead (ug/g)	Ionic Alkyl lead(ng/g)	Percent Burden[d]
Craney Island	07.91	75.3	0.95	04.79	41.6	0.87
CardinalPoint	15.39	60.9	0.40	10.88	65.1	0.60
Hampton South	14.03	45.5	0.32	10.88	24.2	0.22
Hampton North	11.76	30.4	0.26	09.45	18.5	0.20
Fishers Creek	14.21	29.4	0.21	10.92	17.6	0.16
Point Lookout	14.96	33.0	0.22	13.77	05.1	0.04

[a]Mean total lead, ug/g, based on 2 replicate assays.
[b]Mean total ionic alkyllead, ng/g, based on 2 replicate injections of 3 separate determinations.
[c]Burdens have been expressed on a dry weight basis.
[d]Percent Burden = Mean total ionic alkyllead/Mean total lead x 100.

terms of the burdens of total ionic alkylleads the following trend was observed: Norfolk sites (CI,CP) > Hampton sites (HN,HS) > southern Maryland sites (FC,PL).

2.3.5 Individual Ionic Alkyllead Burdens:

Individual alkyllead concentrations in these samples were very low - not detected to 8 ppb on an "as received" basis. A trend to decreasing concentrations from the right to the left side and from the top to the bottom of Table 4 is evident. Ethyllead concentrations in these samples were higher than the mixed alkylleads which, in turn, were higher than trimethyllead concentrations. An

analysis of variance indicated significant site-sex interactions for all analytes excepting Et_3Pb^+ and $EtMe_2Pb^+$, which were both site and sex dependent (Table 6).

Table 6. Analyses of variance of individual alkyllead concentrations in periwinkles with respect to site and sex.

Analyte	Site	Sex	Site-Sex[a]
	Level of Significance		
Me_3Pb^+	0.0213	0.5246	0.0043**
$EtMe_2Pb+$	0.0112*	0.0086**	0.3454
Et_2MePb+	0.0001	0.0237	0.0001**
Et_3Pb+	0.0001**	0.0302*	0.0890
Et_2Pb+2	0.0001	0.0002	0.0034**
Unknown	0.0001	0.1218	0.0061**

[a] * Site-sex interaction
** Significant at $p < 0.05$ level
** Significant at $p < 0.01$ level

Generally males were burdened with higher concentrations of alkylleads compared to females. The simple effects of sex were investigated further using a contrast analysis (Steel and Torrie, 1980). Each of the analytes was sex dependent except for trimethyllead (Table 7).

Table 7. Analysis of variance using contrast analysis for the simple effects of sex.

Analyte	Level of Significance
Me_3Pb^+	0.2535
$EtMe_2Pb+$	0.0031**
Et_2MePb+	0.0155*
Et_3Pb+	0.0016**
Et_2Pb+2	0.0001**
Unknown	0.0022**

* Significant at $p < 0.05$ level
** Significant at $p < 0.01$ level

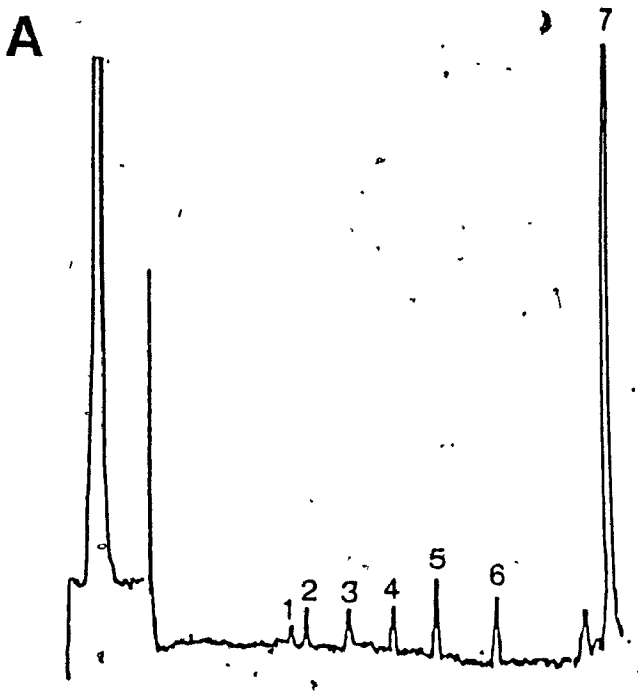
2.3.6 Unknown Ionic Alkyllead Species:

All the samples contained an unidentified lead-containing compound, which co-chromatographed with authentic Et_3MePb, under our instrumental conditions. Further experiments were undertaken to characterize this product. This analyte was not detected in either an initial hexane extract of the enzymatically digested sample homogenate (prior to complexometric extraction), or in hexane-dithizonate extracts which had been back-washed with dilute acid. However, it was observed consistently in the normal hexane-dithizonate extracts. This behaviour precludes the possibility of the unknown being an uncharged tetraalkyllead species. Identification of this product by gas chromatography - mass spectrometry (GC-MS) was not possible because of the very low levels of this analyte present in these samples. Typical chromatograms of a snail sample recorded at 217 nm and 283.3 nm, are presented in Figure 2.

2.3.7 Ethylleads in Periwinkle Samples

Ionic ethylleads formed the major fraction of the total ionic alkyllead burdens in these samples. The concentrations of diethyllead were significantly correlated ($p < 0.01$) to the levels of triethyllead (Table 8) consistent with a metabolic dealkylation and indicative of a common origin, for these two analytes.

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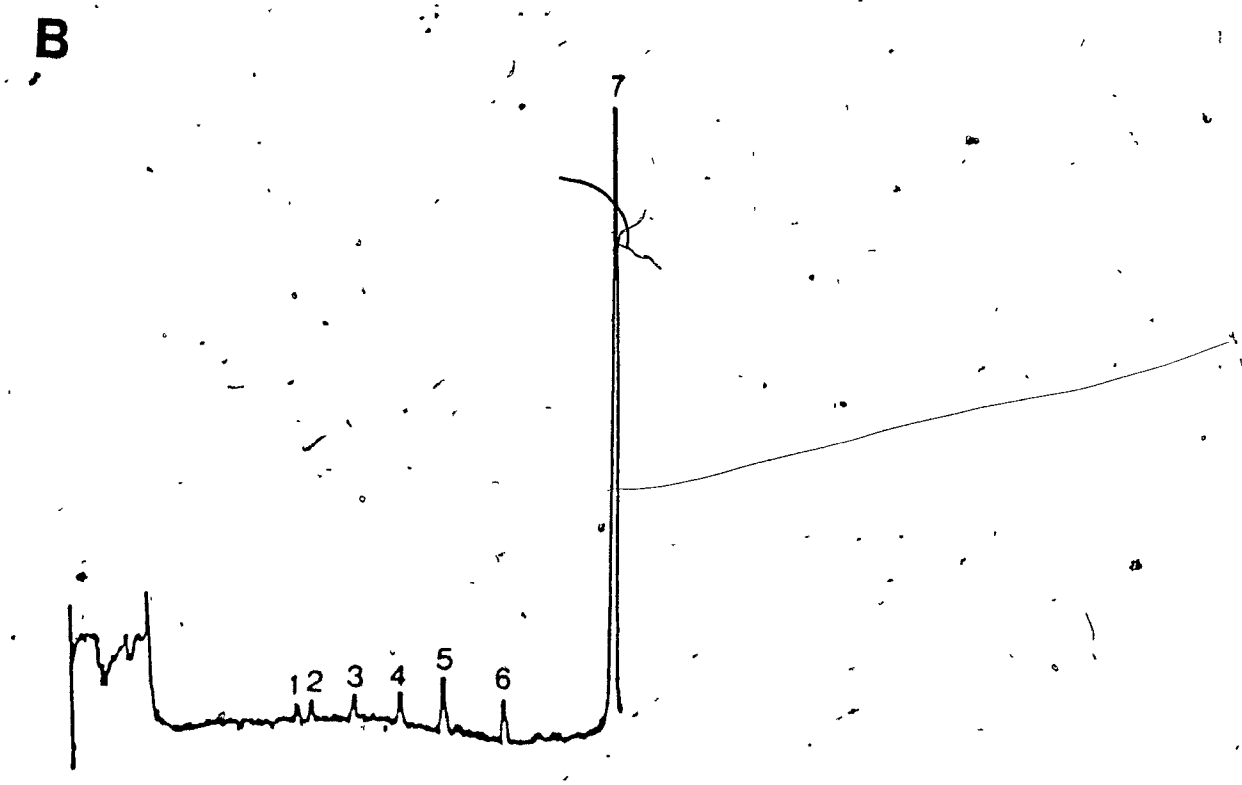
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2.3.8 Trimethyllead in Periwinkle Samples:

In an effort to detect differences in the origin of individual alkyllead species, relationships among the various analytes were studied. A simple correlation of the variables, in which the correlation is solely the result of both variables varying along with some other primary variable, can lead to spurious conclusions (Choi, 1978). In this case, since the analyte levels were significantly influenced by site, sex and their interaction (Table 6), partial correlation would prove useful in determining the relationship among the analytes. This approach allows one to correlate the levels of one analyte with levels of another, if adjustments for the influencing factors are made. With the exception of trimethyllead, all the analytes were significantly correlated ($p < 0.01$) with the total ionic alkyllead levels (Table 8). Concentrations of diethylmethyllead, triethyllead and diethyllead were also correlated to each other significantly ($p < 0.01$). Whereas ethyldimethyllead was significantly correlated to total ionic alkyllead levels ($p < 0.01$), trimethyllead lacked a statistically significant correlation ($p > 0.05$) with any other analyte or with the total ionic alkyllead levels.

Further treatment of the data was performed in which correlations were examined for analyte levels where only concentrations above the limit of detection were considered. The rationale for this approach was that the initial set of data contained several "non detected"

Table 8. Pearson's partial correlations between alkyllead concentrations of Periwinkle tissue.

For 23 degrees of freedom

Analytes	$EtMe_2Pb^+$	Et_2MePb^+	Et_3Pb^+	Et_2Pb^{+2}	Total[a]
Me_3Pb^+	0.3410	0.1288	0.3130	0.1408	0.3812
Prob > |r|	0.0953	0.5395	0.1276	0.5019	0.0601
$EtMe_2Pb^+$		0.3771	0.3160	0.3377	0.5614
Prob > |r|		0.0631	0.1237	0.0987	0.0035**
Et_2MePb^+			0.6085	0.7118	0.8664
Prob > |r|			0.0013**	0.0001**	0.0001**
Et_3Pb^+				0.5809	0.8136
Prob > |r|				0.0023**	0.0001**
Et_2Pb^{2+}					0.8561
Prob > |r|					0.0001**

[a] Total = Sum of the tissue levels of all ionic alkylleads.

** Significant at $p < 0.01$ level

values which may have influenced the analysis for correlations appreciably. Thus an analyte level for which the levels of the second analyte was unavailable, was ignored. This explains the varying degrees of freedom recorded in Table 9. Again diethyllead was significantly correlated with levels of triethyllead ($p < 0.05$) and diethylmethyllead ($p < 0.01$). In addition ethyldimethyllead was correlated significantly with diethyl- ($p < 0.05$) and highly with diethylmethyllead ($p = 0.0503$) concentrations. However, there was a more pronounced lack of correlation between the concentrations of trimethyllead and the concentrations of any other ionic alkyllead. This analysis suggests a difference in the origin of this analyte and the origin of the ethylleads.

In an attempt to clarify the relationships and check whether a true difference in origin of the analytes existed, further analysis of the data included a pooling of the mixed alkyllead concentrations (Et_2MePb^+ plus $EtMe_2Pb^+$), and of the ethyllead concentrations (Et_3Pb^+ plus Et_2Pb^{2+}). This approach enabled the use of all the individual analyte values and thus allowed consideration of all the data points. An analysis of variance (Table 10) of the pooled data showed that the ethyllead concentrations varied significantly among the different sites and between sexes but without significant site-sex interaction; combined mixed alkylleads were site but not

Table 9. Summary of the Pearson's partial correlations between alkyllead concentrations of Periwinkle tissue.

Analyte	$EtMe_2Pb^+$	Et_2MePb^+	Et_3Pb^+	Et_2Pb^{2+}
Me_3Pb^+	0.1510	0.2856	0.3130	0.0987
Prob > | r |	0.5627	0.2835	0.1458	0.7160
df	(16)	(14)	(21)	(14)
$EtMe_2Pb^+$		0.4967	0.2901	0.4584
Prob > | r |		0.0503	0.2282	0.0420*
df		(14)	(17)	(18)
Et_2MePb^+			0.5482	0.6459
Prob > | r |			0.0649	0.0021**
df			(10)	(18)
Et_3Pb^+				0.4856
Prob > | r |				0.0410*
df				(16)

df Degrees of freedom associated with each analysis
* Significant at $p < 0.05$ level
** Significant at $p < 0.01$ level

sex dependent and the interaction between the site and sex was significant in case of trimethyllead. Whereas the levels of the combined mixed alkylleads were significantly correlated ($p < 0.01$) to levels of the sum of the ethyllead salts (Table 11), the trimethyllead concentrations were not correlated significantly ($p > 0.05$) with either the combined ethyllead or with the combined mixed alkyllead levels.

The fraction of trimethyllead in the total ionic alkyllead burdens was then compared with the fraction of ethyllead and mixed alkyllead salts. A statistically significant ($p = 0.0001$) negative ($r = -0.9664$) relationship was obtained. This correlation reflects the significant correlations of individual ethylleads with the total ionic alkyllead burden, the high correlation of mixed alkylleads with total alkyllead burden and the lack of variability of trimethyllead levels among the sites. Again the lack of a common source (origin) for trimethyllead and ethyllead salts or mixed alkylleads is indicated.

2.3.9 Possible origin of Trimethyllead:

After the initial introduction of tetramethyllead in gasolines in 1960 (Grandjean and Nielsen, 1979), the use of this antiknock agent was generally restricted because of its high volatility. Whereas Canadian gasolines are known to contain only tetraethyllead as the additive (Chau

Table 10. Analyses of variance of combined alkyllead concentrations and trimethyllead concentrations with respect to site and sex.

Analyte(s)	Site	Sex	Site-Sex[a]
	Level of Significance		
Me_3Pb^+	0.0401	0.4507	0.0103*
Et_2MePb^+ & $EtMe_2Pb^+$	0.0001**	0.2819	0.1270
Et_3Pb^+ & Et_2Pb^{+2}	0.0001**	0.0029**	0.1953

[a] * Site-Sex interaction
** Significant at $p < 0.05$ level
** Significant at $p < 0.01$ level

Table 11. Pearson's partial correlations between the combined mixed alkyllead concentrations and combined ethyllead concentrations with trimethyllead levels in saltmarsh periwinkle samples.

DF = 23

Analyte(s)	Mixed Alkyls[1]	Mixed Ethyls[2]
Me_3Pb^+	0.2617	0.2653
Prob > | r |	0.2064	0.2000
Mixed Alkyls		0.6398**
Prob > | r |		0.0006**

[1] Mixed Alkyls = Sum of Et_2MePb^+ plus $EtMe_2Pb^+$
[2] Mixed Ethyls = Sum of Et_3Pb^+ plus Et_2Pb^{+2}
** Significant at $p < 0.01$ level

et al., 1976; Radziuk et al., 1979; Forsyth, 1985), American gasolines may contain methylleads or mixed alkylleads as minor components (Du Puis and Hill, 1979; Estes et al., 1982).

Interestingly, the analytical data from the present study suggests different origins for the methyl- and ethyllead compounds in the periwinkles. Whereas ethyllead concentrations in snails increased with increasing pollution stress at the collection sites, a similar trend was not observed with trimethyllead. A lack of statistically significant correlation was observed between the burdens of Me_3Pb^+ and each of the alkyllead analytes. This supports the hypothesis that the burdens of Me_3Pb^+ in these samples originated primarily from source(s) other than the demethylation of tetramethyllead in gasolines. In making these conclusions, it may be reasonable to consider that the composition of the gasoline and the nature of the additives in it in the sampling areas are homogeneous.

In addition, the fact that the burdens of Me_3Pb^+ were sex independent whereas burdens of each of the ethyllead salts were sex dependent, also indicates separate origins for the Me_3Pb^+ and the ethyllead salts. Even if tetramethyllead formed a fraction of the alkyllead additives in the gasolines, it is clear that the entry of trimethyllead from source(s) other than directly from the gasoline is predominant in these samples.

In vitro methylation of various inorganic tin and organotin compounds by microbes isolated from the Chesapeake Bay have been demonstrated by Huey *et al.* (1974), Nelson *et al.* (1973) and Hallas (1981). Later Jackson *et al.* (1982) demonstrated the methylation of tin by the pure cultures of *Penicillium* (strain 244) isolated from the Chesapeake Bay. These authors postulated that the methylstannanes present in the Chesapeake Bay may have arisen in a microbially mediated pathway. This was further supported by the observations reported by other researchers (Chau, 1980; Hallas, 1981; Guard *et al.*, 1981).

With existing chemical similarities between lead and tin, it is possible that the trimethyllead originated from an environmentally-mediated methylation of inorganic lead. Recently, Forsyth and Marshall (1986) reported significant correlation between the tissue levels of trimethyllead and the mean lake inorganic lead levels in samples of Herring gulls culled from several different colonies in Great Lakes, consistent with a methylation of inorganic lead in the environment (possibly microbially mediated). Over the past decade, researchers have reported biological (Wong *et al.*, 1975; Schmidt and Huber, 1976) and abiotic (Craig, 1980) formation of volatile tetramethyllead in aquatic media and its apparent transport into the atmosphere over the coastal flats (Harrison and Laxen, 1978).

2.3.10 Possible Origin of the Mixed Alkyllead Species:

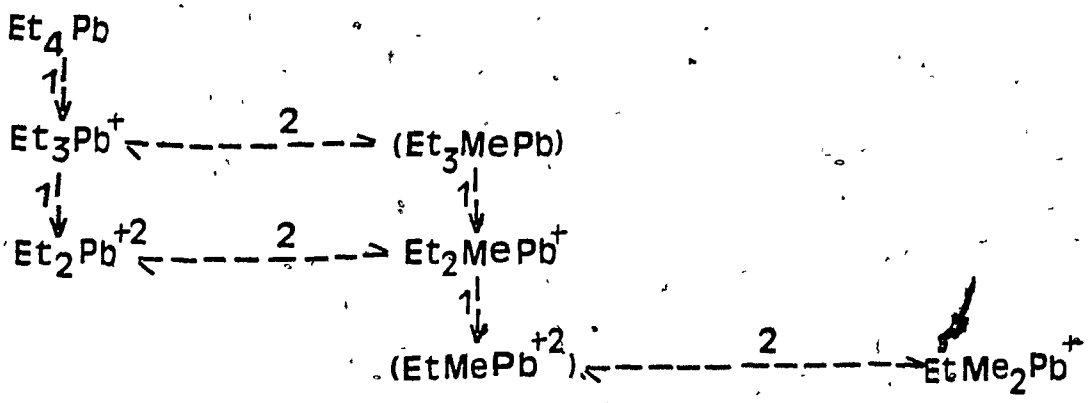
Biomethylation (presumably as a defence mechanism) has been clearly established for other trace elements [mercury (Jensen and Jernelov, 1969; Kivimae et al., 1969; Bertilsson and Neujahr, 1971; Imura, 1974; Neville and Berlin, 1974; Berman and Bartha, 1986), selenium (McConnell and Portman, 1952; Dransfield and Challenger, 1955; Byard, 1969; Palmer et al., 1969, 1970; Fleming and Alexander, 1972), arsenic (Braman and Foreback, 1973; Cox and Alexander, 1973; Wood, 1975; Shirachi et al., 1981; Rowland and Davis, 1982; Buchet and Lauwerys, 1985), and tin (Nelson et al., 1973; Huey et al., 1974; Dizikies et al., 1978; Hallas, 1981; Hallas et al., 1982)] and for the alkyl mercury (Wood et al., 1968; Landner, 1971; Tonomura et al., 1972; Rudd and Furutani, 1980), and alkyl tin species (Guard et al., 1981; Jackson et al., 1982; Rapsomanikis and Weber, 1985; Maguire, 1984).

Huey et al. (1974) observed the methylation of monomethyltin by a Pseudomonas species collected from Chesapeake Bay. Subsequently, the occurrence of mono-, di-, and trimethyltin compounds in the water of Tampa Bay (Braman and Tompkins, 1979) and Lake Michigan (Hodges, 1979) was reported. Later, Guard et al. (1981) demonstrated the methylation of trimethyltin compounds by estuarine sediments from tidal flats in San Fransisco Bay. Methylation of trimethyllead acetate to volatile

tetramethyllead has also been reported (Wong et al., 1975; Schmidt and Huber, 1976). Chau et al. (1984, 1985) speculated the methylation of ionic ethyllead salts, to be the source of ethylmethyllead toxicants to aquatic organisms. The present study shows a possibility of methylation of ethyllead salts as a source of Et_2MePb^+ and $EtMe_2Pb^+$ to the periwinkles.

High levels of correlation between burdens of Et_3Pb^+, Et_2Pb^{+2}, Et_2MePb^+ and $EtMe_2Pb^+$ were demonstrated. Yet the levels of correlation with either ethyllead salt burden decreased as the number of methyl groups on the mixed alkyllead increased (Tables 8 and 9). This pattern is not inconsistent with an environmentally mediated methylation of ethyllead salts as suggested in Figure 3. In this proposed decomposition scheme the gasoline additive Et_4Pb is degraded sequentially to Et_3Pb^+ and to Et_2Pb^{+2}. Methylation of the latter product results in Et_2MePb^+ whereas methylation of the former would result in Et_3MePb, a volatile tetraalkyllead. De-ethylation of Et_2MePb^+ would result in $EtMePb^{+2}$ (which has not been detected in these samples). However, a second methylation would result in $EtMe_2Pb^+$. This scheme would predict that levels of correlation of the burdens of individual alkyllead species should decrease as the number of intervening transformations increases.

Figure 3. Schematic summary of proposed alkylation - dealkylation of ethyllead salts as interpreted from the periwinkle samples. 1, De-ethylation; 2, Methylation.


Et4Pb
1
Et3Pb+
2
(Et3MePb)
1
1
Et2Pb+2
2
Et2MePb+
1
(EtMePb+2)
2
EtMe2Pb+

2.3.11 Summary:

1. Six ionic alkyllead analytes (Et_3Pb^+, Et_2Pb^{+2}, Et_2MePb^+, $EtMe_2Pb^+$, Me_3Pb^+, and an unknown lead containing peak) were quantitated in the tissue of Saltmarsh Periwinkles collected from six separate sites in the lower Chesapeake Bay, U.S.A.

2. The total ionic alkyllead burdens constituted less than 1% of the total lead levels, and the burdens of individual alkylleads, except that of trimethyllead, were sex dependent.

3. Ethyllead salts constituted a major fraction of the total ionic alkyllead burden. Their concentration varied significantly among the sites.

4. The lack of a statistically significant correlation of trimethyllead burdens with any of the other alkyllead analytes, suggests a difference in origin of methylleads in the samples.

5. The identical behaviour of the mixed alkyllead analytes with that of the ethylleads, and not with methylleads, together with the positive and significant correlation of their burdens with ethyllead salts, is consistent with a methylation of ethylleads.

Overall, these results suggest environmental methylation of lead.

3. TOXICITY OF ETHYLLEAD SALTS TO JAPANESE QUAIL

3.1 Introduction

Lead poisoning has been recognized as a factor in wildlife mortality since the turn of the century. Birds are constantly exposed to sublethal quantities of lead in their diet, resulting in measurable quantities of this toxicant in their bones, soft tissues and eggs. Alkylleads were recognized as the toxic principle responsible for the death and sickness of 2,400 birds in the Mersey estuary, U.K., during 1979-1981 (Osborn et al., 1983). Urban pigeons had been found to contain up to 0.3 ppm of lead in the form of trialkyls in their tissues (Johnson et al., 1982).

The toxicity of trialkylleads have been reported by Osborn et al. (1983). In this study starlings, which had received 2.8 or 28 mg Et_3PbCl/kg body weight, daily for up to eleven days, displayed overt signs of severe toxicity. For the higher dosage, the study was terminated before the planned time because of the severe toxic effects of triethyllead. Chronic and subchronic toxicity of tri- and diethyllead to avian species have not been reported previously.

The present work involved the study of the pharmacokinetics, metabolism, and accumulation of triethyllead and diethyllead cations in a suitable avian indicator species. In addition, the transfer of the toxicants into egg was also monitored throughout the

experiment and the possibility of biomethylation of host-ingested inorganic lead investigated.

The Japanese quail (Coturnix coturnix japonica) was chosen for this study. This species is considered to be a much better model for avian wildlife than is the domestic hen (Edens and Garlich, 1983). Based on experimental results with Japanese quail and comparisons made with mammalian studies (Morgan et al., 1975; Stone et al., 1977, 1978), the quail was suggested to be an appropriate model for the study of plumbism. Additionally it represented a species that laid eggs throughout the year.

3.2 Materials and Methods

3.2.1 Chemicals and Reagents

Alkyllead chlorides and butylates were prepared as described by Forsyth and Marshall (1983). Lead nitrate was of ACS reagent grade (Allied Chemicals, New Jersey) as were the other chemicals used in the study.

3.2.2 Feeding Trials

Six to eight month old female Japanese quail purchased from a local breeder (Faisanbec Inc, St. Jacques le mineur, Quebec) were used in this study. The birds were housed in groups of seven in chick batteries (68 x 72 x 20 cm) in a room maintained at a temperature of approximately 24°C and 14L:10D photoperiodic conditions. The birds were provided feed (Turkey purina chow) and water ad libitum. During a two-week acclimatization period, the egg laying

and weight change patterns were established. Two separate feeding trials were conducted. In the first trial, treatments consisted of amending the water with either 0 or 250 ppm of $Pb(NO_3)_2$ or with 25 ppm of Et_2PbCl_2. In the second trial, treatments consisted of amending the water with 0, 0.25 or 2.5 ppm of Et_3PbCl. The amended water was provided afresh every day, and the previous day's consumption was noted. Each trial was conducted for a period of eight weeks. Eggs were collected daily from the control and from each of the treated groups, pooled (within each group) after removal from their shells, and frozen to await analysis. At the termination of the trial, the quail were sacrificed by cardiac puncture, and the soft organs (brain, liver, muscle and kidney) were excised and weighed. Whereas the liver and muscle samples from all birds were analysed separately, the brain samples of each of the treatments were pooled for analysis. The kidney samples were pooled for analysis in all cases, except in 2.5 ppm triethyllead feeding group, in which the kidney of the individual birds was analysed separately.

3.2.3 Analyses for Alkylleads

Pooled eggs from each treatment and individual tissues were analysed in triplicate. Three separate samples from each tissue or pool were placed in a 50 mL Nalgene centrifuge tube and enzymatically hydrolysed (section 2.2.3.1) for 24 h and the crude hydrolysates were extracted

with hexane:dithizone (section 2.2.3.2.2). The concentrated extracts were butylated (section 2.2.3.3) and analysed by GC-QT-AAS as described in section 2.2.3.4. Each sample was quantified twice by comparison with the external standards containing Me_3PbBu, Me_2PbBu_2, Et_3PbBu and Et_2PbBu_2. Mixed methylethyllead compounds were identified by predicted Kovats' retention indices (Forsyth and Marshall, 1986) and from retention time for each of the transalkylated products. The quantitation was achieved by comparison with a similar analyte for which standards were available. Thus the quantitation of Et_2MePb^+ and $EtMePb^{+2}$ were performed by comparison with Et_3Pb^+ and Et_2Pb^{2+} respectively.

3.2.4 Recovery Experiments

Recoveries of ionic alkylleads were calculated by spiking the appropriate tissue from a control bird (which has not previously been exposed to ethylleads) with a standard mixture of Me_3PbCl, Me_2PbCl_2, Et_3PbCl and Et_2PbCl_2 at a level of 5-6 ppb (as Pb). Three separate recovery experiments were conducted as described in sections 2.2.3.2 to 2.2.3.5. The percent recovery was determined by dividing the mean peak area of the recovered butylate by the mean peak area of the butylated spike solution diluted to the expected concentration (assuming 100 % recovery) for each of the analytes.

3.2.5 Statistical Analysis

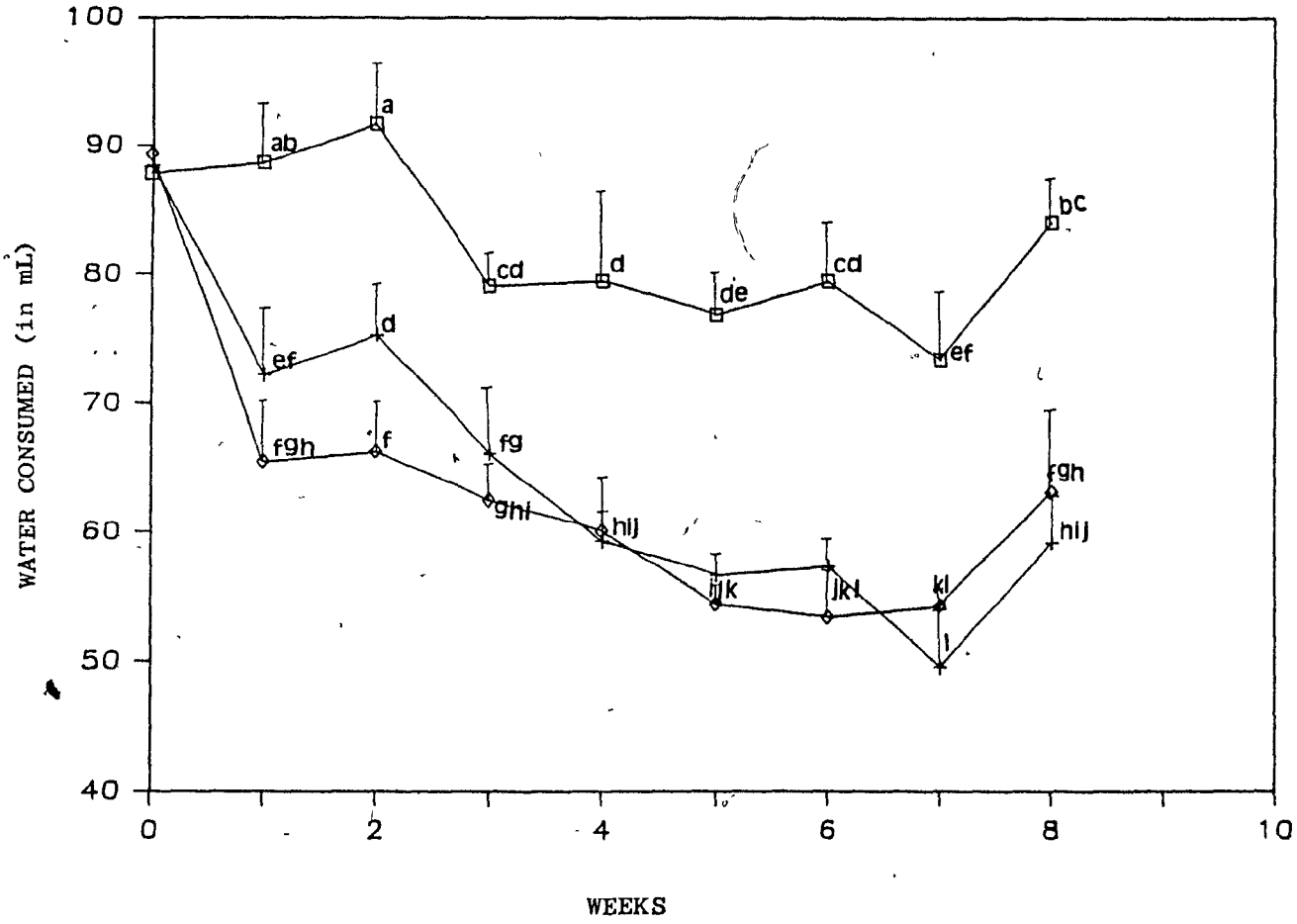
The data were analysed using the method of covariance, taking into account the dependent variables associated with each of the independent variables. When the F ratio indicated a significant interaction, the treatment means were compared using Duncan's New Multiple Range Test (Steel and Torrie, 1980).

3.3 Results and Discussion

3.3.1 Effect on Water Consumption

Treated birds consumed significantly less water ($p < 0.05$) than did the control birds in Trial 1 (Figure 4), however there was no significant difference ($p > 0.05$) in mean water consumed between the group exposed to lead nitrate and the group which had been exposed to diethyllead dichloride. Mean water consumption was the average daily consumption for the previous seven days. In Trial 2, the mean water consumption by the birds on 250 ppb Et_3PbCl feeding was not significantly different ($p > 0.05$) from the mean consumption of controls (Figure 5). The birds on 2.5 ppm triethyllead chloride feeding consumed significantly less water ($p < 0.05$) than the other two groups of birds. The average daily intake of the toxicant per bird in treated groups were as follows: 61.2 ± 11.5, 5.6 ± 0.9 mg/kg body weight, 425 ± 36, 61 ± 5 ug/kg body weight for the $Pb(NO_3)_2$, diethyllead dichloride, high and low triethyllead chloride treatments respectively.

Figure 4. Mean water consumed (daily average for each week) by Japanese quail which were given drinking water amended with 0.0 (□), 25 ppm Et_2PbCl_2 (◇), or 250 ppm $Pb(NO_3)_2$ (+).


100
90
80
70
60
50
40
0
2
4
6
8
10
WATER CONSUMED (in mL)
WEEKS
a
ab
cd
d
de
cd
bc
ef
d
ef
fgh
f
fg
ghi
hij
ijk
jkl
kl
l
fgh
hij

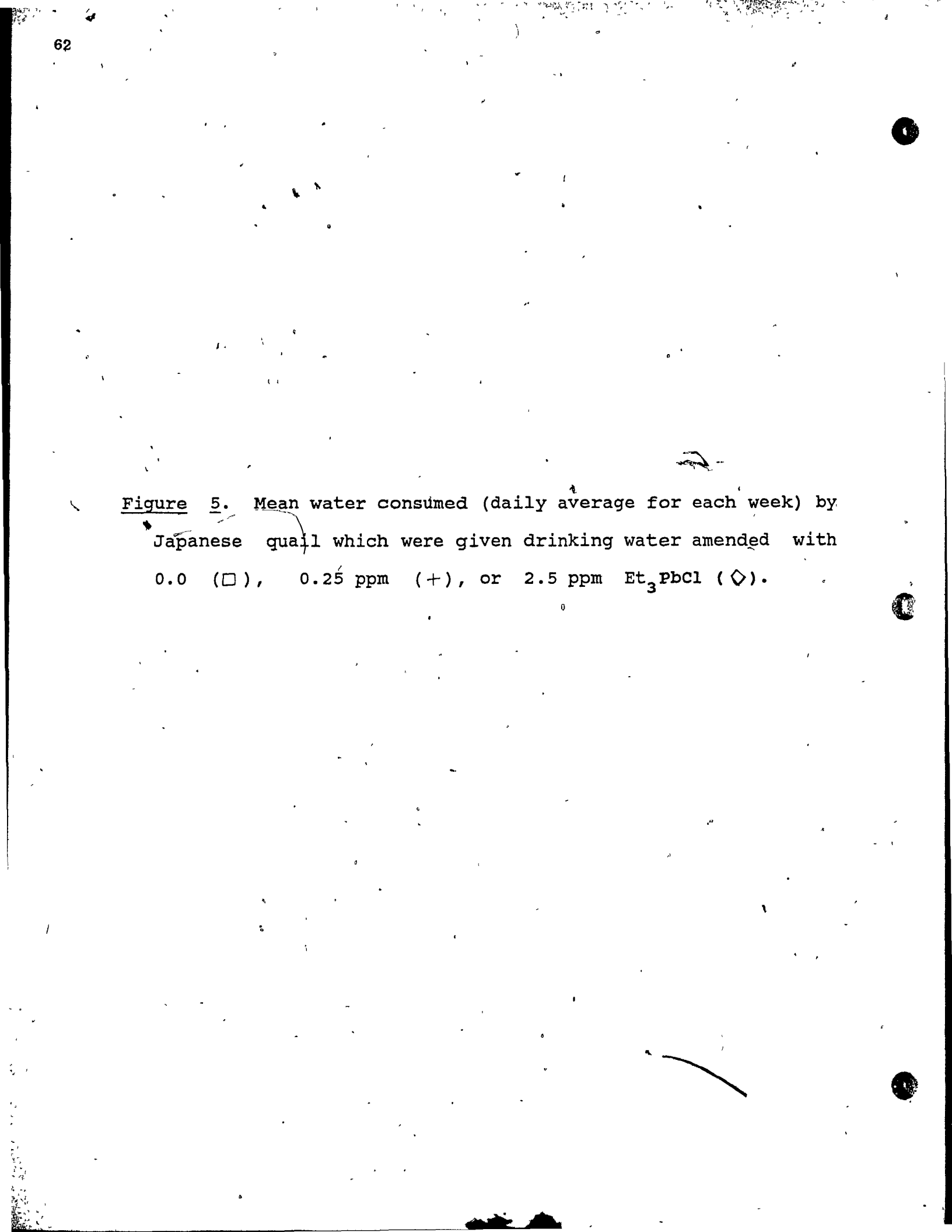
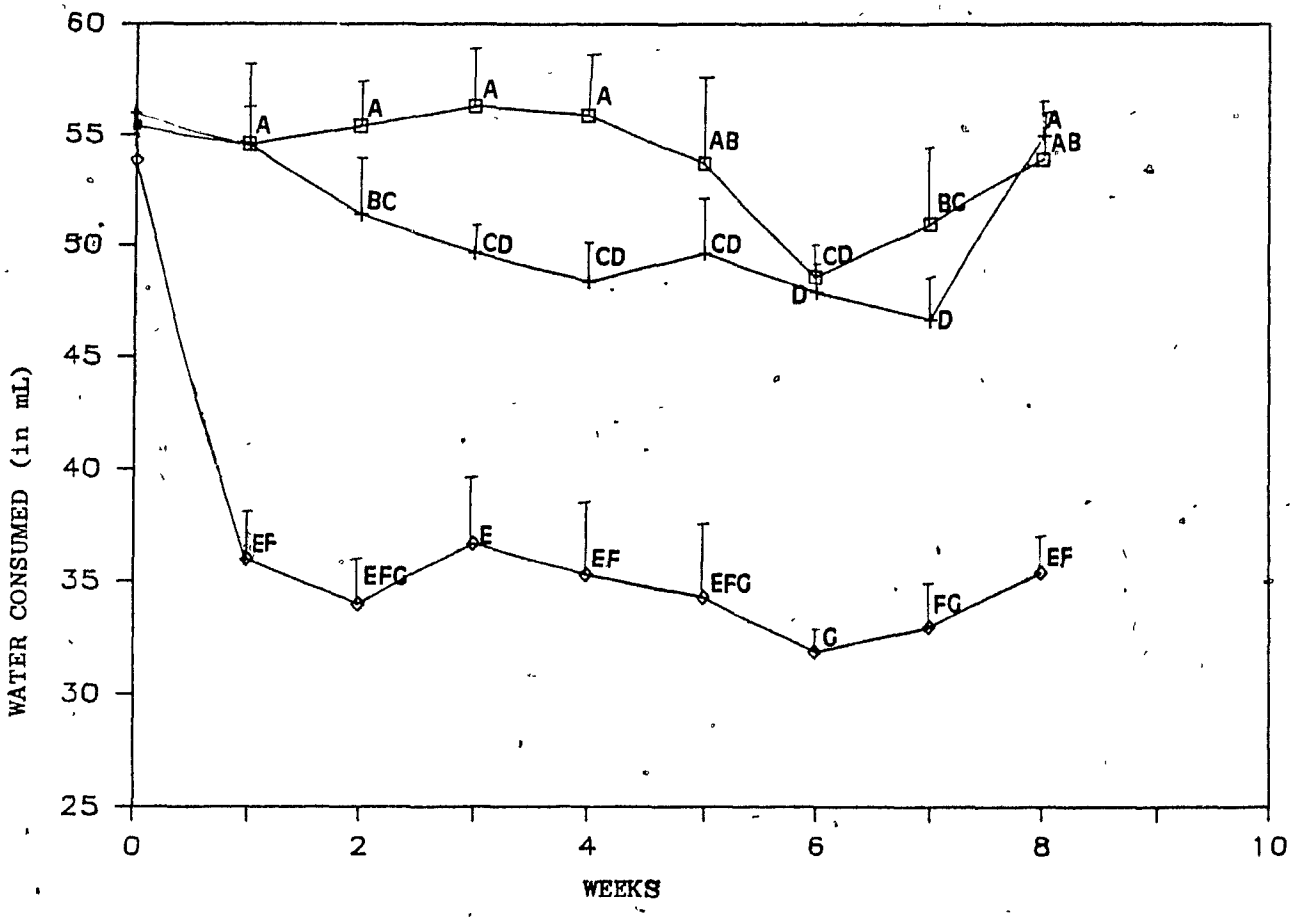


Figure 5. Mean water consumed (daily average for each week) by Japanese quail which were given drinking water amended with 0.0 (□), 0.25 ppm (+), or 2.5 ppm Et_3PbCl (◇).


60
55
50
45
40
35
30
25
0
2
4
6
8
10
WATER CONSUMED (in mL)
WEEKS
A
A
A
A
AB
A
AB
BC
CD
CD
CD
CD
D
BC
D
EF
EFG
E
EF
EFG
G
FG
EF

3.3.2 Effect on Body Weight

Exposure of quail to lead nitrate or to diethyllead dichloride did not result in a statistically significant ($p > 0.05$) change in body weight relative to the controls (Figure 6). The birds on both 0.25 and 2.5 ppm of triethyllead, lost considerable weight over the period of the trial (Figure 7), but not significantly ($p = 0.0503$).

3.3.3 Effect on Organ Weights

The only significant difference between the treated and control groups in terms of the relative organ weight to the body weight was observed for kidney (Table 12). A similar observation has been reported by Stone and Soares (1974) for Japanese quail, which were fed with diets containing a minimum of 500 ppm of inorganic lead for 32 days. It is interesting to note that an exposure of quail to 25 ppm of diethyllead dichloride (daily intake level 6-7 ppm) has resulted in a similar effect.

Table 12. Means of relative organ-weight to body weight ratio ($\times 10^3$) for treated and control birds.

Treatment	Relative kidney weight	Relative liver weight	Relative brain weight
Control	5.0 ± 0.9*	36.1 ± 8.5	3.1 ± 0.4
$Pb(NO_3)_2$ (250 ppb)	7.9 ± 1.0*	37.6 ± 9.5	2.6 ± 0.4
Et_2PbCl_2 (25 ppm)	8.3 ± 3.8*	32.9 ± 4.3	2.8 ± 0.1
Control	7.3 ± 0.9	32.1 ± 4.8	4.0 ± 0.3
Et_3PbCl (250 ppb)	7.0 ± 0.7	29.3 ± 4.8	3.7 ± 0.4
Et_3PbCl (2.5 ppm)	8.3 ± 1.2	30.7 ± 3.3	3.9 ± 0.2

* Significantly different from mean control value ($p < 0.01$)

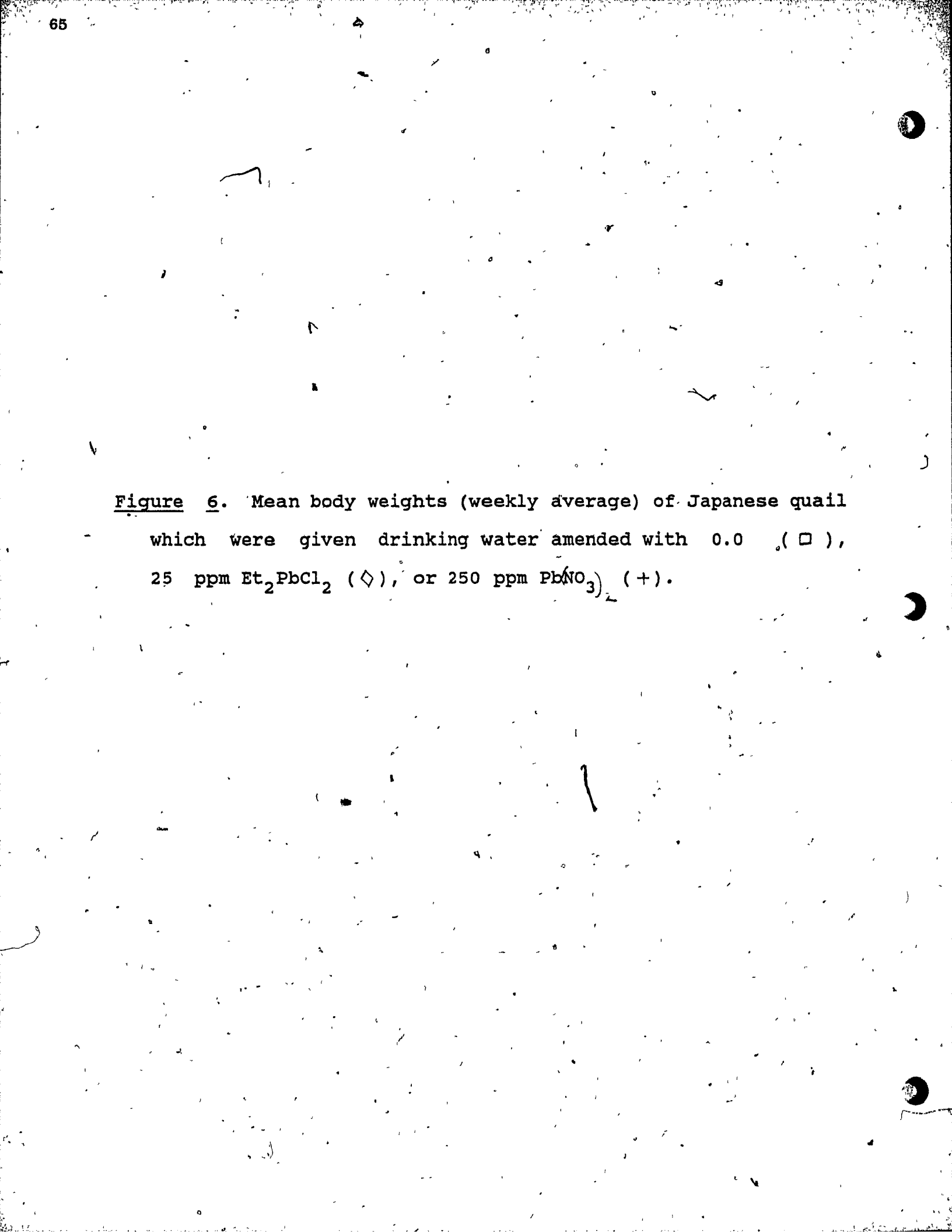


Figure 6. Mean body weights (weekly average) of Japanese quail which were given drinking water amended with 0.0 (□), 25 ppm Et_2PbCl_2 (◇), or 250 ppm $Pb(NO_3)_2$ (+).

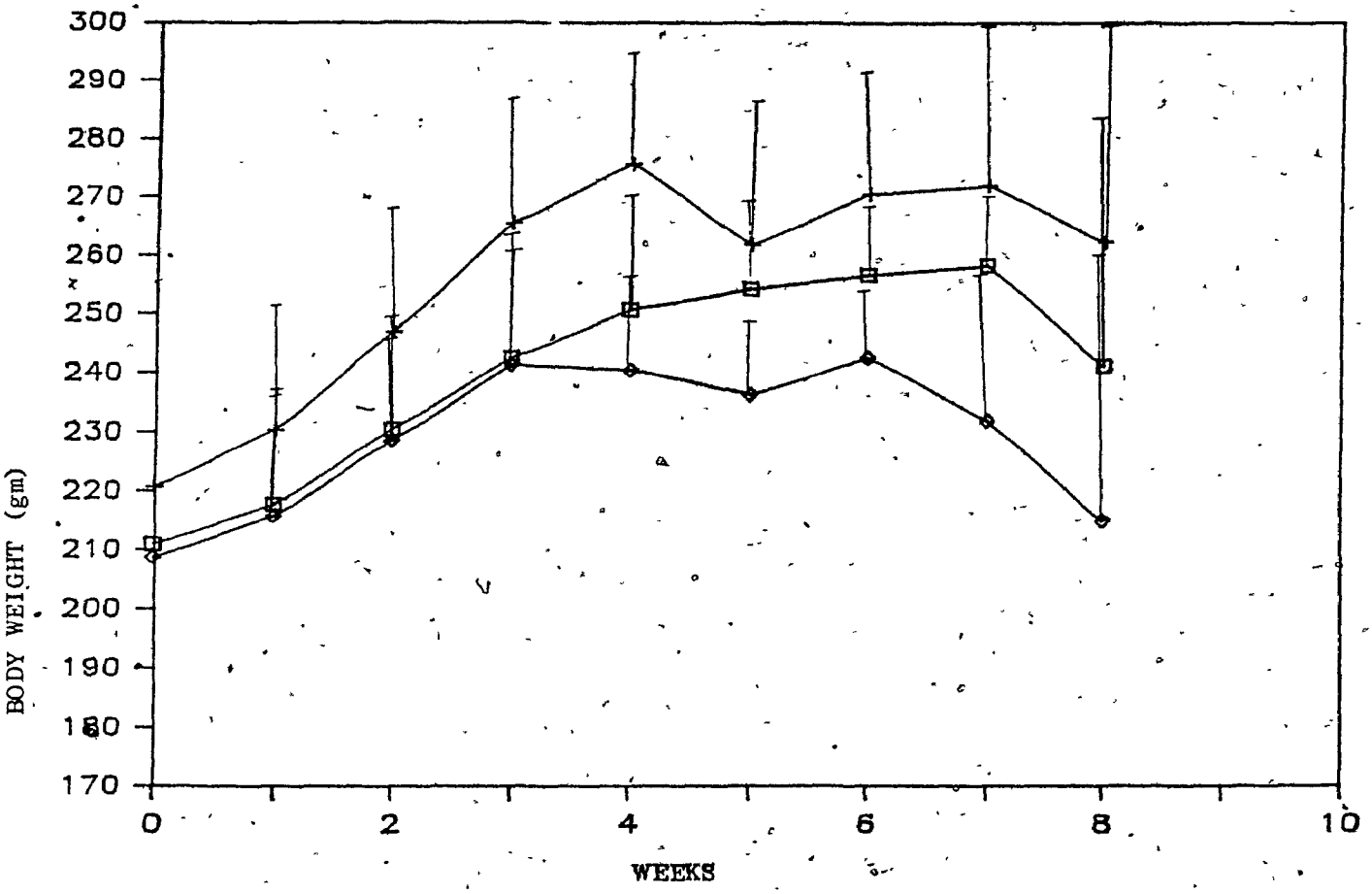
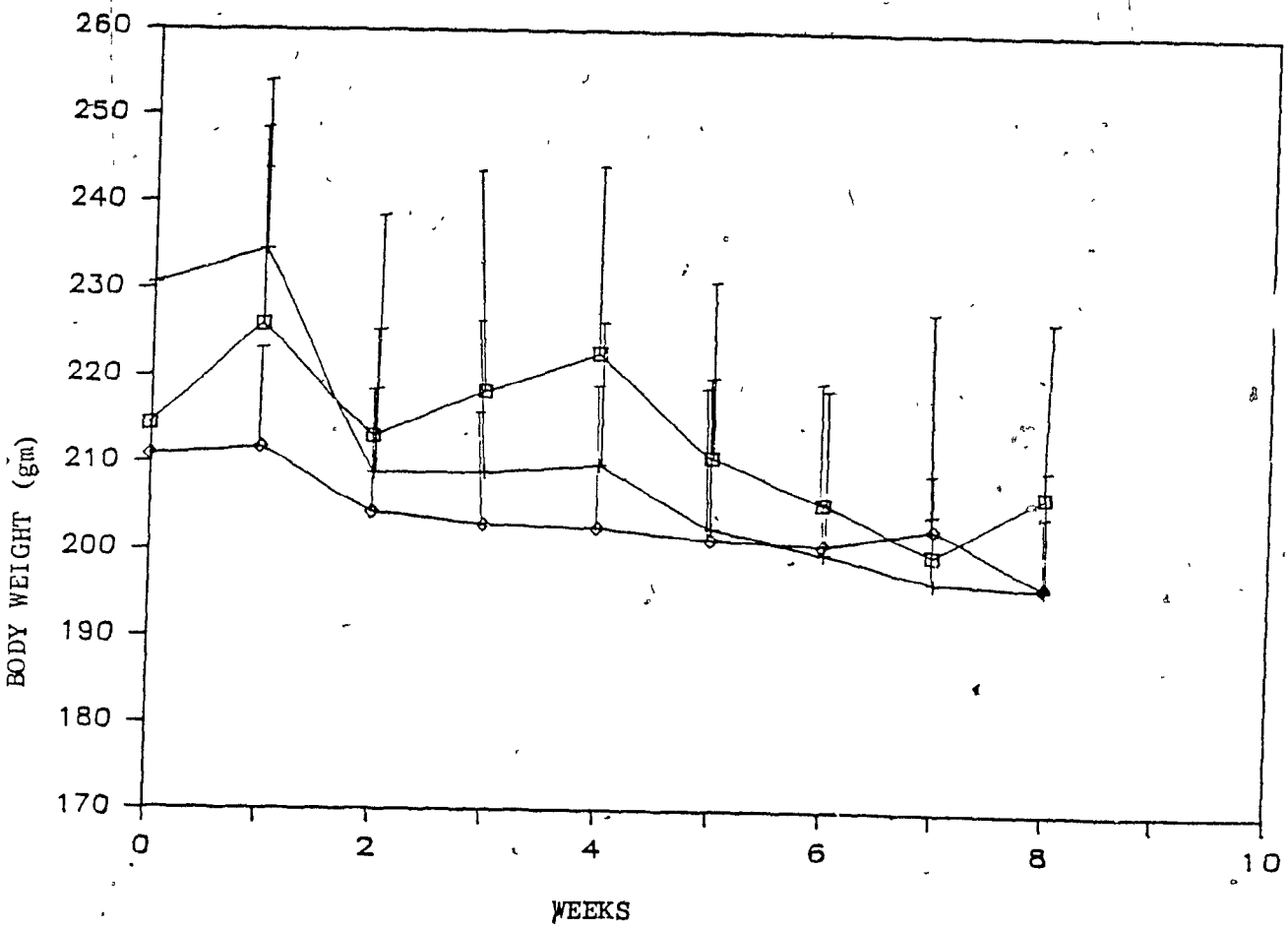

300
290
280
270
260
250
240
230
220
210
200
190
180
170
BODY WEIGHT (gm)
0
2
4
6
8
10
WEEKS

Figure 7. Mean body weights (weekly average) of Japanese quail which were given drinking water amended with 0.0 (□), 0.25 ppm (+), or 2.5 ppm Et_3PbCl (◇).


260
250
240
230
220
210
200
190
180
170
BODY WEIGHT (gm)
0
2
4
6
8
10
WEEKS

3.3.4 Transfer of Toxicants into Egg in Trial 1

Exposure of birds to water amended with Et_2PbCl_2 resulted in a prompt transfer of the toxicant into egg. The level of diethyllead in the daily egg pools is presented in Figure 8 as a function of the day of the trial. Each data point represents the average of three replicate determinations of separate samples from the pool of eggs which had been collected each day. Error bars have been included to provide a measure of reproducibility of the analyses. Eggs laid 24 h after the initial exposure of birds to Et_2PbCl_2 were burdened with diethyllead cation concentration of 9.8 $\pm$ 0.8 ppb. The level of diethyllead in the eggs was directly proportional to the toxicant during the first 33 days (Figure 8) reaching a maximum of 1.04 $\pm$ 0.11 ppm in egg. During this period, egg burdens of Et_2Pb^{+2} were positively ($r = 0.9400$) and significantly ($p = 0.0001$) correlated to the cumulative intake of the toxicant. Although a steady-state condition was not reached during the trial (in terms of amounts of diethyllead cation in egg), it was observed that an average 0.14 $\pm$ 0.024 % of the ingested toxicant was transferred into the eggs. On day 27 and subsequent days triethyllead cation was also detected in the egg pools (Figure 9). The level of this toxicant increased with time, however it remained at less than 3.2 % of the Et_2Pb^{2+} burden in egg. After day 50, traces of Me_2Pb^{2+} were detected and on subsequent days Me_3Pb^{+} was also detected.

Figure 8. Levels of Et_2Pb^{2+} cation in egg of birds which were given drinking water amended with 25 ppm of Et_2PbCl_2, as a function of day of the trial.

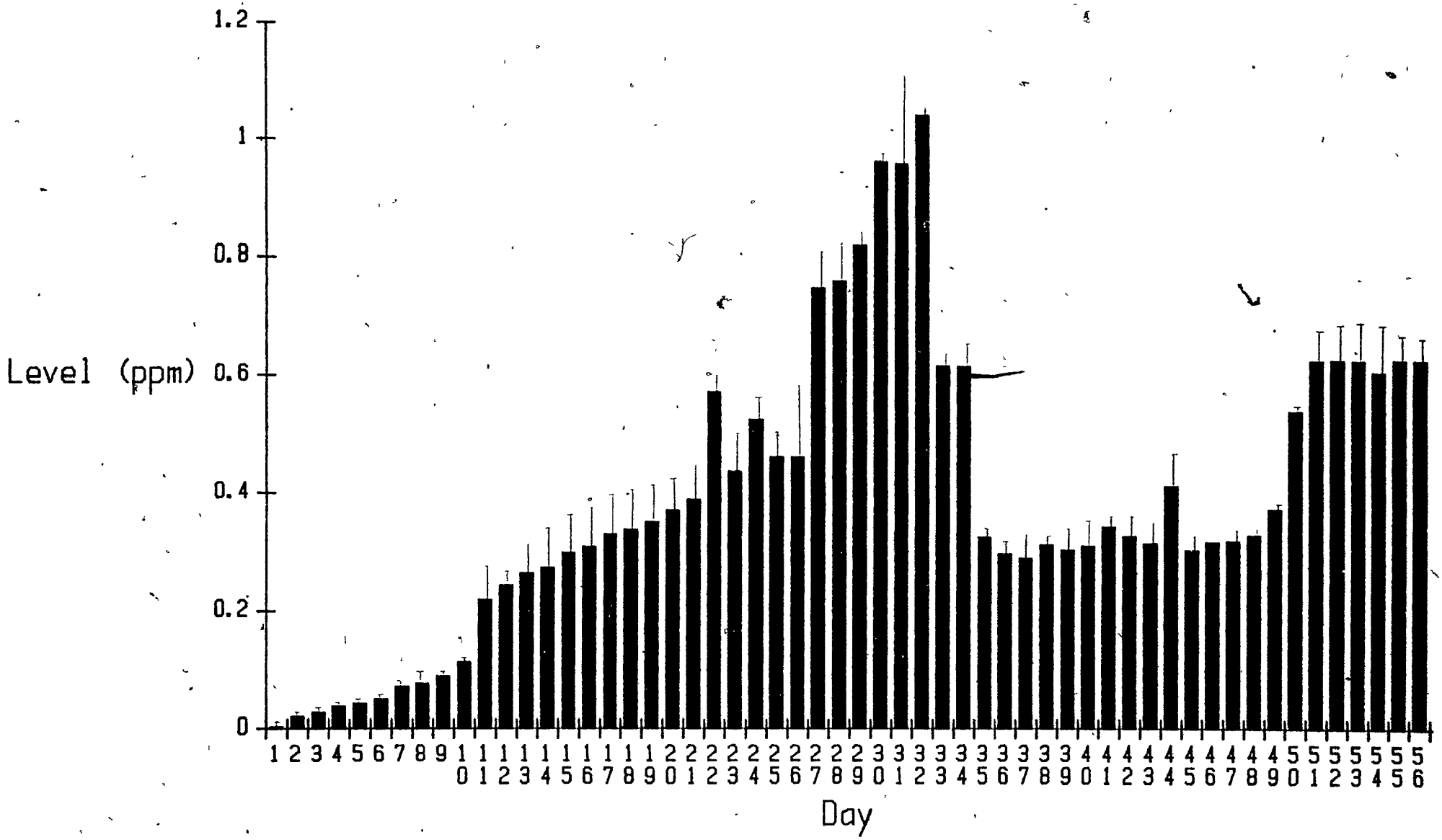
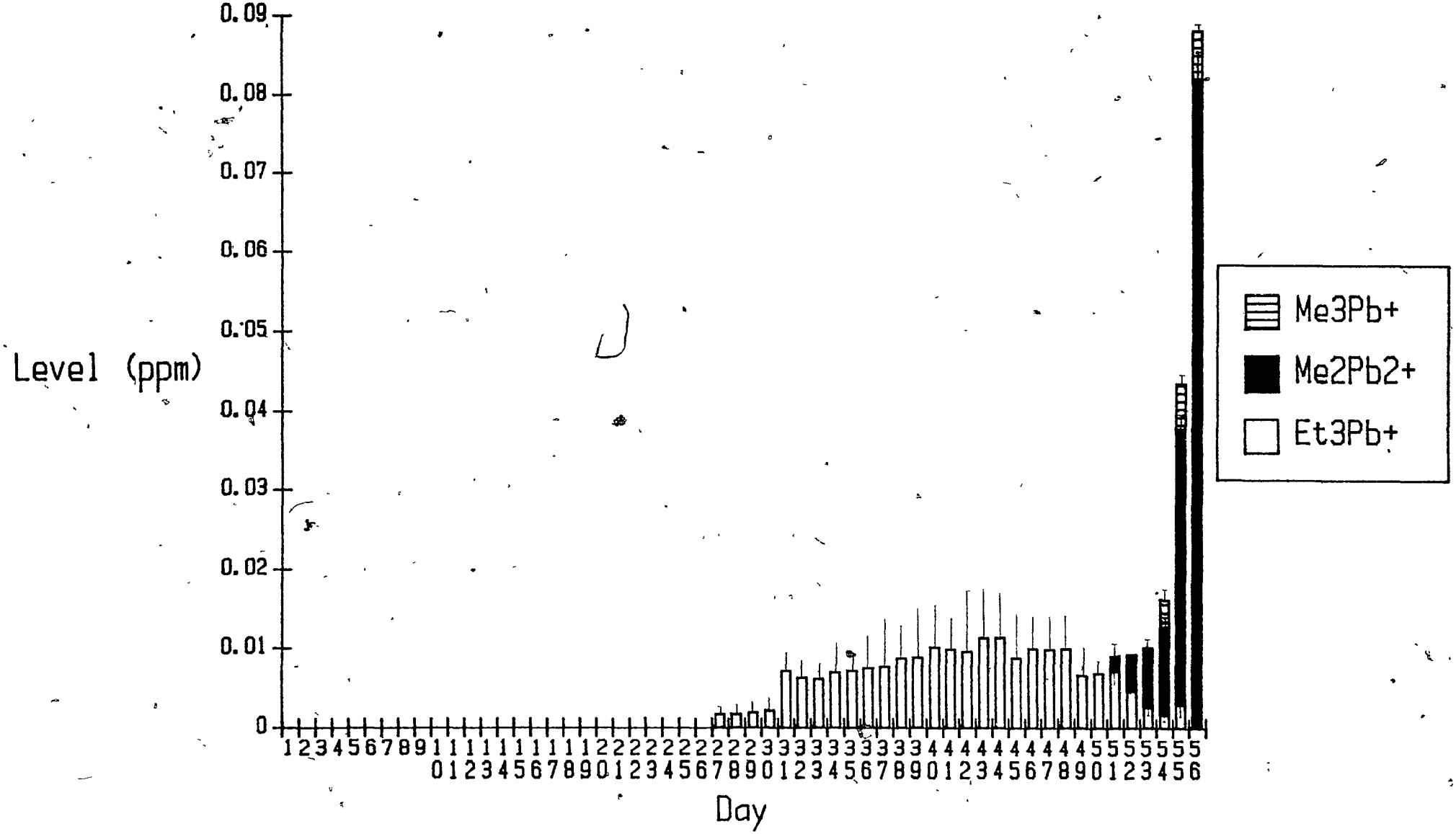

Level (ppm)
1.2
1
0.8
0.6
0.4
0.2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56
Day

Figure 9. Levels of ionic alkylleads (other than Et_2Pb^{2+}) in egg of birds which were given drinking water amended with 25 ppm of Et_2PbCl_2, as a function of day of the trial. (a) Et_3Pb^+ (□); (b) Me_2Pb^{2+} (■); (c) Me_3Pb^+ (目).


0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Level (ppm)
1 2 3 4 5 6 7 8 9 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5
0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6
Day
Me3Pb+
Me2Pb2+
Et3Pb+

These contaminants always remained at trace levels relative to the diethyllead burdens in the eggs (On day 56, the concentration of trimethyllead in egg was 6.5 ± 1.6 ppb and the concentration of dimethyllead was 82.1 ± 6.6 ppb). Since the methyllead salts were not detected in any of the soft tissues at sacrifice, it is hypothesised that the methylation occurs within the egg production machinery and once formed it is not circulated throughout the body. Representative chromatograms of the egg pools from different days are presented in Figure 10.

3.3.5 Tissue Residues of Alkylleads in Trial 1

The levels of alkyllead analytes detected in the various tissues have been corrected for the percentage recovery, for each analyte in each of the tissues (Table 13).

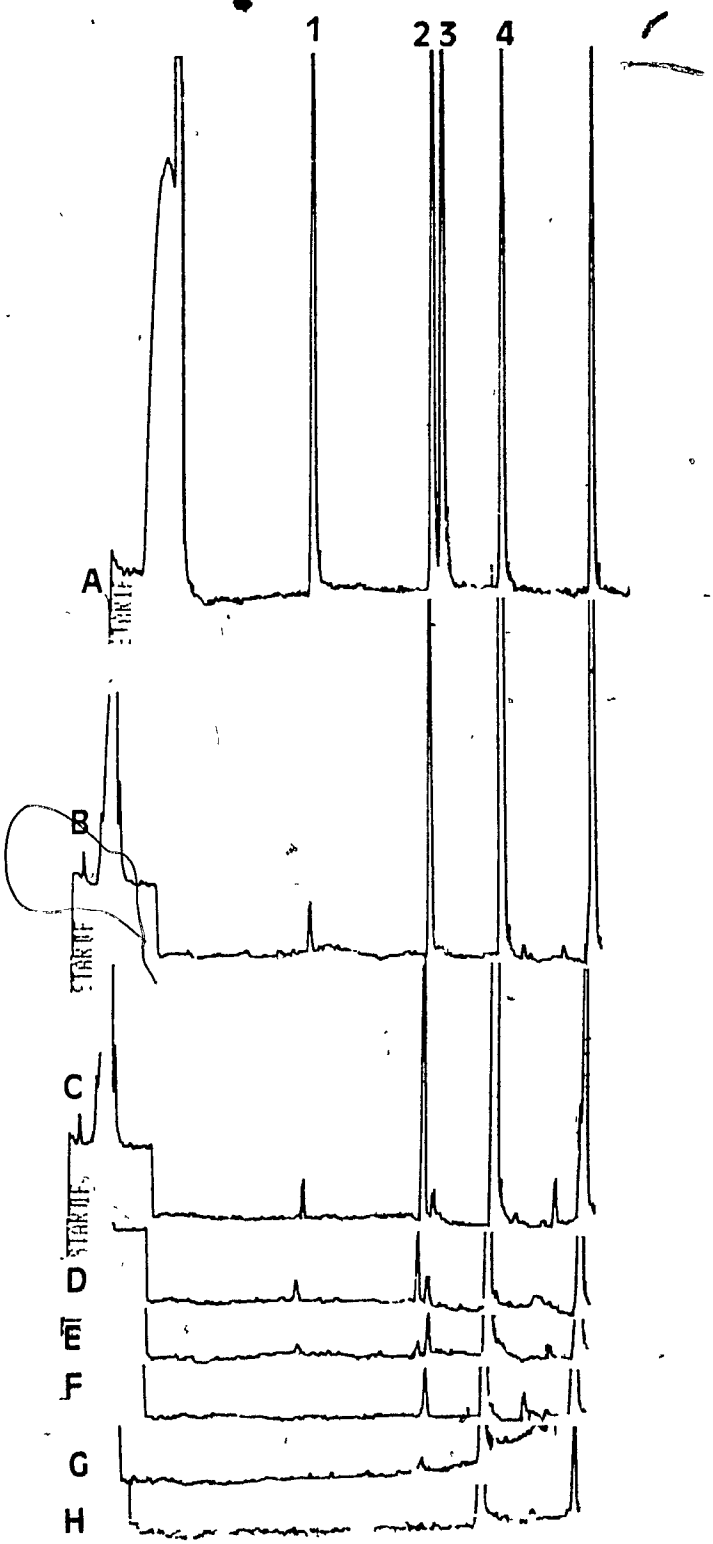
Table 13. Mean recoveries of ionic alkyllead compounds from quail tissues[a].

Tissues	Mean[b] analyte percent recovery ± 1 SD			
	Me_3Pb^+	Me_2Pb^{+2}	Et_3Pb^+	Et_2Pb^{+2}
Egg	92 ± 3	75 ± 6	78 ± 8	89 ± 7
Liver	83 ± 7	18 ± 6	71 ± 7	66 ± 9
Kidney	84 ± 6	22 ± 7	74 ± 5	74 ± 6
Brain[c]	72 ± 2	17 ± 4	61 ± 2	50 ± 2

[a] spiked at 5-6 ppb (as Pb), with a standard mixture of Et_3PbCl, Et_2PbCl_2, Me_3PbCl and Me_2PbCl_2.
[b] N=3 replicate determinations.
[c] calculated from three replicate injections of a single determination.

Analyses of tissues (liver, kidney, brain and breast muscle) of the birds indicated that these tissues were

Figure 10. Representative GC-AAS chromatograms of egg homogenates from quail given drinking water amended with Et_2PbCl_2, for various periods of time. (A) Ionic alkyllead Standards, 1, Me_3Pb^+; 2, Me_2Pb^{2+}; 3, Et_3Pb^+, and 4. Et_2Pb^{2+} respectively. (B) day 56, (C) day 54 , (D) day 53, (E) day 50, (F) day 29, (G) day 22, and (H) day 4.


1
2 3
4
A
B
C
D
E
F
G
H

burdened with higher levels of triethyllead than diethyllead (Table 14).

Table 14. Mean organolead cation concentrations (ppb) in soft tissues from diethyllead dichloride feeding to Japanese quail.

Tissue	Analyte Mean[a] ± 1 S.D. Et_3Pb^+	Et_2Pb2^+	Analyte Range Et_3Pb^+	Et_2Pb^{2+}
Brain Pool[b]	16.2 ± 0.05	5.1 ± 0.01		
Kidney Pool[c]	161.6 ± 32.3	52.6 ± 14.6		
Liver[d]	49.6 ± 44.9	22.0 ± 7.8	14.9 - 150.1	11.9-31.9
Muscle[d]	15.3 ± 19.8	18.2 ± 4.8	2.3 - 55.7	9.2-27.1
	Et_2MePb^+	$EtMePb^{2+}$		
Liver[f]	0.6 ± 0.3	1.4 ± 0.4		
Kidney[f]	1.5 ± 0.3	1.9 ± 0.6		

[a] corrected for recoveries. [b] three replicate injections of one pooled sample. [c] two replicate injections of 3 separate samples. [d] two replicate injections of 3 separate samples from each individual. [f] three replicate injections of a single pooled sample.

This is surprising because the birds had been treated with diethyllead. Whereas a metabolic dealkylation has been reported for several mammalian species a metabolic conversion of dialkyl- to trialkyllead has not been reported previously. Thus diethyllead was the major analyte present in egg, and triethyllead formed the major burden in the tissues of the birds exposed to diethyllead. The levels of triethyllead were significantly correlated to the levels of diethyllead in liver and kidney of the treated quails ($r = 0.6346$, $p = 0.0148$). Clearly a metabolic toxification mechanism is operative in quail. It is interesting to note that the trialkylleads formed the

major fraction of the total alkyllead burdens in urban pigeons (Johnson et al., 1982), Herring gulls (Forsyth and Marshall, 1986) and in dead and sick birds collected from Mersey estuary during 1979-1981 (Bull et al., 1983). The proportion of tri- to diethyllead was much higher in the soft tissues than in eggs. Surprisingly, the metabolised toxicant (Et_3Pb^+) was not transferred into egg as efficiently as was the parent compound (Et_2Pb^{2+}). Concentrated samples of liver and kidney also contained low levels of mixed methylethylleads (Table 14). The trace levels of these toxicants show that methylation of ionic ethyllead salts is operative but is not a major metabolic process in quail.

The results of the inorganic lead trial, in which lead nitrate was added at 250 ppm to distilled water, were less spectacular. No alkylleads were detected in the soft tissues or eggs of treated quail. This indicated that at a daily intake level of 60-70 ppm for 2 months, an alkylation of inorganic lead was not observed, and thus methylation of host ingested inorganic lead is not a detectable metabolic process in quail.

3.3.6 Transfer of Toxicants into Egg in Trial 2

As with the diethyllead feeding trial, alkylleads were transferred promptly into egg during these studies as well. Both triethyllead (the parent toxicant) and diethyllead (its degradation product) were detected in egg samples of treated birds throughout the trial. The burdens of

triethyllead in egg increased gradually during the experimental period. The total ionic ethyllead burdens of egg constituted 0.77 ± 0.5 %, and 0.48 ± 0.2 % of the daily intake of triethyllead in 2.5 and 0.25 ppm triethyllead-feeding groups respectively. Levels of tri- and diethyllead cations in egg samples over the period of the trial are presented in Figures 11 and 12, for 0.25 ppm and 2.5 ppm Et_3PbCl feeding groups respectively. Each bar represents the average of three separate determinations and error bars have been included to provide an estimate of the reproducibility of the analyses. The concentration of Et_3Pb^+ into egg was significantly correlated with the cumulative intake of toxicant (in 2.5 ppm Et_3PbCl feeding birds $r = 0.9495$, $p = 0.0001$, and in birds on 0.25 ppm Et_3PbCl feeding $r = 0.6745$, $p = 0.0212$). Similarly the diethyllead levels in egg were significantly correlated to the daily intake of the toxicant (in the 2.5 ppm Et_3PbCl feeding group $r = 0.3073$, $p = 0.0212$; in 0.25 ppm Et_3PbCl feeding group $r = 0.3987$, $p = 0.0023$). During the latter half of the trial, eggs were burdened with higher concentrations of triethyllead than of diethyllead, in both treatments. But in the low dose group, the levels of Et_2Pb^{2+} relative to triethyllead seemed to be higher (Figure 11) than in the high dose group (Figure 12). Also the levels of triethyllead were significantly correlated ($r = 0.6981$, $p = 0.0001$) to the levels of diethyllead, over the period of the trial.

Figure 11. Concentrations of tri- and diethyllead cation in egg samples of quail which were given drinking water amended with 0.25 ppm of Et_3PbCl, as a function of day of the trial. (1) Et_3Pb^+ (■); (2) Et_2Pb^{2+} (□).

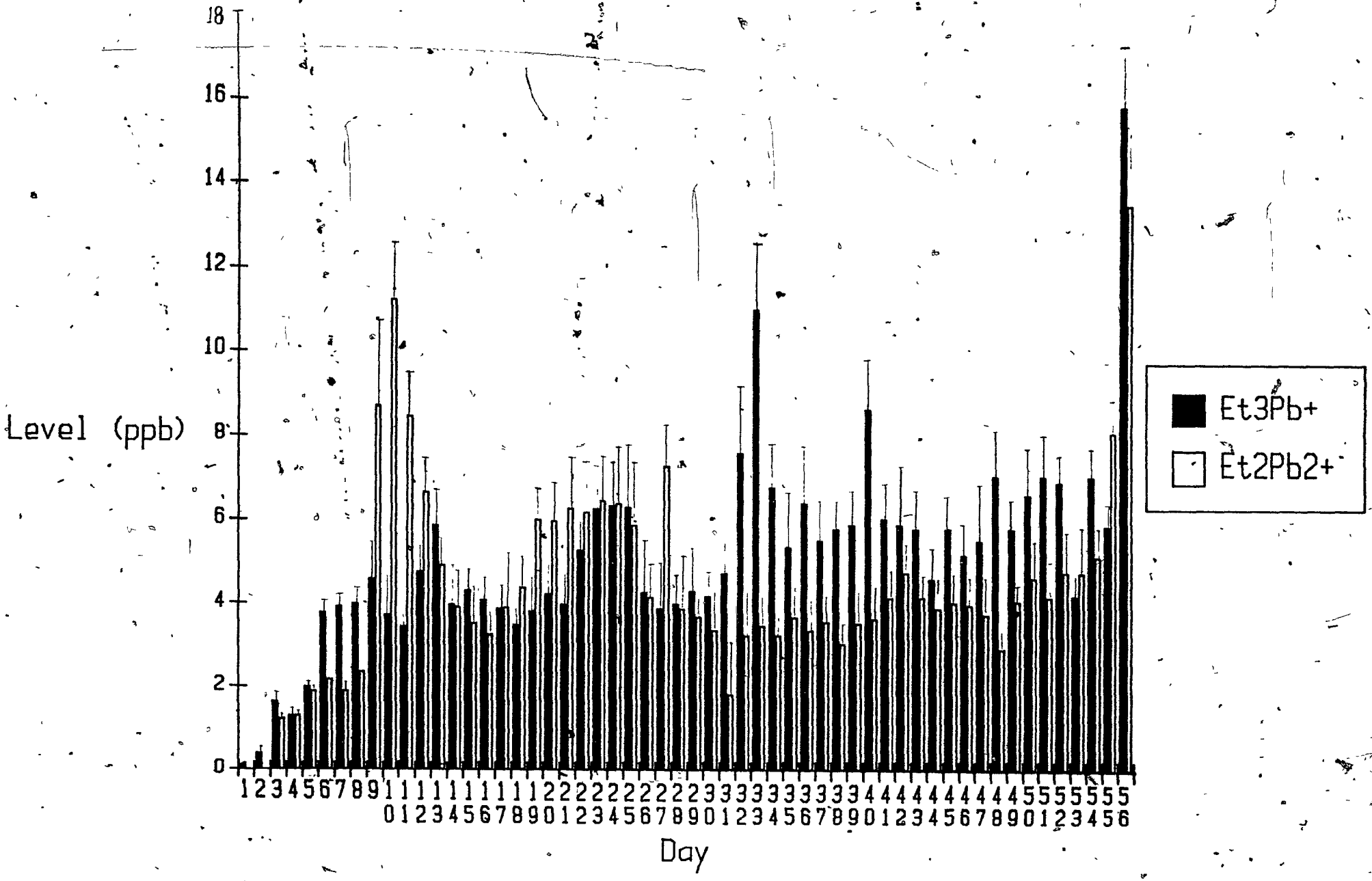
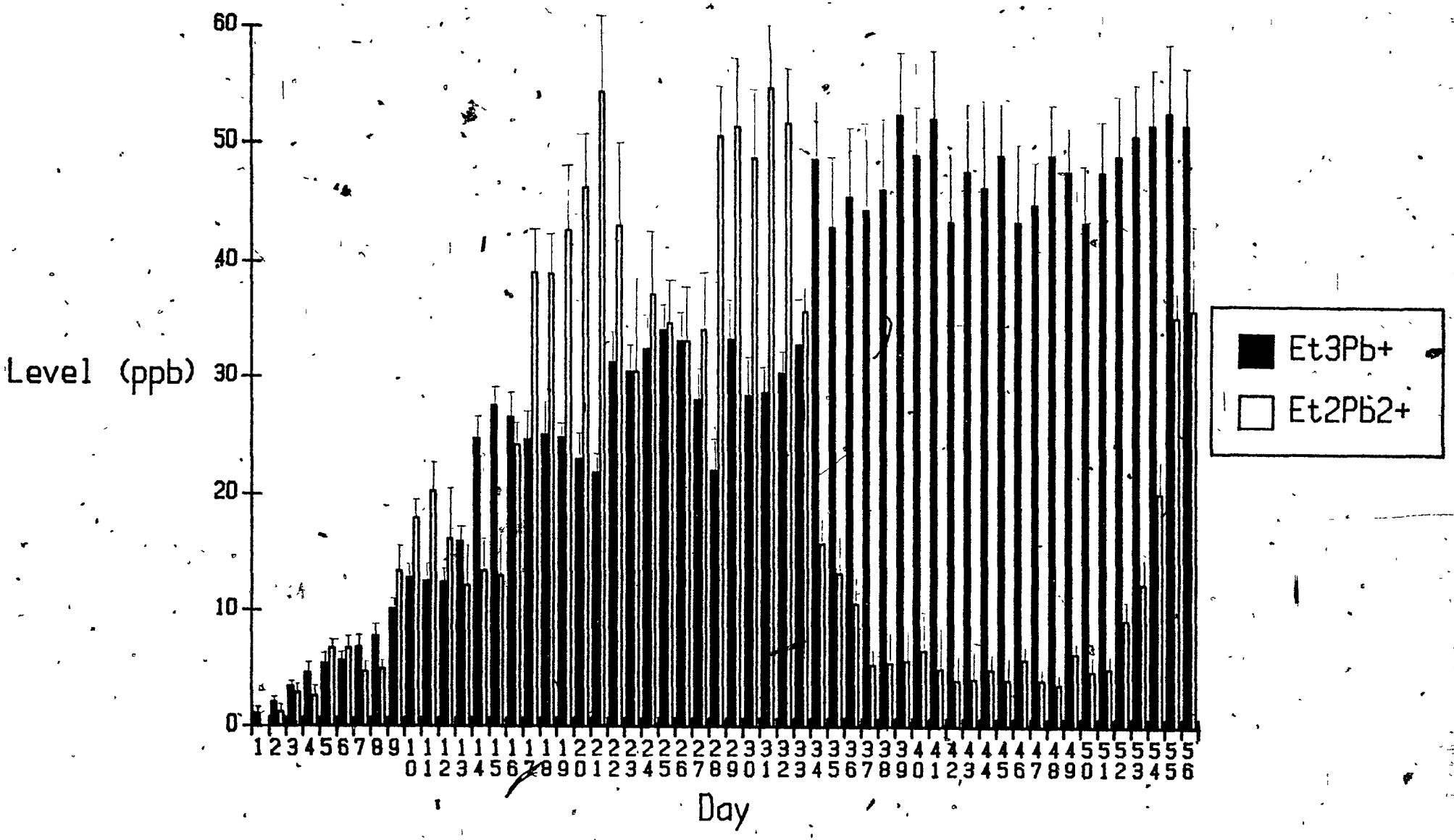

Level (ppb)
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5
0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6
Day
Et3Pb+
Et2Pb2+

Figure 12. Concentrations of tri- and diethyllead cation in the egg of quail given drinking water amended with 2.5 ppm of Et_3PbCl, as a function of day of the trial. (1) Et_3Pb^+ (■); (2) Et_2Pb^{2+} (□).


Level (ppb)
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5
0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6
Day
Et3Pb+
Et2Pb2+

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3.3.7 Tissue Residues of Alkylleads in Trial 2

The analyses of tissues (liver, kidney, brain and muscle) of treated birds from the triethyllead chloride feeding trial demonstrated higher levels of triethyllead relative to diethyllead (Table 15). Diethyllead was ubiquitous in the tissues of the treated birds. Overall, the tissue levels of triethyllead and diethyllead were significantly correlated with each other, in both treatment groups ($r = 0.9049$, $p = 0.0001$) consistent with a metabolic dealkylation. Dealkylation of tetraethyllead to triethyllead in mammals has been demonstrated (Cremer, 1958, 1959; Stevens et al., 1960; Bolanowska, 1968). The principal site for this metabolic activity is the liver microsomal fraction (Cremer, 1958, 1959; Aldridge et al., 1962; Casida et al., 1971), and the microsomal monooxygenase systems have been identified as a mediator of this process (Casida et al., 1971; Kimmel et al., 1977). Based on studies with microsomal preparations, Casida et al. (1971) demonstrated a sequential dealkylation of tetraalkyltins and proposed a similar mechanism for metabolism of tetraethylleads in mammals. The microsomal fractions in liver cells of avian species are comparitively less active than in mammals (Tucker et al., 1973). Nicholson et al. (1979) demonstrated that the activity of certain liver enzymes in immature and adult male quail was very low, compared to females. The

Table 15. Alkyllead cation concentrations in soft tissues of Japanese quail provided with drinking water amended with 0.25 or 2.5 ppm triethyllead chloride.

Tissue and treatment	Analyte Mean[a] ± 1 S.D. Et_3Pb^+ (ug/g)	Analyte Mean[a] ± 1 S.D. Et_2Pb^{2+} (ng/g)	Analyte Range Et_3Pb^+ (ug/g)	Analyte Range Et_2Pb^{2+} (ng/g)
Brain[b]				
250 ppb	0.06 ± 0.007	2.4 ± 0.4		
2.5 ppm	0.45 ± 0.009	23.1 ± 1.1		
Kidney[c]				
250 ppb	0.30 ± 0.1	31.3 ± 14.3	0.42 - 0.53	11.0 - 48.0
2.5 ppm	1.83 ± 0.6	239.8 ± 13.3	1.06 - 2.96	107.2 - 418.2
Liver[d]				
250 ppb	0.29 ± 0.1	21.3 ± 17.9	0.20 - 0.34	11.0 - 48.0
2.5 ppm	1.11 ± 0.03	70.5 ± 34.9	0.66 - 2.33	48.8 - 153.8
Muscle[d]				
250 ppb	0.08 ± 0.03	6.1 ± 2.9	0.03 - 0.13	2.3 - 9.7
2.5 ppm	0.34 ± 0.1	21.0 ± 7.4	0.27 - 0.61	15.0 - 38.6

[a] corrected for recoveries. [b] three replicate injections of one pooled sample. [c] two replicate injections of each individual kidney sample separately. [d] two replicate injections of three separate samples from each individual.

efficiency of dealkylation in both male and immature birds may be lower than in females (the subjects in the present study).

Additionally significant correlations among the ionic ethyllead levels in muscles and livers of the individual birds in both the treated groups were identified (Tables 16, 17).

Table 16. Statistical correlations between ionic ethyllead concentrations of liver and muscles from quail which had been provided with water amended with 250 ppb Triethyllead chloride.

Tissue & Analyte	Muscle Et_2Pb^{+2}	Liver Et_3Pb^{+}	Liver Et_2Pb^{+2}
Muscle Et_3Pb^{+}	0.8703	0.8669	0.7945
Prob > l r l	0.0023**	0.0025**	0.0105*
Muscle Et_2Pb^{2+}		0.8596	0.8720
Prob > l r l		0.0030**	0.0022**
Liver Et_3Pb^{+}			0.9396
Prob > l r l			0.0002**

* significant at < 0:05 level. ** significant at < 0.01

The ubiquity of diethyllead in tissues of triethyllead-treated birds and its significant correlation with the levels of triethyllead in the various body tissues is consistent with a recirculation or redistribution of the dealkylated metabolite among the body tissues. Interestingly, the relative proportion of diethyllead to the triethyllead cation was virtually constant (0.074 $\pm$ 0.03 %) in all soft tissues of both treated groups.

Table 17. Statistical correlations between ionic alkyllead concentrations in liver and muscles of quail which had been provided water amended with 2.5 ppm triethyllead chloride.

Tissue and Analyte	Muscle Et_2Pb^{2+}	Liver Et_3Pb^{+}	Liver Et_2Pb^{2+}	Liver Et_2MePb^{+}	Liver $EtMePb^{2+}$
Muscle Et_3Pb^{+}	0.9530	0.9796	0.9777	0.5326	0.8328
Prob > |r|	0.0003**	0.0001**	0.0001**	0.1741	0.0103*
Muscle Et_2Pb^{2+}		0.9054	0.9885	0.5165	0.8073
Prob > |r|		0.0020**	0.0001**	0.1900	0.0154*
Liver Et_3Pb^{+}			0.9481	0.6049	0.8443
Prob > |r|			0.0003**	0.1121	0.0084**
Liver Et_2Pb^{2+}				0.5180	0.8191
Prob > |r|				0.1885	0.0129*
Liver Et_2MePb^{+}					0.8645
Prob > |r|					0.0056**

* significant at < 0.05 level. ** significant at < 0.01 level.

All the tissues of treated birds from the 2.5 ppm triethyllead feeding were burdened with trace levels of methylethyllead analytes (Table 18). Whereas monoethylmethyllead and diethylmethyllead analytes were

Table 18. Mixed alkyllead concentrations (as alkylbutylleads) in soft tissues[a] from quail which had been provided water amended with 2.5 ppm triethyllead chloride.

Tissue	Analyte Mean[b] ± S.D.		Analyte Range	
	Et_2MePb^+ (ng/g)	$EtMePb^{2+}$ (ng/g)	Et_2MePb^+ (ng/g)	$EtMePb^{2+}$ (ng/g)
Brain[c]	3.15 ± 0.4			
Liver[d]	4.47 ± 2.7	6.30 ± 5.6	nd - 8.2	nd - 18.5
Kidney[e]	5.84 ± 1.8	7.22 ± 5.5	3.8 - 8.5	2.9 - 19.2

[a] none detected in muscle. [b] corrected for recoveries. [c] three replicate injections of one pooled sample. [d] two replicate injections of three separate samples from each individual. [e] two replicate injections of each individual kidney sample analysed separately.

detected in the tissue samples, $EtMe_2Pb^+$ was not detected. The levels of these toxicants were highly correlated to the levels of the ethylleads (Table 18,19), showing a common origin, presumably a metabolic methylation of ethyllead

Table 19. Correlations between ionic alkyllead concentrations in kidney from quail which had been provided water amended with 2.5 ppm triethyllead chloride.

Analyte	Et_2Pb^{2+}	Et_2MePb^+	$EtMePb^{2+}$
Et_3Pb^+	0.8585	0.9086	0.9123
Prob > 1 r 1	0.0064**	0.0018**	0.0016**
Et_2Pb^{2+}		0.8924	0.7640
Prob > 1 r 1		0.0029**	0.0273*
Et_2MePb^+			0.8902
Prob > 1 r 1			0.0030**

* significant at < 0.05 level.
** significant at < 0.01 level.

salts in this species. The levels of these analytes are probably not toxicologically significant, relative to the concentration and toxicity of triethyllead cation in the tissues.

The fact that the levels of mixed alkylleads remained trace, even at these levels of intake of ethyllead salts indicates the absence of an efficient biomethylation mechanism in avian species. The mixed alkyllead burdens reported in tissue of Mallard ducks and Herring gulls (Forsyth, 1985), might well have resulted from the ingestion of these toxicants rather than from a methylation of ingested ethyllead salt. This is again indicative of an environmentally-mediated methylation of ethyllead salts, in regions where only ethyllead gasoline is in use.

Another interesting observation, is the ratio of triethyllead to diethyllead in tissue of the treated birds. The ratio was much higher in tissue of quail exposed to triethyllead than in the ones exposed to diethyllead. The ratio of di- to triethyllead was 0.35 in kidney and 0.44 in liver of Et_2PbCl_2-treated quail (Trial 1). The ratios (Et_2Pb^{2+}/Et_3Pb^{+}) in the liver and kidney of Herring gulls were 0.31 ± 0.10 and 0.35 ± 0.24 respectively (calculated from, Forsyth and Marshall, 1986). This is more characteristic of the (Et_2Pb^{2+}/Et_3Pb^{+}) ratio in liver and kidney of quail which were exposed to Et_2PbCl_2, and is indicative of the exposure of gulls to Et_2PbCl_2 rather than Et_3PbCl, as the major contaminant.

3.3.8 Summary

1. The transfer of ionic ethylleads into egg has been demonstrated by feeding the adult quail with diethyllead dichloride and triethyllead chloride. Soft tissues have been identified to be highly suitable as indicators for the exposure of adults to ionic ethyllead toxicants.

2. A metabolic conversion of diethyllead to the more toxic triethyllead has been demonstrated in Japanese quail. Although the efficiency of this conversion was low relative to the quantity of Et_2PbCl_2 ingested, it does clearly represent a metabolic toxification mechanism.

3. Metabolic dealkyation of tri- to diethyllead has been demonstrated in this indicator species.

4. Methylation of ionic ethylleads is not a major metabolic process and the levels of mixed alkyllead analytes in avian tissues (environmental samples) would appear to have resulted from the intake of these toxicants from source(s) other than the methylation of the ingested ethyllead salts by the avian species themselves.

5. Biological methylation of ingested inorganic lead is not a detectable process in quail.

4. SUGGESTIONS FOR FUTURE WORK

1. It is recommended that further studies be directed at elucidating the mechanism of conversion of di- to triethyllead in biological systems. It would be interesting to further note whether such a conversion takes place with methyllead salts too, and in other aquatic species and mammalian systems.

2. The toxicity of the mixed methylethyllead analytes to various biological species should be determined, and their mode of entry into the environment should also be determined in places where tetraethyllead is exclusively used as the gasoline antiknock additive.

3. Since Penicillium (strain 244) has been shown to methylate organic and inorganic forms of tin and mercury (especially in Chesapeake Bay), it may be worthwhile to pursue further-experimentation with the microbial cultures, isolated from Chesapeake Bay, to find out about their capacity to methylate inorganic lead and ionic ethyllead salts.

4. There is a need to delineate the processes of alkylation - dealkylation in the terresterial and aquatic compartments of the ecosystem. Once the major processes occurring under each of these environmental conditions are identified, understanding the environmental fate and transformation of alkyllead toxicants, would be much easier.

5. APPENDICES

Appendix A. Calculation for alkylbutyllead levels.

Example. Calculation for the triethyllead levels (as butylleads) in the female samples of periwinkles from Hampton North site.

The average of area counts for each of the three separate determinations were calculated as 21832, 17712, and 18988. These area counts correspond to 0.201, 0.165, and 0.177 ng of triethylbutyllead. These were calculated from the concentration and area-response of the known alkylbutyllead standards that were run periodically. The area counts observed in the blank were used for the determination of the background levels, which were subtracted from the total levels in the samples.

The total amount of Et_3BuPb was calculated by including the two factors - the amount injected (20 uL) and the sample volume (1200 uL).

$$\text{Total } Et_3BuPb = \begin{aligned} &0.201 \times (1200/20) = 12.06 \\ &0.165 \times (1200/20) = 9.90 \\ &0.177 \times (1200/20) = 10.62 \end{aligned}$$

Taking into account the weight of the sample matrix (2.5 g) and the mean percent recovery of the triethyllead from the periwinkle tissues (72 %),

$$Et_3BuPb\ (ng/g) = \begin{aligned} &12.06/(2.5 \times 0.72) = 6.7 \\ &9.90/(2.5 \times 0.72) = 5.5 \\ &10.62/(2.5 \times 0.72) = 5.9 \end{aligned}$$

$$= 6.0 \pm 0.6$$

Appendix B. Transalkylation reaction and products.

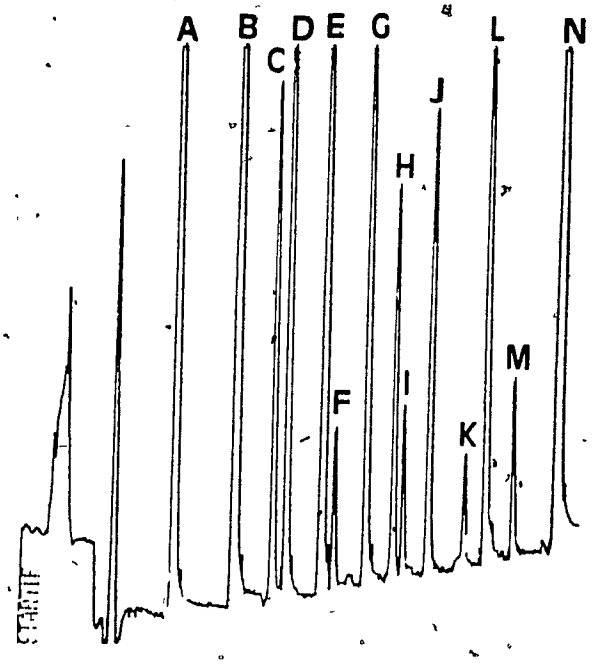
The various methylethyllead compounds can be prepared by heating equimolar quantities of Me_2Bu_2Pb and Et_2Bu_2Pb (both in iso-octane) together, at 150 C for 21 h. The analysis of the mixture of compounds resulting from the reaction indicates the extensive exchange of alkyl groups, resulting in various tetraalkyllead compounds (Figure A1), with retention times between the retention times of the known alkyllead standards. The predicted retention times (Forsyth and Marshall, 1986) for these mixed alkyllead compounds are given in Table A1.

Table A1. Absolute retention times and retention indices of mixed methylethylleads (based on retention times) relative to alkylbutyllead standards. (Forsyth and Marshall, 1986)

Analyte	N	Absolute retention time (RT), min	Retention[a] Index (I)
Me_3BuPb	7	9.21	700
Me_2Bu_2Pb	8	11.22	800 (805)b
$MeEt_2BuPb$	9	13.04	900 (906)b
Me_2Bu_2Pb	10	14.23	1000
Et_3BuPb	10	14.65	1000
$EtMeBu_2Pb$	11	15.77	1100 (1105)b
Et_2Bu_2Pb	12	17.15	1200
Bu_4Pb	16	21.09	1600

[a] predicted retention index (Kovats, 1965). [b] $I = 100N + 100n\,[(RT_{(A)} - RT_{R(N)}) / (RT_{R(N)})]$ where N = number of carbons in analyte, n = carbon number difference between R(N) and R(N+n), A = analyte for which retention index was calculated, R(N) = analyte containing N carbons, and R(N+n) = analyte containing N+n carbons.

Figure A1 GC-AAS chromatogram of the transalkylation reaction products containing A, Me_3EtPb; B, Me_2Et_2Pb; C, Me_3BuPb, D, $MeEt_3Pb$; E, $Me_2EtBuPb$; F, Et_4Pb; G, $MeEt_2BuPb$; H, Me_2Bu_2Pb; I, Et_3BuPb; J, $MeEtBu_2Pb$; K, Et_2Bu_2Pb; L, $MeBu_3Pb$; M, $EtBu_3Pb$ and N, Bu_4Pb.


A
B
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Appendix C. Analyses of variance

(a) Data Set: Tissue levels of alkylleads in Saltmarsh Periwinkles.

Source	SS	DF	MS	F	Pr>F
1. Trimethyllead					
Total	52.6353	35			
Model	38.2700	15	2.5513	3.55	0.0046
Error	14.3652	20	0.7183		
2. Ethyl dimethyllead					
Total	163.4815	31			
Model	124.0830	15	8.2722	3.36	0.0107
Error	39.3985	16	2.4624		
3. Diethyl methyllead					
Total	1270.3503	32			
Model	1184.0919	15	78.9395	15.56	0.0001
Error	86.2585	17	5.0740		
4. Triethyllead					
Total	1121.8301	35			
Model	988.7634	15	65.9176	9.91	0.0001
Error	133.0666	20	6.6533		
5. Diethyllead					
Total	2726.8215	32			
Model	2551.2257	15	170.0817	16.47	0.0001
Error	175.5957	17	10.3291		
6. Unknown lead-containing analyte					
Total	109.6813	35			
Model	99.0565	15	6.6038	12.43	0.0001
Error	10.6813	20	0.5312		

(b) Data Set: Levels of trimethyllead, combined ethyllead and combined mixed alkyllead levels in periwinkles.

Source	SS	DF	MS	F	Pr>r
1. Trimethyllead					
Total	52.7739	35			
Model	33.1101	13	2.5469	2.85	0.0147
Error	19.6638	22	0.8938		
2. Sum of Et_2MePb^+ plus $EtMe_2Pb^+$					
Total	2119.4923	35			
Model	1629.0726	13	125.3133	5.62	0.0002
Error	490.4197	22	22.2918		
3. Sum of Et_2Pb^{2+} plus Et_3Pb^+					
Total	6246.2051	35			
Model	5348.3966	13	411.4151	10.08	0.0001
Error	897.8085	22	40.8095		

(c) Data Set: Mean water consumed by, and body weight change in quail (Trial 1).

Source	SS	DF	MS	F	Pr>F
1. Water consumed - Trial 1					
Total	30590.6012	191			
Source	28936.9870	71	407.5632	29.58	0.0001
Error	1653.6142	120	13.7801		
2. Body weight - Trial 1					
Total	179409.0798	187			
Model	120818.1942	90	1342.4234	2.22	0.0001
Error	58590.8856	97	604.0297		

(d) Data Set: Mean water consumed by, and body weight change in quail (Trial 2).

Source	SS	DF	MS	F	Pr>F
1. Body weight (Trial 2)					
Total	179409.08	187			
Model	152456.78	90	1693.96	6.10	0.0001
Error	26952.30	97			
2. Water consumed (Trial 2)					
Total	13513.71	167			
Model	12646.79	31	407.96	64.00	0.0001
Error	866.92	136	6.37		

Appendix D. Mean water consumed by quail.

Table A2. Mean water consumed by quail provided with drinking water amended with 0.0, 25 ppm Et_2PbCl_2 or 250 ppm $Pb(NO_3)_2$ for two months.

Week	Controls	$Pb(NO_3)_2$	Et_2PbCl_2
0	87.8 ± 4.7	88.5 ± 3.9	89.3 ± 2.9
1	88.6 ± 5.3	72.2 ± 5.3	65.4 ± 5.2
2	91.6 ± 4.8	75.3 ± 2.9	66.2 ± 3.5
3	79.0 ± 2.8	66.1 ± 5.9	62.4 ± 3.3
4	79.4 ± 6.5	59.3 ± 1.6	60.1 ± 3.6
5	76.8 ± 3.4	56.7 ± 1.8	54.4 ± 1.2
6	79.4 ± 5.0	57.4 ± 3.3	53.4 ± 3.9
7	73.3 ± 5.0	49.6 ± 4.3	54.3 ± 1.1
8	84.0 ± 2.8	59.2 ± 3.9	63.1 ± 5.2

Table A3. Mean water consumed by quail provided with drinking water amended with 0.0, 0.25, or 2.5 ppm Et_3PbCl for two months.

Week	Controls	Low Treat[a]	High Treat[b]
0	55.3 ± 2.6	56.1 ± 1.8	54.8 ± 1.4
1	54.6 ± 2.8	54.6 ± 1.3	36.0 ± 1.8
2	55.4 ± 2.8	51.4 ± 2.2	34.0 ± 2.3
3	56.3 ± 2.4	49.7 ± 1.8	36.7 ± 2.1
4	55.9 ± 1.3	48.4 ± 3.0	35.3 ± 3.0
5	53.7 ± 3.9	49.7 ± 2.6	34.3 ± 3.0
6	48.6 ± 2.8	47.9 ± 2.6	31.9 ± 2.3
7	51.0 ± 3.7	46.7 ± 2.4	33.0 ± 2.8
8	53.9 ± 3.3	55.0 ± 1.9	35.4 ± 2.4

a Treatment consisted of amending the water with 250 ppb of triethyllead chloride.

b Treatment consisted of amendig the water with 2.5 ppm of triethyllead chloride.

Appendix E. Body weight change in quail.

Table A4. Mean weekly body weights of quail provided with water amended with 0.0, 25 ppm Et_2PbCl_2, or 250 ppm $Pb(NO_3)_2$ for two months.

Week	Control	$Pb(NO_3)_2$	Et_2PbCl_2
0	211.0 ± 12.4	220.7 ± 20.7	208.7 ± 17.0
1	217.8 ± 18.9	230.5 ± 19.9	215.8 ± 18.9
2	230.5 ± 19.3	247.0 ± 19.4	228.6 ± 16.6
3	242.5 ± 15.0	265.6 ± 21.2	241.3 ± 20.0
4	250.7 ± 14.9	275.7 ± 17.1	240.4 ± 11.3
5	254.3 ± 13.2	261.9 ± 23.3	236.3 ± 8.0
6	256.7 ± 10.5	270.6 ± 21.7	242.6 ± 11.4
7	258.3 ± 11.8	272.0 ± 28.0	231.9 ± 25.3
8	241.2 ± 38.5	262.3 ± 50.3	215.0 ± 44.0

Table A5. Mean body weights of quail provided with drinking water amended with 0.0, 0.25, or 2.5 ppm Et_3PbCl/kg for two months.

Week	Controls	Low Treat[a]	High Treat[b]
0	214.5 ± 24.7	230.7 ± 13.5	211.0 ± 08.3
1	226.0 ± 23.0	234.7 ± 19.6	211.9 ± 11.2
2	213.3 ± 26.1	209.1 ± 15.2	204.5 ± 12.9
3	218.5 ± 25.7	209.2 ± 15.1	203.1 ± 12.1
4	222.9 ± 19.2	210.1 ± 13.0	202.8 ± 12.3
5	211.0 ± 20.8	202.8 ± 15.7	201.5 ± 17.3
6	205.8 ± 17.5	200.0 ± 13.4	201.0 ± 19.4
7	200.0 ± 17.5	196.8 ± 13.5	203.0 ± 16.8
8	206.9 ± 21.6	196.2 ± 12.8	196.4 ± 16.6

a Treatment consisted of amending the water with 250 ppb of triethyllead chloride.

b Treatment consisted of amending the water with 2.5 ppm of triethyllead chloride.

Appendix F. Ionic alkyllead levels in egg in trial 1.

Table A6. Mean alkyllead concentrations in egg homogenates from adult quail provided with drinking water amended with 25 ppm Et_2PbCl_2 for two months.

Day	Et_2Pb^{2+}	Et_3Pb^{+}	Me_3Pb^{+}	Me_2Pb^{2+}
01	0.0098 ± 0.006			
02	0.0202 ± 0.003			
03	0.0279 ± 0.003			
04	0.0393 ± 0.004			
05	0.0441 ± 0.003			
06	0.0525 ± 0.0004			
07	0.0745 ± 0.008			
08	0.0799 ± 0.061			
09	0.0923 ± 0.010			
10	0.1150 ± 0.008			
11	0.2208 ± 0.054			
12	0.2435 ± 0.032			
13	0.2640 ± 0.028			
14	0.2734 ± 0.030			
15	0.2988 ± 0.022			
16	0.3100 ± 0.026			
17	0.3315 ± 0.029			
18	0.3389 ± 0.039			
19	0.3518 ± 0.020			
20	0.3721 ± 0.032			
21	0.3900 ± 0.031			
22	0.5731 ± 0.002			
23	0.4393 ± 0.054			
24	0.5273 ± 0.032			
25	0.4636 ± 0.022			
26	0.4635 ± 0.114			
27	0.7695 ± 0.050	0.00178 ± 0.00081		
28	0.7613 ± 0.078	0.00180 ± 0.00082		
29	0.8200 ± 0.007	0.00200 ± 0.00102		
30	0.9633 ± 0.001	0.00223 ± 0.00188		
31	1.0420 ± 0.169	0.00708 ± 0.00260		
32	0.6184 ± 0.001	0.00617 ± 0.00198		
33	0.6174 ± 0.045	0.00602 ± 0.00224		
34	0.3270 ± 0.035	0.00690 ± 0.00243		
35	0.2973 ± 0.010	0.00713 ± 0.00121		
36	0.2894 ± 0.013	0.00749 ± 0.00310		
37	0.3138 ± 0.022	0.00765 ± 0.00285		
38	0.3042 ± 0.029	0.00882 ± 0.00147		
39	0.3113 ± 0.113	0.00893 ± 0.00489		
40	0.3438 ± 0.020	0.01028 ± 0.00312		
41	0.3287 ± 0.031	0.01002 ± 0.00212		

Day	Et_2Pb^{2+}	Et_3Pb^+	Me_3Pb^+	Me_2Pb^{2+}
42	0.3157 ± 0.026	0.00972 ± 0.00398		
43	0.4139 ± 0.039	0.01143 ± 0.00224		
44	0.3033 ± 0.008	0.01147 ± 0.00198		
45	0.3179 ± 0.019	0.00889 ± 0.00260		
46	0.3200 ± 0.009	0.01012 ± 0.00213		
47	0.3299 ± 0.021	0.01002 ± 0.00188		
48	0.3441 ± 0.012	0.01011 ± 0.00190		
49	0.3741 ± 0.003	0.00655 ± 0.00281		
50	0.4234 ± 0.005	0.00683 ± 0.00163		
51	0.5419 ± 0.040	0.00712 ± 0.00287		
52	0.6277 ± 0.048	0.00460 ± 0.00112		0.01921 ± 0.0087
53	0.6292 ± 0.054	0.00269 ± 0.00108		0.00481 ± 0.0000
54	0.6277 ± 0.061	0.00178 ± 0.00106	0.00340 ± 0.0012	0.00763 ± 0.0034
55	0.6073 ± 0.028	0.00299 ± 0.00048	0.00603 ± 0.0015	0.01081 ± 0.0046
56	0.6292 ± 0.038		0.00650 ± 0.0016	0.03482 ± 0.0028
				0.08210 ± 0.0066

Appendix G. Ionic ethyllead levels in egg in Trial 2.

Table A7. Mean alkyllead concentrations in egg homogenates from adult quail provided with drinking water amended with 250 ppb triethyllead chloride for two months.

Day	Et_3Pb^+	Et_2Pb^{+2}
1	0.13 ± 0.07	
2	0.44 ± 0.18	
3	1.64 ± 0.21	1.25 ± 0.09
4	1.31 ± 0.18	1.32 ± 0.10
5	2.01 ± 0.27	1.89 ± 0.14
6	3.77 ± 0.26	2.22 ± 0.19
7	3.92 ± 0.41	1.91 ± 0.24
8	4.01 ± 0.47	2.42 ± 0.17
9	4.62 ± 0.52	8.69 ± 0.90
10	3.72 ± 0.62	11.17 ± 1.21
11	3.44 ± 0.44	8.48 ± 1.04
12	4.79 ± 0.52	6.68 ± 0.47
13	5.88 ± 0.49	4.94 ± 0.53
14	3.98 ± 1.17	3.92 ± 0.82
15	4.35 ± 0.38	3.54 ± 0.78
16	4.09 ± 0.39	3.29 ± 0.48
17	3.92 ± 0.45	3.92 ± 1.02
18	3.49 ± 0.31	4.43 ± 0.51
19	3.83 ± 0.40	6.03 ± 0.58
20	4.26 ± 0.71	5.99 ± 0.69
21	3.99 ± 0.55	6.31 ± 0.98
22	5.28 ± 0.59	6.21 ± 0.88
23	6.31 ± 0.64	6.50 ± 0.79
24	6.38 ± 0.93	6.43 ± 0.76
25	6.34 ± 0.79	5.89 ± 1.02
26	4.32 ± 0.78	4.17 ± 0.73
27	3.92 ± 0.45	7.29 ± 0.69
28	4.01 ± 0.53	3.93 ± 0.91
29	4.34 ± 0.46	3.71 ± 0.44
30	4.20 ± 0.41	3.39 ± 0.89
31	4.78 ± 0.52	1.84 ± 1.01
32	7.57 ± 0.95	3.30 ± 0.67
33	11.01 ± 1.11	3.50 ± 0.57
34	6.82 ± 0.92	3.31 ± 0.54
35	5.36 ± 0.68	3.70 ± 0.61
36	6.45 ± 0.88	3.42 ± 0.44
37	5.51 ± 0.59	3.61 ± 0.46
38	5.79 ± 0.68	3.11 ± 0.42
39	5.90 ± 0.72	3.57 ± 0.51
40	8.69 ± 1.02	3.67 ± 0.47
41	6.06 ± 0.62	4.17 ± 0.52

Day	Et_3Pb^+	Et_2Pb^{+2}
42	5.91 ± 0.69	4.17 ± 0.98
43	5.80 ± 0.61	3.92 ± 1.00
44	4.65 ± 0.51	4.05 ± 0.69
45	5.79 ± 0.53	4.05 ± 0.81
46	5.22 ± 0.55	4.00 ± 0.58
47	5.52 ± 0.46	3.78 ± 0.59
48	7.10 ± 0.68	2.98 ± 0.31
49	5.79 ± 0.60	3.08 ± 0.44
50	6.67 ± 0.53	4.68 ± 0.39
51	7.11 ± 0.67	4.18 ± 0.48
52	6.96 ± 0.63	4.81 ± 0.52
53	4.21 ± 0.34	4.81 ± 0.43
54	7.09 ± 0.66	5.19 ± 0.54
55	5.87 ± 0.73	8.10 ± 0.67
56	15.86 ± 1.23	13.51 ± 1.09

Appendix G. Ionic ethyllead levels in egg in Trial 2.

Table A8. Mean alkyllead concentrations in egg homogenates from adult quail provided with drinking water amended with 2.5 ppm triethyllead chloride for two months.

Day	Et_3Pb^+	Et_2Pb^{+2}
1	1.11 ± 0.12	
2	2.09 ± 0.19	1.27 ± 0.21
3	3.48 ± 0.27	2.90 ± 0.35
4	4.68 ± 0.38	2.91 ± 0.34
5	5.51 ± 0.67	2.63 ± 0.41
6	5.79 ± 0.46	6.89 ± 0.45
7	6.98 ± 0.49	4.81 ± 0.56
8	7.90 ± 0.87	5.06 ± 0.59
9	10.19 ± 0.92	13.50 ± 1.23
10	12.99 ± 0.82	17.97 ± 1.44
11	12.65 ± 1.04	20.32 ± 1.89
12	12.58 ± 1.00	16.13 ± 2.18
13	15.92 ± 1.49	12.33 ± 2.11
14	24.79 ± 1.57	13.48 ± 1.84
15	27.63 ± 1.38	18.12 ± 2.19
16	26.71 ± 2.19	24.19 ± 1.78
17	24.67 ± 2.40	39.01 ± 3.03
18	25.12 ± 3.30	38.92 ± 3.71
19	24.89 ± 1.40	42.68 ± 4.59
20	22.90 ± 2.11	46.27 ± 3.98
21	21.79 ± 1.85	54.59 ± 4.91
22	31.30 ± 2.59	43.04 ± 3.89
23	30.48 ± 1.64	39.29 ± 4.76
24	32.50 ± 3.13	37.12 ± 3.28
25	34.12 ± 2.89	34.73 ± 3.01
26	33.18 ± 1.87	38.45 ± 2.80
27	28.16 ± 2.54	34.17 ± 3.92
28	22.01 ± 3.33	50.63 ± 3.89
29	33.32 ± 2.46	51.40 ± 4.44
30	28.50 ± 3.41	48.95 ± 4.99
31	28.73 ± 2.52	54.99 ± 3.91
32	30.37 ± 1.95	51.71 ± 4.76
33	32.88 ± 3.11	35.76 ± 2.96
34	48.89 ± 3.29	15.77 ± 1.32
35	43.01 ± 4.86	13.35 ± 1.73
36	45.51 ± 3.88	10.67 ± 1.24
37	44.42 ± 5.59	5.45 ± 0.58
38	46.14 ± 3.68	5.57 ± 0.64
39	52.52 ± 4.72	5.77 ± 0.53
40	49.27 ± 2.02	6.71 ± 1.47
41	52.17 ± 3.62	5.06 ± 1.52

Day	Et_3Pb^+	Et_2Pb^{+2}
42	43.47 ± 3.29	4.05 ± 0.98
43	47.83 ± 4.01	4.18 ± 1.00
44	46.38 ± 4.53	5.02 ± 0.69
45	49.28 ± 5.03	4.08 ± 0.81
46	43.48 ± 4.51	5.89 ± 0.68
47	44.93 ± 2.89	4.09 ± 0.49
48	49.28 ± 3.39	3.68 ± 0.62
49	47.83 ± 3.92	6.43 ± 0.34
50	43.48 ± 4.13	4.85 ± 0.67
51	47.81 ± 3.69	5.12 ± 1.01
52	49.27 ± 4.67	9.31 ± 1.12
53	50.87 ± 4.31	12.48 ± 1.87
54	51.72 ± 4.36	20.25 ± 2.62
55	52.84 ± 5.07	35.37 ± 2.77
56	51.72 ± 4.43	35.98 ± 4.19

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