THE METABOLISM OF TRIPHENYLLEAD ACETATE IN THE RAT

by

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ABSTRACT

The metabolic fate of triphenyllead acetate, a potential molluscicide, has been studied in the rat. Three isotopically labelled forms of the compound were synthesized namely tri(U-^{14}C)phenyllead acetate, tri(^{3}H)phenyllead acetate and triphenyl(^{203}Pb)lead acetate. These were given orally at a dose level of 25mg/kg and intraperitoneally at 2mg/kg.

The excretion pattern of the label of tri(U-^{14}C)phenyllead acetate was followed after its oral administration. Benzene was identified in the expired air whilst the conjugates of phenol and quinol were found in the urine. Reverse isotope dilution suggested that unchanged triphenyllead acetate was probably present in the faeces.

A similar metabolic pattern was found after the intraperitoneal administration of tri(^{3}H)phenyllead acetate, but the radioactivity was excreted more slowly than after oral administration.

Radioactive lead was detected in the faeces after both oral and intraperitoneal administration of triphenyl(^{203}Pb)lead acetate. Lead was also detected in the faeces by a colorimetric method after oral administration of non-radioactive triphenyllead acetate at a dose level of 200mg/kg. The distribution of ^{203}Pb in tissues 24 hours after the oral or intraperitoneal administration of triphenyl(^{203}Pb)lead acetate showed that the radioactive label was associated mainly with the gut tissue and gut contents. After injection however, little of the compound appears to be excreted in the bile.

The role played by hydrogen ions, cysteine and the gut bacteria in the breakdown of triphenyllead acetate to benzene has been investigated in some preliminary experiments and discussed.

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CHAPTER 1

INTRODUCTION

Lead is almost ubiquitous in nature and has been mined for use by man since the time of the Greek and Roman civilizations. Nicander, the Greek poet and physician, described the effects of lead poisoning more than 2000 years ago as did Dioscorides in the first century A.D. The decline in culture of the Roman empire has been attributed to a decrease in number of the upper classes. These alone could afford lead-lined pots for cooking and thus it is believed that widespread lead poisoning occurred among them (Gilfillan,1965).

Lead, one of the main group four elements is a malleable metal resistant to corrosion. It has many uses, as piping for carrying water supplies, in batteries, as lead shot and as a constituent of solder, glazes and paint. However, the greatest modern day use of lead is in the manufacture of the anti-knock agent tetraethyllead.

The two universal sources of lead to which man is exposed are lead in his food and in his drinking water. It has been estimated that in the United States of America an adult person consumes about 0.3mg of lead per day in his food and drink. Under normal conditions most of this traverses the alimentary tract and is evacuated in the faeces (Kehoe, 1961).

The only natural source of lead in water is that absorbed from the rocks with which water comes in contact and thus the level of lead in fresh water varies greatly with location. Rain water may contain a significant level of lead as a result of airborne lead being washed out of the atmosphere. In the United States of America a nationwide sampling of rain water has shown the average level of lead to be 34µg per litre (Lazrus et al. 1970). Another source of lead in water is from the use of lead in old plumbing systems, the levels being greatest in soft water areas. The mean lead content of drinking water in

Glasgow is 160µg per litre compared to the lead limit of 100µg per litre recommended by the World Health Organisation (Chow, 1973).

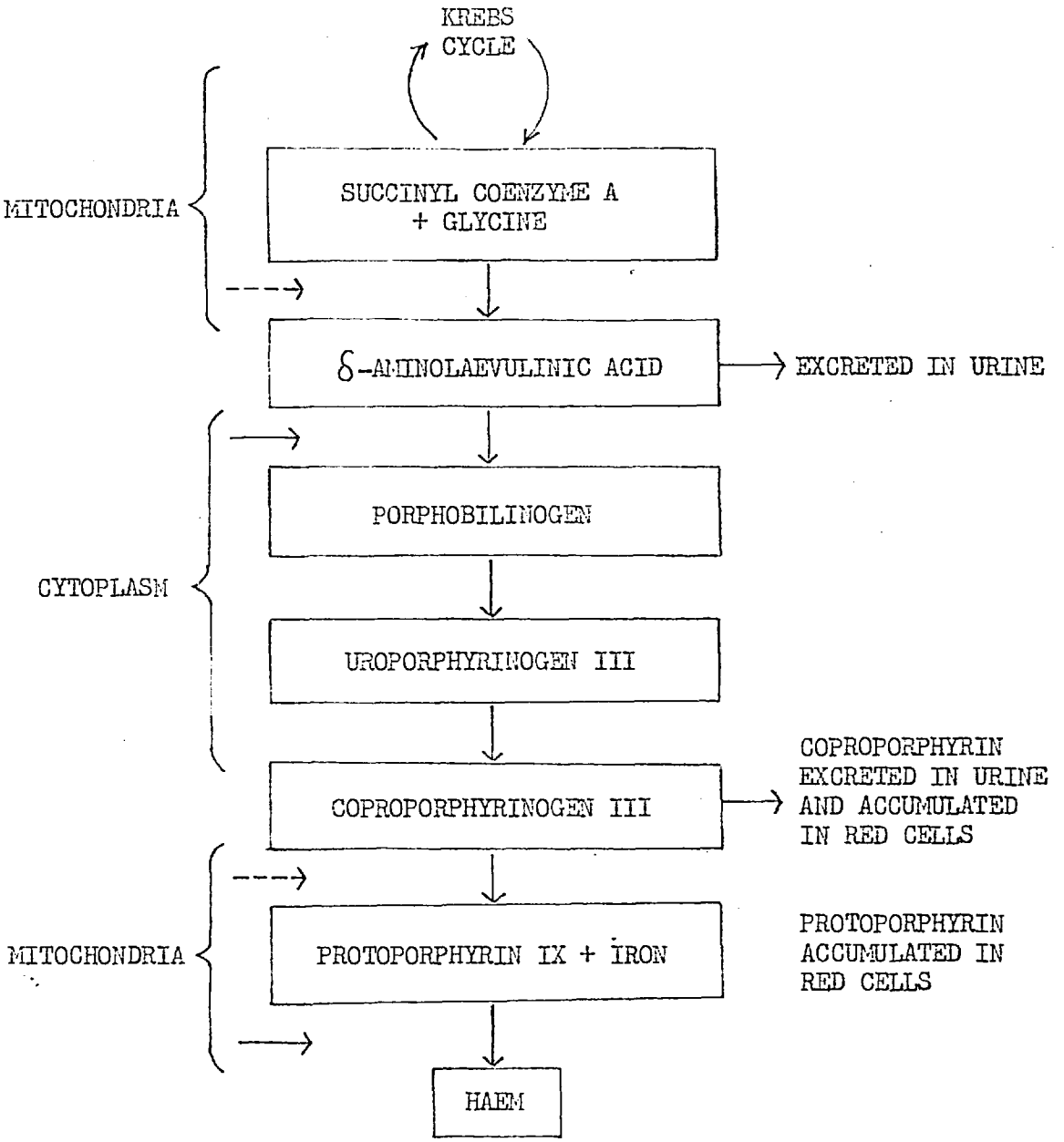
The symptoms and pathology of lead poisoning are well documented (e.g. Goodman and Gilman, 1965a). Often the symptoms present are non-specific making diagnosis difficult. Those found include insomnia, pallor, constipation, slight anaemia, abdominal pain, basophilic stippling of the red blood cells and a black or purplish line at the margin of the gums due to a precipitate of lead sulphide. The more severe symptoms of chronic nephritis, lead palsy and lead encephalopathy may also be present.

Lead interferes in the biosynthesis of haem inhibiting two reactions in the biosynthetic pathway. It severely depresses the activity of δ-aminolaevulinic acid dehydratase (Haeger-Aronsen et al., 1971) and inhibits the formation of haem from protoporphyrin IX and iron (Goldberg, 1968) (Fig. 1). This results in the excretion of δ-aminolaevulinic acid and coproporphyrin in the urine. However, although anaemia may be present the pallor of the skin is often greater than one would expect.

Severe acute plumbism leads to kidney disfunction. In this syndrome, the Fanconi syndrome, there is an increased loss of amino acids, glucose and phosphate in the urine due to low reaborption of these substances by the tubular cells. Chronic lead poisoning, however, leads to nephritis and increase in the number of deaths, due to this, among plumbers and painters compared with the general public has been reported (Lane, 1964).

Prolonged sub-acute poisoning gives rise to "lead palsy". Muscular weakness and fatigue occur in frequently used muscles such as the extensors of the forearm, wrist and fingers. Degenerative changes

Fig. 1 The inhibition of haem biosynthesis by lead



Biosynthesis of haem, a constituent of haemoglobin, is inhibited by lead resulting in accumulation of intermediates in the synthetic pathway. Of six steps in the pathway, the first and the last two take place in the mitochondria, the others elsewhere in the cell cytoplasm. Lead inhibits two steps (solid arrows) and may inhibit two others (broken arrows) (Chisolm, 1971).

follow leading to paralysis, atrophy and contractures.

Intestinal symptoms are one of the early signs of lead poisoning. Constipation, nausea, vomiting and occasionally diarrhoea are experienced. Colic is frequently found and the abdominal pain is usually severe.

Lead encephalopathy is the most serious result of lead poisoning. Although occurring rarely in adults its incidence in children is much higher. The first symptoms seen are clumsiness, vertigo, ataxia, headache, insomnia, restlessness and irritability. These may be followed by visual disturbances, delirium, convulsions and coma. Increased intracranial pressure due to oedema is present.

Lead poisoning is much more serious in children than in adults. It is usually due to the habit of pica and in the United States of America is prevalent in areas of bad housing where children eat pieces of old flaking plaster containing high amounts of lead. On X-ray examination radio-opaque lines are seen at the ends of the long bones due to the deposition of lead.

Byers and Lord (1943) followed up twenty children eight years after they had been in hospital with lead poisoning. Although none of them had acute encephalopathy, eight years later most of them showed evidence of intellectual and emotional difficulties. Since then the sequelae of lead poisoning in children have been well documented (Thurston et al., 1955; Chisolm & Harrison, 1966; Smith et al., 1963; Perlstein & Attala, 1966). The most common sequelae found include mental retardation, recurrent seizures, cerebral palsy and optic atrophy.

Confirmation of the diagnosis of lead poisoning is usually obtained by a determination of the blood lead level. In adults,

symptoms of lead poisoning are said to occur when the blood lead level reaches 0.8ppm. In contrast, this level in a group of normal men, varied from 0.1 to 0.6ppm with a mean of 0.3ppm (Kehoe, 1961). Children are more susceptible to lead and symptoms of poisoning occur when the blood lead levels are much lower. The upper limit of normal has usually been regarded as 50-60µg/100ml (Chisolm & Harrison, 1956; Bradley et al., 1956). However this level may be set too high. Blood lead determinations carried out on 80 children who had no history of mental retardation or pica showed that all but two had a blood lead level of 36µg or less per 100ml of blood (Moncrieff et al., 1964). In contrast a study carried out on 73 children from the Manchester and Salford area showed that thirteen of them had blood lead levels above 50ug per 100ml blood, though the mean level was 30.9µg lead per 100ml blood (Gordon et al., 1967).

The body burden of lead increases with age. In a study of the lead concentration in human tissues at post-mortem it was shown that this accumulation occurred mainly in bone, rising from a concentration of about 1ppm in infants to more than 40ppm in persons over the age of fifty years. Lead concentrations in soft tissues rose until the age of about twenty and thereafter remained steady. In adults concentrations in soft tissues varied between 0.06ppm in muscle and 1.35ppm in liver. It was calculated that nearly 95% of the total body burden was represented by the lead content in bone of which more than 70% was in dense bone (Barry & Mossman, 1970). In a similar study it was calculated that 91% of the total body burden of lead is found in the skeleton (Schroeder & Tipton, 1968). In a series of classical experiments a subject ingested one milligram of lead daily in addition to that which occurred in his food and drink,

over a period of 1456 days. Records of his ingestion and elimination of lead showed that he retained 78 milligrams of lead. Urinary excretion of lead increased during this period. A second subject who ingested 2mg of lead a day took half the length of time to retain the same amount of lead. Following termination of the experiment there was greater excretion the ingestion of lead, the rate of excretion appearing to vary inversely with the length of time over which the accumulation had occurred (Kehoe, 1961).

Controversy exists as to whether or not the current level of lead absorption in the general population presents some subtle risk to health (Bryce-Smith, 1971 & 1972; Chow, 1973). A recent examination by Japanese and American workers of annual snow strata in Greenland has shown that levels of airborne lead have increased since the Industrial Revolution with a sharp increase dating from about 1940. The latter was attributed mainly to the use of alkylleads in petrol (Murozumi et al., 1969). Man has evolved in the presence of a certain amount of inorganic lead to which he may have adapted. Although it cannot be disputed that lead is a cumulative poison no firm decision regarding the danger presented by today's widespread usage of lead can be arrived at.

THE TREATMENT OF LEAD POISONING

Formerly lead poisoning was treated by administration of large amounts of calcium and phosphate promoting the deposition of lead in bone. This procedure relieved the toxic symptoms but as lead could be released from the bone plumbism could recur. The use of chelating agents has revolutionised the treatment of lead poisoning. Three of these are dimercaprol (2,3-dimercaptopropanol or British

Anti-Lewisite), disodium calcium edetate and D-penicillamine (β,β-dimethyl cysteine)(Fig. 2).

Sodium calcium edetate

Sodium calcium edetate is the dihydrate of the calcium chelate of the disodium salt of ethylenediamine NNN'N'-tetra-acetic acid. Its pharmacological action is due to its ability to exchange the calcium atom for a lead one thereby forming a stable non-ionizable lead compound which is water-soluble and readily excreted unchanged by the kidneys.

Calcium disodium edetate is poorly absorbed from the gastro-intestinal tract. After oral administration of the ^{14}C-labelled compound, the rat eliminated 80-95% of the dose in the faeces (Foreman et al., 1953) while man eliminated 91% of the radioactivity in the faeces and 4.2% in the urine (Foreman & Trujillo, 1954). Some authors consider that oral administration of calcium disodium edetate may precipitate or increase the symptoms of lead poisoning by increasing the absorption of lead from the gut (Kehoe, 1955; Smith, 1964; Byers, 1959).

After intravenous or intramuscular administration, calcium disodium edetate is rapidly excreted, mainly in the urine. In human volunteers who received (^{14}C)-disodium calcium edetate, 50% of the radioactivity was excreted by one hour after intravenous administration and by two and a half hours after intramuscular administration. After twenty-four hours the urinary excretion accounted for 98.8% of an intravenously administered dose (Foreman & Trujillo, 1954). In the dog disodium calcium edetate appears to be excreted by glomerular filtration and not to undergo tubular secretion or reabsorption (Forland et al., 1966).

Repeated administration of the compound sometimes leads to a toxic effect on the kidney. Severe hydropic degeneration of the proximal tubules with almost total destruction of their epithelia has been observed (Dudley et al., 1955; Foreman et al., 1956; Altman et al., 1962).

Dimercaprol

Dimercaprol (2,3-dimercaptopropanol, British Anti-Lewisite) was discovered during World War II as an antidote to the arsenical war gas, Lewisite. Its side effects are nausea, vomiting, lachrymation, salivation, headache, a burning sensation of the lips, mouth, throat and eyes, a feeling of constriction of the throat and an increase in the systolic and diastolic blood pressures. Dimercaprol was formerly used for the treatment of lead poisoning (Chisolm & Harrison, 1956; Smith, 1964), but with the advent of the more effective and less toxic disodium calcium edetate it has fallen into disuse and is not recommended for this purpose by the British Pharmaceutical Codex 1973.

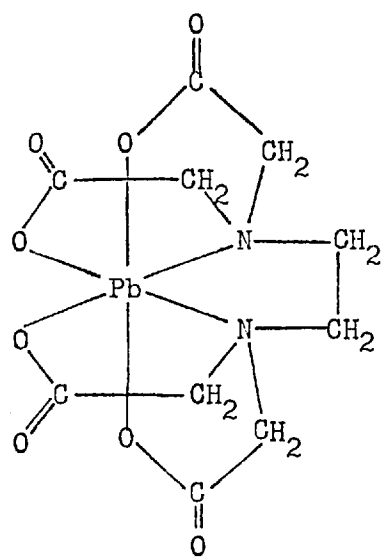
D-Penicillamine

Penicillamine is a white crystalline water soluble compound with a characteristic odour and a slightly bitter taste. It has the advantage that it is effective when given orally but it does not increase the excretion of lead as much as intravenously administered disodium calcium edetate (Selander, 1967; Moncrieff et al., 1964).

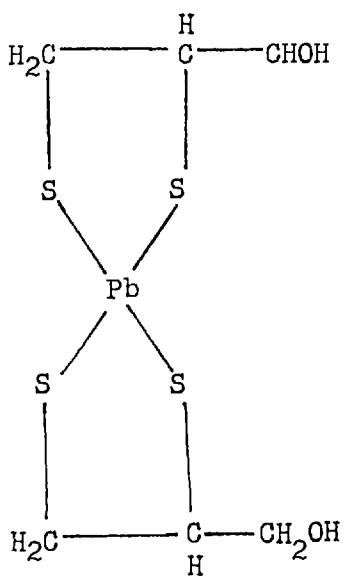
The mechanism of action of these chelating agents is not understood. It was generally assumed that disodium calcium edetate acts to remove lead from the soft tissues rather than from the skeleton (Rieders et al., 1955; Miller, 1959). However, studies on the excretion of ^{210}Pb by rats following administration of disodium calcium edetate or D-penicillamine showed there was mainly a reduction of the lead content of bone

Fig. 2 Chelating agents used in treating lead poisoning

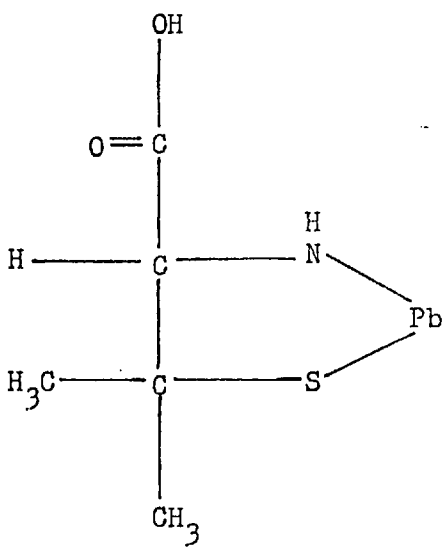
EDTA
(ethylenediaminetetraacetic acid)



BAL
(Dimercaprol)



D-PENICILLAMINE



Chisolm, 1971

rather than of the soft tissues (Hammond, 1971 & 1973).

USES OF ORGANOLEAD COMPOUNDS

Several excellent reviews on the applications of organolead compounds have been written (Harwood, 1963; Willemsens, 1964; Shapiro & Frey, 1968).

The only organolead compound that is being produced commercially on a large scale is tetraethyllead whose potential as an antiknock agent in motor fuels was discovered by Midgeley and Boyd in 1922. It is manufactured by the reaction of ethyl chloride with lead-sodium alloy according to the equation:-

$$4NaPb + 4C_2H_5Cl = (C_2H_5)_4Pb + 3Pb + 4NaCl$$

Tetramethyllead has gained considerable interest as an antiknock agent due to its greater volatility and several comparative studies have been carried out (Richardson *et al.*, 1961; Goodacre *et al.*, 1962; Farnsworth & Boddy, 1962). Many theories have been put forward to explain the mechanism of the antiknock properties of tetraethyllead (Ross & Rifkin, 1956). It has been established that tetraethyllead must decompose to be effective and that its action is possibly due to the formation of finely divided lead oxide which inhibits the precombustion reactions responsible for the knock effect.

The other commercial use of organolead compounds is as alkylating agents in the manufacture of mercury fungicides. Ethylmercury chloride, for instance, is made by the following reaction:-

$$2HgCl_2 + Et_4Pb = Et_2PbCl_2 + 2EtHgCl$$

Although organolead compounds are not applied commercially for any other purpose, other uses are patented. These fall into two main categories - as polymerization catalysts and as pesticides.

Organolead compounds are thermally unstable, decomposing on heating to free radicals and metal. Initiation of chain reactions can be brought about by the free radicals formed. An example is the polymerization of ethylene to a high boiling liquid in the presence of tetraethyllead at a temperature of 250-350° (Taylor & Jones, 1930).

The biocidal effects of organolead compounds were noted as early as 1929 by Krause who described the effectiveness of trialkyl- and triaryllead halides against mouse carcinoma. The simple organolead salts seem to be effective as fungicides and bacteriocides (Sijpesteijn et al., 1962). Other uses reported are as a component of antifouling paints (Guillen et al., 1969), as a molluscicide for the control of the vector snail of bilharzia (Hopf et al., 1967), as a herbicide (de Pree, 1959), as a bacteriostat for cotton and as a moth-proofing agent (Willemsens, 1964).

GENERAL CHEMISTRY OF ORGANOLEAD SALTS

Under the heading of organolead salts are grouped compounds of the type R_3PbX, R_2PbX_2 and $RPbX_3$ where R is an organic group attached with a carbon-lead bond to the lead atom and X is an anionic group. There are a great number of compounds of the type R_3PbX and R_2PbX_2 but of the $RPbX_3$ type only the aryllead tricarboxylates are known. There are several excellent reviews on their chemistry (Willemsens, 1964; Willemsens & van der Kerk, 1965; Shapiro & Frey, 1968).

Although these compounds are called salts their salt like nature is questionable. Organolead salts of strong acids are stable to hydrolysis and exhibit a high degree of ionic character but organolead derivatives of the very weak acids e.g. alkoxides, amides and phosphides exhibit a high degree of covalent character. They are readily hyd-

rolysed and tend to be less thermally stable than salts of strong acids. Thus salts of weak acids are more similar in their properties to tetravalent lead salts of carboxylic acids such as lead tetra-acetate than to divalent lead salts.

A number of organolead salts are known in which two or more different organic groups are present e.g R_2R^1PbX or RR^1PbX_2. A few organolead salts are also known in which two different anions are present.

Synthesis of tri- and di- organolead salts

Most of the methods of synthesis of R_3PbX and R_2PbX_2 compounds involve the use of an organolead compound as starting material.

a) Reaction of tetraorganolead compounds with halogens or acids

$$R_4Pb + X_2 = R_3PbX + RX$$
$$R_4Pb + 2X_2 = R_2PbX_2 + 2RX$$
$$R_4Pb + HX = R_3PbX + RH$$
$$R_4Pb + 2HX = R_2PbX_2 + 2RH$$

This is the most common method for the synthesis of organolead salts of halogens or of carboxylic acids.

b) From Hexaorganodileads

Hexaorganodilead compounds react readily with halogen acids and metal salts but a complex mixture of products is usually formed. The hexaorganodilead can however be reacted with an oxidising agent and by a further reaction the R_3PbX compound is formed.

c) Metathesis reactions

The desired organolead salt can be prepared from another organolead salt by exchange of anions. Typical reactions are:-

$$R_3PbOH + HX = R_3PbX + H_2O$$
$$R_3PbCl + MX = R_3PbX + MCl$$

$$R_3PbCl + AgX = R_3PbX + AgCl$$

Factors which govern metathesis reactions of inorganic lead salts usually apply to the above reactions.

d) Reaction of teraorganolead compounds with metal salts

Tetraorganolead compounds react with a wide variety of metal halides and metal carboxylates.

$$R_4Pb + nMX = R_{4-n}PbX_n + nRM$$

e) Synthesis from inorganic lead salts

Few direct syntheses of organolead salts from inorganic lead salts or metal salts are known. However one well known reaction of this type is the formation of aryllead triacetate from lead tetra-acetate and diaryllead mercury compounds

$$Pb(OOCR)_4 + Ar_2Hg = ArPb(OOCR)_3 + ArHgOOCR$$

(Panov et al., 1956; Criegee et al., 1957).

CHEMICAL PROPERTIES OF ORGANOLEAD SALTS OF THE TYPE R_3PbX AND R_2PbX_2

Organolead salts are thermally unstable. Both R_3PbX and R_2PbX_2 compounds decompose at elevated temperatures to give RX, PbX_2 and R_4Pb.

$$2R_3PbX = R_4Pb + R_2PbX_2$$

$$2R_2PbX_2 = R_3PbX + (RPbX_3)$$

$$(RPbX_3) = RX + PbX_2$$

(Austin, 1932; Calingaert, et al.,1948; Evans, 1938).

Organolead halides decompose during steam distillation to yield a hydrocarbon

$$2R_3PbX = R_4Pb + PbX_2 + R\text{-}R$$

$$R_2PbX_2 = PbX_2 + R\text{-}R$$

(Calingaert et al., 1948).

Certain triorganolead carboxylates undergo decarboxylation on

heating.

$$R_3PbOOCCHR^1COOR^{11} = R_3PbCHR^1COOR^{11} + CO_2$$

(Kocheshkov & Aleksandrov, 1934)

The majority of organolead salts are stable to hydrolysis since they can easily be prepared in aqueous solution. However diaryllead dicarboxylates undergo hydrolysis under relatively mild conditions. Trialkyllead and triaryllead salts react readily with halogens.

$$R_3PbX + X_2 = R_2PbX_2 + RX$$

Similarly they will react with anhydrous hydrogen halides.

$$R_3PbX + HX = R_2PbX_2 + RH$$

Weak acids such as acetic acid will undergo reaction but then elevated temperatures and/or catalysts are often necessary. Dilute aqueous acids attack organolead salts only slowly or not at all (Willemsens, 1964).

PHYSICAL PROPERTIES OF ORGANOLEAD SALTS

The organolead salts of the types R_3PbX and R_2PbX_2 vary from crystalline high-melting compounds to low melting solids and liquids. The nature of both the organic group R and the acid anion X influences the melting point. Aryllead salts are generally higher melting than their alkyllead analogues. With alkyllead salts, the melting points decrease with increasing chain length of the alkyl group.

The salts of the lower alkyl derivatives of simple acids exhibit good solubility in water but this decreases with increasing chain length. Aryllead salts tend to be more soluble in organic solvents such as chloroform than in water.

Usually organolead salts are stable and can be stored for unlimited periods of time, although the trialkyllead halides and the

dialkyllead dihalides decompose slowly, especially in solution, while the di- and trialkyllead hydroxides are unstable (Shapiro & Frey, 1968).

Dipole moment measurements

The dipole moments of several organolead halides have been reported in two papers. Lewis et al. (1940) reported the following values (in Debyes): trimethyllead chloride 4.47, triethyllead chloride 4.39, diethyllead dichloride 4.70, diethyllead dibromide 4.46, triphenyllead chloride 4.21, triphenyllead bromide 4.21 and triphenyllead iodide 3.73. The values obtained by Malatesa and Pizzotti (1943) agreed in only one case, that of triethyllead chloride which had a dipole moment of 4.66 Debyes. Other values reported were triethyllead bromide 4.88, triphenyllead bromide 0.81 and triphenyllead chloride 2.32 Debyes. These large values for the dipole moments have been interpreted as meaning that the organolead halides are about 25-34% ionized making them comparable to the lead II halides (Smyth, 1941; Lewis et al., 1940).

Conductiometric measurements

Conductiometric measurements on triphenyllead chloride dissolved in pyridine and dimethylformamide showed it was not an electrolyte in these systems (Thomas & Rochow, 1957a & 1957b). From the conductivity of triphenyllead chloride in liquid hydrogen chloride it was concluded that ionization to Ph_3Pb^+ did not occur to any great extent (Peach & Waddington, 1961).

Infrared Spectra

It has been proposed from the infrared spectra of trimethyllead acylates that these compounds probably have a planar $MePb^+$ cation and a carboxylate anion (Okawara & Sato, 1961). However Janssen et al.

(1963) concluded from the infrared spectra of trialkyltin and trialkyllead acylates that these compounds exist in the solid state as coordination polymers in which each lead atom is penta-coordinated by three alkyl groups and two oxygen atoms belonging to different acylate groups. In dilute solution they behaved like normal esters giving indications neither for an ionic nor a polymeric structure.

THE TOXICOLOGY, PHYSIOLOGY AND BIOCHEMISTRY OF ORGANOLEAD COMPOUNDS

Tetraethyllead is manufactured on a large scale and distributed throughout the world. Soon after its introduction the symptoms of its intoxication were documented. Kehoe in 1925 and Machle in 1935 described in detail the clinical picture. Mild intoxication causes headache, irritability, restlessness, anxiety, fatigue, insomnia with bad dreams, anorexia, vomiting and diarrhoea. There is sometimes a persistent metallic taste. In more severe poisoning these symptoms are followed by hallucination and delusions, followed by coma and death. The physical signs present are tremors, increased tendon reflexes, hypothermia, bradycardia, hypotension and loss of weight. The usual symptoms of inorganic lead poisoning - abdominal colic, stippling of the red blood cells, anaemia and an elevation of the urinary porphyrins are not usually present.

Observations from animal studies have described the toxic actions of a large number of organolead compounds. The signs of illness induced in rats by oral administration of tetraethyllead and triethyllead chloride were similar. One to three days after receiving the compound the animals became hyperirritable and fine continuous tremors of the whole animal developed. Convulsions occurred, sometimes spontaneously and sometimes in response to auditory or tactile

stimulations (Springman et al., 1963). Intravenous infusion of tetraethyllead and intraperitoneal administration of triethyllead chloride produced similar symptoms. Of the two tetraethyllead was the less toxic with an LD_{50} value of 15.4mg/kg, while that for triethyllead chloride was 11.2mg/kg. The symptoms described are very similar to those produced by oral administration of the compounds. After lethal doses of either compound the animals became quiet and uneasy immediately after injection. Twenty-four hours later the rats behaved very excitably especially in their reactions to sudden noise and movement in the proximity of their cages. During the following twenty-four hours they developed, in addition, severe continuous tremors throughout the body. These were accompanied by violent convulsions leading to death. Rats given just sublethal doses also became very excitable and developed a continuous tremor of the lower jaw only. Pairs of rats were observed facing each other as if sparring (Cremer, 1959). Davies et al. (1963) also observed this combative posture in rats chronically inhaling an atmosphere containing tetraethyllead. Dogs exposed similarly gradually developed tremors and muscular twitchings, first in their extremities and then in the trunk. The extremities moved continuously when the animals were lying down. When standing their legs were stiff and widespread.

Non-specific lesions involving the brain and spinal cord, the liver, the kidneys and the lungs were seen in both species. The lesions in the brain were distributed fairly diffusely through the cerebral cortex, the brain stem and medulla. Clumping and shrinkage of cells, vacuolar degeneration and cellular necrosis were seen. The lesions of the liver consisted of swelling of hepatic cells, nuclear enlargement, prominence of the nucleoli and vacuolar degeneration.

The Küpffer cells were generally swollen. Similar lesions occurred in the kidney with swelling and necrosis in the convoluted tubules and vacuolar degeneration of collecting tubules. Multiple small haemorrhagic lesions and general oedema were observed in the lungs. Most of the animals had some degree of pneumonitis (Davies et al., 1963).

Evidence would suggest that the toxic effect of tetraethyllead is due to the formation of the triethyllead ion, thus accounting for the identical symptoms observed after parenteral administration of the two compounds (Cremer, 1959). The triethyllead ion has been detected in the liver of rats following inhalation of tetraethyllead (Stevens et al., 1960) and in the liver, kidney, blood, brain and muscle after intravenous administration (Cremer, 1959; Bolanowska, 1968). The conversion of tetraethyllead to triethyllead occurs in the presence of liver slices but the brain and kidney had negligible activity. The activity of the liver cells was associated with the microsomal and soluble material fraction and was inhibited by the substance SKF 525A (Cremer, 1959). Bolanowska (1968) showed that triethyllead was excreted in both urine and faeces after intravenous administration of tetraethyllead but the rate of excretion never exceeded 1% of the dose per day. Twenty-four hours after receiving 62μmol/kg of tetraethyllead, triethyllead accounted for 50% of the lead in the internal organs. Seven days after administration of 31μmol/kg however, almost all the lead in the internal organs was found in the form of triethyllead.

When triethyllead chloride was added to slices of brain cortex in vitro, the oxygen consumption was lowered and the production of lactic acid increased, showing that the complete combustion of glucose

was inhibited (Cremer, 1959 & 1962; Aldridge et al., 1962). This occurred in the presence of small amounts of triethyllead chloride (7×10^{-7}M and 2×10^{-6}M). Tetraethyllead at a concentration a hundred times greater had no effect on glucose metabolism. Further evidence that the triethyllead ion was the toxic agent was an identical alteration of glucose metabolism by brain slices from rats which had received triethyllead chloride in vivo. Furthermore brain slices from rats that had received tetraethyllead in vivo showed this same alteration in metabolism (Cremer, 1959). Although triethyllead chloride inhibited glucose metabolism of brain slices it had no effect on kidney slices and had little effect on the metabolism of pyruvate or glutamate by brain slices (Cremer, 1962). Trialkyllead compounds including triethyllead chloride were shown to be inhibitors of oxidative phosphorylation in mitochondria isolated from liver (Aldridge et al., 1962).

Galzigna et al. (1973) showed that triethyllead chloride inhibits the in vitro cholinesterase activity of rat diaphragm and in vivo the serum cholinesterase of the dog. The authors proposed that the action of Et_3Pb^+ on the central nervous system could be explained by a combination of effects resulting from an upset of cholinergic and adrenergic pathways due to the formation of endogenous psychotogenic complexes.

McClain & Becker (1972) found tetraethyllead, tetramethyllead and trimethyllead chloride to be essentially non-teratogenic in Sprague-Dawley rats when administered either in early organogenesis or in late organogenesis. An enhanced incidence of lymphomas was noticed in female mice dosed subcutaneously with tetraethyllead one, seven, fourteen and twenty-one days after birth. These tumours however,

developed late in life in contrast to the general tendency to earlier development of such tumours following administration of strong carcinogens to neonatal mice (Epstein & Mantel, 1968).

Some comparative studies have been made between the toxicity of tetramethyllead and tetraethyllead. The results of an inhalation study with rats showed that tetraethyllead was more toxic by this route. Exposure to an atmosphere containing 0.77mg/l of tetraethyllead for sixty minutes caused 100% mortality, while after exposure to a concentration of tetramethyllead of 9.84mg/l no obvious signs of intoxication apart from slight hyperexcitability were seen (Cremer & Callaway, 1961). However, Davies et al. (1963) found that similar symptoms were seen after either compound. The animals became irritable, then uncoordinated with an apparently combative posture, followed by convulsions and death. After exposure to an atmosphere containing tetramethyllead at 1.2mg/l for 150 periods each of 7 hours, four out of ten animals had died, while with tetraethyllead none had died. At concentrations above this, the rats that inhaled tetramethyllead survived two to three times longer than those exposed to tetraethyllead. A corresponding experiment on dogs showed that they responded in a different manner to rats in that those exposed to tetramethyllead at concentrations of 1.2 and 2.3mg/l succumbed much sooner than did those that inhaled tetraethyllead at the same concentration.

Tetramethyllead is less toxic than tetraethyllead when given by either the oral or the intravenous route. Orally tetraethyllead is several fold more toxic than tetramethyllead (Springman et al., 1963). When given intravenously, tetraethyllead has an LD_{50} value of 15.4mg/kg (Cremer, 1959) whereas no sign of poisoning was seen in rats which had received 34.4mg/kg of tetramethyllead (Cremer & Callaway, 1961).

The symptoms produced by tetramethyllead, tetraethyllead and the trialkyllead chlorides were similar, hyperexcitability, tremors and convulsions occurring (Cremer & Callaway, 1961; Springman et al., 1963).

Two rabbits after receiving trimethyllead chloride by intraperitoneal injection at either 7.5 or 15.0 mg/kg, responded immediately by lying stretched out with a wriggling body movement. The rabbit which received the higher dose died twelve hours after injection. Tetramethyllead also appears less toxic to the rabbit than tetraethyllead when injected intravenously. A dose of 40mg/kg of tetramethyllead produced no signs of poisoning in two rabbits while 31mg/kg of tetraethyllead when given to a rabbit caused death in sixteen hours.

Trimethyllead chloride inhibited the in vitro oxidation of glucose by slices of brain cortex while tetramethyllead at a concentration ten times higher caused no inhibition. There was evidence of a slow rate of conversion of tetramethyllead to trimethyllead chloride by rats in vivo.

Oral administration of both tetrapropyllead and tripropyllead chloride to rats produced an unsteady gait and generalised weakness leading to death. Like trimethyllead chloride, tripropyllead inhibited the oxidation of glucose by slices of rat brain cortex, while tetrapropyllead was a hundred times less active (Cremer & Callaway, 1961).

Diethyllead dichloride given intraperitoneally at doses of 20 and 40mg/kg apart from causing initial uneasiness and loss of appetite during the first few days, had no behavioural effects over a twelve week observation period. Orally it was much less toxic to rats than triethyllead chloride. It caused weakness in the animals which

eventually led to their death (Springman et al., 1963). The utilization of lactate by rat brain brei was affected by diethyllead dichloride, the oxygen uptake decreased and the levels of pyruvate increased. Inclusion of dimercaprol or thioglycollic acid in the incubation medium almost completely prevented these effects. There was some alteration of the metabolism of glucose by brain slices taken from rats killed twenty-four hours after receiving 40mg/kg of diethyllead dichloride by intraperitoneal injection. The oxygen uptake was slightly lowered and the level of lactic acid raised (Cremer, 1959). In a study of the metabolism of diethyllead dichloride, 62% of the lead found in a whole body homogenate of rats twenty-four hours after they had received an intravenous dose of 48.3μmol/kg of the compound, was found to be in an organic form. In an excretion study, during the first day 80% of the urinary lead was found to be organic, while after three days this value had dropped to 50% (Bolanowska, 1968).

The sternutatory properties of a large number of organolead salts were investigated in a series of papers. The activity of the trialkyllead salts was found to increase with increasing chain length of the alkyl group going from methyl to propyl, then to decrease with butyl. Trimethyllead salts were found to be almost without sternutatory activity, as were the dialkyllead salts (Saunders & Heap, 1949; Saunders & Stacey, 1949). The most potent sternutator found was tri-n-propyllead methane sulphonamide (Saunders, 1950).

The antimicrobial and antifungal activity of trialkyl- and triphenylmetal acetates of germanium, tin and lead have been compared. Certain of the organolead compounds were active against fungi and Gram-positive bacteria, the highest activity being found with tributyllead and tripentyllead acetate. Increase in the chain length

above this caused decrease in activity. Much higher concentrations of the compounds were required to inhibit growth of the two Gram-negative bacteria tested (Sijpesteijn et al., 1962).

THE PROPOSED APPLICATIONS OF TRIPHENYLLEAD ACETATE

Triphenyllead acetate is an effective molluscicide and two possible applications of this property have been investigated, namely the incorporation of the compound into an anti-fouling paint and as a molluscicide for the control of the vector snail of bilharzia.

During a series of tests carried out in the Bay of Cadiz on the anti-incrustation properties of various vinyl and oleo-resinous paints in which the cuprous oxide had been partially or totally replaced by an organolead compound, it was observed that paint containing triphenyllead acetate showed excellent anti-incrustation properties (Guillen et al., 1969). This was confirmed by Dick and Novacki (1970) who carried out static raft type exposures in several climates and an accelerated testing programme on anti-fouling paints containing organolead compounds including triphenyllead acetate.

After it had been shown that triphenyltin acetate had the low LC_{50} value of 0.05ppm against Biomphalaria glabrata, one of the vector snails of bilharzia (Hopf & Muller, 1962; Deschiens & Floch, 1962) a screening programme was established to find an organotin or organolead compound which was equally effective as a molluscicide but which was less phytotoxic. Triphenyllead acetate filled both these requirements and had the further advantage of being cheaper to produce. In a field trial it was found that the compound had an LC_{50} value of 0.05-0.1ppm after 24 hours exposure and 224 hours recovery against Biomphalaria glabrata, a value comparable with that of established

molluscicides (Hopf et al., 1967). De Souza and Paulini (1966) tested the molluscicidal activity of triphenyllead acetate towards the eggs of Biomphalaria glabrata 0-1 and 4-5 days after they had hatched. Newly laid eggs showed the highest susceptibility with an LC_{50} value of 0.13ppm after 24 hours exposure. The susceptibility decreased in older eggs (LC_{50} = 0.23ppm) and in the mature snail (LC_{50} = 0.48ppm). Biomphalaria glabrata, when exposed to sublethal concentrations (0.04ppm, 0.03ppm and 0.02ppm) of triphenyllead acetate, died from the effects of the compound over a period of time. However a sub-lethal concentration of 0.01ppm did not affect the infection rate by Schistosoma mansoni (Hira & Webbe, 1972).

In addition to its molluscicidal properties triphenyllead acetate when incorporated in poly(vinyl acetate) emulsion paint and poly(alkyl-resin) lacquer confers fungicidal properties to them (Giesen, 1966)

TOXICITY TESTING OF TRIPHENYLLEAD ACETATE

Investigation of the toxicity of triphenyllead acetate and triphenyllead chloride to mammals was carried out by the University of Exeter and by the Hine Laboratories. The work carried out by Dr. P.J. Walker of the Department of Zoology, University of Exeter included:

(1) Acute toxicity in mice following intraperitoneal administration
(2) Acute toxicity in mice following oral administration
(3) Acute toxicity in rats following oral administration
(4) Acute toxicity in rabbits after oral administration
(5) Acute toxicity in guinea-pigs after oral administration
(6) Acute toxicity in goats after oral administration
(7) Breeding trials on mice fed on a diet containing triphenyllead

acetate.

(1) Acute toxicity of triphenyllead acetate to mice following intraperitoneal administration.

Mice of Parkes' strain weighing between 25 and 39g received an intraperitoneal injection of triphenyllead acetate suspended in olive oil (5mg triphenyllead acetate per 10ml of oil). Five groups of mice, each group containing six mice, received doses of 3.5, 5.0, 7.0, 10.0 and 14.0mg/kg of the compound. After six weeks observation an acute LD_{50} value of 8.0mg/kg was obtained.

(2) Acute toxicity in mice following oral administration.

One hundred and sixty mice were used in this study. Deaths occurred in both experimental and control groups and thus a definite value for the LD_{50} was not obtained. However it was estimated that the acute LD_{50} value for triphenyllead acetate in mice after oral administration lies between 400 and 600mg/kg.

(3) Acute toxicity in rats following oral administration.

Hooded rats of the Lister strain weighing 160-240g were used and they received a suspension of triphenyllead acetate in gum tragacanth. Dose levels of 100, 143, 204, 292, 418 and 600mg/kg were administered and the animals observed for fourteen days. An LD_{50} value of 318mg/kg was obtained, deaths occurring in the higher dosage groups between five and twelve days after dosing.

(4) Acute toxicity in rabbits after oral administration.

Adult rabbits of various strains received doses of 100 and

400mg/kg of triphenyllead acetate or 200 and 400mg/kg of triphenyllead chloride. It was concluded that the LD_{50} value lies between 100 and 400mg/kg for triphenyllead acetate and between 200 and 400mg/kg for triphenyllead chloride.

(5) Acute toxicity in guinea-pigs after oral administration.

Adult guinea-pigs weighing 640-1150g were dosed orally with suspensions of triphenyllead acetate or triphenyllead chloride in gum tragacanth at dose levels of 100, 200 or 400mg/kg. Two guinea-pigs were used in each group. No animals given triphenyllead acetate died suggesting that the LD_{50} value is above 400mg/kg. One animal given triphenyllead chloride at 400mg/kg died suggesting that it is more toxic than triphenyllead acetate to guinea-pigs.

(6) Acute toxicity to goats after oral administration of triphenyllead acetate.

Young goats were dosed with a slurry of triphenyllead acetate in an ethanolic solution of gum tragacanth. A male and a female goat were dosed at three levels, 95, 180 and 342mg/kg. No ill effects were observed in any animal.

(7) Feeding and breeding trials on mice fed on a diet containing triphenyllead acetate.

Mice of Parkes' strain were fed for two weeks on diets containing triphenyllead acetate while the sexes were kept separate. While still receiving the diets the animals were paired and all provisions given for successful breeding. The weights of the parents, the timing, number and growth of young, haematological changes and general con-

dition of all mice were recorded over a period of six months.

The diets given consisted of a powdered standard breeding diet, to a kilo of which was added 50ml of molasses. Triphenyllead acetate dissolved in either olive oil or ethanol was added to this mixture. The following concentrations were used: triphenyllead acetate in oil - control, 80ppm, 100ppm, 120ppm, 156ppm and 195ppm; triphenyllead acetate in ethanol - 100ppm and 195ppm.

The production of young and the frequency of having litters was not affected by triphenyllead acetate in the diet. No abnormalities were observed in the young. The growth rates of the offspring were noted and they showed that even at the highest dose the presence of triphenyllead acetate in the diet had no observable effect on the initial weight, the rate of weight gain or on the final adult weight. Male and female parent mice were examined haematologically after six weeks on the diets. The total number of cells per millilitre of blood decreased with increasing amounts of triphenyllead acetate in the diet. Both the lymphocyte and the polymorphonuclear leucocyte count dropped reaching 72% and 65% of the control group readings in rats maintained on the diet containing 195ppm of triphenyllead acetate.

At the end of six months the parents and offspring were weighed and examined externally. At the higher levels (156ppm and 195ppm) there was a general loss of condition in the fur and well-being of the animals. The principle lesions were loss of hair and inflammation of the underlying skin of the head and neck. This effect was less noticeable in the lower level test diets but was not entirely absent in the controls.

A sample of the control and the 195ppm groups were dissected. All the viscera were of normal size, colour and texture and no tumours

were found.

Prolonged toxicity testing of triphenyllead acetate was carried out by the Hine Laboratories on behalf of the International Lead Zinc Research Organisation. This consisted of:

(a) 90-day aerosol inhalation in rats

(b) 180-day oral feeding in rats

(c) 45-day dermal toxicity testing in rabbits

(d) Skin sensitivity in guinea-pigs

(e) Repeated insult patch in humans

(a) 90-day aerosol inhalation in rats.

This was carried out on groups containing ten male and ten female rats of the Long-Evans strain weighing 100 to 110g. The animals were exposed to aerosols of triphenyllead acetate in ethanol in a specially designed inhalation chamber. The aerosols were stabilized at 50, 150, and 450$\mu g/m^3$ of triphenyllead acetate while the control rats received only ethanol. The animals were exposed to the aerosol for 7 hours a day, 5 days a week, for 90 days. Body weights were measured during the course of the experiment and at its end the animals were autopsied. Blood samples were taken for haematological examination, clinical chemistry determinations and lead analyses. The organs were examined "in situ" and the body to organ weight ratio for certain organs determined. Half of the organs were examined histologically while the lead content of the remainder was measured.

At the end of the experiment no difference could be seen between the general health of the test animals and the controls nor was there any significant difference in weight gain. Comparison of the organ weights of the test and control animals showed a decreased weight of

the kidneys of female rats from all three test groups. Histological examination of tissues showed an increased incidence of kidney lesions in the test animals. There was no depression of the haemoglobin content of the blood or of the haematocrit while the red and white blood cell counts were within normal limits as were the clinical chemistry determinations. Lead analyses of tissues showed differences between certain organs of the highest exposure test group and those of the control animals. There was increased deposition of lead in the kidney, brain, liver and bone but not in the blood and lung. The highest concentration of lead was found in the femur.

(b) 180-day oral feeding in rats.

Male and female rats of the Long-Evans strain with starting weights of 100 to 110g were used in this experiment. Four groups of rats, each group containing ten rats of each sex, were used and these were allowed free access to experimental diet and water. The diet consisted of a standard granular rat food containing either 50, 150 or 450ppm by weight of triphenyllead acetate while the control group received untreated food. During the course of the experiment the animals were weighed every fortnight while the food consumption, mortalities and general health of the animals were noted. At the end of the experiment rats were placed in metabolism cages for sixteen hours and maintained on a triphenyllead acetate free diet. Lead analyses were carried out on the faecal and urine samples produced. All the animals were killed and examined. Blood samples were taken for a determination of the haemoglobin content, haematocrit and red and white cell counts. Organ weights were determined for liver, kidney, heart and testes. Lead analyses were carried out on

the liver, blood, brain and femur while the adrenals, brain, heart, gonads, kidney, liver, lung, small intestine, large intestine, spleen, stomach, thyroid and bladder were examined histologically.

No deaths occurred during the course of this feeding programme and no difference was observed in the general activity and alertness of the test and control groups. There was, however, an increased consumption of food by female rats maintained on the 450ppm triphenyllead acetate diet. Rats maintained on the 50 and 150ppm triphenyllead acetate diet gained weight at a comparable rate to control rats but there was a reduction in weight gain by male and female rats maintained on the 450ppm diet. The following organ to body weight ratios were raised: male and female liver ratios of rats on the 450 ppm diet; the heart ratio of female rats on the 150ppm diet and the kidney ratio of male rats on the 450ppm diet. Pathological examination of the organs showed no change which could be attributed to diet, either at the gross level or upon microscopical examination.

Haematological examination showed a decrease in the percentage of haemoglobin present in the blood of male and female rats maintained on the 450ppm of triphenyllead acetate diet and of male rats fed on the 150ppm diet. Clinical chemistry determinations on blood showed all values to be within normal limits.

Lead analyses showed increased storage of lead in all rats on test diets. The storage increased with increasing concentration of triphenyllead acetate in the diet. The highest concentration of lead was found in the femur. The lead content of urine and faeces from female rats fed on test diets was elevated above that of control animals. At the highest test diet the urinary output of lead was about ten times that of control rats while the faecal excretion was

increased about forty times.

(c) 45-day dermal toxicity in rabbits.

Four groups of New Zealand White rabbits were used, each group containing three male and three female animals. The animals were shaved and the following day received 1ml of either 0.01%. 0.033% or 0.10% solution of triphenyllead acetate in alcohol applied over half the shaved area. Control animals received the same volume of ethanol. Applications were made daily five times a week for nine weeks. The degree of erythema and oedema were recorded three times a week, the untreated skin area being used for comparison. After the last application blood samples were drawn and the animals killed. Gross inspection of the organs was made and sections of liver, heart, kidney and skin taken for microscopic examination. Lead analyses were carried out on blood and liver.

All animals showed a progressive weight gain during the course of the experiment and at its termination there were no significant differences in the terminal weights of the various groups. Microscopic examination showed no significant changes in the viscera examined while lead analyses of the liver and blood showed increasing accumulation of lead with increasing dose of triphenyllead acetate.

Flaking and scaling of the skin was seen in all animals which received triphenyllead acetate. This led to cracking and bleeding of the skin in some animals. After the initial application pronounced oedema was seen in many of the animals, especially those which had received the highest concentration solution. This subsequently reduced following further applications. In general, the area of skin affected was greater with the high dose than with the low one.

(d) Skin sensitization in guinea-pigs

Twenty Dunkin Hartley guinea-pigs of weight 300-500g were shaved on the back and flanks. The following day ten of the animals had an area of the skin, about 2cm in diameter painted with a 0.05% solution of triphenyllead acetate in alcohol while the other ten animals were treated with a positive control solution of 0.25% dinitrochlorobenzene in dimethylformamide. This treatment was repeated for four days and the challenge application made one week after the initial application. The presence or absence of a red flare was noted after twenty-four hours.

Of the ten animals which received the triphenyllead acetate solution, two had borderline areas of redness but the other eight animals showed no reaction. It was concluded that a 0.05% solution of triphenyllead acetate is either non-sensitizing or very weakly sensitizing.

(e) Repeated insult patch test on humans

Two solutions of triphenyllead acetate, 0.01% and 0.05% in alcohol, were used in this experiment. Ten female subjects had two Elastoplast coverlets, each containing 0.25ml of one of the solutions, applied to their forearms. The subjects were asked to leave the plasters on for twenty-four hours. At the end of this time they were removed, the skin wiped with iso-propyl alcohol and the degree of oedema and erythema read off. The reading was repeated one hour later.

At the end of twenty-four hours four of the subjects had erythema and oedema. Three of the subjects experienced irritation of sufficient intensity to cause premature removal of the test

patches. At the 25 hour check the erythema persisted in two of these subjects and a third subject also had developed slight reddening of the skin.

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND METHODS

CHEMICALS

Triphenyllead acetate was supplied by Pure Chemicals Ltd. (Kirby, Liverpool) as a white powder m.p. 204° (uncorr.) and was recrystallized from acetone before use. Diphenyllead diacetate (m.p. 201-210°) and phenyllead triacetate (m.p. 77-86°) were gifts from the Institute for Organic Chemistry TNO (Utrecht, Holland). Hexaphenyldilead (Krause & Grosse, 1937) was prepared as described in the literature.

Phenol (m.p. 42°), catechol (m.p. 103°), resorcinol (m.p. 109°) and quinol (m.p. 170°) were purchased and purified. Phenylglucuronide, m.p. 161-162°, $(\alpha)_D^{17}$ -78.5 (c, 2 in water) was a sample previously prepared in this laboratory (cf. Garton et al., 1949). Potassium phenyl sulphate, potassium 4-hydroxyphenyl sulphate (quinol monosulphate) and 4-hydroxyphenylglucuronide (quinol monoglucuronide) were also samples previously prepared in this laboratory (Capel et al., 1972). Phenyl toluene p-sulphonate and quinol ditoluene p-sulphonate were prepared as described by Vogel, (1956a).

Tri(U-^{14}C)phenyldilead acetate

(a) Hexa(U-^{14}C)phenyldilead was made by a modification of the method of Krause and Grosse (1937).

(U-^{14}C)Bromobenzene (22.42mg; 22.3μCi/mg) was purchased from the Radiochemical Centre, Amersham, Bucks. This was diluted with 70mg of non-radioactive bromobenzene.

Dry magnesium turnings (0.045g) were placed in a three-necked flask fitted with a reflux condenser and a magnetic stirrer. Anhydrous ether (0.11ml) and (U-^{14}C)bromobenzene (0.09ml) were added, together with one small iodine crystal. The flask was heated gently until the reaction started, then 0.13ml of (U-^{14}C)bromobenzene in 0.89ml of

anhydrous ether was added. The mixture was stirred and refluxed for 1h after which $PbCl_2$ (0.278g) was added. The mixture turned brown as diphenyllead formed and metallic lead precipitated soon after. Stirring was continued for 3h at room temperature. Crushed ice (1.5g) was added rapidly to decompose the Grignard complex and the ether was removed *in vacuo*. The resulting mass was filtered off and dried *in vacuo* over P_2O_5. Hot chloroform (3 x 2ml) was used to extract the dried solid and the bulked extracts were concentrated to 0.6ml. Ethanol (1ml) was added whereupon yellow crystals of hexaphenyldilead precipitated. The crystals were filtered off and dried in air.

The yield of hexaphenyldilead was 0.086g, m.p. 149-152° (decomp.) (lit. m.p. 155° decomp.), a 30% chemical yield with respect to bromobenzene.

(b) (^{14}C)bis(triphenyllead) oxide was prepared from the (^{14}C)hexaphenyldilead by the method of Bahr (1949) as adapted by Willemsens and van der Kerk (1965) as follows:-

The hexa(U-^{14}C)phenyldilead was suspended in acetone (4ml) and a solution of potassium permanganate (0.012g) in acetone (3ml) was added dropwise. A brown precipitate of $K_2O.2MnO_2$ formed. Addition of potassium permanganate was continued until a permanent pink colour was obtained. The reaction mixture was allowed to stand for $\frac{1}{2}$h, then the precipitate of $K_2O.2MnO_2$ was filtered off leaving a solution of bis(triphenyllead) oxide in acetone.

(c) Tri(U-^{14}C)phenyllead acetate was made from the bis(triphenyllead) oxide by the method of Willemsens and van der Kerk (1965).

The calculated quantity of glacial acetic acid (0.11ml) was added to the solution of (^{14}C)bis(triphenyllead) oxide and the mixture was allowed to stand in the cold (5°) until white crystals of triphenyl-

lead acetate appeared (about 30min). After evaporation to dryness *in vacuo* the crystals were washed into a test-tube with ether and the ether removed with a stream of nitrogen. The yield of impure tri-(U-^{14}C)phenyllead acetate was 0.087g, m.p. 197-198°. Thin-layer chromatography in two systems, hexane/benzene (1:1 v/v) and carbon-tetrachloride/ethanol (20:1 v/v) showed the compound to be impure. Therefore the triphenyllead acetate was recrystallized from acetone to give 0.058g of white needle-shaped crystals m.p. 200°, of specific radioactivity 0.58μCi/mg (an overall chemical yield of 18% based on bromobenzene). It was shown to be radiochemically pure by thin-layer chromatography (see above) and to have a purity of 100±1.4% by reverse isotope dilution.

Triphenyl(^{203}Pb)lead acetate

The ^{203}Pb was a gift from the M.R.C. Cyclotron Unit (Hammersmith Hospital, London W.12, U.K.) and was supplied as carrier free $^{203}PbCl_2$ in normal saline having an activity of 1.0mCi/ml. Lead nitrate (0.3310g) was added to the solution of $^{203}PbCl_2$ in saline (1.5ml) and lead chloride was precipitated by addition of an excess of 10M-HCl. The precipitate was filtered, washed with acetone and dried in an oven at 110°. Non-radioactive lead chloride (0.0111g) was added to the precipitate. Triphenyl(^{203}Pb)lead acetate was then prepared by the method given for tri(U-^{14}C)phenyllead acetate. The triphenyl(^{203}Pb)lead acetate formed was recrystallized three times from acetone to give 0.0412g of white needle-shaped crystals (m.p. 194°). It was shown to be radiochemically pure by thin-layer chromatography and to have a purity of greater than 98% by reverse isotope dilution.

Tri(^{3}H)phenyllead acetate

(a) Preparation of p-tritiobromobenzene

This was synthesized by adapting the method of Melander (1951). p-Dibromobenzene was purchased (Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.) and was recrystallised from ethanol to give pale yellow platelets m.p. 87°. Tetrahydrofuran was dried using lithium aluminium hydride and redistilled, the fraction b.p. 65-66° being collected.

A Grignard complex was formed from magnesium turnings (0.4068g) and p-dibromobenzene (4g) with tetrahydrofuran (15ml) as solvent. Tritiated water (0.3ml, 5Ci/ml, The Radiochemical Centre, Amersham, Bucks., U.K.) was added while the reaction mixture was stirred rapidly and external cooling applied to the flask, followed by 2N-HCl (1ml) to complete the hydrolysis. The tetrahydrofuran was distilled off and the mixture extracted with ether (2x15ml) which was washed with water (2x25ml), with 2N-sodium carbonate solution (2x25ml), again with water (2x25ml) and finally dried over anhydrous sodium sulphate. The ether was distilled off and the brown viscous fluid remaining was distilled in a micro-distillation apparatus (Quickfit and Quartz Ltd., Stone, Staffs., U.K.). Four fractions were collected of b.p. (34-90°), (90-138°), (138-148°) and (148-118°).

(b) Determination of the purity of the distilled p-tritiobromobenzene

Portions (2µl) of a 10% v/v solution of each distillate in ether were analysed by g.l.c. as follows. For g.l.c. an F & M model 402 gas-liquid chromatograph (Hewlett-Packard Inc., Pasadena, Calif., U.S.A.) with flame ionization detector was used. The glass column (1.83m, internal diameter 3mm) was packed with AW-DMCS treated Chromosorb G (100-120 mesh) coated with SE-30 (3% w/w). The conditions used

were: injection port temperature 70°, detector temperature 150°, N_2 flow rate 15ml/min, N_2, air and H_2 pressures 275, 165 and 140kN/m^2 (40, 24 and 20lbf/in^2) respectively. The temperature programming facility was used, the initial column temperature being 50° and the temperature raised by 5°/min. The retention times for benzene, bromobenzene and p-dibromobenzene were 4.6min, 15.7min and 24.4min respectively (Fig. 3). The percentage of bromobenzene in each fraction was determined by cutting out the area of paper under each peak of the gas-liquid chromatogram trace and weighing it. The three highest boiling point fractions were combined and used for the remaining part of the synthesis. These contained 80%, 93% and 90% bromobenzene respectively.

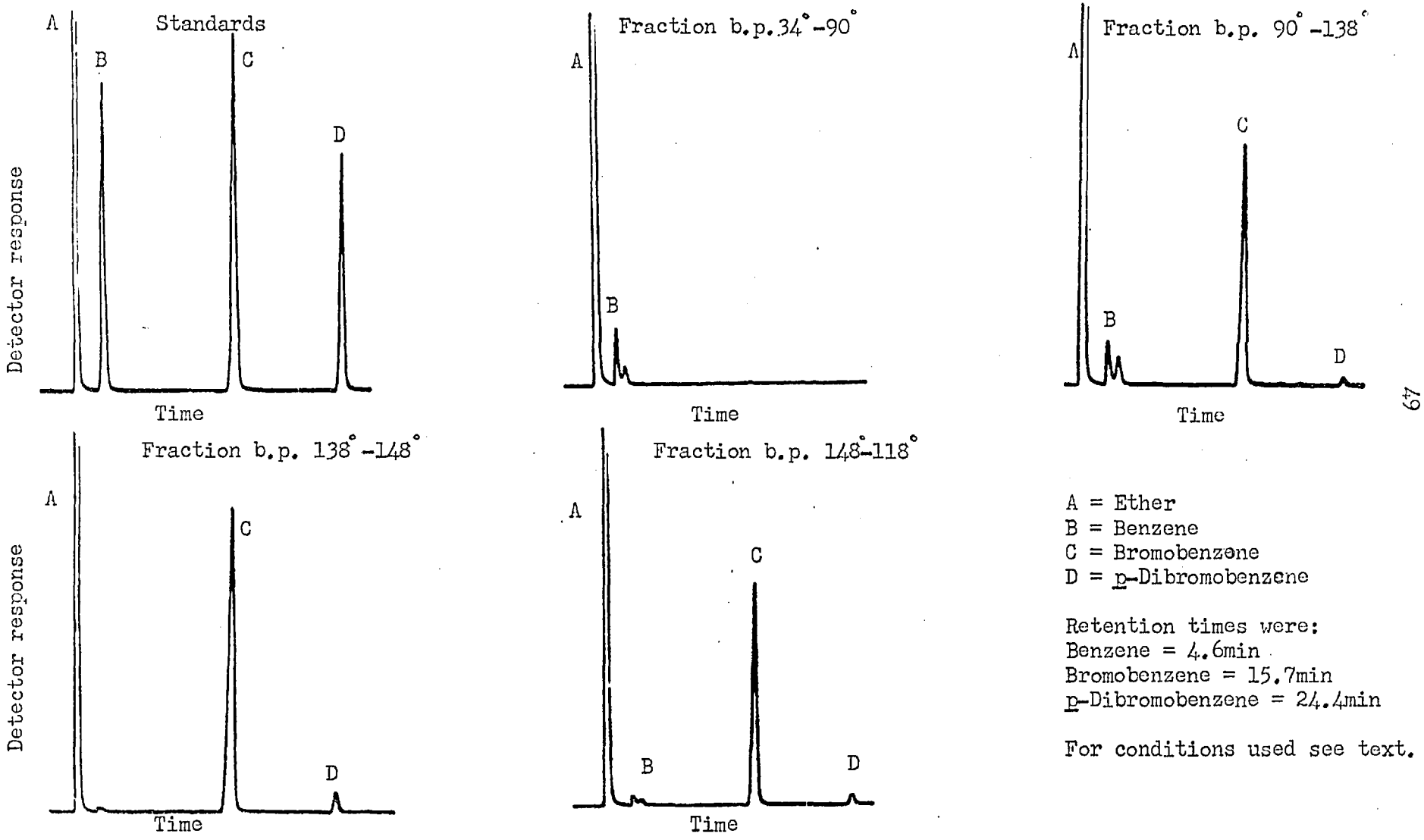
(c) Preparation of tri(^{3}H)phenyllead acetate

This was carried out as described for the synthesis of tri-(U-^{14}C)phenyllead acetate. The final product was recrystallized firstly from acetone/hexane and then ethanol/water to give 0.1134g of small white crystals m.p. 195° (uncorr.) of specific radioactivity 173μCi/mg. It was shown to be at least 98% radiochemically pure by thin-layer chromatography (see chromatography section) and by reverse isotope dilution.

ANIMALS

Female Wistar albino rats (wt. 180-250g) were used throughout and these had free access to standard laboratory rat diet and water in all experiments. Metabolism cages were used for the separate collection of urine and faeces. When the expired air was examined for metabolites the animals were kept in glass metabolism cages (Metabowl, Jencons Ltd., Mark Road, Hemel Hempstead, U.K.).

Fig. 3. Gas liquid chromatography of the distilled p-tritiobromobenzene.



A = Ether
B = Benzene
C = Bromobenzene
D = p-Dibromobenzene

Retention times were:
Benzene = 4.6min
Bromobenzene = 15.7min
p-Dibromobenzene = 24.4min

For conditions used see text.

Cannulation of the common bile duct of rats was carried out as described by Abou-El-Makarem et al. (1967).

RADIOCHEMICAL TECHNIQUES

^{14}C-compounds

(1) Collection of $^{14}CO_2$.

$^{14}CO_2$ in the expired air was determined by the method of Jeffay and Alvarez (1961). Air was drawn through the metabolism cage at a constant rate and passed through two traps each containing a solution of ethanolamine in ethylene glycol monomethyl ether (1:2 v/v)(180ml). Aliquots (2ml) were assayed for radioactivity using 15ml of a scintillant consisting of a mixture of toluene and ethylene glycol monomethyl ether (2:1 v/v) containing 2,5-diphenyloxazole (5.5g/l). Ethanol (3ml) was added where necessary to form a homogeneous mixture. Counting efficiencies were determined by the "internal standard" method (Wang & Willis, 1965). Solutions in the traps were renewed at 24h intervals.

(2) Collection of ^{14}C-labelled volatile compounds.

Air was drawn through the metabolism cage at a constant rate by means of a pump, passed through one Dreschel bottle containing calcium chloride and one containing magnesium perchlorate, to remove moisture and then through two 200ml tubes in each of which was methanol (75ml) and 3mm glass beads (50ml). The tubes were kept at a temperature of -70° by means of an acetone/solid CO_2 mixture. At the end of each 24h period a portion of the methanol (1ml) was assayed for ^{14}C activity after Bridges et al. (1967).

(3) Quantitation of radioactivity in urine and faeces.

^{14}C in urine and faeces was determined with a Packard Tri-Carb scintillation spectrometer (model 3214 or 3320) as described by Bridges et al. (1967).

(4) Quantitation of urinary metabolites.

Urinary metabolites were detected by scanning paper strip chromatograms of urine (0.05-0.30ml) using a Packard Radiochromatogram Scanner Model 7200. Identification of the ^{14}C peaks was made by comparison of Rf values with those of authentic samples. The radioactivity in each spot was quantified as given by Caldwell et al. (1972). Urine was hydrolysed by either acid (refluxing for 3h with an equal volume of 10M-HCl), sulphatase (Type H-2, Sigma Chemical Co., Kingston-upon-Thames, U.K.)(incubation at 37° for 18h with an equal volume of sodium acetate - acetic acid buffer pH5.2, containing 0.15ml of enzyme), or β-glucuronidase (William R. Warner & Co. Ltd., Eastleigh, U.K,)(incubation at 37° for 18h with an equal volume of the enzyme preparation). Radiochromatogram scans were prepared of the hydrolysed samples.

Thin-layer chromatography of free phenols was carried out as follows. Urine (2ml) was hydrolysed by refluxing for 3h with 10M-HCl (2ml). The urine was extracted with ether (3 x 10ml), the extract concentrated to 1ml with a stream of nitrogen and subjected to thin-layer chromatography (see chromatography section).

(5) Determination of radioactivity in rat carcasses.

Whole rats were left to dissolve in one litre of a 20% w/v solution of sodium hydroxide in 30% aqueous ethanol for 24h. A 50ml

aliquot was neutralized with hydrochloric acid, diluted to 100ml and the ^{14}C activity determined by liquid scintillation counting (Bridges et al., 1967).

(6) Reverse isotope dilution procedures.

(a) Benzene Methanol (15ml) from the cooled tubes for the collection of volatile substances in the expired air, was taken and benzene (1g) added. The nickel cyanide clathrate complex as described by Evans et al. (1950) was made as follows:-

To a cold suspension of nickel sulphate (4.36g) in water (17.4ml) was added a solution of potassium cyanide (2.16g) in water (8.7ml) and then ammonia solution (sp.gr. 0.88, 17.3ml). This mixture was left in ice for thirty minutes, filtered through Whatman No. 1 filter paper and 60% acetic acid added until a slight turbidity appeared. To this was added the benzene methanol mixture (see above). This solution was shaken for 1h in a stoppered flask and then cooled in ice for a further hour. The solid violet coloured clathrate complex which formed was filtered, washed with water, alcohol and ether and dried in air (Yield = 1.88g = 71.4%).

This clathrate complex (1.5g) was dry distilled on an oil-bath at 190-210° and the benzene given off was condensed and passed into fuming sulphuric acid (containing 20% w/w SO_3)(1.2ml) at 40° Vogel, 1956b). When all the benzene had reacted, water (5.5ml) was added to the mixture. Diphenyl sulphone, a by-product, was filtered off. The mixture was partially neutralized with sodium bicarbonate (0.68g), sodium chloride (1.45g) added and the mixture heated until the salts dissolved. On cooling, sodium benzene sulphonate crystallized out. This was filtered, recrystallized from ethanol and dried in vacuo

over P_2O_5. The salt was recrystallized to constant specific radioactivity. The sodium benzene sulphonate had an infra-red spectrum identical with an authentic sample and the p-toluidine salt had m.p. 195-198°(lit. 205°).

(b) Total phenol in urine Phenol (0.5g) was added to urine (10ml) and the solution refluxed for 3h with an equal volume of 10M-HCl. The mixture was steam distilled to remove the phenol which was converted to the toluene p-sulphonate as described by Parke and Williams (1953b). The phenyl toluene p-sulphonate m.p. 93° was recrystallized from ethanol to constant specific radioactivity.

(c) Total quinol in urine Quinol (0.251g) was added to urine (10ml), the solution refluxed for 3h with an equal volume of 10M-HCl, and then extracted with ether (3 x 20ml). The ether was removed in vacuo and the dark brown sludge remaining washed into a stoppered flask. Toluene p-sulphonyl chloride (1g), 40% (w/v) NaOH (20ml) and acetone (100ml) were added. After shaking for 1h the quinol ditoluene p-sulphonate was isolated by dilution with water and recrystallized to constant specific radioactivity from ethanol (m.p. 160°).

(d) Triphenyllead acetate in faecal homogenates Triphenyllead acetate (0.25-1.00g) was added to a homogenate of faeces in water (1.0-3.0ml). Tetrahydrofuran was added until the triphenyllead acetate dissolved. Faecal material was filtered off and water added to the filtrate until triphenyllead acetate precipitated. This was filtered off and recrystallized from acetone or acetone/hexane to constant specific radioactivity.

(^{203}Pb)Compounds.

Samples of urine, faeces and tissue were counted in a Packard

Auto-Gamma Spectrometer. Decay of the isotope ($t_{\frac{1}{2}}$ = 52h) was corrected for by counting a standard dose solution prior to counting the samples, with intervals of not more than one hour between standards.

(^{3}H)-Compounds.

(1) Determination of radioactivity in urine, faeces and bile.

Tritium in samples was determined using a Packard Tri-Carb scintillation spectrometer (Model 3320). The scintillation fluid used consisted of naphthalene (200g), 2,5-diphenyloxazole (10g), dioxan (1440ml), toluene (270ml) and methanol (90ml). Counting efficiencies were determined by the channels ratio method.

Scintillation fluid (15ml) was added to urine (0.5-1.0ml) and bile (0.1-0.2ml). Air dried faeces were ground to a powder and samples (0.05-0.5g) were combusted in a Packard Tri-Carb Sample Oxidizer (model 305) dispensing the above scintillation fluid.

(2) Collection of (^{3}H)-labelled volatile compounds.

Air from the metabolism cage was passed through a cooled methanol trap as described in the section on radiochemical techniques using ^{14}C. The methanol was assayed for tritium activity by adding 1ml to the above scintillation fluid.

(3) Quantitation of urinary metabolites.

Urinary metabolites were quantified by scanning paper strip chromatograms of urine (0.3ml) and the peaks were identified by comparison of Rf values with those of authentic samples as described in the (^{14}C)compound section. The radioactivity in each spot was deter-

mined by the method of Caldwell et al. (1972).

(4) Reverse isotope dilution procedures.

(a) Benzene Methanol (15ml) from the cooled methanol trap was taken and benzene (1g) added. The nickel cyanide clathrate complex was made as described in the ^{14}C section. Samples (0.01-0.05g) were combusted in a Packard Tri-Carb Sample Oxidizer (see determination of tritium in faeces).

(b) Triphenyllead acetate in faeces Samples of faeces (about 0.1g) were taken and triphenyllead acetate (about 0.4g) added. Acetone was added and the mixture heated until the triphenyllead acetate dissolved. The mixture was filtered to remove undissolved faecal material, the filtrate reduced in volume and cooled until triphenyllead acetate crystallized out. This was filtered off and recrystallized from acetone/hexane to constant specific radioactivity.

ESTIMATION OF LEAD

Total lead was determined by the method of Irving and Butler (1953) whereby an aliquot of urine or faeces was ashed using concentrated nitric, sulphuric and perchloric acids and the lead estimated colorimetrically as a complex with dithizone (diphenylthiocarbazone).

CHROMATOGRAPHY

The Rf values of triphenyllead acetate and its possible metabolites are given in table 1. For paper chromatography Whatman No. 1 paper and the descending technique was used; for thin-layer chromatography, plates prepared from Silica Gel G (E. Merck A.G., Darmstadt,

W. Germany) of 0.25mm thickness were used.

The following solvents were used for chromatography:-

A Propan-1-ol / ammonia (sp. gr. 0.88)(7 : 3 v/v)

B Butan-1-ol / ammonia (sp. gr. 0.88) / water (10 : 1 : 1 by vol.)

C Benzene / dioxan / glacial acetic acid (90 : 25 : 4 by vol.)

D Hexane / benzene (1 : 1 v/v)

E Carbon tetrachloride / ethanol (20 : 1 v/v).

The following detecting reagents were used:

Diazotised p-nitroaniline (DP) p- Nitroaniline (0.25g) was dissolved by gentle heating in 25ml of N-HCl and the solution diluted with ethanol to 50ml. To 10ml of this stock solution was added 0.1g of sodium nitrite. Immediately after the nitrite had dissolved the chromatogram was sprayed with the solution. After 5min the chromatogram was resprayed with a 0.5M solution of KOH in ethanol.

Naphtharesorcinol spray (NR) This consisted of naphtharesorcinol (1% w/v) in aqueous trichloroacetic acid (33% w/v) followed by heating the paper at 120°C for 5-10min.

Gibbs reagent (GR) This is a spray of ethanolic 2,6-dichloroquinone-chloroimide (1%) followed by saturated $NaHCO_3$ (Dawson et al., 1959).

HCl then Gibbs reagent (HGR) The paper was sprayed with 5M-HCl, dried and then sprayed with Gibbs reagent and $NaHCO_3$.

Prussian blue spray (PB) The plate was sprayed with an aqueous solution of ferric chloride (5-10% w/v) (Randerath, 1966).

Dithizone spray (D) The plates were sprayed with a solution of dithizone (diphenylthiocarbazone) in chloroform (6% w/v).

Table 1. Chromatography of triphenyllead acetate and possible metabolites

	Rf values in solvents					Colour reactions					
	Paper		Thin-layer								
	A	B	C	D	E	DP	NR	GR	HGR	PB	D
Triphenyllead acetate	-	-	-	0	0.96	-	-	-	-	-	Yellow
Diphenyllead diacetate	-	-	-	0	0.36	-	-	-	-	-	Pink
Phenyllead triacetate	-	-	-	0	s	-	-	-	-	-	None
Hexaphenyldilead	-	-	-	0.99	0.95	-	-	-	-	-	Pink
Phenol	-	-	0.74	-	-	Yellow	-	-	-	Blue	-
Quinol	-	-	0.52	-	-	Red	-	-	-	Blue	-
Catechol	-	-	0.60	-	-	Brown	-	-	-	Blue	-
Resorcinol	-	-	0.54	-	-	Orange	-	-	-	Blue	-
Pyrogallol	-	-	0.42	-	-	None	-	-	-	None	-
Phenyl sulphate	0.75	0.52	-	-	-	None	None	None	Blue	-	-
Quinol sulphate	0.53	0.18	-	-	-	Red	None	None	Blue	-	-
Phenyl glucuronide	0.43	0.07	-	-	-	None	Blue	Blue	Blue	-	-
Quinol glucuronide	0.11	0	-	-	-	Red	Blue	Blue	Blue	-	-

s = streaks
- = not determined

CHAPTER 3

THE FATE OF ORALLY ADMINISTERED TRIPHENYLLEAD ACETATE

THE FATE OF ORALLY ADMINISTERED TRIPHENYLLEAD ACETATE

During the manufacture and use of a compound both the manufacturers and the users are likely to become exposed to the compound. Absorption could occur by inhalation, ingestion or skin absorption. The only organolead compound produced in large quantities is tetraethyllead and extremely stringent safety precautions are enforced for its handling (Barry, 1957; Boyd et al, 1957). This metabolic study of the fate of triphenyllead acetate was undertaken to ascertain likely routes of metabolism in man since experimentation on man himself with such a substance is unethical.

As ingestion is a likely route of absorption, the fate of the compound after oral administration was examined. In addition it has been shown that inhalation of particles larger than two microns results in entrapment of some of the particles in the upper respiratory tract whence they pass into the alimentary canal (Kehoe, 1961). Acute oral LD_{50} values for triphenyllead acetate have been obtained for various species (Table 2).

Results

In a preliminary experiment tri(U-^{14}C)phenyllead acetate was administered orally to female rats as a solution in dimethylsulphoxide. Urine, faeces and carbon dioxide were collected for six days. At the end of this period recovery of the label was low (52.9%) while little radioactivity remained in the carcass (Table 3). This suggested that radioactivity had been lost in the expired air. When the experiment was repeated to include the collection of volatile metabolites in the expired air recovery of radioactivity was higher (75.0%). Of this 25.1% was in the urine, 28.9% in the faeces and 20.1% in the ex-

Table 2. Acute LD_{50} values for the oral administration of triphenyllead acetate to various species.

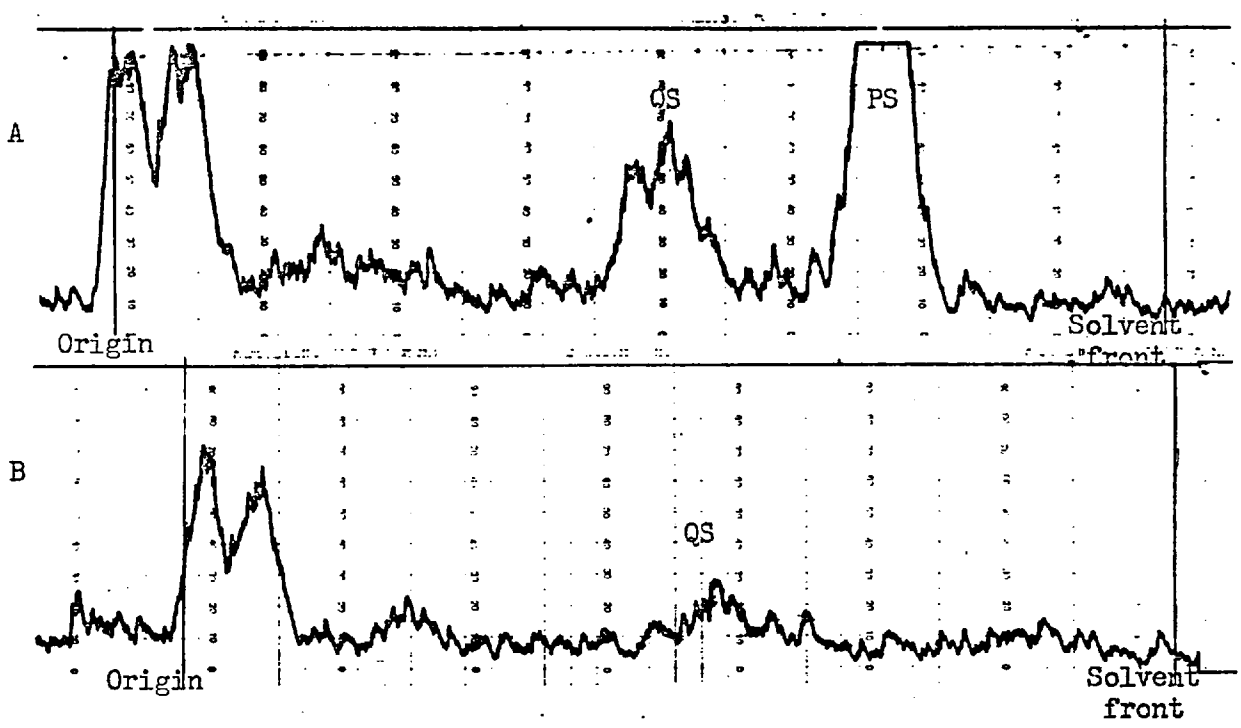
LD_{50} (mg/kg)	Animal	Reference
228	Albino rats of the Sprague-Dawley strain (♂ & ♀)	Fowler, D.G.
318	Hooded rats of the Lister strain	Walker, 1967
200-400	Various strains of rabbit	Walker, 1967
400-600	Mice of Parkes' strain (♂ & ♀)	Walker, 1967
600	Albino rats of the Sprague-Dawley strain	Hine Laboratories, 1966

pired air as volatile metabolites (Table 4). Reverse isotope dilution showed that all the radioactivity in the trap for volatile compounds was present as benzene.

The main urinary metabolite found during the first two days was phenyl sulphate (12.9% dose ^{14}C) with smaller amounts of quinol (2.3% dose ^{14}C). In addition two unknown metabolites, X and Y were found mainly in the urine collected in the second twenty-four hour period (Table 5). The unknown metabolite X had an Rf of 0.07 in solvent A and 0.02 in solvent B, while Y had an Rf of 0.02 in solvent A and 0.00 in solvent B. Incubation of the urine with β-glucuronidase showed no alteration of the radioactive peaks while incubation with sulphatase caused a disappearance of the phenyl sulphate peak (Fig. 4). After acid hydrolysis there was almost complete disappearance of the phenyl sulphate and quinol sulphate peaks. Reverse isotope dilution for total phenol and quinol in the 0-24 hour urine from rat 5 confirmed the presence of these compounds accounting for 8.3% and 0.3% of the dose respectively. Peaks corresponding to phenol and quinol could be detected after thin-layer chromatography of an ether extract of hydrolysed urine but quantitation in this case was not possible due to the volatility of the phenol.

After oral administration of triphenyl(^{203}Pb)lead acetate a large proportion of the radioactivity was found to be associated with the faeces (33%) whereas less than 1% of the dose was recovered in the urine in four days (Table 6). Overall recovery of radioactivity was low (34%) and was probably due to the errors incurred using the isotope ^{203}Pb with its short half-life of 52h. A large proportion of the lead (75%) was also recovered in the faeces following oral administration of non-radioactive triphenyllead acetate at 200mg/kg (Table 7). The

Fig. 4 Radiochromatogram of rat urine after dosing orally with tri(U-^{14}C)phenyllead acetate.



A = Radiochromatogram of untreated urine

B = Radiochromatogram of urine after incubation with sulphatase

PS = Phenyl sulphate

QS = Quinol sulphate

bulk of the administered lead was recovered in the faeces on days 3, 4 and 5. No lead was detected in the urine using this colorimetric method. Reverse isotope dilution for triphenyllead acetate in the faeces following oral administration of tri(^{14}C)phenyllead acetate suggested that part of the radioactivity was excreted as the unchanged compound (Table 5). A tissue distribution study twenty-four hours after an oral dose of triphenyl(^{203}Pb)lead acetate (Table 8) showed most of the radioactivity to be associated with the gut and its contents. Low levels of radioactivity were found in the liver, kidney and lungs while there was a trace of radioactivity in the brain.

Discussion

It would appear that following an oral dose of triphenyllead acetate breakdown of the compound occurs in the gut. Benzene is released, some of which passes out in the expired air. The remainder is metabolised to phenol and quinol which are excreted in the urine after conjugation with sulphuric acid. Some unchanged triphenyllead acetate appears to pass through the gut and is eliminated in the faeces along with lead or lead containing metabolites. Some lead appears to be absorbed but the chemical nature of this material is not known.

Parke and Williams (1950) showed that rabbits, after an oral dose of benzene eliminated a part unchanged via the lungs. At levels of 0.25 and 0.5g/kg, of the administered dose 40% is eliminated unchanged while this figure rises to 64% at 1g/kg. This elimination in the expired air was confirmed when the fate of (^{14}C)benzene in rabbits was studied by the same authors (Parke & Williams, 1953a). In two days about 45% of the dose was eliminated via the lungs of which 43% was unchanged benzene and about 1.5% was carbon dioxide. Nearly 35% of

the benzene was eliminated as metabolites in the urine. Phenol (23%) was the major urinary metabolite with smaller amounts of quinol (4.8%) catechol (2.2%) and hydroxyquinol (0.3%).

In this metabolic study on the fate of triphenyllead acetate, phenyl sulphate was the major urinary metabolite found. Earlier work on the metabolic fate of (^{14}C)benzene and (^{14}C)phenol has shown that both phenyl sulphate and phenylglucuronide are excreted in the urine. Of an oral dose of benzene (150-500mg/kg) the rabbit excretes 6-8% as phenylglucuronide and 11-14% as phenyl sulphate. Similarly, after an oral dose of phenol at 50-60mg/kg the urinary metabolites found for the rabbit were mainly phenylglucuronide (40-50%) and phenyl sulphate (about 45%) (Parke & Williams, 1953b). In the first twenty-four hours after an oral dose of phenol (25mg/kg) the rat excreted 54% of the dose as phenyl sulphate and 42% as phenylglucuronide (Capel et al., 1972).

This difference in the pattern of the metabolites of benzene may be due to a difference in the dose given. Triphenyllead acetate was administered orally at 25mg/kg. If this was entirely degraded it would be equivalent to a dose of 11.8mg/kg of benzene. As some triphenyllead is excreted in the faeces unchanged, the amount of benzene absorbed by the animal would be considerably less than this figure. Degradation of the organolead compound is unlikely to be instantaneous and this would result in the animal receiving a low dose of benzene over a period of time. Under these conditions the rat may metabolise an oral dose of benzene mainly to phenyl sulphate. It has been shown that the rate of sulphate conjugation is limited by the availability of sulphate whereas the rate of glucuronide formation is proportional to the body level of the phenol (Bray et al., 1952a & b).

It would appear that breakdown of triphenyllead acetate occurs

in the gut and therefore there could be several reasons for the degradation of the compound.

1) The enzymes of the intestinal tract could catalyse the decomposition of the compound.

2) The gut bacteria may metabolize the compound resulting in the release of benzene.

3) The compound may decompose spontaneously under acidic conditions.

Table 3. Excretion of radioactivity by rats receiving tri(U-^{14}C)phenyllead acetate orally.

Three ♀ rats were given tri(U-^{14}C)phenyllead acetate 25mg/kg (2.9uCi) in 0.3ml of dimethyl sulphoxide.

Day	% of dose of ^{14}C								
	In Urine			In Faeces			In expired air as CO_2		
	Rat 1	Rat 2	Rat 3	Rat 1	Rat 2	Rat 3	Rat 1	Rat 2	Rat 3
1	10.4	3.2	12.6	5.7	9.9	4.4	0.1	1.3	0.1
2	11.3	13.3	20.4	19.0	10.4	15.9	0.2	0.4	0.4
3	1.1	0.8	4.3	4.0	1.4	0	<0.1	0.1	0.3
4	0.6	0.4	0.7	0.6	0.2	1.5	<0.1	0.1	0.1
5	0.2	0.1	0.2	0.6	<0.1	0.1	-	-	0.1
6	0.1	0.3	0.1	0.3	<0.1	0.2	-	-	<0.1
Total	23.7	18.1	38.3	30.2	21.9	22.1	0.3	1.7	1.0

Total recoveries Rat 1 = 55.6%
Rat 2 = 41.7%
Rat 3 = 61.5%

Activity remaining in carcass of Rat 1 = 1.4%

- = not determined

Table 4. Excretion of radioactivity by rats receiving tri(^{14}C)phenyllead acetate orally.

Three rats were given tri(^{14}C)phenyllead acetate 25mg/kg (2.9μCi) in 0.3ml of dimethyl sulphoxide. Urine, faeces and volatile products in the expired air were collected.

Day	% dose of ^{14}C								
	In urine			In faeces			Caught in cooled methanol trap from expired air		
	Rat 5	Rat 6	Rat 7	Rat 5	Rat 6	Rat 7	Rat 5	Rat6	Rat 7
1	11.0	7.1	6.0	9.9	3.4	0.9	29.4	2.9	19.6
2	15.6	3.7	18.3	10.9	5.6	18.0	0.9	3.1	3.4
3	0.6	3.3	2.3	1.4	17.3	5.1	0.1	0.5	0.1
4	-	4.1	0.9	-	9.9	2.1	-	0.5	-
5	-	1.7	0.5	-	1.7	0.5	-	-	-
6	-	-	0.3	-	-	0.2	-	-	-
Total	27.2	19.9	28.3	22.2	37.9	26.8	30.3	7.0	23.0

Total recoveries Rat 5 = 79.7%
Rat 6 = 67.3%
Rat 7 = 78.1%

Activity recovered in carcass of Rat 6 = 2.5%

Table 5. The metabolites of tri(U-^{14}C)phenyllead acetate.

Substance	% Dose of ^{14}C													
	In Urine								In Faeces					
	Day 1				Day 2				Day 1			Day 2		
	Rat 1	Rat 2	Rat 3	Rat 5	Rat 1	Rat 2	Rat 3	Rat 5	Rat 1	Rat 2	Rat 3	Rat 1	Rat 2	Rat 3
Phenyl sulphate	6.9	2.1	7.3	7.9	4.4	7.3	9.6	6.1	-	-	-	-	-	-
Quinol sulphate	1.4	0.4	1.8	0.2	1.2	1.1	2.0	0.9	-	-	-	-	-	-
Unknown X	n.d.	n.d.	1.4	0.2	3.4	1.5	3.3	4.5	-	-	-	-	-	-
Unknown Y	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.2	n.d.	-	-	-	-	-	-
Triphenyllead acetate	-	-	-	-	-	-	-	-	3.5	-	1.1	-	7.1	1.9
Total	8.3	2.5	10.5	8.3	9.0	9.9	17.1	11.5	3.5	-	1.1	-	7.1	1.9
Total ^{14}C in 24h sample	10.4	3.2	12.6	11.0	11.3	13.3	20.4	15.6	5.7	9.9	4.4	19.0	10.4	15.9

n.d. = not detected

Table 6. Excretion of radioactivity by rats dosed orally with triphenyl(^{203}Pb)lead acetate.

Three ♀ rats were given triphenyl(^{203}Pb)lead acetate orally at a dose level of 25mg/kg in 0.3ml of dimethyl sulphoxide.

Day	% dose of ^{203}Pb					
	In Urine			In Faeces		
	Rat 17	Rat 18	Rat 19	Rat 17	Rat 18	Rat 19
1	0.1	trace	trace	0.1	3.8	0.1
2	0.1	trace	0.2	12.7	8.1	12.5
3	0.1	trace	0.6	5.5	4.2	11.9
4	0.6	0.2	0.6	16.7	23.5	-
Total	0.9	0.2	1.4	35.0	39.6	24.5

Total recoveries Rat 17 = 36%
Rat 18 = 40%
Rat 19 = 26%

trace = less than o.1% of the dose
\- = not determined

Table 7. Excretion of 'total' lead by rats dosed orally with non-radioactive triphenyllead acetate.

Three female rats were each dosed orally with a suspension of triphenyllead acetate (200 mg/kg) in a 2% w/v solution of carboxymethyl cellulose. The total lead excreted was determined for the bulked samples. Results of duplicate determinations are shown in parentheses.

Day	% recovery of lead	
	In Urine	In Faeces
1	n.d.	2 (2.1, 2.6)
2	n.d.	2 (2.8, 2.4)
3	n.d.	15 (14.6, 15.5)
4	n.d.	24 (25.5, 23.6)
5	n.d.	24 (23.3, 24.3)
6	n.d.	6 (6.0, 5.6)
7	n.d.	2 (2.4, 2.0)

Total recovery = 75%

n.d. = not detected

Table 8. Tissue distribution of ^{203}Pb 24h after administration of triphenyl(^{203}Pb)lead acetate orally.

Three female rats were given triphenyl(^{203}Pb)lead acetate (25mg/kg) orally by intubation as a solution in dimethyl sulphoxide (0.3ml per rat). After 24h the animals were killed by decapitation.

	% recovery ^{203}Pb in entire organ			µg equivalents of triphenyllead acetate per gram wet wt tissue		
	Rat 29	Rat 30	Rat 31	Rat 29	Rat 30	Rat 31
Heart	0.1	0.1	0.1	1.0	2.0	1.0
Uterus	trace	0.1	trace	0.5	2.8	0.7
Brain	trace	0.1	trace	0.3	0.6	0.5
Lung	0.1	0.2	0.1	2.4	2.6	2.7
Kidney	trace	0.6	0.6	10.4	7.9	7.4
Spleen	0.1	0.1	0.1	1.9	3.0	1.2
Liver	3.1	2.4	2.2	7.4	6.8	3.6
Large Intestine	5.2	0.4	1.2	1.7	3.1	1.1
Small Intestine	1.5	2.3	3.6	4.2	10.0	13.8
Stomach	6.3	10.8	3.0	53.6	140.0	41.3
Contents of large intestine	38.0	21.2	24.2	157.5	162.9	165.7
Contents of small intestine	5.9	5.4	6.3	16.1	24.4	24.0
Contents of stomach	33.6	43.8	22.2	344.8	849.5	555.4
Fat	-	-	-	0.3	0.4	0.4
Muscle	-	-	-	0.5	0.6	0.4
Blood	-	-	-	1.2	0.9	1.0
24h urine	n.d.	n.d.	n.d.	-	-	-
24h faeces	n.d.	3.4	12.3	n.d.	6.8	11.0

trace = less than 0.1% dose ^{203}Pb
n.d. = not detected
- = not determined

CHAPTER 4

THE FATE OF INTRAPERITONEALLY ADMINISTERED TRIPHENYLLEAD ACETATE

THE FATE OF INTRAPERITONEALLY ADMINISTERED TRIPHENYLLEAD ACETATE

The route of administration of a foreign compound often alters its metabolism. After oral administration some compounds may be absorbed from the mouth (Gibaldi & Kanig, 1965), others from the stomach (Brodie, 1967) but the majority are absorbed from the intestine. Thus prior to absorption a foreign compound will come in contact with and may be chemically changed by the contents of the gastro-intestinal tract, such as the gut flora (Drasar et al., 1971), the gut enzymes and by acid present in the stomach (Goodman & Gilman, 1955b). During absorption the compound may be changed by the enzymes present in the intestinal mucosa (Gingell & Bridges, 1971). Once absorbed from the gastro-intestinal tract compounds are largely transported via the hepatic-portal vein directly to the liver (Hathway, 1970).

In contrast, after intraperitoneal administration a compound will usually pass directly to the liver via the hepatic-portal vein (Hathway, 1970). Thus a comparison of the metabolism of a compound following administration by these two routes may indicate the part played by the gut contents and the gut wall in the biotransformation of an orally administered compound.

A large difference has been observed between the acute LD_{50} values of triphenyllead acetate given by the oral and intraperitoneal routes. Walker (1967) reported that in mice of Parkes' strain the acute intraperitoneal LD_{50} was 8.0mg/kg while for oral administration the acute LD_{50} value lay between 400 and 600mg/kg.

Triphenyllead acetate appears to be broken down in the gut on oral administration. However when doses near the LD_{50} value are given to rats they develop symptoms of intoxication. The large difference

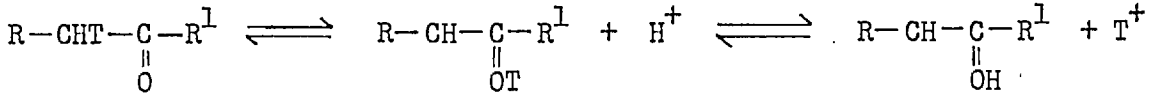
in the LD_{50} values for the two routes of administration would suggest that if triphenyllead acetate were absorbed on oral administration, only a small percentage of the administered dose passes across the intestinal wall. The fate of intraperitoneally administered triphenyllead acetate was investigated to see if the compound was metabolised after administration by this route and whether the compound accumulated in the tissues.

Carbon-14 is the more desirable isotope to incorporate in foreign compounds for metabolic work due to its long half-life and low energy of the radiation. In this case, because of its high toxicity only small amounts of triphenyllead acetate could be given intraperitoneally, making it economically unfeasible to obtain a sufficiently high specific radioactivity using radiocarbon. In radioactive tracer studies it is necessary to assume that the radioactive atom remains an integral part of the molecule under study. However, with tritium this assumption is not always valid as exchange of tritium often occurs in vivo (Evans, 1966)(Fig. 5). In spite of this difficulty, as a high specific radioactivity could be obtained at a low cost, triphenyllead acetate was labelled with tritium.

Results

Rats given lethal doses of triphenyllead acetate intraperitoneally showed ataxia, rapid breathing, cyanosis of the limbs and convulsions leading to death. Three rats given the compound suspended in a 2% w/v solution of carboxymethyl-cellulose at a dose level of 10mg/kg all died within twenty-four hours. At a dose level of 7.5mg/kg, one rat died within twenty-four hours, another after five days and the third after ten days. Two of the animals showed chromo-

Fig. 5. An example of labile tritium in a molecule.



Carbon-bound tritium may be labilized if the tritium atoms are in positions which may undergo tautomerism. Such an example is tritium attached to a carbon atom next to a keto group; tautomerism to the enol form occurs under alkaline conditions and the tritium atoms are lost by exchange with the hydrogen ions in the solvent (Evans, 1966).

dachryia and the longest surviving animal showed weakness of the hind limbs prior to death. Three animals dosed at 5mg/kg showed no symptoms after fourteen days observation.

After intraperitoneal injection of tri(^{3}H)phenyllead acetate radioactivity was detected in the expired air, the urine and the faeces (Table 9 & Fig. 7). Although the radioactivity was not excreted rapidly, by the fifth day 55.1% of the dose had been accounted for (Table 9 & Fig. 6). During the first twenty-four hours 5.6% of the radioactivity was found in the expired air. However, after this period excretion by this route decreased, until by the fifth day only 0.1% of the dose was found in the expired air (Table 9 & Fig. 7). Urinary excretion was highest on the second day after dosing when it accounted for 4.6% of the dose of tritium. Faecal excretion was very low until the third day after dosing but thereafter became the major route of excretion, accounting for 33.3% of the dose by day five (Table 9 & Fig. 7).

Comparison of the excretion after intraperitoneal administration of tri(^{3}H)phenyllead acetate and tri(U-^{14}C)phenyllead acetate showed a similar excretion pattern (Tables 9 & 10). The excretion of ^{14}C was however very low and the faecal excretion of the two isotopes did not compare well. This was probably due to the low amount of radioactivity used with the (^{14}C)-labelled material and the high quenching obtained with faecal samples.

Reverse isotope dilution for benzene in the trap for volatile metabolites of Rat 62 during the first twenty-four hours showed that part of the radioactivity (24% of the tritium) was due to benzene. The remainder of the radioactivity was not investigated but was possibly due to tritiated water.

Paper chromatography showed the main urinary metabolite was phenyl sulphate with smaller amounts of quinol sulphate and quinol glucuronide. Two unknown metabolites X and Z were also found. Z had an Rf value of 0.89 in solvent A and 0.87 in solvent B (Table 15). Reverse isotope dilution suggested that part of the faecal radioactivity was due to unchanged triphenyllead acetate (Table 15).

When rats were given triphenyl(^{203}Pb)lead acetate intraperitoneally, no radioactivity was detected in the urine while 25% of the dose was recovered in the faeces during the first four days after dosing (Table 11). A tissue distribution study carried out twenty-four hours after an intraperitoneal dose of triphenyl(^{203}Pb)lead acetate showed that a large part of the radioactivity was found associated with the gut, the gut contents and the liver. Lower amounts were also detected in the kidney, the spleen, uterus and fat. Low levels were also found in the brain (0.2µg equivalents triphenyllead acetate per gram wet weight tissue)(Table 12).

In spite of the association of radioactivity with the gut and its contents little radioactivity was recovered in the bile after administration of either tri(^{3}H)phenyllead acetate or triphenyl(^{203}Pb)-lead acetate. Twenty-four hours after a dose of either triphenyl-(^{203}Pb)lead acetate or tri(^{3}H)phenyllead acetate the radioactivity in the bile accounted for 3.9% of the lead-203 and 1.5% of the tritium (Tables 13 & 14). Four days after the administration of tri(^{3}H)phenyl-lead acetate 6.7% of the radioactivity had been recovered in the bile (Table 14).

Discussion

A similar metabolic pattern is seen after both oral and intra-

peritoneal administration of triphenyllead acetate. In both cases benzene was detected in the expired air and conjugates of phenol and quinol in the urine. After oral administration, breakdown of the compound probably occurs in the gastro-intestinal tract. The large difference in the LD_{50} values for the two routes of administration would suggest that triphenyllead acetate as such is not absorbed to any great extent. In addition lead was not detected in the urine after oral administration whereas a large part was recovered in the faeces.

After intraperitoneal administration metabolism of a foreign compound can occur in several tissues. The compound could be broken down by the liver and kidneys or it could pass into the gut where biotransformation could occur. Thus benzene could be released from triphenyllead acetate in the liver and kidneys, further metabolism occurring in these organs or benzene could be released in the gut and on absorption pass to the liver for metabolism to phenol, quinol and their conjugates.

Unchanged triphenyllead acetate was found in the faeces. Its presence could be due to its biliary excretion, to passage directly across the gut wall or to its excretion in pancreatic juice or intestinal juice. Low levels of radioactivity were found in the bile after intraperitoneal administration of either tri(^{3}H)phenyllead acetate or triphenyl(^{203}Pb)lead acetate. The nature of this material was not determined.

Castellino et al. (1966) showed that fifty hours after an intravenous dose of 100µg of lead-210 8.4% of the dose had been recovered in the bile while little lead was recovered in the intestinal contents and the faeces. This value for the biliary excretion of lead in the

rat was confirmed by Cirkt (1972) who observed that 6.7% of a dose of 125μg Pb^{++} was recovered in the bile in twenty-four hours.

Millburn et al. (1967) suggested that for anionic compounds to be appreciably excreted in the bile of the rat they should have a molecular weight of about 350. The molecular weight threshold for appreciable biliary excretion appears to be different for cations. Hughes et al. (1973a) have suggested that for the biliary excretion of monoquaternary nitrogen cations, in amounts greater than 10% of the dose, the cation should have a molecular weight of not less than about 200 ± 50. The authors suggested from data on the biliary excretion of diquaternary nitrogen cations that the minimum molecular weight for dications is different and is probably in the region 500-600 (Hughes et al., 1973b). For substances to be eliminated in the bile they must be polar. Thus the lipid soluble dieldrin (molecular weight = 381) is only slowly eliminated in the bile, only 1% of a 5mg/kg dose being recovered in the bile from rats in twenty-four hours (Williams et al., 1965).

Benzene and its metabolites, all molecules of low molecular weight, are unlikely to be excreted in the bile to any great extent. Abou-El-Makarem et al. (1967) showed that in the rat, after an intraperitoneal dose of 50mg/kg of (^{14}C)benzene, only 0.8% of the radioactivity was recovered in the bile in twenty-four hours. Similarly, 4.6% of a 50mg/kg dose of (^{14}C)phenol and 7.0% of a 100mg/kg dose of quinol were recovered by this route. It is thus feasible that following intraperitoneal administration of triphenyllead acetate low amounts of inorganic lead and phenolic conjugates could be excreted in the bile. Due to the uncertainty of the physico-chemical nature of triphenyllead acetate it is difficult to suggest whether it would be likely to

undergo biliary excretion. Its high solubility in chloroform and benzene would suggest that it is probably lipid soluble. Dieldrin is absorbed from the gastro-intestinal tract in the rat but nevertheless is almost entirely excreted unchanged in the faeces (Hunter et al., 1960). It is not excreted in the bile to any great extent (0.8% in twenty-four hours). On injection or absorption through the skin, rats still excreted it mainly in the faeces (Rosen & Williams, 1965). This suggests that dieldrin is secreted into the intestine by some other route than through the bile. In this study, the total recovery of tritium in the faeces four days after administration of triphenyllead acetate (25% of the dose) was higher than the recovery of tritium in the bile at this time (6.7% of the dose). This would suggest the possibility of a mechanism other than biliary excretion for the passage of the radioactivity into the intestinal contents. This discrepancy could however be due to other factors such as damage to the liver by the cannulation of the bile duct or the enhanced toxicity of the compound when given in conjunction with thiopentone.

Appreciable levels of radioactivity were observed in fat after intraperitoneal administration of triphenyl(^{203}Pb)lead acetate. Long term storage of organochlorine insecticides in fat has been observed (Hayes, 1965). In view of the high toxicity of intraperitoneally administered triphenyllead acetate, the possibility of accumulation in the fat of the compound or its metabolites on prolonged administration should be investigated.

Table 9. Excretion of radioactivity by rats receiving tri(^{3}H)phenyllead acetate intraperitoneally.

Three ♀ rats were given 2mg/kg (69.2μCi) of tri(^{3}H)phenyllead acetate intraperitoneally in dimethyl sulphoxide. Each rat received 0.2ml of solution.

Time in Days	% dose of tritium								
	In urine			In faeces			In air as volatile metabolites		
	Rat 60	Rat 61	Rat 62	Rat 60	Rat 61	Rat 62	Rat 60	Rat 61	Rat 62
1	4.2	3.0	3.9	0.1	0.1	0.1	5.4	6.2	5.2
2	4.2	5.2	4.5	0	0	0.3	0.6	0.8	1.2
3	2.5	3.3	2.5	18.3	8.4	13.0	0.2	0.1	0.2
4	2.6	2.4	2.0	10.2	17.3	7.9	0.1	0.1	0.1
5	1.6	1.5	1.6	9.4	4.2	10.8	0.1	<0.1	<0.1
6	1.4	0.7	1.0	3.5	4.0	6.0	<0.1	<0.1	<0.1
7	1.2	0.7	0.6	3.4	3.7	2.1	-	-	-
8	0.9	0.9	0.8	2.2	1.1	0.9	-	-	-
9	0.7	0.5	1.0	2.3	4.4	1.1	-	-	-
10	0.5	0.4	0.4	2.2	1.4	2.2	-	-	-
11	0.4	0.4	0.3	1.3	1.2	1.2	-	-	-
12	0.4	0.2	0.4	1.1	0.8	1.1	-	-	-
13	0.3	0.3	0.3	0.7	0.7	0.8	-	-	-
14	0.2	0.3	0.4	0.8	0.7	0.8	-	-	-
Total	21.1	19.8	19.5	55.4	47.9	48.2	6.4	7.2	6.7

Total recoveries Rat 60 = 82.9%
Rat 61 = 74.9%
Rat 62 = 74.4%

- = not determined

Fig. 6 Histogram showing the total daily excretion of tritium by rats dosed intraperitoneally with tri(^{3}H)phenyllead acetate. (Figures are the average for three rats.)

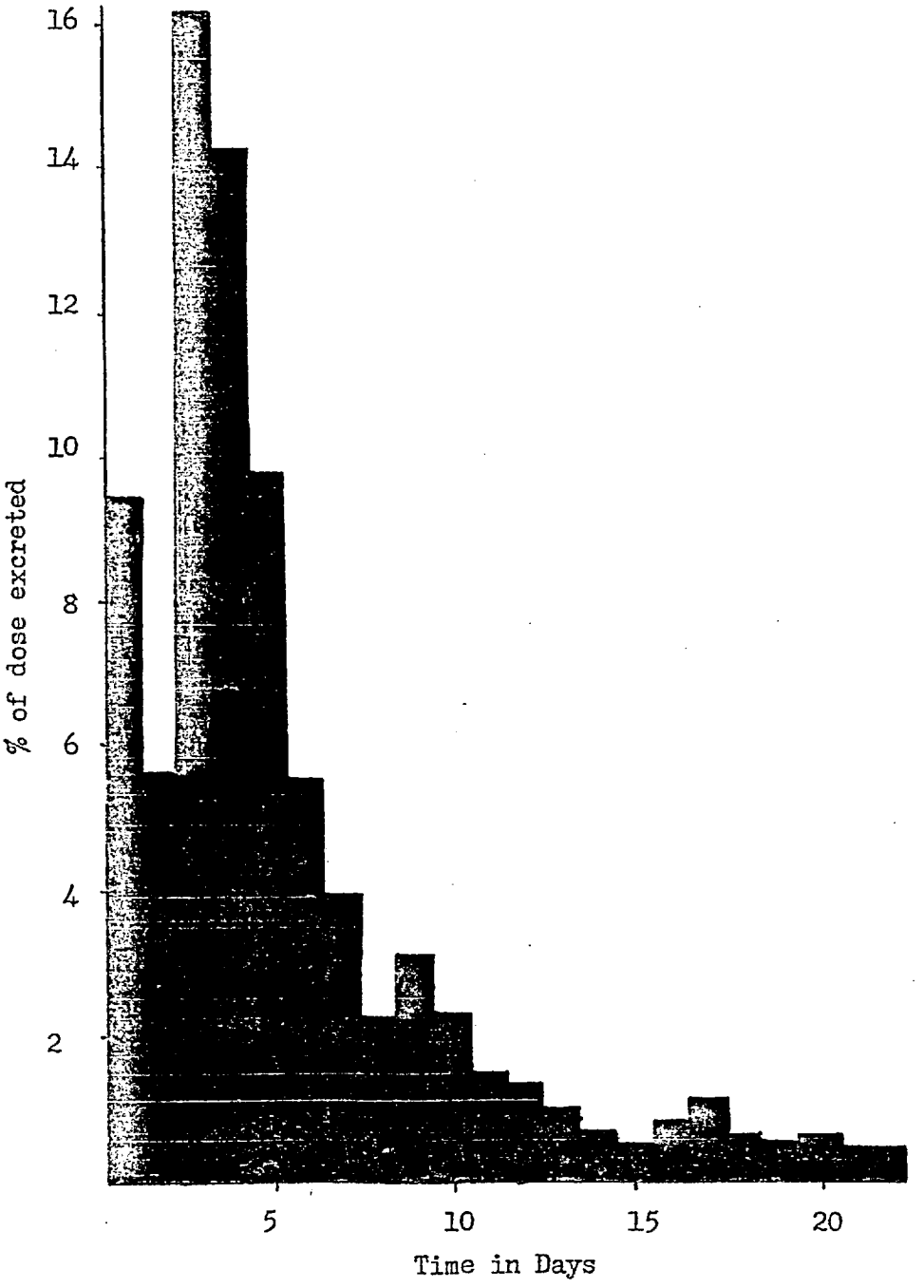


Fig. 7 Histogram showing the routes of excretion of radioactivity by rats following intraperitoneal administration of tri(^{3}H)phenyllead acetate. (Figures are the averages for three animals).

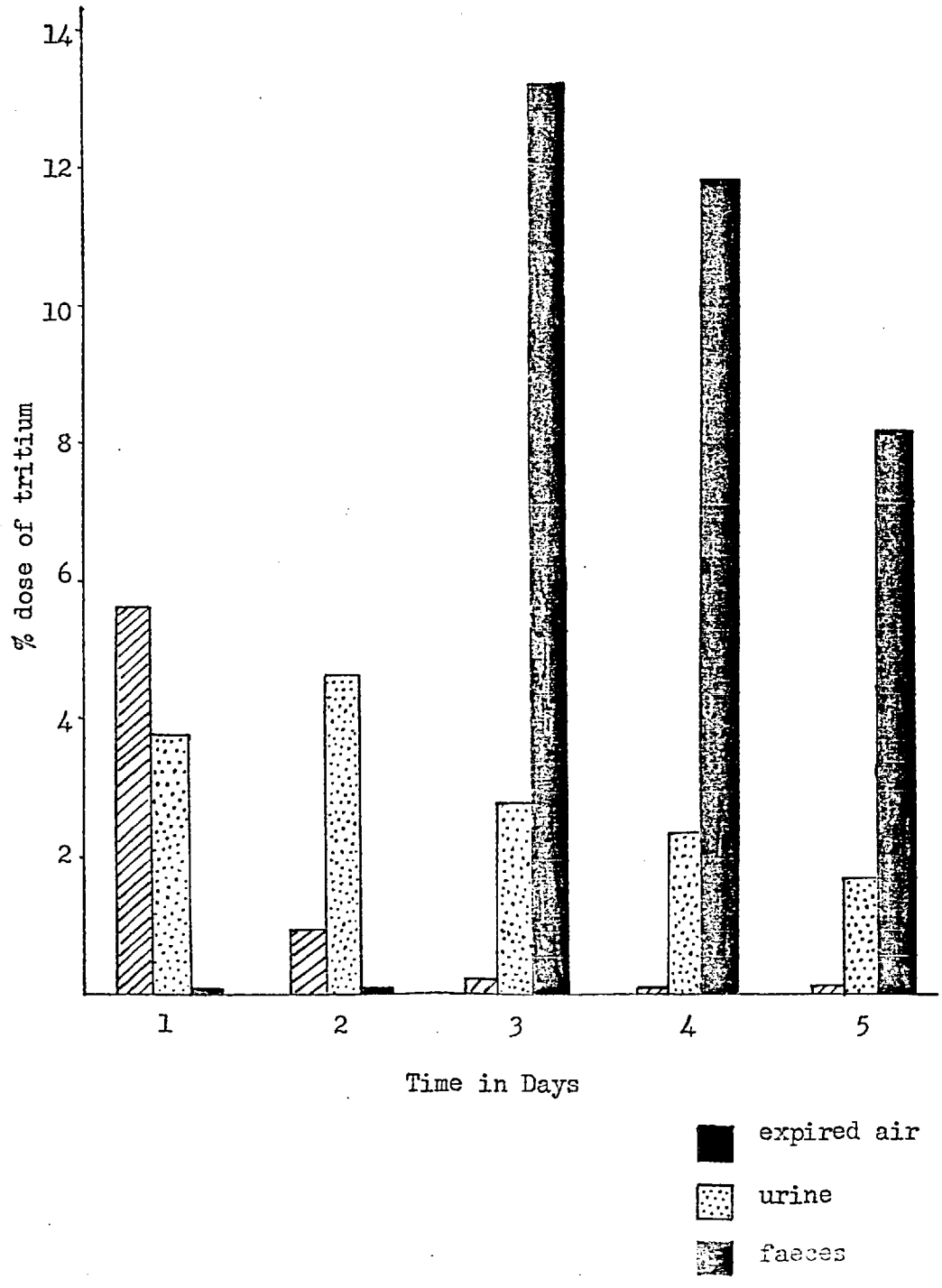


Table 10. Excretion of radioactivity by rats receiving tri(U-^{14}C)phenyllead acetate intraperitoneally.

Three ♀ rats were given 2mg/kg (0.24uCi) of tri(U-^{14}C)phenyllead acetate intraperitoneally as a solution in dimethyl sulphoxide. Each rat received 0.2ml of solution.

Time in Days	% dose of ^{14}C								
	In Urine			In Faeces			In air as volatile metabolites		
	Rat 32	Rat 33	Rat 34	Rat 32	Rat 33	Rat 34	Rat 32	Rat 33	Rat 34
1	2.9	2.0	0.9	0	0	0	4.5	2.2	2.6
2	4.6	3.1	1.7	0	0.9	1.0	1.0	1.3	-
3	3.3	1.6	2.3	11.4	1.5	trace	trace	trace	trace
4	1.9	trace	1.5	6.1	3.4	2.3	trace	trace	-
5	1.2	2.1	1.6	2.6	2.2	1.1	-	-	-
6	1.4	1.3	1.0	1.1	1.5	1.2	-	-	-
Total	15.3	10.1	9.0	21.2	9.5	5.6	5.5	3.5	2.6

Total recoveries Rat 32 = 42.0%
Rat 33 = 23.1%
Rat 34 = 17.2%

Table 11. Excretion of radioactivity by rats receiving triphenyl(^{203}Pb)lead acetate intraperitoneally.

Three ♀ rats were given triphenyl(^{203}Pb)lead acetate intraperitoneally 2mg/kg in 0.2ml dimethyl sulphoxide.

Day	% recovery of dose ^{203}Pb					
	In urine			In faeces		
	Rat 20	Rat 21	Rat 22	Rat 20	Rat 21	Rat 22
1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	n.d.	n.d.	n.d.	2.4	9.8	8.3
3	n.d.	n.d.	n.d.	10.4	9.9	8.3
4	n.d.	n.d.	n.d.	10.3	3.4	12.2
Total				23.1	23.1	28.8

Total recoveries Rat 20 = 23%
Rat 21 = 23%
Rat 22 = 29%

n.d. = not detected

Table 12. Tissue distribution of ^{203}Pb 24h after administration of triphenyl(^{203}Pb)lead acetate intraperitoneally.

Three female rats were each given triphenyl(^{203}Pb)lead acetate (2mg/kg) intraperitoneally as a solution in dimethyl sulphoxide (0.2ml per rat). After 24h the rats were killed by decapitation.

	% recovery ^{203}Pb in entire organ			µg equivalents triphenyllead acetate per gram wet wt tissue		
	Rat 23	Rat 24	Rat 25	Rat 23	Rat 24	Rat 25
Heart	0.3	2.0	0.4	0.7	0.6	0.9
Uterus	3.1	0.5	1.1	5.5	1.2	2.5
Brain	0.2	0.2	0.3	0.2	0.2	0.3
Lungs	0.9	0.4	0.9	0.8	0.3	1.1
Kidney	1.6	2.0	2.5	1.7	2.1	2.2
Spleen	0.3	0.9	1.4	1.2	3.4	4.8
Liver	16.7	13.6	18.3	4.1	3.3	4.1
Large intestine	19.7	17.4	7.8	6.8	4.2	2.7
Small intestine	5.4	13.0	5.7	2.7	3.4	2.1
Stomach	1.1	1.3	2.2	0.7	1.5	2.0
Contents of large intestine	2.8	2.2	7.8	0.9	0.8	2.1
Contents of small intestine	2.9	1.9	4.9	3.7	3.5	1.6
Contents of stomach	0.1	trace	n.d.	trace	trace	n.d.
Fat	-	-	-	1.9	2.8	2.1
Muscle	-	-	-	0.2	0.3	0.1
Blood	-	-	-	0.3	0.3	0.3
Intraperitoneal fluid	-	-	-	0.7	3.0	-
24h urine	0.1	0.2	0.2	-	-	-
24h faeces	0.1	n.d.	n.d.	-	-	-

trace = less than o.1% dose ^{203}Pb
n.d. = not detected

Table 13. The biliary excretion of radioactivity after intraperitoneal administration of triphenyl(^{203}Pb)lead acetate.

The bile ducts of three female rats were cannulated and they were administered a dose of triphenyl(^{203}Pb)lead acetate (2mg/kg) intraperitoneally in dimethyl sulphoxide (0.2ml per rat).

Sample	Time of collection in hours	% recovery of ^{203}Pb		
		Rat 17	Rat 26	Rat 28
Urine	0-24	0.7	n.d.	n.d.
Bile	0-3	0.2	0.3	0.1
Bile	3-24	3.7	3.0	4.4

n.d. = not detected

Table 14. The biliary excretion of radioactivity after intraperitoneal administration of tri(^{3}H)phenyllead acetate.

Three female rats were dosed intraperitoneally with tri(^{3}H)phenyllead acetate (2mg/kg) in dimethyl sulphoxide. Each rat received 0.2ml of the solution.

Period of collection in days	% dose of tritium					
	In Urine			In Bile		
	Rat 80	Rat 81	Rat 82	Rat 80	Rat 81	Rat 82
1	4.7	1.8	0.5	2.3	2.0	0.3
2	3.8	1.8	1.0	2.2	2.2	0.1
3	7.5	5.8	0.2	2.1	2.8	0.1
4	2.9	1.3	0.1	1.3	2.0	3.0
Total	18.9	10.7	1.8	7.9	9.0	3.5

Table 15. The metabolites of tri(^{3}H)phenyllead acetate after intraperitoneal administration.

For dose see Table 9.

Substance	% dose of tritium														
	In Urine									In Faeces					
	Day 1			Day 2			Day 3			Day 3			Day 4		
	Rat 60	Rat 61	Rat 62	Rat 60	Rat 61	Rat 62	Rat 60	Rat 61	Rat 62	Rat 60	Rat 61	Rat 62	Rat 60	Rat 61	Rat 62
Phenyl sulphate	2.3	1.1	0.6	0.7	2.1	0.6	0.8	trace	1.0	-	-	-	-	-	-
Quinol sulphate	0.2	0.2	0.1	0.3	0.3	0.3	0.3	trace	0.2	-	-	-	-	-	-
Quinol glucuronide	n.d.	n.d.	n.d.	0.1	0.2	0.1	n.d.	n.d.	n.d.	-	-	-	-	-	-
Unknown X	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	-	-	-	-	-	-
Unknown Z	n.d.	trace	0.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-	-	-	-	-	-
Triphenyllead acetate	-	-	-	-	-	-	-	-	-	7.7	3.4	13.0	4.4	5.5	2.8
Total	2.5	1.3	0.7	1.1	2.6	1.0	1.1	0.1	1.2	7.7	3.4	13.0	4.4	5.5	2.8
% dose in 24h sample	4.2	3.0	0.9	4.2	5.2	4.5	2.5	3.3	2.5	18.3	8.4	13.0	10.2	17.3	7.9

n.d. = not detected
trace = less than 0.1% dose
- = not determined

CHAPTER 5

INVESTIGATIONS ON THE STABILITY OF TRIPHENYLLEAD ACETATE

INVESTIGATIONS ON THE STABILITY OF TRIPHENYLLEAD ACETATE

The breakdown of triphenyllead acetate appears to take place in the gastro-intestinal tract following oral administration. However although a similar metabolic pattern is seen after intraperitoneal administration the site of metabolism, in this case, is not so clear. The mechanism of the reaction which occurs might shed some further light on the toxicity of the compound.

Methods

Incubation of triphenyllead acetate with solutions of varying pH.

Triphenyllead acetate (1g) was incubated at 37° with either M-H_2SO_4, M-NaOH, or 0.1M-KH_2PO_4-NaOH buffer pH 7 (20ml). The mixture was aerated with a slow intermittent current of air. In the case of the last two mixtures the air was first passed through 40% w/v NaOH to remove carbon dioxide. After passage through the incubation mixture the air was bubbled through a nitrating mixture in order to estimate benzene as described by Parke & Williams (1950). Suspension of the triphenyllead acetate was maintained by agitation of the tube at intervals.

Incubation of tri(U-^{14}C)phenyllead acetate with the gut contents of the rat.

Portions of the gut contents taken from a freshly killed rat were added to a sterile medium of 1% Tryptone and Yeast Extract (Oxoid Ltd., Southwark Bridge Road, London, U.K.) in distilled water (10ml) under an atmosphere of CO_2 and H_2. The tubes were either left untreated, boiled for 30min or autoclaved at 15lbf/in^2 for 15min. A solution of tri(U-^{14}C)phenyllead acetate (1.0mg) in dimethylsulphoxide

(0.1ml) was added to the medium which was incubated at 37° for 18h. At the end of this period, (U-^{14}C)benzene was estimated by extracting the mixture with non-radioactive benzene (2x10 and 2x5ml), drying overnight with anhydrous sodium sulphate, distilling and counting aliquots.

Investigation of the in vitro acid lability of tri(^{3}H)phenyllead acetate.

This was carried out by modification of the method of Weiner et al. (1962) for the determination of the in vitro acid lability of various organo-mercury compounds. A solution of tri(^{3}H)phenyllead acetate (approx. 0.0005g) in acetone (1ml) was quickly poured into water and the resulting solution made up to 100ml. Aliquots (3ml) of this solution were incubated with Britton-Robinson buffer pH4 in 0.1M KCl (27ml) at 37°. In one case the incubation mixture contained 1ml of an oxygen free solution of cysteine ($6x10^{-2}$M). Unchanged triphenyllead acetate was estimated by reverse isotope dilution. Triphenyllead acetate (0.5g) was added to the incubation mixture, extracted with toluene (2x35ml) which was dried with anhydrous sodium sulphate. The toluene was removed by distillation in vacuo to give triphenyllead acetate which was recrystallized to constant specific radioactivity from acetone/hexane.

Triphenyllead acetate was estimated after 4h with and without the addition of cysteine and under an atmosphere of nitrogen. Triphenyllead acetate and benzene were estimated after 24h of incubation with buffer under an atmosphere of air and triphenyllead acetate estimated after 24h under an atmosphere of nitrogen.

Results

Incubation of triphenyllead acetate with solutions of varying pH.

As can be seen from table 16 there was a very slow release of benzene at pH 1. The reaction did not appear to occur at pH 7 or at pH 14.

Incubation of tri(U-^{14}C)phenyllead acetate with the gut contents of the rat.

As shown in table 17 there was no indication that the bacteria present in the intestinal tract of the rat catalysed the breakdown of triphenyllead acetate to benzene under the conditions used. However appreciable decomposition of the compound appears to occur on incubation in the medium used. The extent of this breakdown is increased by the addition of food or the contents of the gastro-intestinal tract.

Investigation of the in vitro acid lability of tri(^{3}H)phenyllead acetate.

As shown in table 18, at the end of the twenty-four hour period only about 70% of the radioactivity could be accounted for as triphenyllead acetate. The detection of radioactivity in the distilled benzene suggests that there was decomposition of triphenyllead and not a migration of tritium into the surrounding water.

Discussion

The gut bacteria are known to play a part in the metabolism of orally administered compounds (Drasar *et al.*, 1971) and those which undergo biliary excretion (Smith, 1973). Bridges *et al.* (1967) showed that the rat metabolizes di(1-^{14}C)ethyltin dichloride to give the

ethyltin^{3+} ion. Since there was no formation of (^{14}C)CO_2 the authors suggested that the ethyl group was lost as ethane. Conversion of diethyltin to ethyltin was shown to occur in the presence of the contents of the caecum.

Incubation of tri(^{14}C)phenyltin acetate with non-sterile soil resulted in a slow release of (^{14}C)CO_2. A series of four experiments indicated that 50% of the label was recovered in 140 days. In comparable experiments with sterile soil only very little evolution of (^{14}C)CO_2 occurred (0.4% in 60 days). Two filamentous moulds and a Gram-negative rod capable of bringing about the above reaction were isolated (Barnes et al., 1973). Tonomura and Kanzuki (1969) have shown that a cell-free extract of a mercury resistant pseudomonad is capable of splitting phenylmercury acetate, ethylmercury phosphate and methylmercuric chloride to form metallic mercury and releasing benzene, ethane and methane respectively.

Cleavage of the carbon-mercury bond in methylmercuric salts in vivo to give inorganic mercury occurs in the rat (Norseth & Clarkson, 1970a). However as no difference in the metabolism of the compound by germ free and control rats could be detected, the theory that microorganisms caused the release of the inorganic mercury in the gastrointestinal tract, was rejected (Norseth, 1971). The results obtained for triphenyllead acetate would indicate that as in the case of methylmercury chloride, although degradation of the organometallic compound occurs in the gastro-intestinal tract, the microorganisms present do not catalyse the reaction.

Triphenyllead acetate appears to decompose in aqueous solution. After subcutaneous administration of methoxy(^{14}C)ethylmercury chloride to the rat, ethylene was exhaled in the breath and inorganic mercury

was excreted in the urine. Ethylene was rapidly released when methoxyethylmercury chloride was incubated at 37° at pH 4 in the presence of cysteine. There was considerable release of ethylene at pH 5 but much less at pH 6. The authors concluded that it was possible that the breakdown of methoxyethylmercury chloride was not catalysed by an enzyme system (Daniel et al., 1971). Cysteine did not appear to increase the breakdown of triphenyllead acetate in aqueous solution. A different mechanism to that which yields ethylene from methoxyethylmercury chloride must therefore cause the release of benzene from triphenyllead acetate. Barnes et al. (1973) showed that photochemical degradation of triphenyltin acetate to inorganic tin via diphenyltin- and monophenyltin- compounds, occurs. However ultraviolet light is unlikely to have played a part in the breakdown of triphenyllead acetate in vivo.

Although the results would indicate that some decomposition of triphenyllead acetate occurs in aqueous solution, the rate of breakdown is slow but might account for the release of benzene on intraperitoneal administration. The liver, however, is a well known site for the metabolism of foreign compounds (Parke, 1968). Phenylmercury acetate is stable in weakly acidic solutions at 37° in the presence of cysteine (Weiner et al., 1962). Incubation of the compound in vitro with liver homogenates however, resulted in the release of inorganic mercury and benzene. The reaction was catalysed by the soluble but not the microsomal fraction and did not require NADPH or NADH (Daniel et al., 1972). It is thus likely that on intraperitoneal administration triphenyllead acetate could be metabolised by the liver.

The release of benzene from triphenyllead acetate on oral administration may be the result of a spontaneous decomposition. Barnes

et al. (1973) using triphenyltin 1,2(^{14}C)acetate showed that rapid hydrolysis of the compound occurs giving possibly triphenyltin hydroxide. It is thus possible that in aqueous solution triphenyllead acetate may give triphenyllead hydroxide. This compound could then decompose, the reaction proceeding by removal of one of the products. Other factors, such as the enzymes secreted into the gastro-intestinal tract or those present in the gastric mucosa could, however, cause the release of benzene.

Table 16. Incubation of triphenyllead acetate with solutions of various pH.

Triphenyllead acetate (1g) was incubated at pH 1, 7 and 14. Benzene was removed by aerating the mixture and then estimated according to Parke and Williams (1950).

Time in Hours	µg benzene released		
	pH 1	pH 7	pH 14
0-3	4510	n.d.	n.d.
3-24	2750	trace	n.d.
24-48	6650	n.d.	n.d.
48-72	7000	n.d.	n.d.

n.d. = not detected
trace = less than 1000µg

Table 17. Incubation of tri(U-^{14}C)phenyllead acetate with the gut contents of the rat.

Tri(U-^{14}C)phenyllead acetate was incubated for 18h in a medium containing portions of the gut contents of the rat. At the end of the period (U-^{14}C)benzene was estimated.

Region of intestinal tract from which contents were taken	Treatment of intestinal contents	% of radioactivity recovered as (U-^{14}C)benzene
-	-	7
Food †	Sterilized	28
Stomach	None	30
Stomach	Boiled	27
Stomach	Sterilized	32
Small Intestine	None	20, 23*
Small Intestine	Boiled	7
Small Intestine	Sterilized	19
Caecum	None	7, 8*
Caecum	Boiled	17
Caecum	Sterilized	26

* results of duplicate experiments

† Standard laboratory rat diet was homogenized with water and an aliquot was added to 10 ml of a 1% solution of Tryptone and Yeast extract (Oxoid Ltd., Southwark Bridge Road, London, U.K.). The mixture was sterilized under an atmosphere of CO_2 and H_2 at 15 lbf/m^2 for 15 min. A solution of tri(U-^{14}C)phenyllead acetate (1.0 mg) in dimethylsulphoxide (0.1 ml) was added. The medium was incubated at 37° for 18 h.

Table 18. Investigation of the in vitro acid lability of tri(^{3}H)phenyllead acetate.

A solution of tri(^{3}H)phenyllead acetate was incubated at pH 4 and at 37° with and without the presence of cysteine and under an atmosphere of either nitrogen or air. Unchanged triphenyllead acetate was estimated at the end of the incubation period.

Time of incubation in hours	Presence or absence of cysteine	Gas present in atmosphere	% tritium added	
			As triphenyllead acetate	As benzene
4	+	Nitrogen	94	-
4	-	Nitrogen	89	-
24	-	Nitrogen	74	-
24	-	Air	70	6

CHAPTER 6

DISCUSSION

DISCUSSION

It has been suggested that triphenyllead acetate may have two commercial uses, firstly as a molluscicide for the control of the vector snail of schistosomiasis (Hopf et al., 1967) and secondly as an ingredient of anti-fouling paints (Guillen et al., 1969). Both uses will involve man coming into contact with the compound during its manufacture and subsequent usage. The treating of large expanses of water with the compound could be especially hazardous since there are dangers not only from the contamination of drinking water downstream from the site of application but also from the ingestion of residues of the compound remaining on crops. Conversely the application of triphenyllead acetate to ships, incorporated in an anti-fouling paint is unlikely to lead to widespread contamination but rather to constitute a hazard to a small group of people involved in the manufacture and application of the paint. This metabolic study has been carried out in order to assess the possible fate of triphenyllead acetate in man and thus gain some idea of any possible toxic consequences.

Since the discovery of organometallic compounds in the middle of the last century, many people have died as a result of receiving fatal doses of them. Kehoe in 1925 and Machle in 1935 described in detail the clinical picture of tetraethyllead poisoning. Cases of poisoning are still reported though, occurring mainly as a result of tank cleaning operations (Cassells & Dodds, 1946).

"Stalinon" was a preparation sold in capsules throughout France in 1954 for the treatment of furuncles and other staphyloccal skin infections, osteomyelitis, anthrax and acne. It was said to contain diethyltin diiodide (15mg per capsule) and Vitamin F (linoleic acid) (100mg per capsule). The recommended dose was six capsules a day for

the adult and half that amount for children. Two hundred and seventeen people are known to have been poisoned by this preparation and one hundred of these died. It was found that the main impurities were monoethyltin and triethyltin. The latter compound which accounted for up to 10% of the theoretical amount of diethyltin is ten times as toxic as diethyltin to rats by the oral route (Barnes & Stoner, 1958). The whole episode has been described by Barnes and Stoner (1959).

The toxic effects of organomercurials were reported as long ago as 1865 when two laboratory technicians in St. Bartholomew's Hospital who were engaged in preparing "mercuric methides" developed the characteristic symptoms of alkyl mercury poisoning and subsequently died (Edwards, 1865). However, the significance of organomercury poisoning was not recognised until the two large scale incidents which occurred at Minamata between 1953 and 1960 and at Niigata in 1963. In Minamata Bay high concentration of methylmercury in fish caused many cases of poisoning amongst the inhabitants, cats and waterfowl who ate the fish or shellfish, caught from the bay. Effluent from a process for the manufacture of acetaldehyde that used mercury as a catalyst contained trace amounts of methylmercury (Lofroth, 1970; Report from an Expert Group 1971; Mellpine and Araki, 1958). Jalili and Abasi (1961) reported 600 cases, many fatal, after consumption of home-made bread prepared from flour made from grain, intended for use as seed, which had been treated with the organomercury fungicide, ethylmercury toluene sulphonilide.

In spite of these fatalities organometallic compounds have been used successfully and are of economic importance. The anti-knock tetraethyllead has been in widespread use for about the last fifty years. Stringent, but successful precautions are enforced for its

use (Barry, 1957; Boyd et al., 1957). The arylmercury compound, phenylmercury acetate, has been used successfully as a slimicide in paper pulp mills (Gage, 1964). Triphenyltin acetate is used in agriculture as a fungicide (Barnes et al., 1973). Numerous patents have been published on the uses of tetraalkyltins as polymerization catalysts (Harwood, 1963).

Thus, as with all organometallic compounds any difficulty in working safely with triphenyllead acetate must be weighed against the advantages, both social and economic, that its use would bring. Schistosomiasis is an exceedingly serious disease in rice eating people of the East, where the combination of poor sanitation and the planting of rice by bare-legged people in flooded paddy fields, provides ideal conditions for the spread of the parasite from its secondary host, a water snail, to man. The presence of schistosomes in man causes a disease characterized by body pains, a rash, a cough in the early stages, severe dysentery and anaemia (Buchsbaum, 1961). It is estimated that over two hundred million people are seriously affected by the disease (Vines & Rees, 1974).

Much work has been carried out to provide an explanation of the toxic symptoms produced by organometallic compounds. A great deal of information has been obtained on organomercurial toxicity, following the wide-spread poisoning that occurred in Japan. The short chain alkylmercury compounds - the ethyl and methyl derivatives, are exceedingly toxic. The clinical picture is one of a neurological disorder. In advanced cases serious disability occurs due to severe generalized ataxia, disarthria and concentric constriction of the visual fields (Swensson & Ulfvarson, 1963). In contrast neither symptoms of involvement nor histological damage to the central nervous

system was observed in rats given repeated small doses of phenylmercury acetate at five times the dosage rate which produced the characteristic signs with methylmercury dicyandiamide (Gage & Swan, 1961). Although there is little information in the literature on the toxicity of alkoxyalkylmercury salts, Daniel et al. (1971) suggested that methoxyethylmercury chloride does not show the marked neurotoxic action of alkylmercury salts. In their review Swensson and Ulfvarson (1963) reported that the usual symptoms of alkoxyalkylmercury poisoning came from the intestinal tract with loss of appetite, flatulence, diarrhoea and loss of weight. Neurological symptoms may occur in the form of numbness of fingers and toes, ataxia and paresis.

The neurotoxic action of the short chain alkylmercury compounds is believed to be partly due to the stability of the carbon-mercury bond in these compounds. Both phenylmercury acetate and methoxyethylmercury chloride are rapidly broken down by the rat. When the metabolism of (U-^{14}C)phenylmercury acetate was investigated it was shown that cleavage of the carbon-mercury bond occurred releasing inorganic mercury which was excreted mainly in the faeces and conjugates of phenol and quinol which were excreted in the urine. Metabolism of the compound was rapid, 57% of the radioactivity being recovered in twenty-four hours. In experiments in vitro phenylmercury acetate was broken down to release inorganic mercury and benzene. The reaction was effected by the soluble but not the microsomal fraction of the liver cell and did not require NADPH or NADH (Daniel et al., 1972). Methoxy(^{14}C)ethylmercury chloride is rapidly broken down after subcutaneous administration to the rat. Ethylene is exhaled in the breath and inorganic mercury is excreted in the urine. The half-life of the compound in the tissues was estimated to be about one day

(Daniel et al., 1971).

Cleavage of the carbon-mercury bond occurs during the metabolism of methylmercury chloride after intravenous administration to rats. Inorganic mercury was detected in plasma, brain, kidney and liver while mercury was excreted mostly in the faeces (Norseth & Clarkson, 1970b). However the carbon-mercury bond in methylmercury appears to be more stable than it was in phenylmercury acetate or methoxyethylmercury chloride. Although no accurate figures could be given for the overall rate of biotransformation Norseth (1969) calculated that about 20% of the dose may have undergone this reaction during the first ten days after injection of the methylmercury chloride. Slow excretion of other alkylmercury compounds by the rat has also been observed. Miller et al. (1961) detected ethylmercuric chloride in the liver and kidney of rats twenty-eight days after they had received an oral dose of the compound. Takeda et al. (1968) showed that the alkylmercury compounds ethylmercuric chloride, S-ethylmercuric cysteine and n-butylmercuric chloride were excreted more slowly than the arylmercury compound, phenylmercury chloride. A study of the fate of ethyl(^{203}Hg)mercuric chloride in rats showed that eight days after a subcutaneous dose of the compound, 93% of the mercury in the liver and 63% of that in the kidney was present as ethylmercuric chloride (Takeda & Ukita, 1970).

The difference in the distribution of alkyl- and aryl- mercury compounds in the brain of rats seems to correlate with the specific neurotoxicity of the short-chain alkylmercury compounds (Takeda et al., 1968). It has been suggested that the toxic agent is the intact organomercurial (Norseth & Clarkson, 1970a).

Symptoms of tetraethyllead poisoning are likewise unlike those of inorganic lead poisoning. The usual symptoms produced by inorganic

lead i.e. abdominal colic, stippling of the red blood cells, anaemia and an elevation of the urinary porphyrins are not usually present. The symptoms produced by tetraethyllead in man include headache, anxiety, insomnia with bad dreams, hallucinations and tremors, all suggesting involvement of the central nervous systen (Kehoe, 1925; Machle, 1935). The toxicity of tetraethyllead is believed to be due to the formation of the triethyllead ion (Cremer, 1959). Triethyllead was detected in the internal organs seven days after rats had received an intravenous dose of 31μmols tetraethyllead per kilogram of body weight (Bolanowska, 1968). As with methylmercury the brain appears to be the target organ, because triethyllead inhibited the complete combustion of glucose by brain slices in vitro (Cremer, 1959 & 1962).

The use of triphenyllead acetate must therefore be considered in the light of the known toxicity of methylmercury and tetraethyllead. In both cases the toxic agent appears to be an organometallic compound which is slowly excreted and the target organ is the brain. A stable carbon-metal bond is probably present in the toxic agent.

Consideration must be given to the different ways triphenyllead acetate could produce its toxic action. Firstly, the toxic agent could be unchanged triphenyllead acetate, or it could be an organometallic metabolite. Secondly, the toxic agent could be inorganic lead. The tissue distribution of this metal could be different to that obtained after administration of an inorganic lead salt, due to prior distribution of the organolead salt followed by decomposition to the inorganic metal.

There is a large difference in the LD_{50} values of triphenyllead acetate given orally (200-300mg/kg) and intraperitoneally (approx.

6mg/kg). After oral administration, a large proportion of the radioactivity was recovered in the air as benzene while conjugates of phenol and quinol were detected in the urine. Little lead was found in the urine whereas a large part of the dose was recovered in the faeces. These facts suggest that triphenyllead acetate is broken down in the gastro-intestinal tract. Metabolism to benzene also occurs after intraperitoneal administration but it would appear to take place at a slower rate.

As the LD_{50} value for triphenyllead acetate on intraperitoneal administration is low (approx. 6mg/kg), it is possible that on oral administration a small amount of unchanged triphenyllead acetate is absorbed into the body and that this is the toxic agent.

High instability of the carbon-lead bond in triphenyllead acetate would reduce the hazard from using the compound. Benzene is released after both oral and intraperitoneal administration showing that cleavage of the carbon-lead bond occurs readily in conditions found in vivo. One would expect organolead compounds to be relatively unstable from the position of lead in the Periodic Table. The elements of the fourth main group of the Periodic Table are from top to bottom carbon, silicon, germanium, tin and lead. In this order the atoms of the elements increase in size and the outer electrons are less firmly held. Their increasing freedom lends the element a more metallic character. Thus an element lower down the Periodic Table is more electropositive. From carbon to lead the element-carbon bond length increases. The carbon-lead bond is thus the weakest of the bonds between carbon and other group four elements (Table 19). This compares with the bond strength of the carbon-mercury bond which has been estimated to be in the region of 23 to

27kcal/mol (Cottrell, 1958) and a bond length of 2.07A° (Sutton, 1965).

Decomposition of triphenyllead acetate in aqueous solution would increase its potential as a molluscicide for use in open water. The studies here, indicated that triphenyllead acetate was unstable in aqueous solution. It is of interest that in field trials it was found that the two usual methods of estimating triphenyllead acetate, namely by thin-layer chromatography and colorimetrically after reaction with dithizone, were not satisfactory due to changes which occurred when the compound was added to water. Instead total organic-soluble lead was estimated (Hopf et al., 1967). Barnes et al. (1973) showed that photochemical degradation of triphenyltin acetate to inorganic tin via diphenyltin and monophenyltin compounds occurs. Hopf et al. (1967) reported however, that photochemical degradation was not a significant factor in the loss of organic-soluble lead. If triphenyllead acetate were to undergo similar reactions this would increase its potential as a molluscicide for use in the treatment of freshwater, especially in tropical regions. The resulting inorganic lead would be of little danger as the lead intake of people living in areas where bilharzia is rife would probably be low. In screening tests on the toxicity of organolead compounds tetraphenyllead, diphenyllead diacetate and phenyllead triacetate were all found to be less toxic than triphenyllead acetate when given orally to rats (Fowler, D.G.).

In view of the known neurotoxic actions of methylmercury chloride and tetraethyllead the possibility of triphenyllead acetate or an organolead metabolite crossing the blood-brain barrier must be considered. It would be expected that triphenyllead acetate

Table 19. Distances and strengths of bonds between carbon and group four elements

Element	M-C distance (A) (Sutton, 1965)	M-C bond strength (kcal) (Cottrell, 1958)
C	1.54	83
Si	1.54-1.87	60
Ge	1.95	-
Sn	2.18	54
Pb	2.30	31

would be lipid soluble as it is highly soluble in non-polar solvents and only sparingly soluble in water (Willemsens & van der Kerk, 1965). Traces of lead in the brain were detected after both oral and intraperitoneal administration but the chemical nature of the material was not determined. The metal lead can usually be detected in the brain of man (Barry & Mossman, 1970; Schroeder & Tipton, 1968) but the highest concentration is found in bone (Kehoe, 1961). Barry & Mossman (1970) showed that there was little evidence of increased deposition of lead in the brain between occupationally exposed and non-occupationally exposed men of the age range 56–70 years. There was however increased deposition of lead in the bones. If an organometallic compound is rapidly broken down in the tissues one would expect a difference in the tissue distribution of the metal to that found after administration of the metal itself. It has been shown that prolonged administration of triphenyllead acetate in the diet of rats caused increased levels of lead in the liver, brain and bone. Likewise after rats were exposed to an aerosol of triphenyllead acetate at a level of $450\mu g/m^3$ there was increased deposition of lead in the kidney, liver, brain and bone (Hine Laboratories, 1972). As a result of metabolism of triphenyllead acetate to inorganic lead one would expect deposition of the metal in the kidney, liver and bone. However the increased levels of lead in the brain might indicate that triphenyllead acetate or an organolead metabolite is capable of crossing the blood-brain barrier.

Finally, consideration must be given to the effect triphenyllead acetate would have on the general population if it were to be used. Triphenyllead acetate is only sparingly soluble in water having an approximate solubility of 0.15g/l (Willemsens & van der Kerk, 1965).

The daily water intake of an average man is about two litres per day. Saturation of this water with triphenyllead acetate would result in a daily intake of about 300mg/day, which for an average man of 70kg would mean the ingestion of a daily dose of 4.3mg/kg. This figure is a factor of 50 below the oral LD_{50} of triphenyllead acetate to the rat. Treatment of water to kill the molluscs would only be required at intervals and provided that stagnant water was not treated it is unlikely that triphenyllead acetate would be ingested for long periods of time.

When a compound is widely dispersed in water there is a possibility that it will be ingested by a diverse population which will include pregnant women. Autoradiography showed the presence of radioactivity in the foetus after administration of methyl(^{203}Hg)-mercury dicyandiamide to the mouse (Berlin & Ullberg, 1963). In Japan some children, born to mothers who themselves showed no sign of methylmercury poisoning, were found to display the clinical symptoms of intoxication (Matsumoto et al., 1965). Two organolead compounds, tetraethyllead and tetramethyllead are essentially non-teratogenic when fed to rats (McClain & Becker, 1972). In a breeding study on mice fed on diets containing triphenyllead acetate, no abnormalities were found in the offspring (Walker, 1967). However, species react differently to known teratogens. For instance, thalidomide which has pronounced teratogenic effects in man was found to be only moderately teratogenic to the rabbit, mouse and rat (Lister, 1974). Thus before widespread use of triphenyllead acetate occurs, further teratogenic studies should be undertaken.

During its manufacture and use, triphenyllead acetate might be absorbed unchanged, either through the skin or by inhalation. It is

believed that tetraethyllead is absorbed through the skin (Kehoe & Thamann, 1931). The physical properties of tetraethyllead and triphenyllead acetate are however quite different, the former being a colourless liquid and the latter a solid with a high melting point. Nevertheless, in two separate studies triphenyllead acetate had a pronounced effect on the skin. It caused flaking and scaling when applied to the skin of rabbits and lead analyses of the liver and blood showed increasing accumulation of lead with increasing dose of triphenyllead acetate. Female volunteers developed erythema and oedema of the skin after contact with the compound. It has also been noted that one laboratory worker involved in the preparation of triphenyllead acetate showed evidence of absorption of lead (Lyle, personal communication).

In conclusion, the organolead compound triphenyllead acetate is toxic when given orally to rats and highly toxic when given intraperitoneally. After both routes of administration the compound breaks down to release benzene, but the rate of metabolism would appear to be slower after intraperitoneal administration. In view of the high toxicity of triphenyllead acetate extremely stringent precautions to prevent its absorption by ingestion, inhalation or percutaneously, during its manufacture and use, would be required. Once applied to water as a molluscicide, the hazard to the general population would probably be acceptable owing to the low concentrations that would be encountered.

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APPENDIX I

ESTIMATION OF ^{203}Pb

ESTIMATION OF ^{203}Pb

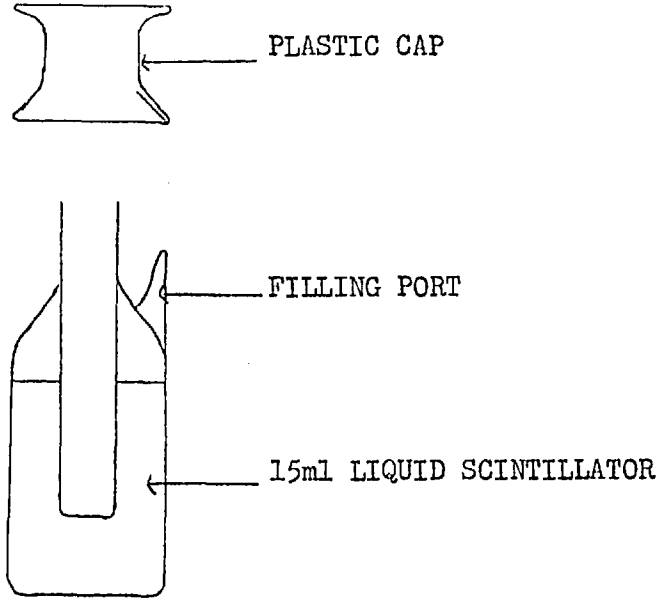
The lead isotope ^{203}Pb has great potential in metabolic studies on lead. Although this isotope emits gamma radiation, the low energy of this radiation (energies of 0.279, 0.401 and 0.680 MeV)(Heath, 1967) and the fact that it decays to non-radioactive thallium means that it has several advantages over the more widely available ^{210}Pb. Its only disadvantage is its short half-life of 52h.

This isotope can be readily estimated by gamma detectors which employ a sodium iodide crystal and as a minimum of sample preparation is required, this method is advantageous. However, the isotopes which are widely used in metabolic work, namely ^{14}C, ^{3}H, ^{35}S and ^{32}P, are all β-emitters. Thus a method that would allow ^{203}Pb to be estimated in a liquid scintillation counter would be of great use. Such a method has been described for the counting of ^{125}I (Ashcroft, 1970). Moreover, the method has the advantage of ease of sample preparation obtained with a conventional gamma-detector. A novel sealed counting vial containing a scintillator incorporating tetrabutyltin was used (Fig. 8).

Such a vial is now marketed (Gammavial, Koch-light Laboratories, Colnbrook, England). The sample to be counted is placed in a disposable plastic tube which is inserted in the well of the counting vial. The vial is then counted in the usual manner.

However, even this system has the disadvantage of being rather costly, so some preliminary investigations were carried out to see if a modified system could be devised.

Fig. 8 A counting vial for routine gamma counting of iodine-125 using a metal-loaded liquid scintillator Ashcroft, 1970.



METHODS

Evaluation of the Gammavial for counting ^{203}Pb

A sample of blood containing ^{203}Pb was placed in a Gammavial A (filled with a toluene / PPO / POPOP scintillator loaded with tin). The sample was counted in a Packard Autogamma Spectrometer (40% amplification, 150-450 window, high voltage 798 volts) and in a Packard Tri-Carb scintillation spectrometer model 3320 (13.5% gain, 50-1000 window).

Investigation of the use of normal vials for counting ^{203}Pb

Scintillator consisting of 9ml of the scintillator used for counting tritium (see Materials and Methods section) and 6ml of tetrabutyltin was placed in a plastic capped counting vial (dimensions 2.7 x 5.6 cm). A sample of blood containing the isotope ^{203}Pb was placed in a glass tube with a polythene closure (dimensions, 2 x $\frac{1}{2}$in) (C.E. Payne, Iveley Rd., London) which was placed centrally in the glass vial. The optimum gain setting was determined. The sample was then counted in a scintillator containing varying percentages of tetrabutyltin.

RESULTS

Evaluation of the Gammavial for counting ^{203}Pb

The counts from the Gammavial were very similar to those obtained on counting the sample in the gamma-counter, being 159440 and 158870 respectively.

Investigation of the use of normal vials for counting ^{203}Pb

It was found that the optimum gain setting was 32% and the

optimum concentration of tetrabutyltin mixed with scintillator was 35% v/v. Using these figures it was found that the counts obtained from the sample compared favourably with those obtained from the conventional gamma-counter. In no case was the efficiency of counting determined as a standard sample of ^{203}Pb was not available.

Table 20. Comparison of the counts obtained from the liquid scintillation counter and the gamma-counter.

	Cpm	Background
Packard Autogamma Spectrometer	6300	95
Liquid Scintillation Counter	6380	67

DISCUSSION

It would appear that a system using a tin-loaded scintillator would be successful for the counting of the isotope ^{203}Pb. Both systems have several disadvantages. The Gammavial is rather expensive. While this fact is overcome in the modified system two other disadvantages become apparent. Firstly, there is the risk of absorbing tetrabutyltin on changing the sample tube. Like most organometallic compounds tetrabutyltin is toxic (Stoner et al., 1955; Cremer, 1958). Secondly, it is very difficult to centre the sample in the counting vial which may upset the efficiency of counting. However, in view of the savings that would be made on instrumentation, such a method has many advantages.

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APPENDIX II

THE MASS SPECTRA OF SOME ORGANOLEAD COMPOUNDS

THE MASS SPECTRA OF SOME ORGANOLEAD COMPOUNDS

The mass spectra were prepared using a Varian MAT CH5 mass spectrometer at an ionization voltage of 70eV. The organolead compounds were prepared as solutions in methanol (1mg/ml).

Fig. 9 The mass spectrum of triphenyllead acetate.

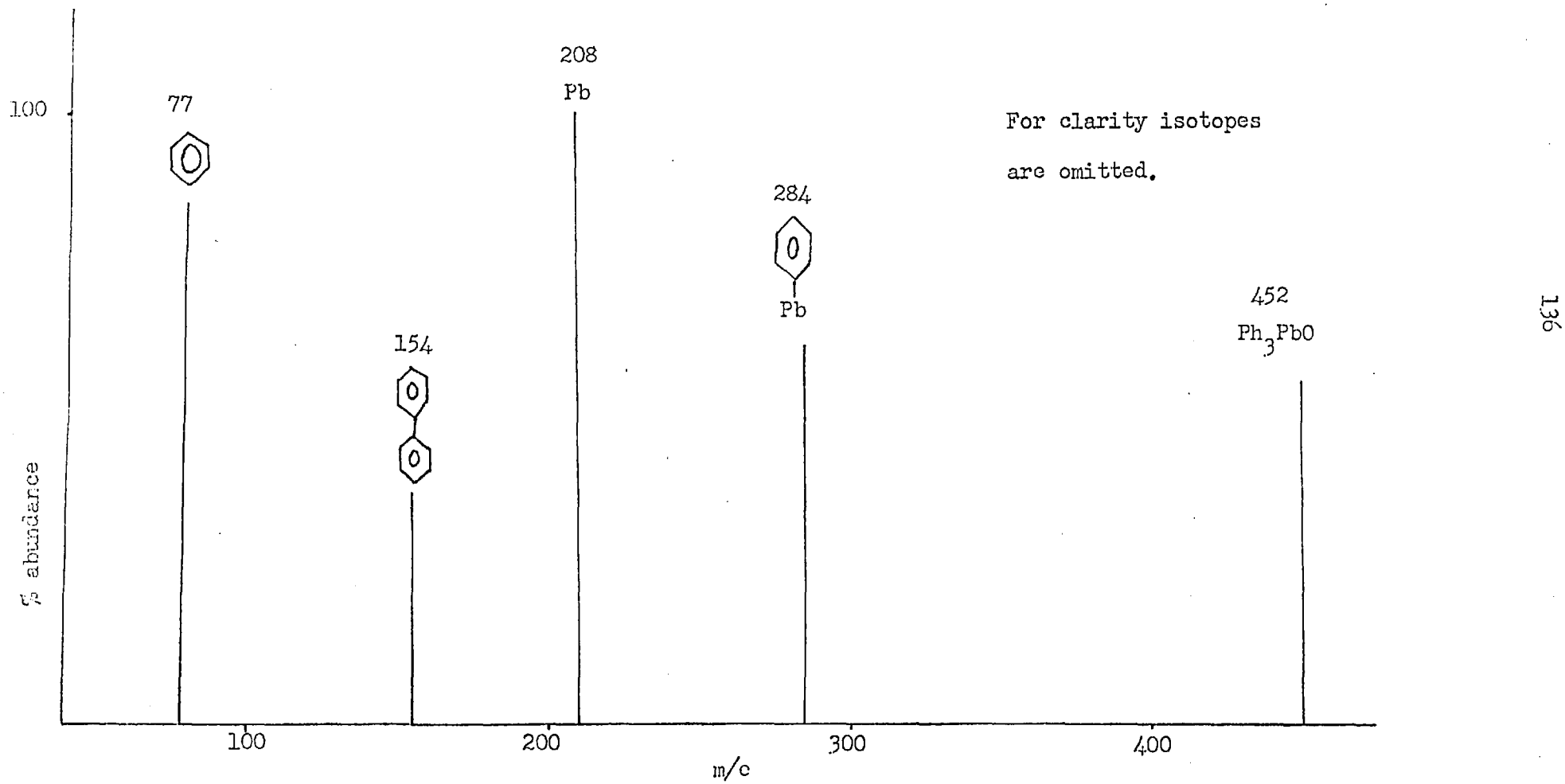
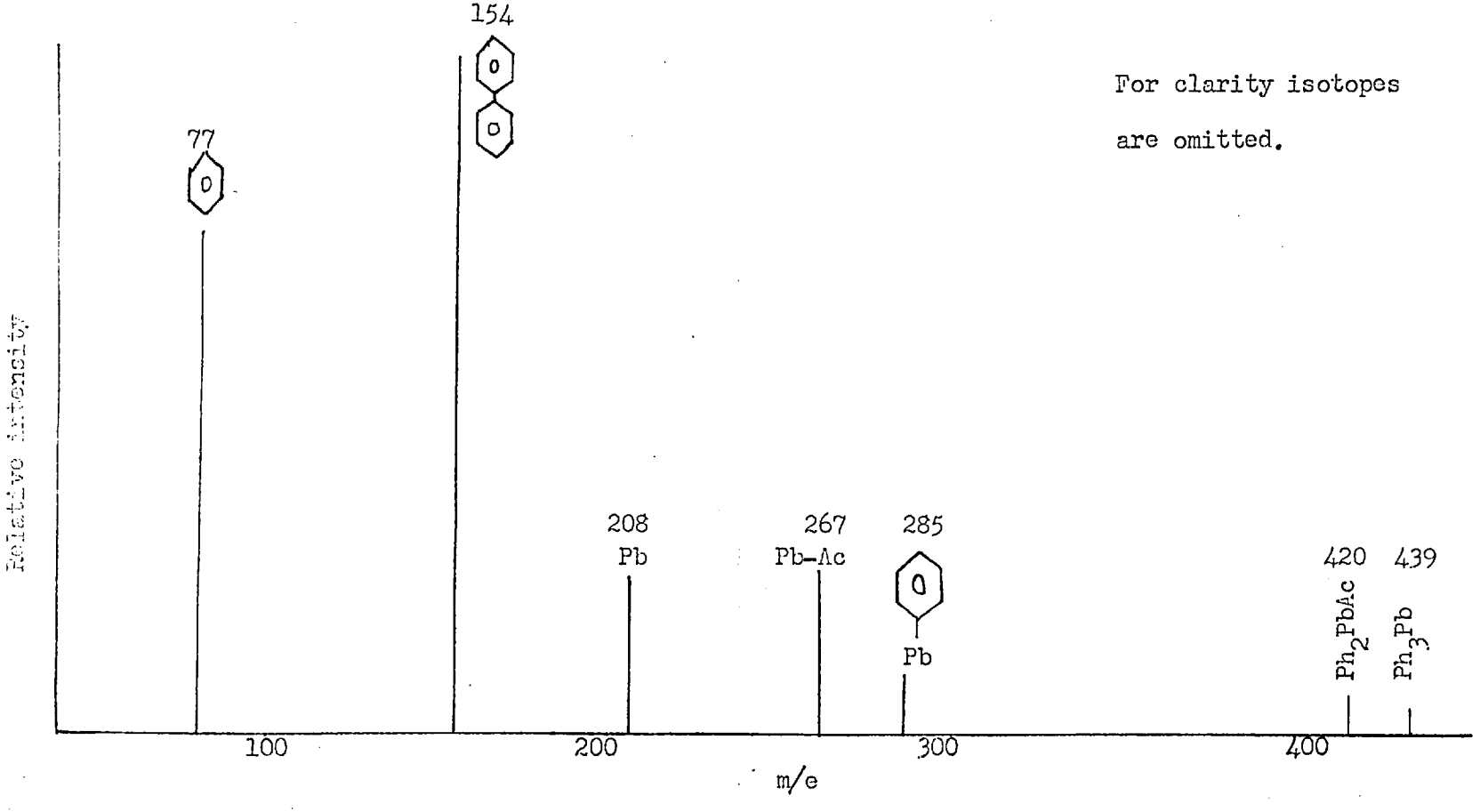


Fig. 10. The mass spectrum of diphenyllead diacetate.



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Benzene as a Metabolite of Triphenyl-lead Acetate in the Rat

By BARBARA WILLIAMS, L. G. DRING and R. T. WILLIAMS (*Department of Biochemistry, St. Mary's Hospital Medical School, London W.2, U.K.*)

Triphenyl-lead acetate has been proposed as an anti-fouling agent in paint (see, e.g., Guillen *et al.*, 1969) and as a molluscicide for the control of bilharzia (Hopf *et al.*, 1967).

This compound is quite toxic to rats when injected intraperitoneally in dimethyl sulphoxide (approx. LD_{50} 6mg/kg), but is considerably less toxic by mouth (approx. LD_{50} 200–300mg/kg). Tri[U-^{14}C]-phenyl-lead acetate was synthesized from bromo-[U-^{14}C]benzene and lead dichloride and administered orally dissolved in dimethyl sulphoxide to rats (dose 25mg/kg). The urine, faeces and expired air of the animals were collected daily for 7 days. During this period 25% of the ^{14}C was recovered in the urine, 29% in the faeces and 20% in the expired air, these being average values for three rats. With an oral dose (200mg/kg) of unlabelled triphenyl-lead acetate, no lead could be detected in the urine, but 75% was found in the faeces in 7 days.

The ^{14}C of the expired air was found to be present almost entirely as benzene, with a little $^{14}CO_2$ (1%). The benzene was trapped in methanol at −70°C and isolated as the nickel cyanide ammonia clathrate (Parke & Williams, 1953*a*) after addition of non-radioactive benzene. The benzene was released from the clathrate by heating and sulphonated in fuming H_2SO_4. The sodium benzenesulphonate was recrystallized to constant specific radioactivity, and the benzenesulphonic acid was identified as its *p*-toluidine salt, m.p. 195–198°C, and by the i.r. spectrum of the sodium salt. By radiochromatogram scanning, the ^{14}C of the urine was found to be present as mainly phenyl sulphate, with a smaller amount of quinol sulphate. The faecal ^{14}C has not yet been identified.

These experiments suggest that triphenyl-lead acetate as such is not absorbed from the intestine. It appears that the compound is at least partly decomposed in the gastrointestinal tract, possibly by the gut flora, to give benzene, which is then absorbed. Some, possibly nearly a half, of the absorbed benzene is eliminated as such in the expired air and the rest is metabolized to known benzene metabolites (Parke & Williams, 1953*b*), mainly phenol, which is excreted in the urine as conjugates.

This work was supported by grants from the Medical Research Council and Pure Chemicals Ltd., Liverpool, U.K.

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