

REVIEW OF LEAD TOXICOLOGY RELEVANT TO THE SAFETY ASSESSMENT OF LEAD ACETATE AS A HAIR COLOURING

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Summary—The literature on lead toxicology has been critically reviewed to provide a safety assessment of lead acetate as a hair colouring. The main objectives were: (i) to determine the additional lead contribution from hair-colouring use to the total daily environmental lead intake; and (ii) to assess the toxicological significance of this additional contribution. The review also focuses attention on newer issues of concern over the effects of environmental lead on human health. Data available in animals and humans (including occupational exposure), mainly on lead acetate and other inorganic lead salts, have been presented and evaluated in respect of the following: absorption, distribution and excretion following ingestion; percutaneous absorption; carcinogenicity; genotoxicity; reproductive toxicity; neurological/behavioural status with particular reference to neuropsychological effects in children; and effects on other systems (e.g. cardiovascular). It is concluded that the absorption of lead from hair-colouring use represents about 0.5% of the lead absorption from the current average daily environmental lead intake. No convincing evidence could be found of any deleterious effect of current environmental lead levels on human health and thus the tiny contribution of lead acetate exposure from hair-colouring use can be regarded unequivocally as being toxicologically insignificant.

Introduction

The principal aims of this review are to establish whether or not lead exposure from hair-colouring use makes a material contribution to the daily amount of absorbed lead from all sources of lead intake (namely food, water, air, etc.) and to assess the toxicological significance of the contribution. Since lead acetate hair colourings were first marketed and gained regulatory approval, the debate on the potential effects of environmental lead on human health has raised new issues and areas of concern, and this review will also focus attention on these more recent developments.

Four types of hair dyes—permanent (oxidation dyes), semi-permanent (acidic and basic dyes), temporary and progressive—have been marketed for over 30 years. The progressive hair colourings usually contain lead acetate designed to darken greying hair on exposure to the atmosphere. Commercial products typically contain 0.57–0.75% (w/w) of lead acetate (as trihydrate) so that the lead concentration is 0.31–0.41% (w/w) in the product.

By their very nature, lead acetate hair colourings are used by adults and not children. Exposure

is intermittent, with once- or twice-weekly applications being the norm. The volume of the product normally applied is about 6 ml. Of this, approximately 0.18 ml reaches the scalp. The amount of lead reaching the scalp after each application has been reported to be about 612 μg (Moore *et al.*, 1980). The use of the product is discouraged on scalp hair where there are cuts, abrasions or disorders of the skin of the scalp. Also, the use of the product on hair other than that of the scalp is not recommended.

The efficacy of lead acetate hair colourings relies on a biochemical mode of action. Lead ions readily form complexes with anionic ligands such as SH groups in proteins. These reactions provide the rationale for the use of lead acetate as a hair colouring in that lead ion combines with available SH groups only in the hair to form black insoluble lead sulphides thereby darkening the hair. Sulphur, also present as an ingredient of hair colourings, will undergo reduction, the subsequent reaction with lead acetate thereby contributing to the dyeing process.

Regulatory approval has been granted in various countries. In the USA, the FDA (1980 and 1981) concluded that lead acetate was safe for use in cosmetics that colour the hair of the scalp and approved its use, subject to a maximum content of 0.6% (w/v) lead in the product. Lead acetate hair colourings have also been approved in Australia,

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Abbreviations: CNS = central nervous system; SCE = sister chromatid exchange.

Brazil, Canada and in the European Community (EEC, 1990).

It has been calculated that the commercial use of lead acetate hair colourings in West Germany (as it used to be known) would give rise to an annual consumption of 162 kg lead, which compares with a total annual lead consumption of 340,000 tons in that country and 4.5 million tons worldwide (Ippen *et al.*, 1981). In Belgium, about 46 kg of lead has been estimated to be used annually in lead acetate hair colourings, compared with an estimated annual total of 550,000 kg of lead from airborne emission relating to the use of leaded petrol in the same country (M. K. Taylor, personal communication, 1990). The eventual discharge of lead from lead acetate hair colourings into the water supply has been estimated to give rise to lead concentrations in the range 0.0004 to 0.01 µg/litre (M. K. Taylor, personal communication, 1990), compared with an EEC prescribed limit of 50 µg/litre for lead in the water supply. These data show that the contribution from lead acetate hair colourings to the total environmental lead burden is negligible.

There is a massive database on the toxicology of lead in laboratory animals and humans covering classical lead toxicology and all facets of environmental and occupational lead exposure. In this review we draw heavily on this database, concentrating on those areas relevant to the safety assessment of lead acetate as a hair colouring. In addition, data available on lead acetate hair-colouring preparations will be summarized and assessed.

The nature of the product's use is such that particular attention will be given to the absorption of lead salts through the skin of animals and humans under normal and exaggerated exposure conditions.

In the assessment of the toxicological findings in the rat and humans, particular attention will be given to a major species difference in that the primary target for lead toxicity is the kidney in the rat and the central nervous system (CNS) in heavily exposed humans.

The finding of lead-induced renal tumours in rodents, albeit at doses substantially above those that are tolerated by humans, poses a need for careful consideration in terms of the mechanism of carcinogenic action on the target organ and of the significance of the finding to humans. Genotoxicity data, dose-response relationships for renal effects in laboratory animals and epidemiological evidence play a crucial role in this consideration.

For the most part, the toxic effects of lead and lead compounds correlate with blood lead levels. Hence, although the focus of interest in this review is lead acetate, data concerning other lead salts such as lead subacetate or phosphate are also relevant to our assessment. Far less relevant are other lead compounds such as lead arsenate, lead chromate or lead dimethyldithiocarbamate where the non-lead moiety may be an important determinant of the compound's toxicity.

Toxicokinetics in humans and laboratory animals

Assays to estimate body lead levels

The body burden of lead has been estimated by measurement of the lead concentration in blood, shed

teeth and hair. Although none of these is an ideal index of the body lead burden, the blood lead concentration, which reflects both recent lead exposure and steady-state prolonged exposure, is the most reliable marker. Other indices of lead exposure include urinary lead and biochemical indicators of absorbed lead such as zinc protoporphyrin concentration or δ -aminolaevulinic acid dehydratase activity in the blood, but such measurements are considered less reliable or are not suitable for epidemiological studies, as compared with blood lead levels (MRC, 1984 and 1988). More recently, lead has been measured in human bone using an X-ray fluorescence technique (McGregor and Mason, 1990).

Lead intake in humans

For most adults in the UK (as indeed in other industrialized countries), food is the major source of lead intake, other sources such as drinking-water and air making only a small contribution; most lead in food is believed to be in the inorganic form (MAFF, 1989). Normal hand-to-mouth activity or pica in children can contribute significantly to the total lead intake (Table 1) and in such children about 50% of the blood lead level may be of non-dietary origin, that is to say from the oral intake of lead-bearing dust and dirt (ATSDR/EPA, 1988; EPA, 1986; US Nutrition Foundation, 1982).

In the UK and the USA, recent estimates of the total daily intake of about 100 µg contrast with intakes of up to 500 µg a decade or more ago (Table 1). Additional exposure above baseline levels is, however, not uncommon (ATSDR/EPA, 1988; EPA, 1986; Table 1) and consumption of 2 litres beer/day could add a further 110 µg of lead to the daily intake (MAFF, 1989). Currently in the UK, the average daily intake of lead from food, water and air was reported by MAFF (1989) to be below the FAO/WHO (1972) provisional tolerable daily lead intake derived from 3 mg/week, although in some areas with high lead levels in tap-water this limit has been exceeded. For other countries, the average dietary lead intake has been estimated to be 60–80 µg/day in the USA, Finland and Denmark and 210–290 µg/day in Austria, Belgium and Italy (MAFF, 1989).

Absorption, distribution and excretion of lead

Following dietary or oral exposure, the extent of gastro-intestinal absorption of lead depends on various factors including age, fasting, nature of lead-containing source and nutritional status.

Whilst only 10–15% of dietary lead is absorbed in adults, as much as 50% is absorbed in children (ATSDR/EPA, 1988; EPA, 1986; US Nutrition Foundation, 1982). Similarly in laboratory rats, the young absorb 40–55% of an oral dose and adults only 1–15% (ATSDR/EPA, 1988; EPA, 1986; Kostial *et al.*, 1971). Young rats seemingly soon lose their high absorption profile. Lactating rats placed on a basic diet containing 0.5% lead acetate for 7 weeks showed a blood lead level of 100 µg/100 ml; their offspring given the same diet after weaning showed three times this blood lead level at 3–5 weeks of age before returning to the maternal level by 7 weeks of age (Mykkanen *et al.*, 1979).

Table 1. Estimates of daily lead intake ($\mu\text{g/day}$) from the general environment and of amount absorbed ($\mu\text{g/day}$) in humans

Food	Water	Air	Dust, soil, paint	Amount of lead absorbed	Reference
300	—	30–40	—	30–50	Kehoe, 1964
200	20*	—	—	—	MAFF, 1972
—	—	—	—	30 (food) + 7.5 (air)	MAFF, 1975
100–500	50 (upper limit)	3 (non-urban) 30–60 (urban)	—	—	WHO, 1977
250 (range 100–500)	100 (max)	2 (non-urban) 20–400 (urban)	—	35 (total)	FDA, 1980
100	—	—	—	—	MAFF, 1982
77–132	16.3	1.5–62.5	—	—	US Nutrition Foundation, 1982
20–83†‡	0.8–8†	0.2–4.5†	20–500†	—	US Nutrition Foundation, 1982
34.9–54.7§	—	1§	4.5§	—	Elias, 1985
25.8 ¶	—	0.5¶	21¶	—	Elias, 1985
20–70	10**	—	—	—	UK MAFF, 1989

*Pb intake from beverages.

†In infants up to 6 years old.

‡Equivalent to 3–8 $\mu\text{g/kg}$ body weight/day.§Data for adults—total intake 40.4–60.2 $\mu\text{g/day}$, equivalent to 0.59–0.76 $\mu\text{g/kg}$ body weight/day (estimate of baseline scenario intake based on person living and working in non-urban environment, consuming food from a typical grocery shelf and no habits/activities tending to increase lead exposure). Additional intakes ($\mu\text{g/day}$) from exposure to urban environment—dust (7–28), residence near smelter (250), interior lead paint (17), garden vegetables (120), wine consumption of 1 litre/day (100), smoking of 30 cigarettes/day (30), primary occupational source (1100) and secondary occupational source (44) (ATSDR/EPA, 1988; Elias, 1985; EPA, 1986).

||Data for food, water and beverages combined.

¶Data for 2-year-old children—total intake 47.3 $\mu\text{g/day}$, equivalent to 3.6 $\mu\text{g/kg}$ body weight/day (baseline intake estimate) to be supplemented by additional intake ($\mu\text{g/day}$) from exposure to urban environment—dust (91), residence near smelter (2200), interior lead paint (110), garden vegetables (48) and secondary occupational source (150) (ATSDR/EPA, 1988; Elias, 1985; EPA, 1986).

**Value represents water and air intake combined.

Fasting also has a pronounced effect on gastrointestinal absorption of lead (ATSDR/EPA, 1988; EPA, 1986) and studies with ^{203}Pb have shown that fasting human subjects administered lead in water absorb 40–50% (MAFF, 1989).

The absorption of ^{203}Pb is greater when taken with beverages such as tea, coffee or beer than when taken with food (14–19% *v.* 7%) (MAFF, 1989).

Nutritional factors can dramatically influence the degree of lead absorption from the gastrointestinal tract (ATSDR/EPA, 1988; EPA, 1986; US Nutrition Foundation, 1982). In laboratory animals, depletion or deficiency in calcium, iron, zinc, copper or phosphate results in enhanced absorption and tissue uptake of lead; supplementation with vitamin D, lipids or milk components (particularly lactose) enhances lead absorption (ATSDR/EPA, 1988; EPA, 1986) as does placing rats on various human-type diets (Kostial and Kello, 1979). Calcium and phosphate supplementation retards lead absorption (ATSDR/EPA, 1988; EPA, 1986). In deprived children with suboptimal nutritional states for iron and calcium, elevated lead absorption has been reported (ATSDR/EPA, 1988; EPA, 1986).

About 30–50% of inhaled lead is retained and this undergoes extensive and rapid absorption in humans and laboratory animals (ATSDR/EPA, 1988; EPA, 1986).

In the USA, blood lead levels are dependent on age, sex, race and degree of urbanization—being 1–5 $\mu\text{g}/100\text{ ml}$ in remote areas and 10–20 $\mu\text{g}/100\text{ ml}$ in populated areas. During 1976–1980, blood lead levels were 11.9, 12.8 and 14.8 $\mu\text{g}/100\text{ ml}$ in rural, small urban and large urban areas, respectively. A

downward trend in blood lead levels continued from the 1970s into the 1980s (ATSDR/EPA, 1988; EPA, 1986). Blood lead levels in the USA were also reported to be 17–18 $\mu\text{g}/100\text{ ml}$ (FDA, 1980; US Nutrition Foundation, 1982). More recently in the UK, steady-state blood levels averaged 12.2 and 8.5 $\mu\text{g}/100\text{ ml}$ in men and women, respectively (MAFF, 1989). In the UK, average blood lead levels fell by 0.5–1 $\mu\text{g}/\text{ml}$ for each of the years in the period 1984–1987; by 1987 the levels in both adults and children had fallen to below 10 $\mu\text{g}/100\text{ ml}$ (DoE, 1990). The EEC (1977) set an acceptable upper blood lead level of 35 $\mu\text{g}/100\text{ ml}$ but more recently it has been recommended that where the blood lead levels exceeds 25 $\mu\text{g}/100\text{ ml}$, steps should be taken to reduce environmental exposure, especially in children (ATSDR/EPA, 1988; DoE, 1990; MAFF, 1989).

The relationship between the lead intake from the diet, water and air and the blood lead level is reported to be curvilinear such that successive increases in lead intake will yield progressively smaller increases in the blood lead level (US Nutrition Foundation, 1982). This does not necessarily mean that lead uptake into the body is proportionately lower at higher exposure levels as the blood lead level may not reliably reflect the tissue lead burden with increasingly high exposure (ATSDR/EPA, 1988; EPA, 1986).

In the carcinogenicity study of lead acetate in rats (Azar *et al.*, 1973), blood lead levels at 2 yr in groups on 0, 10, 50, 100 and 500 ppm (as Pb in the diet) were 12.7, 11.0, 18.5, 35.2 and 77.8 $\mu\text{g}/100\text{ ml}$, respectively, and in satellite groups on 0, 1000 and 2000 ppm (as Pb) 16.4, 98.6 and 98.4 $\mu\text{g}/100\text{ ml}$, respectively.

These data also show the existence of a curvilinear relationship.

It has been estimated (ATSDR/EPA, 1988; EPA, 1986) that exposure to lead from different sources would contribute to blood lead levels on an incremental basis as follows: 0.02–0.05 $\mu\text{g}/100\text{ ml}$ in blood (adults) and 0.16 $\mu\text{g}/100\text{ ml}$ (children) per $\mu\text{g}/\text{day}$ in diet; 1.25–2.46 $\mu\text{g}/100\text{ ml}$ per $\mu\text{g}/\text{m}^3$ in air; 0.06 $\mu\text{g}/100\text{ ml}$ per $\mu\text{g}/\text{litre}$ in water; 0.6–6.8 $\mu\text{g}/100\text{ ml}$ per 1000 ppm in soil. Other estimates (MAFF, 1983 and 1989; US Nutrition Foundation, 1982; WHO, 1977) reached similar conclusions. It has also been estimated that for 2-yr-old children, a blood level of 4.32 $\mu\text{g}/100\text{ ml}$, based on an intake from food, water, beverages and dust and a zero air lead concentration, increased to 16.72 $\mu\text{g}/100\text{ ml}$ as air lead increased from 0 to 1.5 $\mu\text{g}/\text{m}^3$ (ATSDR/EPA, 1988; EPA, 1986). Heavy smoking and alcohol consumption can each add about 2 $\mu\text{g}/100\text{ ml}$ to the blood lead level (Quinn, 1985).

The biological half-life of lead has been estimated to be 28–36, 40 and 10⁴ days in the blood, soft tissues and bone, respectively, in human adults; the half-life in the blood of 2-yr-old children is 10 months. The half-life of newly absorbed lead in blood may extend to several months depending on the mobile lead burden in the body (ATSDR/EPA, 1988; EPA, 1986).

In humans, absorbed lead that is not excreted is stored in the bone, which accounts for 95% of the total body burden of lead in adults and 73% in children. Bone lead appears to exist in two biological compartments—an inert compartment with a half-life of several decades and a labile compartment that maintains equilibrium with lead in soft tissues and blood. Increased mobilization of lead from human bone may occur during the physiological stress of pregnancy or lactation. The bone lead level increases up to 60 years of age, attaining $\geq 200\text{ mg}$ from environmental exposure in 60–70-yr-old men, corresponding to a lifetime average retention rate of 9–10 $\mu\text{g}/\text{day}$; bone lead in children under 16 years is reported to be 8 mg (ATSDR/EPA, 1988; EPA, 1986).

In contrast to bone, soft tissues generally show no age-related increase in lead content in humans after age 20, two possible exceptions being the kidney and aorta (ATSDR/EPA, 1988; EPA, 1986).

Kidney lead levels in human adults, whether occupationally exposed to lead or not, are in the region of 0.8 ppm. This compares with 30 or 300 ppm in the kidneys of rats fed on a 0.1 or 1% diet of lead acetate (Moore and Meredith, 1979).

From studies of tissue lead levels in rats given 1% lead acetate in the diet for 4 months (Goyer *et al.*, 1970) and occupationally exposed humans (Barry, 1975), it is apparent that kidney/blood ratios are markedly increased in lead-treated rats but not in occupationally exposed humans, when compared with corresponding unexposed control groups. Kidney/bone ratios were lower in lead-treated rats and in occupationally exposed workers when compared with controls. These data indicate the preferential uptake of lead in rat kidney and human bone.

Tissue lead levels of 69 human subjects at post mortem showed: (a) levels of 0.0–1.35 ppm in soft tissues by the second decade of life with no further

increases with advancing age; (b) an increase in bone lead from 1 ppm (infants/children) to 40 ppm in adults aged 50 yr; (c) nearly 95% of total body burden of lead located in bone; and (d) soft-tissue levels of lead in occupationally exposed humans tended to be similar to those in unexposed humans, although bone uptake was greater in the former group (Barry and Mossman, 1970).

In humans, lead undergoes placental transfer with blood levels in the foetus (umbilical cord), infants and mothers being 6.6, 12 and 14 $\mu\text{g}/100\text{ ml}$, respectively (ATSDR/EPA, 1988; EPA, 1986).

In humans and laboratory animals, dietary lead that is not absorbed is eliminated in the faeces, whilst absorbed lead that is not retained is excreted through the kidneys or to a lesser extent in the gastro-intestinal tract by way of biliary excretion. About 50–60% of that absorbed is excreted; the balance is initially taken up primarily in bone and then half of this bone lead fraction (half-life 20–25 days) is excreted so that the overall bone retention represents 25% of the absorbed amount (ATSDR/EPA, 1988; EPA, 1986).

The mean daily excretion of lead in the urine and faeces of 10 human volunteers over 1 wk was 70 $\mu\text{g}/\text{day}$; the figure for alcohol beverage drinkers was 160 $\mu\text{g}/\text{day}$ (MAFF, 1989).

To summarize the major findings: a limited proportion (about 10–15%) of dietary lead is absorbed in human adults, but the extent of absorption is much higher in children; blood lead levels have declined over the last 2 decades in the general population; nutritional factors can markedly influence the absorption of dietary lead; lead is stored in the bone with levels increasing with age.

Percutaneous absorption of lead compounds in animals

Evidence of minimal absorption of inorganic lead salts through intact or scarified rat skin was reported by Laug and Kunze (1948). Several inorganic lead salts including lead acetate were applied in different vehicles to the clipped skin in doses of 77–148 mg/rat. Lead levels were assayed in tissues, but surprisingly not in the blood, up to 48 hr after dosing. Kidney lead rose from 0.82 ppm (untreated skin) to 1.8 ppm (intact skin) and 5.9 ppm (scarified skin). The degree of absorption was considered to be too low to have been detected by conventional urine assays. Tetraethyllead showed much greater penetration, about 6% of the dose applied being found in the tissues and carcass.

Lead naphthenate, in four different oily vehicles with lead contents of 0.55–1.35% (w/w), was applied in a volume of 1 ml/kg, 5 times/week, for 4 wk to clipped rabbit skin. Percutaneous absorption was evidenced by the rise in blood lead levels up to 100 $\mu\text{g}/100\text{ ml}$ (Hine *et al.*, 1969).

Rats received five dermal applications on alternate days of 0 or 500 μl of lead acetate (0.24 M-Pb in water) or lead naphthenate (0.24 M-Pb in ether/ethanol). Lead acetate treatment resulted in 3–7-fold increases in the lead content of kidneys, liver and muscle; tissue uptake was lower with lead naphthenate. Blood levels were, surprisingly, not determined (Rastogi and Clausen, 1976).

These studies show that lipophilic lead compounds, such as tetraethyllead, are absorbed to a greater

extent than water-soluble lead salts (lead acetate being the most soluble lead salt in aqueous solution), which are absorbed very inefficiently.

Percutaneous absorption of lead compounds in humans

Dermal absorption of inorganic lead compounds is much less significant than absorption by inhalation or oral exposure; dermal absorption of alkyl lead compounds, however, is rapid (ATSDR/EPA, 1988; EPA, 1986).

Lead naphthenate in gear oil (lead content 1.35%, w/w) was applied to the forearm skin of 10 volunteers, 1–2 ml/application, twice daily on 20 occasions over 28 days (total lead applied 0.81 g). The mean group lead levels rose from 37 (pretreatment level) to 72 $\mu\text{g}/100\text{ ml}$ for blood and from 7 (pretreatment level) to 13 $\mu\text{g}/100\text{ ml}$ for urine. The increases in blood and urinary lead levels, seen after five applications, persisted at the same level throughout the 20 applications, providing evidence of absorption and attainment of steady-state conditions (Hine *et al.*, 1969).

Absorption of lead salts through intact human skin has been reported (Kennedy and Lynas, 1949; Singhal and Thomas, 1980). Marzulli *et al.* (1978) claimed evidence of absorption by demonstrating increased lead levels in the pubic and axillary hair following applications of a lead acetate hair colouring to the hair of the scalp for 6–24 months. This study has, however, been criticized on the grounds that no blood or urine parameters were investigated and that pubic and axillary hair lead levels are not necessarily reliable indicators of systemic uptake; the shortcomings of this study were noted by the FDA (1981).

No evidence of percutaneous absorption of lead was obtained with hair-colouring preparations containing up to three times the normal lead acetate content (Ippen *et al.*, 1981). In this study, 53 human volunteers received hair applications to the scalp hair of 0, 0.6 or 1.8% lead acetate as aqueous preparations (5 ml/application) or 0.6% lead acetate as a cream preparation (1 ml/application). Treatment was daily for 3 wk, then twice weekly for 5 months. Hair was washed twice weekly before dyeing. Regular assays over 5 months and in the 1-month post-treatment period revealed no effects on the blood levels of lead, δ -aminolaevulinic acid dehydratase or porphyrin, on the urinary levels of lead, δ -aminolaevulinic acid, total porphyrins or porphobilinogen or on haematological status (red blood cells, white blood cells, haemoglobin).

By far the most reliable assessment of the percutaneous absorption potential of lead acetate derives from the radiotracer technique employed by Moore *et al.* (1980). Commercial hydroalcoholic or cream preparations of lead acetate hair colourings spiked with ^{203}Pb were applied to the forehead skin of eight human volunteers for 12 hr before being washed off. Treatment (mainly lotion) involved specially developed types of application, described as "wet cell, dry cell, dry plus scratch cell and cream cell", with appropriate controls. ^{203}Pb was assayed in blood (over 12-hr application period), urine (up to 48 hr after dosing) and by whole-body monitoring around the calves. It was found that 0.01–0.18% of the dose was absorbed through intact skin and 0.01–0.26%

through scratched skin. It was calculated that only 0.35 μg of lead per application was actually absorbed through intact skin.

When the highly sensitive method involving radio-labelling with ^{203}Pb is used and is capable of detecting sub- μg quantities of lead in biological samples, it has provided unequivocal evidence that percutaneous absorption of lead from lead acetate hair colourings is negligible. It is not surprising, therefore, that the use of less sensitive techniques (blood levels of unlabelled lead, δ -aminolaevulinic acid dehydratase or protoporphyrin and urinary levels of δ -aminolaevulinic acid and coproporphyrin) have failed to detect such traces of lead at the limits of their sensitivity (Moore *et al.*, 1980).

The studies in humans have given results consistent with the view that whereas percutaneous absorption of lipophilic lead compounds such as lead naphthenate occurs readily, there is negligible absorption of water-soluble lead salts such as lead acetate. Even in scarified skin, the percutaneous absorption of lead acetate is increased only very slightly.

The FDA (1980 and 1981) also noted that the lead contribution from hair-colouring use to the total body burden of lead was inconsequential. Moore *et al.* (1980) have shown that the weekly absorption of lead from hair-colouring use is about 0.7 μg (equivalent to 0.1 $\mu\text{g}/\text{day}$).

Evaluation of carcinogenic risk of lead salts to man

Experimental carcinogenicity studies in rodents

Carcinogenicity studies on lead acetate and other inorganic lead salts have been conducted mainly in the rat and to a limited extent in the mouse and hamster. Details of treatment and the principal findings obtained are summarized in Table 2.

Renal tumours in rodents

High levels of lead salts (acetate, subacetate, phosphate) induce benign (adenomas) and malignant (mainly adenocarcinomas) tumours in the kidneys of rats (both sexes) following dietary or parenteral administration (Table 2).

A study with lead subacetate demonstrated the nephrocarcinogenicity of lead in mice but not in hamsters (Van Esch and Kroes, 1969; Table 2). In mice, renal tumours occurred in 6/25 males and 1/25 females on the 0.1% dietary level and in 0/25 males and 1/25 females on 1.0/0.5%, at which dietary level only 2 males and 6 females survived 1 year. In the absence of any renal tumours in control mice there is thus reasonable evidence of an effect in males and limited evidence of an effect in females.

With other lead compounds, renal tumours have been induced in mice treated dermally with lead naphthenate (Baldwin *et al.*, 1964) and in rats given lead chromate intramuscularly (Furst *et al.*, 1976), but negative results have been reported in other studies on lead chromate (Furst *et al.*, 1976) or lead dimethyldithiocarbamate (NCI, 1979) in mice, lead powder (Furst *et al.*, 1976), lead arsenate (Fairhall and Miller, 1941; Kroes *et al.*, 1974) or lead dimethyldithiocarbamate (NCI, 1979) in rats and lead oxide (Kobayashi and Okamoto, 1974) in hamsters.

Table 2. Carcinogenicity studies on inorganic lead salts

Lead salt	Route of administration	Treatment	Principal findings	Reference
Mouse				
Pb subacetate	Diet	0, 0.1 and 1.0/0.5% for 2 yr, 25 M + 25 F/group	Kidney tumours in 0/50, 7/50 and 1/50 mice on 0, 0.1 and 1.0/0.5%, resp; high early mortality at 1%; no increase in extrarenal tumours; intranuclear inclusions in renal tubules	Van Esch and Kroes, 1969
Pb subacetate	Oral	10 mg thrice weekly for 6 wk to 16 M + 13 F (total dose 190 mg/mouse); observed 6 months	Lung tumours in 3/16 M & 5/13 F in treated group & 52/136 M & 33/131 in control group	Stoner <i>et al.</i> , 1986
Pb subacetate	ip	2, 5 or 10 mg/kg thrice weekly for 5 wk (total dose 30, 75 & 150 mg/kg bw) to 10 M + 10 F/group; 10 M + 10 F controls given vehicle thrice weekly for 8 wk; study lasted 30 wk	Lung adenomas in 8/18, 6/17, 5/12 and 11/15 surviving mice on 0, 30, 75 and 150 mg/kg bw total dose, resp.	Stoner <i>et al.</i> , 1976
Pb subacetate	ip	2, 5 or 10 mg, thrice weekly to 3-16 M & 13-15 F for 6 wk (total dose 38, 95 & 190 mg/mouse); observed 6 months)	Lung tumours in 9/31, 5/30 & 2/16 on total dose 38, 95 & 190 mg/mouse, resp.	Stoner <i>et al.</i> , 1986
Rat				
Pb acetate	Diet	1% for 1 yr to 20 M; no control group; study lasted 21 months	Renal tumours (mainly carcinomas) in 15/20 rats; 16 rats survived at least 322 days	Boylard <i>et al.</i> , 1962
Pb acetate	Diet	0 or 3-4 mg/day for 18 months to 19 M + 13 F & 94 M + 32 F, resp; study lasted 26 months	Renal adenomas/carcinomas in 0/32 controls & 72/126 test rats; Leydig-cell tumours of testis in 23/94 males (control incidence not given)	Zawirska and Medraś, 1968
Pb acetate	Diet	10, 50, 100 or 1000 ppm Pb (as lead acetate) to 50 M + 50 F/dose; 100 M + 100 F in control group	Vacuolation, degenerative and regenerative change and intranuclear inclusions in proximal convoluted renal tubule cells at 100 and 1000 ppm & no changes at lower levels; increase in kidney tumours at 1000 ppm in males only	Jessup and Shott, 1969a
Pb acetate	Diet	0 or 3 mg/day for 60-504 days to 31 M + 31 F & 47 M + 47 F, resp.	Of 94 treated rats 12 showed renal adenomas, 10 cerebral gliomas, 17 pituitary tumours, 11 thyroid adenomas, 5 parathyroid adenomas, 15 adrenal adenomas, 15 lung adenomas, 11 prostatic adenomas & 8 mammary adenomas; no survival data given for controls and absence of any tumours in 32 control suspect	Zawirska and Medraś, 1972
Pb acetate	Diet	0, 10, 50, 100 or 500 ppm (calculated as Pb) for 2 yr to 50 M + 50 F/dose	Increase in kidney tumours at 500 ppm in males; no renal pathological changes at ≤ 100 ppm	Azar <i>et al.</i> , 1973
Pb acetate	Diet	0, 1000 or 2000 ppm (calculated as Pb) for 2 yr to 20 M + 20 F/group	Increase in kidney tumours at both levels in males and at top level in females	Azar <i>et al.</i> , 1973
Pb acetate	Diet	0, 500, 2000 or 8000 ppm for 2 yr to 24 M + 24 F/dose	Renal tumours increased at 2 top levels in M & F but not at lowest dose; no increase in extrarenal tumours	NCI, 1978
Pb acetate	Drinking-water	0 or 5 ppm for lifespan to groups of 50 rats	No effect on tumour incidence	Schroeder <i>et al.</i> , 1965
Pb acetate	Drinking-water	0 or 5 ppm to 32 rats for lifespan	No effect on tumour incidence	Kanisawa and Schroeder, 1969
Pb acetate	Drinking-water	0, 26 or 2600 ppm to 16 M for 19 months; no control group	Kidney tumours in 13/19 rats on 2600 ppm	Koller <i>et al.</i> , 1985
Pb carbonate	Diet	0.1% to 55 rats for 2 yr	Non-neoplastic but no neoplastic changes in kidneys	Fairhall and Miller, 1941
Pb nitrate	Drinking-water	0 or 25 μ g/l to 50-52 M for lifespan	No increase in kidney tumours; tumour response in controls and treated group comparable	Schroeder <i>et al.</i> , 1970
Pb phosphate	sc	20 mg weekly for 16 months, totalling 40-760 mg/rat to 270 rats; 40 rats in control group	Renal tumours in 19/29 surviving at least 10 months of treatment (total doses 120-650 mg/rat); no renal tumours in controls	Zollinger, 1953

[contd]

Table 2—continued

Lead salt	Route of administration	Treatment	Principal findings	Reference
Pb phosphate	sc	20 mg weekly or fortnightly for 18 months to 80 rats (total dose 1.3 g/rat); 20 rats in control group	Renal adenomas in 0/20 controls & 29/80 treated rats	Baló <i>et al.</i> , 1965
Pb phosphate	sc + ip	0, 29, 145 or 450 mg/rat (total dose) for 6.75–8.5 months to 24 M/group; observed for 2 yr	Kidney tumours (mainly adenomas/carcinomas) in 0/23, 14/23 & 2/3 surviving 200 days on 29, 145 and 450 mg/rat (total dose); 2/24 controls developed renal tumours (not adenomas or adenocarcinomas)	Roe <i>et al.</i> , 1965
Pb subacetate	Diet	0, 0.1 or 1% for 24–29 months to 13–16 M + 11–16 F per group	Kidney tumours in 5/16 M + 6/16 F and 6/13 M + 7/11 F at 0.1 & 1%, resp; no renal tumours in controls; mortality increased at 1%; intranuclear inclusions in renal tubules	Van Esch <i>et al.</i> , 1962
Pb subacetate	Diet	0 & 0.5–1.0% for 92 wk	Renal tumours increased in treated rats, none in controls	Shakerin and Paloucek, 1965
Pb subacetate	Diet	0 or 1% for 20 months to 20 M & 40 M, resp.	Kidney tumours in 31/40 rats over 9–20 months & in 1/20 controls; intranuclear inclusions in renal tubules in test group	Mao and Molnar, 1967
Pb subacetate	Diet	0 or 1% to 285 M + F and 17 M, resp. for about 1 yr; observed 18 months	Renal tumours in 13/17 & cerebral gliomas in 2/17 on 1%; 1/285 controls showed a cerebral glioma; survival in controls and test group averaged 50–60 wk	Oyasu <i>et al.</i> , 1970
Pb subacetate	Diet	1.5% to 10 M for 48 wk; no control group	Renal tumours in 10/10 M; no extrarenal tumours	Ito <i>et al.</i> , 1971
Pb subacetate	Diet	1%; 90-wk study	Increase in kidney tumours	Coogan <i>et al.</i> , 1972
Pb subacetate	Diet	1.5% to 11–13 M for up to 48.5 wk; no controls	Renal tumours in 0/13 at 23 wk & in 9/11 at 48.5 wk	Ito, 1973
Pb subacetate	ip	No details given; 90-wk study	Renal tumours	Coogan <i>et al.</i> , 1972
Pb subacetate	sc	No details given; 90-wk study	Renal tumours and injection-site sarcomas	Coogan <i>et al.</i> , 1972
Hamster				
Pb subacetate	Diet	0, 0.1 & 0.5% for 2 yr to 22 M + 23–24 F/group	High early mortality at 0.5%; no evidence of renal neoplasia/hyperplasia or of extrarenal neoplasia; pleomorphic cells and intranuclear inclusion bodies in proximal renal tubules	Van Esch and Kroes, 1969

bw = body weight

Lack of tumours at extrarenal sites in rodents

Studies on lead and lead compounds have led to reports of the occurrence of the tumours at sites other than the kidneys but these reports do not withstand critical assessment, either because the quality of the design or data obtained is poor or because the method of exposure is unreliable. The sites in question are: (i) brain tumours (gliomas) in rats; (ii) injection-site sarcomas in rats; (iii) endocrine tumours in rats; and (iv) lung tumours in mice.

Brain tumours. The reported occurrence of cerebral gliomas in rats on diets containing lead acetate for up to 2 yr is flawed by the highly suspect finding of few or no tumours of any kind or site in the control groups in the two studies concerned (Zawirska and Medraś, 1968 and 1972).

In the study by Hass *et al.* (1967) involving dietary administration of 0.5–1.0% lead subacetate with other compounds to rats, the incidence of gliomas was 2/24 for lead subacetate plus indole (1.6% in diet), 4/50 for lead subacetate plus indole plus linseed

oil and 11/74 for lead subacetate plus indole plus linseed oil plus 2-acetylaminofluorene. No control group was used and lead subacetate was not tested alone. Hence, no valid conclusions can be drawn from this study.

The only other class of glioma production stems from the study in rats by Oyasu *et al.* (1970), which involved diets containing lead subacetate and/or 2-acetylaminofluorene and/or indole. The incidence of gliomas was 5/58 in the lead subacetate group and 2/354 in the untreated control group. The validity of this study can be questioned on several grounds: (i) the lead treated and control rats were not contemporaneous and there is known to be a wide variation in the control incidence of gliomas in rats (Svenberg, 1986); (ii) the test group comprised males whilst the control group comprised both sexes, which is particularly relevant as brain tumours are more common in male than in female rats (Gopinath, 1986); and (iii) the possibility of accidental cross-contamination of the lead acetate only group by 2-acetylaminofluorene, which alone was capable of inducing gliomas.

No evidence of increased brain tumours was obtained in various adequately controlled studies (Azar *et al.*, 1973; NCI, 1978 and 1979; Roe *et al.*, 1965; Van Esch and Kroes, 1969; Van Esch *et al.*, 1962). On the other hand, in some of these studies examination of the brain at autopsy and histologically only took place if prompted by the presence of neurological signs.

Injection-site sarcomas in rats. The appearance of sarcomas at the site of repeated intramuscular injections of lead powder or lead chromate in rats (Furst *et al.*, 1976) is no longer regarded as evidence of risk of carcinogenesis for the same substances administered by a non-parenteral route.

Repeated injections of substances such as salt, glucose and xylose have similarly induced local sarcomas. Physicochemical properties of the test substance are influential in governing the nature of the local tissue reaction, which ultimately may proceed to sarcoma development. In view of the non-specific nature of this response, local sarcoma induction has been ruled out as a valid index of carcinogenic potential (Grasso and Golberg, 1966). Moreover, the chromate moiety of lead chromate may be responsible for the local sarcomas in the rat study as calcium chromate produced similar tumours (Roe and Carter, 1969).

Endocrine tumours in rats. Zawirska and Medraś (1968 and 1972) reported an increased incidence of tumours of the testis, thyroid, adrenals, prostate and pituitary in rats given a diet containing lead acetate. The control group in both studies is suspect because of the absence in one and the virtual absence in the other of any endocrine tumours whatsoever. The reported excess of thyroid tumours in lead-treated rats (Coogan *et al.*, 1972) was subsequently attributed to a magnesium-deficient diet (P. S. Coogan, personal communication, 1979).

In view of the deficiencies of these studies and the absence of any evidence of lead-related endocrine neoplasia in any of the major carcinogenicity studies on lead and in particular when combined with thiouracil treatment (NCI, 1978), the induction of endocrine neoplasia by lead in rodents can be ruled out.

Lung tumours in mice. Increased incidences of lung tumours in strain A mice have been reported following treatment with lead subacetate given intraperitoneally (Stoner *et al.*, 1976) or orally or intraperitoneally (Stoner *et al.*, 1986). In the former report, the incidences of lung adenomas were 6/17, 5/12 and 11/15 in mice receiving total doses of 30, 75 and 150 mg/kg body weight *v.* 8/18 in controls, with an effect being claimed at the top dose. However, inappropriate statistical analysis was applied to the results in this study. Moreover, interpretation of lung tumour findings in this mouse strain, which is prone to a high spontaneous incidence of such tumours, is problematical and positive findings are not regarded as definitive evidence of carcinogenicity by many regulatory authorities.

It is noteworthy that no evidence of increased lung tumour risk was obtained with lead oxide given intratracheally to hamsters (Kobayashi and Okamoto, 1974) or when lead acetate was added to the drinking-water of mice pretreated with urethane, a powerful inducer of lung tumours (Blakley, 1987).

Mechanism of rodent nephrocarcinogenicity of lead salts and significance to man

The early or acute phase of lead intoxication in the rat kidney is characterized by the appearance of distinctive intranuclear inclusion bodies as well as degenerative changes (e.g. necrosis in the proximal renal tubules); after prolonged and long-term exposure, more severe and irreversible changes occur in the renal tubular epithelia including degenerative, proliferative (hyperplasia and cystic changes) and neoplastic lesions (ATSDR/EPA, 1988; EPA, 1986; US Nutrition Foundation, 1982; WHO, 1977).

Intranuclear inclusions which consist of lead-protein complexes (Carroll *et al.*, 1970; Goyer *et al.*, 1970; Moore and Goyer, 1974) have also been found in the proximal tubular cells of other species including humans, pig, dog and rabbit after exposure to lead (Goyer and Rhyne, 1973). In non-rodent species, inclusions also occur in liver cells. The inclusions at both sites serve as a sequestration mechanism for lead.

In various rat studies, renal tumours occur only at dose levels that are also associated with overt renal toxicity (degenerative or regenerative lesions in the proximal renal tubules with the presence of intranuclear inclusion bodies) (Azar *et al.*, 1973; Jessup and Shott, 1969a; NCI, 1978). Moreover, when the dose levels of lead in the rat are too low to cause histological change to the proximal renal tubules, no renal tumours develop (Azar *et al.*, 1973; Jessup and Shott, 1969a; NCI, 1978 and 1979).

As discussed elsewhere in this review, there is persuasive evidence that lead salts are not genotoxic. Their nephrocarcinogenic action is almost certainly mediated therefore by a non-genotoxic mechanism.

It is possible to arrive at a "no-effect" dose level for renal neoplasia in the rat from three dietary studies on lead acetate. Renal tumours were evident at dietary levels of 2000 and 8000 ppm but not at 500 ppm (NCI, 1978), at 1000 ppm but not at 10–100 ppm (Jessup and Shott, 1969a) and at 500 ppm but not at 10–100 ppm (as lead) (Azar *et al.*, 1973). Hence the no-effect carcinogenic dose level is 100 ppm in the diet (equivalent to 5 mg/kg body weight/day as lead).

The NCI (1979) study on lead dimethyldithiocarbamate would provide an underestimate of the no-effect dose level for renal neoplasia in the rat as no renal pathology or tumours occurred at the highest dietary level of 50 ppm used (equivalent to 1 mg/kg body weight/day, calculated as lead).

Roe *et al.* (1965) observed renal tumours in rats given a combination of sc + ip doses totalling 145 mg or 450 mg but not 29 mg lead phosphate. The total dose of 29 mg (given over 8 months) corresponds approximately to 50 mg/kg body weight or 0.07 mg/kg body weight/day averaged over the lifespan. This no-effect level is considerably lower than the dietary no-effect level of 5 mg/kg body weight/day.

The rat can tolerate exceptionally high levels of lead for prolonged periods without showing the acutely toxic manifestations (especially encephalopathy) seen in humans (Van Esch *et al.*, 1962). Doses causing renal cancer in rats are about 200 times the

Table 3. Inadequate carcinogenicity studies in non-rodent species on inorganic lead salts

Lead salt	Route of administration	Treatment	Principal findings	Reference
Rabbit				
Pb subacetate	Diet	0 or 0.5% to 34–39 animals for 3–55 wk	Changes in proximal renal tubules but no renal tumours	Hass <i>et al.</i> , 1964
Pb subacetate	No details	No details	No renal or extrarenal tumours	Coogan <i>et al.</i> , 1975
Dog				
Pb acetate	Diet	0, 10, 50, 100 or 1000 ppm to 6 M + 6 F/dose for 22 months	No renal pathology apart from thickening of glomerular tufts at 1000 ppm in F	Jessup and Shott, 1969b
Pb acetate	Diet	0, 10, 50, 100 or 500 ppm (calculated as Pb) for 2 yr to 4 M + 4 F/dose	Cytomegaly in proximal convoluted renal tubules at 500 ppm	Azar <i>et al.</i> , 1973
Monkey				
Pb acetate	Oral	0, 1.25 or 25 mg/kg/day to 2 M + 2 F/dose for 22 months	Degenerative changes in proximal renal tubules; intranuclear inclusions in kidney and liver	Jessup and Busey, 1970

dose that would be acutely toxic to humans (FDA, 1979). Van Esch *et al.* (1962) calculated that the 0.1% lead subacetate diet given to rats would correspond to an intake of 550 mg lead/day in humans. Thus, the high acute toxicity of lead in humans would preclude chronic exposure levels being attained which could present a carcinogenic risk.

It has been suggested that, as lead is not genotoxic, rodent neoplasia arises from chronic renal tubular damage and regenerative hyperplasia (US Nutrition Foundation, 1982). Lead can exert a mitogenic action on renal tubules in rodents (Choie and Richter, 1974).

New light on the mechanism of renal neoplasia in the rat stems from the finding that certain non-genotoxic agents (decalin, 2,2,4-trimethylpentane and *d*-limonene), which induce renal tumours in male rats, inhibit the lysosomal protease action on the protein $\alpha_2\mu$ -globulin in the proximal renal tubules (Kanerva *et al.*, 1987; Kitchen, 1984; Phillips and Cockrell, 1984). The accumulation of this protein causes the death of the proximal renal tubular cells, two consequences of which are compensatory reparatory hyperplasia (a recognized precursor of neoplasia) and serious interference with calcium regulation.

The " $\alpha_2\mu$ -globulin nephropathy/renal neoplasia syndrome", which appears to be specific to male rats, cannot readily explain lead-induced neoplasia, which is manifest in both sexes of rats as well as in mice. However, both lead and the non-genotoxic agents, known to induce the $\alpha_2\mu$ -globulin nephropathy, share a common pathway in producing a regenerative hyperplasia response following necrosis of the proximal tubular cells. Swenberg *et al.* (1989) also consider that the nephrocarcinogenic response in the rat is not indicative of a carcinogenic risk in humans. It is being increasingly recognized that prolonged hyperplasia may non-specifically predispose to increased cancer risk in the rodent (Ames and Gold, 1990; Cohen and Ellwein, 1990; Grasso *et al.*, 1991; Roe, 1989; Roe and Lee, 1991).

Experimental carcinogenicity studies in non-rodent species

Studies in the rabbit, dog and monkey, as summarized in Table 3, provide no evidence of neoplasia in

the kidney or other sites. These studies are, however, too short in duration for carcinogenic evaluation.

Rabbits did, however, show renal changes, as seen in the rat (Hass *et al.*, 1964), dogs showed minimal non-specific histological changes in the kidney (Jessup and Shott, 1969b), whilst monkeys exhibited degenerative changes and intranuclear inclusions in the proximal renal tubules (Jessup and Busey, 1970).

Human studies of cancer risk of lead

Evaluation of kidney cancer risk. Epidemiological studies investigating lead exposure (mainly occupational) and the risk of various cancers including kidney cancer are summarized in Table 4. No valid evidence of excess kidney cancer has been obtained (ATSDR/EPA, 1988; EPA, 1986; IARC, 1987b).

Studies claiming an excess of kidney cancer in lead smelter workers in the USA (Selevan *et al.*, 1985 and 1988) either did not take into account confounding variables such as smoking and/or the association was marginal and statistically non-significant with poor correlation with the duration of lead exposure. Other studies failed to show an association between excess kidney cancer and lead exposure in smelter and battery workers (Cooper *et al.*, 1985; Gerhardtsson *et al.*, 1986; Malcolm and Barnett, 1982) or in workers exposed to lead chromate (Davies, 1984; Sheffet *et al.*, 1982). Attempts to associate Wilms's tumour (a renal neoplasm of childhood) with paternal exposure to occupational lead have been unsuccessful (Kantor *et al.*, 1979; Wilkins and Sinks, 1984).

Evaluation of extrarenal cancer risk. None of the studies summarized in Table 4 provide valid evidence of an association between lead exposure and a significant excess of extrarenal cancer risk in man. Those studies that are suggestive of such an association are either confounded by simultaneous exposure to other chemicals of proven carcinogenicity or are inadequately controlled. IARC (1980), in its evaluation of human data, could find no evidence associating lead exposure with excess cancer risk in humans but drew attention to the absence and inadequacy of human studies, which prevented its evaluation from reaching any firm conclusion. In studies claiming an excess of lung cancer, the excess was small with no clear-cut trend with duration or degree of exposure

Table 4. Epidemiological studies on lead exposure and cancer risk in humans

Findings of study	Comments	Reference
Lung Pb levels similar in groups of 4 patients with/without lung cancer	—	Jecklin, 1956
Cerebral tumour in lead worker	Anecdotal—no evidence of causation	Portal, 1961
337 workers classified according to Pb exposure levels—no excess of cancer deaths	Pb exposure assessed by urinary Pb, which reflects current rather than cumulative exposure	Dingwell-Fordyce and Lane, 1963
24 cases of Pb poisoning followed up for 10 yr. No cancers found	Inadequate study to draw conclusions	Rakow and Lieben, 1968
Pb battery workers with high exposure up to 1 mg/m ³ —no excess of cancer deaths in England & Wales	—	Malcolm, 1971
Correlations found between trace metals in USA water supplies (1962–1967) and cancer mortality rates (1950–1967). Pb implicated in renal cancer. As implicated in larynx and eye cancer	Design of study prevents establishment of cause and effect relationship; 50% of 28 positive correlations were of chance occurrence on authors' own admission. Study failed to associate Cr or Fe with lung cancer, Cd with prostatic cancer or As with skin or lung cancer	Berg and Burbank, 1972
Copper, lead or zinc smelting or refining industries in 36 US Counties. Excess of lung cancer deaths in general population—environmental arsenic incriminated	Pb confounded by arsenic; no conclusions on Pb possible	Blot and Fraumeni, 1975
Follow-up of 7032 lead battery and smelter workers in USA exposed for 1–23 yr between 1947–1970. No excess of deaths from kidney or brain tumours. No correlation between degree of lead exposure and cancer incidence. Excess of deaths from all malignant tumours in smelter but not battery workers	Arsenic exposure in smelters probably accounts for slight excess of respiratory tumours. IARC (1980) found no statistically significant excess of cancer deaths in re-analysis	Cooper and Gaffey, 1975; Cooper, 1976
153 workers exposed to tetraethyllead for ≥20 yr. No excess of cancer with matched control group not exposed occupationally to Pb	Inadequacies in follow-up	Robinson, 1976
No association found between Pb levels in tap-water and mortality from cancer or cardiovascular disease in Wales 1969–1973	—	Elwood <i>et al.</i> , 1976
Copper smelter mine workers showed 3-fold excess of lung cancer associated with increased exposure to Cu, SO ₂ , H ₂ SO ₄ mist, As and Pb	Simultaneous exposure to other agents precludes incrimination of Pb; arsenic is a known lung carcinogen in man; not controlled for smoking	Rencher <i>et al.</i> , 1977
149 cases of Wilms's tumour 1935–1973 in USA—paternal group versus matched controls without hydrocarbon-related occupations. Wilms's tumour more prevalent in offspring of fathers in hydrocarbon-related occupations and lead-related occupations	Inadequate assessment of Pb exposure in fathers precludes any conclusion on Pb's association with Wilms's tumour	Kentor <i>et al.</i> , 1979
Lead battery workers. No excess of kidney cancer or other cancer deaths or correlation between Pb exposure level and cancer risk	—	Malcolm and Barnett, 1982
Workers exposed to mixture of lead and zinc chromates. Excess of lung cancer attributed to chromate; no excess of kidney cancer reported	—	Sheffet <i>et al.</i> , 1982
Workers exposed to lead chromate. No excess of kidney or lung cancer	—	Davies, 1984
Lead smelter workers. No excess of all cancer deaths; non-significant excess of kidney and bladder cancer	Confounding variables such as smoking not taken into account. Prior history of exposure in mining (mainly Pb, Zn, Ag)	Selevan <i>et al.</i> , 1985
Lead battery and lead producing workers—no excess deaths from kidney cancer. Excess of lung and stomach cancers statistically significant in battery plant workers but not smelter workers. Downward trend in standardized mortality ratio with duration of employment	No correlation between death rates and cumulative exposure; confounding variables such as ethnicity, smoking, diet and alcohol not taken into account	Cooper <i>et al.</i> , 1985
Swedish smelter workers. No excess of kidney cancer or other cancer deaths and no differences between high and low lead exposure groups	Lead exposure assessed by blood Pb—mean levels 58.2 & 33.61 g/100 ml in 1950 & 1974, respectively	Gerhardsson <i>et al.</i> , 1986
Lead workers exposed 1926–1965; no excess of cancer deaths	—	Fanning, 1988
Lead smelter workers (also exposed to Cd, Zn & As). Excess mortality from kidney cancer (6 observed, 3 expected)	Marginal, non-significant association in high Pb exposure group; poor correlation with duration of Pb exposure	Selevan <i>et al.</i> , 1988

and could have been confounded with factors such as arsenic or smoking (IARC, 1987b). Similarly, EPA (1986) and ATSDR/EPA (1988) could draw no definitive conclusions from the epidemiological studies in lead-exposed workers.

Assessment of carcinogenic risk of lead acetate in hair-colouring users

The outcome of animal and human studies can be summarized as follows:

- (i) lead salts are carcinogenic for the kidney of rats and mice at dietary levels of 500 ppm (25 mg/kg body weight/day) or higher. Dietary levels at or below 100 ppm (5 mg/kg body weight/day) were not nephrocarcinogenic to rats;
- (ii) hamsters showed no kidney tumours even at dietary levels as high as 0.5%.
- (iii) lead salts, when tested by parenteral administration in the rat, induced kidney tumours. Lead salts have not been tested by the dermal route in laboratory animals;
- (iv) none of the animal studies provided valid evidence of increased tumour risk at sites other than the kidney;
- (v) epidemiological studies have provided no valid evidence to suggest that lead is a human carcinogen.

A typical intake of environmental lead in humans from various sources (food, water and air) is of the order of 1.5 µg/kg body weight/day. This intake is at least three orders of magnitude lower than the no-effect carcinogenic dose by the dietary route in the rat. Because of this exceptionally wide margin and because humans would succumb to the high doses used in the rat carcinogenicity studies, it is inconceivable that the typical daily intake of lead would present a carcinogenic risk to man. Even higher lead intakes from occupational exposures have not been found to be associated with increased cancer risk.

In humans, Moore *et al.* (1980) estimated that of a 200-µg/day intake of lead, about 38 µg/day would be absorbed. Twice-weekly application of lead acetate hair colourings would give rise to absorption from the skin into the circulation of 0.7 µg/week or 0.1 µg/day, which represents only about 0.25% of the lead absorption from the 200-µg/day lead intake. Based on current lead intakes of, say, 100 µg/day the proportion would increase to about 0.5%. The lead contribution from hair-colouring use to the total daily intake is thus infinitesimally small and must be regarded as insignificant in terms of human cancer risk (amount of lead acetate absorbed from hair-colouring use is over five orders of magnitude lower than the no-effect carcinogenic dose by the dietary route in rats).

Applying "worst case" risk estimates extrapolated from animal carcinogenicity studies, the FDA (1980) arrived at two estimates of 1 in 5 million lifetimes and 1 in 18.5 million lifetimes for the upper limit lifetime cancer risk for lead exposure from lead acetate hair colourings. These risk estimates were not deemed to present any significant risk to public health by the FDA (1980), who consider risk estimates of at least 1 in 1 million lifetimes to be acceptable and exposure

to such a risk to be reasonable. Since 1980, the methodology for quantitative risk assessments has been refined but not to an extent that could permit valid application to the lead data obtained in animals (ATSDR/EPA, 1988). In any event the UK DoH (1991) does not support the routine use of quantitative risk assessment for chemical carcinogens because of the lack of validation. We therefore attach more importance to the comparison of the no-effect carcinogenic dose for kidney tumours in laboratory animals in relation to the human exposure level in assessing human risk.

It should be pointed out that the blood lead level associated with the no-effect carcinogenic dose in rats (see toxicokinetics section) is only about 2-fold greater than the blood level found in environmentally exposed humans. However, in view of the curvilinear relationship between lead exposure and blood lead levels, which understates the lead body burden at high exposure levels and the fact that kidney/blood ratios and kidney lead concentrations are dramatically increased in rats on high lead exposure, the use of comparative blood levels in assessing renal cancer risk would be misleading. Of particular importance is the lead concentration at the target site (namely, kidney); the high exposure levels used in the rat carcinogenicity studies would almost certainly have led to high kidney lead levels.

Genotoxicity studies on lead salts

In view of the ubiquitous nature of environmental lead contamination, genotoxicity studies are important from two standpoints: assessing the potential for genetic damage in humans and furthering our knowledge of the mechanism of renal carcinogenesis in rodents. Genotoxicity studies in non-mammalian and mammalian animal systems and in humans have been reviewed (ATSDR/EPA, 1988; EPA, 1986; IARC, 1980 and 1987a; US Nutrition Foundation, 1982; WHO, 1977).

Experimental assay systems

A commercial 0.57% lead acetate hair-colouring preparation gave negative results for microbial mutagenicity when assayed at concentrations of 1–500 µg/plate in tester strains TA98, 100, 1535, 1537 and 1538 of *Salmonella typhimurium* (Ames test) and in *Saccharomyces cerevisiae* strain D₄ in the presence/absence of a rat-liver metabolic activation system (Combe Inc., 1975).

About 40 tests have been conducted on inorganic lead salts, mainly with negative results (IARC, 1987a). Tests for DNA damage or gene mutation in bacteria, gene mutation or mitotic recombination in yeast, and aneuploidy in *Drosophila* all proved negative (Hollstein and McCann, 1979; IARC, 1987a). Tests *in vivo* for unscheduled DNA synthesis in rodents were also negative (IARC, 1987a).

Conflicting results have been obtained in tests for sister chromatid exchanges (SCEs). Negative results were reported *in vitro* (Abe and Sasaki, 1982) and *in vivo* (IARC, 1987a), but a positive result was reported *in vivo* by Sharma *et al.* (1985). In the latter study a small but statistically significant increase in SCEs was observed in mice given an ip dose of

200 mg/kg body weight, but this weak response needs to be weighed against the negative response at the lower dose of 50 mg/body weight and against the variability of spontaneous control frequencies of SCEs. When these facts are taken into account the finding of Sharma *et al.* (1985) is not convincing.

Cell transformation assays also gave conflicting results—positive results being reported in cultured BALB/c3T3 or SA7/SHE cells but negative results with SHE, clonal assay and RLV/Fischer rats (IARC, 1987a). More recent cell transformation tests (Dunkel *et al.*, 1988) with lead acetate at the limit of solubility (10 µg/ml) and in the insolubility range (20 µg/ml) gave negative results. The cell transformation assay system may be flawed because some chemicals with mitogenic properties, such as inorganic lead salts, may enhance the frequency of transformation through selection for pre-existing transformed cells (Brusick, 1980). Currently, the reliability of this assay as well as its relevance to genetic effects *in vivo* is suspect (Brusick, 1989), and it is not included in the list of recommended tests for genotoxicity (DoH, 1989).

The mouse spermhead abnormalities assay gave positive findings (Holstein and McCann, 1979; IARC, 1987a) but a non-genetic cellular effect may account for these findings, especially at the high doses of lead acetate used, and this assay system cannot be regarded as providing unequivocal evidence of genotoxicity (Brusick, 1980; DoH, 1989).

Of four micronucleus tests conducted *in vivo*, three gave negative results and one a questionable positive result (IARC, 1987a).

Of five tests conducted *in vivo* for chromosomal aberrations, two were negative and three questionably positive (IARC, 1987a). Lead acetate can inhibit DNA and RNA synthesis readily in isolated nuclei but with difficulty in intact cells (Frenkel and Middleton, 1987); thus, inhibition is dependent on cell membrane permeability. It is conceivable that at very high exposure levels *in vivo* or under *in vitro* conditions, where membrane barriers may be only partially effective, the resultant cellular entry would result in chromosomal damage. As this could be regarded as an indirect effect, the existence both of a threshold dose level and of a complete reversibility of effect are real possibilities. The *in vivo* studies suggest that acute exposures to high concentrations of lead salts may result in small but statistically significant elevations of chromosomal aberrations but chronic exposures to lower, but still high, levels of lead salts appear to have no effect on mammalian chromosomes (Brusick, 1987).

Schaaper *et al.* (1987) investigated various metals and metal salts for their ability to produce apurinic sites in DNA (a mechanism that could explain why some metals are mutagenic) and in a separate study they treated ϕ X174 phage DNA with metals followed by transfecting the DNA into *Escherichia coli* spheroblasts, survival of the DNA being directly dependent upon the number of apurinic sites. It was found that lead acetate did not produce the number or type of reactions common to genotoxic metals or metal salts.

In its review of lead genotoxicity, the classification scheme adopted by IARC (1987a) did not assign a

positive endpoint to any of the submammalian and mammalian systems *in vitro* or *in vivo*. The overall results indicate that lead salts are not directly genotoxic and, at low exposure levels, pose no risk for somatic or germ cell mutation. At high exposure levels there is evidence of a slight elevation of chromosomal aberrations but whether or not this represents an indirect effect of lead exposure remains to be established (Brusick, 1989). The EPA (1986) and ATSDR/EPA (1988) have also drawn attention to the conflicting results in mammalian systems *in vitro* and *in vivo* but consider that the weight of evidence suggests a clastogenic effect, possibly associated with the status of calcium nutrition.

Human cytogenetic studies

Studies, mainly in workers occupationally exposed to lead for chromosomal abnormalities, have been reviewed (ATSDR/EPA, 1988; EPA, 1986; IARC, 1980 and 1987a; Léonard, 1986; US Nutrition Foundation, 1982; WHO, 1977).

No evidence was obtained *in vitro* of DNA damage or chromosomal aberrations in lymphocytes (IARC, 1987a). Conflicting results have been obtained in tests for SCE and chromosomal aberrations *in vivo* but IARC (1987a) classification did not assign a positive response for the overall results of either of these endpoints. Increased incidences of SCEs have been reported in the lymphocytes of workers exposed to lead but not in children exposed to high levels of lead in the environment (IARC, 1987a).

Of 18 studies, 11 have reportedly shown chromosomal changes (blood lead 10–100 µg/100 ml) whilst seven have shown no association (blood lead 4–50 µg/100 ml) (ATSDR/EPA, 1988; EPA, 1986; Forni *et al.*, 1980; IARC, 1980 and 1987a). However, in the case of most of these studies there has been exposure to a cocktail of other metals and chemicals, particularly in the case of smelter workers. The evidence incriminating occupational exposure to lead *per se*, even at high levels, is far from convincing. It is most unlikely that exposure to much lower levels of environmental lead presents any genotoxic risk to man.

Effects of lead on reproduction in animals and man

Reproductive toxicity studies in laboratory animals

Negative findings were obtained in studies on lead acetate for teratogenicity in rats and mice given oral doses of 3.9–390 mg/kg body weight (calculated as lead) given throughout the period of organogenesis (Kennedy *et al.*, 1975) and in rabbits given dietary levels up to 546 ppm on days 6–16 of gestation (Jessup, 1967) (Table 5). Evaluation of 23 teratogenicity studies on lead acetate or nitrate given orally (gavage, diet, drinking-water) to rats or mice gave no evidence of teratogenicity (ATSDR/EPA, 1988; EPA, 1986).

A three-generation reproduction study in rats, given dietary levels of lead acetate up to 1000 ppm Pb, also proved negative (Jessup, 1969) (Table 5). Various other studies, especially those using massive exposure levels, have led to reported effects on reproductive performance and peri- and postnatal development, and incidence of foetal abnormalities (Table 5).

Table 5. Reproductive toxicology studies on inorganic lead salts in animals

Pb salt administered	Study and treatment	Principal findings	Reference
Mouse			
Pb acetate	Reproduction: 5 ppm in drinking-water (0.95 mg/kg/day Pb) during gestation & lactation	Delayed reflex development in offspring	Reiter <i>et al.</i> , 1975
Pb acetate	Teratogenicity: Daily oral doses of 0, 3.9, 139 or 390 mg/kg (as Pb) on days 5–15 of gestation	No evidence of teratogenicity at doses up to MTD; maternal toxicity, reduced foetal weight, and increased foetal resorption at top dose (discontinued after 3 days)	Kennedy <i>et al.</i> , 1975
Pb acetate	Teratogenicity: 0–0.5% (as Pb) in diet throughout pregnancy	Maternal deaths at top dose, evidence of embryotoxicity but no gross malformations	Jacquet <i>et al.</i> , 1975
Pb acetate	Teratogenicity: ip dose of 15 or 35 mg/kg on day 8, 9, 10 or 12 of gestation	Increases in foetal mortality and skeletal anomalies (effects exacerbated by Ca-deficient diet)	Jacquet and Gerber, 1979
Pb chloride	Embryotoxicity: Single iv dose 40 mg/kg on day 3, 4 or 6 of gestation	Embryonic changes	Wide and Nilsson, 1977
Pb (soluble salt)	Reproduction (3-generation): 25 ppm (as Pb) in drinking-water plus 0.2 ppm (as Pb) in diet	Reproductive performance impaired in F1 and F2 generations	Schroeder and Mitchener, 1971
Rat			
Pb acetate	Reproduction: 64 or 512 ppm (as Pb) in diet to males and females	No effect on reproductive performance	Morris <i>et al.</i> , 1938
Pb acetate	Reproduction (3-generation): 10–1000 ppm in diet to 3 parental generations	Negative findings	Jessup, 1969
Pb acetate	Reproduction: 1% (as Pb) in diet—untreated males × untreated females, treated males × untreated females, untreated males × treated females, treated males × treated females design	Reproductive performance mainly impaired with Pb treatment of females; no malformations	Stowe and Goyer, 1971
Pb acetate	Reproduction: 0.014 & 0.28 mg/kg/day orally for 30 days	Irregular oestrous cycles at both doses; blood Pb levels 30 & 53 µg/100 ml	Hilderbrand <i>et al.</i> , 1973
Pb acetate	Reproduction: 0.015 or 0.29 mg/kg/day orally for 30 days to males	Testicular change at higher dose	Hilderbrand <i>et al.</i> , 1973
Pb acetate	Teratogenicity: 25 mg/kg ip on day 9 of pregnancy	Increased resorption and malformations; no control data given	Zegarska <i>et al.</i> , 1974
Pb acetate (plus ²¹⁰ Pb)	Placental transfer: 5 mg/kg ip during gestation or on day of delivery	Rapid transfer of Pb from mother to foetus during gestation and to young during lactation	Green and Gruener, 1974
Pb acetate	Teratogenicity: Daily oral doses of 0, 3.9, 39 or 390 mg/kg (as Pb) on days 6–16 of gestation	No evidence of teratogenicity at doses up to MTD; maternal toxicity, reduced foetal weight and increased foetal resorption at top dose (discontinued after 3 days)	Kennedy <i>et al.</i> , 1975
Pb acetate	Teratogenicity: 400 or 750 mg/day in water during gestation to weaning and/or post-weaning	Histological damage to brain, even after post-weaning exposure only	Murray <i>et al.</i> , 1977
Pb nitrate	Teratogenicity: Single iv dose 25–70 mg/kg on days 8–17 of pregnancy	Evidence of embryotoxicity, teratogenicity and reduced postnatal survival at and above 50 mg/kg	McLain and Becker, 1975
Pb (soluble salt)	Reproduction (3-generation): 25 ppm (as Pb) in drinking-water plus 0.2 ppm (as Pb) in diet	Reproductive performance impaired in F1, F2 & F3 generations	Schroeder and Mitchener, 1971
Pb acetate	Reproduction/teratogenicity: 0.5, 5, 25, 50 or 250 ppm in drinking-water over gestation, lactation and directly to offspring	Delayed vaginal opening at ≥25 ppm; delayed development of righting reflexes in offspring at 50 & 250 ppm; no evidence of foetotoxicity or teratogenicity at any level	Grant <i>et al.</i> , 1980; Kimmel <i>et al.</i> , 1980
Hamster			
Pb acetate*	Teratogenicity: iv dose of 50 mg/kg on days 7, 8 or 9 of pregnancy	Increased foetal mortality and foetal abnormalities; no control data given	Ferm and Carpenter, 1967

[contd]

Table 5—continued

Pb salt administered	Study and treatment	Principal findings	Reference
Pb acetate	Teratogenicity: 25 or 50 mg/kg given iv with/without Cd sulphate (2 mg/kg) during pregnancy	Increased resorption and tail malformations at top dose of Pb acetate; synergistic and antagonistic effects with Cd	Ferm, 1969
Pb nitrate	Teratogenicity: iv dose 25 or 50 mg/kg on day 8 or 9 of pregnancy	Skeletal abnormalities	Ferm and Ferm, 1971
Pb nitrate	Teratogenicity: iv dose 50 mg/kg on day 8 of pregnancy	Neural tube defects seen on days 9–10	Carpenter and Ferm, 1974
Rabbit			
Pb acetate	Teratogenicity: 54.6 or 546 ppm (as Pb) in diet on days 6–16 of gestation	No evidence of teratogenicity or embryotoxicity	Jessup, 1967
Vole			
Pb acetate	Teratogenicity: 8–64 mg/kg iv on days 7–10 of pregnancy	No effects at 8 or 16 mg/kg; exencephaly, spina bifida and increased resorption at 32 or 64 mg/kg	Kruckenberger <i>et al.</i> , 1976
Cow			
Pb acetate	Teratogenicity: 5 mg/kg orally on days 22–90 or 51–120 of pregnancy to 3 cows	Normal calves from two cows and normal foetus from one cow which died from Pb poisoning	Shupe <i>et al.</i> , 1967
Sheep			
Pb powder	Teratogenicity: 0.5–16 mg/kg bw in diet over entire pregnancy	Abortions but no malformations	Sharma and Buck, 1976

bw = body weight MTD = maximum tolerated dose

*Nitrate and chloride also administered.

Positive effects were invariably obtained at dose levels above 25 mg/kg body weight and also by high parenteral dosage, which is even less meaningful in terms of environmental or occupational exposure.

Human studies

The effects of lead on the human reproductive system have been reviewed (ATSDR/EPA, 1988; EPA, 1986; IARC, 1980; US Nutrition Foundation, 1982; WHO, 1977).

Lead readily crosses the human placenta giving rise to similar maternal and foetal blood levels (ATSDR/EPA, 1988; EPA, 1986; US Nutrition Foundation, 1982).

In past decades females, occupationally exposed to very high lead levels, showed an increased risk of abortions and stillbirths at blood lead levels of 75–317 µg/100 ml (US Nutrition Foundation, 1982; WHO, 1977). More recent studies, reporting increases in abortions (Saric, 1984), menstrual disorders (Yang, 1986) or malformations (Nordström *et al.*, 1979a, b), were either inadequately controlled for confounding variables or rendered uninterpretable because of the simultaneous exposure to other occupational pollutants. Pre-natal lead exposure has no effect on the incidence of congenital malformations or a consistent effect on birth weight, but is claimed to reduce the duration of pregnancy at maternal blood lead levels down to 15 µg/100 ml (ATSDR/EPA, 1988; EPA, 1986), although Bellinger *et al.* (1987) found no correlation between the length of pregnancy and low and high and blood levels of <3 and 10–25 µg/100 ml, respectively. Where there is occupational exposure, both prospective fathers and prospective mothers planning to have a child have been advised to keep blood lead levels below

30 µg/100 ml in order to minimize the risk of impaired reproductive performance (OSHA, 1978).

In occupationally exposed males, oligospermia and abnormalities in sperm structure, unaccompanied by changes in sexual behaviour patterns, increased as blood lead levels increased above 60 µg/100 ml (Assennato *et al.*, 1987; Cullen *et al.*, 1984; Lancranjan *et al.*, 1975). No effect on semen quality was seen in workers with a blood lead level of 46 µg/100 ml (Wildt *et al.*, 1985), and fertility was restored in a worker on reduction of the blood lead level from 88 to 36 µg/100 ml (Fisher-Fischbein *et al.*, 1987).

Effects on sperm counts and on testicular endocrine function have been reported at blood lead levels in the range 40 to 70 µg/100 ml (Winder, 1989). Serum follicle stimulating hormone was only increased at blood lead levels in excess of 47 µg/100 ml, with serum testosterone and luteinizing hormone being unaffected (McGregor and Mason, 1990).

Threshold blood lead levels for male and female reproductive effects were tentatively set at 40–50 and 60 µg/100 ml, respectively (ATSDR/EPA, 1988; EPA, 1986).

There is no convincing evidence to indicate reproductive disorders in males or females from current environmental lead exposure levels.

Relevance to lead acetate hair-colouring use

There is a negligible risk of reproductive or teratogenic effects arising in humans from the average daily lead intake from the general environment. Doses and exposure levels at which effects have been reported in laboratory animals or humans are several orders of magnitude greater than the systemic exposure level derived from lead acetate hair-colouring use. It is inconceivable that the miniscule contribution of lead

from hair-colouring use to the daily body burden could have any adverse effect on any aspect of reproductive performance in humans.

Neurotoxicity of lead in humans and animals

Effects from occupational and environmental exposure in humans

High-level lead exposure can adversely affect the central and peripheral nervous systems, the most severe manifestation being encephalopathy, which is associated with blood levels in excess of 120 and 80–100 $\mu\text{g}/100\text{ ml}$ in adults and children, respectively (ATSDR/EPA, 1988; EPA, 1986; US Nutrition Foundation, 1982). Less severe signs and symptoms of neurotoxicity have been reported at blood lead levels down to 40–60 $\mu\text{g}/100\text{ ml}$ in adults (ATSDR/EPA, 1988; EPA, 1986).

Clinical evidence of lead neurotoxicity, especially from heavy occupational exposure, is well documented but in the last decade neuropsychological (intelligence, cognitive ability) and electrophysiological (electroencephalogram, nerve conduction velocity) studies have attempted to determine whether lead exerts subclinical effects, especially from low-level environmental exposure.

In lead-exposed workers, neuropsychological tests have revealed slight changes at blood lead levels in excess of 50 $\mu\text{g}/100\text{ ml}$ (Arnvig *et al.*, 1980; Hogstedt *et al.*, 1983; Jeyaratnam *et al.*, 1986; Parkinson *et al.*, 1986; Ryan *et al.*, 1987) but convincing evidence of significant effects, causally related to lead, below 50 $\mu\text{g}/100\text{ ml}$ is lacking.

Nerve conduction velocity tests (a measure of peripheral nerve function) in occupationally exposed workers have yielded conflicting findings—negative results at blood lead levels of 60–80 $\mu\text{g}/100\text{ ml}$ (Spivey *et al.*, 1980) and $\geq 70\text{ }\mu\text{g}/100\text{ ml}$ (Triebig *et al.*, 1984) but slight impairment at 30–48 $\mu\text{g}/100\text{ ml}$ (Seppäläinen *et al.*, 1983) and 50–80 $\mu\text{g}/100\text{ ml}$ (Bordo *et al.*, 1982). Although there was no convincing evidence of an effect below 50 $\mu\text{g}/100\text{ ml}$, especially as the observed changes fell within the range of normal variation and arguably therefore 50 $\mu\text{g}/100\text{ ml}$ could be deemed to be an approximate threshold level, ATSDR/EPA (1988) concluded that in adults the effect could occur possibly down to 30 $\mu\text{g}/100\text{ ml}$.

Some EEG studies have revealed changes at blood lead levels of $\leq 30\text{ }\mu\text{g}/100\text{ ml}$, but overall the findings between studies were inconsistent (ATSDR/EPA, 1988).

In human adults, therefore, there is no convincing evidence that current environmental lead levels giving rise to blood levels below 20 $\mu\text{g}/100\text{ ml}$ present a risk of neurotoxicity—clinical or subclinical.

Neuropsychological effects of lead in children

This controversial and complex area has been reviewed (ATSDR/EPA, 1988; Bornschein *et al.*, 1980a,b; DHSS, 1980; DoE, 1990; EPA, 1986; Lee and Moore, 1990; MRC, 1984 and 1988; Pocock and Ashby, 1985; Royal Commission on Environmental Pollution, 1983; US Nutrition Foundation, 1982; WHO, 1977).

Neuropsychological deficits have been reported in children with blood lead levels above 40 $\mu\text{g}/100\text{ ml}$ (US Nutrition Foundation, 1982) and in the range 30 to 70 $\mu\text{g}/100\text{ ml}$ (ATSDR/EPA, 1988; EPA, 1986). Despite considerable effort in the last decade to establish whether neuropsychological function in childhood is impaired by low-level environmental lead exposure, only conflicting evidence has emerged and it is generally accepted that, even where lead-associated effects have been claimed, they are dwarfed by the impact of confounding variables such as socio-economic status or parents' IQ (ATSDR/EPA, 1988; EPA, 1986; Fulton *et al.*, 1987; Lee and Moore, 1990).

US studies in 1979–1983, showing a negative association between lead exposure and neuropsychological function including the much-cited paper by Needleman *et al.* (1979), were critically reviewed by an Expert Committee on Pediatric Neurobehavioral Evaluations for the EPA; these studies were criticized for serious deficiencies in design and analysis and the Committee concluded that, after proper control for confounding variables, there was no valid evidence of a relationship between low-level lead exposure and neuropsychological deficits in children (Pocock and Ashby, 1985). Moreover, key studies in the UK covering 1979–1984 all showed no evidence of a significant association between body lead burden and IQ deficits after allowance for confounding factors (MRC, 1984; Pocock and Ashby, 1985).

Interpretation of such neuropsychological studies is complex as methodological deficiencies include the possibility of selection bias, difficulties in measuring body lead burden, inadequate allowance for numerous confounding factors (hereditary and environmental) known to be associated with IQ, inappropriate statistical analysis, inadequate size of the study and the difficulty in finding a statistical association in cross-sectional surveys to be causally related to lead (Pocock and Ashby, 1985). The UK MRC (1988) also emphasized that, even when a statistical association between body lead burden and some neuropsychological parameter is obtained, it should not be interpreted as necessarily establishing a causal relationship as the association could be due to chance, confounding factors, biases in study methodology or reverse causality (i.e. increase in lead burden due to neuropsychological deficiency).

Further review of UK studies in 1984–1988 found that any neuropsychological effects in children from urban lead level exposure were small in magnitude and difficult to detect with certainty and, because of the limitations of the studies, it was not possible to conclude that such exposure was harmful (MRC, 1988). Two key studies in the UK gave conflicting results—significant associations with IQ being reported by Fulton *et al.* (1987) but none being found by Harvey *et al.* (1988).

Despite suffering fewer limitations than cross-sectional studies, the UK MRC (1988) found that overall the data from prospective studies in the UK and the USA were inconclusive.

In contrast, the ATSDR/EPA (1988) accepted the reported findings of an inverse relationship between neuropsychological deficits and blood lead levels in the ranges 6 to 46 $\mu\text{g}/100\text{ ml}$ (Hawk *et al.*, 1986).

and 5.6 to 22.1 $\mu\text{g}/100\text{ ml}$ (Fulton *et al.*, 1987) and of the absence of a clear threshold and concluded that neuropsychological function can be impaired at blood lead levels as low as 10 $\mu\text{g}/100\text{ ml}$ in children. However, in prospective longitudinal studies (Bellinger *et al.*, 1987 and 1988), postnatal blood levels of 10–25 $\mu\text{g}/100\text{ ml}$ were not associated with neuropsychological deficits monitored during 12–24 months of age in children of lower socio-economic background or during 6–24 months of age in children from a more favoured background—a deficit was observed only at 6 months in the former group.

An 11-yr follow-up study reported that neuropsychological impairment persisted from childhood into young adulthood (Needleman *et al.*, 1990) but the study's methodology was criticized by Good (1991) and Ernhart *et al.* (1991). Schroeder *et al.* (1985) found that previously observed IQ deficits had disappeared 5 yr later when blood lead levels had declined to below 30 $\mu\text{g}/100\text{ ml}$. The issue of reversibility or persistence of alleged neuropsychological effects remains unresolved.

Pre-natal lead exposure did not result in neuropsychological deficits during a 3-yr period post-partum (Ernhart *et al.*, 1987), but deficits associated with umbilical cord blood lead levels of 6–7 $\mu\text{g}/100\text{ ml}$ and $\geq 10\text{ }\mu\text{g}/100\text{ ml}$ have been reported (Bellinger *et al.*, 1987 and 1988). Although US studies indicated that pre-natal exposure had a greater impact than post-natal exposure to lead, the reverse was found in an Australian study (MRC, 1988).

In summary, the prospective studies have not provided clear-cut evidence of neuropsychological impairment to low-level lead exposure *per se* and methodological deficiencies render interpretation difficult. The EPA (1986) and ATSDR/EPA (1988), however, consider that neuropsychological effects in children are associated with blood lead levels down to 10–15 $\mu\text{g}/100\text{ ml}$ for either pre-natal or postnatal lead exposure, but the UK authorities do not support this view (DoE, 1990; MRC, 1988). Children from a low socio-economic background appear to be more susceptible to the effects of lead than children from more favoured backgrounds (Lee and Moore, 1990).

Attempts to associate smoking (Fogelman and Manor, 1988) or vitamin/mineral status (Benton and Butts, 1990; Benton and Roberts, 1988; Crombie *et al.*, 1990) with children's performance have encountered similar problems of interpretation, especially with regard to taking full account of the confounding factors.

Animal studies

The poor absorption of ingested lead in adult rats may partly explain why high doses are required to cause CNS damage in this species. Hyperactivity in the rat and rabbit occur with blood lead levels usually above 80 $\mu\text{g}/100\text{ ml}$. Tests on learning (e.g. active avoidance and visual discrimination) have shown impairment in species such as the rat and monkey at blood levels close to 40 $\mu\text{g}/100\text{ ml}$ (US Nutrition Foundation, 1982), but more recent studies suggest neurobehavioural deficits at levels down to 15–20 $\mu\text{g}/100\text{ ml}$ (ATSDR/EPA, 1988).

The age of the rat can have a striking influence on CNS toxicity in that a single dose giving rise to a

blood lead level of 1000 $\mu\text{g}/100\text{ ml}$ with attendant cerebral haemorrhage and oedema in young animals was without effect in rats, aged more than 20 days (Press, 1977).

Relevance to lead acetate hair-colouring use

At environmental lead exposure levels, there is no conclusive evidence that the nervous system is adversely affected in the human adult. Any neuropsychological impairment in children from low-level environmental lead is small and relatively insignificant when compared with the effects of confounding variables.

Based on a weekly absorption of 0.7 μg lead from lead acetate hair-colouring use, such use can hardly be expected to cause any measurable increase in the potential risk of neurological or neuropsychological disturbances from environmental or occupational lead exposure.

Effects of lead on other systems

Tests on lead acetate hair-colouring preparations for acute oral toxicity and skin/eye irritation in laboratory animals and skin sensitization in humans

In rats, the single dose oral LD_{50} of a commercial 0.75% lead acetate hair-colouring preparation exceeded 100 ml/kg body weight, demonstrating an extremely low order of acute toxicity of the product (Combe Inc., 1972).

Tests carried out on a commercial 0.75% lead acetate hair-colouring preparation for primary skin irritation in rabbits (0.5 ml/patch) (Combe Inc., 1971) and for eye irritation in rabbits (0.1 ml/eye) using the Draize technique (Combe Inc., 1973) gave negative results.

Two commercial 0.75% lead acetate hair-colouring preparations, examined for skin sensitization in 201 healthy human volunteers by the occlusive repeated insult patch test method, gave negative results (Combe Inc., 1989).

Contact dermatitis in humans

Over many decades, despite the frequency of cases of lead poisoning, case reports of contact dermatitis from lead exposure are extremely rare. Two cases are described by Cronin (1980): in one, eczema developed on the fingers of a truck driver after supposedly changing battery terminals (patch tests to aqueous solutions of lead acetate or chloride were positive) and in the other, a painter, after using lead paints for 25 years, presented with dermatitis (patch tests with lead oxide and lead acetate were positive)—his occupational exposure was heavy enough to have resulted in neurological disease.

Haemopoietic system in humans

The haemopoietic system shows effects at lower blood lead levels than any other system. The inhibitory effect of lead on several enzymes involved in haem synthesis causes biochemical changes (e.g. stimulation of mitochondrial δ -aminolaevulinic acid synthetase leading to elevated formation and urinary excretion of δ -aminolaevulinic acid; inhibition of the cystolic enzyme, δ -aminolaevulinic acid dehydratase, which catalyses formation of porphobilinogen from

δ -aminolaevulinic acid; inhibition of incorporation of bivalent iron into protoporphyrin IX to form haem, a process catalysed by ferrochelatase, resulting in accumulation of zinc protoporphyrin in erythrocytes). Although such changes may occur at blood lead levels below $30 \mu\text{g}/100 \text{ ml}$ and thus fall within the range of the general population, the toxicological significance of such subclinical effects has not been established (FDA, 1981; MAFF, 1983; US Nutrition Foundation, 1982; WHO, 1977). Recent evidence suggests that the changes occur in lead-exposed humans at blood lead levels down to $<10 \mu\text{g}/100 \text{ ml}$ and it has been suggested that disturbances in haem biosynthesis may be involved in the mechanism of lead's action on the nervous system as well as on other physiological processes, including vitamin D metabolism (ATSDR/EPA, 1988; EPA, 1986). However, no clinical manifestations from low-level environmental lead due to disturbances in haem synthesis are evident.

Renal system in humans

Two types of renal damage in humans are known. One involves reversible proximal tubular effects in children and adults with subtle signs of lead poisoning from short-term exposure. The other, which is irreversible, arises from prolonged heavy exposure to lead (blood lead level above $70 \mu\text{g}/100 \text{ ml}$) and is characterized by sclerotic changes and interstitial fibrosis that may progress to renal failure. This chronic irreversible nephropathy is rarely encountered under present-day working conditions. Signs of renal damage are likely to develop only with blood lead levels greater than $40\text{--}50 \mu\text{g}/100 \text{ ml}$ sustained for prolonged periods (US Nutrition Foundation, 1982; WHO, 1977). Epidemiological studies in occupationally exposed workers show that chronic nephropathy is associated with blood lead levels of $40\text{--}>100 \mu\text{g}/100 \text{ ml}$, although the more relevant past exposure levels may have been higher. In children suffering from lead poisoning, nephropathy occurs only at blood lead levels $>80 \mu\text{g}/100 \text{ ml}$ and usually $>120 \mu\text{g}/100 \text{ ml}$ (ATSDR/EPA, 1988; EPA, 1986).

It should be emphasized that none of the proliferative changes seen in the rat kidney has been described in cases of heavy lead exposure in humans.

Immune system in humans

Immune function studies have hitherto not indicated any effect of lead on the immune system in occupational workers with blood lead levels of $25\text{--}53 \mu\text{g}/100 \text{ ml}$ (ATSDR/EPA, 1988; EPA, 1986; Kimber *et al.*, 1986; Jaremin, 1983a,b; Yoshida *et al.*, 1980) or in children with blood lead levels of $\geq 40 \mu\text{g}/100 \text{ ml}$ (ATSDR/EPA, 1988; EPA, 1986). In laboratory rats, evidence of immunosuppressive effects was obtained at a blood lead level of $29.3 \mu\text{g}/100 \text{ ml}$ (ATSDR/EPA, 1988; EPA, 1986).

Cardiovascular/cerebrovascular system in humans

Heavy occupational exposure to lead in the past has led to renal damage followed by hypertension. Excess mortality from cerebrovascular disease at high levels of occupational exposure to lead during 1946–1965 has disappeared at lower exposure levels experienced during 1966–1985 (Fanning, 1988).

Recent general population studies have suggested a modest increase in blood pressure at blood lead levels of $30 \mu\text{g}/100 \text{ ml}$ with small but significant associations down to $<10 \mu\text{g}/100 \text{ ml}$ (ATSDR/EPA, 1988; EPA, 1986). Lead is a relatively minor contributor to hypertension (Lee, 1990) and accounts for only 1–2% of the variation in blood pressure when other factors (e.g. age, body build, smoking) are accounted for (ATSDR/EPA, 1988; EPA, 1986). Males aged 40–59 yr are deemed to be at most risk, although uncertainty surrounds the statistical analyses leading to this conclusion (ATSDR/EPA, 1988; EPA, 1986).

Other systems in humans

Effects on the gastro-intestinal tract and endocrine organs have been described in cases of lead poisoning but are not relevant to normal lead exposure levels of the general population (US Nutrition Foundation, 1982; WHO, 1977).

Evidence for an association between lead levels and slight impairment in growth in children has been presented, after controlling for confounding variables, together with corroborative data from animal studies (ATSDR/EPA, 1988; EPA, 1986).

Conclusions

Assessment of lead toxicology

The average human intake of lead from food, water and air is in the region of $100 \mu\text{g}/\text{day}$. About 10–15% of that ingested, the main source of intake, is absorbed (about 50% absorption in the case of children) and excreted in the urine, that unabsorbed being excreted in the faeces. In humans, lead is retained in significant but seemingly innocuous amounts in bones, and, unlike the rat, not in the kidneys. Lipophilic lead compounds, such as tetraethyllead, are far more readily absorbed through the skin than water-soluble salts such as lead acetate.

We have reviewed the genotoxicity studies including some conflicting evidence and conclude that daily exposure to normal background lead levels would not present a mutagenic or clastogenic risk to humans.

That lead is non-genotoxic is of importance also in interpreting the significance to humans of lead nephrocarcinogenicity in rodents, the kidneys being the only site for which there is valid evidence of increased tumour risk in rodents given high doses of lead. We regard the rodent finding as being of no relevance to man because: (i) carcinogenic doses in rodents exceed those that would be acutely lethal to man; (ii) the no-effect carcinogenic dose (5 mg/kg body weight/day) in rodents by the dietary route represents over 1000 times the current daily intake of environmental lead in humans; (iii) dose levels in rodents insufficient to induce damage to the proximal renal tubule cells similarly fail to induce renal tumours; and (iv) the mechanism of renal tumour development in lead-treated rats is mediated by a non-genotoxic mechanism, seemingly involving a prolonged reparative hyperplasia that is peculiar to the rat and has no counterpart in man. These considerations, coupled with the lack of any valid epidemiological evidence connecting environmental or occupational lead exposure with excess kidney

tumour risk in humans, lead us to the firm conclusion that the daily lead burden in humans presents no carcinogenic risk to the human kidney.

Whilst high occupational exposure levels of lead may increase the risk of disturbances to the reproductive system, there is no evidence to suggest that the background lead burden would pose any risk of teratogenicity or reproductive toxicity in humans.

Certain sectors of the general population have been identified as being at relatively higher risk from environmental lead exposure—foetuses, pre-school-age children and males aged 40–59 years (ATSDR/EPA, 1988; EPA, 1986). The evidence showing impaired neuropsychological function from low-level pre-natal or postnatal lead exposure is controversial and the magnitude of any effect is small when compared with the prominent effects of confounding variables such as socio-economic status. Effects of low-level lead on birth weight are not consistent, but an association with reduced gestational age appears to be stronger. The association of low-level lead exposure with hypertension in middle-aged men is weak when compared with the effects of other factors such as age, body build, smoking, etc.

Assessment of safety-in-use of lead acetate hair colourings

Of the estimated 612 μg of lead reaching the scalp for each application of lead acetate hair colourings, 0.35 μg of lead enters the systemic circulation (Moore *et al.*, 1980). Under conditions of twice-weekly use, the amount absorbed would average 0.1 $\mu\text{g}/\text{day}$, which is about 200 times less than the total amount of lead absorbed from a current intake of 100 $\mu\text{g}/\text{day}$ from food, water and air. Hair-colouring lead absorption thus represents about 0.5% of the total lead absorption from the current average daily environmental intake. To put the matter into perspective, the amount of lead absorbed into the body after drinking a single glass of port stored in a lead crystal decanter for 4 months (Graziano and Blum, 1991) or after drinking 7 pints of beer (MAFF, 1989) would be similar to that resulting from lead acetate hair-colouring use over a whole year.

The FDA (1980) concluded that the additional contribution of lead from lead acetate hair-colouring use was minuscule and of no toxicological consequence.

Apart from variations in the background lead intake that govern the level of absorption, dietary components such as milk and minerals can dramatically influence the degree of absorption. Any increase in absorption from such dietary components is likely to be of much greater significance than the minuscule increase in the blood lead level due to lead acetate hair colourings.

Adults are less susceptible than children to lead toxicity and it is noteworthy that hair-colouring preparations are targeted on the adult population. Lead acetate hair colourings fulfil a social need, especially in those consumers concerned with the greying with age of their hair. However, consideration needs to be given to pregnant women users, especially in relation to foetal and postnatal development. At current environmental lead levels, there is

no evidence of increased risk of foetotoxicity or malformations. Evidence for an effect on birth weight is not conclusive and that for neuropsychological impairment in young children from pre-natal lead exposure remains highly controversial, in view of the impact of confounding variables. As lead acetate hair-colouring use contributes about 0.5% to the daily amount of lead absorbed, it is inconceivable that foetal or postnatal development would be adversely affected from such use by pregnant women.

This review concludes that the use of lead acetate hair-colouring preparations, involving once- or twice-weekly applications to the scalp, would give rise to a weekly absorption of up to 0.7 μg , which can be regarded unequivocally as being toxicologically insignificant, especially as current environmental lead levels do not present a significant risk to human health.

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