4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Inorganic lead compounds*

(*a*) *Humans*

(i) *Absorption*

Absorption of lead is influenced by the route of exposure, the physicochemical characteristics of the lead and the exposure medium, and the age and physiological status of the exposed individual (e.g. fasting, concentration of nutritional elements such as calcium, and iron status). Inorganic lead can be absorbed by inhalation of fine particles, by ingestion and, to a much lesser extent, transdermally.

Inhalation exposure

Smaller lead particles (< 1 μm) have been shown to have greater deposition and absorption rates in the lungs than larger particles (Hodgkins *et al.*, 1991; ATSDR, 1999). In adult men, approximately 30–50% of lead in inhaled air is deposited in the respiratory tract, depending on the size of the particles and the ventilation rate of the individual. The proportion of lead deposited is independent of the absolute lead burden in the air. The half-life for retention of lead in the lungs is about 15 h (Chamberlain *et al*., 1978; Morrow *et al*., 1980). Once deposited in the lower respiratory tract, particulate lead is almost completely absorbed, and different chemical forms of inorganic lead seem to be absorbed equally (Morrow *et al*., 1980; US EPA, 1986).

In two separate experiments, male adult volunteers were exposed to aerosols of lead oxide (prepared by bubbling propane through a solution of tetraethyl lead in dodecane and burning of resulting vapour) containing 3.2 μg/m^3 lead (15 subjects) or 10.9 μg/m^3 lead (18 subjects) in a room for 23 h/day for up to 18 weeks (Griffin *et al.,* 1975a). Six unexposed controls were included in each experiment. For those volunteers who remained until the end of the study, blood lead concentrations increased for about 12 weeks and then remained at ~37 μg/dL and ~27 μg/dL for the groups with higher and lower exposure, respectively. The concentration in the blood of controls was ~15 μg/dL. Lead content in blood declined after cessation of exposure, returning to near pre-exposure concentrations after 5 or 2 months for higher and lower exposure, respectively. Chamberlain *et al.* (1978)

suggested that some residual unburnt tetraethyl lead vapour may have been present in these experiments.

Twelve volunteers exposed to 150 μg/m^3 lead as lead oxide for 7.5 h per day on 5 days per week for 16–112 weeks exhibited elevated concentrations of lead in blood and urine, with one subject achieving a blood lead concentration of 53 μg/100 g (Kehoe, 1987). An average respiratory intake of 14 μg lead per day was reported for five male volunteers while exposed to an ambient concentration of 0.4–2.1 μg/m^3 airborne lead (Rabinowitz *et al*., 1977).

Oral exposure

Most data on gastrointestinal absorption of lead are available for adults; there have been very few studies in children. Absorption of lead occurs primarily in the duodenum (reviewed in Mushak, 1991). The mechanisms of absorption have yet to be determined but may involve active transport and/or diffusion through intestinal epithelial cells (transcellular) or between cells (paracellular), and may involve ionized lead (Pb^{2+}) and/or inorganic or organic complexes of lead (Mushak, 1991). The extent and rate of gastrointestinal absorption are influenced by physiological conditions of the exposed individual such as: age, fasting, the presence of nutritional elements including calcium, phosphorus, copper and zinc, iron status, intake of fat and other calories; and physicochemical characteristics of the medium ingested, including particle size, mineral species, solubility and lead species.

Studies in adults

The experimental studies in adults have mainly employed lead chloride and radioactive tracers (^{212}Pb and later ^{203}Pb). Other evidence, often indirect, comes from stable lead isotope methods and epidemiological studies. Representative studies in adults are summarized in Table 81. The experimental studies generally had small numbers of subjects, ranging from one to 23 and the studies with ^{203}Pb were very short-term, due to the short half-life of the tracer (52 h). There was a wide variation in absorption between individuals in most studies; absorption was up to 96% in subjects who ingested lead with alcohol whilst fasting (Graziano *et al*., 1996) but was generally less than 10% in subjects who received lead with food.

Studies in children

Dietary data for very young infants (< 6 months old) are scarce; results of some studies are listed in Table 82. For example, of the eight children investigated by Alexander *et al.* (1974), only one was aged less than 6 months. In the study by Ziegler *et al.* (1978), only one infant was studied from 14 days of age, two were studied from 72 and 83 days of age, respectively, and the rest were over 118 days (~4 months) old. In a study by Gulson *et al.* (2001a), 15 newborn infants were monitored for at least 6 months postpartum. Infants were breastfed or formula-fed or both and, aged about 91–180 days, usually fed solid foods (baby food called beikost). Daily lead intake ranged from 0.04 to 0.83 μg/kg bw with a geometric mean of 0.23 μg/kg bw and the excretion/intake ratio ranged from 0.7 to 22 with a geometric mean of 2.6. In a stable-isotope study, the mean value of blood

Table 81. Absorption of lead in adults after ingestion

No. and sex of subjects	Percentage ± SD absorption (range)	Exposure conditions	Reference
Radioactive tracer $^{203}PbCl_2$[a] or $^{203}PbAc$[a]			
11 men +	65	Fasting	James *et al.* (1985)
12 women	3.5	Meal with calcium + phosphorus	
	16	3 h after breakfast	
	43	5 h after breakfast	
10 men	21 ± 16 (10–67)	2 h after meal	Blake (1976)
6 men	8 (4–11)	Meal	Chamberlain *et al.*
	45 ± 17 (24–65)	Fasting	(1978)
7 men	21 (10–48)	2 h after meal	Moore *et al.* (1979)
4 women	70 (67–74)	Fasting	Blake & Mann (1983)
	17 (7–26)	Fasting + 175 mg calcium/250 mg phosphorus	
	2 (1–5)	Fasting + 1750 mg calcium/2500 mg phosphorus	
8 men	63 (59–67)	Fasting	Heard & Chamberlain
6 men	3 (2–5)	In liver and kidney of lamb[b]	(1982)
2 men	44 (37–56)	Fasting	Heard *et al.* (1983)
9 men	5.5 (2–14)	In spinach[c]	
8 men	10 (5–15)	Normal meal + 200 mg calcium/ 140 mg phosphorus	
2 men	14 (3–28)	With coffee and tea (and normal meal) (nine tests)	
4 men	19.5 (11–23)	With beer 10 min before light lunch	
9 men	14 ± 3 (8–18)	Tracer given 2 h after meal	Newton *et al.* (1992)
Stable lead isotopes[d]			
9 men	13.8 ± 3.1	'Contaminated' beer, 0.5 L/d for 5 d	Newton *et al.* (1992)
5 men	10 ± 2.7 (6.5–14)	With food: lead nitrate	Rabinowitz *et al.* (1976)
4 men	8.2 ± 2.8 (6–10)	With food: lead nitrate/cysteine	Rabinowitz *et al.* (1980)
4 men	35 ± 13 (30–37)	Fasting: lead nitrate/sulfide/cysteine	
2 men, 4 women	76 (46–96)	Fasting: Sherry in lead crystal decanter	Graziano *et al.* (1996)
1 man	34	Fasting: Wine doped with tracer ^{207}Pb	Gulson *et al.* (1998c)
	2.3	Wine doped with ^{207}Pb consumed with meal	

[a] Volunteers ingested ^{203}Pb as lead chloride or lead acetate in various media, e.g. in water, beverages and meal, with varying amounts of calcium and phosphorus under fasting and non-fasting conditions. Venous blood samples were taken at various times, e.g. 24 and 48 h. Body burden was measured by γ-ray counting, 4–9 days after dosing.

[b] Portions of liver or kidney from lamb injected with ^{203}Pb as lead chloride and butchered after 6 days were cooked and served in meals to volunteers.

[c] The plants of spinach were placed in water containing the ^{203}Pb as lead chloride for 48 h. The tops were then harvested, cooked and eaten with normal meal.

[d] Volunteers ingested non-radioactive enriched lead isotopes ^{204}Pb, ^{206}Pb or ^{207}Pb as lead nitrate, lead cysteine or lead sulfide in various media, e.g. in water, beverages and meal. Lead in samples (blood, urine or faeces) was determined by thermal ionization mass spectrometry.

Table 82. Daily lead intakes in children and absorption of lead after ingestion

Study group	Exposure	Daily intakes (μg/kg bw per day) mean (range)	Mean absorption[a] (%)	Mean retention[b] (%)	Reference
4 boys and 4 girls, aged 3 months to 8 years	11 balance studies in own homes. One child was studied 4 times from 3 months to 1.08 years.	10.6 (5–17)	53	18	Alexander *et al.* (1974)
6 boys and 6 girls, aged 14–746 days	2 separate balance studies 11–18 days apart in metabolic unit; 61 studies with variable lead intakes > 5 μg/kg bw per day	> 5	42	32	Ziegler *et al.* (1978)
9 children in hospital, aged 3–13 weeks; part of group of 29 children aged 3 weeks–14 years	104 balance studies with 29 children	6.5 [1.5–17]	Between –79% and 12% for the 9 subjects, but high inter-subject variability, some in negative balance; –40% for all 29 children		Barltrop & Strehlow (1978)

[a] Absorption denotes total intake minus faecal excretion
[b] Retention denotes total intake minus total excretion

lead coming from diet was 50% (Ryu *et al.*, 1983, 1985). This value was consistent with earlier estimates of uptake of lead in blood in newborn infants when environmental lead concentrations were much higher (Alexander *et al.*, 1974; Ziegler *et al.*, 1978). In contrast, Manton *et al.* (2000) suggested that the absorption in one child aged 4 months was only 1–5%. [The Working Group noted that the percentage absorption observed in this study is at variance with the majority of observations in infants.]

It should be noted that no absorption studies have been conducted in children older than 8 years. However, the changes in stable isotope tracers of blood lead in mothers and their children present similar profiles; both reach equilibrium with a unique exogenous lead isotope profile suggesting that children aged 6–11 years and their mothers may absorb a similar percentage of ingested lead from dietary sources (Gulson *et al.*, 1997a).

Nutritional factors affecting absorption

Mineral content is one factor that may lower the absorption of lead when it is ingested with food. For example, the presence and amount of calcium and phosphorus in a meal depress the absorption of ingested lead. The effect is greater for the two elements together than for either alone, with calcium showing a stronger effect than phosphorus (Blake & Mann, 1983; Heard *et al.*, 1983; James *et al.*, 1985). In children, an inverse relationship has been observed between dietary calcium intake and retention of lead, suggesting that children who are deficient in calcium may absorb more lead than calcium-replete children (Ziegler *et al.*, 1978). Several studies have drawn attention to the potential toxicity of lead in calcium or vitamin supplements (Capar & Gould, 1979; Roberts, 1983; Boulos & von Smolinski, 1988; Bourgoin *et al.*, 1993; Rogan *et al.*, 1999; Scelfo & Flegal, 2000). However, a study using isotope differences between lead in two types of calcium supplements and that in the blood of adults showed that the supplements did not increase blood lead concentration over a 6-month trial (Gulson *et al.*, 2001b).

A higher dietary intake of iron is associated with lower blood lead concentrations among children and iron deficiency may result in higher absorption of lead (Watson *et al.*, 1980; Mahaffey & Annest, 1986; Watson *et al.*, 1986; Marcus & Schwartz, 1987; Hammad *et al.*, 1996; Wright *et al.*, 2003). Evidence for the effect of iron deficiency on lead absorption has been provided also from animal studies (see Section 4.1.1(*b*)).

In a metabolic study of 10 adult subjects who ingested copper, zinc or iron supplements incorporated into a basal diet, higher faecal lead losses and lower blood lead concentrations were observed only with the copper supplements (Kies & Ip, 1991). [This could be an effect on either absorption or retention.]

A positive correlation has been observed between blood lead in children and total and saturated fat and caloric intake (Lucas *et al.*, 1996; Gallichio *et al.*, 2002). No relationship between intake of fat and protein and lead concentrations in bone and blood was found in middle-aged to elderly men in the Normative Aging Study (Cheng *et al.*, 1998).

Ascorbic acid is known to enhance the urinary elimination of lead from blood, liver and kidney in rats (Flora & Tandon, 1986). However, evaluation of the data from the Third National Health and Nutrition Examination Survey showed that there is no signi-

ficant relationship between ascorbic acid intake in diet and blood lead concentrations in humans (Simon & Hudes, 1999; Houston & Johnson, 2000).

Absorption of lead from soil

In a study to mimic the soil ingestion habits of children, six adult subjects ingested soil (particle size less than 250 μm) from the Bunker Hill (ID, USA) mining site, resulting in a dose of 250 μg lead/70 kg bw. Based on stable lead isotope analysis, the subjects absorbed 26 ± 8% of the lead in the soil when they were in the fasted state and 2.5 ± 1.7% when the same soil lead dose was ingested with a meal (Maddaloni *et al*., 1998). There are no reported measurements of the absorption of soil-borne lead in infants or children. Evidence for a lower absorption of soil-borne lead compared with dissolved lead is provided from studies in laboratory animals (see Section 4.1.1(*b*)). Experiments with lead-bearing mine waste soil suggested that surface area characteristics determine dissolution rates for particles < 90 μm in diameter, whereas dissolution of 90–250-μm particles appeared to be controlled more by surface morphology (Davis *et al*., 1994). Similarly, in-vitro experiments showed that the solubility of 30-μm particles of lead sulfide in real gastric fluid [origin not specified] was much greater than that of 100-μm particles (Healy *et al*., 1982).

Dermal exposure

Little information is available regarding absorption of lead in humans after dermal exposure. Moore *et al.* (1980a) conducted a study in which commercially-available lead acetate solution (6 mmol/L lead acetate) or skin cream (9 mmol/kg lead), labelled with [^{203}Pb]acetate, was applied to the forehead skin of eight male volunteers for 12 h and then washed off. Blood and urine samples were collected. The percentage of absorption was estimated by measuring the ^{203}Pb activity in blood samples, by counting over the subject's calf region using a whole-body monitor, and also by counting 24-h and 48-h urine samples. Absorption through intact skin was 0.18 ± 0.15% of the dose applied; that through scratched skin was 0.26 ± 0.46%. Lead exposure from the use of hair-colouring agents containing lead acetate was reported to be insignificant (Moore *et al*., 1980a; Cohen & Roe, 1991). However, this assumes that only adults will be in contact with the colouring agents and ignores human behaviour in the home environment (Mielke *et al*., 1997b). Measurements of lead on hands and surface wipes (including combs, hair dryer, faucet) from subjects using hair-colouring agents showed between 150 and 700 μg lead per hand and more than 100 μg/9.3 dm^2 [~10 μg/dm^2] on the surfaces. At such concentrations, there is a potential for hand-to-mouth and hand-to-surface transfer of lead not only to adults but also to children (Mielke *et al*., 1997b).

The dermal absorption studies of Florence and colleagues (1988), although limited in subject numbers (nine workers), remain the most comprehensive to date. Following observations that workers in a lead battery factory exhibited high concentrations of lead in sweat, Florence *et al*. (1988) and Lilley *et al*. (1988) showed that finely-powdered lead metal and lead oxide (20 mg; particle size < 0.45 μm) or 60 μL of 0.5 M lead nitrate solution (6 mg lead) placed on the skin of one arm was rapidly absorbed. The absorbed lead

appeared in sweat (induced by pilocarpine iontophoresis) on the other arm and in saliva, but was not detectable in blood or urine. The authors found that the rate of lead absorption through the skin increased with increased sweating and, as observed by Moore *et al.* (1980a), suggested that the mechanism was one of rapid diffusion through filled sweat ducts followed by a slower diffusion through the stratum corneum (Lilley *et al*., 1988). The authors (as also observed by Moore *et al.*, 1980a) noted that the absorbed lead must be transported in the plasma and concentrated quickly into the extracellular pool (sweat and saliva), that its mean residence time in the plasma is very short and that little lead enters the erythrocytes (Lilley *et al.*, 1988). [No quantification of the amount of lead absorbed was undertaken and there were inconsistencies between the concentrations of lead in sweat from the two arms on certain days.]

In later experiments using compounds made with ^{204}Pb tracer and employing the sensitive thermal ionization–mass spectrometry (TIMS) and ICP–MS methods, lead acetate or lead nitrate was applied to the skin of four volunteers and perspiration induced by either pilocarpine iontophoresis or thermally in a sauna (Stauber *et al*., 1994). The lead compounds were rapidly absorbed through the skin and detected in sweat, blood and urine within 6 h of application. In one subject, 4.4 mg lead (as lead nitrate) was applied to the skin under a patch and perspiration induced by iontophoresis. Of the applied dose, 1.3 mg lead was not recovered from skin washings, indicating that 29% of the applied dose was absorbed into or through the skin. The authors suggested that some of the absorbed lead was still present in the epidermis and had not entered the circulatory system as the other experiments indicated that an equivalent of only 0.2% of the ^{204}Pb applied to the skin was detected in blood. However, no measurable increase of total lead in blood or urine was found in this study. [The Working Group agreed with the authors in their concern about this lack of increase in total lead in blood or urine, since blood lead is the accepted biomarker of exposure.]

(ii) *Distribution*

Lead enters and leaves most soft tissues reasonably freely. The clearance from the blood into both soft tissues and bone dominates lead kinetics during the first few weeks after an exposure, with an apparent half-life of several weeks (Table 83). Once an approximate equilibrium is reached between soft tissues and blood, the concentration of lead in blood is determined almost entirely by the balance among absorption, elimination, and transfer to and from bone. In the absence of continuing exposure, the whole-body half-life represents the loss of lead from bone. Lead enters and leaves bone by physiologically-distinguishable mechanisms (reviewed and summarized in O'Flaherty, 1991a, 1992, 1993), which include rapid exchange between blood plasma and bone at all bone surfaces, incorporation of lead into forming bone and its loss during bone resorption, and very slow diffusion of lead throughout undisturbed bone. Slow diffusion accounts for the gradual build-up of large quantities of bone-seeking elements such as lead in quiescent, largely cortical bone (Marshall & Onkelinx, 1968).

Table 83. Kinetic parameters for lead in blood of non-occupationally exposed adults and young children

Study group and exposure	No. and sex of subjects	Age range (years)	Lead half-life in days ± SD (range)	Comments	Reference
Adults					
Inhalation of lead oxide	24 men 21 men	24–49 24–50	~1 month ~1 month	10.9 μg/m^3 lead 3.2 μg/m^3 lead	Griffin *et al.* (1975a)
Ingestion of stable ^{204}Pb and ^{207}Pb as nitrate	5 men	25–53	25 ± 3	With meals	Rabinowitz *et al.* (1976)
Ingestion of lead-contaminated beer for 28 days	9 men	23–65	30 ± 4 (19–46)	No standardization of meals	Newton *et al.* (1992)
Ingestion of wine doped with ^{207}Pb tracer	1 man	NR	23	With meals	Gulson *et al.* (1998c)
Exposed to environments with different lead isotopes	7 (of 8) women (immigrants[a])	26–36	59 ± 6 (50–66)	Isotopic changes in blood lead monitored monthly	Gulson *et al.* (1995, 1999)
Children					
Newborn infants of immigrant mothers[a]	9	0–0.5	91 ± 19 (65–131)	Isotopic changes in blood lead monitored every 2 months	Gulson *et al.* (1999)

NR, not reported

[a] The Working Group noted the longer half-lives of lead in blood for the immigrant women and their newborn infants. The timing of sampling for the immigrant women, usually at least 1 month after their arrival in Australia, suggests that the longer half-life observed was a whole-body half-life, reflecting a primary contribution from bone.

Bone formation and bone resorption are generally tightly coupled. During infancy and childhood, the bones grow rapidly and they are continually reshaped. Although the formation rate may greatly exceed the resorption rate, both processes are active throughout bone. When full growth is reached in the late teens, bone formation and resorption rates are equal. Subsequently, resorption of old bone and formation of new bone, which take place throughout the entire bone volume, serve to maintain healthy bone tissue and to restructure the bone in response to changing physical demands. The bulk of this activity takes place in trabecular bone. The coupling of bone formation and bone resorption is a two-edged sword: it is necessary to think of bone as both a sink for lead and a source of endogenous lead, since both processes operate simultaneously. During childhood, when the formation rate is

high, so is the resorption rate, so that little bone lead from an early childhood exposure will persist into adulthood. On the other hand, generally whenever resorption is high, so is formation, so that return of lead to blood plasma with resorbing bone will be partially compensated by its redeposition into forming bone. Since trabecular bone generally turns over more rapidly than cortical bone, the lead content of trabecular bone should respond more rapidly than the lead content of cortical bone to changes — either increases or decreases — in lead exposure (O'Flaherty, 1993).

Beginning as early as at age 25–30 years, bone resorption rate rises slightly while bone formation rate does not change, so that slow net bone loss begins in early adulthood (Jowsey *et al.*, 1965; Mazess, 1982). There are also physiological states in which bone resorption and formation become temporarily partially uncoupled. During the first five or more years following menopause in women, the bone resorption rate is temporarily increased without a compensatory increase in bone formation rate, after which bone resorption rate drops back to a level about the same as that observed in older men (and in women before menopause) (Mazess *et al.*, 1987; Nilas & Christiansen, 1988). During pregnancy and lactation, the bone resorption rate is increased in order to supply calcium to the fetus and neonate.

The distribution of lead in various body compartments is considered in greater detail below.

Blood

Lead in blood is found primarily in the red blood cells (> 99%) rather than the plasma (Hursh & Suomela, 1968; Everson & Patterson, 1980; DeSilva, 1981; US EPA, 1986; Bergdahl *et al.*, 1997a). Bergdahl *et al.* (1997b,c, 1998a) showed that the principal lead-binding protein was delta-aminolevulinic acid dehydratase (ALAD), also known as porphobilinogen synthase (PBGS). Human ALAD has two alleles, ALAD-1 and ALAD-2, with three phenotypes (and their percentages in Caucasian populations): ALAD 1-1 (80%), ALAD 1-2 (19%) and ALAD 2-2 (1%) (Battistuzzi *et al*., 1981; Benkmann *et al.*, 1983). It has been proposed that this polymorphism causes differential sensitivity to lead exposure (see Section 4.2.2).

Half-life of lead in blood

The half-life of lead in human blood has been determined experimentally, primarily in adult men. These studies were carried out on small numbers of subjects, usually fewer than ten, exposed only for up to 124 days. There are very limited data for children. A summary of estimated half-lives from experimental studies is given in Table 83. The mean half-lives of loss of lead from the blood immediately following an exposure are similar across studies and are independent of the route of exposure, although there are large differences between individuals. The mean half-lives for adult men in these studies ranged from 19–30 days. However, in lead workers exposed for periods of up to 10 years, with high blood lead concentrations, the half-life of initial loss from the blood following cessation of exposure is of the order of 20–130 days; the half-life in lead workers was a function of cumulative occupational exposure (O'Flaherty *et al*., 1982).

When equilibrium is reached between soft tissues and blood, the net rate of loss of lead from the blood decreases. Subsequently, the rate-determining step for whole-body loss is the return of lead from the bone; in environmentally exposed subjects at equilibrium with their environment, 40–70% of lead in blood derives from bone (Manton, 1985; Gulson *et al*., 1995; Smith *et al*., 1996). Because of the nature of the mechanisms responsible for return of lead from bone to blood, the overall process is not correctly described by a half-life; however, it is convenient to continue to use half-lives to characterize whole-body loss as expressed by the decline of blood lead concentrations. In adult women of child-bearing age, Gulson *et al*. (1995, 1999) determined a mean whole-body half-life of 59 ± 6 days. Infants born to mothers immigrant to Australia had whole-body half-lives of 65–131 days, considerably longer than the 50–66-day half-lives observed for the adult women (Gulson *et al*., 1999). Manton *et al*. (2000) observed longer whole-body half-lives of lead during the first 2 years of life in the blood of two groups of children who had been exposed to lead from residential remodelling over varying periods of time. Half-lives of lead in children exposed for unspecified brief periods of time were between 8 and 11 months, while half-lives in those with longer exposures varied from 20 to 38 months.

Whole-body half-lives of lead in blood estimated for workers occupationally exposed to lead are commonly much greater than those shown in Table 83 for non-occupationally exposed individuals, and reflect a much greater loading of the skeleton with lead (O'Flaherty *et al*., 1982; Hryhorczuk *et al*., 1985; Schütz *et al*., 1987; Nilsson *et al*., 1991; Fleming *et al*., 1997, 1999). They are comparable to half-lives of lead measured in cortical bone (Christoffersson *et al*., 1986; Erkkilä *et al*., 1992).

Serum–whole blood relationships

Several authors have proposed that measurement of lead in serum may better reflect the fraction of lead that is available in the circulation for exchange with target organs such as the central nervous system and kidneys, and with the developing fetus (Manton & Cook, 1984; Schütz *et al*., 1996; Hernandez-Avila *et al*., 1998; Hu, H. *et al*., 1998; O'Flaherty, 1998; O'Flaherty *et al*., 1998; Smith *et al*., 1998; Bergdahl *et al*., 1999). A stronger association was found between the ratio plasma lead/blood lead with bone lead concentrations (measured by X-ray fluorescence) than with whole blood lead concentrations (Cake *et al*., 1996; Hernandez-Avila *et al*., 1998). Using urine as a proxy for plasma, Tsaih *et al*. (1999) observed significant associations between bone lead and urinary lead.

The low concentration of lead in plasma, relative to red blood cells, has made it extremely difficult to measure accurately plasma lead concentrations in humans, particularly at blood lead concentrations less than 20 μg/dL (Schütz *et al.*, 1996; Hernandez-Avila *et al.*, 1998). Serum analyses, especially at lower blood lead concentrations, are complicated by erythrocyte contamination (haemolysis), sampling and laboratory contamination, measurement error and misinterpretation of the data (Manton *et al*., 2001).

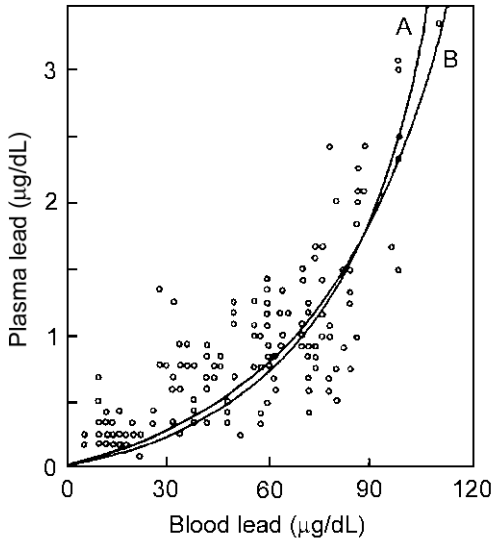
Plasma is generally accepted as the source of lead available to distribution and excretion processes. Urinary lead excretion in humans is directly proportional to plasma lead concentration but not to blood lead concentration (O'Flaherty, 1993). Breast milk lead has been

considered an indirect measure of plasma lead. A number of studies have shown significant linear relationships between lead in human breast milk and whole blood collected at delivery (cord blood) or post partum (Moore *et al*., 1982; Ong *et al*., 1985; Rabinowitz *et al*., 1985; Namihira *et al*., 1993; Palminger Hallén *et al*., 1995a; Gulson *et al*., 1998a).

Physiologically-based kinetic models in which transfers of lead (other than into bone) are assumed to be proportional to plasma lead concentration have been successful in a variety of different applications (O'Flaherty, 1998, 2000).

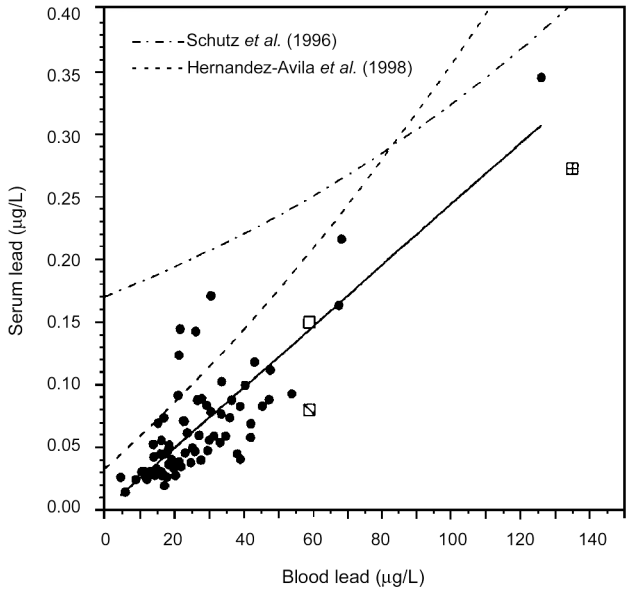
The relationship between serum lead and blood lead concentrations is not linear, due at least in part to limited availability of lead binding sites in the erythrocyte (DeSilva, 1981; Marcus, 1985; O'Flaherty, 1993). This binding is highly variable among individuals; it is influenced by extrinsic factors, such as iron nutritional status; and there is some evidence for its inducibility (Raghavan *et al.*, 1980; Marcus & Schwartz, 1987). The saturable binding of lead to erythrocytes has been interpreted as binding to three principal components, the tightest binding of which is to ALAD (Bergdahl *et al*., 1998a). Thus, the fraction of blood lead in the plasma, the driving compartment for transfer into tissues, increases disproportionally with increasing blood lead concentration (Figures 1 and 2). The disproportionality becomes more pronounced as blood lead concentrations increase above about 40 μg/dL. Below this concentration, the relationship of serum lead concentration to blood lead concentration can be approximated by a straight line (Manton *et al*., 2001).

Figure 1. The relationship between plasma lead and blood lead



Adapted from O'Flaherty (1993)
Data points are from DeSilva (1981) as shown in Marcus (1985). Curve A is the model simulation. Curve B is Marcus' fit to the data, using a no-intercept model (Marcus' Model 4) consistent with the concept of a maximum erythrocyte binding capacity for lead.

Figure 2. Plot of serum lead concentration *vs* blood lead concentration for 73 subjects



Adapted from Manton *et al.* (2001)
The line fitted to the points has the equation y = 0.00246 + 0.00236x (r = 0.82). Curves for workers obtained by Schütz *et al.* (1996) and Hernandez-Avila *et al.* (1998) are those calculated by Manton *et al.* (2001). The curves of both groups of workers were defined down to blood lead concentrations of 23 µg/dL so that at the lower end of their ranges, the observations for the workers overlap with those of Manton *et al.* (2001). See Manton *et al.* (2001) for further details.

In summary, in more recent investigations within this apparently linear range, there is convergence towards a percentage of serum lead/whole blood lead of < 0.3% (Cake *et al.*, 1996; Bergdahl & Skerfving, 1997; Bergdahl *et al.*, 1997a; Hernandez-Avila *et al.*, 1998; Bergdahl *et al.*, 1999; Manton *et al.*, 2001; Smith *et al.*, 2002; see Table 84). These investigations confirm earlier studies with radioactive ^{203}Pb tracers that showed that 0.2% of the ^{203}Pb was present in plasma at 50–100 h after exposure (Heard & Chamberlain, 1984).

Soft tissues

Lead has been measured in a variety of tissue samples in humans but care needs to be taken when comparing results because of the different reporting of measures for wet, dry and ashed weights.

In a study of lung tissues collected at autopsy from individuals with no known occupational exposure to lead [no details given], an average lead concentration of 0.22 ± 0.11 µg/g tissue was found (Barry, 1975). In 42 non-occupationally exposed subjects, Gross *et al.* (1975) detected 0.36 ± 0.12 µg/g wet weight (ashed, 23.9 ± 10.6 µg/g) in lung tissue. In a

Table 84. Analyses of blood lead concentrations in serum and whole blood samples

Subjects (no.) exposed or not to lead	Analytical method[a]	Blood lead concentration (µg/dL)	% serum/whole blood (mean or range)	Curvilinear relationship	Reference
Women, non-exposed (73)	TIMS	0.6–12.6	0.1–0.4	Linear < 10 µg/dL	Manton *et al.* (2001)
Women and men, non-exposed (26)	ICP-MS	2.3–41.6	0.2–0.7	Yes	Hernandez-Avila *et al.* (1998)
Women, non-exposed (63)	ICP-MS	< 25	0.099–0.48	For samples > 10 µg/dL	Smith *et al.* (2002)
Men, exposed (143)	ICP-MS	10–30 30–90	0.4 (read from the graph) 0.35 (read from the graph)	Yes	Bergdahl *et al.* (1997a); Bergdahl & Skerfving (1997)
Men, exposed (49)	TIMS	16.5–55.2	0.8–2.5	Not stated	Cake *et al.* (1996)
Children (44), exposed (31) Considerably lower exposed: 'Unexposed' (13)	ICP-MS	9.9–92 3.9–12	0.24–2.0 (median, 0.79) 0.23–0.49 (median, 0.36)	Yes (for exposed and unexposed children)	Bergdahl *et al.* (1999)

[a] TIMS, thermal ionization–mass spectrometry; ICP–MS, inductively-coupled plasma mass spectrometry

similar study, Mylius and Ophus (1977) reported an average of 0.56 µg/g dry weight (range, 0.28–1.14 µg/g) in lung tissue from 10 non-occupationally exposed individuals.

Gerhardsson *et al.* (1995b) showed that in 32 deceased smelter workers with known lead exposure history, the major soft tissue organs of lead accumulation were: liver > kidney > lungs > brain. Lyon *et al.* (2002) measured lead in liver tissue of 157 subjects aged < 1 day to 6 years. Lead concentrations ranged from 0.0083 to 0.407 µg/g wet weight. The median fetal liver concentration in 10 subjects was 0.0256 µg/g dry weight (Lyon *et al.*, 2002), comparable with the value in a Canadian study of 21 fetal livers of 0.061 ± 0.023 µg/g dry weight as calculated by Lyon *et al.* (2002) from the reported value, 0.243 ± 0.092 µg/g wet weight (Gélinas *et al.*, 1998). These values are considerably lower than those found in adults before 1994 (0.25–2.30 µg/g; Caroli *et al*., 1994), in 73 adults in Canada (0.01–1.2 µg/g; Treble & Thompson, 1997) and in children aged 0–10 years for the period 1975–89 (0.08–1.37 µg/g; Patriarca *et al*., 2000).

Barregård *et al.* (1999) measured lead in the renal cortex from 36 living healthy kidney donors in Sweden and found mean values of 0.18 µg/g dry weight. This was the first study of heavy metals in kidney cortex of living, healthy subjects.

Al-Saleh and Shinwari (2001b) measured concentrations of lead in tumour tissue from 23 patients (17 women, six men) with malignant brain tumours and 21 patients (11 women,

10 men) with benign brain tumours who were undergoing treatment at a Saudi Arabian hospital. Mean lead concentrations were similar in malignant and benign tumours (0.65 ± 1.7 and 0.61 ± 1.7 μg/g, respectively). In a study of a population in the USA, however, the concentration of lead in brain was below the limit of detection of 0.0008 μg/g (Bush *et al.*, 1995). [The Working Group noted the lack of a proper control group and other indices of cumulative lead exposure, and the limited statistical analyses of this study.]

Bone

In human adults, more than 90% of the total body burden of lead is found in the bone, whereas bone lead accounts for ~70% of the body burden in children (Barry, 1975). Lead is not distributed uniformly in bone (Somervaille *et al*., 1986; Wittmers *et al*., 1988; Aufderheide & Wittmers, 1992; Hoppin *et al*., 2000; Todd *et al*., 2000b, 2001c,d, 2002).

Estimates of the half-life of lead in trabecular bone are partly dependent on the tissue analysed and the 'purity' of the trabecular component [patella, calcaneus, finger bone (phalanx)]; current estimates range from about 12–16 years although earlier estimates ranged from 2–7 years (Christoffersson *et al.*, 1986; Schütz *et al.*, 1987; Gerhardsson *et al*., 1993; Bergdahl *et al*., 1998b). Earlier estimates for the half-life of lead in cortical bone were of the order of 13–27 years (Rabinowitz, 1991; Gerhardsson *et al*., 1993; Bergdahl *et al*., 1998b).

Studies over the past two decades using X-ray fluorescence methods have shown that trabecular bone — which has a faster turnover rate — (measured at the calcaneus or patella) has higher concentrations of lead/mg bone mineral than cortical bone (measured at the tibia) in the same subjects. The ratio of the concentration of lead in trabecular *vs* cortical bone generally ranges from 1.1 to 2.0; it appears to be independent of duration of exposure, occupation, age, sex, life-stage, pregnancy status, trabecular bone site, or blood lead concentration (Hu *et al*., 1996b,c; Bergdahl *et al*., 1998b; Fleming *et al*., 1998; Hernandez-Avila *et al*., 1998; Tsaih *et al*., 1999; Brown *et al*., 2000; Hu *et al*., 2001; Elmarsafawy *et al*., 2002; Korrick *et al*., 2002; Rothenberg *et al*., 2002; Hernandez-Avila *et al*., 2003; Garrido Latorre *et al*., 2003). A ratio of 3.6 in trabecular/cortical bone lead was measured in active workers exposed to lead in Finland (Erkkilä *et al*., 1992). The higher ratio is consistent with the more rapid turnover of trabecular bone, which would be expected to be responsive to current exposure. [There may be a possible bias in the studies reported here since the majority of the subjects were from the Normative Aging Study].

Maternal patella bone lead concentrations have been shown to be superior to tibia bone lead concentrations in predicting lower infant birth weight (Gonzalez-Cossio *et al*., 1997) and reduced growth rate from birth to 1 month of age (Sanín *et al*., 2001).

In two of three adult males studied after cessation of occupational exposure to lead, lead concentrations in the patella (representative of trabecular bone) decreased more rapidly than those in the tibia (representative of cortical bone), consistent with the estimates of a shorter lead half-life in trabecular bone (Hu *et al*., 1991).

Fleming *et al* (1997) and, in a follow-up study, Brito *et al* (2001) observed non-linear relationships between cumulative blood lead index (CBLI) and bone lead concentrations

in groups of 367 and 519 active lead-smelter workers. By subdividing their study groups by date of hire, the authors showed that the apparent half-life of bone lead increased with length of employment (Brito *et al.*, 2001). They suggested that the increase was attributable to the age-dependence of bone turnover; i.e. that turnover was lower in the older men with longer employment histories. However, since blood lead concentrations in both groups of smelter workers exceeded 60 µg/dL during their earlier employment years (before the mid-1970s) and declined thereafter, the curvilinearity in the bone lead concentration/CBLI relationship could also be explained simply as a reflection of that of the plasma lead/whole blood lead relationship. This curvilinearity would have led to a disproportionate loading of bone with lead relative to blood lead concentrations (but not relative to plasma lead concentrations) during the early employment years when blood lead concentrations were high (Fleming *et al.*, 1997).

Cortical bone lead concentrations gradually increase with age whereas concentrations in trabecular bone (rib, vertebrae) level-off in the fifth decade of life and then may decrease (Gross *et al*., 1975; Drasch *et al*., 1987; Wittmers *et al*., 1988; Kosnett *et al*., 1994; Hu *et al*., 1996c).

Analyses of bone and teeth can provide an integrated biomarker of previous lead exposure and can be used in a variety of investigations. For example, K-X ray fluorescence (K-XRF) analysis of bone has shown strong associations between bone lead and hypertension, cognitive functioning (e.g. Korrick *et al*., 1999; Cheng *et al*., 2001; Gerr *et al*., 2002; Rothenberg *et al*., 2002) and delinquency (Needleman *et al*., 2002).

(iii) *Metabolism*

Ionic lead in the body is not known to be metabolized or biotransformed. It does form complexes with a variety of proteins and non-protein ligands (US EPA, 1994; ATSDR, 1999).

(iv) *Excretion*

Lead in the faeces includes both lead that has not been absorbed in the gastrointestinal tract and lead excreted in the bile (endogenous faecal excretion). When lead exposure is by ingestion, more than 90% of excreted lead is found in the faeces (Kehoe, 1987; Smith *et al*., 1994). Biliary clearance is also a major route of excretion of absorbed lead. Excretion of lead does not appear to depend on exposure pathway (ATSDR, 1999), but the ratio of urinary to faecal excretion is variable. Values of from 1:1 to 3:1 have been reported for the ratio of urinary lead clearance to endogenous faecal lead clearance in adult humans after injection, inhalation or ingestion of ^{203}Pb-lead (Chamberlain *et al*., 1978; Campbell *et al*., 1984).

Excretion of lead through sweat is a minor process. Concentrations of lead in sweat vary depending on exposure and can be significantly elevated in workers in the lead industry (Stauber & Florence, 1988; Omokhodion & Howard, 1991; Omokhodion & Crockford, 1991a) compared with unexposed subjects (Omokhodion & Howard, 1991; Omokhodion & Crockford, 1991b). In healthy subjects who volunteered to ingest small

amounts of lead, the lead concentrations in sweat were less than 10 µg/L and were about 20% of the concentrations found in urine and 6% of those in blood (Rabinowitz *et al*., 1976; Omokhodion & Crockford, 1991b). [The Working Group noted the possibility of contamination of samples during collection and/or the lack of baseline lead concentrations reported in some of these studies.]

(v) *Mobilization of lead*

Although earlier investigators (Brown & Tompsett, 1945; Ahlgren *et al.*, 1976) had suggested that the skeleton was a potential endogenous source of lead poisoning, the opposing concept of the skeleton as a 'safe' repository for lead persisted until the mid-1980s and early 1990s. Potential mobilization of lead from the skeleton can occur at times of physiological stress associated with enhanced bone remodelling, such as during pregnancy and lactation (Manton, 1985; Silbergeld, 1991; Hertz-Picciotto *et al*., 2000), menopause (Silbergeld *et al*., 1988; Silbergeld, 1991), extended bed rest (Markowitz & Weinberger, 1990), hyperparathyroidism (Kessler *et al*., 1999) and weightlessness. The lead deposited in the bone of adults can serve to maintain blood lead concentrations long after exposure has ended (O'Flaherty *et al*., 1982; Manton, 1985; Kehoe, 1987; Schütz *et al*., 1987; Nilsson *et al*., 1991; Gulson *et al*., 1995; Inskip *et al*., 1996; Smith *et al*., 1996; Fleming *et al*., 1997).

Pregnancy and lactation

During pregnancy, the mobilization of bone lead increases. The increase in blood lead concentrations during the third trimester has been attributed to increased bone resorption to meet the calcium requirements of the developing fetal skeleton (Manton, 1985; Rothenberg *et al*., 1994; West *et al*., 1994; Lagerkvist *et al*., 1996b; Schuhmacher *et al*., 1996b; Gulson *et al*., 1997b; Hertz-Picciotto *et al*., 2000).

Manton (1985) monitored blood lead of one woman by use of high-precision measurement of stable lead isotopes and attributed the almost doubling of blood lead concentrations during pregnancy to skeletal sources. In subjects who had been exposed in their earlier life to lead from sources different from their current environment, Gulson *et al.* (1995) and Smith *et al.* (1996) — using the same methods — estimated that 40–70% of lead in blood is derived from the skeleton. In Australia, Gulson *et al*. (1997b) monitored two immigrant cohorts longitudinally during and after pregnancy over a 10-year period using the same study design and monitoring protocols. The first cohort (Gulson *et al*., 1997b, 1998a), comprising 16 pregnant immigrants, six long-term Australian women and six non-pregnant immigrant controls, showed that concentrations of blood lead increased during pregnancy by an average of about 20% compared with the non-pregnant immigrant controls. The increases were attributed to release of lead from the skeleton associated with increased bone remodelling, and were possibly related to the low calcium intake of most of the subjects.

Berglund *et al.* (2000) determined lead in blood and urine in relation to bone turnover in pregnant and lactating women in Stockholm, Sweden. In contrast to many of the studies

cited above, no increase in blood lead during pregnancy was detected. The authors suggested that this could be attributed to normal physiological haemodilution (Hytten, 1985), a diet relatively high in calcium and low in lead, transfer of lead to the fetus and a possibly relatively low body burden of lead in younger women in Sweden. However, significant increases in concentrations of blood during pregnancy have been observed in women whose blood lead concentrations were also low (Gulson *et al*., 1997b, 1998a; Rothenberg *et al.*, 2000). In addition to increases in blood lead during later stages of pregnancy, blood lead concentrations have been observed to decrease in the early stages of pregnancy. The mechanisms for these changes are not understood, although increased mobilization of bone lead during pregnancy may contribute partly to the increase (Lagerkvist *et al*., 1996b; Schuhmacher *et al*., 1996b; Gulson *et al*., 1997b, 1998a). Increased blood volume and haemodilution may contribute to the decrease observed in the first half of pregnancy, whereas increased absorption of lead during pregnancy or decreased elimination may also occur (Rothenberg *et al.*, 1994; Franklin *et al*., 1997; Gulson *et al*., 1997b).

Transplacental transfer/breast milk

Transplacental transfer of lead in humans has been demonstrated in a number of studies indicating that the ratio of cord/maternal blood lead concentration at delivery ranges from about 0.6 to 1.0 (Barltrop, 1969; Rabinowitz *et al*., 1984; McMichael *et al*., 1986; Goyer, 1990a; Graziano *et al*., 1990; Al-Saleh *et al*., 1995; Schuhmacher *et al*., 1996b; Gulson *et al.*, 1997b). Diffusion has been proposed as the primary mechanism for transplacental lead transport (Goyer, 1990a).

Evidence for maternal-to-fetal transfer of lead in humans can be gained from stable lead isotope measurements. For example, a 0.99 correlation in lead isotopic ratios for maternal and cord blood (Manton, 1985; Gulson *et al*., 1997b) and similarity of isotopic ratios in maternal blood and in blood and urine of newborn infants provide strong evidence of placental transfer (Gulson *et al*., 1999; Gulson *et al.*, 2004). The presence of lead in neonatal liver provides further direct evidence that it crosses the human placental barrier (Lyon *et al*., 2002).

Breast milk can also be a vehicle for maternal excretion of lead. However, given the very low lead concentrations and the analytical difficulties arising from the high fat content of breast milk, lead analyses require careful attention (Gulson *et al*., 1998a). For breast milk collected serially, the mean lead concentration was found to be 0.73 ± 0.70 μg/L for mothers whose blood lead concentration was less than 5 μg/dL. For the first 60–90 days postpartum, the contribution from breast milk to blood lead in the infants varied from 36–80%. Gulson *et al.* (1998a) evaluated studies published over the last 15 years of lead concentrations in breast milk and suggested that studies in which the ratio of lead concentration in breast milk to lead concentration in maternal whole blood were greater than 0.15 should be viewed with caution because of potential contamination during sampling and/or laboratory analyses. Several studies appear to show a linear relationship between lead in breast milk and maternal whole blood lead. The percentage of lead in breast milk was comparable with that in whole blood in subjects with blood lead concentrations ranging from

2–34 µg/dL. Gulson *et al.* (1998a) suggested that breastfed infants are only at risk if the mother is exposed to high concentrations of lead contaminants either from endogenous sources such as the skeleton or exogenous sources.

Reduction of lead mobilization during pregnancy and lactation

Studies that focused on the reduction of lead mobilization during pregnancy and lactation in humans have usually employed calcium supplementation. Increased intake of calcium has been suggested as a measure to prevent mobilization of extra lead during pregnancy and lactation (Farias *et al*., 1996; Hernandez-Avila *et al*., 1996; Gulson *et al*., 1998d; Hertz-Picciotto *et al*., 2000; Gulson *et al*., 2003). Calcium supplementation at the recommended level of approximately 1000 mg/day (NIH, 1994) was found to almost halve the extra lead released during pregnancy but offered no benefit during lactation (Gulson *et al*., 2004). In contrast, calcium carbonate supplementation of 1200 mg/day elemental calcium during lactation gave a modest reduction of 16% in blood lead concentrations amongst women with relatively high bone lead burdens (Hernandez-Avila *et al*., 2003). In an earlier report that apparently used the same cohort but with smaller numbers, there did not seem to be any benefit from calcium supplementation during lactation (Téllez-Rojo *et al*., 2002). Using concentrations of cross-linked N-telopeptides of type I collagen (NTX), a sensitive biomarker of bone resorption, Janakiraman *et al.* (2003) observed that a 1200-mg calcium supplement taken at bedtime during the third trimester of pregnancy reduced maternal bone resorption by an average of 14%.

Menopause

Increases in blood lead in postmenopausal women have been attributed to release of lead from the skeleton associated with increased bone resorption during menopause (Silbergeld *et al*., 1988; Symanski & Hertz-Picciotto, 1995; Muldoon *et al*., 1994; Weyermann & Brenner, 1998; Hernandez-Avila *et al*., 2000). Most of these studies were based on blood lead concentrations. More recent investigations employing bone X-ray fluorescence measurements as well as blood lead concentrations have supported an endogenous contribution of bone lead to blood (Webber *et al.*, 1995; Korrick *et al*., 2002; Garrido Latorre *et al*., 2003). Postmenopausal women using hormone replacement therapy may have lower blood lead concentrations and higher bone lead values than nonusers (Webber *et al*., 1995; Garrido Latorre *et al*., 2003). In contrast, in a cross-sectional study of 264 women (46–74 years old) in Boston, USA, both tibia and patella lead values were significantly and positively associated with blood lead but only among postmenopausal women not using estrogen (Korrick *et al*., 2002). In a pilot study of immigrant women in Australia, Gulson *et al.* (2002) found a decrease in blood lead concentrations and changing lead isotopic composition in women treated for 6 months with a powerful anti-bone resorptive bisphosphonate drug. Upon cessation of treatment, the blood lead concentrations increased and the isotopic composition changed.

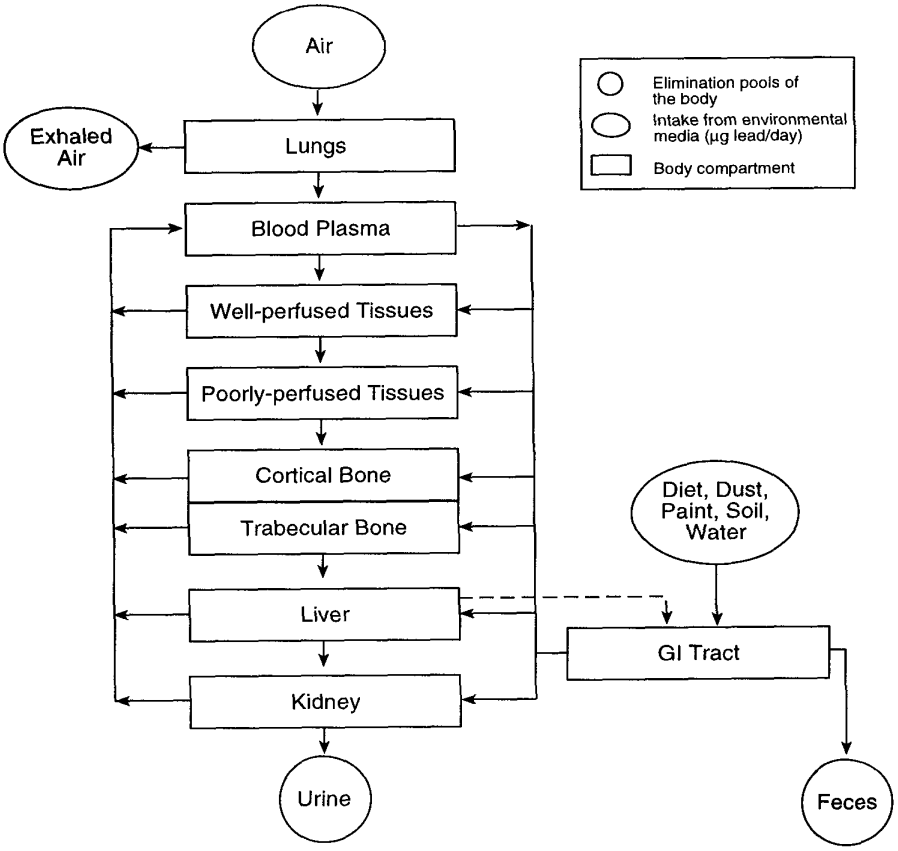
Preferential partitioning of bone lead into plasma

Several authors have proposed that lead released from the skeleton is preferentially partitioned into serum rather than erythrocytes; one explanation being that the lead from endogenous sources was in a different form to that from exogenous sources (Cake *et al*., 1996; Hernandez-Avila *et al*., 1998; Tsaih *et al*., 1999). In a study employing similar methods, Bergdahl and Skerfving (1997) disputed the findings of Cake *et al.* (1996). Chettle *et al.* (1997) also challenged the hypothesis of Cake *et al.* (1996) that the ratio of serum lead to whole blood lead increases with increasing amounts of lead released from bone. Using urine as a proxy for serum, Gulson *et al.* (2000) compared lead isotopic ratios and lead concentrations in 51 matched blood and spot urine samples from 13 subjects, covering the interval from before pregnancy through 180 days postpartum. There was no evidence for preferential partitioning of lead into serum compared with whole blood.

Pharmacokinetic models

Several pharmacokinetic models for lead have been proposed to explain and predict physiological processes, including intercompartmental lead exchange rates, retention of lead in various pools, and relative rates of distribution among the tissue groups. Comprehensive discussions of these models have been published by ATSDR (1999). One of the earliest was a three-compartment model based on stable lead isotope tracer experiments and balance data from five healthy men (Rabinowitz *et al*., 1976). A physiologically-based pharmacokinetic (PBPK) model developed initially for rats by O'Flaherty (1991a,b,c, 1993, 1995) uses physiologically-based parameters to describe the volume, composition and metabolic activity of blood and tissues that determine the disposition of lead in the human body. The compartments and pathways in the O'Flaherty model are shown in Figure 3 (ATSDR, 1999). Two other models in current use are compartmental pharmacokinetic models; the integrated exposure uptake biokinetic (IEUBK) model for lead in children (Figure 4) (US EPA, 1994) and the Leggett model (Leggett, 1993), which simulate the same general processes as those in the PBPK model, although transfer rate constants and kinetic coefficients may not have precise physiological correlates. All three models have been calibrated, to varying degrees, against empirical physiological data from animals and humans, and blood lead concentrations observed in exposed populations of children and adults. Pharmacokinetic models have been used to estimate the probability distribution of blood lead concentrations in children potentially exposed to lead via multiple exposure pathways at hazardous waste sites. The O'Flaherty and Leggett models have accurately reproduced adult blood lead concentrations, and may be modified to reflect changes in lead associated with pregnancy, ageing, or disease states (Pounds & Leggett, 1998; O'Flaherty, 2000).

Figure 3. Compartments and pathways of lead exchange in the O'Flaherty model



From ATSDR (1999), derived from O'Flaherty, 1991b, 1993, 1995

(*b*) *Animals*

(i) *Absorption*

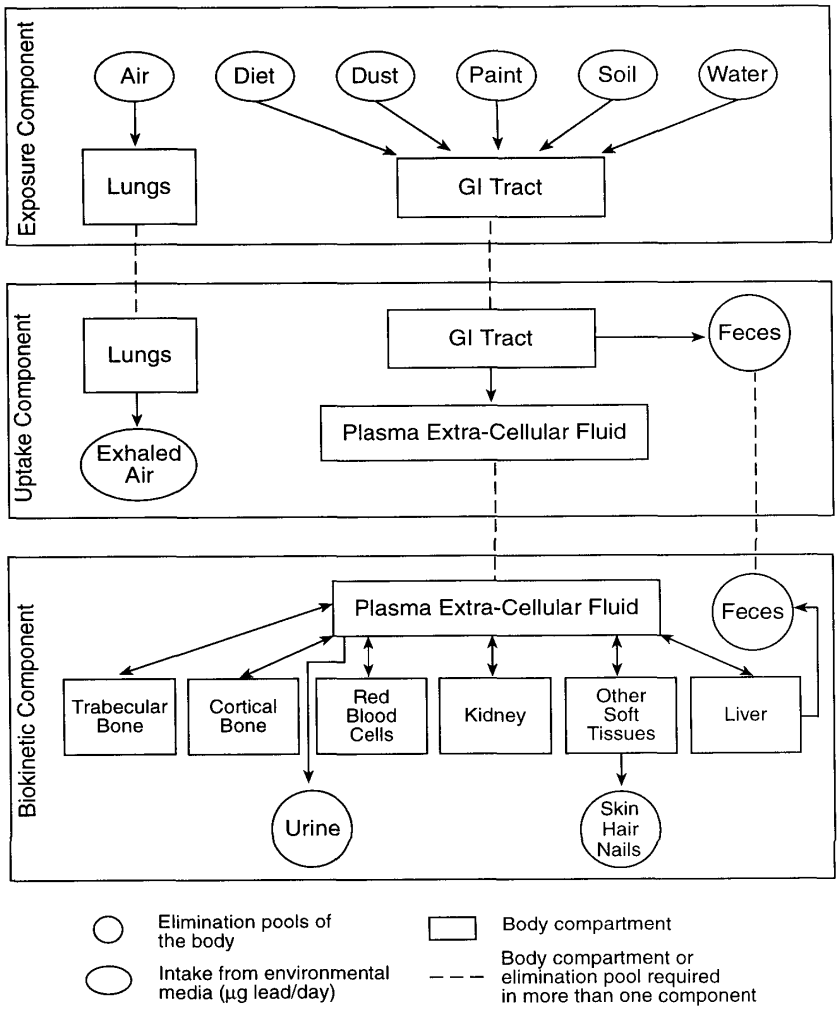
Ingestion

Absorption of lead from the gastrointestinal tract in experimental animals is age-dependent and is influenced by the amount of food intake.

Prior to weaning, rodents absorbed from 50% to more than 80% of a single oral dose of radiolabelled lead, while older rodents absorbed < 1–15% (Forbes & Reina, 1972; Garber & Wei, 1974; Kostial *et al*., 1978; Flanagan *et al*., 1979).

In rats receiving a carrier-free oral dose of 0.02 μCi ^{212}Pb, absorption of lead from the gut declined steadily from 74–89% in animals 16–22 days of age to 15–42% in animals 24–32 days old and to only 16% in 89-day-old animals (Forbes & Reina, 1972). [It was

Figure 4. Structure of the IEUBK model for children



From ATSDR (1999)

unclear whether the study was conducted with fasted animals.] A single oral dose of ^{203}Pb-lead chloride resulted in 52% absorption in 1-week-old suckling rats compared with 0.4% in 6-week-old adults on a standard diet (Kostial *et al.*, 1978). Lead absorption from an intragastric dose of 2 µCi ^{210}Pb-lead acetate was 5.4% and 9.7% in adult C56Bl/6 Jax mice that were given an iron-supplemented diet or an iron-deficient diet, respectively (Flanagan *et al.*, 1979).

The presence of food in the intestine was shown to reduce by more than 80% the absorption of a 10-mg/kg oral dose of lead acetate in Sprague-Dawley rats (Aungst & Fung, 1981). In Swiss-Webster mice, food reduced lead absorption from 14% to 7.5%, when a single tracer dose of ^{210}Pb-lead acetate (3 µg/kg bw) was administered. However, the absorption rate (4–5%) was similar in fasted and non-fasted mice receiving a higher dose of lead (2 mg/kg bw) (Garber & Wei, 1974).

In non-human primates, the absorption of lead ranged from 38–65% in young animals and from ~3–40% in mature animals (Willes *et al*., 1977; Pounds *et al*., 1978; O'Flaherty *et al*., 1996; Cremin *et al*., 2001).

Fasted young monkeys (*Macaca fascicularis*; 10 days of age) absorbed 64.5% of an oral dose of 10 µg/kg bw ^{210}Pb-lead nitrate, while only 3.2% was absorbed by fasted mature adults (Willes *et al*., 1977). Similarly, the gastrointestinal absorption of an oral dose of 72.6 µg ^{206}Pb-lead acetate (352 nmol) in 12 mL apple juice was ~65% in fasted infant rhesus monkeys (Cremin *et al*., 2001). Fasted adult cynomolgus monkeys absorbed 22–44% of a single dose of lead given as ^{210}Pb-lead nitrate, depending on the dose (O'Flaherty *et al*., 1996). In fed juvenile rhesus monkeys (5–7 months old), lead absorption was 38% versus 26.4% in fed adults following a single gavage dose of 10 mg/kg bw ^{210}Pb-lead acetate (Pounds *et al*., 1978).

Experiments in mice and rats provide evidence that lead absorption is increased in the later stages of pregnancy and during lactation (Donald *et al*., 1986; Maldonado-Vega *et al*., 1996). The gastrointestinal absorption of ^{203}Pb in lactating rats was found to be about 2–3.5 times that in controls (Kostial & Momcilovic, 1972; Momcilovic, 1979).

There is experimental evidence that gastrointestinal absorption of lead is a saturable process. In mice, single administrations of 0.2, 2 or 20 mg/kg ^{210}Pb-lead acetate resulted in similar absorption rates (Garber & Wei, 1974). Duodenal perfusate experiments in mice have shown that lead uptake from the lumen increased in proportion to lead concentration in the perfusate but that the transfer of lead across isolated mouse duodenum to the carcass was saturable (Flanagan *et al*., 1979).

The extent of absorption decreased from 42% in fasted adult Sprague-Dawley rats administered a single oral dose of 1 mg/kg bw lead (as lead acetate) to 2% when the dose was increased to 100 mg/kg bw. Furthermore, the percentage of the dose recovered from the tissues (brain, liver, kidneys, blood) decreased from 6.9% after 1 mg/kg bw lead to 0.6% after 100 mg/kg bw in adults and from 11.0% to 0.6% in pups (Aungst *et al*., 1981). A 100-fold increase in a single oral dose from 10 µg to 1 mg ^{203}Pb-lead chloride was accompanied by an increase of only 20-fold in the quantity of lead absorbed in fasted Wistar rats (Conrad & Barton, 1978). Studies by Polák *et al.* (1996) demonstrated a dose-dependent biovailability in rats of both soluble lead and lead in mine waste or in mine waste-contaminated soils. Fractional absorption decreased as lead intake increased, regardless of the source of the lead, but the magnitude of this dose dependence was lead source-dependent. Fractional absorption varied from 4–5% at low exposure rates (1–2 mg/kg bw lead per day) when lead acetate was added to the diet, to 0.24% at a high

exposure rate (24 mg/kg bw lead per day) when a test soil of mine waste contaminated with lead was added to the diet (Polák *et al*., 1996).

The absorption of an intraluminal dose of ^{203}Pb-lead acetate in chicks was impaired by the presence of lead (as lead chloride) in the diet, in a dose-dependent fashion from 43% at a dietary concentration of 0.1% lead to 16 % at a dietary concentration of 0.8% (Fullmer, 1991).

Fractional absorption of ^{210}Pb-lead nitrate decreased from 44% of a single oral dose of 750 µg/kg bw to 22–28% of a dose of 1500 µg/kg bw in fasted adult cynomolgus monkeys (O'Flaherty *et al*., 1996).

The bioavailability of lead is dependent on its chemical form and its particle size as well as the matrix and the source of environmental lead. Absorption of lead from the gastrointestinal tract of Wistar rats (30-day-old) varied greatly with chemical form; lead carbonate administered in the diet showed a 12-fold greater absorption coefficient than metallic lead. Relative to the absorption of lead acetate taken as 100%, the absorption coefficients of lead salts were: 44% for lead chromate, 62% for lead octoate, 64% for lead naphthenate, 67% for lead sulfide, 121% for lead tallate and 164% for lead carbonate (basic) (Barltrop & Meek, 1975). In studies performed by Dieter *et al.* (1993), rats were fed ≤ 38-µm size particles of lead sulfide, lead oxide, lead acetate and a lead ore concentrate from Skagway, Alaska, USA, mixed into the diet at doses of 0, 10, 30 and 100 ppm for 30 days. Bioavailability was found to be highest for lead acetate, intermediate for lead oxide and lowest for lead sulfide and Alaskan mixed-ore concentrate. Lead concentrations in bone and kidney were about 20- and 10-fold greater, respectively, in rats fed the more soluble compared with the less soluble lead compounds (Dieter *et al*., 1993). Experimental studies on fed young rats showed that the mean relative bioavailability (compared with lead acetate) of lead in the Butte mining waste soil was 20%, 9% and 8% based on measurements of lead in blood, bone and liver, respectively (Freeman *et al*., 1992). In further studies by these authors, the absolute bioavailability of ingested lead acetate in feed was estimated to be 15% based on measurements of blood lead concentrations after oral administration. The addition of control soil to the diet with lead acetate resulted in a significant decrease in lead bioavailability. The absolute bioavailability to rats of mining waste lead in soil administered in feed was approximately 3% based on blood lead concentration and less than 1% based on bone and liver lead concentrations (Freeman *et al*., 1994). The bioavailability of lead sulfide was found to be approximately 10% that of lead acetate (Freeman *et al*., 1996).

In immature swine, the relative bioavailability (compared with lead acetate) of lead from soil samples from the Smuggler Mountain Superfund Site in Aspen (CO, USA) was shown to range from 57% based on blood lead area-under-the-curve (AUC) to about 80% based on liver lead concentration. The absolute bioavailability was estimated to be from 28% (via blood AUC) to about 40% (via liver uptake) (Casteel *et al*., 1997).

An inverse relationship was found between the particle size of metallic lead (6–250 µm) administered in the diet and absorption in rats. This relation was more marked in the 6–100-µm range; a fivefold enhancement of absorption was observed when

rats were fed with lead particles of mean size 6 μm compared with 197-μm particles. A marked enhancement of absorption (1.5–1.8-fold) was also found on feeding lead chromate and lead octoate when particle size was reduced from 500–1000 μm to less than 50 μm (Barltrop & Meek, 1979).

The bioavailability of lead is influenced by dietary habits. Regular rat chow attenuates the absorption of lead by the strong binding or precipitative action of the chow diet (Freeman *et al.*, 1996). Lead absorption of a single oral dose of ^{203}Pb-lead chloride in adult rats fed several 'human' diets ranged from about 3% to more than 20% above that in controls receiving regular rat chow food. Highest absorption values were observed in animals fed fruit and cow's milk (Kello & Kostial, 1973; Kostial *et al.*, 1978; Kostial & Kello, 1979).

Palminger Hallén and Oskarsson (1995) studied the effects of milk on lead absorption in rat pups. At 2 h after gastric intubation of various liquid diets labelled with ^{203}Pb, the lead bioavailability was 47% from water, 42% from human milk, 40% from infant formula, 31% from cows' milk and 11% from rat milk. After 6 h, the bioavailability of lead was about 50% from water and human milk, 45% from infant formula and cow's milk and 36% from rat milk. Rat pups given lead in human milk had lead concentrations in blood and brain approximately twice as high as those of pups given lead in rat milk.

Other investigators have not found any effect of milk on lead absorption in suckling or adult rodents (Garber & Wei, 1974; Meredith *et al*., 1977; Henning & Leeper, 1984). Kinetic analysis of pups' blood lead concentration revealed a rate-limited absorption in suckling mice exposed to milk from mothers administered lead, with a slower absorption of lead in the offspring compared with dams. The conflicting evidence on whether milk influences absorption of lead in infant rodents might be resolved, at least in part, by measurements of lead absorption at different time periods after its administration to the animals (Palminger Hallén *et al*., 1996a).

Nutritional status has been shown to influence lead absorption and/or retention in experimental animals. Vitamin D, calcium and phosphorus have complex and interrelated effects on lead absorption (Fullmer, 1990, 1991, 1997). Diets deficient in calcium and/or phosphate are associated with increased intestinal absorption and/or retention of lead in experimental animals (mice, rats, chicks, monkeys) (Six & Goyer, 1970; Quarterman & Morrison, 1975; Jacobson & Snowdon, 1976; Barton & Conrad, 1981; Mykkänen *et al*., 1984; Aungst & Fung, 1985; Van Barneveld & Van den Hamer, 1985). Simultaneous reduction of both dietary calcium and phosphate content produced an additive effect on absorption of lead (Quarterman & Morrison, 1975; Barltrop & Khoo, 1976). However, experimental studies in chicks have shown that variations in the extent and duration of lead ingestion and calcium deficiency may result in increases or decreases in lead absorption (Fullmer, 1991). In chicks fed standard diet but administered a single injection into the lumen of the intestine of ^{203}Pb-lead acetate, lead absorption increased from 18.8% in animals with adequate calcium content in the diet to 54.5% in animals fed a severely calcium-deficient diet. In calcium-deficient chicks on a diet containing lead, a biphasic response was observed; intestinal absorption of lead was enhanced by calcium deficiency

initially, in a manner similar to the groups not fed lead, but this response was inhibited by prolonged dietary lead intake (Fullmer, 1991).

Calcium supplemention has been shown to reduce lead absorption in several animal species when administered at the same time as lead (Barltrop & Khoo, 1976; Meredith *et al*., 1977; Barton *et al*., 1978a; Varnai *et al*., 2001) but not when administered separately (Quarterman *et al.*, 1978; Aungst & Fung, 1985; Van Barneveld & Van den Hamer, 1985). Calcium supplementation caused a statistically significant dose-related decrease in lead in tissues (liver, kidneys, brain and carcass) of suckling rats exposed to lead orally but had no effect on lead incorporated in tissues after parenteral exposure to lead, suggesting that calcium primarily reduced lead absorption from the gastrointestinal tract (Varnai *et al*., 2001).

Administration of cholecalciferol (vitamin D3) or 1,25-dihydroxycholecalciferol ($1,25\text{-}(OH)_2D$), the active metabolite of vitamin D3, was found to increase gastrointestinal absorption of lead in rats and chicks (Smith *et al*., 1978; Hart & Smith, 1981; Mykkänen & Wasserman, 1982; Edelstein *et al*., 1984; Fullmer, 1990). Dietary vitamin D deficiency or depletion resulted in increased intestinal absorption of lead in intact animals, but the manipulation of dietary phosphate and vitamin D3 content had no significant effect upon the absorption of lead from isolated gastrointestinal segments of rats. Hence, this increased absorption was attributed to a decrease of gastrointestinal motility with a prolonged transit time (Barton *et al*., 1980; Barton & Conrad, 1981). However, the administration of cholecalciferol to rachitic chicks resulted in an increase in the transepithelial transport of ^{203}Pb in the intestine (Mykkänen & Wasserman, 1982). The vitamin D-induced intestinal calcium-binding proteins bind lead with higher affinity than calcium suggesting a co-transport mechanism whereby lead absorption would be increased by calcium deficiency (Fullmer *et al*., 1985; Fullmer, 1997). However, the effect of $1,25\text{-}(OH)_2D$ appeared to be dependent upon the duration of exposure to lead and the magnitude of lead stores in the body. The efficiency of intestinal ^{203}Pb absorption was significantly diminished by dietary lead in an apparently dose-dependent fashion (Fullmer, 1990).

Iron status also influences the absorption and/or retention of dietary lead in rodents (Six & Goyer, 1972; Ragan, 1977; Barton *et al*., 1978b; Conrad & Barton, 1978; Robertson & Worwood, 1978; Flanagan *et al*., 1979; Morrison & Quarterman, 1987; Crowe & Morgan, 1996). Lead absorption was found to be promoted by iron deficiency and inhibited by iron loading (Barton *et al*., 1978b). Rats fed iron-deficient diets had increased concentrations of lead in kidney and bone (femur) when compared with rats ingesting equivalent quantities of lead (as lead acetate) in drinking-water while being fed an iron-adequate diet (Six & Goyer, 1972). The degree of iron deficiency does not need to be severe to increase lead retention. A sixfold increase in tissue lead was demonstrated in rats when body iron stores were reduced, but before frank iron deficiency developed (Ragan, 1977). ^{203}Pb absorption in fasted rats was found to be increased by a short period of severe iron restriction before any change in haematological parameters became apparent. An extended period of moderate iron restriction, causing a reduction in haemoglobin concentration, resulted in increased iron and lead absorption. When iron dietary concentrations

were made adequate to meet essential requirements produced by blood loss or hypoxia, lead absorption was similar to that in controls (Morrison & Quarterman, 1987). The ion lead Pb^{++} is a substrate for the divalent-cation metal transporter 1 (DMT1). This transporter is expressed most significantly in the proximal duodenum in the rat and is upregulated by dietary iron deficiency (Gunshin *et al*., 1997). In a yeast model, it was demonstrated that DMT1 transports lead and iron with similar affinity and that iron inhibits the transport of lead (Bannon *et al*., 2002).

Other dietary factors reported to influence absorption of lead in experimental animals are lipids (Barltrop & Meek, 1975; Barltrop & Khoo, 1976; Quarterman *et al*., 1977; Ku *et al*., 1978), amino acids and proteins (Conrad & Barton, 1978; Quarterman *et al*., 1980), citrate and ascorbic acid (Garber & Wei, 1974; Conrad & Barton, 1978; Spickett *et al*., 1984) and lactose (Bushnell & DeLuca, 1983).

Blood lead concentrations measured in rats after controlled oral exposure to lead as lead acetate are given in Table 85.

Table 85. Blood lead concentrations in rats during chronic oral exposure to lead acetate

Strain of rat	Age at start of exposure	Duration of exposure (days)	Lead acetate added to diet (ppm as lead)	Blood lead concentration (μg/dL ± SD)	Reference
Fischer 344/N	6–7 weeks	30	0	–	Dieter *et al*. (1993)
			10	16 ± 1.7	
			30	31.8 ± 3.8	
			100	84.8 ± 8.9	
Fischer 344	4 weeks	7	0	5.0 ± 0.9	Freeman *et al*. (1996)
			17.6	32.0 ± 6.9	
			42.8	37.6 ± 5.4	
			127	77.1 ± 11.2	
		15	0	2.7 ± 0.3	
			17.6	20.5 ± 1.6	
			42.8	42.4 ± 8.4	
			127	67.6 ± 8.1	
		44	0	1.2 ± 0.1	
			17.6	24.9 ± 1.9	
			42.8	40.5 ± 2.8	
			127	70.6 ± 3.4	
Sprague-Dawley	24 days	31	*In drinking-water*	*Read from graph*	O'Flaherty (1991c)
		60	1000	~100	
			1000	~100	
	22 days	418	*In drinking-water*	*Read from graph*	
			1000	~120	
			2000	~135	

Intratracheal instillation, inhalation

The bioavailability of lead from inhaled particles depends on the particle size and is affected by the particle matrix.

A study by Eaton *et al.* (1984) showed that five weeks after exposure by intratracheal instillation, the vast majority of a lead chromate paint particulate suspension (median particle diameter, 6 μm) remained in the lung of rats (ratio lung lead:bone lead, 540). In contrast, after exposure to lead acetate, little remained in the lung, but significant elevations were found in bone (ratio lung lead:bone lead, 0.5) and kidney. Intratracheal instillation of lead tetraoxide showed an intermediate absorption rate (ratio lung lead:bone lead, 73).

Grobler *et al.* (1988) showed that in rats exposed to aerosols of lead chloride (0.05, 77, 249 and 1546 μg lead/m³) (mass median diameter (MMD), < 5.8 μm; 56% of the total particles at 77 μg/m³, 44% at 249 μg/m³, 37% at 1546 μg/m³) for 28, 50 and 77 days, blood lead concentrations reached relatively stable plateaux, which differed significantly with lead exposure. Stability was reached after 10 days at the highest exposure concentration and after 30 days when rats were exposed to 249 μg/m³. When exposure ceased, blood lead concentrations declined with a half-life of 3–5 days.

In baboons, the rate of absorption of lead into the bloodstream after exposure to coarse (mean particle size, 1.6 μm; MMD, 5.9 μm) airborne particles of lead oxide was faster and the concentration reached was higher than that after exposure to fine (mean particle size, 0.8 μ; MMD, 2 μm) particles (Rendall *et al*., 1975). This finding contrasts with the greater deposition and absorption rates of fine particles (< 1 μm) reported in humans exposed by inhalation (ATSDR, 1999; see also Section 4.1.1(*a*)(i)).

Blood lead concentrations measured in the studies by Rendall *et al*. (1975) and Grobler *et al*. (1988) are given in Table 86.

Skin absorption

After application of a solution of lead acetate or nitrate (6.4 mg of lead) to the skin of female BALB/c mice, an analysis of the organs, faeces and urine showed that 0.4% of the applied dose was absorbed through the skin and entered the circulatory system. In less than 24 h significant increases in lead concentrations were observed in the skin, muscle, pancreas, spleen, kidney, liver, caecum, bone, heart and brain but not in the blood (Florence *et al.*, 1998).

Sun *et al.* (2002) measured the urinary lead content of rats after application of a patch of linen cloth containing lead compounds (100 mg lead in petrolatum on a 2 × 6-cm cloth), under occlusive conditions, for 12 days. Total amounts of lead in urine increased from 10.8 ng in the controls to 3679.3 ng for lead naphthenate, 146.0 ng for lead stearate, 736.6 ng for lead nitrate, 123.1 ng for lead sulfate, 115.9 ng for lead oxide and 47.8 ng for lead metal powder.

In studies by Bress and Bidanset (1991), in-vivo absorption was measured by applying 300 mg/kg bw tetrabutyl lead, lead nuolate, lead naphthenate, lead acetate or lead oxide to the shaved backs of guinea-pigs for 7 days under occluded wrappings. Tetrabutyl lead was

Table 86. Blood lead concentrations in baboons and rats during and after inhalation exposure

Species	Exposure protocol	Mean blood lead concentration (μg/dL ± SD)	Reference
Baboon (*Papio ursinus*)	Dust clouds of Pb_3O_4; target conc., 2 mg/m^3 lead; mean particle size: group A, 1.6 μm; group B, 0.8 μm; 5 days/week for 4 weeks	At the end of exposure: group A, ~50*; group B, ~18* 6 weeks after end of exposure: group A, 40 ± 19.7; group B, 20 ± 7.7	Rendall *et al*. (1975)
BD IX rats	Aerosol of lead chloride; 4 groups; 0.05 μg/m^3 lead (controls) 77 μg/m^3 lead for 77 days 249 μg/m^3 lead for 28 days 1546 μg/m^3 lead for 50 days All groups, 22 h/day, 7 days/week	During exposure (plateau conc.) 0.5–4 ~15* ~25* ~60*	Grobler *et al*. (1988)

*Read from the graph

present in blood, brain, liver and kidney in the highest quantities. Lead nuolate was found in greater amounts than lead naphthenate in the liver and kidneys. Lead acetate was poorly absorbed while lead oxide showed no absorption (see also Section 4.1.2(*b*)(i)).

(ii) *Distribution*

Experimental studies have shown that lead is rapidly distributed into soft and mineralizing tissues after acute and chronic exposures. The initial distribution of lead into soft tissues has a half-life of 3.5 days in rats (O'Flaherty, 1991c).

In rodents and non-human primates, 98–99% of the blood lead content is associated with erythrocytes, the remainder being found in the plasma (Morgan *et al.*, 1977; Willes *et al.*, 1977; Keller & Doherty, 1980a; Palminger Hallén & Oskarsson, 1993). As in humans, plasma lead is the source of lead available to distribution and excretion processes. Keller and Doherty (1980a) found that milk lead concentration in lactating mice was linearly related to plasma lead concentration but not to blood lead concentration. Similarly, Oskarsson *et al.* (1992) were able to fit the relationship between lead concentrations in whole blood and milk in cows with an exponential expression, demonstrating its nonlinearity.

A physiologically-based model in which soft-tissue distribution and excretion of lead are assumed to be proportional both to the rate of blood flow to the tissue (which is proportional to plasma flow) and to the concentration of lead in blood plasma has successfully

reproduced the time-profile of concentration changes in blood lead after cessation of chronic feeding of lead to rats (O'Flaherty, 1991c). Because of the high dose rates used in these studies (blood lead concentrations in excess of 100 μg/dL) and the disproportionality between plasma lead and blood lead, a model that assumed distribution to be proportional to whole blood lead could not have duplicated the observed blood lead concentration profiles.

After acute exposure by inhalation, lead content expressed as percentage of the dose in rats has been shown to be highest in kidneys, liver and lung, with concentrations increasing in bone as those in soft tissues declined and stabilized (Morgan & Holmes, 1978). After oral exposure, lead concentrations in rats were highest in kidneys (Aungst *et al.*, 1981). After intravenous injection of ^{203}Pb-lead chloride to rats, 20% of the dose was found initially in the kidney; subsequently, long-term deposition of 25–30% occurred in bone (Morgan *et al.*, 1977). At steady state, the pattern of distribution of lead is bone > kidney > liver > brain (Griffin *et al.*, 1975b; Morgan *et al.*, 1977; Conrad & Barton, 1978; Kostial *et al.*, 1978; Aungst *et al.*, 1981; Rader *et al.*, 1981; Mykkänen *et al.*, 1982; Cikrt *et al.*, 1983; Miller *et al.*, 1983; Cory-Slechta *et al.*, 1989; P'An & Kennedy, 1989).

When Sprague-Dawley rats (44–48 days old at the beginning of the study, mature adult at the end) were given intraperitoneal injections of lead acetate (10 or 20 mg/kg bw) at 1, 2, 4, 8, 12, 16, 20 and 24 weeks, lead accumulated steadily in the bone, kidney and brain and reached high concentrations in bone and kidney, but remained low in brain. The authors suggested that although brain lead values increased, some regulatory mechanisms limited access of lead to the adult brain (P'An & Kennedy, 1989). In a study reported by Crowe and Morgan (1996), rats were exposed to lead acetate from 3 days prior to birth (day 18 of pregnancy) until 15, 21 or 63 days postpartum, via the placenta, and then via the milk. This was achieved by giving a diet containing 0 or 3% lead acetate to pregnant Wistar rats, as well as 0.2% lead acetate in their drinking-water. After weaning, 0.2% lead acetate in the drinking-water became the sole source of dietary lead for the offspring. To study the effect of iron deficiency on lead absorption, low-iron diets were given to mothers from day 18 of pregnancy and were continued with the young offspring rats after weaning. It was found that iron deficiency did not increase lead deposition in the brain and brain lead concentrations were relatively low (< 0.1 μg/g) in all rats. Lead concentrations in the liver were below 2 μg/g, whereas kidneys had almost 20-fold higher concentrations. Compared with other tissues, the blood–brain barrier appeared to restrict lead uptake by the brain independent of the iron status of the animals; the functional blood-brain barrier is present very early in development, possibly before birth (Crowe & Morgan, 1996). In other studies in rats, following intravenous administration, lead has been shown to cross the blood–brain barrier (Bradbury & Deane, 1993).

In rats exposed almost continuously (22 h/day, 7 days/week) to lead oxide particles (MMD, 86% ≤ 0.18 μm) at an average concentration of 21.5 μg/m^3 for 12 months, the concentrations of lead in blood, kidney and liver were found to reach a maximum at 3–4 months of exposure and did not increase significantly after that time. The concentration of lead in soft tissues decreased after the exposure, but remained elevated in bone (Griffin

et al., 1975b). In a subsequent study in which rats were exposed to lead oxide particles (20 μg/m^3) for 15 months, a small increase in tissue lead was found between the sixth and the fifteenth months of exposure, but in the femur the increase during this period was nearly 70% (Russell *et al.*, 1978).

In studies conducted by Maldonado-Vega *et al.* (1996), rats were given 100 ppm [100 μg/mL] lead acetate in distilled water either before and during lactation (during 158 days), or before lactation only (144 days), or during lactation only (14 days). Results were compared with those obtained from non-pregnant lead-exposed matched rats and non-exposed pregnant and non-pregnant control rats. During lactation, lead concentrations in blood, liver and kidney increased while those in bone decreased. The increase in tissue concentrations was shown to result from increased intestinal absorption (exogenous exposure) and bone resorption (endogenous exposure). Significant deposition of lead in bone was observed in rats exposed to lead only during lactation indicating that both processes (deposition and bone resorption) take place in this period (Maldonado-Vega *et al.*, 1996, 2002).

There is experimental evidence of lead mobilization from bones to blood (Grobler *et al.*, 1991). In studies in monkeys, 17–20% of the total blood lead originated from historical bone stores (Inskip *et al.*, 1996; O'Flaherty *et al.*, 1998). Increased lead release from the skeleton occurs during pregnancy and lactation (Buchet *et al.*, 1977; Maldonado-Vega *et al.*, 1996; Franklin *et al.*, 1997; Maldonado-Vega *et al.*, 2002). Maternal-to-fetal transfer of lead appears to be related partly to the mobilization of lead from the maternal skeleton. Evidence for transfer of maternal bone lead to the fetus has been provided by studies with stable lead isotopes in cynomolgus monkeys (*Macaca fascicularis*). The study by Franklin *et al.* (1997) showed that 7–39% of the maternal lead burden that is transferred to the fetus appears to derive from the maternal skeleton (see Section 4.1.1(*a*)(v)).

The mean half-life of lead in bone was found to be 3.0 ± 1.0 years in the rhesus monkey (McNeill *et al.*, 1997). Injection of 25 μCi of an aqueous solution of ^{210}Pb and its daughters into adult rats and analysis of bone tissue over the subsequent 140 days showed a half-life of lead in bone of 64–109 days (Torvik *et al.*, 1974).

Age-related differences in the distribution of lead have been reported in experimental animals. After intraperitoneal injection of ^{203}Pb, marked differences were observed in the kinetics of lead retention and distribution in suckling as compared with adult rats. Compared with older rats, suckling rats showed 2.3-fold higher whole-body retention, higher blood concentrations and an almost 8-fold greater accumulation in the brain. Retention in the kidneys was one third lower in the suckling rats (Momcilovic & Kostial, 1974; Kostial *et al.*, 1978).

Similar findings have also been reported for kidney and bone in neonatal monkeys exposed to a single oral dose of ^{210}Pb lead nitrate. Bone lead concentrations and bone:blood lead ratios were significantly higher in infant monkeys than in adults. Brain:blood lead ratios were significantly greater in 10-day-old infants than in adult monkeys. The liver lead concentration was also higher in neonates and young monkeys than in adults (Willes *et al.*,

1977). Lead concentrations in fetal bone of monkeys have been reported to exceed maternal bone lead concentrations (Franklin *et al.*, 1997).

Ageing has also been shown to alter the pattern of distribution of lead in rats administered lead acetate in drinking-water. In studies reported by Cory-Slechta *et al.* (1989), blood lead concentrations in adult (8-month-old) and old (16-month-old) rats showed different trends over the course of exposure; values in adults declined, while those of old rats tended to increase. Brain lead concentrations and, to a marginally significant extent, liver lead concentrations were higher in old rats than in adult rats, while bone lead concentrations were significantly lower in old rats than in adult rats. The pattern of distribution, namely femur > liver > brain, was similar in all age groups, but age-related increases in lead concentrations in brain and kidney were noted, along with decreases in femoral bone lead content. This shift did not appear to reflect enhanced lead uptake from the gastrointestinal tract but rather a change in bone physiology with age, combined with altered patterns of urinary lead excretion over time (Cory-Slechta, 1990).

The intracellular bioavailability of lead in major target organs such as the kidney and brain appears to be determined largely by formation of complexes with a group of low-molecular-weight proteins. Several distinct high-affinity cytosolic lead-binding proteins (PbBP) have been identified in the rat kidney and brain that appear to act as receptors for lead (Oskarsson *et al.*, 1982; DuVal & Fowler, 1989). The PbBP from rat kidney has been shown to be a specific cleavage product of α_{2u}-globulin, produced most extensively in the livers of male rats and to a much lesser extent in female rats of breeding age. The PbBP was shown to migrate to the nucleus and form complexes with nuclear chromatin (Mistry *et al.*, 1985; 1986; Fowler & DuVal, 1991). The renal PbBP is selectively localized in only certain nephrons and only specific segments of the renal proximal tubule. Short-term, high-dose lead exposure (1% or 7% lead acetate in drinking-water for 7 weeks) resulted in increased excretion of this protein in the urine with a concomitant decrease in renal concentrations of PbBP (Fowler & DuVal, 1991). The brain PbBP appears to be a chemically similar but distinct molecule (DuVal & Fowler, 1989). High-affinity PbBPs have also been identified in the kidney and brain of monkeys (Fowler *et al.*, 1993).

(iii) *Excretion*

Excretion of lead occurs mainly in the faeces and urine (WHO, 1985). Adult mice were found to excrete about 62% of intravenously injected lead within 50 days; cumulative lead concentrations in faeces were 25–50% of the administered dose (Keller & Doherty, 1980b). Adult rats excreted 24.4% and 9.5% of intravenously injected lead in faeces and urine, respectively, within 48 h (Kostial & Momcilovic, 1974). In rats and monkeys exposed by inhalation to lead oxide (21.5 μg/m^3) for 1 year, lead excretion was greater in faeces than in urine, but wide variations between individual animals were noted (Griffin *et al*., 1975b). Five days after a single intravenous dose of ^{203}Pb in rats, total lead excretion was found to amount to 53%, with similar amounts being excreted in urine and faeces, except on day 2 (ratio faeces:urine, 2) (Morgan *et al*., 1977). Studies on rats exposed for 30–45 min to an ‘urban-like’ aerosol of ^{210}Pb-dibenzoylmethane (added to

gasoline and burned in a tubular furnace heater at 600 °C) showed that, 6 days after inhalation, less than 1% of the total absorbed dose of lead was retained in lung, 40% had been eliminated in faeces and 15% in urine, 40% was fixed in the skeleton and 4–5% in soft tissue (Boudene *et al*., 1977).

Studies on dogs after intravenous administration of ^{210}Pb showed that 56–75% of the total dose of lead was excreted in the faeces (Hursh, 1973; Lloyd *et al*., 1975).

Adult monkeys have been shown to excrete more absorbed lead in faeces than young animals (13% versus 3.45%), while urinary excretion was similar (5.31% versus 3.84%) (Pounds *et al*., 1978).

Marked species differences in the biliary excretion of lead have been reported (Castellino *et al.*, 1966; Klaassen & Shoeman, 1974; Conrad & Barton, 1978; Cikrt *et al*., 1983; Gregus & Klaassen, 1986). A relatively high biliary excretion of lead was reported in rats (Klaassen & Shoeman, 1974). About 6.5–8.5% of a dose of ^{210}Pb-lead nitrate or ^{203}Pb-lead chloride administered intravenously to rats was excreted in the bile within 24 h; biliary excretion thus plays an important role in the enterohepatic circulation of lead in rats (Cikrt, 1972; Cikrt & Tichy, 1975). In a further study, biliary excretion of lead was analysed in three groups of rats given drinking-water containing lead acetate (at 100, 250 and 2500 mg lead/L) for 80 days. Biliary excretion of lead in the exposed groups reached 0.08 ± 0.01, 0.20 ± 0.04 and 1.46 ± 0.09 µg/mL, respectively, compared with 0.05 ± 0.04 µg/mL in a control group (Cikrt *et al.*, 1983). Rabbits have been shown to excrete lead in the bile at < 50% and dogs at < 2% of the rates of biliary excretion of lead in rats (Klaassen & Shoeman, 1974).

Studies on the renal handling of lead (^{203}Pb) in dogs showed that plasma lead is filtered and reabsorbed but that there is no evidence of tubular secretion of lead (Vander *et al*., 1977). Urinary clearance of lead was calculated to be 19% of the estimated glomerular filtration rate in two cynomolgus monkeys (O'Flaherty *et al*., 1996).

In rodents, lead is transferred across the placenta to fetuses and during lactation to the litter (Kostial & Momcilovic, 1974; McClain & Siekierka, 1975; Hackett *et al*., 1982a,b; Donald *et al*., 1986; Maldonado-Vega *et al*., 1996, 2002). The lactational transfer after current or recent exposure of dams to lead is considerably higher than the placental transfer (Kostial & Momcilovic, 1974; Palminger Hallén *et al.*, 1995b). A high transfer of lead into milk was demonstrated in rodents, as well as a high uptake of lead in the tissues of suckling pups. About 20–33% of an initial maternal dose of lead was transferred to suckling rats or mice (Momcilovic, 1978; Keller & Doherty, 1980a; Palminger Hallén *et al*., 1996b). In a study by Palminger Hallén & Oskarsson (1993), rat and mouse dams were administered a single intravenous dose of ^{203}Pb on day 14 of lactation in four or five doses ranging from 0.0005 to 2.0 mg/kg bw. The concentration of ^{203}Pb in plasma was linearly correlated with that in milk. The milk:plasma ratios were 119 and 72 in mice and 89 and 35 in rats at 24 and 72 h after administration, respectively. Excretion into milk appeared more efficient in mice than in rats, but rat pups had higher tissue concentrations than mouse pups; this may be due to a higher bioavailability and/or a lower excretion of lead in rat pups (Palminger Hallén & Oskarsson, 1993). Continuous exposure of rats to

lead in drinking-water during gestation and lactation resulted, at day 15 of lactation, in milk lead concentrations about 2.5-fold higher than blood lead concentrations. When exposure to lead was terminated at parturition, the milk lead concentrations were similar to those of blood lead at day 15 of lactation, and were only 10% of the milk lead concentrations found after continuous exposure to lead during gestation and lactation. Exposure of offspring to lead via placenta and milk from dams exposed continuously resulted in blood and brain lead concentrations sixfold higher than those in offspring exposed via the placenta only. Exposure of offspring via milk only from dams exposed to lead until parturition resulted in blood lead concentrations that were higher than those in offspring exposed to lead via the placenta only (Palminger Hallén *et al*., 1995b).

(*c*) *Experimental systems* in vitro

In vitro, intestinal permeability to lead has been shown to be similar in intestines from fasted and fed rats (Aungst & Fung, 1981).

Healy *et al.* (1982) showed that the rate of dissolution of lead sulfide in gastric acid *in vitro* was dependent on particle size, being much greater for particles of 30 μm diameter than for particles of 100 μm diameter.

Studies in kidneys of rats *in vitro* have shown that the kidney PbBP facilitates the nuclear uptake of lead followed by its binding to chromatin (Mistry *et al*., 1985, 1986; Fowler & DuVal, 1991; Goering, 1993; Fowler, 1998).

Bress and Bidanset (1991) measured the degree of in-vitro penetration of lead acetate and lead oxide, using diffusion tubes in excised guinea-pig skin and human skin from autopsy. The percentage recovery of lead acetate in guinea-pig skin and human skin was 0.03% and 0.05%, respectively. There were no measurable amounts of lead oxide absorbed in either species.

Steady-state kinetic analyses of ^{210}Pb in hepatocytes from rats demonstrated three compartments, more than 85% of the lead being found in the kinetic pool associated with mitochondria (Pounds *et al.*, 1982). In osteoclastic bone cells from mouse calvaria, three similar kinetic pools of intracellular lead containing approximately 10%, 12% and 78% of total cellular lead were identified; as in hepatocytes, the bulk of cellular lead was associated with mitochondria. The half-times for isotopic exchange were 1, 27 and 480 min, respectively (Pounds & Rosen, 1986).

4.1.2 *Organic lead compounds*

The toxicity of organic lead compounds is generally high, but varies widely between animal species and according to the chemical structure of the compound. Most of the information available concerns tetraethyl lead, but the toxicity of tetramethyl lead and some of its metabolites is also well described. Organic lead compounds are toxicokinetically distinct from inorganic lead compounds in terms of absorption and distribution and, owing to their greater lipophilicity, they are rapidly partitioned into soft tissues.

(*a*) *Humans*

(i) *Absorption*

Inhalation exposure

Inhaled tetraethyl and tetramethyl lead vapours behave as gases in the respiratory tract and, as a result, their pattern and extent of deposition and absorption differ from that of inhaled inorganic lead particles (US EPA, 1994; ATSDR, 1999). These differences result in a higher fractional absorption: approximately 60–80% of the deposited tetraethyl and tetramethyl lead was absorbed by the lungs (Heard *et al*., 1979).

Dermal exposure

Tetraethyl lead is a lipophilic substance that can penetrate intact skin in lethal quantities. The amount absorbed is proportional to the surface area exposed and the concentration. Accidents involving transdermal absorption of tetraethyl lead and tetramethyl lead in humans have been described (Hayakawa, 1972; Gething, 1975). Due to its higher lipophilicity, tetraethyl lead is more readily absorbed than tetramethyl lead.

(ii) *Distribution*

Inhalation of tetraethyl lead results in much higher concentrations of lead in the brain than does inhalation exposure to inorganic lead.

Distribution of organic lead in humans has been observed to be highly variable and measurements are complicated by metabolism of the alkyl lead to inorganic lead. For example, in a man who ingested a chemical mixture containing 59% tetraethyl lead (38% lead w/w), the highest concentrations of triethyl lead and inorganic lead were found in the liver and kidneys followed by the brain, pancreas and heart (Bolanowska *et al*., 1967). In another report in which a man and a woman accidentally inhaled a solvent containing 31% tetraethyl lead (17.6% lead w/w), concentrations of triethyl lead and inorganic lead were highest in the liver and lower in the kidney, brain, pancreas, muscle and heart (Bolanowska *et al*., 1967), although the liver/kidney ratio for triethyl lead was 5:1 in the woman compared with that of 1.3:1 in the man. Trialkyl lead metabolites have also been detected in brain tissue of subjects not occupationally exposed to air pollution (Nielsen *et al.*, 1978).

Organic lead compounds are ultimately metabolized to inorganic lead and the latter is stored in the bones (Schwartz *et al*., 1999, 2000a).

(iii) *Metabolism*

Alkyl lead compounds are actively metabolized in the liver through oxidative dealkylation catalyzed by cytochrome P-450. Relatively few human studies that address the metabolism of alkyl lead compounds were found in the available literature (Bolanowska *et al.*, 1967; Nielsen *et al.*, 1978; ATSDR, 1999).

(iv) *Excretion*

Tetraethyl lead is excreted in the urine as diethyllead and inorganic lead (Turlakiewicz & Chmielnicka, 1985; Vural & Duydu, 1995). Following inhalation exposure, exhalation of tetraalkyl lead compounds is a major pathway of elimination in humans. Heard *et al.* (1979) showed that 48 h after inhalation exposure, 40% and 20% of inhaled tetramethyl and tetraethyl lead doses, respectively, that were initially deposited in the lung, were exhaled, and there was little urinary excretion.

(*b*) *Animals*

(i) *Absorption*

Bress and Bidanset (1991) measured absorption *in vivo* by applying 300 mg/kg tetrabutyl lead, lead nuolate, lead naphthenate, lead acetate or lead oxide to the shaved backs of guinea-pigs for 7 days under occluded wrappings. Tetrabutyl lead was present in tissues in the highest quantities: mean (± SD) total lead concentration reached 7.46 (± 0.68) µg/g in blood, 8.52 (± 0.46) µg/g in kidney, 4.31 (± 0.21) µg/g in liver and 4.02 (± 0.29) µg/g in brain (see also Section 4.1.1(*b*)(i)).

(ii) *Distribution*

Previous monographs (IARC, 1972; 1980) have summarized many studies on the distribution of lead published before 1980. In more recent studies in rabbits (Arai *et al.*, 1998), total lead in the brain 1 day after intravenous injection of triethyl neopentoxy lead consisted of triethyl lead alone; total lead in liver and kidney was about 72–78% triethyl lead, about 14–19% inorganic lead and about 8–9% diethyl lead. Lead in blood was about 34% triethyl lead, about 38% inorganic lead and about 28% diethyl lead. In bile, it was about 2% triethyl lead, about 9% inorganic lead and about 89% diethyl lead. These ratios of lead species in the organs were similar 7 days after injection, but only inorganic lead was detected in blood.

Studies by Morgan and Holmes (1978) using adult rats exposed for 40–60 min by inhalation to an aerosol containing ^{203}Pb-tetraethyl lead added to lead-free petrol showed that less than 2% of the dose was present in the lungs after 1 week. Mean total deposition of lead was calculated to be 30.5%. At least half of the ^{203}Pb deposited in the lungs was absorbed with a half-life of less than 1 h. To investigate whether lead in ingested exhaust particles is absorbed from the gastrointestinal tract, exhaust particles from an engine running on ^{203}Pb tetraethyl-enriched gasoline were collected on millipore filters, which were then fed to rats. Less than 0.5% of the ^{203}Pb lead associated with the particles was found to be absorbed.

(iii) *Metabolism*

Tetraethyl and tetramethyl lead undergo oxidative dealkylation and are metabolized to the highly neurotoxic metabolites triethyl and trimethyl lead, respectively. In rabbit liver, the reaction is catalysed by a cytochrome P-450-dependent monoxygenase system

(Kimmel *et al.,* 1977). Complete oxidation of alkyl lead to inorganic lead also occurs in rat, mouse and rabbit (Bolanowska, 1968; ATSDR, 1999).

(iv) *Excretion*

Previous monographs have summarized many studies on the excretion of lead published before 1980 (IARC, 1972; 1980). Kozarzewska and Chmielnicka (1987) studied the excretion of tetraethyl lead in rabbits. After intragastric or intravenous administration to rabbits of 12 mg/kg bw tetraethyl lead, diethyl lead constituted 70–90% and 50%, respectively, of the total lead excreted in urine during the first seven days, and 70% and 40%, respectively, after 30 days. Maximum diethyl lead excretion occurred on the first three days regardless of the route of the administration. After administration of a 3 mg/kg bw dose, excretion of diethyl lead did not vary so much between the intragastric and the intravenous routes of administration; in this case, during 30 days of observation, diethyl lead constitued about 40% of the total lead excreted in urine. In rabbits exposed for 5 h to tetraethyl lead by inhalation at a concentration of 200 µg/m³ in air, maximum diethyl lead excretion was recorded on day 2 after exposure and constituted about 20% of total lead excreted in the urine. On day 7, only trace quantities of this metabolite were found.

Arai and Yamamura (1990) showed that in rabbits, after a single intravenous dose of 9.9 mg/kg bw tetramethyl lead (7.7 mg/kg bw lead), the mixture of lead compounds excreted in urine was composed of about 73% dimethyl lead, 19% trimethyl lead, 6% inorganic lead and 2% tetramethyl lead on the day following injection. The excretion on day 7 was entirely composed of trimethyl lead. In rabbits injected with 39.7 mg/kg bw tetramethyl lead (30.8 mg/kg bw lead), total urinary lead excretion was composed of about 67% dimethyl lead, 14% trimethyl lead, 17% inorganic lead and 2% tetramethyl lead on the day following administration and about 8% dimethyl lead, 74% trimethyl lead, 17% inorganic lead and 1% tetramethyl lead on day 7 after dosing. In both groups of rabbits, total lead excretion in faeces during the 7 days after injection was entirely composed of inorganic lead. During the same period, 1–3% of either administered dose of tetramethyl lead was excreted in the urine and 7–19% in the faeces.

Further studies (Arai *et al.*, 1998) showed that about 4% of an intravenous dose of triethyl neopentoxy lead (10 mg/kg bw; 4 mg/kg bw lead) administered to rabbits was excreted in the urine within 7 days and about 68% in the faeces. Urinary excretion of total lead was composed of about 85% diethyl lead, 8% triethyl lead and 7% inorganic lead. The 7-day faecal excretion was composed of about 92% inorganic lead, 4% diethyl lead and 4% triethyl lead. Hence, the major chemical species of lead excreted in the urine was diethyl lead, while the major species excreted in the faeces was inorganic lead.

(*c*) *Experimental systems* in vitro

Bress and Bidanset (1991) measured the degree of in-vitro penetration of tetrabutyl lead, lead naphthenate, lead nuolate, lead acetate and lead oxide, using diffusion tubes in excised guinea-pig skin and human skin from autopsy. The percentage recovery of lead in guinea-pig skin ranged from 1.3% with tetrabutyl lead, demonstrating the highest skin

penetration, followed by lead naphthanate (0.45%), lead nuolate (0.25%), lead acetate (0.03%) and lead oxide (< 0.01%). The same rank order of recovery was seen in excised human skin where recovery of tetrabutyl lead was 6.3%.

4.2 Toxic effects

The extensive literature on the toxic properties of lead up until 1998 has been reviewed in the Toxicological Profile for Lead (ATSDR, 1999) and earlier by the National Research Council (NRC, 1993).

4.2.1 *Overt symptoms of lead intoxication*

Mankind has been using lead for over 6000 years and the widespread contamination of the environment with lead is solely the result of anthropogenic activities. In 370 BC, the Greek physician Hippocrates was probably the first to recognize lead as the cause of colic in a man who was a metal worker. In the 1st century AD, Dioscorides, another Greek physician, noted that exposure to lead could cause paralysis and delirium in addition to intestinal problems and swelling (Cilliers & Retief, 2000; Hernberg, 2000). In Roman times winemakers used lead pots or lead-lined kettles to boil the crushed grapes, and added lead acetate to their wine as a sweetener (Aitchinson, 1960). References to paralysis in miners exposed to lead increased in Europe in the 1600s, as did reports of colic in wine-drinkers (Lin-Fu, 1992).

One of the earliest manifestations of lead intoxication in adults is so-called lead-induced colic, which is a syndrome characterized by a combination of abdominal pain, constipation, cramps, nausea, vomiting, anorexia and weight loss. Various pathogenic mechanisms have been proposed for this syndrome: it may result from changes in visceral smooth muscle tone secondary to the action of lead on the visceral autonomic nervous system; from lead-induced alterations in sodium transport in the small-intestinal mucosa; and from lead-induced interstitial pancreatitis. The possible presence of this syndrome should be considered in the differential diagnosis of abdominal pain of obscure etiology, and whenever a disparity is observed between the symptoms and the abdominal findings in a patient with abdominal pain, especially in the presence of a history of occupational exposure to lead (Janin *et al.*, 1985).

Many cases of acute lead intoxication have been described in the literature (for reviews, see Srianujata, 1998; Vig & Hu, 2000; Matte, 2003). Only a few studies are discussed below.

The effects on health of occupational exposure to lead have been investigated in 92 exposed workers in a lead–acid battery factory and 40 non-exposed workers who served as a control group. The two groups were closely similar in age, stature, body weight and socioeconomic status. In the factory, concentrations of lead in air varied between 1.8 and 2.2 mg/m^3. In 46 workers, average concentrations of lead in blood were 48–81 μg/dL, depending on job title. In 12 controls, an average blood lead concentration of 21 μg/dL

was measured. A highly significant increase ($p < 0.01$) was also recorded in urinary coproporphyrin and basophilic stippled red blood cells of the exposed group in comparison with the control group. Central nervous system symptoms (insomnia, fatigue, weakness and drowsiness) were reported by 50% of the workers, and other symptoms such as abdominal colic and constipation were noted by 41% of the exposed group (Awad el Karim *et al.*, 1986).

Three cases of acute lead poisoning in adults were reported to be caused by exposure to old leaded paint. Initial concentrations of lead in blood in the three subjects were 84.2, 85.2 and 87.1 µg/dL, respectively, and all complained of abdominal pain, malaise and nausea. The patients received sodium calcium edetate and/or succimer for three weeks, which reduced their blood lead concentrations by 50–75%. Despite removal from the source of exposure, lead concentrations remained elevated in two cases, which may be explained by release of lead from the skeleton (Gordon *et al.*, 2002).

A case of severe lead poisoning in a young woman was reported to be caused by prolonged use of eye make-up ('kohl') made of lead sulfide. Clinically, the patient presented with abdominal cramps, anxiety and irritability, and microcytic sideropenic anaemia. Emergency chelate treatment improved her condition and decreased lead concentrations in blood from their initial value of 490 µg/dL to 49 µg/dL 6 weeks after treatment (Bruyneel *et al.*, 2002).

4.2.2 *Effects on haeme-containing systems*

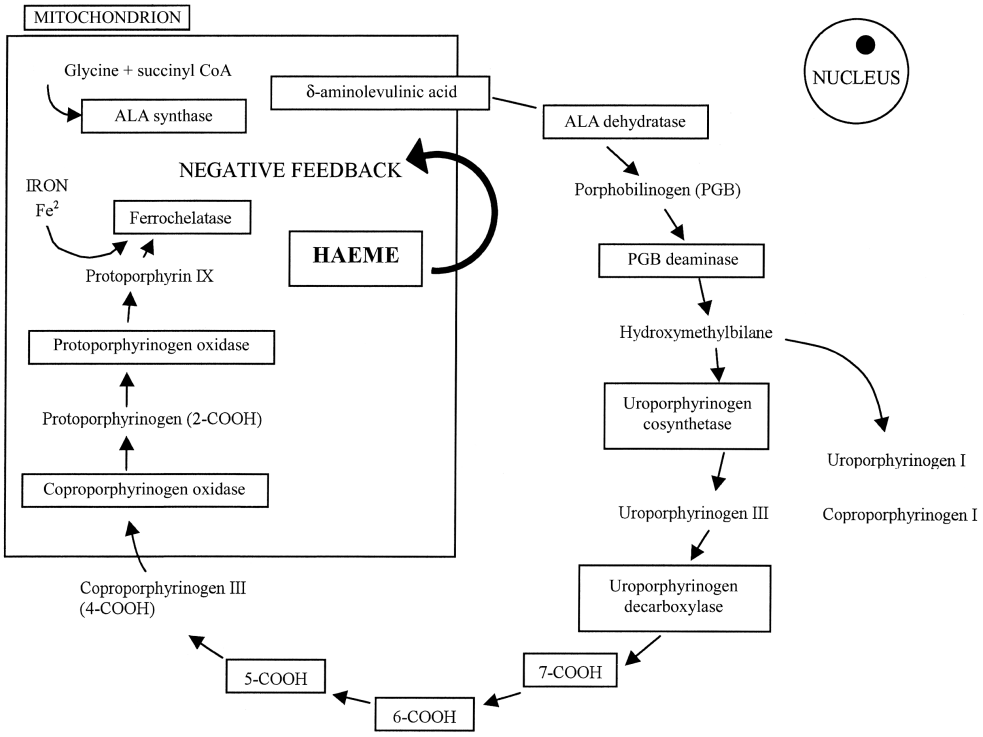
(*a*) *Humans*

Interaction with enzymes and high-affinity metal-binding proteins is probably the most important mechanism of lead toxicity. This interaction usually consists of reversible binding of lead to sulfhydryl groups or to other protein sites capable of binding divalent cations. In this regard, the most well-known example is the inhibition by lead of δ-aminolevulinic acid dehydratase (ALAD) (also known as porphobilinogen synthase, PBGS), a key enzyme in the pathway of haeme synthesis (Figure 5). This enzyme has a unique zinc binding site (Jaffe *et al*., 2001) that confers a very high affinity for Pb^{2+} (Simons, 1995).

Over the past 10 years, attention has been paid increasingly to early, subtle (subclinical) effects of lead on haeme systems, the hypothesis being that these form a physiopathogenetic continuum with the clinical or overt effects. The only difference between early and clinical effects of a given type depends on their degree and not on their nature (Goyer, 1990b). The effects on haeme synthesis and haematological effects are dose-dependent (Table 87) and become manifest at blood lead concentrations < 10 µg/dL (ALAD inhibition), at 15–30 µg/dL (inhibition of iron chelation in haeme), and 50–60 µg/dL (reduction of haemoglobin concentrations).

Effects of lead, such as inhibition of ALAD, elevation of aminolevulinic acid (ALA) in urine and increase of free erythrocyte protoporphyrin in blood, have been observed in humans exposed to lead (Lauwerys *et al*., 1973; Alessio *et al.*, 1976; Tomokuni & Ogata, 1976; Horiguchi *et al.*, 1981; Moore, 1988). These biological indicators correlate more or

Figure 5. Haeme biosynthesis in the cell



Modified from Moore (1988)

Lead inhibits ALA dehydratase, coproporphyrinogen oxidase and ferrochelatase with consequent increases in the urinary excretion of ALA and coproporphyrin III together with accumulation of protoporphyrin 9 in the erythrocyte. Primary control of the rate-limiting enzyme, 5-aminolaevulinate synthase is vested in the end product of the pathway, haeme through the 'pool' of free haeme in the cell. This pool is depleted in lead poisoning through diminished synthesis of haeme and enhanced catabolism of the haeme by haeme oxygenase. The net result is increased synthesis of ALA synthase and excessive production of pathway intermediates. There is, in addition, some deleterious influence on haemoprotein synthesis because of the lack of haeme substrate for their formation.

less closely with the concentration of lead in peripheral blood (see Section 1.6.2), and are therefore useful in evaluating the effect of lead in individuals exposed occupationally or environmentally to the metal. They have also been used for assessing exposure to lead.

In addition to effects on haeme in erythropoiesis, attention has also been paid to the possible effects of lead on other haeme-containing enzymatic systems, such as P450 cytochromes or systems involved in the metabolism of vitamin D (Silbergeld *et al.* 1988; Goyer, 1990b). Such effects result in a decreased availability of cytochromes for the respiratory chain and the accumulation of toxic metabolites such as ALA.

Table 87. Effects of lead on haeme synthesis in adults and relative concentrations of lead in blood at which they become manifest

Lowest observed effect level (blood lead concentration, μg/dL)	Haeme synthesis and haematological effects
80	Frank anaemia
50	Reduced haemoglobin production
40	Increased urinary ALA and elevated coproporphyrins
25–30	EP elevation in men
15–20	EP elevation in women
< 10	ALAD inhibition

Adapted from US EPA (1986)
ALA, delta-aminolevulinic acid; ALAD, delta-aminolevulinic acid dehydratase; EP, erythrocyte protoporphyrin

(i) *Inhibition of ALAD by lead*

Inhibition of ALAD by lead is discussed in Section 1.6.2. It is responsible in part for increases in ALA in plasma, in blood and in urine. ALA synthase is induced by negative feedback from the depression of haeme synthesis. ALA in plasma and blood should reflect the effects of lead more directly than ALA in urine (Moore *et al.*, 1980b; WHO, 1980; see Section 1.6.2 for analytical methods).

In subjects recently exposed to lead, there is a latency period of about 2 weeks before ALA in urine increases. After cessation of exposure, the excretion of ALA in urine returns to normal relatively quickly; thus this parameter is not suitable for detecting past exposure to lead (Tola *et al.*, 1973; Haeger-Aronsen *et al.*, 1974; Benson *et al.*, 1976).

(ii) *ALAD gene polymorphism*

ALAD is a well-known enzyme that is essential to tetrapyrrole biosynthesis (e.g. haeme, chlorophyll and vitamin B12). In humans, ALAD is a polymorphic enzyme with two common alleles, $ALAD_1$ and $ALAD_2$ (Battistuzzi *et al.*, 1981; Petrucci *et al.*, 1982). The enzyme is polymorphic due to a G-to-C transversion of nucleotide 177 in the coding region, which results in replacement of lysine by asparagine at position 59 in the protein (Wetmur *et al.*, 1991a).

Numerous studies have investigated the role of the ALAD polymorphism in relation to blood lead concentrations. The data indicate that at equivalent exposures, homozygotes or heterozygotes for $ALAD_2$ have significantly higher mean blood lead concentrations, and lower concentrations of ALAP (only at high blood lead concentrations, 40–60 μg/dL) than homozygotes for $ALAD_1$ (Wetmur *et al.*, 1991b; Smith *et al.*, 1995; Sithisarankul *et al.*, 1997; Sakai, 2000). However, no differences in the net accumulation of lead in bone were found (Fleming *et al.*, 1998). Lead (Pb^{2+}) has been shown to bind the ALAD enzyme

by displacement of zinc (Zn^{2+}) (Simons, 1995; Bergdahl *et al*., 1998a). The difference in haeme precursor concentrations in people carrying different ALAD genotypes is thought to be due to a difference in binding affinity of lead for the ALAD isoenzymes (Bergdahl *et al*., 1997b). However, the model of human ALAD based on homologous crystal structure showed no obvious structural variation that would affect either metal binding to, or catalytic function of, the different ALAD isoenzymes. In in-vitro binding experiments, no differential displacement of Zn^{2+} by lead (Pb^{2+}) was found between the $ALAD_1$ (K59) and $ALAD_2$ (N59) protein variants (Jaffe *et al.*, 2000), but the two allozymes show a small difference in the kinetics of lead displacement by zinc (Jaffe *et al*., 2001). This implies that differences in susceptibility to lead of subjects carrying different ALAD genotypes may be related to a difference in direct binding of lead to the gene products. However, other indirect mechanisms leading to differences in lead retention in carriers of the different genotypes cannot be ruled out.

(iii) *Other gene polymorphisms*

An additional polymorphism that may modify the toxicity of lead involves the vitamin D receptor (*VDR*) gene. This gene can exist in two alleles (B and b) and experimental data suggest that bone calcium content increases with increasing copy number of the b allele. Because lead can substitute for calcium in many biological systems, and since both lead and calcium are divalent cations, it has been suggested that the toxicity of lead may be modified by polymorphisms in the *VDR* gene which could explain the increased concentrations of lead in dense cortical bone in populations occupationally exposed to lead (Schwartz *et al*., 2000b). The *VDR* gene is involved in the absorption of calcium through the gut and into calcium-rich tissue such as bone. However, effects of *VDR* polymorphism on a number of parameters of lead toxicity were not observed in a recent study of 798 workers exposed to lead (Weaver *et al.*, 2003).

Schwartz *et al.* (2000b) evaluated the association of tibial lead concentration with polymorphisms in the vitamin D receptor (*VDR*) gene in 504 former organolead manufacturing workers (mean age, 57.4 years). Tibial lead concentrations were measured by X-RF spectrometry in subjects with different *VDR* genotypes, adjusting for confounding variables. All study subjects had low tibial lead concentrations (mean, 14.4 μg/g bone mineral) and there were only small differences by *VDR* genotype. In a multiple linear regression model, the *VDR* genotype modified the relation between tibial lead concentration and age or years since last exposure. Although the influence of the *VDR* genotype on bone mineral density is a matter of debate, the data suggest that variant VDR alleles modify lead concentrations in bone.

Another gene that may influence the absorption of lead is the haemochromatosis gene encoding the HFE protein. Mutations in the *HFE* gene give rise to haemochromatosis in homozygous individuals. Because of the associations between iron and lead transport, it is possible that polymorphisms in the *HFE* gene may also influence the absorption of lead. Patients homozygous for the *HFE* mutation accumulate more lead than those who

do not carry two mutated alleles (Barton *et al.*, 1994). The role of these genes in the effects of lead is not fully understood (Onalaja & Claudio, 2000).

(iv) *Lead and coproporphyrins*

Lead may inhibit other enzymes in the metabolic pathway of haemoglobin synthesis. Inhibition of coproporphyrinogen decarboxylase results in accumulation of coproporphyrins and their increased urinary excretion. Urinary coproporphyrin is not, however, a specific indicator of exposure to lead, since it may also result from porphyria cutanea tarda, liver disease, haemolytic anaemia, infectious disease and alcohol consumption. The influence of lead on disorders of porphyrin metabolism, which are more evident in women than in men, have been documented among lead-exposed workers.

No effects are detected on urinary coproporphyrin at blood lead concentrations ≤ 40 µg/dL and, in constantly exposed subjects, blood lead and urinary coproporphyrin correlate well, with a positive linear relation (Williams *et al*., 1969; US EPA, 1986). Increased excretion of urinary coproporphyrin occurs with a latency of about 2 weeks, when blood lead concentrations are slightly higher than those at which ALAU increases (Tola *et al*., 1973; Benson *et al*., 1976). After cessation of exposure, the urinary coproporphyrin concentrations normalize with a few weeks (sometimes within a few days). The validity of urinary coproporphyrin to predict different blood lead concentrations is rather modest, so its use as a screening test is limited. In addition, subjects with severe lead exposure may in some cases show normal concentrations of urinary coproporphyrin (Alessio *et al*., 1976).

(v) *Lead and free erythroprotoporphyrin*

Lead causes an increase in free protoporphyrin IX in blood, which is measured as zinc protoporphyrin (ZPP). This is possibly due to the interrelationship between iron availability and haeme biosynthesis (Labbé *et al*., 1999). An increase in ZPP results from the ferrochelatase enzyme inserting Zn^{2+} in place of Fe^{2+} (Bloomer *et al*., 1983).

ZPP is a normal metabolite that is formed in trace amounts during haeme biosynthesis. During periods of iron insufficiency or impaired iron utilization, zinc becomes an alternative metal substrate for ferrochelatase, leading to increased ZPP formation. Evidence suggests that this zinc-for-iron substitution occurs predominantly within the bone marrow, and the ZPP:haeme ratio in erythrocytes reflects the iron status in the bone marrow. In addition, ZPP may regulate haeme catabolism through competitive inhibition of haeme oxygenase, the rate-limiting enzyme in the haeme degradation pathway that produces bilirubin and carbon monoxide (Labbé *et al*., 1999).

Roh *et al*. (2000) showed that ZPP concentrations measured by haematofluorometry were consistently higher than those measured by HPLC and spectrofluorometry in non-exposed adults, but were lower in exposed workers. They also found a positive correlation between blood lead and ZPP in workers exposed to high concentrations of lead, but not in non-exposed controls. The increase in ZPP is observed only at exposures resulting in blood

lead concentrations > 20 µg/dL and good correlations have been found with blood lead concentrations > 40 µg/dL (Leung *et al*., 1993; Froom *et al*., 1998) (see also Section 1.6.2).

The best-fitting correlation between blood lead and ZPP is an exponential curve, with an *r*-value ranging from 0.38 to 0.69. In a group of 97 subjects selected in a stratified sample, in whom the blood lead values ranged from 10–120 µg/dL, there was a very good correlation between blood lead and log ZPP ($r = 0.87$). When the diagnostic validity of the ZPP test was analysed in various groups of workers, a high number of false negatives was observed at various ZPP cut-off values. ZPP cannot be applied in screening of workers with medium or low exposures to lead. In such situations, it is considered advisable to use blood lead as an indicator (Apostoli & Maranelli, 1986).

At blood lead concentrations < 20 µg/dL, ZPP concentrations were not found to be significantly different between the genotypes $ALAD_1$ and $ALAD_2$. Furthermore, ALAD genotypes did not affect the concentrations of haeme precursors at low blood lead concentrations (Alexander *et al*., 1998; Zhang *et al*., 1998). At blood lead concentrations of 20–60 µg/dL, ZPP concentrations in $ALAD_1$ homozygotes were significantly higher than those in $ALAD_2$ carriers (Schwartz *et al*., 1995; Alexander *et al*., 1998).

(vi) *Lead and pyrimidine 5′-nucleotidase*

Lead may affect haematocrit and haemoglobin concentrations also via the haemolytic effect of pyrimidine nucleotide accumulation due to the inhibition of pyrimidine 5′-nucleotidase (P5N) (Sakai, 2000). Following initial observations on the inhibitory effects of lead on P5N (Paglia *et al*., 1975; Angle & McIntire, 1978), a number of studies have been carried out mainly to develop adequate analytical methods for measuring P5N activity in the general population and to study its relationship with blood lead concentrations and with other enzymes such as deoxy-P5N and arginase (Cook *et al*., 1985, 1986; Sakai & Ushio, 1986). It was reported that ALAD is more sensitive to lead than P5N (Tomokuni & Ichiba, 1988b; Ong *et al*., 1990; Pagliuca *et al*., 1990; Kim *et al*., 1995a). It has been suggested that P5N is the 45-kDa protein component in the lysate from erythrocytes of exposed workers that is seen to bind Pb^{2+} (Bergdahl *et al*., 1998a).

(vii) *Lead and indicators of anaemia*

Anaemia following exposure to lead is caused by the decreased synthesis of both haeme and globin and by a haemolytic mechanism that is due partly to inhibition of P5N (Sakai, 2000). Anaemia induced by lead poisoning is normocytic in children and women and commonly associated with iron deficiency, which may produce a more severe microcytic hypochromic anaemia (Clark *et al*., 1988). Anaemia may also result in part from the inhibitory action of lead on erythropoietin (Graziano *et al*., 1991).

A threshold lead-in-blood concentration resulting in a decrease in haemoglobin has been estimated to be 50 µg/dL for occupationally exposed adults (US EPA, 1986).

A cross-sectional epidemiological study was conducted to assess the association between blood lead concentration (11–164 µg/dL) and hematocrit value in 579 children (age, 1–5 years) living near a primary lead smelter. There was a non-linear dose–response

relationship between blood lead concentration and hematocrit, which was influenced by age. In one-year-olds, the age group most severely affected, the risk of having an hematocrit < 35% — indicative of anemia — was 2% at a blood lead concentration of 20–39 μg/dL, 18% at 40–59 μg/dL, and 40% at a PbB > 60 μg/dL. The data suggest that lead-induced anemia is an important consequence of lead absorption, even at low exposure levels (Schwartz *et al.*, 1990).

(viii) *Lead and other haeme-containing systems*

Lead inhibits the synthesis of cytochromes, such as cytochrome C, in both animal and human systems (Bull *et al*., 1983). It also affects other haeme-requiring enzymes, such as cytochrome C oxidase in muscle (Goldberg *et al*., 1985).

A decrease in haeme will also alter the activity of other haeme-requiring proteins (Figure 6).

(*b*) *Animal studies*

The effects of lead on the haematopoietic system in experimental animals have been studied extensively (see WHO, 1995; ATSDR, 1999).

A decrease in ALAD activity in erythrocytes was observed in rats given lead acetate at 1000 ppm in the drinking-water for 6 days. Blood lead concentrations increased to 44 μg/dL after the first day and remained within 10 μg/dL of that value until the end of the exposure period (Simmonds *et al.*, 1995).

In subchronic exposure studies, decreased haematocrit values and impaired haeme synthesis were reported, the lowest effective dose being dependent on the exposure route and on the chemical form of lead. When administered to rats by gavage, lead acetate caused a decrease in haematocrit values at a dose of 30 mg/kg bw per day given for 19 days (Overmann, 1977). When rats received 1% lead acetate in the diet for 7 weeks, a similar decrease in haematocrit value was seen (Walsh & Ryden, 1984). In contrast, rats that received lead acetate in their drinking-water at 34 mg lead/kg bw per day did not show adverse effects on haematocrit (Fowler *et al.*, 1980).

Urinary excretion of ALA was significantly increased in rats that received lead acetate or lead oxide at 5 mg lead/kg bw per day for 30 days, but no effect was seen with lead sulfide or a lead ore (Dieter *et al.*, 1993). Likewise, ALAD activity in serum was more strongly inhibited by lead acetate than by lead sulfide or lead-contaminated soil (Freeman *et al.*, 1996).

The effect of ingestion of lead on haematological parameters was investigated in male and female Swiss mice. Eight different doses of lead were administered through preparation of different feeds. The amounts of lead in the diet were designed to provide blood lead concentrations below (0.6–< 2.0 μg/dL) and above (> 2.0–13 μg/dL) the normal background. One litter of mice was exposed to each dose by feeding the mother with the lead-containing diet starting 1 day after mating, and mother and offspring continued to receive the feed until the litter was 90 days old. Male and female mice receiving concentrations of dietary lead below normal background displayed enhanced erythrocyte produc-

tion as measured by higher cell numbers and increased haemoglobin and haematocrit values. However, as the blood lead concentrations approached 10 μg/dL, there was a marked decrease in erythrocyte production. These findings are significant since lead appeared to stimulate erythrocyte production at low concentrations (2.0 μg/dL) while adversely affecting red cell synthesis at higher concentrations (7.0–13 μg/dL) (Iavicoli *et al.*, 2003).

(*c*) *Experimental systems* in vitro

Acute toxic effects of lead (at 0.5, 1.0, 2.5 and 5.0 μM for 24 h) on coproporphyrinogen oxidase activity have been evaluated in an in-vitro model using HepG2 cells, a hepatoma cell line of human origin (Hernández *et al*., 1998). The cells were treated with 150 μM ALA to induce porphyrins. Cellular protoporphyrin increased in a dose-dependent manner, reaching a maximum at 2.5 μM lead, but no changes in extracellular protoporphyrin were found. Extracellular coproporphyrin concentration was increased two-fold at all concentrations of lead, without changes in cellular content. The coproporphyrinogen oxidase activity was depressed in a dose-dependent manner to 62% of control activity at 5.0 μM lead. The dose-dependent increase in coproporphyrin secretion accompanied by the depression of coproporphyrinogen oxidase activity supports the hypothesis that lead inhibits coproporphyrinogen oxidase.

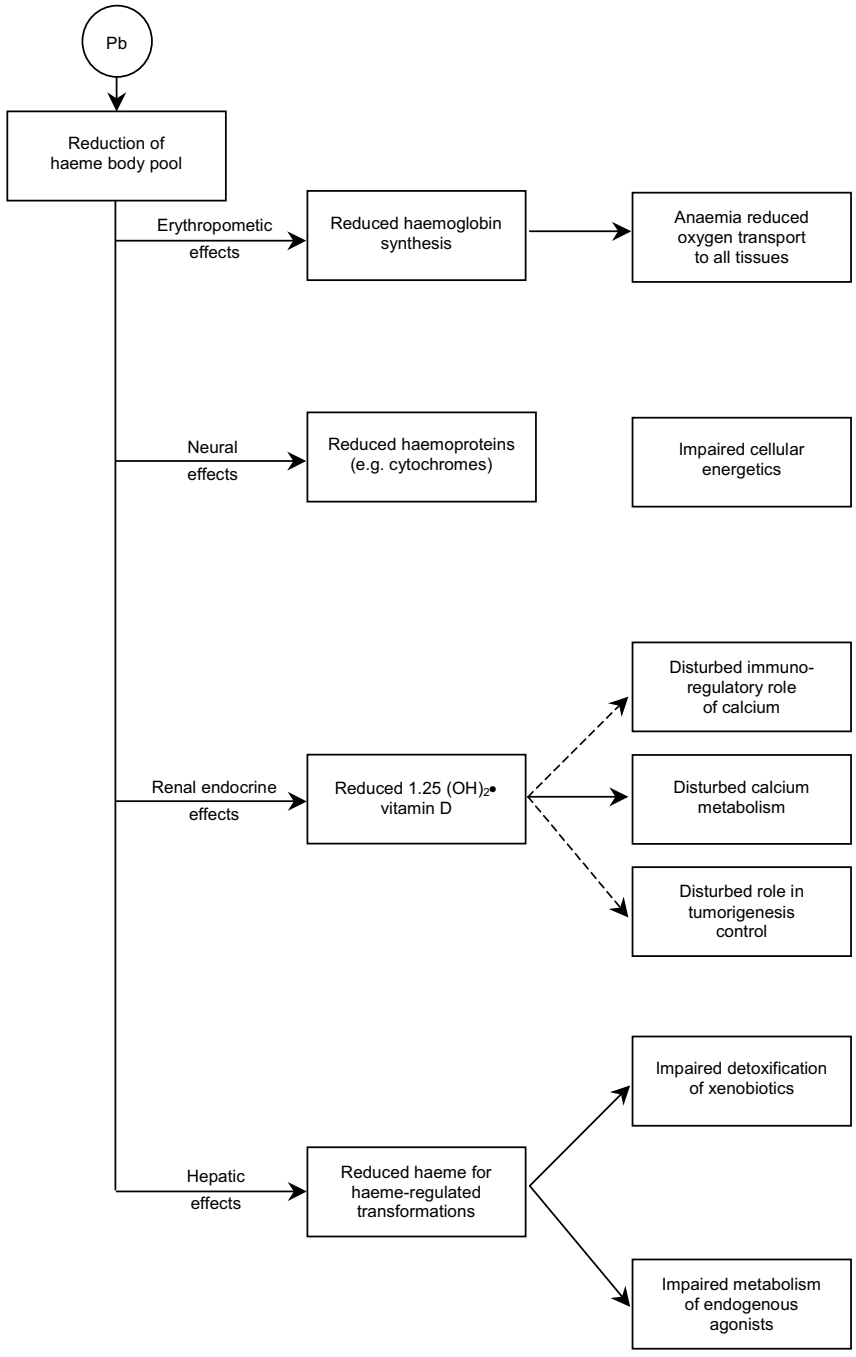
4.2.3 *Nephrotoxicity*

The renal effects of lead in humans and experimental systems have been reviewed (Goyer, 1989; Nolan & Shaikh, 1992; Goyer, 1993; WHO, 1995; Loghman-Adham, 1997). Acute and chronic effects of lead on the kidney are summarized in Figure 7. Acute exposure to high concentrations of lead results in disruption of proximal tubular architecture with disturbances in proximal tubular function. Histological changes include intranuclear inclusions in proximal tubular cells and mitochondrial swelling. Renal manifestations of acute lead poisoning include glycosuria, aminoaciduria and phosphaturia, collectively presented as the Fanconi syndrome. Chronic exposure to low concentrations of lead is also associated with increased urinary excretion of low-molecular-weight proteins and lysosomal enzymes. Chronic exposure to high concentrations of lead results in irreversible changes in the kidney, including interstitial fibrosis, tubular atrophy, glomerular sclerosis and ultimately chronic renal failure. It has also been implicated in the development of gout and hypertension secondary to nephropathy.

It has become evident that concentrations of lead as low as 10 μg/dL in blood, previously considered to be safe, may also be associated with renal function abnormalities, such as changes in serum creatinine concentration or in creatinine clearance (Staessen *et al*., 1992; Kim *et al*., 1996b). Whether such small changes in renal function result in clinically-significant health problems is uncertain.

The renal effects of lead in humans and experimental systems reviewed below are summarized in Table 88.

Figure 6. Multiorgan impact of reduction of haeme body pool by lead



Modified from ATSDR (1999)

Figure 6 (contd)

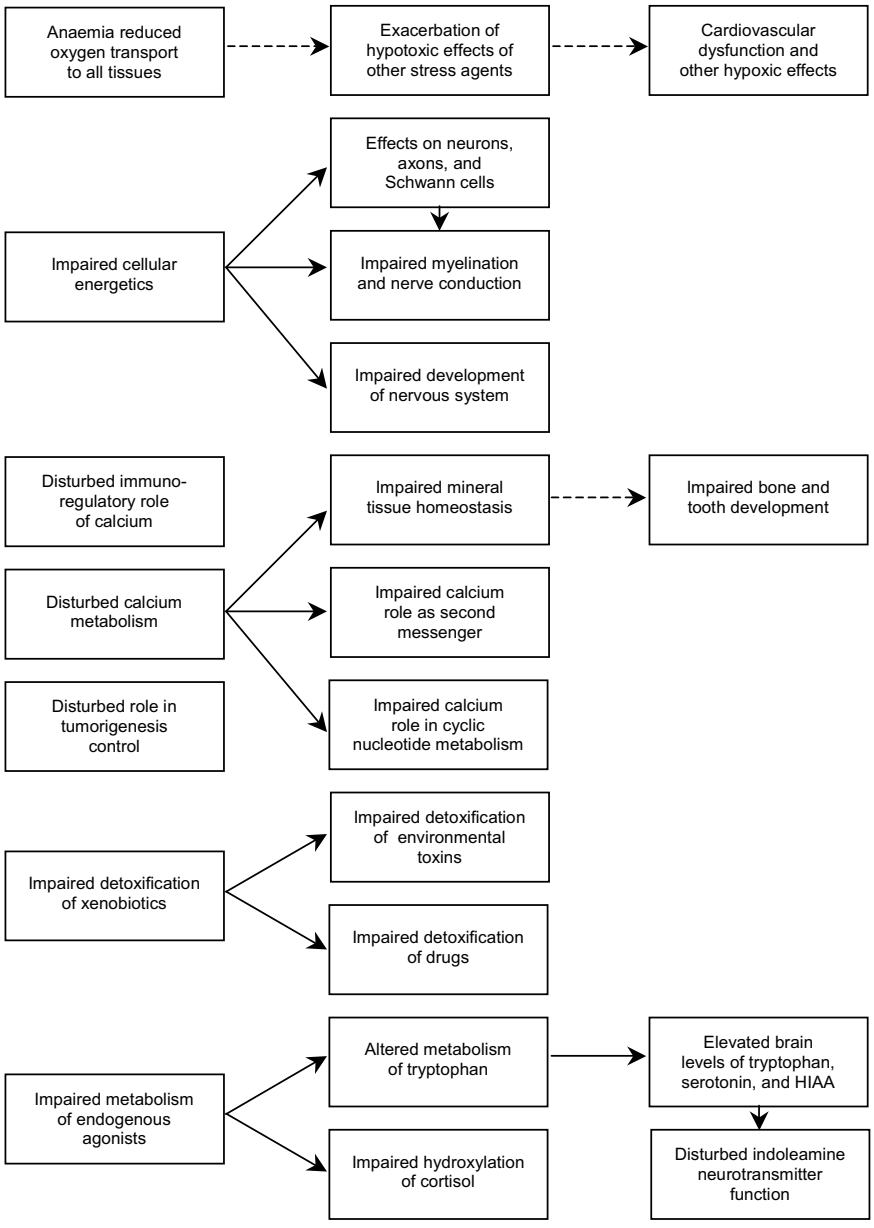
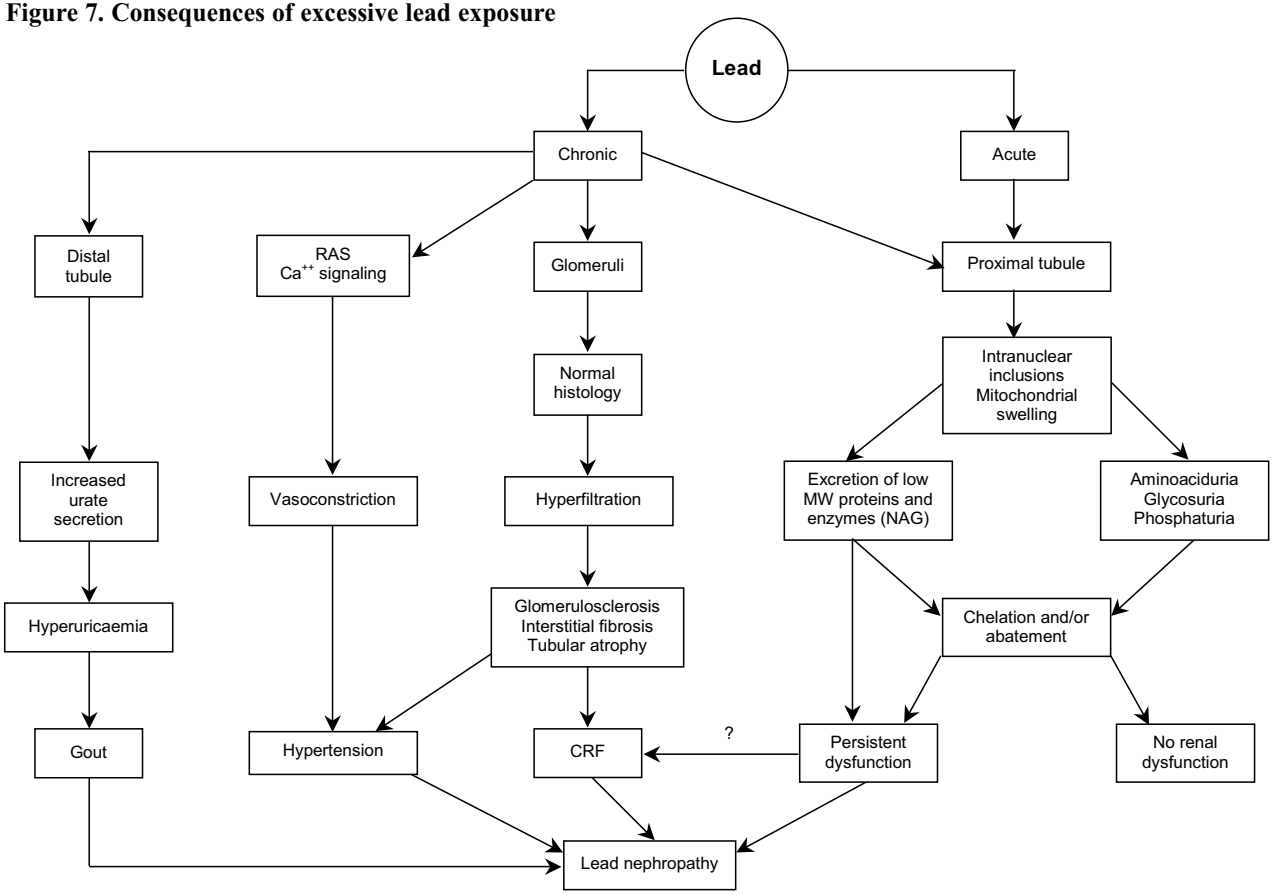


Figure 7. Consequences of excessive lead exposure



Modified from Loghman-Adham (1997); CRF, chronic renal failure; NAG, *N*-acetyl-β-D-glucosaminidase

Acute lead poisoning results in proximal tubular dysfunction; these changes usually disappear with chelation therapy or removal from lead sources. Chronic lead poisoning can affect glomerular function when blood lead levels exceed 60 µg/dL. After an initial period of hyperfiltration, the glomerular filtration is reduced and nephrosclerosis and chronic renal failure ensue. Prolonged lead exposure also interferes with distal tubular secretion of urate, leading to hyperuricaemia and gout. Finally, chronic lead exposure may cause hypertension, resulting from vasoconstriction due to the action of lead on the renin–angiotensin system (RAS) and on calcium signaling.

Table 88. Summary of published studies of the renal effects of lead

Effects	General population	Occupational exposure	Clinical studies	Animal studies
Acute				
Hypophosphaturia, aminoaciduria, glycosuria (Fanconi syndrome)	–	–	Chisholm (1962)	–
Glomerular filtration rate	–	–	Wedeen *et al*. (1979)	Khalil-Manesh *et al*. (1992a, 1994)
γ-Glutamyl transferase natriuria	–	–	–	Huguet *et al*. (1982)
Tubular change (inclusion bodies, mitochondria)	–	Cramér *et al*. (1974)	Biagini *et al.* (1977)	Moore & Goyer (1974); Goyer & Wilson (1975); Fowler *et al*., (1980)
Chronic				
S-Creatinine	Staessen *et al*., (1990); Kim *et al*. (1996)	Ong *et al*. (1987)	–	–
Creatinine clearance	Staessen *et al*. (1992)	Ong *et al*. (1987)	–	–
S-Urea (BUN)	Campbell *et al*. (1977)	Baker *et al*. (1979); Maranelli & Apostoli (1987); Ong *et al*. (1987)	–	–
Hyperuricaemia	Campbell *et al*. (1977)	Maranelli & Apostoli (1987)	–	–
α1-Microglobulin		Chia *et al*. (1995)	–	–
β2-Microglobulin	Staessen *et al*. (1992)	Huang, J.-X. *et al.* (1988)	–	–
N-Acetyl-β,D-glucosaminidase	Verberk *et al*. (1996)	Meyer *et al*. (1984); Ong *et al*. (1987); Verschoor *et al*. (1987)	–	–
Glutathione *S*-transferase	–	–	–	Khalil-Manesh *et al*. (1992a); Moser *et al.* (1995)
Serum proline	–	Cramér *et al*. (1974)	–	–
6-kPGF1α, TXB2, tubular antigen	–	Cárdenas *et al*. (1993)	–	–
Gout	–	Batuman *et al*. (1981); Pollock & Ibels (1988)	–	–
Renal mortality	–	McMichael & Johnson (1982)	–	–

–, no data; BUN, blood urea nitrogen; 6-kPGF1α, 6-ketoprostaglandin F1 alpha; TXB2, thromboxane B2

(*a*) *Humans*

(i) *General population*

In a longitudinal study of 459 men, Kim *et al*. (1996b) reported a positive correlation between blood lead concentration and impairment of renal function measured by serum creatinine concentrations. A weak positive correlation between serum creatinine and blood lead concentrations had also been found by Staessen *et al*. (1990) in a study conducted among civil servants not subject to industrial exposure to heavy metals. Staessen *et al*. (1992) examined a random population sample, including 965 men and 1016 women (geometric mean blood lead concentrations, 11.4 μg/dL and 7.5 μg/dL, respectively), and reported that creatinine clearance was inversely correlated with blood lead concentrations. A positive correlation was found in this study between serum β2-microglobulin and blood lead concentrations in men.

Verberk *et al*. (1996) reported a positive relationship between concentration of lead in blood (mean ± standard deviation, 34.2 ± 22.4 μg/dL) and the activity of *N*-acetyl-β-D-glucosaminidase (NAG) in urine in 151 children (3–6 years old) who resided at different distances from a lead smelter in Romania. There was a 13–14% increase of urinary NAG activity per 10 μg/dL increase in blood lead concentration, which was indicative of renal tubular damage. Campbell *et al*. (1977) found that increased blood lead concentrations were associated with increased serum urea concentrations and hyperuricaemia in 283 people living in houses known or believed to have lead plumbing systems, with lead concentrations in the drinking-water > 0.1 mg/L.

(ii) *Occupational exposure*

Buchet *et al*. (1980) examined 25 male lead-smelter workers (blood lead concentration range, 33.8–61.3 μg/dL; mean (range) duration of exposure, 13.2 (3.1–29.8) years) and 88 male controls (blood lead concentration range, 5.5–34.2 μg/dL), and found no differences in renal function between the groups and no clinical signs of renal impairment. The authors concluded that blood lead concentrations less than 62 μg/dL were not associated with renal toxicity.

Ong *et al*. (1987) examined renal function in 158 male and 51 female lead-exposed workers (age range, 17–68 years) with mean (± SD) blood lead concentrations of 42.1 (± 16.6) and 31.9 (± 14.3) μg/dL, respectively. Serum creatinine, blood urea nitrogen and creatinine clearance were significantly correlated with blood lead concentrations. After adjusting for age of the subjects, the increase in NAG excretion with increasing blood lead concentration was found to be statistically significant ($p < 0.001$).

Meyer *et al*. (1984) found significant increases in median urinary NAG activity in 29 workers exposed to lead, but there was no correlation with blood lead concentrations. In a later study by Verschoor *et al.* (1987), the excretion of NAG was reported to be a consistent and sensitive parameter of early effects on renal tubular function in workers occupationally exposed to low concentrations of lead. No significant differences were found in various indicators of renal function between 148 male workers exposed to lead (blood lead, 2.29 μM (geometric mean); range, 1.63–3.21 μM) [47.4 μg/dL; range, 33.8–66.5 μg/dL]

and 125 non-exposed workers (blood lead, 0.40 μM (geometric mean); range, 0.27–0.58 μM) [8.3 μg/dL; range, 5.6–12.0 μg/dL] matched for age, smoking habits, socioeconomic status and duration of employment. There were no differences in protein excretion patterns and no signs of renal impairment. However, regression and matched-pair analyses suggested that renal tubular parameters as measured by NAG excretion might be more strongly influenced by exposure to lead than the glomerular parameters. Changes in renal function parameters may occur at blood lead concentrations below 60 μg/dL.

Chia *et al*. (1995) suggested that time-integrated blood lead indices were the most important descriptors of the variability in urinary α1-microglobulin, urinary β2-microglobulin and urinary retinol binding protein in 128 workers exposed to lead (current blood lead concentration range, 7.6–66.2 μg/dL). Urinary α1-microglobulin was the only marker that was significantly higher in the lead-exposed group than in controls, with a good dose–response and dose–effect relationship with the time-integrated blood lead indices.

No clinical signs of renal impairment were observed among active and retired lead-smelter workers with long-term exposure whose blood lead concentrations were below 70 μg/dL (Gerhardsson *et al.,* 1992; see also Roels *et al.,* 1999)

Elevated concentrations of blood urea nitrogen (≥ 20 mg/dL) were reported in 28 of 160 lead-exposed workers whose blood lead concentrations ranged from 16–280 μg/dL (Baker *et al*., 1979). Maranelli and Apostoli (1987) reported significantly higher concentrations of blood urea nitrogen and serum uric acid in 60 workers with lead poisoning (mean ± SD of blood lead, 71.9 ± 16.5 μg/dL) compared with 76 control subjects.

Cramér *et al*. (1974) found significantly lower plasma concentrations of proline, valine, tyrosine and phenylalanine, but no excessive aminoaciduria in five men with heavy occupational exposure to lead (blood lead concentration range, 71–138 μg/dL) compared with non-exposed controls. Typical lead-induced intranuclear inclusion bodies were found only in renal biopsies of the workers with short exposure. Mitochondrial changes were found in all subjects.

Cárdenas *et al*. (1993) reported interference of lead (mean blood lead concentration, 48 μg/dL) with the renal synthesis of eicosanoids, resulting in lower urinary excretion of 6-keto-prostaglandin $F_{1\alpha}$ and an enhanced excretion of thromboxane. As this was not associated with any sign of renal dysfunction, it may represent a reversible biochemical effect or contribute to the degradation of renal function after the onset of clinical lead nephropathy. The urinary excretion of some tubular antigens (BBA, BB50 and HF5) was positively associated with duration of exposure to lead.

(iii) *Clinical studies*

Chisholm (1962) examined renal tubular injury in 23 lead-intoxicated children and compared the pattern of aminoaciduria with that seen in 56 patients with other diseases that impair renal function. Acute lead intoxication in children produced disorders of renal tubular function similar to those of Fanconi syndrome. Hypophosphataemia, aminoaciduria and glycosuria were found in 8/23 children, and most frequently in those with severe

clinical manifestations. The abnormalities disappeared within 2 months, showing that the effect of lead was reversible.

Biagini *et al*. (1977) studied renal morphology in eight patients with chronic lead poisoning (blood lead concentration range, 90–200 μg/dL). The ultrastructural changes, which mainly involved the proximal tubules, were (1) a degenerative pattern (swollen mitochondria, dilated endoplasmic reticulum and scanty microvilli), (2) signs of metabolic hyperactivity (intranuclear granular inclusions, oddly shaped nuclei) and (3) a regenerative pattern (poorly differentiated cells with few microvilli, shallow infoldings of basal cell membranes). In the glomeruli, the most characteristic finding was a mesangial reaction. In some cases, the basement membrane appeared to be thickened and the visceral epithelial cells were hypertrophic. Interstitial fibrosis was present, as well as a certain degree of arteriolar hyperplasia. These findings appear to confirm chronic lead nephropathy.

Wedeen *et al*. (1979) reported reduced glomerular filtration rates (GFR; < 90 mL/min/1.73 m^2; see McIntosh *et al.*, 1928) in 21 of 57 workers with excessive lead body burdens (urinary lead > 1000 μg/24 h, after edetate disodium calcium lead mobilization test). In seven of eight renal biopsy specimens examined by immunofluorescence microscopy, the finding of glomerular and tubular immunoglobulin deposition raises the possibility that an autoimmune response may contribute to the interstitial nephritis that occurs in occupational lead nephropathy.

Batuman *et al*. (1981) examined 44 male patients with gout by using the ethylenediaminetetracetic acid (EDTA) lead-mobilization test. The amount of mobilizable lead was significantly greater in patients with gout who had renal impairment than in patients with gout who had normal renal function, although lead blood concentrations were not significantly different between the groups (26 ± 3 and 24 ± 3 μg/dL, respectively). Renal function (determined by the serum creatinine concentration) correlated with mobilizable lead in all 44 patients. The data indicate that lead plays a role in gout nephropathy. If lead nephropathy with gout or hypertension is suspected, the diagnosis may be confirmed using an EDTA chelation test (Pollock & Ibels, 1988).

An age-standardized proportional mortality analysis was conducted among 241 lead-smelter workers diagnosed with 'lead poisoning' between 1928 and 1959. Among the 140 deaths in this group, the study showed a substantial excess in the numbers of deaths from chronic renal disease, particularly prior to 1965 (see also Section 2.1.2). A moderate excess was also apparent for other smelter workers, not diagnosed with lead poisoning. In recent years, these excesses of mortality in lead-exposed workers have largely disappeared (McMichael & Johnson, 1982).

(*b*) *Animal studies*

Chronic intoxication with lead is associated with the presence of characteristic intranuclear inclusions in proximal tubular epithelial cells of the kidney. Chemical analysis of these inclusion bodies has indicated the presence of lead as well as of protein, presumably of the non-histone type. The inclusion bodies are eosinophilic and do not appear to contain DNA or RNA. Lead-induced formation of nuclear inclusion bodies has been observed in

kidneys of rabbits (Hass *et al*., 1964), rats (Goyer *et al*., 1970; Choie & Richter, 1972a,b), monkeys (Allen *et al*., 1974) and dogs (Stowe *et al*., 1973).

Moore and Goyer (1974) used differential centrifugation to isolate inclusion bodies from renal tubular cells of rats exposed to lead and studied their biochemical composition. The inclusion bodies contain about 40–50 μg lead/mg protein and may function as an intracellular depot of non-diffusible lead. Further studies indicated that protein-bound lead in renal tubular cells may be partitioned between insoluble and non-diffusible, morphologically-discrete inclusion bodies and a soluble, extractable fraction that is presumably diffusible.

Goyer and Wilson (1975) demonstrated that the nuclear inclusion bodies formed in lead-treated rats could be disrupted and removed from the nuclei by the administration of EDTA and that this removal corresponded to peak urinary excretion of lead. The sharp increase in urinary lead following EDTA therapy is the result, at least in part, of chelation and excretion of sequestered lead bound to nuclear protein and indicates that the formation of inclusion bodies is reversible.

The lowest chronic exposure to lead resulting in a detectable renal effect in rats has been reported to be 5 mg/L in drinking-water, which resulted in a median blood lead concentration of 11 μg/dL (Fowler *et al*., 1980). At this exposure level, cytomegaly and karyomegaly were found in renal proximal tubular cells. Proximal tubular cells from rats exposed to 50 and 250 ppm lead for 6 or 9 months showed intranuclear inclusion bodies. Inhibition of renal mitochondrial respiration and swollen mitochondria were seen at 9 months of exposure, but these changes were not evident at 6 months.

Huguet *et al*. (1982) reported acute kidney damage following intraperitoneal administration of lead acetate (0, 0.05, 0.15 and 0.30 mmol Pb^{2+}/kg bw) to groups of five male and five female rats. Minimal kidney damage shown by increased urinary γ-glutamyl transferase activity was observed only in males given the highest dose. In all animals and at all doses, natriuria was significantly decreased on the first day (from 4 h after administration). Such changes evoke mild tubular abnormalities but glomerular disturbances may also be involved.

Khalil-Manesh *et al*. (1992a) studied the progression of lead nephropathy in rats given lead acetate at a high dose (5% in drinking water) for 1–12 months. Control animals were pair-fed. In the exposed rats, the glomerular filtration rate (GFR) was significantly higher than in the controls after 3 months of lead exposure, but was lower than the controls after 12 months. Lead inclusion bodies were found in nuclei of proximal convoluted tubules and the pars recta in all lead-treated animals from 1 month onwards. Tubular atrophy and interstitial fibrosis first appeared at 6 months, and increased in severity thereafter. Brush borders of proximal tubules were disrupted at 1 and 3 months, but recovered thereafter. After 3 and 6 months of lead exposure, urinary NAG and glutathione *S*-transferase (GST) concentrations were elevated in the exposed rats compared with controls, but at 9 and 12 months the differences were not all significant. Concentrations of urinary brush border antigens were also increased above controls at 1 and 3 months, but were decreased at 6 and 12 months, correlating with morphological changes in the brush border. The authors concluded

that a high dose of lead in rats may initially stimulate both renal cortical hypertrophy and an increase in GFR. Later, the adverse effects of lead on the tubulointerstitium predominate, and the GFR decreases. The urinary marker, NAG, was found to be abnormal in the early stages post-exposure, but age-related changes obscured this abnormality at later stages and urinary GST appeared to be a more consistent marker of injury.

In the same experimental system, administration of the chelator dimercaptosuccinic acid (DMSA) resulted in an improvement in GFR and a decrease in albuminuria, together with a reduction in size and number of nuclear inclusion bodies in proximal tubules (Khalil-Manesh *et al*., 1992b). Overall, treatment with DMSA improved renal function but had less effect on pathological alterations.

In rats exposed via drinking-water to 5000 mg/L or 100 mg/L lead acetate for 1–12 months, GFR and blood lead concentrations correlated positively during the first 6 months of treatment. GFR and red blood cell membrane Na-K-ATPase correlated negatively at 6 and 12 months in rats given the high dose (Khalil-Manesh *et al*., 1994).

Moser *et al*. (1995) reported effects of acute and chronic exposure to lead on GST isoforms during kidney development in rats. In the acute exposure experiment, rats of 14 and 50 days of age were given three daily intraperitoneal injections of lead acetate (114 mg/kg bw) for 3 days and were sacrificed 24 h after the third injection. In the chronic exposure studies, rats received lead acetate in drinking-water (50–500 ppm) from the day after conception. Acute and chronic lead exposure were found to have similar effects, causing increases in all but one GST isoform (Yb3); these increases were markedly higher under conditions of dietary calcium depletion. Lead-related increases in GSTs were partially reversed by transferring the animals to lead-free water for a 4-week period.

4.2.4 *Neurological and neurotoxic effects*

(*a*) *Humans*

(i) *Neurological symptoms of high-level exposure to lead*

Neurological and neurotoxic effects of lead are well recognized in both adults and children. High-level exposure to lead causes symptomatic lead poisoning.

Both the peripheral and the central nervous system are targets for lead, although peripheral neural effects (wrist drop and slowing of nerve conduction velocities) have, so far, been described largely in adults in occupational settings. Lead encephalopathy has been reported to occur in cases of acute symptomatic lead poisoning and its severity depends on a combination of factors, including the intensity and duration of exposure.

Children are more vulnerable than adults to the effects of lead for several reasons: a greater proportion of ingested lead is absorbed from the gastrointestinal tract of children than of adults, more lead gains access to the brain of children than of adults, and the developing nervous system is far more vulnerable to the toxic effects of lead than the mature brain (Leggett, 1993).

The symptoms of severe lead poisoning in children are typically associated with a blood lead concentration of 70 μg/dL, but can occur in some children at a concentration of 50 μg/dL (Adams & Victor, 1993). The early symptoms include lethargy, abdominal cramps, anorexia and irritability. Over a period of days or weeks, in children younger than 2 years of age, there is progression to vomiting, clumsiness and ataxia; then to alternating periods of hyperirritability and stupor; and finally coma and seizures. Children who survive are either severely cognitively compromised or frankly mentally retarded (reviewed by Lidsky & Schneider, 2003).

Rahman *et al.* (1986) described six infants, three of them neonates, diagnosed as having acute lead poisoning; four had acute encephalopathy. All had been given an indigenous preparation, 'Bint Al Zahab' (Daughter of Gold), for abdominal colic and early passage of meconium after birth. Chemical analysis of this powder revealed a lead content of 82.5%. The index case had anaemia with punctate basophilia, dense metaphysial lines on X-ray and markedly raised blood lead concentrations, arousing a strong index of suspicion for the early diagnosis of subsequent cases. Computerized axial tomography (CAT) scan in three cases showed signs of early cerebral cortical atrophy. The picture of cerebral oedema was absent in the four cases of acute lead encephalopathy.

In a later study by Al Khayat *et al.* (1997b), a group of 19 infants (mean age, 3.8 months) showed symptoms consistent with acute lead encephalopathy following the use of traditional medicines. All children presented with convulsions, and CAT scans of the brain showed oedema in four patients and atrophy in four others. Cerebrospinal fluid of nine children was analysed and showed pleocytosis in six and a high protein content in eight cases. The median lead concentration in the blood of these 19 infants was 74.5 μg/dL, and seven children had a mean lead concentration of 57 μg/dL which is below the proposed threshold (70 μg/dL) for encephalopathy. The children received chelation therapy. During follow-up 13 infants were observed to have developed brain damage. The results indicate that acute encephalopathy may occur in very young infants at lead concentrations lower than previously reported.

Blood concentrations of lead below that which produces clear clinical symptoms are also neurotoxic in children and have lasting effects on neurobehavioural function. Lead poisoning at these lower levels of exposure is far more common and is particularly insidious because of its lack of diagnostically-definitive physical signs. Some children complain of stomach pains and loss of appetite and may or may not have anaemia. Neurobehavioural deficit resulting from exposure to lead can occur in the absence of clinical symptoms (reviewed by Lidsky & Schneider, 2003).

The characteristic acute and predominantly cerebellar encephalopathy associated with high exposure to lead in neonates contrasts to the subtle, axo-dendritic disorganization shown to be associated with low-level exposure of infants to inorganic lead. In both low-level exposure to inorganic lead and exposure to organolead, there is a preferential involvement of the hippocampus, and the clinical syndromes of irritability, hyperactivity, aggression and seizures are common features of disturbed hippocampal function. Neurotransmitter system abnormalities and changes in glutamate, dopamine and/or γ-aminobutyric acid (GABA) uptake, efflux and metabolism have been described following expo-

sure to inorganic lead. Among these effects, abnormalities of GABA and glutamate metabolism are also found after exposure to organolead. While inorganic lead produces a clinically-definable encephalopathy and neuropathy dependent upon age, route of exposure and dose, the clinical syndrome caused by organolead — i.e. triethyl lead, the neurotoxic metabolite of tetraethyl lead — is characterized by lethargy, tremors, hyperexcitability, hypermotility, aggression, convulsions, ataxia, paralysis and death (Verity, 1990).

(ii) *Impact on hearing induced by low-level exposure to lead*

Lead-induced impairment of the auditory brain and cochlea is believed to contribute substantially to the cognitive disorders and learning disabilities associated with low-level exposure to lead. However, the specific effects of elevated blood lead concentrations on central nervous system physiology and sensory systems, particularly the auditory system have not been clearly elucidated. Furthermore, earlier studies on the effects of lead intoxication on brainstem physiology and auditory sensory-neural functions have resulted in conflicting results (Otto & Fox, 1993).

Several investigations have reported that humans exposed to lead develop auditory brainstem abnormalities and significant hearing loss.

Holdstein *et al.* (1986) recorded auditory brainstem evoked potentials (ABEP; in response to 75-dBHL (decibels hearing level) clicks presented at rates of 10/sec and 55/sec) from 29 adults and children (age range, 8–56 years) (blood lead concentration range, 30–84 μg/dL) who were accidentally exposed to lead in food until approximately 1 year prior to the study. A prolonged interpeak latency difference (between peaks I and III) was the most significant recurring result, with longer intervals in lead-exposed children compared with the control group. Increasing stimulus rate, on the other hand, affected exposed adults to a greater extent than the children. The results may imply an impairment of the peripheral portion of the auditory system with axonal and myelin involvement.

Otto *et al.* (1985) evaluated 49 children aged 6–12 years for residual effects of lead exposure using the ABEP test. The initial blood lead concentration range in these children was 6–59 μg/dL, the range at the time of ABEP testing was 6–30 μg/dL. A linear relationship between blood lead concentration and slow brain wave voltage during sensory conditioning was observed at initial evaluation and at follow-up after 2 years. No significant relationship between blood lead concentration and slow wave voltage during passive conditioning was found at the 5-year follow-up. A significant linear relationship between the original blood lead concentrations and the latency of waves III and V of the ABEP was also reported. The latency of both waves increased as a function of the initial blood lead concentration, which is suggestive of subclinical pathology of the auditory pathway.

Schwartz and Otto (1987) used NHANES data to confirm the relationships previously observed between blood lead concentration and hearing threshold and found that the probability of elevated hearing thresholds at 500, 1000, 2000 and 4000 Hz increased significantly for both ears with increasing blood lead concentration. However, others have reported a lack of effects on auditory sensory-neural function.

Counter *et al.* (1997a) investigated blood lead concentrations and auditory sensory-neural function in 62 schoolchildren living in a lead-contaminated area of Ecuador and 14 children in a neighbouring area with no known lead exposure. The median blood lead concentration in the lead-exposed group was 52.6 µg/dL (range, 9.9–110.0 µg/dL) compared with 6.4 µg/dL (range, 3.9–12.0 µg/dL) in the non-exposed group ($p < 0.001$). Auditory thresholds for the lead-exposed group were normal at the pure tone frequencies of 2500–8000 Hz over the entire range of blood lead concentrations. Auditory tests in seven of the children with high blood lead concentrations showed normal absolute peak and interpeak latencies. In a more extensive neurophysiological and audiological study conducted by the same research group, the exposed children showed normal wave latencies and neural transmission times, with no statistical correlation between blood lead concentrations and interpeak latencies. Audiological tests indicated normal cochlear function and no statistical relation between auditory thresholds and blood lead concentration (Counter *et al.*, 1997b).

(iii) *Visual functions affected by low-level exposure to lead*

In 19 gun metal founders occupationally exposed to lead (initial blood lead concentrations, 16–64 µg/dL), Araki *et al.* (1987) found that the N2 latency — conduction time from the retina to the visual cortex — of the visual-evoked potential (VEP) was significantly prolonged. Twelve months later, after improvement of the work environment, the N2 latency had returned to the normal level. This change was correlated positively with absorption indicators of lead and inversely with those of zinc and copper. This suggests that lead interferes with visual function, and that this interference is antagonized by zinc and copper. In another study, an increase in P100 latency — i.e. the latency of the VEP-positive peak 100 msec after stimulus onset — was reported in 17 lead-exposed workers (non-smokers, blood lead concentrations, 25–52 µg/dL) compared with 27 unexposed controls, while the N75 latency — i.e. the latency of the VEP-negative peak 75 msec after stimulus onset — was not affected. However, no significant effects of lead were observed for the smokers or for the total subject population (31 exposed, 54 controls) (Solliway *et al.*, 1995). The results indicate that lead affects neural function even at permitted levels of exposure.

Altmann *et al.* (1998) investigated 384 children (age, 5.0–7.8 years) from lead-polluted areas for the impact of lead on the visual system. The range of blood lead concentrations in these children was 1.4–17.4 µg/dL. Statistically significant lead-related changes were found only for some of the VEP interpeak latencies after adjusting for confounding effects. All other outcome variables were not significantly related to lead concentrations.

(iv) *Peripheral nervous functions affected by low-level exposure to lead*

Nerve conduction studies have been carried out in chronically-exposed industrial workers with elevated blood lead concentrations but with no clinical evidence of neuropathy. In one of the first studies of this kind, Seppäläinen *et al.* (1975) found evidence for

asymptomatic slowing of the motor nerve conduction velocity in 26 exposed workers whose blood lead concentrations had never exceeded 70 μg/dL. In a further study among 78 workers with maximal blood lead concentrations (ever recorded) in the range ≤ 40 μg/dL up to ≥ 70 μg/dL, Seppäläinen *et al.* (1979) found that ulnar nerve conduction velocity was depressed in workers whose blood lead concentrations had never exceeded 50 μg/dL. A third study reported slowing of ulnar nerve conduction velocity at blood lead concentrations just above 30 μg/dL (Seppäläinen *et al.*, 1983). Jeyaratnam *et al.* (1985) found that mean maximum motor conduction velocities of the median nerve were significantly lower in workers exposed to lead (mean blood lead concentration, 48.7 μg/dL) than in controls (mean blood lead concentration, 15.8 μg/dL). Other studies have also reported reduction in peripheral nerve conduction velocities in workers exposed to lead (Chen *et al.*, 1985; Murata *et al.*, 1987).

Triebig *et al.* (1984) studied 148 male workers exposed to lead from the manufacture of storage batteries, and 66 non-exposed controls. Statistically significant differences in nerve conduction velocities were seen only for the distal sensory fibres of the ulnar and median nerves. In contrast to the reports mentioned above, the authors concluded that at blood lead concentrations below 70 μg/dL, no functionally significant lead-induced reduction of nerve conduction velocity is to be expected. These findings repeated and confirmed the earlier results of Spivey *et al.* (1980) and Nielsen *et al.* (1982).

Schwartz *et al.* (1988) demonstrated a negative correlation between blood lead concentration and motor nerve conduction velocity in 202 asymptomatic children aged 5–9 years living near a lead smelter in Idaho, USA, whose blood lead concentrations ranged from 13–97 μg/dL. The authors found evidence for a threshold in three regression analyses: at a blood lead concentration of 30 μg/dL in 'hockey stick' regression, at 20 μg/dL in logistic regression and at 25–30 μg/dL in quadratic regression. Age, sex, socioeconomic status or duration of residence near the smelter did not significantly modify the relationship. The study confirmed that, in the absence of symptoms, increased lead absorption caused slowing of nerve conduction in children, but also indicated that measurement of maximal motor nerve conduction velocity is an insensitive screen for low-level lead toxicity.

All the above studies relate the findings on nerve conduction velocity to 'current' blood lead concentrations in humans. This practice is not ideal as the toxicity of lead to the peripheral nerves progresses over time. Chia *et al.* (1996a) studied 72 workers in a lead-battery manufacturing plant and 82 non-exposed referents. At the time of the study, mean blood lead concentrations in these groups were 36.9 (range, 7.3–68.5) and 10.5 (range, 4.4–19.8) μg/dL, respectively. Past blood lead measurements were available for 62 workers, for whom the mean cumulative blood index was 136.8 (range, 6.7–1087.0) μg-year/dL. There was a significant reduction in sensory conduction velocity of the median nerve of the dominant forearm for the group of 49 workers with mean cumulative blood lead index > 40 μg-years/dL. Current blood lead concentrations, however, did not show any trends against the nerve conduction parameters.

Ishida *et al.* (1996) studied 58 male and 70 female ceramic painters, aged 29–75 years, with lead concentrations in blood ranging from 2.1–69.5 μg/dL. They examined maximal

conduction velocity in the median nerve of the forearm as a measure of motor nerve function, the variation in the cardiac cycle time in electrocardiography as a measure of parasympathetic function, and changes in finger blood-flow volume and drop velocity with change in posture from the supine to standing position as a measure of sympathetic function. No significant association was found between blood lead concentrations and the results of these neurophysiological tests.

(v) *Neurotoxicity of lead in children*

The neurotoxicity of lead was recognized as early as the 1st century AD when Dioscorides, physician to Nero, wrote that "Lead makes the mind give way." Childhood lead poisoning was first reported at the end of the 19th century (Lockhart Gibson *et al*., 1892). Until the 20th century, it was generally thought that lead-exposed individuals who did not die during the acute illness were left without any trace of their exposure. When a study of children who had recovered from acute lead poisoning showed impaired cognition, poor school performance and increased antisocial behaviour (Byers & Lord, 1943), the long-term effects of lead toxicity were established and the modern era of lead toxicology began. Until the 1970s, it was thought that these residua were found only in children who had displayed clinical signs of encephalopathy. Among studies in the early 1970s of children in the USA who had no overt symptoms, four found lead-associated deficits in intelligence quotient (IQ) (David, 1974; Perino & Ernhart, 1974; De la Burdé & Choate, 1975; Landrigan *et al*., 1975c), while three found no significant differences between exposed and unexposed children (Kotok, 1972; Lansdown *et al*., 1974; Baloh *et al*., 1975). These early studies tended to have small sample sizes and low statistical power; many used insensitive measures of cognition; covariate control was limited; and the exposure measure was lead in blood, which is a short-term storage system for lead. Later studies, using larger samples, more appropriate and sensitive outcome measures and better covariate control, tended to report impaired cognition at concentrations of lead in blood well below those associated with clinical symptoms. Not all studies reported significant effects, and the issue of silent lead exposure has continued until quite recently to be a source of contention.

Cross-sectional studies

Byers and Lord (1943) were alerted to the possibility of long-term effects of lead poisoning when two children were referred for aggressive behaviour. They were recognized as children who had in the past been treated for lead poisoning and discharged as recovered. A further 20 children with similar histories were then identified and it was found that 19 had school failure, behavioural disorders or mental retardation.

David (1974) compared blood and penicillamine-provoked urinary lead concentrations in 54 children with hyperactivity with corresponding values in 37 controls and found that the hyperactive children had increased lead concentrations in blood and urine.

Perino and Ernhart (1974) compared 30 children with blood lead concentrations $> 40\,\mu g/dL$ with 50 children with concentrations ranging from 10–30 $\mu g/dL$. Using

multiple regression to control for age, parental intelligence and birth weight, they found a significant inverse relationship between blood lead concentrations and McCarthy intelligence scores.

De la Burdé and Choate (1975) compared 67 children who had been exposed to lead, but displayed no acute symptoms, with a group of 70 controls with no known exposure. Exposed children had deficits in global IQ and associative abilities, visual and fine motor coordination and behaviour. School failure due to learning and behavioural problems was more frequent in the lead-exposed than in the control group.

Landrigan *et al*. (1975c) compared a group of children who lived in the vicinity of a smelter and had blood lead concentrations > 40 μg/dL with children of similar socioeconomic status with blood lead concentrations < 40 μg/dL. The children with the higher lead concentrations were found to have significantly lower scores in performance and full-scale IQ tests, as well as in a finger–wrist tapping test, which measures fine motor function.

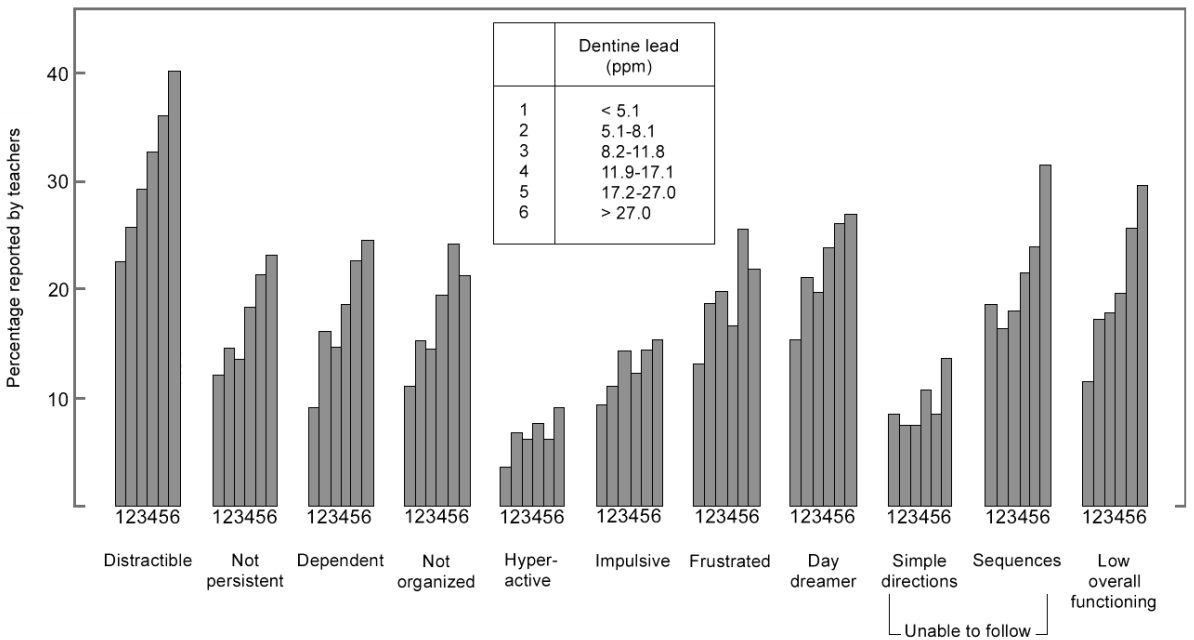
At the end of the 1970s and the beginning of the 1980s, studies were conducted with larger sample sizes, better covariate control and more sophisticated use of statistics. Needleman *et al*. (1979), using lead in shed deciduous teeth as marker of exposure, compared 58 children with high concentrations of lead in their dentine (> 24 μg/g) with 100 children with low concentrations (< 6 μg/g). After control for covariates, children with high lead concentrations had significantly lower IQ scores, impaired attention and reduced language function than those with low concentrations. Teachers' negative ratings of 2146 children on a forced-choice classroom behavioural rating scale were related to higher dentine lead concentrations (Figure 8).

Yule *et al*. (1981) classified 166 children by their blood lead concentrations (range, 7–33 μg/dL) and found significant negative associations with IQ, reading and spelling. A later study used the teachers' rating scale employed by Needleman (see Figure 8) and found the same results (Yule *et al*., 1984).

Winneke *et al.* (1982) studied 458 school-age children whose dentine lead concentrations had been measured (range, 1.4–12.7 μg/g). From this group, two subgroups of 26 children each (mean age, 8.5 years) were chosen with low (means, 2.4 μg/g) and high (means, 9.2 μg/g) tooth lead concentrations, respectively. The groups were matched for age, sex and father's occupational status. The high-lead group scored significantly lower ($p < 0.05$) in two perceptual motor-integration tests and had a 5–7 point lower IQ (nearly significant, $p < 0.1$) than the low-lead group. In a further study (Winneke *et al.*, 1983) of 115 school-age children living in a lead-smelter area (mean tooth lead concentration, 6.2 μg/g; range, 1.9–38.5 μg/g), inverse associations — some of which were significant, $p < 0.05$ — were found between tooth lead values and outcomes of perceptual motor-integration and reaction-performance tests. After correction for confounding, there remained a tendency for children with tooth lead > 10 μg/g to have on average a 4.6-point lower IQ than children with tooth lead ≤ 4 μg/g.

Smith *et al*. (1983) measured dentine lead in 402 schoolchildren in London and reported that, after covariate adjustment, children with high lead concentrations (mean, 11 μg/g) had lower verbal IQ, performance IQ and full-scale IQ scores than those with

Figure 8. Teachers' ratings on forced-choice behavioral items classified by ascending dentine lead level



Modified from Needleman *et al.* (1979)
The group boundaries were chosen to obtain symmetrical cell sizes for the median (groups 1 and 6 = 6.8 per cent, groups 2 and 5 = 17.6 per cent, and groups 3 and 4 = 25.6 per cent).

intermediate (mean, 6 μg/g) and low dentine lead (mean, approx. 3 μg/g). The exact *p*-values were not given, and the differences were reported as 'not significant'. Children with high lead concentrations also had lower scores (also reported as 'not significant') on a word reading test.

Lansdown *et al*. (1986) found no significant associations between lead and IQ in a study of 194 children classified by blood lead concentrations in the range 7–24 μg/dL.

Fulton *et al*. (1987) evaluated 501 primary-school children aged 6–9 years in Edinburgh, United Kingdom, using the British Ability Scales combined scores. Lead burden was measured by blood lead concentrations (range, 3.3–34 μg/dL), and 33 covariates were controlled for in the multiple regression model. A significant inverse relationship between lead and cognitive scores was found with no evidence of a threshold.

Silva *et al*. (1988) studied 579 children and found no association between blood lead concentration (mean ± SD, 11.1 ± 4.9 μg/dL; range, 4–50 μg/dL) and IQ, but a significant association with behavioural problems, including inattention and hyperactivity as reported by teachers and parents.

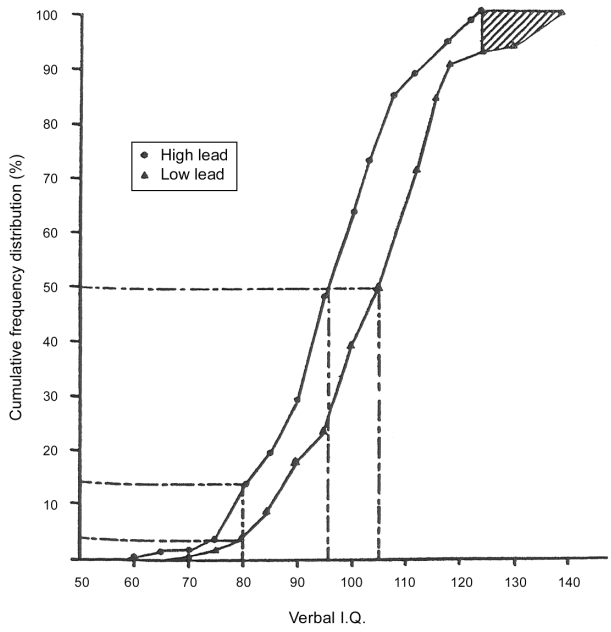
Hansen *et al*. (1989) studied the relationship between dentine lead concentration (average, 10.7 μg/g; range, 0.4–168.5 μg/g) and IQ in 162 schoolchildren in Denmark. After adjustment for covariates, significant inverse associations were found between lead and IQ ($p < 0.01$) and visual motor performance ($p < 0.001$).

Wang *et al*. (1989) studied 180 elementary-school children in China and found a significant inverse relationship between blood lead concentration (mean, 21.1 μg/dL; range, 4.5–52.8 μg/dL) and IQ as measured by the revised Wechsler intelligence scale for children (WISC-R).

Greene and Ernhart (1993) obtained IQ scores of 164 children aged 4 years and 10 months and measured lead concentrations in blood and in dentine of shed deciduous teeth. Using multiple regression, the association was measured with and without controlling for Home Observation for Measurement of the Environment (HOME) scores. Verbal IQ and performance IQ were inversely related ($p < 0.001$ and $p = 0.025$, respectively) to dentine lead concentration in the absence of HOME score adjustment. When HOME was entered into the model, the relationship between lead and performance IQ was no longer significant (at $p = 0.590$). An errors-in-variables analysis was applied, and verbal IQ continued to be significantly (but inversely) related with dentine lead ($p = 0.011$).

Some critics of the association between lead and intelligence have argued that the effect of lead is small and therefore inconsequential (Ernhart *et al*., 1989). Figure 9 shows the cumulative frequency distribution of IQ scores in subjects with high and low lead concentrations in the study of Needleman *et al.* (1982). It can be seen that a median difference of six points is associated with a fourfold increased rate of severe deficit (IQ < 80). In addition, 5% of the subjects with high lead concentrations were prevented from achieving superior function (IQ > 125).

Figure 9. Cumulative frequency distribution of verbal IQ scores in subjects with low or high levels of lead



Modified from Needleman *et al.* (1982)

Prospective studies

McMichael *et al*. (1988) followed a cohort of 537 children living in the vicinity of a lead smelter in Australia from birth onwards. At 4 years of age, an inverse association was found between body lead burden and mental development, as measured according to the McCarthy Scales of Children's Abilities. At 11–13 years of age, the inverse association of blood lead concentrations with WISC scores continued to be significant (Tong *et al*., 1996).

Ernhart *et al*. (1989) studied a group of 242 infants, and reported no significant covariate-adjusted associations between intelligence test scores of these preschool children and lead concentrations in maternal blood, umbilical cord blood or venous blood of the children up to 4 years of age. The strength of the negative inference is lessened by the use of a sample in which half of the mothers were alcohol abusers.

Needleman *et al*. (1990) followed-up 132 adolescents (mean age, 18.4 years) from the group first tested 11 years before (see above). At the time of the re-examination, blood lead concentrations were measured for 48 subjects; all were < 7 µg/dL. Subjects were grouped in quartiles according to their earlier dentine lead concentrations (< 5.9, 6.0–8.2, 8.3–22.2 and > 22.2 µg/g). Higher lead concentrations (> 20 µg/g) were associated with lower class

standing in the senior year in high school, increased absenteeism, lower vocabulary and grammatical reasoning scores, poorer eye–hand coordination, longer reaction times and slower finger tapping. Having an elevated dentine lead concentration in childhood was associated with a sevenfold increased risk for failing to graduate from high school and a sixfold risk for reading disability.

Bellinger *et al*. (1992) studied a group of 148 infants at birth and at 6,12, 18, 57 and 120 months of age. Blood lead concentrations at 2 years, but not at other ages, were significantly associated with a reduced IQ score at both 57 and 120 months of age. Over the range 0–25 μg/dL, a 10-μg/dL increase in blood lead concentration at 24 months was associated with a 5.8-point decline in WISC-R score.

Fergusson *et al*. (1997) followed a birth cohort of 1265 children in New Zealand until 18 years of age. Lead burden was measured at age 6–8 years by deciduous teeth analysis. At age 18, after adjustment for confounders and errors in measurement, subjects with elevated dentine lead concentrations had significantly poorer reading scores, lower levels of success in school examinations and greater likelihood of failure to graduate.

Schnaas *et al*. (2000) followed a group of 112 children in Mexico at 6-month intervals from 6 to 60 months. After adjustment for covariates, lead was significantly related to the general cognitive index on the McCarthy scales. The postnatal lead concentrations (mean value of measurements at 6, 12 and 18 months) had a maximum effect on the cognitive index at 4–5 years of age.

Wasserman *et al*. (2000) followed 442 children in a lead-exposed and a non-exposed area in Serbia and Montenegro from before birth until 7 years of age and found that elevations in both prenatal and postnatal blood lead concentrations were related to reduced scores in cognitive ability tests (McCarthy scales; WISC).

Coscia *et al*. (2003) followed a cohort of 196 children from birth to 15 years of age, and applied growth-curve analysis to study the association between exposure to lead and cognition parameters measured at 6.5, 11 and 15 years of age. Blood was collected prenatally from the mother near the end of the first trimester of pregnancy, approximately 10 days after birth, every 3 months until the age of 5 years, at 66, 72 and 78 months, and at approximately 15 years of age. The highest mean blood lead concentration for this group of children was 17.03 ± 8.13 (SD) μg/dL, measured at 2 years of age. Children with higher lead concentrations showed lower verbal IQ scores over time, and greater decline in the rate of vocabulary development.

Recent studies

Following the removal of lead from gasoline, blood lead concentrations in the general population — first in the USA and then in Europe — began to decline. Mean blood lead concentrations in the USA were 15 μg/dL in 1975, 9 μg/dL in 1980 and 2 μg/dL in 2000 (NHANES IV). This decline has permitted investigators to compare exposed subjects to reference groups at 1 μg/dL, an opportunity that was foreclosed when the mean blood lead concentration in the general population was 15 μg/dL.

Two recent studies of the effects of extremely low concentrations of lead have been published. Lanphear *et al*. (2000b) examined data from the NHANES III on 4853 children between the ages of 6 and 16 years. The association between scores on arithmetic and reading skills, achievement scores, and blood lead concentration was measured; adjustments were made in the multiple regression analysis for age, race, sex, region of the country, parental marital status and education, poverty level and serum cotinine concentration. The geometric mean blood lead concentration was 1.9 μg/dL. Significant inverse relationships were found for arithmetic and reading tests at blood lead concentrations < 5 μg/dL, for block design at blood lead concentrations < 7.5 μg/dL and for digit span at blood lead concentrations < 10 μg/dL.

Canfield *et al*. (2003) examined the association between blood lead concentrations and Stanford-Binet Intelligence Scale (SBIS) scores in a prospective study of 172 children aged 6–60 months. A longitudinal analysis, adjusting for sex, birth weight, blood iron status, mother's IQ, education, race, tobacco use, income and HOME scores, was conducted. Mean blood lead concentrations were 3.4 μg/dL at 6 months, 9.7 μg/dL at 24 months and 6.0 μg/dL at 60 months. A significant inverse relationship between these average blood lead concentrations and SBIS scores was found.

The studies by Lanphear *et al.* (2000b) and Canfield *et al.* (2003) support the meta-analysis of Schwartz (1994) who reanalysed the data from the Boston prospective study (Bellinger *et al*., 1992) and several others. Using non-parametric regression, Schwartz (1994) found an inverse relationship between IQ and blood lead concentrations below 5 μg/dL.

Lead and antisocial behaviour

Suggestions that lead exposure may have a role in antisocial behaviour are not new. Parents of lead-poisoned children have frequently complained that, after recovery from the acute illness, their children became oppositional, aggressive or violent. In the first follow-up study of lead-poisoned children, Byers and Lord (1943) found that 19/20 subjects had severe behavioural problems or learning disorders. Denno (1990) found that the strongest predictor of arrest of youths enrolled in the Collaborative Perinatal Disease Study in Philadelphia, USA, was a history of lead poisoning.

Needleman *et al*. (1996) studied a cohort of 301 boys in the school system in Pittsburgh, USA. Bone lead concentrations, measured by X-RF spectrometry at 12 years of age, were significantly related to parents' and teachers' child behaviour checklist ratings of aggression, attention and delinquency. The subjects' self-reports of delinquent acts were also positively associated with bone lead concentrations. Dietrich *et al*. (2001) studied 195 urban youths and found that prenatal exposure to lead was significantly related with covariate-adjusted increases in parental reports of delinquent and antisocial behaviour. Prenatal and postnatal exposure to lead was associated with self-reports of such behaviour.

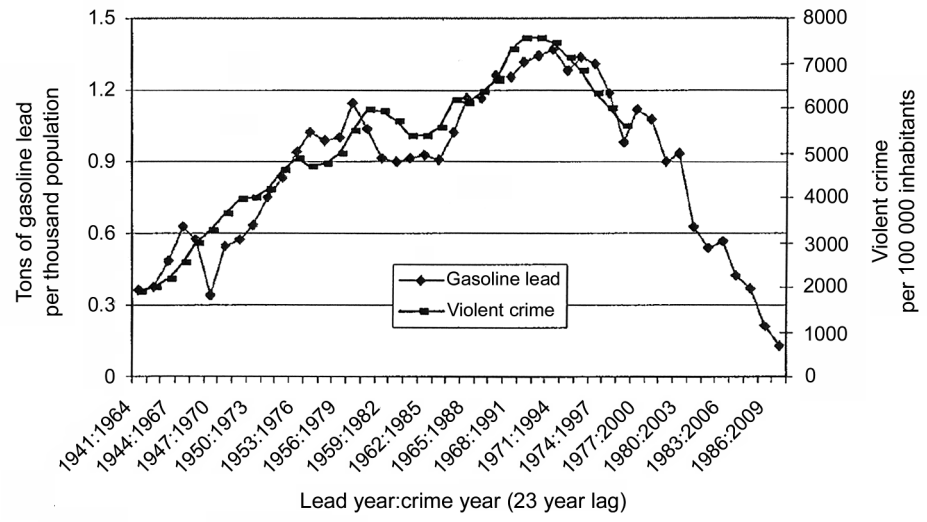
Needleman *et al*. (2002) conducted a case–control study of bone lead concentrations in 194 male youths arrested and adjudicated as delinquents. Cases had significantly higher mean concentrations of lead in their bones than controls (11.0 ± 32.7 μg/g versus

1.5 ± 32.1 µg/g). Logistic regression analysis found an unadjusted odds ratio of 1.9 (95% CI, 1.1–3.2) for a lead concentration ≥ 25 µg/g versus < 25 µg/g. After adjustment for covariates and interactions, adjudicated delinquents were four times more likely to have bone lead concentrations > 25 µg/g (odds ratio, 4.0; 95% CI, 1.4–11.1). In addition, self-reports of delinquency were positively associated with bone lead concentrations.

Two recent ecological studies have reported positive associations between environmental concentrations of lead and antisocial behaviour. Stretesky and Lynch (2001) measured the association between estimated air lead concentrations for all 3111 contiguous counties in the USA (range, 0–0.17 µg/m^3) and homicide data (average over the period 1989–91) from the National Center for Health Statistics. After adjusting for 15 confounding variables, they reported a fourfold increase in homicide in the counties with the highest lead concentrations compared with those with the lowest lead concentrations.

Nevin (2000) reported a statistically significant association between sales of leaded gasoline and violent crime after adjustment for unemployment and percentage of population in the high-crime age group. Figure 10 shows the rates of violent crime in the USA by year in relation to the yearly sales of leaded gasoline.

Figure 10. Violent crime rates and sales of lead gasoline in the USA



Modified from Nevin (2000)

(vi) *Other effects*

Since the late 1980s, several studies have explored the effect of lead exposure on postural stability. Motivated by a positive pilot study with 33 children (Bhattacharya *et al.*, 1988), Bhattacharya *et al.* (1990, 1993; with 63 and 109 children, respectively) confirmed an association between postural sway abnormalities and lead intoxication. There was a

significant relationship between postural sway response recorded at 6 years of age and maximum blood lead concentrations during the second year of life. Chia *et al.* (1994) initially reported lead-induced postural instability among a group of workers exposed to lead compared with non-exposed workers. However, a significant relation between current blood lead concentrations and postural sway parameters could not be established. In a later study (Chia *et al.*, 1996b), there was a significant association between most of the postural sway parameters and the cumulative blood lead concentrations in the 2 years prior to the date of the postural assessment. Current blood lead concentrations were poorly correlated with most of the postural sway parameters. It was concluded that the adverse effect of lead on postural stability is the result of chronic rather than acute exposure to lead.

(vii) *Neurobehavioural effects of organic lead*

To investigate the relationship between bone lead concentration after exposure to organic lead compounds and neurobehavioural test scores, a study was conducted with 529 former organolead workers of mean age 57.6 years. The mean time since last exposure was 16 years. X-RF spectrometry of the tibia was used to estimate accumulated bone lead concentration. Lead-exposed workers had significantly lower scores on visuoconstruction tasks, verbal memory and learning. Peak tibial lead concentrations were associated with decline in verbal and visual memory, executive function and manual dexterity. These effects of lead were reported to be more pronounced in individuals who had at least one ε4 allele of the apolipoprotein E4 gene (Stewart *et al*., 2002).

(*b*) *Experimental systems*

(i) *In-vivo studies with inorganic lead*

Several of the neurological and neurotoxic effects of lead described in humans have been investigated and confirmed in animal model systems. This section reviews a limited number of relevant studies in this area.

Acute lead encephalopathy has been induced experimentally in various animal species, such as the rat, the guinea pig, the baboon and the rhesus monkey.

Five-day-old rats that received the highest non-lethal dose of aqueous lead acetate (1 mg lead/g bw per day) on two consecutive days were found to develop haemorrhagic encephalopathy (Toews *et al*., 1978).

Lead administered to newborn rats postnatally on days 1–15 by daily intraperitoneal injections of 10 mg lead nitrate/kg bw was found to cause haemorrhagic encephalopathy in the cerebellum at 15 days (Sundström & Karlsson, 1987).

Bouldin and Krigman (1975) induced acute lead encephalopathy in adult guinea-pigs that received 2, 3, 5 or 6 consecutive daily oral doses of lead carbonate (155 mg per dose). Clinical signs of intoxication started after 2–3 doses and became more severe with higher doses.

Lead encephalopathy in the baboon (*Papio anubis*) was reported by Hopkins and Dayan (1974). Two adult animals received 6–8 injections of lead carbonate (1 g per injection;

approx. 90 mg/kg initial weight) over a period of 8–12 months. Seizures were observed to begin at 3 and 5 months, respectively, in these two animals.

In rhesus monkeys, encephalopathy was induced by doses of 0.5 g lead subacetate, given by gastric gavage on alternate days, three times a week, for 6–18 weeks (Clasen *et al.*, 1974). Vitamin D (1000 units) was given together with each dose to enhance the alimentary absorption of lead (Sobel & Burger, 1955).

Effects on learning

Experimental studies, primarily with rodent and non-human primate models, have provided evidence that chronic low-level exposure to lead affects learning abilities and behaviour, in particular in the developing animal. The magnitude of these effects appears to be strongly dependent on the developmental period in which exposure takes place (for a review, see Cory-Slechta, 2003). Since learning requires the remodelling of synapses in the brain, lead may specifically affect synaptic transmission, and it has been proposed that the learning deficits caused by lead are due to events regulated by a calcium-dependent protein kinase C (PKC), most likely at the synapse (Bressler *et al*, 1999). However, the effects of lead on PKC studied in brain homogenates *in vitro* may not accurately reflect effects of chronic in-vivo exposure to lead (Cremin & Smith, 2002).

In a study by Altmann *et al.* (1993), rats were exposed chronically to low concentrations of lead at different stages of development, and tested with respect to active-avoidance learning and hippocampal long-term potentiation. When exposure comprised the prenatal and the early postnatal period and was continued into adulthood, both processes were impaired. However, when exposure started at 16 days after birth, neither learning nor hippocampal potentiation was affected. These results reflect the higher vulnerability of the immature hippocampus to lead-induced functional deficits compared with the mature hippocampus.

Effects on visual function

In a study by Kohler *et al.* (1997), rhesus monkeys were exposed pre- and postnatally to 0, 350 or 600 ppm lead acetate in the diet for 9 years. Lead exposure was followed by a 35-month period of lead-free diet. During this period, blood lead concentrations of the treated animals declined to nearly equal those of untreated controls. Lead exposure affected the dopaminergic amacrine cells in the retina by reducing the tyrosine hydroxylase content in these neurons. This neurotoxic effect persisted beyond the end of exposure.

Rice and Hayward (1999) exposed monkeys to lead acetate at 500 or 2000 μg lead/kg bw per day from birth onwards. Spatial and temporal contrast-sensitivity functions were assessed at adulthood and during ageing, by measuring the frequency and amplitude at peak sensitivity and the high-frequency cut-off value. Compared with controls, lead-exposed monkeys exhibited reduced temporal visual function at the first assessment but not the second. There was no evidence of an accelerated decline in contrast sensitivity as a result of exposure.

Effects on hearing

Yamamura *et al.* (1984) gave guinea-pigs intraperitoneal injections of 1% lead acetate once a week for 5 weeks. The animals were examined electrophysiologically using cochlear microphonics and action potential. There were no significant changes in the thresholds of cochlear microphonics. The thresholds of maximum voltage of N1 in the action potential of the animals injected with a total of 100 mg lead acetate were elevated by about 15 dB and increased N1 latency was also observed.

Rice (1997) determined pure tone detection thresholds in a group of six monkeys (*Macaca fascicularis*) dosed with lead acetate (2.8 mg lead/kg bw, 5 days per week) from birth until testing at 13 years of age. Blood lead concentrations at the time of testing were 60–170 µg/dL. Pure tone detection thresholds were determined at six frequencies between 0.125 and 31.5 kHz. Three lead-exposed monkeys had thresholds outside the control range at some frequencies. The findings are consistent with reports of elevated pure tone detection thresholds in lead-exposed humans, although the effect is smaller than might have been predicted given the concurrent blood lead concentrations of the monkeys in this study.

Effects on nerve conduction velocity

Conduction velocity of the optic nerve has been studied in rats that received 7.6 or 15.8 µg lead/kg bw daily by intraperitoneal injection during the first 2 weeks of postnatal life. Optic nerve conduction velocity was examined at 30 days of age in 14 rats taken from 10 different litters. The mean conduction velocities for the two faster axonal groups were 16.8 and 5.4 m/s in control rats, 10.3 and 5.8 m/s in rats given the lower dose and 9.4 and 5.2 m/s in rats given the higher dose of lead. The reduction in conduction velocity for the fastest axons was significant in both dose groups (Conradi *et al*, 1990).

Purser *et al.* (1983) maintained five cynomolgus monkeys at blood lead concentrations of 90–100 µg/dL for 9 months by daily oral dosing with lead acetate (12–15 mg lead/kg bw). The animals showed no clinical or behavioural evidence of lead poisoning at any time during the study, although there was a decrease in packed cell volume, haemoglobin and erythrocyte concentration in the blood. The maximal motor nerve conduction velocity of the ulnar nerve remained constant throughout the study, although changes were observed in the conduction velocity of slowly-conducting nerve fibres. At the end of the study, focal areas of myelin degeneration were found in the ulnar and sciatic nerves.

Effects on motor function and aggressive behaviour

Two groups of rats were given 50 ppm sodium acetate and 50 ppm lead acetate, respectively, in the drinking-water for 3 months. Ocular motor function was tested by rotating the animals on a platform at an increasing angular velocity and measuring ocular nystagmus when the rotation is abruptly stopped. The lead-exposed animals showed a reduction in post-rotatory nystagmus that was significantly related to blood lead and brain lead concentrations, while no such alterations were observed in animals treated with sodium acetate. The results show that low concentrations of lead may impair both sensory and motor

functions, and indicate that these measurements provide a screening tool for neurotoxic effects of lead even in the absence of clinical signs of lead intoxication (Mameli *et al*., 2001).

Young rats (3–4 weeks old) were treated with lead acetate (daily oral doses of 10 mg lead/kg bw) and ethanol (10% v/v in drinking-water), either alone or in combination, for 8 weeks. Motor activity, the number of fighting episodes and several lead-sensitive biochemical indices were measured. Spontaneous locomotor activity and aggressive behaviour were significantly increased in the group ingesting ethanol plus lead compared with the controls. The lead concentrations in blood, liver, kidney and brain were significantly higher in rats exposed simultaneously to lead and ethanol compared with the group treated with lead alone (Flora *et al.*, 1999).

The effects of lead exposure on a feline model of aggression were investigated by Li *et al.* (2003). Five cats were stimulated with a precisely controlled electrical current via electrodes inserted into the lateral hypothalamus. The response measure was the predatory attack threshold, i.e. the current required to elicit an attack response in 50% of the trials. Lead was mixed (as lead acetate) into cat food at doses of 50–150 mg/kg bw lead per day for 4–5 weeks. Blood lead concentrations were < 1, 21–77 and < 20 µg/dL before, during and after lead exposure, respectively. The predatory attack threshold decreased significantly during lead exposure in three of the five cats and increased after cessation of exposure in four of the five cats ($p < 0.01$). There was a significant ($p = 0.0019$) negative association between threshold current and blood lead concentration. These data show that lead exposure enhances predatory aggression in cats.

Effects on neurochemical parameters

While neurological and neurotoxic effects are difficult to define and quantify precisely, neurochemical effects are easy to define and to quantify but their interpretation remains elusive. Most neurochemical studies have been conducted since the 1970s and 1980s (see Tables 89 and 90); in this section, only the most recent and important findings are reported.

Neurochemical parameters were measured in discrete brain areas of rat pups whose mothers were intoxicated with lead in drinking-water (300 ppm) from day 1 of pregnancy until postnatal day 12. This treatment produced a significant reduction in the activity of alkaline phosphatase and ATPase in the brain, and reduced the concentration of adenine nucleotides, most notably in the striatum, but not in the hypothalamus. Lead also reduced the concentration of neurotransmitters throughout the brain, especially in the hippocampus (Antonio & Leret, 2000).

In a study to investigate the effects of lead on antioxidant enzyme activities in the developing brain, female Wistar rats were given drinking-water containing 500 ppm lead (as lead acetate) or 660 ppm sodium acetate during pregnancy and lactation. The activities of superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase were determined in the hypothalamus, hippocampus and striatum of male pups at 23 or 70 days of age. In 23-day-old pups, the activity of SOD was decreased in the hypothalamus. There

Table 89. Effects of lead on catecholamines

Reference	Animal	Major results
Silbergeld & Goldberg (1975)	Mouse	Increase in norepinephrine in forebrain, midbrain, brainstem
Silbergeld & Chisolm (1976)	Mouse	Increase in VMA in whole brain
Sauerhoff & Michaelson (1973)	Rat	No change in norepinephrine, decrease in dopamine in whole brain
Golter & Michaelson (1975)	Rat	Increase in norepinephrine, no change in dopamine in whole brain
Sobotka & Cook (1974)	Rat	No change in norepinephrine in brain
Hrdina *et al.* (1976); Dubas *et al.* (1978); Jason & Kellog (1981)	Rat	Decrease in norepinephrine in hypothalamus, striatum, brainstem
Sobotka & Cook (1974)	Rat	No change in dopamine in cortex, brainstem, hypothalamus, striatum and forebrain

VMA, vanillylmandelic acid

Table 90. Cholinergic effects of lead

Reference	Animal	Cholinergic effects
Silbergeld & Goldberg (1975)	Mouse	No change in acetylcholine in whole brain
Modak *et al.* (1975); Shih & Hanin (1978)	Rat	No change in choline and acetylcholine in cerebellum, hippocampus, midbrain, pons-medulla, cortex, and striatum
Modak *et al.* (1975); Hrdina *et al.* (1976)	Rat	Increase in acetylcholine in diencephalon and cortex
Modak *et al.* (1978)	Rat	Decrease in acetylcholine in whole brain and cerebellum, medulla, diencephalon, cerebrum, striatum, and midbrain.

was no significant effect of the treatment on any of the enzymes and brain regions evaluated in adult (70-day-old) animals. Oxidative stress due to decreased antioxidant function may occur in lead-treated rats at weaning (23 days) but it is not likely to be the main mechanism involved in the neurotoxicity of lead (Moreira *et al.*, 2001).

(ii) *In-vivo studies with organic lead*

The neurotoxic properties of organic lead compounds and their neurobehavioural effects have been reviewed by Walsh and Tilson (1984). The behavioural effects produced by organic lead compounds resemble the sequelae of damage in the limbic system. Alterations in sensory responsiveness or behavioural reactivity and task-dependent changes in avoidance learning are observed following both exposure to organic lead and experimental disruption of the limbic system. In addition, the neurochemical changes induced by organic lead compounds are site-specific and restricted to the limbic forebrain and frontal cortex.

Rat pups (age, 5 days) received 15% ethanol or 3 or 6 mg/kg bw triethyl lead chloride by subcutaneous injection (LD_{50}, 13 ± 1 mg/kg bw). Controls were sham-injected with ethanol. Transient effects included reduced olfactory discrimination on day 7, decreased incidence of nipple attachment on day 9, and fine whole-body tremor on day 10. Persistent hypo-activity was observed on days 15, 22, 24, 26 and 29 in males that received the high dose. A reduction in number, but not in magnitude, of startle responses was also noted in the lead-treated animals. Thus a single postnatal injection of triethyl lead produced transient effects possibly reflecting direct pharmacological activity, as well as long-term effects suggesting potentially permanent alterations in behavioural function (Booze *et al.*, 1983).

Cragg and Rees (1984) administered tetramethyl lead dissolved in olive oil to pregnant rats by subcutaneous injection on days 7, 14 and 21 of gestation. Pups were born on day 22 and received similar injections 7 and 14 days after the last prenatal dose. The total lead concentration in the brain was about 1 μg/g at 28 days. Birth weight was unaffected, but postnatal brain growth was reduced relative to body growth, resulting in a higher body:brain weight ratio. Brain myelination, dendritic growth, granule cell production and retinal receptor development were unchanged. The body:brain weight ratio appeared to be a sensitive parameter for detecting effects on neurological development of exposure to low concentrations of tetramethyl lead, which is neurotoxic at higher concentrations.

(iii) *In-vitro studies*

Various in-vitro studies implicate second-messenger metabolism and protein kinase activation as potential pathways for the disruptive action of lead on nervous system function. These reactions could contribute to the subtle defects in brain function associated with low-level lead poisoning.

To investigate the effects of acute lead exposure on evoked responses in the hippocampus of the rat *in vitro*, field potentials in response to paired-pulse stimulation were measured in rat hippocampal slices perfused with medium containing 0.2–53 μM lead. The evoked population excitatory postsynaptic potentials and the orthodromically-evoked population spike showed a dose-dependent decrease during lead exposure, whereas the presynaptic fibre volley remained unchanged. Within 20 min after the start of exposure, the recorded responses had reached the control level again in spite of further lead perfusion. These results show that lead acts pre-synaptically in the hippocampus, and that it interferes with non-synaptic processes at the pyramidal neurons (Altmann *et al.*, 1988).

Excessive glutamate release in the brain and subsequent neuronal stimulation cause increased production of reactive oxygen species (ROS), oxidative stress, excitotoxicity and neuronal damage. The interaction between glutamate and lead may result in neuronal damage, as glutamate-induced production of ROS is greatly amplified by lead in cultured neuronal cells. Alterations in the activity of protein kinase C seem to play an important role in this process. The neurotoxic effects of lead may be amplified through glutamate-induced neuronal excitation (Savolainen *et al*., 1998).

Lead can substitute for calcium in several intracellular regulatory events associated with neurological function. At nanomolar concentrations, lead activates calmodulin-dependent phosphodiesterase and calmodulin inhibitor-sensitive potassium channels. At picomolar concentrations it activates calmodulin-independent protein kinase C. There is evidence to support the hypothesis that activation of PKC underlies some aspects of lead neurotoxicity (Goldstein, 1993).

4.2.5 *Cardiovascular toxicity*

Cardiovascular effects of lead in humans and experimental systems have been reviewed (Victery, 1988; Goyer, 1993; Hertz-Picciotto & Croft, 1993; WHO, 1995).

(*a*) *Humans*

(i) *Blood lead concentrations and blood pressure*

The literature discussed in the reviews mentioned above can be divided into studies on the general population and occupational cohort studies. Surveys of the general population have been conducted in Belgium, Canada, Denmark, the United Kingdom and the USA. Results of most of the studies suggest positive associations between blood lead concentrations and blood pressure, but some of the studies do not show any significant association.

General population

Staessen *et al*. (1995) carried out an extensive meta-analysis including 23 studies with a total of 33 141 subjects. Among the studies were 13 surveys of the general population and 10 of occupational groups. Most studies took into account confounding factors. The association between blood pressure and blood lead was similar in men and women. For all groups and both sexes combined, a twofold increase in blood lead concentration was associated with a 1.0-mmHg rise in systolic pressure (95% CI, 0.4–1.6 mmHg; $p = 0.002$) and with a 0.6-mmHg increase in diastolic pressure (95% CI, 0.2–1.0 mmHg; $p = 0.004$). A recent update comprising 31 studies (19 surveys in the general population, 12 occupational studies) largely confirmed these results (Nawrot *et al.*, 2002).

Occupational exposure and lead poisoning

In a longitudinal study of > 500 lead-foundry workers who had been examined annually for periods of up to 14 years, Neri *et al*. (1988) found an association between short-term changes in an individual's blood lead concentration and contemporary changes in diastolic

pressure. The average increase in diastolic blood pressure per 1-μg/dL increase in blood lead concentration was 0.3 mm Hg. The association remained significant after allowance for age or time trends and for effects related to changes in body weight.

Parkinson *et al*. (1987) examined the relationship between occupational exposure to lead and diastolic and systolic blood pressure in randomly-selected samples of 270 exposed and 158 non-exposed workers. After controlling for other known risk factors such as age, education, income, cigarette usage, alcohol consumption and exercise, the associations between exposure and blood pressure were small and non-significant.

(ii) *Blood pressure and renal function*

Batuman *et al.* (1983) used the EDTA lead-mobilization test to study the etiological role of lead burden in 48 men diagnosed as having essential hypertension. Patients who had hypertension and a reduced renal function (i.e. serum creatinine > 1.5 mg/dL) had significantly larger amounts of mobilizable lead than did patients who had hypertension without renal impairment. The increase in mobilizable lead was not due to the renal disease itself.

(iii) *Coronary risk of lead exposure*

Silver and Rodriguez-Torres (1968) studied electrocardiograms in 30 children (aged 17–60 months) with lead poisoning (blood lead concentration range, 60–200 μg/dL). Twenty-one patients (70%) had at least one abnormal electrocardiographic finding (mostly myocardial damage) before treatment [details of this treatment were not reported], which persisted in only four (13%) after treatment. The most significant findings were increased heart rate (six patients), and atrial arrhythmia (five patients). More frequent abnormalities were found in children with higher blood lead concentrations.

Kirkby and Gyntelberg (1985) studied the coronary risk profile in 96 heavily-exposed workers (mean ± SD blood lead concentration, 51 ± 16 μg/dL) employed at a lead smelter for 9–45 years. The reference group (mean blood lead, 11 ± 3 μg/dL) was not exposed to lead but was comparable with respect to age, sex, height, weight, social grouping, occupational status and alcohol and tobacco consumption. The exposed workers had slightly higher diastolic blood pressure, significantly more ischaemic electrocardiographic changes, and lower high-density lipoprotein levels than the reference group. The exposed workers with electrocardiographic changes had higher blood pressure than the referents with corresponding changes. These findings indicate a higher coronary risk profile for lead smelter workers, and support the hypothesis of a positive association between lead exposure and arteriosclerosis and high blood pressure.

(*b*) *Experimental systems*

(i) *Cardiovascular effects of lead*

A number of animal experiments have suggested a biphasic response of blood pressure to lead dose (Victery *et al.*, 1982; Victery, 1988; Staessen *et al.*, 1994). Rats were exposed to lead *in utero* and after birth until weaning by giving their mothers 100 or 500 ppm lead (as lead acetate) in drinking-water. This regimen was then continued for the offspring after

weaning. Male rats receiving 100 ppm developed a significant elevation of systolic blood pressure at 3.5 months and remained hypertensive until sacrifice at 6 months; male rats exposed in this way to 500 ppm lead and female rats exposed to 100 or 500 ppm lead remained normotensive. At 6 months, plasma renin activity was significantly reduced in the low-dose male group but was normal in the high-dose group (Victery *et al*., 1982).

In several experiments involving high doses of lead, hypertension was observed, but the nephrotoxicity of lead may have contributed to its development. However, in other high-dose experiments, no hypertension was seen. In contrast, the experiments conducted with lower doses of lead consistently demonstrated a hypertensive effect (Victery, 1988).

Evis *et al*. (1985) reported that chronic (3 or 12 months) low-level exposure of spontaneously hypertensive rats to lead (25 ppm lead (as lead acetate) in the drinking-water) enhanced the susceptibility of the heart to ischaemia-induced arrhythmias at 3 but not at 12 months. In contrast, chronic (3 months) high-level exposure of these rats to lead (250 or 1000 ppm in the drinking-water) resulted in slightly enhanced susceptibility of the heart to arrhythmias induced by myocardial ischaemia (Evis *et al*., 1987).

In experiments in which rats were exposed to lead (0.25, 0.5 and 1.0% lead acetate in the drinking-water) for 90 days, Lal *et al*. (1991) found that the two higher doses of lead resulted in increased arterial blood pressure and calcium influx in atrial trabeculae and papillary muscles. No marked pathological or histochemical changes were observed in heart tissue except congestion (build-up of fluid) and a slightly reduced activity of succinic dehydrogenase in the high-dose group.

(ii) *Studies on the etiology of lead-induced hypertension*

Chai and Webb (1988) reviewed a number of animal studies on the possible role of lead in the etiology of hypertension. The main results indicate that the response of isolated vascular smooth muscle to adrenergic agonists is increased in rats with lead-induced hypertension, and that alterations in the regulation of intracellular calcium concentration may contribute to the abnormal vascular function associated with lead-induced hypertension.

Boscolo and Carmignani (1988) reported that blood pressure was increased in rats receiving 30 and 60 ppm lead (as acetate) in drinking-water for 18 months. The contractile activity of the heart was augmented only in those animals receiving the higher dose of lead, and the heart rate was not modified. Exposure to lead affected the renin-angiotensin system and induced sympathetic hyperactivity by acting on central and peripheral sympathetic junctions and by increasing the reactivity to stimulation of cardiac and vascular β-adrenergic and dopaminergic receptors.

4.2.6 *Immunological effects*

In a recent review by Singh *et al.* (2003), the immunomodulatory role of lead on cellular and humoral components of the immune system is discussed, with particular reference to effector cells such as B cells, T cells, natural killer (NK) cells and soluble mediators such as cytokines, chemokines and nitric oxide (NO).

(*a*) *Humans*

Studies in exposed workers

Ewers *et al.* (1982) examined the sera of 72 male lead-exposed workers (mean age, 36.4 years; range, 16–58 years; blood lead concentration range, 18.6–85.2 μg/dL) and of 53 reference subjects (mean age, 34.8 years; range, 21–54 years; blood lead concentration range, 6.6–20.8 μg/dL) for immunoglobulins IgM, IgG and IgA and complement C3 by radial immunodiffusion. IgA in the saliva was measured in samples from 33 workers and 40 controls. The workers had a mean duration of exposure of 10.2 years (range, 1–34 years). Lead-exposed workers had lower serum IgM ($p = 0.008$) and lower salivary IgA concentrations ($p = 0.008$) than the controls. A significant negative correlation was found between blood lead concentrations and serum concentrations of IgG and complement C3 in the lead-exposed group.

Jaremin (1990) studied the effects on the humoral immune response of exposure to lead in 77 men (mean age, 38.1 years) occupationally exposed to lead for 0.5–24 years. The ambient concentration of lead in air ranged from 0.06 to 1.6 mg/m^3. Three subgroups were distinguished: Group 1 (mean blood lead concentration, 40.1 μg/dL) without traits of lead poisoning; Group 2 (mean blood lead, 72.2 μg/dL) with biochemical features of lead poisoning; and Group 3 (mean blood lead, 106.7 μg/dL) with clinical signs of lead poisoning. Decreased concentrations of IgG and IgM in serum and reduction of the peripheral B lymphocyte pool were observed in Groups 2 and 3.

Queiroz *et al*. (1994a) examined the immunological status of 33 male lead acid–battery workers (mean age, 32.4 years; range, 18–56 years; mean exposure period, 5.8 years; range, 0.5–20 years) compared with that of 20 non-exposed, age-matched controls, all with blood lead concentrations < 10 μg/dL. The workers' blood lead concentrations ranged from 12–80 μg/dL, with 21 of them having concentrations between 40–60 μg/dL. Serum concentrations of IgG, IgA and IgM did not differ between the groups and there was no correlation between blood lead concentrations or urinary ALA concentrations and serum immunoglobulin levels. In addition, there was no difference between the groups in the capacity of peripheral blood mononuclear cells (PBMCs) to respond to the mitogen phytohaemagglutinin (PHA), a correlate of T-cell function. There was also no correlation between mitogenic response and blood lead concentration. These data suggest that chronic exposure to lead does not compromise lymphocyte function.

In a further study, Queiroz *et al*. (1994b) investigated phagocytosis and intracellular killing of *Candida albicans* and *C. pseudotropicalis* by neutrophils and splenic phagocytic function in blood samples from a similar group of lead-exposed workers (see above). The *Candida* assay is used to identify myeloperoxidase-deficient subjects who have neutrophils that are unable to kill *C. albicans*, whereas *C. pseudotropicalis* can be effectively lysed. Lysis of *C. albicans*, but not *C. pseudotropicalis*, was impaired in lead-exposed workers with blood lead concentrations and urinary ALA concentrations below 60 μg/dL and 6 mg/L, respectively, as well as in toxic ranges. This suggests that lead exposure may result

in myeloperoxidase deficiency. There was no difference between the groups in any of the other parameters examined.

Ündeger *et al*. (1996) compared peripheral blood lymphocytes, serum immunoglobulins (IgG, IgA and IgM), and C3 and C4 complement protein concentrations of 25 male lead-exposed workers (mean age, 33 years; range, 22–55 years) employed in storage-battery plants (mean exposure period 6 years; range, 0.5–15 years; average blood lead concentration, 74.8 μg/dL) with those of 25 male controls with no history of lead exposure (mean age, 33 years; range, 22–56 years; average blood lead concentration, 16.7 μg/dL). The numbers and the percentage of T, T-suppressor, B, and NK cells, were not different between the groups, but the numbers of T-helper lymphocytes and the serum concentrations of IgG, IgM, C3 and C4 complement components were significantly lower in lead-exposed workers compared with controls ($p < 0.05$). These results suggest that chronic exposure to lead may be detrimental to the human immune system.

Pinkerton *et al.* (1998) evaluated a number of immune parameters in 145 lead-exposed workers (mean age, 32.9 ± 8.6 years) with a median blood lead concentration of 39 μg/dL (range, 15–55 μg/dL) and 84 unexposed workers (mean age, 30.1 ± 9.3 years; mean blood lead, < 2 μg/dL; range, < 2–12 μg/dL). After adjusting for covariates, no major differences were found between the two groups in the percentage of CD3+ cells, CD4+ T cells, CD8+ T cells, B cells, NK cells, serum immunoglobulin levels, salivary IgA, serum C3 complement levels or lymphoproliferative responses. However, among exposed workers, serum IgG was negatively associated with cumulative lead exposure, and the percentage and number of CD4+/CD45RA+ cells were positively associated with cumulative lead exposure. This study found no evidence of a marked immunotoxic effect of lead, although subtle differences in some immunological parameters were noted.

The immunological effects of occupational exposure to lead have been studied by measurement of lymphocyte proliferation, NK cell cytotoxicity and interferon (IFN)-γ production in PBMCs of three groups of lead-exposed workers: drivers of three-wheelers (30, eight of whom had blood lead > 10 μg/dL; average blood lead, 6.5 ± 4.7 μg/dL), battery workers (34, all with blood lead > 10 μg/dL; average blood lead, 128.11 ± 104 μg/dL) and silver-jewellery makers (20, 12 with blood lead > 10 μg/dL; average blood lead, 17.8 ± 18.5 μg/dL). Unexposed healthy volunteers (30, none with blood lead > 10 μg/dL; range, 1.6–9.8 μg/dL) served as controls. Lymphocyte proliferation in response to PHA stimulation was lower in lead-exposed individuals than in controls, but there was no correlation with blood lead concentrations. NK cell cytotoxicity was not different between groups. In contrast, the concentration of IFN-γ was significantly elevated in culture supernatants collected from PHA-stimulated PBMCs of lead-exposed individuals, showing a significant positive correlation with blood lead concentrations. This study demonstrates that lead can affect the immune response of exposed workers (Mishra *et al*., 2003).

(*b*) *Experimental systems*

Swiss Webster mice that received 130 or 1300 mg/L lead as lead acetate in drinking-water for 70 days showed decreased β-lymphocyte responsiveness and humoral antibody

titres (Koller & Kovacic, 1974; Koller & Brauner, 1977). Similar findings were obtained by Luster *et al.* (1978) in rats exposed to 25 or 50 mg/L lead acetate in drinking-water for 35–45 days.

In CBA mice exposed to lead in drinking-water (13–1300 mg/L, as lead acetate) for 10 weeks, the ability of the mitogens lipopolysaccharide and purified protein derivative to induce lymphocyte proliferation in the kidney was inhibited, but the response to concanavalin A was not significantly affected (Koller *et al.*, 1979).

To analyse the effect of lead on the immune system and to determine the ability of α-tocopherol to reverse lead-induced immunotoxicity, Fernandez-Cabezudo *et al.* (2003) treated groups of six TO mice intraperitoneally for 2 weeks with saline alone, lead acetate alone, lead acetate plus α-tocopherol or with α-tocopherol alone. Spleens were then analysed for (i) cellular composition by flow cytometry, (ii) cellular response to B and T cell mitogens and (iii) production of NO. The treatment with lead acetate resulted in a significant splenomegaly associated mainly with an influx of CD11b+ myeloid cells, but these cells exhibited no up-regulation of activation markers and did not produce NO. The mitogenic responses of the lymphocytes were inhibited by ≥ 70% in the lead-treated group. Concurrent treatment with lead acetate and α-tocopherol resulted in an almost complete reversal of the lead-induced splenomegaly, but the mitogenic response in this case was approximately 50% of that observed in saline-treated controls.

The effects of lead on the immune system of the developing embryo were assessed by Miller *et al.* (1998) in 9-week-old female Fischer 344 rats exposed to lead acetate (0, 100, 250 and 500 ppm lead) in their drinking-water during breeding and pregnancy. Exposure was discontinued at parturition and offspring received no additional lead treatment. At 13 weeks, tumour necrosis factor (TNF)-α and NO production were elevated in the female offspring of dams exposed to 250 ppm lead, while cell-mediated immune function was depressed, as shown by a decrease in delayed-type hypersensitivity (DTH) reactions. IFN-γ concentrations were lower in the offspring of the 500-ppm treatment group than in controls. Serum IgE levels were increased in rats exposed *in utero* to 100 ppm lead. The lead-exposed dams did not show chronic immune alterations. These results indicate that exposure of pregnant females to moderate levels of lead produces chronic immune modulation in their offspring.

Bunn *et al.* (2001) gave adult female Sprague-Dawley rats 500 ppm lead as lead acetate in the drinking-water early (days 3–9) or late in gestation (days 15–21). Significantly depressed DTH responses and elevated interleukin (IL)-10 production, higher relative monocyte numbers and increased relative thymic weights were observed when female offspring exposed during late gestation were assessed as adults. In contrast, male offspring had increased IL-12 production and decreased IL-10 production, while the DTH response, relative monocyte numbers and thymic weights were unchanged. Exposure during early gestation decreased NO production in lead-treated male, but not female offspring. These results suggest that the rat embryo may be more sensitive to lead-induced immunotoxic effects when exposed during late gestation, with the effects on DTH function being more pronounced in females.

Differential embryonic sensitivity to lead-induced immunotoxicity was studied by Lee, J.-E. *et al.* (2001) by injection of sublethal doses of lead (5–400 μg) into fertilized Cornell K Strain White Leghorn chicken eggs via the air sac on days 5, 7, 9 and 12 of incubation, designated as E5, E7, E9 and E12, respectively. In 5–6-week-old chickens, splenic lymphocyte production of IFN-γ was significantly suppressed (measured for E7 and E9 exposures only, $p < 0.05$) among lead-treated groups compared with controls. Production of NO by macrophages (measured as nitrite production) was significantly depressed ($p < 0.05$) after E5, E7 and E9 lead exposures but not following E12 lead exposure. In contrast, DTH function was unaltered following the E5, E7 and E9 exposures, but was significantly depressed ($p < 0.05$) after E12 exposure. The findings indicate that lead exposure during different stages of embryonic development results in different immunotoxic outcomes in the juvenile chicken.

In turkey poults fed 100 ppm dietary lead acetate, the concentration of arachidonic acid in macrophage phospholipids increased to twice that of controls. In-vitro production of eicosanoids by these macrophages was substantially increased, and this effect was most pronounced following lipopolysaccharide stimulation: prostaglandin F2α increased 11-fold, thromboxane B2 3-fold and prostaglandin E2 1.5-fold. The in-vitro phagocytic potential of these macrophages was only half that of control macrophages. The results show that lead influences immunological homeostasis in birds (Knowles & Donaldson, 1997).

The combined effects of a non-pathogenic immunological challenge and exposure to lead shot were investigated in three groups of 24 Japanese quail chicks (*Coturnix coturnix japonica*) that were given either one lead shot (0.05 g) or four lead shots (0.2 g) orally, at the age of 8 days. Controls did not receive lead. As immunological challenge, a third of each group of chicks was injected intraperitoneally with either 0.075 mL 10% chukar partridge (*Alectoris graeca*) red blood cells, Newcastle disease virus, or a placebo vaccine at 13 and 35 days of age. Lead did not affect antibody production or cell-mediated immune response. Granulocyte numbers were significantly higher in the lead-treated birds than in controls, and both antigen-treated groups had lower granulocyte numbers than controls. At the 0.2-g dose, lead increased haematocrit values, lowered plasma protein concentrations and increased granulocyte numbers in the quail (Fair & Ricklefs, 2002).

The effects of lead nitrate (0.1 μM–1 mM) on proliferative responses of B and T lymphocytes of mouse, rat and human origin were investigated. T cells were stimulated by PHA or by monoclonal antibodies directed at the T cell receptor/CD3 complex, while B cells were activated by T-independent mitogens (*Staphylococcus aureus* cells, *Escherichia coli* lipopolysaccharide and *Salmonella typhimurium* mitogen for human, mouse and rat lymphocytes, respectively). Large differences in proliferative responses were observed for lead nitrate across species; rat lymphocytes were very sensitive to immunomodulation by lead, whereas human cells were found to be relatively resistant (Lang *et al.*, 1993).

4.2.7 *Other toxic effects*

(*a*) *Lead-induced mitogenesis*

Endogenous DNA damage is present in every living cell and may remain relatively harmless — even when it is not, or very slowly, repaired — as long as the cell does not replicate its DNA prior to mitosis. However, when cell proliferation is induced, endogenous DNA damage may be converted into mutations, some of which may lead to disturbance of cellular growth control and, ultimately, to carcinogenesis. Stimulation of cell proliferation (mitogenesis) may, therefore, play an important role in the mode of action of carcinogens that do not directly interact with DNA (Cohen & Ellwein, 1990). The mitogenic activity of lead and lead compounds has been studied extensively.

Choie and Richter (1974a) showed that a single dose of 5 mg lead/kg bw, given as lead acetate by intracardiac injection produced a 45-fold increase in DNA synthesis in the kidney of mice, followed by a wave of mitoses. This effect was found to be preceded by a general increase in synthesis of RNA and protein (Choie & Richter, 1974b). A single intracardiac dose of lead acetate (40 mg lead/kg bw) induced a 25-fold increase in mitosis of mouse hepatocytes within 5 hours. The prompt appearance of a mitotic wave and the relatively large number of mitoses suggest that the mitotic cells were from a hepatocyte sub-population arrested in the G2 phase (Choie & Richter, 1978).

The effects of a single intraperitoneal dose of lead acetate (0.04 mg lead/kg bw) on the proliferation of the proximal tubule epithelium of the rat kidney were investigated by autoradiographic analysis of [^{3}H]thymidine incorporation, over a 3-day period after injection. Within 2 days, the labelling index increased approximately 40-fold compared with controls. Three days after injection of lead 14.5% of the proximal tubular epithelial cells were labelled (Choie & Richter, 1972c).

In a subsequent study, the same authors investigated the effects of chronic administration of lead. Rats received intraperitoneal injections once a week for 6 months, at doses of 1–7 mg lead per rat. At the end of this period, the proliferative activity of the proximal tubular epithelium was 15 times higher in treated than in untreated rats. Epithelial hyperplasia was seen in some proximal tubules, with occasional atypia. The results suggest that the renal carcinogenicity of lead (see Section 3) may be due to lead-induced stimulation of renal cell proliferation (Choie & Richter, 1972a).

Stevenson *et al.* (1977) showed that a single intraperitoneal injection of 10 mg/kg bw lead chloride into rats caused a transient twofold increase in synthesis of RNA and DNA in the kidney after 1 and 3 days, respectively; RNA synthesis in liver and lung was also increased twofold, but DNA synthesis was decreased in these organs.

Columbano *et al.* (1983, 1984) gave groups of seven male Wistar rats a single dose of lead nitrate (100 μmol/kg bw) by intravenous injection and sacrificed them 1, 2, 3, 4 and 7 days later. The treatment caused a marked enlargement of the liver, which reached a maximum of 71% at the third day after treatment. This effect was accompanied by an increase in total hepatic protein and DNA content, with a maximum at 3 and 4 days, respectively. An increase in DNA synthesis, as monitored by the incorporation of [^{3}H]labelled thy-

midine, was observed at 24 h, reaching a maximum at 36 h after administration of lead nitrate, with a 30-fold higher level than in control rats. DNA synthesis returned to normal within 3 days. The lead-induced stimulation of liver-cell proliferation was reflected in a significant increase in the number of parenchymal and non-parenchymal cells entering mitosis, with a peak at 48 h. No histologically detectable liver-cell necrosis was seen, which suggested that the cell proliferation induced by lead is not due to a regenerative response. The stimulatory effect of lead on liver growth was reversible; during return to normal size, cell death, morphologically similar to apoptosis, was observed in histological sections of liver from animals sacrificed 4–7 days after treatment.

A series of studies by the same research group investigated the effect of different types of cell proliferation on the development of enzyme-altered preneoplastic hepatic foci in male Wistar rats. In the first experiment, animals were given a single intraperitoneal injection of *N*-nitrosodiethylamine (NDEA; 100 mg/kg bw). After a 2-week recovery period liver cell proliferation was induced by repeated doses of carbon tetrachloride (2 mg/kg bw, by intragastric intubation), or by repeated mitogenic treatments with lead nitrate (100 μmol/kg bw, by intravenous injection). Histologically-altered hepatocytes were monitored as γ-glutamyltransferase-positive or adenosine triphosphatase-negative foci. The results indicated that compensatory cell proliferation induced by carbon tetrachloride enhanced the growth of NDEA-initiated hepatocytes to enzyme-altered foci. On the contrary, repeated waves of cell proliferation induced by lead nitrate did not result in any significant number of enzyme-altered foci (Columbano *et al*., 1990).

In follow-up studies, the same authors determined the efficacy of different types of cell-proliferative stimuli given during several liver tumour-promoting regimens, with respect to the formation of enzyme-altered hepatocyte foci. Male Wistar rats were initiated with NDEA (150 mg/kg bw, by intravenous injection). After recovery, the animals were subjected to different promoting regimens, i.e. the resistant hepatocyte model (Solt & Farber, 1976), the phenobarbital model (Peraino *et al.*, 1971) and the orotic acid model (Laurier *et al.*, 1984). While the rats were on these regimens, they received different types of liver cell-proliferative stimuli, either a compensatory type (two-thirds partial hepatectomy or a necrogenic dose of carbon tetrachloride) or a direct hyperplastic stimulus by lead nitrate. Initiated cells thus promoted were monitored as foci of enzyme-altered hepatocytes. While compensatory cell regeneration induced by carbon tetrachloride and partial hepatectomy stimulated the promoting ability of the regimens used, direct hyperplasia induced by lead nitrate did not stimulate the formation of foci and/or nodules from initiated hepatocytes. Incorporation of [^{3}H]thymidine showed that there was no significant difference in the extent of DNA synthesis resulting from the different proliferative stimuli, irrespective of the promoting procedure used. These results suggest that the two types of cell-proliferative stimuli may involve different cell growth and signal-transduction pathways, or they may act on different cell populations (Ledda-Columbano *et al*., 1992; Coni *et al*., 1993a).

An enhanced susceptibility of renal tubular epithelial cells in rats to lead-induced mitogenicity was reported at doses comparable to those used in the cancer bioassay. This

may contribute to the carcinogenic response seen in the kidney following exposure to lead. It is of interest to note that the liver — an organ that is not susceptible to lead-induced carcinogenicity — showed a significantly lower mitogenic response towards lead exposure (Calabrese & Baldwin, 1992).

To evaluate the effect of pre-exposure to mitogens on carbon tetrachloride-induced hepatotoxicity, Calabrese *et al.* (1995) gave male Wistar rats a single intraperitoneal injection of carbon tetrachloride (0.3 mL/kg bw in corn oil) 48 h after either a single intravenous injection of lead nitrate (0.33 mg/kg bw) or distilled water. The rats pre-treated with lead nitrate showed markedly lower serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities at 24, 48 and 72 h after administration of carbon tetrachloride than rats pre-treated with distilled water. However, treatment with the anti-mitotic agent colchicine did not alter the lead-induced protection. These findings suggest that the lead-induced protection is not associated with the major mitogenic response of lead, despite its strong temporal association.

Bell *et al.* (1993) tested lead nitrate and lead acetate for mitogenic effects in the liver of adult male and female rainbow trout. Groups treated with a single intraperitoneal injection of lead nitrate or lead acetate (up to 375 mg/kg bw) or a single intravenous injection of lead nitrate (up to 5 mg/kg bw) showed no statistically significant alterations in liver:body weight ratio. There was no change in hepatic DNA content of the fish that received the intraperitoneal injections. The results suggest significant interspecies differences between the mitogenic response of the liver in rainbow trout and Wistar rats exposed to lead.

(*b*) *Effects on regulatory proteins*

The steady-state levels of *c-fos* and *c-jun* messenger RNA have been investigated in rat liver tissue after various proliferative stimuli, i.e. compensatory cell regeneration induced by partial hepatectomy or carbon tetrachloride, and direct hyperplasia induced by different hepatomitogens, one of which was lead nitrate. Whereas *c-fos* and *c-jun* expression increased soon after partial hepatectomy or administration of carbon tetrachloride, an increased expression of *c-jun* in the absence of *c-fos* expression was seen during direct hyperplasia induced by lead nitrate. These results suggest that, depending on the nature of the proliferative stimulus, an increased expression of these regulatory genes may not be necessary for in-vivo induction of liver cell proliferation (Coni *et al*., 1993b).

The influence of lead on various protein factors involved in cell signalling has been studied in relation to its neurotoxic effects. Male Long-Evans rats (aged 21 days; $n = 40$) received lead acetate (50 ppm) in drinking-water for 90 days. Control animals ($n = 40$) received sodium acetate. After this period, mean ± SD blood lead concentrations in the control and lead-exposed groups were 4 ± 0.2 and 18 ± 0.2 µg/dL, respectively. Compared with controls, the lead-exposed animals showed a significantly higher accumulation of lead in the frontal cortex, brain stem, striatum and hippocampus, as well as a three- to fourfold increase in the concentrations of NF-κB and activator protein 1, a four- to 10-fold activation of c-Jun N-terminal kinase, a five- to sixfold activation of mitogen-activated protein kinase kinase (MAPKK) and an enhanced activity of caspases in these four brain

regions, which is consistent with apoptosis. These effects may contribute to the neurotoxicity of lead (Ramesh *et al*., 2001).

To identify genes that are upregulated in PbR11 cells (a lead-resistant variant of rat glioma C6 cells), Li and Rossman (2001) applied the method of suppression subtractive hybridization between mRNAs of C6 and PbR11 cells. Three upregulated genes were identified, i.e. thrombospondin-1, heparin sulfate 6-sulfotransferase, and neuropilin-1, which play important roles in angiogenesis and axon growth during neuronal development. It is of interest to note that all these genes are functionally related to heparin sulfate. The effects of short-term lead exposure (24 h, up to 600 μM) on the expression of these genes were examined in C6 cells. While thrombospondin-1 is repressed by lead in a dose-dependent manner, neuropilin-1 and heparin sulfate 6-sulfotransferase showed low constitutive expression in C6 cells, which was not altered by exposure to lead. Since low concentrations of lead inhibit the sulfation of heparin (Fujiwara & Kaji, 1999), the results suggest that heparin sulfate 6-sulfotransferase may be the lead-sensitive enzyme responsible for this inhibition. In addition to this enzyme, neuropilin-1 and thrombospondin-1 may also be targets for lead-induced developmental neurotoxicity (Li & Rossman, 2001).

Bouton *et al.* (2001) used cDNA microarrays to analyse the effects of acute lead exposure (10 μM lead acetate, 24 h) on large-scale gene expression patterns in immortalized rat astrocytes. Control cells were treated with 10 μM sodium acetate. Many genes previously reported to be differentially regulated by lead exposure were identified in this system. In addition, novel putative targets of lead-mediated toxicity were identified, including calcium/phospholipid binding annexins, angiogenesis-inducing thrombospondins, collagens, and t-RNA synthetases. In a biochemical assay, the phospholipid binding activity of the protein annexin A_5 was shown to be induced by nanomolar concentrations of lead.

Lead acetate (100 nM–100 μM) stimulated DNA synthesis and cell-cycle progression in human astrocytoma cells through selective lead-induced activation of protein kinase Cα (PKCα) (Lu *et al*., 2001). In a further study, the same authors investigated the ability of lead to activate the mitogen-activated protein kinase (MAPK) cascade. Exposure of these astrocytoma cells to lead acetate (1–50 μM) resulted in a concentration- and time-dependent activation of MAPK, as was evident from increased phosphorylation and increased kinase activity. This effect was significantly reduced by specific inhibition or down-regulation of PKCα. Lead also activated MAPK MEK1/2 kinase, an effect that was mediated by PKCα. Addition of specific MEK inhibitors blocked lead-induced MAPK activation and inhibited lead-induced DNA synthesis, as measured by [^{3}H]thymidine incorporation. The results of this study suggest that lead may act as a tumour promoter in transformed glial cells (Lu *et al.*, 2002).

The effect of divalent lead on protein phosphorylation in bovine adrenal chromaffin cells and human SH SY5Y cells has been examined. Cells were incubated with inorganic [^{32}P] for 1 h in the presence of lead acetate (1, 5 and 10 μM) and proteins were separated by two-dimensional polyacrylamide gel electrophoresis. Among the spots that were indicative of increased protein phosphorylation, three proteins, with an apparent molecular weight of 25 kDa and iso-electric points in the range 4.0–4.5, were immuno-identified as

isoforms of the heat-shock protein 27 (Hsp27). The effect of lead on Hsp27 phosphorylation was blocked by the p38MAPK inhibitor SB203580 (1 μM) and phosphorylation of p38MAPK was increased by lead. The results were similar for both cell types studied. Thus lead can modulate the phosphorylation state of Hsp27 via activation of the p38MAPK pathway. Since Hsp27 in its non-phosphorylated form confers resistance towards oxidative stress (Rogalla *et al*., 1999) this effect of lead may result in a higher vulnerability of cells to oxidative damage (Leal *et al*., 2002).

The zinc finger, a major structural motif involved in protein–nucleic acid interactions, is present in the largest super-family of transcription factors (Zeng & Kagi, 1995). Zinc (Zn^{2+}) ions coordinate this finger-like structure through interaction with cysteine and histidine residues. Factors containing such motifs are potential targets for perturbation by divalent lead (Büsselberg, 1995; Guilarte *et al*., 1995; Tomsig & Suszkiw, 1996). Lead has been shown to interfere with the DNA-binding properties of the zinc finger-containing transcription factors Sp1 and Egr-1, both *in vivo* and *in vitro*. More recently, the inhibitory effects of lead on the DNA-binding of the zinc finger protein transcription factor IIIA (TFIIIA) have been demonstrated (Hanas *et al*., 1999). The interaction of lead with Sp1, Egr-1, and TFIIIA shows that lead can also target other cellular proteins that contain the zinc-finger motif and that this protein domain is a potential mediator for lead-induced alterations in protein function. Thus by specifically targeting zinc-finger proteins, lead is able to produce multiple responses through its action on a common site that is present in enzymes, channels and receptors (Zawia *et al*., 2000).

(*c*) *Apoptosis*

(i) *In-vivo studies*

Apoptosis or programmed cell death is induced by various physiological or pathological stimuli. Mitochondria and a specific class of proteins, the caspases, play an important role in this process. At an early stage of apoptosis, the mitochondrial permeability transition pore is opened, which leads to depolarization of the mitochondrion and to the release of cytochrome C. Subsequently, caspases activate endonucleases that cleave the genomic DNA into the high-molecular-weight fragments that are characteristic of apoptotic cells. Although the detailed molecular mechanism of lead-induced apoptosis is still unknown, calcium overload and the generation of ROS may be important triggers. These and other mechanistic aspects of lead-induced apoptosis are discussed in recent reviews (Waalkes *et al.*, 2000; Pulido & Parrish, 2003).

Columbano *et al.* (1985) showed that in male Wistar rats, a single intravenous injection of lead nitrate (100 μmol/kg bw) caused liver enlargement associated with hepatic cell proliferation. The subsequent involution of the liver hyperplasia was studied by histological examination of liver sections prepared during regression of the liver. There was no sign of massive lytic cell necrosis, and no change in serum concentrations of glutamate pyruvate transaminase. Apoptotic bodies were observed in the involuting liver by microscopy and ultrastructural examination. A marked increase in the number of apoptotic bodies

was noted 5 days after administration of lead, when the liver was already regressing, while very few were observed in control animals or in rats 2 days after lead injection, when the mitotic index reached its maximum, or at 15 days, when the liver had returned to normal. These findings suggest that the removal of excess liver tissue that follows the initial lead-induced hyperplasia is due to apoptosis.

Fox *et al.* (1998) showed that exposure to lead resulted in the selective apoptotic loss of rods and bipolar cells in the retina of rats. Lead-exposed rats were reared from dams that received 0.02% or 0.2% lead acetate in drinking-water during lactation only. At 21 days of age (weaning), the mean blood lead concentrations in the non-exposed rats and the two dose-groups were 1, 19 and 59 μg/dL, respectively. During and following lead exposure, rod/retinal cGMP phosphodiesterase expression and activity were delayed in onset and decreased, the concentration of calcium was elevated, and mitochondrial ATP synthesis was decreased in the infant rats.

(ii) *In-vitro studies*

The role of apoptosis in the effects induced by lead (lead acetate, 0.01–100 μM) and glutamate (0.1 and 1 mM) has been studied in mouse hypothalamic GT1-7 neurons. Loikkanen *et al.* (2003) found that glutamate alone had no effect on cell viability, but it enhanced neuronal cell death induced by lead (at concentrations 1–100 μM) at 72 h. Glutamate alone did not induce caspase-3-like protease activity or internucleosomal DNA fragmentation which are both biochemical hallmarks of apoptosis. However, combined exposure to lead (10 or 100 μM) and glutamate (1 mM) resulted in more prominent caspase-3-like protease activity than that caused by lead alone, with the highest activity measured at 48 h. Internucleosomal DNA fragmentation caused by lead (10 or 100 μM) was enhanced by glutamate (1 mM). Immunoblotting did not reveal any changes in p53 protein concentration in cells exposed to lead, glutamate, or their combination at any time point (3–72 h). These results suggest that lead-induced neurotoxicity may be mediated partially through p53-independent apoptosis and enhanced by glutamate.

Cultured granule cells from newborn rat cerebellum were used to study whether apoptotic or necrotic death is the major consequence of exposure to low concentrations of lead. At a dose of 1 μM, lead did not affect glutamate-induced neuronal necrosis but promoted neuronal apoptosis, as characterized by cell shrinkage and chromatin condensation, internucleosomal DNA fragmentation and by dependence on de-novo synthesis of macromolecules. The low concentrations of lead that promoted apoptosis in this study were within the range of blood lead concentrations reported to impair the cognitive function in children and to alter synaptogenesis in the neonatal rat brain. These in-vitro results suggest that the highly neurotoxic action of lead may depend on a facilitation of apoptosis (Oberto *et al.*, 1996).

In-vitro studies using rat retinas incubated in the presence of calcium or lead showed increased high molecular weight DNA fragmentation and a higher number of apoptotic rods. In addition, retinal mitochondrial ATP synthesis was decreased, mitochondrial cytochrome C was released and caspase activity was increased. These effects were additive in

the presence of physiological concentrations of both calcium and lead. These results suggest that lead-induced rod and bipolar cell apoptosis is triggered by calcium and lead overload and that mitochondrial alterations play a central role in this process (Fox *et al.*, 1998).

An in-vitro model using isolated rat retinas was used to determine the mechanisms underlying retinal degeneration induced by calcium and/or lead. Confocal microscopy and histological and biochemical analyses established that elevated amounts of calcium and/or lead were concentrated around photoreceptors and produced rod-selective apoptosis. Mitochondrial depolarization, swelling and cytochrome C release were also seen, followed by activation of caspase-9 and caspase-3, but not caspase-7 or caspase-8. The effects of calcium and lead were additive. The concentrations of reduced and oxidized glutathione and pyridine nucleotides in rods were unchanged. These results show that rod mitochondria are the target sites for calcium and lead, and suggest that these metals bind to the internal binding site of the mitochondrial permeability transition pore, which then opens up, initiating the cytochrome C-caspase cascade of apoptosis (He *et al.*, 2000).

The effects of extracellular lead supplementation on the cellular lead content and on cell proliferation and survival have been studied in normal rat fibroblasts. The culture medium contained a background level of 0.060 μM lead and the normal cellular concentration of lead was 3.1 ± 0.1 ng/10^7 cells. Cells were exposed to 0.078–320 μM lead acetate, which caused a dose-dependent inhibition of cell proliferation after 48 h, which was apparent at 0.312 μM ($p = 0.122$) and became statistically significant at concentrations > 0.625 μM ($p = 0.0003$ at 5 μM). DNA fragmentation, a hallmark of apoptosis, increased significantly at lead concentrations from 2.5–10.0 μM. The occurrence of apoptosis was confirmed by flow cytometry, which showed a sub-diploid peak at 5–20 μM lead. There was a dose-dependent accumulation of cells in the G0/G1 phase, mainly compensated by a decrease in the percentage of cells in S phase. These results demonstrate that induction of apoptosis contributes to the lead-induced inhibition of cell proliferation in rat fibroblasts (Iavicoli *et al.*, 2001).

De la Fuente *et al.* (2002) incubated human peripheral blood mononuclear cells with increasing concentrations of cadmium, arsenic or lead, and determined apoptosis by flow cytometry and DNA electrophoresis. Arsenic (15 μM) induced a significant level of apoptosis after 48 h of incubation, while cadmium had a similar effect at higher concentrations (65 μM). In contrast, lead concentrations as high as 500 μM were non-toxic and did not induce a significant degree of apoptosis.

(*d*) *Effects on hepatic enzymes*

Alvares *et al.* (1975) determined metabolizing capacities in 10 normal adults and in 10 children aged 1–8 years with two test drugs, antipyrine and phenylbutazone. Eight children had biochemical evidence but no clinical expression of lead poisoning. Among the children, there were no differences in their capacities to metabolize the two drugs. The mean antipyrine half-life in the children, 6.63 h, was significantly lower than the mean half-life of 13.58 h in adults. The mean phenylbutazone half-lives in the children and adults, 1.68 and 3.16 days, respectively, also differed significantly. In two other children who showed

clinical as well as biochemical manifestations of acute plumbism, antipyrine half-lives were significantly longer than normal.

Saenger *et al.* (1984) investigated the possible inhibitory effects of lead on the metabolism of 6β-hydroxycortisol (6βOHF, a highly polar metabolite of cortisol) by analysis of urinary excretion of 6βOHF in 26 children with mildly to moderately elevated blood lead concentrations (average, 44 μg/dL; range 23–60 μg/mL). The EDTA provocative test was used to assess the size of chelatable and potentially toxic lead stores in these children. Children with elevated urinary lead excretion after an EDTA provocative test, i.e. elevated tissue lead stores, had markedly decreased urinary excretion of 6βOHF (178 ± 15 μg/m^2 body surface area in 24 h) compared with children who had negative tests (333 ± 40 μg/m^2 in 24 h; $p < 0.01$); the urinary cortisol excretion in both these groups of children was not different from that of age-matched controls. These findings suggest that lead, at relatively low concentrations, may interfere with hepatic microsomal formation of a cortisol metabolite.

(*e*) *Effects on endocrine function*

(i) *Human studies*

Gustafson *et al*. (1989) carried out a study in Sweden in a group of secondary lead smelter workers and appropriately selected controls, and found a complex effect on the endocrine system induced by moderate exposure to lead, possibly mediated by changes at the hypothalamic–pituitary level. It should be noted that all the hormone values were within the normal range for the Swedish population.

The possible neuroendocrine effects of lead were studied in six children with high blood lead concentrations (range, 41–72 μg/dL) and in four children with low blood lead (0–30 μg/dL). The first group received EDTA chelation therapy. The growth rate of these children increased considerably after the chelation therapy, from 4.2 ± 0.9 cm/year before treatment to 9.0 ± 0.9 cm/year after treatment (data for 2–3-year-olds, $n = 5$). The children with low blood lead concentrations had a growth rate of 8.9 ± 1.0 cm/year (2–2.3-year-olds, $n = 3$) (Huseman *et al*., 1992)

Thyroid function tests were performed in 58 petrol-pump workers or automobile mechanics (mean age, 31.7 ± 10.6 years; mean duration of exposure to lead, 13 ± 10 years). Their mean blood lead concentration was 51.9 ± 9.4 μg/dL, which was approximately five-fold higher than that in 35 non-exposed control subjects. There was no difference in serum concentrations of triiodothyronine (T3) or thyroxine (T4) between the groups. Interestingly, T3 was significantly lower with longer exposure times (210 versus 29 months). The mean thyroid-stimulating (TSH) concentrations were significantly higher ($p < 0.01$) in exposed workers. This was independent of exposure time, but more pronounced in individuals with higher blood lead values. However, TSH concentrations remained within the normal range. The results suggest that elevated blood lead concentrations could enhance the pituitary release of TSH without having a significant effect on circulating levels of T3 and T4 (Singh *et al.*, 2000).

The effects of lead on the endocrine system were studied in 77 lead-smelter workers (62 active, 15 retired) compared with 26 referents. Lead concentrations were determined in plasma (i.e. giving an index of recent exposure), in blood and in finger-bone (i.e. giving an index of long-term exposure). In addition, the serum concentrations of pituitary hormones, thyroid hormones and testosterone were determined. Nine exposed workers and 11 referents were challenged with gonadotrophin-releasing hormone and thyrotrophin-releasing hormone, followed by measurement of stimulated pituitary hormone concentrations in serum. Median blood lead concentrations were 33.2 μg/dL in active workers, 18.6 μg/dL in retired workers and 4.1 μg/dL in controls. Respective median bone lead concentrations were 21 μg/g, 55 μg/g and 2 μg/g. Concentrations of pituitary hormones, thyroid hormones and testosterone were similar in the three groups. In the challenge test, stimulated follicle-stimulating hormone (FSH) concentrations were significantly lower in lead workers ($p = 0.014$) than in referents, indicating an effect of lead in the pituitary. The results show that moderate exposure to lead was associated with only minor changes in male endocrine function, particularly affecting the hypothalamic–pituitary axis (Erfurth *et al.*, 2001).

(ii) *In-vitro study*

To examine the in-vitro effects of lead on cytochrome P450 aromatase and on estrogen receptor β, human ovary granulosa cells were collected from women undergoing in-vitro fertilization and cultured with 10 μM lead acetate. Lead content in these cells increased to 85 μg/g after 5 h of culture, 390 μg/g after 24 h and 1740 μg/g at 72 h. Aromatase activity was significantly reduced, as were the amounts of P450 aromatase enzyme, estrogen receptor β and their mRNAs. Inhibition of protein synthesis by cycloheximide (10 μg/mL) did not eliminate the effects of lead. The results suggest that the effects of lead on female fertility may result, in part, from the down-regulation of P450 aromatase and estrogen receptor β gene transcription in ovarian granulosa (Taupeau *et al.*, 2003).

4.3 Effects on reproduction

It is generally accepted from the older literature that lead adversely affects the reproductive process in both men and women. The evidence is however mostly qualitative and dose–effect relationships have not been established.

Most of the information is based on studies among workers with high occupational exposure to lead, while low-dose effects have been reported from occupational cohorts or groups in the general population living in polluted areas.

Some factors make it difficult to extrapolate animal data to the human situation. These difficulties are due mainly to differences among species in reproductive end-points and to the level of exposure.

4.3.1 *Humans*

(*a*) *Male fertility*

Studies have focused mainly on the quality of semen, endocrine function and birth rates in occupationally-exposed subjects, and have shown that concentrations of inorganic lead > 40 µg/dL in blood can impair male reproductive function by reducing sperm count, volume and density, and by affecting sperm motility and morphology.

Dose–response relationships, in particular at a threshold level, are poorly understood, and site, mode or mechanism of action are often unknown. Also, the effects were not always the same or associated in the same way, although the prevalent effects were on sperm count and concentration.

The classic study by Lancranjan *et al*. (1975) performed in Romania first provided some evidence of impaired spermatogenesis in men with blood lead concentrations > 40 µg/dL. The subjects were classified into four groups: 'men with lead poisoning' ($n = 23$), men with 'moderate' ($n = 42$), 'slight' ($n = 35$) or 'physiological' ($n = 50$) lead absorption. The major finding of this study was the suggestion of a dose–response relationship for the decrease in sperm count (hypospermia) and sperm motility (asthenospermia) and the increase in abnormal sperm morphology (teratospermia) with increasing lead absorption. The strengths of this study were the use of a standardized questionnaire to collect the data, the relative comparability of controls and the relatively large number of subjects involved. On the other hand, assessment of the dose–response relationship was limited by the overlap between exposure groups, by the relatively high blood lead concentrations in control subjects, by the inclusion of coitus interruptus as a means to collect semen and by lack of information on sperm counts.

Similar findings were reported by Lerda (1992) in Argentina, although no dose–response relationship was found. The result should be noted, mainly because selection of subjects and characterization of exposure to lead were well conducted, as were the collection and analysis of the semen and the statistical analyses of the results.

The cross-sectional study by Alexander *et al*. (1996) showed that blood lead concentrations > 40 µg/dL may affect spermatogenesis by reducing sperm concentration and total sperm count. No association was found between exposure to lead and sperm morphology or motility, or serum concentration of reproductive hormones. The strengths of the study were mainly the size and careful selection of the study population, availability of historical data of lead exposure, the control for all the relevant confounding factors (e.g. age, smoking, a1cohol consumption, period of abstinence before semen collection, blood concentrations of other metals such as cadmium and zinc), the statistical analysis, and the validity of the semen analysis.

A study by Rodamilans *et al*. (1988) in Spain showed no clear correlation between blood lead concentrations and endocrine variables. Smelter workers were divided into three groups according to duration of exposure: < 1 year (group 1, $n = 5$), 1–5 years (group 2, $n = 8$) and > 5 years (group 3, $n = 10$). In group 3, serum testosterone was significantly lower, steroid binding globulin (SBG) was higher and there was a clear reduction

in the free testosterone index (testosterone/SBG). In group 2, there was a significantly lower free testosterone index but there were no clear differences between testosterone and SBG concentrations compared to the controls. There was an increase in serum luteinizing hormone (LH) concentration in the first group, but this did not persist with longer durations of lead exposure. The authors suggested an initial testicular toxicity followed by a dysfunction in the hypothalamus or the pituitary gland, which disrupts the hypothalamic–pituitary feedback mechanism associated with prolonged exposure (Rodamilans *et al.*, 1988).

A study by McGregor and Mason (1990) suggested that lead may cause subclinical primary toxic damage to the seminiferous tubules in the testis at blood lead concentrations > 47 μg/dL. In this study, testosterone concentrations were normal, in contrast to the findings of Rodamilans *et al*. (1988).

Ng *et al*. (1991) carried out a study in Singapore and found that concentrations of LH and FSH showed a moderate increase in relation to blood lead concentrations in the range of 10–40 μg/dL, thereafter reaching a plateau or declining. An increase in concentrations of LH and FSH, with normal testosterone, was noted in subjects with < 10 years of exposure to lead whereas men exposed for 10 or more years had normal FSH and LH and low testosterone concentrations. The main conclusion was that moderate exposure to lead resulted in small changes in endocrine function in a dose-related manner, reflecting primary and secondary effects of lead on the testes and the hypothalamus–pituitary axis.

Gennart *et al*. (1992) assessed the thyroid, testes, kidney and autonomic nervous system function in 98 battery workers in Belgium (mean blood lead concentration, 51 μg/dL; range, 40–75 μg/dL) and found no abnormalities.

Several of the studies described above (Lancrajan *et al.*, 1975; Lerda, 1992; Alexander *et al.*, 1996) and that of Assennato *et al.* (1987) reported effects on testicular function in groups of men with mean blood lead concentrations above 40–50 μg/dL. These results are consistent with a likely threshold of about 45–55 μg/dL (Bonde *et al.*, 2000). In contrast, the findings of a study of semen (Robins *et al*., 1997) in 97 men employed in a South African lead–acid battery plant, with blood lead concentrations ranging from 28–93 μg/dL, did not support an effect of lead on sperm concentration and total sperm count. However, the authors noted that their results should be interpreted with caution because of the relatively high range of current blood lead concentrations, the high prevalence of abnormalities in semen quality and the lack of a control population.

In a cross-sectional survey (Telisman *et al.*, 2000) of workers exposed to lead and non-occupationally exposed controls, a significant negative association was found between sperm count and mean blood lead concentrations in six subgroups stratified by blood lead concentration. The mean blood lead concentrations in the six subgroups ranged from 5–35 μg/dL. In contrast, in a longitudinal study (Viskum *et al*., 1999) of battery workers in Denmark, no improvement was found in sperm concentration or in the proportion of morphological abnormalities with a decline in blood lead concentration from about 40 to 20 μg/dL.

Bonde *et al.* (2002) undertook a cross-sectional survey on some fertility parameters of 503 workers employed by 10 companies in Belgium, Italy and the United Kingdom, as part of the ASCLEPIOS project. Volume of semen and concentration of sperm were determined in a fresh semen sample according to an agreed protocol of quality assurance. Measurement of dose indicators in blood and seminal fluid and its fractions and the sperm chromatin structure assay were all performed by centralized laboratories. Abnormal chromatin structure of the spermatozoa was analysed by flow cytometric measurement of red (denaturated single stranded DNA) and green (native DNA) fluorescence in sperm cells stained with acridine, and expressed by the ratio of red to total (red + green) fluorescence (Garner *et al.*, 1986). Extraneous determinants including centre, period of sexual abstinence and age were taken into account in the statistical analysis. If appropriate, possible thresholds were examined by iterative threshold slope linear regression. The mean blood lead concentration was 31.0 μg/dL (range, 4.6–64.5 μg/dL) in 362 workers exposed to lead and 4.4 μg/dL (range, below the detection limit to 19.8 μg/dL) in 141 workers not exposed to lead. The median sperm concentration was reduced by 49% in men with blood lead concentrations > 50 μg/dL. The findings were consistent across the three centres and the sample size was larger than in earlier studies thus strengthening the findings. However, in this study and in previous ones, the authors noted that the low participation rate at two of the three sites is a major limitation conferring risk of selection bias as men who perceived themselves to be less fertile may have been more motivated to take part (Bonde *et al*., 1996; Larsen *et al*., 1998; Bonde *et al.*, 2002).

The concentration of inorganic lead in blood may not reflect the concentration in the target organs and therefore lead measured in seminal fluid and its fractions might be better correlated with testicular lead and histopathological alterations. Apostoli *et al*. (1999) and Bonde *et al.* (2002) found a high content of lead within spermatozoa and a low concentration in seminal fluid, indicating that lead is either taken up by spermatozoa or is incorporated into the sperm cells during spermatogenesis. The analyses based on lead in semen largely corroborated the findings based on analysis of lead concentration in blood, but men with the highest concentration of lead in spermatozoa also had higher mean αT, and a higher proportion of sperm cells outside the main population, indicating alterations of the sperm chromatin structure (Bonde *et al.*, 2002).

Zinc contributes to sperm chromatin stability and binds to protamine 2. It has recently been shown that lead competes with zinc and binds human protamine 2 (HP2) causing conformational changes in the protein (Quintanilla-Vega *et al*., 2000). This decreases the extent of HP2-DNA binding, which probably results in alterations in sperm chromatin condensation. Alteration of sperm chromatin structure by increased in-situ denaturation is strongly correlated with the presence of sperm DNA strand breaks (Aravindan *et al*., 1997) and is associated with reduced fecundity in humans (Spanò *et al*., 2000).

There appears to be a direct negative correlation between seminal plasma lead concentrations and in-vitro fertilization rates (Benoff *et al.*, 2000, 2003). Lead concentrations are also negatively correlated with standard semen parameters (sperm count, motility and morphology) and sperm function biomarkers (mannose receptor expression and mannose-

stimulated acrosome reaction), and positively correlated with premature acrosome breakdown.

Positive relationships between blood lead concentrations and seminal plasma lead or sperm lead concentrations have been reported after both occupational exposures (Aribarg & Shukcharoen, 1996; Telisman *et al.*, 2000; Bonde *et al*., 2002) and environmental exposures (Telisman *et al*., 2000) to lead.

Another way to verify the possible effect of lead on male fertility is through retrospective evaluation of time to pregnancy. A French cohort study (Coste *et al*., 1991) of 229 workers exposed to lead (mean blood lead concentration, 46.3 µg/dL) compared with 125 unexposed subjects did not provide clear evidence of adverse effects of occupational exposure to lead on male fertility as studied by recording live births.

Apostoli *et al*. (2000) found decreased fertility among men with blood lead concentrations of at least 40 µg/dL, but this was statistically significant only in a subgroup analysis restricted to subjects with just one child. Fertility was not reduced in men with blood lead concentrations in the range 30–40 µg/dL.

Sallmén *et al.* (2000) conducted a retrospective study on time to pregnancy among the wives of men who had been monitored for lead to assess whether paternal occupational exposure to inorganic lead was associated with decreased fertility. Lead exposure was assessed by blood measurements and by questionnaires. The final study population consisted of 502 couples who did not use contraception at the beginning of the pregnancy. The fecundability density ratios, adjusted for potential confounders, were 0.92 (95% CI, 0.73–1.16), 0.89 (95% CI, 0.66–1.20), 0.58 (95% CI, 0.33–0.96) and 0.83 (95% CI, 0.50–1.32) for blood lead categories in men of 0.5–0.9, 1.0–1.4, 1.5–1.8 and ≥ 1.9 µmol/L, respectively. This study provided limited support for the hypothesis that paternal exposure to lead is associated with decreased fertility.

In a study by Joffe *et al*. (2003) as part of the ASCLEPIOS project, a total of 1104 subjects in four European countries took part, of whom 638 were occupationally exposed to lead at the relevant time. Blood lead concentrations were mainly < 50 µg/dL. No consistent association between time to pregnancy and lead exposure was found in any of the exposure models. It may be concluded from this multicentric survey that there are no detectable effects on male fertility at the levels of lead exposure currently measured in European worksites.

Lead may be determined in Leydig cells, thus in possible relation with testosterone levels in serum. Lead may also be detected in germ cells, demonstrating that it passes through the blood–testis barrier, which is functionally very similar to the blood–brain barrier, and affects the germ cells at different degrees of differentiation (spermatogonia, primary spermatocytes, spermatids or spermatozoa). In this regard, it is still an open question whether lead in cells or in fluids is a result of a breakdown of the blood–testis barrier or whether lead normally passes this barrier.

(*b*) *Effects of lead during pregnancy*

Wibberley *et al*. (1977) studied placental lead concentrations in a series of births in Birmingham, United Kingdom, classified by stillbirth, neonatal death or survival beyond one week. Average results showed higher lead concentrations in those neonates who failed to survive both birth and the neonatal period. There was no association of placental lead with impaired birth weight among survivors.

Placental transfer of lead and its effects on newborns were examined by Clark (1977). Following delivery, blood from 122 mothers and cord blood from their infants were taken to measure lead, haemoglobin, packed cell volume and mean corpuscular haemoglobin concentration. All were resident in Kasanda, Zambia, a lead mine and smelter town. The mean blood lead concentrations were 41.2 µg/dL and 37 µg/dL for maternal blood and cord blood, respectively, with a significant correlation ($r = 0.77$, $p < 0.001$). The increased lead transfer, however, did not appear to affect adversely birth weight or red cell values of the newborn.

Nordström *et al.* (1979a) investigated the frequencies of congenital malformations in the offspring of female employees at a smelter in northern Sweden and in a reference population near the smelter. In the population of the area, no significant variation in the total frequency of malformations or in any particular group of malformations was found. Among the women who worked at the smelters, the risk for malformations was about two times as high and the risk for multiple malformations about four times as high as in the reference population.

In a study of the relationship between prenatal lead exposure and congenital anomalies, Needleman *et al.* (1984) measured lead concentration in umbilical cord blood from 5183 consecutive deliveries of at least 20 weeks' gestation. The demographic and socioeconomic variables of the mothers, including exposure to lead, which were shown on univariate analysis to be associated with increased risk for congenital anomalies, were evaluated in a stepwise logistic-regression model with malformation as the outcome. Coffee, alcohol, tobacco and marijuana use, which were associated with lead concentrations, but not with risk for malformation in offspring, were also taken into account. The model was reduced in steps by eliminating the variables with the highest *p*-value, until the most parsimonious model was created. The relative risk for anomalies associated with lead was then calculated while holding other covariates constant. Lead was found to be associated, in a dose-related fashion, with an increased risk for minor anomalies, but the risk for major malformations was not increased.

Bellinger *et al.* (1991) evaluated the relationship between prenatal low-level lead exposure and fetal growth in 4354 pregnancies in which the mean lead concentration in umbilical cord blood was 7.0 µg/dL (SD, 3.3; 10th percentile, 3.4 µg/dL; 90th percentile, 10.9 µg/dL). Higher cord blood lead concentrations were significantly associated with gestations of slightly longer duration. Comparing infants with cord blood lead concentrations ≥ 15 µg/dL with those with < 5 µg/dL, adjusted risk ratios of 1.5–2.5 were observed for low birth weight (< 2500 g) and for fetal growth indices that express birth

weight as a function of length of gestation (e.g. small for gestational age, intrauterine growth retardation). The 95% confidence intervals of these risk ratios included 1, but precluded rejection of the null hypothesis of no association. The authors concluded that the risk for adverse fetal growth is not increased at cord blood lead concentrations < 15 µg/dL but that modest increases in risk may be associated with concentrations ≥ 15 µg/dL.

Factor-Litvak *et al.* (1991) tested the hypothesis that exposure to lead during pregnancy is associated with reduced intrauterine growth and an increase in preterm delivery. The sample comprised women, recruited at mid-pregnancy, residing in Titova Mitrovica, a lead smelter town, or in Pristina, a non-exposed town 25 miles away, in the province of Kosovo, Serbia and Montenegro. Mean blood lead concentrations at mid-pregnancy were 0.92 µmol/L (± 0.38, n = 401) in women in the exposed town and 0.26 µmol/L (± 0.09, n = 506) in women in the comparison town. No differences were found between towns for either birth weight or length of gestation: mean birth weight was 3308 (± 566) g in Titova Mitrovica and 3361 (± 525) g in Pristina; mean length of gestation was 274 (± 18.8) days in Titova Mitrovica and 275 (± 15.6) days in Pristina. After adjustment for the effects of potential confounders, no significant relationships were found between maternal blood lead measured at mid-pregnancy, at delivery or in the umbilical cord, and either birth weight or length of gestation or preterm delivery (< 37 weeks). The authors concluded that exposure to environmental lead does not impair fetal growth or influence length of gestation.

The relation between paternal occupational exposure to lead and low birth weight/prematurity was also examined in a retrospective cohort study (Lin *et al*., 1998). Birth weight and gestational age, obtained from New York State birth certificates (1981–92), were compared for children born to lead-exposed and non-exposed workers. The exposed group *(*n = 4256) consisted of births to male workers of reproductive age reported to the New York State Heavy Metals Registry. The control group (n = 2259) consisted of the offspring of a random sample of male bus drivers, frequency matched by age and residence. There were no statistically-significant differences in birth weight or gestational age between the exposed and the control groups. However, workers who had elevated blood lead concentrations for more than 5 years had a higher risk of fathering a child of low birth weight (risk ratio, 3.40; 95% CI, 1.39–8.35) or who was premature (risk ratio, 3.03; 95% CI, 1.35–6.77) than did controls after adjustment for paternal age, low maternal education, race, residence, gravidity, maternal spontaneous abortion history, perinatal complications, adequacy of prenatal care and sex of the infant.

The effect of maternal bone lead on length and head circumference of newborns and infants aged one month was evaluated by Hernandez-Avila *et al.* (2002). Birth length of newborns was found to decrease as tibia lead concentrations increased. Patella lead was positively and significantly related to the risk of a low head circumference score; this score remained unaffected by inclusion of birth weight.

(*c*) *Effects of lead on abortion*

As a whole, the literature on this topic provides consistent evidence in the form both of case series and epidemiological studies, that the risk for spontaneous abortion (defined

as a pregnancy loss occurring before the 20th week of gestation, but after the stage of unrecognized, subclinical loss) is increased by maternal exposure to high concentrations of lead. The data on male exposures and spontaneous abortions in their partners are more sparse and less consistent.

Torelli (1930) provided data on pregnancies in Milan, where the printing industry was a source of lead exposure. The risk for spontaneous abortion was reported to be 4.5% in the general population, 14% in partners of men employed in the printing industry and 24% in women who themselves were so employed; these data yield relative risks of 3.1 and 5.3. The infant mortality was more than doubled among exposed women as compared with the rate in all of Italy: 320 versus 150 per 1000 livebirths (cited by Hertz-Picciotto, 2000).

Nordström *et al*. (1978a) reported an increased frequency of spontaneous abortion in women living close to a smelter in northern Sweden. In a later report, Nordström *et al*. (1979b) described the responses to a questionnaire completed by 511/662 women who had worked at the smelter and were born in 1930–59. Spontaneous abortion rates were high in those pregnancies in which the mother was employed during the pregnancy (13.9%) or had been employed before and was living close to the smelter (17%); the rate was higher (19.4%) when the father worked at the smelter. It should be noted that the smelter produced copper and lead in addition to a number of other metallurgical and chemical products (Nordström *et al*., 1978a) and that the effects reported may not necessarily be attributable exclusively to lead.

A study of pregnancies in the centre and surrounding areas of the lead smelter town of Port Pirie, Australia, found that incidence of miscarriages (22/23) and stillbirths (10/11) was higher in women living close to the smelter (McMichael *et al*., 1986). Two studies found a decreased length of gestation in women whose blood lead concentrations were > 0.58 μmol/L (12 μg/dL) (Dietrich *et al*., 1986) or 0.68 μmol/L (14 μg/dL) (McMichael *et al*., 1986). However Needleman *et al*. (1984), Bellinger *et al*., (1984) and Factor-Litvak *et al.* (1991) did not find differences in gestational length of pregnancy in women with higher blood lead concentrations.

Murphy *et al*. (1990) analysed the rates of spontaneous abortion among women living in the vicinity of a lead smelter with those of women living in a town where exposure to lead was low. The data were taken from the obstetric histories of both groups of women when they sought prenatal care for a subsequent pregnancy. A total of 639 women (304 exposed, 335 unexposed) had at least one previous pregnancy and had lived at the same address since their first pregnancy. The geometric mean blood lead concentrations at the time of the interviews were 0.77 μmol/L [16 μg/dL] in women in the exposed town and 0.25 μmol/L [5 μg/dL] in women in the unexposed town. The rates of spontaneous abortions in first pregnancies were similar: 16.4% of women in the exposed town and 14.0% in the unexposed town . The adjusted odds ratio relating town of residence to spontaneous abortion was 1.1 (95% CI, 0.9–1.4).

A case–referent study conducted by Lindbohm *et al* (1991) focused on whether occupational exposure of men to inorganic lead is related to their partners' spontaneous

abortion. The cases (213 spontaneous abortions) and referents (300 births) were identified from medical registers. Lead exposure was assessed by blood lead measurements and data obtained from a questionnaire. The results did not show a statistically-significant relationship between spontaneous abortion and paternal exposure to lead among the study subjects.

In a comparison of placental lead concentrations in 71 normal deliveries and 18 births with adverse outcomes (premature birth or premature rupture of membranes) significantly higher placental lead concentrations were found in the adverse birth groups (153.9 ± 71.7 ng/g dry weight compared with the placentas from normal deliveries (103.2 ± 49.5 ng/g dry weight) (Falcón *et al.*, 2003).

Hu (1991) provided data from Boston, MA, USA, on the pregnancies of women who themselves experienced lead poisoning during their childhood in the years 1930–44. The rationale for this study lay in the fact that lead is stored in bone tissue for decades, and the possibility that demineralization of the skeleton takes place during pregnancy. Thirty-five cases of childhood plumbism were identified from hospital records. These women were traced in the 1980s, and interviewed regarding their pregnancy histories. Matched control subjects were included for 22 of the 35 women with childhood plumbism. The proportion of pregnancies reported to have ended in spontaneous abortion or stillbirth was 22% (11/51) among cases with matched plumbism, 29% (8/28) among the cases with non-matched plumbism and 13% (6/48) among matched control subjects. The matched-pairs odds ratio was 1.6 (95% CI, 0.6–4.0) reflecting the small size of the study. Inclusion of unmatched plumbism subjects did not alter the results.

In conclusion, the studies reviewed here show that the effects of lead on fertility and abortion were not always the same either morphologically or quantitatively, neither did they always vary in the same direction. Those on sperm count and concentration were the most frequent in showing effects of lead. It is not yet clear whether the mechanism is a direct effect of lead on reproductive organs or on the endocrine control of reproduction, or both. The mechanism for inducing pregnancy loss is also not clear. Besides preconceptional chromosomal damage to the sperm or a direct teratogenic effect on the fetus, interference with the maternal–fetal hormonal environment is possible, as endocrine-disrupting activity associated with lead has been observed in rodents, primates, and humans. Vascular effects on the placenta are also plausible, given the literature on lead and hypertension (Hertz-Picciotto & Croft, 1993). Developmental toxicity to the fetus is also possible.

(*d*) *Effects on stature and growth*

The effects of low to moderate prenatal and postnatal lead exposure on children's growth in stature were studied by Shukla *et al.* (1989, 1991) in 235 subjects assessed every 3 months for lead exposure (blood lead concentration) and stature (recumbent length) up to 33 months of age. Fetal lead exposure was indexed by maternal blood lead concentration during pregnancy. Adverse effects of lead on growth during the first year of life were observed. Mean blood lead concentrations during the second and third years of life were negatively associated with attained height at 33 months of age ($p = 0.002$), but only among those children who had mean blood lead concentrations above the cohort

median (> 10.77 µg/dL) during the 3–15-month period. The results suggest that the effects of lead exposure (*in utero* and during the first year of life) are transient provided that subsequent exposure to lead is not excessive. An average blood lead concentration of 25 µg/dL or higher during the second and third year of life was detrimental to the child's attained stature at 33 months of age. Approximately 15% of this cohort experienced these levels of lead exposure.

The relationship between blood lead concentration and stature was evaluated for a group of 1454 Mexican-American children (age, 5–12 years), from data sets of the 1982–84 Hispanic Health and Nutrition Examination Survey. An inverse relationship was found between blood lead concentration in the range 0.14–1.92 µmol/L [3–40 µg/dL] and stature, which suggests that growth retardation may be associated even with moderate concentrations of blood lead (Frisancho & Ryan, 1991).

Concentrations of lead, zinc and lysozyme, a factor of non-specific immunity, were determined in blood and placental tissue from 50 pregnant women with intrauterine fetal growth retardation (IUGR) and from 27 pregnant women in a control group. Statistically-significant differences in zinc and lead concentrations were found between the groups, with the IUGR group having lower zinc and higher lead concentrations. A significant negative correlation between zinc and lead concentrations was observed, as well as a statistically significant relationship between placental lead concentrations and the age of the pregnant women. Greater age was associated with higher lead concentrations in placental tissue, whereas zinc concentrations decreased. Higher lysozyme concentrations were found in placental tissues of women in the IUGR group (Richter *et al.*, 1999).

The possible role of environmental pollutants in the incidence of IUGR in India was investigated by measurement of lead and zinc concentrations in blood collected at parturition from mothers and neonates. Both maternal and cord blood lead concentrations were significantly higher in IUGR cases than in normal cases ($p < 0.05$). The mean concentration of zinc was also higher in maternal blood of IUGR cases. The mean cord blood lead concentration was > 10 µg/dL in 54% of newborns. A good correlation ($r = 0.53$; $p < 0.01$) between maternal and cord blood lead concentrations confirmed the transfer of lead from mother to fetus. There was a weak but significant inverse relationship between cord blood lead concentrations and birth weight of newborns ($r = –0.23$, $p < 0.05$) (Srivastava *et al.*, 2001).

4.3.2 *Animal studies*

(*a*) *Male fertility*

Many studies in experimental animals have generated results that are consistent with direct toxic effects of lead on seminiferous tubules or Leydig cells, but one study reported simultaneous impairment of spermatogenesis and reduced pituitary content of FSH, which points to a primary action at the extratesticular level.

The male reproductive organs of Sprague-Dawley rats and NMRI mice are apparently rather resistant to the toxicity of inorganic lead. However, several studies of other rat

strains and other rodent species indicate fairly consistently that exposures to lead that result in blood lead concentrations > 30–40 μg/dL for at least 30 days are associated with impairment of spermatogenesis and reduced concentrations of circulating androgens. The great variations in hormone concentrations, whether they are circadian, age-related, seasonal, individual or even strain-related make it difficult to draw valid conclusions on hormonal effects (Lee *et al*., 1975; Ellis & Desjardins, 1982; Heywood & James, 1985).

Age and sexual maturity of the animal may have a bearing on the results in several ways. It has been shown that prepubertal rats are less sensitive to the toxic effects of lead on testosterone and sperm production than animals exposed to lead after puberty (Sokol & Berman, 1991).

Momcilovic and Kostial (1974) found marked differences in lead distribution in suckling rats compared with adult rats. Age-related changes should also be considered: Heywood and James (1985) showed that up to 7% of rats maintained for 52 weeks showed spermatogenesis not proceeding beyond the spermatocyte stage. At 104 weeks, 20% of rats had developed atrophy of the seminiferous epithelium.

Of the 21 experimental studies reviewed by Apostoli *et al* (1998), 15 mentioned the age of the animals at the start of the experiment. However, animals were sexually mature (i.e. 90 days old) at the start of the experiment in only two studies. In four other studies, age at start was described only as 'mature'. Descriptions of subchronic effects should be interpreted with caution when the test period is shorter than 77 days for rats, 53 days for mice, 64 days for rabbits and 57 days for monkeys. Taking this into account, about half of the animal studies reviewed by Apostoli *et al.* (1998) can be considered to assess only acute effects.

Schroeder and Mitchener (1971) have shown that mice are more vulnerable to the toxic effects of lead on reproduction than rats. Exposure of sexually-mature animals to lead caused varying degrees of impaired spermatogenesis (Chowdhury *et al*., 1984; Barratt *et al*., 1989), premature acrosome reaction and reduction of fertility (Johansson, 1989) or hormonal disorders (Sokol & Berman, 1991) at widely varying (30–187 μg/dL) blood lead concentrations (Apostoli *et al.*, 1998).

Ivanova-Cemišanska *et al*. (1980) reported changes in levels of enzymatic activity and ATP in testicular homogenate of rats given 0.2 and 20 mg/kg bw solutions of lead acetate, over a 4-month period.

Chowdhury *et al*. (1984) found testicular atrophy and cellular degeneration in rats with blood lead concentrations > 70 μg/dL, but not in rats with blood lead concentrations of 54.0 μg/dL.

A comprehensive study in rabbits (Moorman *et al*., 1998) estimated a threshold for effects on total sperm count of 23.7 μg/dL lead in blood.

Groups of cynomolgus monkeys with mean blood lead concentrations of 10 ± 3 μg/dL (n = 4) and 56 ± 49 μg/dL (n = 7) after treatment with lead acetate from birth to the age of 15–20 years had increased abnormal sperm chromatin as expressed by the αT distribution (shift from green to red fluorescence) with a larger SD αT when compared with a reference

group with blood lead < 1 μg/dL. However, there were no effects of treatment on parameters of semen quality such as sperm count, viability, motility (Foster *et al*., 1996).

The results of studies on the lead content of testicular or seminal fluid are inconclusive (Hilderbrand *et al.*, 1973; Der *et al.*, 1976; Chowhury *et al.*, 1984; Sokol *et al.*, 1985; Saxena *et al.*, 1987; Boscolo *et al.*, 1988; Barratt *et al.*, 1989; Saxena *et al.*, 1990; Sokol & Berman, 1991; Nathan *et al.*, 1992; Pinon-Lataillade *et al.*, 1993; Thoreux-Manlay *et al.*, 1995). Although a relation between testicular lead content and histopathological changes has been noted, the lack of uniformity regarding age of the animals, duration of exposure, assessment of internal doses, identification of reproductive end-points, and methods to measure effect indicators, makes it impossible to draw any clear conclusions on mechanisms and dose–response relationships.

(*b*) *Effects on pregnancy, fertility and growth and development in animals*

Many early studies identified effects on spermatogenesis in rats exposed to lead and also indicated that high exposure of dams to lead can reduce numbers and size of offspring. There may also be paternally-transmitted effects resulting in reductions of litter size, weights of offspring and survival rate (for references, see WHO, 1995). Other important topics are the exposure periods, the sites of action, and growth and development.

Lead (as lead acetate) was administered to mouse dams via the drinking-water (at 10 mg/mL) during three periods: (1) when target mice were born (postnatal); (2) after conception of target mice (gestational); or (3) during the mothers' own pre-weaning age (premating). These experiments showed variable effects of lead exposure on brain weight, DNA per brain and protein per brain (Epstein *et al.*, 1999).

Exposure of female rats to lead produced irregular estrous cycles at blood lead concentrations of 30 μg/dL and morphological changes in ovaries including follicular cysts and reduction in numbers of corpora lutea at blood lead concentrations of 53 μg/dL (Hilderbrand *et al*., 1973).

Grant *et al*. (1980) reported delayed vaginal opening in rats whose mothers were given 25, 50 and 250 ppm lead in drinking-water. The vaginal opening delays in the 25-ppm group occurred in the absence of any growth retardation or other developmental delays and were associated with median blood lead concentrations of 18–29 μg/dL.

Testicular homogenates from 2–3-week-old male offspring of lead-exposed female rats (mean blood lead concentration in the pups, 6.3 μg/dL) showed decreased ability to metabolize progesterone (Wiebe *et al*., 1982).

In a study by McGivern *et al.* (1991), Sprague-Dawley dams were given lead acetate (0.1%) in drinking-water from day 14 of gestation until parturition to determine whether exposure of the fetus to elevated lead concentrations during a period of rapid differentiation of the hypothalamic–pituitary–gonadal (HPG) axis would disrupt HPG function in adulthood. Female offspring from lead-treated dams were found to have a significant delay in the day of vaginal opening and prolonged and irregular periods of diestrous accompanied by an absence of observable corpora lutea at 83 days of age. Male offspring

from these dams were found to have decreased sperm counts at 70 and 165 days of age, exhibit enlarged prostates at 165 days and ~35% reduction in the volume of the sexually dimorphic nucleus of the preoptic area of the hypothalamus. Pulsatile release of gonadotropins, measured in castrated male and female adult animals, revealed irregular release patterns of both FSH and LH in some lead-treated animals which were not observed in controls. The overall pattern of data suggested to the authors that multiple functional aspects of the HPG axis can be affected by exposure to lead during a period of gestation when structures related to the HPG axis are undergoing rapid proliferation.

The reproductive toxicity and growth effects of lead exposure in developing rats have also been assessed by Ronis *et al.* (1996). Lead exposure was initiated *in utero*, prepubertally, or postpubertally. In male animals, weights of testis and all secondary sex organs were significantly decreased in animals exposed prepubertally. Serum testosterone levels were significantly suppressed, most severely in animals exposed *in utero*. In female animals exposed prepubertally, delayed vaginal opening and disrupted estrous cycling was observed in 50% of the animals. The group treated *in utero* had suppression of circulating estradiol accompanied by significant decreases in both circulating LH concentrations and pituitary LH protein concentration, but no effect on LHβ mRNA was observed. These findings suggested to the authors a dual site of action for lead: (a) at the level of the hypothalamic pituitary unit; and (b) at the level of gonadal steroid biosynthesis. Prepubertal growth in both sexes was suppressed by 25% in the group exposed *in utero*. The effects of lead on growth are possibly due to a delay in the development of sex-specific pituitary growth hormone secretion rather than a persistent developmental defect.

Studies on female monkeys have shown that pre- and/or postnatal exposure to lead can affect pubertal progression and hypothalamic–pituitary–ovarian–uterine functions. Chronic exposure to lead of nulliparous female monkeys, resulting in blood concentrations of approximately 35 μg/dL, induced subclinical suppression of circulating LH, FSH and estradiol without producing overt effects on general health and menstrual function (Foster, 1992).

4.4 Genetic and related effects

4.4.1 *Human studies* (see Table 91)

In human genotoxicity studies, co-exposures to lead and other compounds could not be discounted and thus it is difficult to attribute genetic and other related effects to lead alone. A general description of lead concentrations in air and blood in various exposure situations is given in Section 1.

The single-cell gel electrophoresis assay (Comet assay) provides data that are indicative of DNA damage; either direct strand breaks or alkali-labile sites. Five studies of DNA damage using the Comet assay on blood leukocytes in lead-exposed workers gave positive results. In workers in a secondary lead smelter in India with blood lead concentrations of 24.8 ± 14.7 μg/dL, there was a significant increase in the percentage of leukocytes showing

Table 91. Genetic and related effects in humans occupationally or non-occupationally exposed to lead

Subjects	No. of exposed/controls	End-point Result[a]	Air lead concentration ($\mu g/m^3$)	Mean blood lead concentration ($\mu g/dL$)	Reference
Occupationally exposed					
		DNA damage (SCGE (Comet) assay)			
Secondary lead smelter workers, Hyderabad, India	45 exposed	% of cells with tail length increased, 44.6 ± 8.5 ($p < 0.05$);	4.2	24.8 ± 14.7	Danadevi *et al.* (2003)
	36 controls	21.1 ± 11.7		2.75 ± 1.52	
Secondary lead smelter workers, China	46 exposed	Significant increase in tail length, dose-related ($p < 0.05$)	–	Range of medians in different subgroups, < 13–> 37; median in controls, 9	Ye *et al.* (1999)
	28 controls				
Battery plant workers, Italy	37 exposed	Significant increase in tail moment ($p = 0.011$), dose-related	–	39.6 ± 7.6	Fracasso *et al.* (2002)
	29 controls			4.4 ± 1.7	
Battery plant workers, Colombia	43 exposed	Significant increase in tail length, no dose–response ($p < 0.05$)	–	98.5 ± 25.3	De Restrepo *et al.* (2000)
	13 controls			5.4 ± 3.6	
Battery plant workers, Poland	44 exposed	% of cells with tail length increased, 15.6 ± 4.1 ($p < 0.05$)	–	50.4 ± 9.2	Palus *et al.* (2003)
	40 controls	11.3 ± 5.0		5.6 ± 2.8	
		Other DNA damage			
		DNA–protein crosslinks			
Battery plant workers, Taiwan, China	23 high exposed	$1.8 \pm 0.7\%^{S}$; 1.4 ± 0.5^{NS} ($p < 0.05$)	0.2–10.3	32.5 ± 14.5	Wu *et al.* (2002)
	34 low exposed	1.2 ± 0.4^{S}; 1.1 ± 0.5^{NS}		9.3 ± 2.9	
	30 controls	1.0 ± 0.2^{S}; 1.0 ± 0.3^{NS}		4.2 ± 1.4	
		DNA single strand break			
Workers exposed at 10 facilities in Hessen, Germany (Cd and Co co-exposed)	78 exposed	No significant effects	1.6–50	2.8–13.7	Hengstler *et al.* (2003)
	22 controls		Median, 3	Median, 4.41	
		Micronuclei (% of cells with micronuclei)			
Battery plant workers, Bulgaria	73 exposed	38.6 ± 16.8% ($p < 0.05$)	193–700	67 ± 23	Vaglenov *et al.* (1997)
	23 controls	19.1 ± 16.2%	60	25 ± 6	

Table 91 (contd)

Subjects	No. of exposed/controls	End-point Result[a]	Air lead concentration ($\mu g/m^3$)	Mean blood lead concentration ($\mu g/dL$)	Reference
Battery plant workers, Pazardzik, Bulgaria	22 exposed 19 external controls 19 internal controls	62 ± 3% ($p < 0.001$) 20 ± 2% 26 ± 3%	447 ± 52 73 ± 22 58 ± 5	61 ± 3 (SE) 18 ± 0.6 (SE) 2.8 ± 1.6 (SE)	Vaglenov *et al.* (1998)
Metal powder factory (exposure to Pb, Zn, Cd), Turkey	31 exposed 20 controls	0.65/cell ($p < 0.01$) 0.24/cell	–	40 ± 18 12 ± 4	Hamurcu *et al.* (2001)
Battery plant workers (may include some subjects from previous study), Pazardzik, Bulgaria	103 workers 78 controls (43 internal, 35 external combined)	43 ± 2% ($p < 0.001$) 22 ± 1%	–	56 ± 2 19 ± 0.8	Vaglenov *et al.* (2001)
Battery plant workers, Poland	30 exposed 42 controls	18.6 ± 5.0% ($p < 0.01$) 6.6 ± 3.9%	–	50.4 ± 9.2 5.6 ± 2.8	Palus *et al.* (2003)
		Chromosomal aberrations			
Lead oxide workers, Germany	8 exposed[b] 14 controls	Significant increase in various types of chromosome damage ($p < 0.01$)	–	74.7 ± 9.4 14.9 ± 4	Schwanitz *et al.* (1970)
Lead manufacturing workers, Germany	32 exposed 20 controls	No significant effect	–	NR (3 with lead intoxication)	Schmid *et al.* (1972)
Ship-breaking workers, UK		Chromatid abs[c] / Chromosomal abs[c]	–		O'Riordan & Evans (1974)
	35 exposed 31 controls 285 other survey controls	5.16% / 0.69% 4.46% / 0.42% 2.18% / 1.16%		Range, 40–> 120 < 40	
Steel plant workers, Germany	105 exposed no control group	No significant correlation with blood lead or urine ALA	–	37.7 ± 20.7	Schwanitz *et al.* (1975)
Battery plant workers (prospective study), Italy	11 exposed (same subjects, pre-employment)	Significant increase in chromosomal aberrations ($p < 0.05$)	< 800	After 1 month: 45 ± 17.3 Pre-employment: 34 ± 12.6	Forni *et al.* (1976)

Table 91 (contd)

Subjects	No. of exposed/controls	End-point Result[a]		Air lead concentration (μg/m^3)	Mean blood lead concentration (μg/dL)	Reference
Lead smelter workers, Finland	18 exposed 12 controls	1.3% 1.8% no significant effect		50–500	48.7 ± 1.7 < 10	Mäki-Paakkanen *et al*. (1981)
Smelter workers (exposed to Pb, As), Rönnskär, Sweden	26 exposed Historical controls	Chromatid abs/cell 0.023 0.019 0.006 0.004	Chromosomal abs/cell 0.027 ($p < 0.001$) 0.004 0.000 0.001	–	 High: 64.77 ± 10.95 Medium: 39.19 ± 7.13 Low: 22.48 ± 1.77	Nordenson *et al*. (1978)
Battery plant workers, Baghdad, Iraq	 19 exposed 9 controls	Chromatid abs 3.4 ± 2.4% 1.5 ± 3.0%	Chromosomal abs 3.3 ± 2.3% 2.0 ± 2.3%	–	NR	Al-Hakkak *et al*. (1986)
Battery plant workers, China	7 high exposed 7 medium exposed 7 low exposed 7 controls	3.71 ($p < 0.01$) 2.71 1.43 1.14		–	86.9 ± 16.5 52.1 ± 7.3 33.7 ± 5.9 7.8 ± 2.3	Huang, X.-P. *et al*. (1988)
		Sister chromatid exchange				
Lead smelter workers, Finland	18 exposed 12 controls	11.7 ± 0.4^S; 9.8 ± 0.7^{NS} ($p < 0.05$ in smokers only) 10.4 ± 0.4^S; 9.2 ± 0.4^{NS}		50–500	48.7 ± 1.7 < 10	Mäki-Paakkanen *et al*. (1981)
Battery plant workers, Denmark	10 long-term exposed 18 new employees	Long-term exposed: lower frequency after a 4-wk vacation New employees: no significant increase after 2–4 months employment		–	29.0–74.5 6.2–29.0	Grandjean *et al*. (1983)
Battery plant workers, Monterrey, Mexico	54 exposed 13 controls	7.9 ± 1.5 7.0 ± 1.2		–	45.2 ± 16.6 25.5 ± 6.4	Leal-Garza *et al*. (1986)
Battery plant workers, China	7 high exposed 7 medium exposed 7 low exposed 7 controls	7.06 ± 0.39 ($p < 0.001$) 4.48 ± 0.75 3.93 ± 0.53 4.04 ± 0.33		–	86.9 ± 16.5 52.1 ± 7.3 33.7 ± 5.9 7.8 ± 2.3	Huang, X.-P. *et al*. (1988)
Printers, India	13 exposed 16 controls	No increase		–	NR	Rajah & Ahuja (1995)

Table 91 (contd)

Subjects	No. of exposed/controls	End-point Result[a]	Air lead concentration ($\mu g/m^3$)	Mean blood lead concentration ($\mu g/dL$)	Reference
Metal-powder factory workers, Turkey	32 exposed 20 controls	8.9 ± 1.4^{S}; 8.2 ± 0.9^{NS} ($p < 0.01$ in nonsmokers only) 8.7 ± 1.0^{S}; 7.2 ± 0.6^{NS}	–	13.8 ± 9.2 2.4 ± 0.9	Donmez *et al.* (1998)
Battery plant workers, Taiwan, China	23 high exposed 34 low exposed 30 controls	6.4 ± 0.5^{S}; 5.9 ± 0.7^{NS} ($p < 0.05$) 5.8 ± 0.4^{S}; 5.5 ± 0.7^{NS} 5.7 ± 0.3^{S}; 4.9 ± 0.4^{NS}	0.2–10.3	32.5 ± 14.5 9.3 ± 2.9 4.2 ± 1.4	Wu *et al.* (2002)
Battery plant workers, Ankara, Turkey	71 exposed 20 controls	Significant increase in group with blood lead > 50 µg/dL ($p < 0.05$)	–	34.5 ± 1.5 10.4 ± 0.4	Duydu & Süzen (2003)
Battery plant workers, Poland	30 exposed 43 controls	7.6 ± 0.9^{S}; 7.1 ± 0.9^{NS} ($p < 0.05$) 6.5 ± 1.1^{S}; 5.9 ± 0.8^{NS}	–	50.4 ± 9.2 5.6 ± 2.8	Palus *et al.* (2003)
Non-occupationally exposed					
		Oxidative DNA damage			
Citizens of Bremen, Germany	141	No increase in oxidative DNA damage (Fpg-sensitive sites)	–	Median, 4.6	Merzenich *et al.* (2001)
		Sister chromatid exchange			
Children living near a lead smelter, Milan, Italy	19 exposed 12 controls	No effect	–	29.3–62.7 10.0–21.0	Dalpra *et al.* (1983)
		Chromosomal aberrations			
Male volunteers, Netherlands	11 ingested[d] 10 controls	No significant effect	–	$40 \pm 5 \times 7$ wks	Bijlsma & de France (1976)
Children living near lead smelter, Germany	20 exposed 20 controls	No significant effect	–	> 30 7–19	Bauchinger *et al.* (1977)

–, No data; S, smoker; NS, nonsmoker; SE, standard error; NR, not reported; ALA, δ-aminolevulinic acid; Fpg, formamidopyrimidine-DNA glycosylase

[a] Dose–response refers to blood lead concentrations.

[b] Exposed workers have significantly increased mitotic index.

[c] Chromatid/chromosomal abnormalities

[d] Daily ingested lead acetate to give mean blood lead concentration of 40 ± 5 µg/dL for 7 wks

DNA damage and increased Comet tail length compared with controls (non-exposed). Blood lead was positively associated with the percentage of DNA-damaged cells (Danadevi *et al*., 2003). [The Working Group noted that the air lead level was unexpectedly low.] Significantly increased percentages of DNA-damaged leukocytes and tail length, as well as increased malondialdehyde concentrations were also seen in workers in a secondary lead smelter in China. The effects were dose-related, with minimal blood lead concentrations of 27–37 μg/dL being associated with genotoxicity (Ye *et al*., 1999). Similar results were seen in workers in battery plants in Italy, Columbia and China, Province of Taiwan (De Restrepo *et al*., 2000; Fracasso *et al*., 2002; Wu, F.-Y. *et al*., 2002) where significant increases in tail moment, tail length and/or DNA in the tail were observed in workers' lymphocytes. In one study, the Comet assay results were correlated with blood lead concentrations, and with decreased concentrations of reduced glutathione (GSH) in blood (Fracasso *et al.*, 2002). The DNA damage occurred at blood lead concentrations > 40 μg/dL in the workers in Columbia and sister chromatid exchange occurred at blood lead concentrations > 15 μg/dL in workers in China, Province of Taiwan.

In a single study, evidence for increased DNA–protein crosslinks was seen at high blood lead concentrations in the highly-exposed group (blood lead, 32.5 ± 14.5 μg/dL) of battery plant workers (Wu, F.-Y. *et al*., 2002). DNA single-strand breaks (measured with the alkaline elution assay) were not increased in lymphocytes of workers with median blood lead concentrations of 4.41 μg/dL (Hengstler *et al*., 2003). [The Working Group noted that the air lead level was unexpectedly low.] However, in the latter study, lead exposure increased the effects of cadmium in inducing DNA strand breaks.

All of five studies of micronuclei in blood lymphocytes of exposed workers found increases. These occurred in battery plant workers exposed to at least 193 μg/m^3 lead in air (resulting in 3.16 μM [65.5 μg/dL] in blood) (Vaglenov *et al*., 1997). A second study confirmed these results and demonstrated a reduction in micronucleus frequency in workers given a vitamin and mineral supplement (Vaglenov *et al*., 1998). The authors suggested that oxidative DNA damage may be responsible for the micronuclei. Battery plant workers in Poland were shown to have increased micronuclei in both centromere-positive and centromere-negative classes, indicating both a clastogenic and aneugenic effect of lead (Palus *et al*., 2003).

Studies of chromosomal aberrations in lead-exposed workers gave mixed results. Chromosomal aberrations were evaluated in 105 lead-exposed workers in Germany and found to be slightly but not significantly increased (Schwanitz *et al*., 1975). In an earlier report from this group with a small number of subjects, chromosomal aberrations were positively correlated to excretion of ALA (Schwanitz *et al*., 1970), but a higher mitotic index in lymphocytes from workers was noted. Negative results for chromosomal aberrations were reported by Schmid *et al*. (1972) and O'Riordan and Evans (1974) for workers in lead manufacturing and ship breaking, respectively. In a prospective study in which 11 battery plant workers acted as their own controls, a doubling of chromosomal aberrations (mostly chromatid and one-break aberrations) was seen after 1 month of employment. There was a further increase in the second month, but then the level remained the same

for at least 7 months. The increased frequency of chromosomal aberrations was correlated to inhibition of ALAD in red blood cells (Forni *et al.*, 1976). The authors speculated that culture conditions may have been responsible for the DNA damage, whose repair is inhibited by lead because, in a previous study in which the bone-marrow cells were not cultured, exposure to lead did not result in increased chromosomal aberrations (Forni & Secchi, 1972). Mäki-Paakkanen *et al*. (1981) also found evidence of 'culture-born aberrations', and noted that these may have influenced the outcome of the study.

In a study in which primary copper and lead smelter workers were stratified by blood lead concentrations, increased frequencies of chromatid-type aberrations were seen in the intermediate group (mean blood lead, 39.19 μg/dL); and chromosome-type aberrations were seen only in the 'high' group (mean blood lead, 64.77 μg/dL) (Nordenson *et al*., 1978). The authors estimated that a blood lead concentration of 25 μg/dL is the minimum required to produce any chromosomal effects. Huang *et al*. (1988) only saw an increased frequency of chromosomal aberrations in their intermediate group (mean blood lead, 52.1 μg/dL).

The results of studies measuring sister chromatid exchange in workers exposed to lead are mostly positive but, in some studies, positive responses were seen only in smokers. For example, a small increase in sister chromatid exchange was seen only in lead smelter workers who smoked (Mäki-Paakkanen *et al*., 1981). No significant increase in sister chromatid exchange was seen in printers (confounded by smoking) (Rajah & Ahuja, 1995), whereas there was a significant increase in battery plant workers after controlling for smoking (Duydu & Süzen, 2003). In these studies, there was also inconsistency in the correlations with blood lead concentrations. In one study, the level of sister chromatid exchange decreased in battery plant workers after a 4-week vacation (Grandjean *et al*., 1983). The same authors also monitored newly-employed workers and found no increases in sister chromatid exchange after 2–4 months of employment.

In general, studies in which a variety of genotoxic end-points were measured in non-occupationally exposed subjects (children living near plants, volunteers, general population) gave negative results (Table 91).

4.4.2 *Effects in animals* (see Table 92)

DNA damage was assessed in kidney cells of male rats by use of the Comet assay (single-cell gel electrophoresis). In cells isolated from the kidneys of rats that had received three doses of lead acetate by oral administration, a larger Comet tail was seen than in cells from animals that had been given the same amount of lead in a single dose. The same study also showed an increased level of sister chromatid exchange in kidney cells, with the single high dose being more effective (Robbiano *et al*., 1999). When mice were exposed for three generations to lead acetate in the drinking-water, Comet tail length increased in blood cells in the F_1 and F_2 generations, but not in the dams (Yuan & Tang, 2001). With an inhalation protocol in mice, DNA damage was detected in the liver and lung after a single exposure to lead acetate, whereas kidney, brain, nasal cells, bone

Table 92. Genetic and related effects of lead compounds in animals *in vivo*

Test system	Result	Dose[a] (LED or HID)	Reference
Lead acetate			
DNA damage, female Kunming mouse leukocytes (SCGE)	–	1 µg/mL water approx. 3–4 mo	Yuan & Tang (2001)
DNA damage, Kunming mouse leukocytes (SCGE), 2nd and 3rd generations of multigeneration study	+	1 µg/mL water *in utero* to sexual maturity	Yuan & Tang (2001)
DNA damage, male CD-1 mouse liver, kidney, nasal cavity, brain, bone-marrow cells (SCGE)	w+	6800 µg/m^3, inhal., 60 min × 2/wk, 4 wk	Valverde *et al*. (2002)
DNA damage, male CD-1 mouse testicle cells, leukocytes (SCGE)	–	6800 µg/m^3, inhal., 60 min × 2/wk, 4 wk	Valverde *et al*. (2002)
DNA damage, male CD-1 mouse lung cells (SCGE)	?*	6800 µg/m^3, inhal., 60 min × 2/wk, 4 wk	Valverde *et al*. (2002)
DNA damage, unilaterally nephrectomized Sprague-Dawley rat kidney (SCGE)	+	78 mg/kg bw po × 3	Robbiano *et al*. (1999)
Sister chromatid exchange, rabbit lymphocytes	–	0.5 mg/kg bw sc 3×/wk, 14 wk	Willems *et al*. (1982)
Micronucleus formation, female C57BL mouse bone marrow	–	25 mg/kg bw ip × 2	Jacquet *et al*. (1977)
Micronucleus formation, female C57BL/6 × C3H/He F_1 mouse bone marrow	–	1000 mg/kg bw ip	Bruce & Heddle (1979)
Micronucleus formation, male and female Sprague-Dawley rat bone marrow	w+	104 mg/kg bw ip	Tachi *et al*. (1985)
Micronucleus formation, rabbit bone marrow erythrocytes	–	0.5 mg/kg bw sc 3×/wk, 14 wk	Willems *et al*. (1982)
Micronucleus formation, unilaterally nephrectomized Sprague-Dawley rat kidney	+	78 mg/kg bw po × 3	Robbiano *et al*. (1999)
Chromosomal aberrations, female C57B1 mouse bone marrow	–	0.5% diet × 1 mo	Jacquet *et al*. (1977)
Chromosomal aberrations, male C57B1 mouse bone marrow	–	Normal diet + 0.5% × 1 mo	Deknudt & Gerber (1979)
Chromosomal aberrations, male C57B1 mouse bone marrow	+	Low Ca diet + 0.5% × 1 mo	Deknudt & Gerber (1979)
Chromosomal aberrations, female Sprague-Dawley rat bone marrow	+	104 mg/kg bw ip	Tachi *et al*. (1985)
Chromosomal aberrations, male Sprague-Dawley rat bone marrow	–	104 mg/kg bw ip	Tachi *et al*. (1985)
Chromosomal aberrations, female Sprague-Dawley rat bone marrow	–	104 mg/kg bw ip × 5	Tachi *et al*. (1985)
Chromosomal aberrations, male Sprague-Dawley rat bone marrow	w+	104 mg/kg bw ip × 5	Tachi *et al*. (1985)
Chromosomal aberrations, Wistar rat bone marrow	–	10 mg/kg bw po 5×/wk, 4 wk	Nehéz *et al*. (2000)
Chromosomal aberrations, male and female A/sw mouse leukocytes	+	1% diet × 2 wk	Muro & Goyer (1969)
Chromosomal aberrations, cynomolgus monkey lymphocytes	±	6 mg/d po × 10 mo	Deknudt *et al*. (1977)
Chromosomal aberrations, cynomolgus monkey leukocytes	–	5 mg/kg bw po/d × 12 mo	Jacquet & Tachon (1981)
Aneuploidy, Wistar rat bone marrow	+	10 mg/kg bw po 5×/wk, 4 wk	Nehéz *et al*. (2000)
Aneuploidy, cynomolgus monkey lymphocytes	±	6 mg/d po × 10 mo	Deknudt *et al*. (1977)
Sperm morphology, C57BL/6 F_1 × C3H/He F_1 mice	+	125 mg/kg bw ip	Bruce & Heddle (1979)
Sperm morphology, rabbits	–	0.5 mg/kg bw sc 3×/wk, 14 wk	Willems *et al*. (1982)
Sperm abnormality, cynomolgus monkey (acid denaturation of DNA)	+	50 µg/kg bw/d for 100–200 d[b]	Foster *et al*. (1996)

Table 92 (contd)

Test system	Result	Dose[a] (LED or HID)	Reference
Lead chloride			
Dominant lethal mutations, NMRI mice	–	1.33 g/L dw	Kristensen *et al*. (1993)
Lead nitrate			
Sister chromatid exchange, pregnant female Swiss Webster mouse bone marrow	+	150 mg/kg bw iv	Nayak *et al*. (1989)
Sister chromatid exchange, liver and/or lung of fetus of maternal Swiss Webster mice	–	200 mg/kg bw iv	Nayak *et al*. (1989)
Sister chromatid exchange, male Swiss albino mouse bone marrow	+	10 mg/kg bw ip	Dhir *et al*. (1993)
Micronucleus test, male and female Swiss albino mouse bone marrow	?	80 mg/kg bw ip	Jagetia & Aruna (1998)
Chromosomal aberrations, maternal bone marrow and fetal liver cells of Swiss Webster mouse	+	100 mg/kg bw iv	Nayak *et al*. (1989)
Aneuploidy, maternal bone marrow and fetal liver cells of Swiss Webster mouse	+	100 mg/kg bw iv	Nayak *et al*. (1989)
Induction of nondisjunction, *Drosophila melanogaster*	–	200 ppm feed	Ramel & Magnusson (1979)

+, positive; –, negative; ±, equivocal; w+, weak positive; ?, significant variation from dose to dose, no clear dose–response relationship; ?*, significant variation from week to week; po, oral; inhal., inhalation; dw, drinking-water; sc, subcutaneous; iv, intravenous; d, day; wk, week; mo, month; SCGE, single-cell gel electrophoresis; bw, body weight

[a] Lowest effective dose or highest ineffective dose

[b] Dose resulted in blood lead concentrations of 6–20 µg/dL.

marrow and leukocytes required more than one exposure before DNA damage was seen. No damage to testicular cells was seen after 4 weeks (Valverde *et al*., 2002).

No increases in sister chromatid exchange in rabbit lymphocytes were seen after subcutaneous injections of lead acetate (Willems *et al*., 1982). The same treatment also failed to cause sperm abnormalities or micronucleus formation in bone-marrow erythrocytes. However, intravenous injection of lead nitrate on day 9 of gestation increased sister chromatid exchange frequency in the bone marrow of F_1 mice, but not in fetal liver and/or fetal lung cells, although the lead was shown to cross the placenta (Nayak *et al*., 1989). In this study, lead nitrate caused chromosomal aberrations, mostly deletions, in both dams and fetal cells, as well as aneuploidy, increased embryonic resorptions and reduced placental weights. Dhir *et al.* (1993) showed that intraperitoneal injection of low doses of lead nitrate caused a significant increase in sister chromatid exchange in bone marrow in male Swiss albino mice. The lowest dose that caused micronucleus formation in bone marrow (but without a dose–response relationship) was 0.63 mg/kg bw lead nitrate. Male mice were found to be more sensitive than females (Jagetia & Aruna, 1998).

Feeding mice a diet containing lead acetate resulted in increased frequencies of chromosomal aberrations in leukocytes, particularly involving single chromatids (Muro & Goyer, 1969). Similar results were seen in a study in female C57BL mice (Jacquet *et al*., 1977) but, in a further study, only when mice were given a low-calcium diet (Deknudt & Gerber, 1979).

Female (but not male) rats given a single intraperitoneal injection of lead acetate had increased chromosomal aberrations (mostly gaps) (Tachi *et al*., 1985). In the same study, both male and female rats showed an increased frequency of micronuclei following treatment with lead acetate. The nature of the micronuclei was not determined, but lead acetate-induced chromatid gaps may reflect mostly clastogenicity rather than aneuploidy. Aneuploidy was induced in pregnant mice and their offspring (maternal bone marrow and fetal liver cells) by intravenous administration of lead nitrate on day 9 of gestation (Nayak *et al*., 1989) and in rats (bone marrow) given lead acetate orally (Nehéz *et al*., 2000), but nondisjunction did not increase in *Drosophila* given lead acetate in feed (Ramel & Magnusson, 1979).

Increased frequencies of chromosomal aberrations (gaps and fragments) and enhanced aneuploidy were seen in lymphocytes of monkeys given lead acetate orally or by intubation in one study (Deknudt *et al.*, 1977) but not in another (Jacquet & Tachon, 1981).

In a single in-vivo mutagenesis study, lead chloride in the drinking-water had no effect in the dominant lethal assay in mice (Kristensen *et al*., 1993).

Increased abnormal sperm morphology was seen in mice given lead acetate intraperitoneally (Bruce & Heddle, 1978). Increased sperm abnormality (analysed by sperm chromatin structure assay) was seen in monkeys given lead acetate resulting in blood lead concentrations of up to 20 µg/dL (Foster *et al*., 1976). However, subcutaneous administration of lead acetate did not induce sperm abnormalities in rabbits (Willems *et al*., 1982).

4.4.3 *Mammalian cells* in vitro (for references, see Table 93)

The genetic effects of lead compounds have been reviewed (Hartwig, 1994; Silbergeld *et al*., 2000). Equivocal results have been published with respect to the mutagenicity of water-soluble lead compounds in mammalian cells in culture; in most classical test systems, the effects were rather weak and/or restricted to toxic doses. Nevertheless, in AS52 Chinese hamster ovary cells carrying a single copy of an *Escherichia coli gpt* gene, lead chloride induced mutations in a dose-dependent manner at non-cytotoxic concentrations of < 1 μM (Ariza & Williams, 1996, 1999; Ariza *et al*., 1998). More detailed studies revealed predominantly point mutations with increasing frequencies of partial and complete deletions, in the dose range 0.5–1.0 μM (Ariza & Williams, 1999). Increased mutant frequencies in the *Hprt* gene were also observed in a study in Chinese hamster ovary K1 cells, but at higher, although still not cytotoxic concentrations of lead acetate, starting at 0.5 mM. Analysis of mutation spectra revealed base substitutions predominantly at G-C sites, as well as small and large deletions resulting from DNA damage induced by ROS (Yang *et al*., 1996). Furthermore, two studies revealed an increase in mutation frequency in combination with ultraviolet (UV) C irradiation or treatment with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) (Roy & Rossman, 1992). One potential mechanism may be the interaction with DNA repair processes; although results are equivocal, different outcomes may depend on incubation conditions. Thus, in human HeLa cell lines, two independent studies showed repair inhibition of X-ray-induced or UVC-induced DNA damage after 24 and 20 h preincubation, respectively (Skreb & Habazin-Novak, 1977; Hartwig *et al.*, 1990), while one study did not show an effect after 30 min preincubation (Snyder *et al.*, 1989).

With respect to the induction of chromosomal aberrations by lead acetate, treatment of human leukocytes showed clearly elevated frequencies of achromatic lesions, chromatid breaks and isochromatid breaks in 72-h cultures but not 48-h cultures (Beek & Obe, 1975); other studies with lead nitrate and lead glutamate were mostly negative. However, consistently positive results were obtained with lead chromate, which the authors related to the probable action of chromate (Wise *et al.*, 1994). Concerning the induction of micronuclei, a recent study reported a dose-dependent increase starting at concentrations of 1.1 μM lead chloride or 0.05 μM lead acetate. Both positive and negative results have been reported for the induction of sister chromatid exchange; nevertheless, similar to the enhancement of UVC-induced mutagenicity noted above, lead acetate also increased the UVC-induced frequency of sister chromatid exchange. More consistently, lead acetate as well as particulate lead chromate induced cell transformation in several studies; in the case of lead chromate, the effect was thought by the authors to be not due solely to the action of chromate (Elias *et al.*, 1989; Sidhu *et al.*, 1991). The induction of DNA damage in mammalian cells by lead acetate and lead nitrate has been investigated repeatedly, yielding negative or (mostly weakly) positive results for DNA strand breaks; one study did not find 8-OH-deoxyguanosine (8-OH-dG) in nuclear DNA, and one suggested the induction of DNA–protein crosslinks. Besides genotoxic effects, there is growing evidence for altered gene

Table 93. Genetic and related effects of lead and lead compounds; in-vitro studies

Test system	Result		Dose[a] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Lead acetate				
DNA strand breaks, isolated plasmid DNA	+[b]	NT	1 mM	Roy & Rossman (1992)
DNA strand breaks, isolated plasmid DNA	+	NT	0.1 mM	Yang *et al.* (1999)
8-OH-dG, calf thymus DNA	+[b]	NT	0.5 mM	Yang *et al.* (1999)
Escherichia coli WP2, rec-assay	–	NT	50 mM	Nishioka (1975)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	NT	333 µg/plate	Dunkel *et al.* (1984)
Salmonella typhimurium TA1535, TA1538, reverse mutation	–	–	250 µg/plate	Rosenkranz & Poirier (1979)
Escherichia coli WP-2 *uvr*A, reverse mutation	–	NT	333 µg/plate	Dunkel *et al.* (1984)
Saccharomyces cerevisiae D3, mitotic recombination	–	–	50 000 µg/mL	Simmon (1979)
Plant cuttings of *Tradescantia* clone 4430 (exposed to lead tetraacetate), micronucleus formation	+		0.44 ppm	Sandhu *et al.* (1989)
DNA strand breaks, primary rat kidney cells *in vitro*	+	NT	560 µM	Robbiano *et al.* (1999)
DNA strand breaks, Chinese hamster ovary (CHO) cells *in vitro*	(+)	NT	1 mM	Robison *et al.* (1984)
DNA strand breaks, transgenic cell lines G12 from Chinese hamster V79 cells *in vitro*	+		1.7 mM	Roy & Rossman (1992)
8-OHdG in nuclear DNA, Chinese hamster ovary (CHO K1) cells *in vitro*	–	NT	100 µg/mL	Yusof *et al.* (1999)
Gene mutation, Chinese hamster ovary (CHO K1) cells, *Hprt* locus *in vitro*	+	NT	0.5 mM	Yang *et al.* (1996)
Gene mutation, Chinese hamster V79 cells, *Hprt* locus *in vitro*	–		5 µM	Hartwig *et al.* (1990)
Gene mutation, transgenic cell lines G12 from Chinese hamster V79 cells, *Gpt* locus *in vitro*	(+)		1.7 mM	Roy & Rossman (1992)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	–		10 µM	Hartwig *et al.* (1990)
Enhancement of UVC-induced sister chromatid exchange, Chinese hamster V79 cells *in vitro*	+		1 µM	Hartwig *et al.* (1990)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+		0.05 µM	Thier *et al.* (2003)
Chromosomal (structural) aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–		1 mM	Bauchinger & Schmid (1972)
Cell transformation, Syrian hamster embryo (SHE) cells	+		10 µM	Zelikoff *et al.* (1988)
DNA strand breaks, human kidney cells *in vitro*	+		1.8 mM	Robbiano *et al.* (1999)
DNA strand breaks, human HeLa cells *in vitro*	–		500 µM	Hartwig *et al.* (1990)
DNA single- and double-strand breaks, human lymphocytes *in vitro*	(+)		1 µM	Wozniak & Blasiak (2003)
DNA-protein cross-links, human lymphocytes *in vitro*	+		100 µM	Wozniak & Blasiak (2003)
Effect on the resealing of X-ray induced DNA single-strand breaks, human HeLa cells *in vitro*	–		100 µM	Snyder *et al.* (1989)

Table 93 (contd)

Test system	Result		Dose[a] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Effect of pyrimidine dimer removal induced by UVC, human	–		10 mM	Snyder *et al.* (1989)
Inhibition of UVC-induced DNA repair, human HeLa cells *in vitro*	+		500 µM	Hartwig *et al*. (1990)
Gene mutation, diploid human fibroblasts, *HPRT* locus *in vitro*	–		2 mM	Hwua & Yang (1998)
Sister chromatid exchange, human leukocytes *in vitro*	–		10 µM	Beek & Obe (1975)
Chromosomal aberrations, human lymphocytes *in vitro*	–		1 mM	Schmid *et al*. (1972)
Chromosomal aberrations, human lymphocytes *in vitro*	?		1 mM	Deknudt & Deminatti (1978)
Chromosomal aberrations, human lymphocytes *in vitro*	–		1 mM	Gasiorek & Bauchinger (1981)
Achromatic lesions, chromatid breaks and isochromatid breaks, human leukocytes *in vitro*	+		10 µM	Beek & Obe (1974)
Cell transformation, diploid human fibroblasts (anchorage-independent growth)	+		0.5 mM	Hwua & Yang (1998)
Lead bromide				
Salmonella typhimurium TA1535, reverse mutation	+		9.0 µg/plate	Maslat & Haas (1989)
Salmonella typhimurium TA1537, reverse mutation	–		68.0 µg/plate	Maslat & Haas (1989)
Serratia marcescens, reverse mutation	+		1.91 mM	Maslat & Haas (1989)
Escherichia coli KMBL 1851, reverse mutation, met^+ and his^+	+		3.27 mM	Maslat & Haas (1989)
Lead chloride				
Escherichia coli WP2, rec-assay	–		50 mM	Nishioka (1975)
Escherichia coli K12, Trp^+ reversion plate test	–		1 mM	Nestmann *et al*. (1979)
Salmonella typhimurium TA98, TA100 reverse mutation	–	–	580 µg/plate	Nestmann *et al*. (1979)
Saccharomyces cerevisiae D7, mitotic cross-over	+		0.3 mM	Fukunaga *et al*. (1982)
Gene mutation, Chinese hamster ovary AS52 cells, *Gpt* locus *in vitro*	+		0.1 µM	Ariza & Williams (1996); Ariza *et al*. (1998); Ariza & Williams (1999)
Micronucleus formation, Chinese hamster V79 cells *in vitro*	+		1.1 µM	Thier *et al*. (2003)
Inhibition of X-ray-induced DNA repair, human HeLa cells *in vitro*	+[c]		250 µM [70 µg/mL]	Skreb & Habazin-Novak (1977)
Lead chromate				
Escherichia coli K12 Gal^+ forward mutation	–		100 µg/mL	Nestmann *et al.* (1979)
Escherichia coli Trp^+ reversion plate test	–		1 mM	Nestmann *et al*. (1979)
Escherichia coli WP2 Uvr^- Trp^+ reversion fluctuation assay	+		5 µM	Nestmann *et al*. (1979)

Table 93 (contd)

Test system	Result		Dose[a] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Salmonella typhimurium TA100, reverse mutation	–	–	200 µg/plate	Nestmann *et al*. (1979)
Salmonella typhimurium TA1535, reverse mutation	–	–	100 µg/plate	Nestmann *et al*. (1979)
Salmonella typhimurium TA1537, reverse mutation	+	–	200 µg/plate	Nestmann *et al*. (1979)
Salmonella typhimurium TA1538, TA98, reverse mutation	+	+	200 µg/plate	Nestmann *et al*. (1979)
Saccharomyces cerevisiae D5, mitotic recombination	+	–	63 µg/mL	Nestmann *et al*. (1979)
DNA strand breaks, DNA–protein crosslinks, Chinese hamster ovary (CHO) cells *in vitro*	+		0.08 µg/cm^2 (1 µM)	Xu *et al*. (1992)
Gene mutation, C3H 10T1/2 mouse cells, ouabain resistance *in vitro*	–		100 µM	Patierno *et al*. (1988)
Gene mutation, Chinese hamster ovary (CHO) cells, 6-thioguanine resistance and ouabain resistance *in vitro*	–		100 µM	Patierno & Landolph (1989); Patierno *et al*. (1988)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	+		0.4 µg/cm^2 (5 µM)	Xu *et al*. (1992)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	+		0.4 µg/cm^2 (5 µM)	Wise *et al*. (1992); Wise *et al*. (1994)
Cell transformation, C3H 10T1/2 mouse cells	+		25 µM	Patierno & Landolph (1989); Patierno *et al*. (1988)
Cell transformation, Syrian hamster embryo (SHE) cells, simian adenovirus SA7 viral enhancement	+		80 µM	Schechtman *et al*. (1986)
Cell transformation, Syrian hamster embryo (SHE) cells	+		~0.8 µg/mL	Elias *et al*. (1989)
Chromosomal aberrations, human foreskin fibroblasts *in vitro*	+		0.08 µg/cm^2 (1 µM)	Wise *et al*. (1992)
Cell transformation, nontumorigenic human osteosarcoma (HOS) TE85 cells	+		2 µg/mL	Sidhu *et al*. (1991)
Lead glutamate				
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	?		1 mM	Wise *et al*. (1994)
Lead nitrate				
Saccharomyces cerevisiae D7, mitotic gene conversion, reverse mutation	–		60 µg/mL	Kharab & Singh (1985)
Allium cepa L, chromosomal aberrations	+		10 ppm	Lerda (1992)
Drosophila melanogaster, non-disjunction	–		200 ppm	Ramel & Magnusson (1979)
Gene mutation, Chinese hamster V79 cells, *Hprt* locus *in vitro*	+		500 µM	Zelikoff *et al*. (1988)
Gene mutation, transgenic cell lines G12 from Chinese hamster V79, *Gpt* locus *in vitro*	–		1.7 mM	Roy & Rossman (1992)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	–		3 mM	Zelikoff *et al*. (1988)

Table 93 (contd)

Test system	Result		Dose[a] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	+		3 µM	Lin *et al*. (1994)
Sister chromatid exchange, Chinese hamster ovary (CHO) cells *in vitro*	+		100 nM	Cai & Arenaz (1998)
Micronucleus formation, Chinese hamster ovary (CHO) cells *in vitro*	–		30 µM	Lin *et al*. (1994)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–		30 µM	Lin *et al*. (1994)
Chromosomal aberrations, Chinese hamster ovary (CHO) cells *in vitro*	–		2 mM	Wise *et al*. (1994)
DNA strand breaks, transgenic cell lines G12 from Chinese hamster V79 cells *in vitro*	+		1.7 mM	Roy & Rossman (1992)
Lead sulfide				
Gene mutation, Chinese hamster V79 cells, *Hprt* locus *in vitro*	+		376 µM	Zelikoff *et al*. (1988)
Sister chromatid exchange, Chinese hamster V79 cells *in vitro*	–		938 µM	Zelikoff *et al*. (1988)
Lead, diethyl dichloride				
Drosophila melanogaster, non-disjunction	+		16 ppm	Ramel & Magnusson (1979)
Lead, triethyl chloride				
Drosophila melanogaster, non-disjunction	+		8 ppm	Ramel & Magnusson (1979)

SCGE, Single-cell gel electrophoresis
+, positive; (+), weakly positive; –, negative; ?, inconclusive; NT, not tested
[a] LED, lowest effective dose; HID, highest ineffective dose unless otherwise stated; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal; po, oral; NG, not given
[b] In the presence of H_2O_2
[c] Incorporation of [^{3}H]thymidine or [^{3}H]uridine triphosphate

expression resulting from low-level exposure to lead (Bouton *et al*., 2001; Li & Rossman, 2001).

4.4.4 *Prokaryotic systems* (for references, see Table 93)

Lead acetate and lead chloride were not mutagenic in bacterial test systems. In contrast, lead chromate was mutagenic in *E. coli* and *Salmonella typhimurium*, indicating that chromate may be the active component. Furthermore, one study demonstrated lead bromide to be mutagenic which may be due to bromination of uracil subsequently incorporated into DNA (Maslat & Haas, 1989).

4.4.5 *Yeast and plants* (for references, see Table 93)

In yeast, lead acetate and lead nitrate usually gave negative results in test systems assessing mitotic recombination. One study demonstrated a limited number of mitotic gene conversions at growth inhibitory concentrations; however, among the convertants there were significantly higher frequencies of mitotic crossing-over. Chromosomal aberrations and micronuclei were observed in plants after exposure of roots to lead nitrate or cuttings to lead tetraacetate, respectively.

4.4.6 *Cell-free systems* (for references, see Table 93)

The effect of lead acetate on isolated DNA has been investigated in detail. A dose-dependent increase in DNA strand breaks was observed in plasmid DNA as well as an increase in 8-OH-dG in calf thymus DNA in the presence of hydrogen peroxide as determined by HPLC-electrochemical detection. Studies with different radical scavengers suggested the participation of singlet oxygen ($^1O_2$) and hydrogen peroxide in DNA damage induction. A Fenton-like reaction, involving the reduction of Pb^{2+} to Pb^{1+} and/or lead-oxygen or lead-peroxide complexes, has been proposed to play a role in this process (Yang *et al.*, 1999). One other mode of action with potential relevance for genetic stability consists of interactions with zinc-binding motifs in DNA-binding proteins. In this context, zinc finger 3 of transcription factor TFIIIA exerts a higher binding constant for Pb^{2+} than for Zn^{2+}, and lead chloride has been shown to inhibit DNA binding of transcription factor TFIIIA and Sp1 (Petering *et al.*, 2000; Razmiafshari *et al.*, 2001). However, the zinc finger-containing repair proteins XPA and Fpg were not inhibited by lead (Asmuss *et al.*, 2000).

4.5 Mechanistic considerations

4.5.1 *Introduction*

A considerable number of experiments have been conducted to elucidate the toxicokinetic and toxicodynamic mechanisms by which exposure to lead may result in cancer. Chemical form and route, patterns and magnitude of exposure are important factors in eva-

luating the toxicokinetic mechanisms relevant to the carcinogenic potential of lead. Issues such as mode of action, genotoxicity and the mitogenic and/or cytotoxic potential of lead must be considered in describing the toxicodynamics of lead carcinogenicity.

4.5.2 *Toxicokinetics and metabolism of lead*

(*a*) *Inorganic lead*

(i) *Absorption*

Lead absorption from the gastrointestinal tract in both humans and experimental animals is strongly influenced by age (neonates and the young absorb a larger fraction than do adults), fasting/fed status (fasting experimental animals and humans absorb much greater fractions), nutritional status (fat and caloric intakes, and phosphorus, copper, zinc and especially iron and calcium status all affect absorption), solubility (soluble compounds are better absorbed) and particle size (in controlled studies in rats, lead absorption from mining wastes was shown to be inversely proportional to particle size).

The fraction of lead absorbed from an inhalation exposure is not known to be dependent on the amount of lead in the lung. Patterns and rates of deposition are highly dependent on particle size and ventilation rate, but all lead deposited deep in the lung is eventually absorbed.

Limited studies indicate that dermal absorption of inorganic lead is negligible, although slightly enhanced by high perspiration rates.

Intravenous, intraperitoneal or subcutaneous administration of lead salts gives no useful information about the kinetics of lead, because metal salts administered by these routes are distributed and excreted very differently from the same salts absorbed by a more physiological or natural route.

(ii) *Distribution*

In both experimental animals and humans, absorbed lead is distributed from blood plasma rapidly and simultaneously into erythrocytes, soft tissues, and bone. Once the lead in soft tissues has reached an approximate equilibrium with that in blood, the concentration of lead in blood is determined almost entirely by the balance among absorption, elimination, and transfers to and from bone. Initially, however, distribution into soft tissues dominates the shape of the blood lead concentration–time curve, with a half-life of 20–130 days in adult humans and 3.5 days in rats. In both humans and rats, the highest soft-tissue concentrations of lead are found in the liver and kidney, with considerably lower concentrations in the brain.

After equilibration with soft tissues and in the absence of continuing exposure, the blood lead concentration–time profile mirrors the return of lead from bone. Because of the nature of the several processes that mediate bone lead uptake and release, loss of lead from bone is not a first-order process, and in principle neither return of lead from bone nor whole-body loss can be characterized by a single half-life. Nonetheless, half-lives are commonly used for this purpose. While the bone can be a significant source of endogenous

lead, exposures must be both moderately high and extended in time to load the bone with lead.

Plasma, rather than whole blood, is generally accepted as the source of lead available for distribution and excretion processes. The fraction of whole blood lead that is in the plasma is substantially larger at high blood lead concentrations than at low blood lead concentrations. Although the relationship of plasma lead to whole blood lead is curvilinear at all points, it can be approximated by a straight line at low blood lead concentrations. In one group of 73 adult women, it has been established that the slope of the plasma lead to whole blood lead regression line is 0.00246 at whole blood lead concentrations below about 6 µg/dL; up to this concentration, the relationship between plasma lead and whole blood lead can be approximated by a straight line, and the mean plasma lead concentration is 0.24% of the whole blood lead concentration. The most marked outlier in this group of women had a plasma lead concentration of 0.017 µg/dL at a whole blood lead concentration of about 3 µg/dL (0.56%). At whole blood lead concentrations exceeding about 40 µg/dL, the fraction of blood lead found in the plasma increases. For example, at a whole blood lead concentration of 60 µg/dL, plasma lead concentration is about 0.8 µg/dL (1.3%); at 80 µg/dL in whole blood, it is about 1.5 µg/dL (nearly 2%); and at 100 µg/dL in whole blood, it may be as high as 3 µg/dL (3%) (Manton *et al.*, 2001).

In certain physiological states, such as pregnancy, lactation and the period just after menopause in women, an increase in bone resorption rate takes place without a fully compensatory increase in bone formation rate. In general, it appears that whenever any of these situations has been studied, significant increases in markers of bone resorption have been observed along with comparable increases in that fraction of blood lead coming from bone.

(iii) *Excretion*

Absorbed lead is excreted both in the urine and in faeces (by secretion in the bile). Excretion in the urine is by filtration and reabsorption, and the rate of excretion is proportional to the concentration of lead in plasma. Excretion in bile is highly variable among experimental animal species. In humans, biliary excretion has been reported to be between 25% and 50% of urinary excretion.

Absorbed inorganic lead is not exhaled from the lung.

(*b*) *Organic lead*

(i) *Absorption*

Organic lead compounds, such as tetraethyl lead and tetramethyl lead, behave as gases in the respiratory tract, and are absorbed to a greater extent than are inorganic lead particles. Organic lead compounds are also absorbed through the skin in both humans and experimental animals.

(ii) *Distribution and metabolism*

Tetraethyl lead and tetramethyl lead are oxidatively dealkylated in the body. Any inorganic lead produced endogenously is distributed in the same pattern as administered

inorganic lead, but the parent compounds and the intermediate dealkylated products are distributed quite differently and in accordance with their lipophilicity. In humans exposed to tetraethyl lead, concentrations of the parent compound and its metabolites, including inorganic lead, are highest in the liver and kidneys followed by the brain and heart. The rates of metabolite production are not known in detail for either humans or experimental animals. In rats, however, production of the toxic metabolite triethyl lead appears to be fairly rapid (in the order of hours), while production of subsequent metabolites is much slower (in the order of weeks). The highest concentrations of total lead in rats after exposure to alkyl leads are found in the kidney and liver, followed by the brain.

(iii) *Excretion*

In humans, tetraethyl lead was found to be excreted in the urine as diethyl lead and inorganic lead. In rats and rabbits, dialkyl lead is the major metabolite found in urine. Tetraalkyl leads would also be excreted in the faeces as inorganic lead, the end product of metabolism.

In humans, exhalation of tetraethyl lead and tetramethyl lead from the lung is a major route of excretion, accounting for 40% (tetramethyl lead) and 20% (tetraethyl lead) of the inhaled dose at 48 h after inhalation.

4.5.3 *Toxicodynamics and mode of action of lead*

(*a*) *Genotoxic mechanisms*

In considering the possible mechanisms whereby lead compounds could be mutagenic, it is important to keep in mind the doses at which different biological responses are seen. Since those mechanisms that occur only at highly toxic doses are not relevant carcinogenesis, the mechanisms discussed below emphasize findings at lower doses.

In most commonly-used test systems, effects were rather weak and/or restricted to toxic lead doses. In two published studies, in Chinese hamster ovary AS52 cells carrying a single copy of an *Escherichia coli gpt* gene, lead chloride induced mutations in a dose-dependent manner, at concentrations less than 1.0 μM. High mutant frequencies after exposure to lead were also observed in a different study in Chinese hamster ovary CHO K1 cells at higher but non-cytotoxic concentrations of lead starting at 0.5 mM. The mutation spectrum included base substitutions predominantly at G-C sites, as well as small and large deletions similar to DNA damage induced by ROS. Furthermore, two studies revealed an increase in mutant frequency when non-mutagenic concentrations of lead were used in combination with UVC irradiation or MNNG. One potential mechanism may be the interference with DNA repair processes. Two independent studies revealed an inhibition by lead of repair of UVC-induced or X-ray-induced DNA damage.

In addition to inducing gene mutations, lead appears to be an effective clastogen *in vivo* (although not consistently) and *in vitro*. Human studies are mostly confounded by the presence of other genotoxic compounds. Lead can induce aneuploidy, chromosomal

aberrations, micronuclei, sister chromatid exchange and DNA damage (as measured most frequently with the Comet assay).

There is some evidence to suggest that one of the mechanisms of the genotoxicity seen after exposure to lead may be mediated by ROS. Lead appears to stimulate lipid peroxidation *in vivo*. ROS can be increased in cells through a number of mechanisms. For example, ALA, the haeme precursor whose levels are increased by lead exposure as a result of inhibition of the enzyme ALAD, can generate free radicals in cells and cause the formation of oxidative DNA lesions. Another mechanism may be depletion of cellular antioxidants such as glutathione. The loss of protection against ROS generated by other events may result in increased free radical and oxidative damage to DNA. Another aspect of lead that will result in oxidative DNA damage is the ability of lead to undergo Fenton-type reactions in the presence of hydrogen peroxide, leading to DNA strand breaks. One study suggested that singlet oxygen may be involved, since singlet oxygen quenchers, but not hydrogen peroxide or hydroxyl radical quenchers, blocked the reaction.

Dose considerations

As indicated earlier (see Distribution, above), the usual concentration of lead measured in blood is almost entirely accounted for by the fraction present within and bound to erythrocytes. Only a small fraction of blood lead is present in plasma, the precise proportion depending on the concentration in whole blood. In people heavily exposed to lead, with blood lead concentrations of about 100 µg/dL, plasma lead may be as high as 3 µg/dL (about 140 nM), whereas human populations in less contaminated environments may have whole blood lead concentrations of about 10 µg/dL, which corresponds to 0.024 µg/dL (about 1 nM) in plasma. These values are important in considering the human and non-human applicability of genetic toxicity data obtained from in-vitro experiments (see Tables 92 and 93).

(*b*) *Cell proliferation by mitogenic and regenerative mechanisms*

Cell proliferation can occur either as a regenerative response to cytotoxicity or by a process termed mitogenesis which does not involve cytotoxicity. Lead can increase proliferation of rat and mouse kidney cells, rat liver cells, vascular smooth muscle cells and spleen cells as well as cultured human astrocytoma cells. Often, these proliferative effects occur in the absence of cytotoxicity, although, at higher doses, lead is clearly causing cell death. Thus, tritiated thymidine incorporation was significantly increased in human astrocytoma cells at 1 µM lead concentrations, while lactate dehydrogenase activity in the medium (a measure of cytotoxicity) was not significantly increased until a concentration of 20 µM lead was reached (10 µM having no effect). In the kidneys of mice treated with intracardiac doses of lead acetate, there was a dose-related increase in tritiated thymidine incorporation into DNA that was evident at 1 mg/kg bw lead and maximal at 5 mg/kg bw lead in the absence of tubular necrosis. It is therefore plausible that lead exposure can induce proliferation by both mechanisms and could act as a tumour promoter. It is also

plausible, given the demonstration of lead-induced proliferation in such a wide variety of cells, that any cell capable of replication in any tissue could be stimulated to do so by lead.

Lead has been shown to activate PKC, which comprises a large family of isozymes. Activation of PKC has multiple consequences, including neurotransmitter release and the induction of cell proliferation or differentiation and apoptosis. In human astrocytoma cells specifically, lead induces the translocation of the PKCα isoform from the cytosolic to the membrane fraction and stimulates DNA synthesis by a signal transduction cascade of the form PKCα → Raf-1 → MEK1/2 → ERK1/2 → $p90^{RSK}$, a protein that stimulates DNA synthesis. The authors of the study stress, however, that this mechanism might not be applicable to other cell types.

In rat kidneys, the early toxic effects of lead appear to be localized primarily in cells of the proximal tubule, where lead is taken up by extensive membrane binding and possibly by a passive transport mechanism. Studies in rats have demonstrated proximal tubular damage, which is characterized by the development of intranuclear inclusion bodies in cells that remain capable of division. Renal tumours have been observed in rodents after high-dose exposure to lead. The inclusion bodies, which mainly consist of lead and lead-binding proteins (PbBPs), are thought to act as intracellular depots of non-diffusible lead. A number of high-affinity renal PbBPs have been identified, one of which is a cleavage product of α_{2u}-globulin, a male rat-specific protein. A cytolethal mechanism in the development of tumours after lead exposure that involves α_{2u}-globulin is, however, unlikely, since lead induces tumours in male and female mice as well as male and female rats. Several other cytosolic PbBPs have been found in kidneys from environmentally exposed humans. These include thymosin β_4 and the 9-kDa acyl-coenzyme A binding protein. Other similar low molecular weight proteins bind lead in brain and analogous proteins exist in several species. A common feature seems to be that they are rich in aspartic and glutamic dicarboxylic acid residues. In-vitro studies using rat cells have shown that the renal PbBP facilitate the intranuclear transport of lead and provide evidence of chromatin binding of the lead binding complex. It has been suggested that this could lead to the altered gene expression associated with the mitogenic effects of lead in the kidney.

Lead can induce significant functional impairments *in vivo* in major target organs at doses below those associated with cytotoxicity. Significant increases in proliferative lesions of the kidneys, including tubular cell carcinomas, were observed after lead acetate exposures that did not result in pathological changes in adjacent tissues. Chronic nephropathy was not observed in any of the studies at doses that produced tumours. Thus, non-specific target organ toxicity, resulting in cell death, does not appear to be responsible for the production of tumours due to lead exposure. On the other hand, other evidence suggests that some form of cytotoxicity might play a role in renal carcinogenesis.

Cystic hyperplasia, a late morphological manifestation of chronic lead nephropathy, is a risk factor for renal cancer. Renal adenocarcinoma in experimental animals occurs against a background of proximal tubular cell hyperplasia, cytomegaly and cellular dysplasia. Cystic hyperplasia was reported to occur prior to adenoma formation in animals treated with renal carcinogens. One hypothesis regarding the progression from hyperplasia to

cancer is that cells lining cysts become transformed and proliferate abnormally in response to increased volumes of intracystic fluid. Both human and experimental studies suggest that renal cyst formation contributes to an increased incidence of renal adenocarcinomas. In the case of lead, adenocarcinoma may be a consequence of the cystic change in the renal cortex that follows chronic lead-induced nephropathy.

More subtle types of cytotoxicity may also play roles in the carcinogenic process. Oxidative stress may contribute to some aspects of the cellular toxicity of lead by disrupting the pro-oxidant–antioxidant balance that exists within cells. For example, lipid oxidation is significantly elevated in animals exposed to inorganic lead. These results suggest that lead exerts its toxic effects by enhancing peroxidative damage to the membranes, thus compromising cellular functions.

(*c*) *Molecular mechanisms of action*

The main mutagenic mechanisms of lead at non-cytotoxic concentrations demonstrated to date are: (1) those involving ROS; and (2) interference with DNA repair processes. It has been shown in many systems that exposure to lead results in altered ROS levels and species. The mechanisms by which this can occur include inhibition of antioxidant defence systems, catalysis of Fenton-type reactions, and via accummulation of ALA. Nucleotide excision repair has been shown to be blocked by exposure to lead. This type of inhibition would be expected to enhance the mutagenicity of agents such as polycyclic aromatic hydrocarbons, UV and other agents causing bulky lesions in DNA. The co-mutagenicity of lead with UVC or MNNG is consistent with the hypothesis that both nucleotide excision repair and base excision repair may be affected by lead.

One mechanism for lead interaction with proteins could be via displacement of metals, such as zinc or calcium, from their respective binding sites. In cell-free systems, lead has been shown to reduce DNA binding of transcription factors TFIIIA and Sp1, presumably by replacing zinc in zinc fingers. However, the zinc finger-containing repair proteins XPA and Fpg were not inhibited by lead. Thus, zinc finger proteins cannot be considered as a general target, but interactions depend on the specific protein. Furthermore, these interactions have not yet been demonstrated in intact cells.

Another mechanism that may be relevant for carcinogenesis by lead is its ability to alter gene expression. One pathway by which this could occur is via activation of PKC, which occurs at low concentrations of lead. PKC activation starts a signaling pathway that leads to upregulation of ‘immediate early response’ genes, which ultimately results in a proliferative response.

In conclusion, lead is a toxic metal and one expression of this property is genetic toxicity. There is, however, little evidence that it interacts directly with DNA at normally encountered concentrations. The genetic toxicity of lead appears to be modified in part by increases in and modulation of ROS. In addition, lead itself can interact with proteins, including those involved in DNA repair. This latter mechanism might be responsible for the enhancement of genotoxicity caused by other agents. These properties could result in

mutation, changes in gene expression and cell proliferation, all of which would contribute to a carcinogenic response if exposure is sustained.