Medical Research Council

**Institute for Environment and Health**

IEH report on

# FACTORS AFFECTING THE ABSORPTION OF TOXIC METALS FROM THE DIET

**1998** **REPORT R8**

The Institute for Environment and Health was established by the Medical Research Council at the University of Leicester in 1993. The Institute is partly funded by the Department of the Environment, Transport and the Regions, the Department of Health and other Government Departments and Agencies by way of specific research and consultancy contracts.

This report incorporates the output of a workshop held in January 1997, and has been prepared by the Institute for Environment and Health under a contract with the Ministry of Agriculture, Fisheries and Food. The Institute gratefully acknowledges the contribution of all those who attended the workshop and provided material for inclusion in the Report, but assumes no endorsement from these scientists for the conclusions and recommendations contained here.

The views expressed here do not necessarily represent those of any Government Department or Agency.

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Published by the Institute for Environment and Health, 1998



Printed by Page Bros, Norwich

ISBN 1 899110 12 7

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# Executive summary

There are many factors that, both independently and in combination, affect the uptake of trace toxic metals from the gut. These include physiological state, the dose and chemical form (species) of the metal, interactions with dietary macronutrients, and social and ethnic dietary patterns. In studying the uptake of the toxic elements, measurements of both availability from the diet and actual absorption are needed to give accurate information for risk assessment.

The Ministry of Agriculture, Fisheries and Food commissioned the Institute for Environment and Health (IEH) to review the literature concerning factors affecting uptake of lead, cadmium, arsenic and mercury from the diet in humans, and methods for monitoring availability and absorption of these metals from the diet.

In this report, factors affecting the uptake of each of the four metals, lead, cadmium, mercury and arsenic, are reviewed. Information on the mechanisms of uptake and metabolic interactions is presented as a background for describing the effects of speciation and dose, other nutrients and dietary constituents. The effects of pregnancy, lactation and extremes of age are then considered, followed by the effects of medication and changes in the gastrointestinal flora. As the relationship between the total amount of a toxic metal in the diet and its bioavailability is important in assessing risk, current knowledge on the bioavailability of the four metals in the diet in relation to intake and uptake from the diet is also described. The report assesses how useful biomarkers of uptake and effect for each of the metals are in assessing uptake and whether such markers would be of special use in susceptible groups.

The scientific literature was searched and reviews prepared by IEH were presented to an expert workshop in January 1997 for assessment and validation.

There was agreement among the experts at the workshop that although there is a large scientific literature concerning the toxicology of each metal, their levels in different diets and biomonitoring methods, surprisingly little is known about the effects on uptake of such factors as pregnancy, lactation, childhood and concurrent medication. Nonetheless, the workshop considered that, at the low

levels of each metal occurring in the current diet in the UK, there is no general concern about population risk. However, the evidence that children can accumulate lead and cadmium at a faster rate than adults requires further investigation. The effects of vitamin D deficiency on lead absorption suggest that such groups as Asian children may be at extra risk and this too should be investigated. Cadmium exists in dietary constituents of animal origin as cadmium-metallothionein, which has different absorption and tissue distribution to the metal alone. More research is needed into how the binding of cadmium to metallothionein affects organ distribution; the usefulness of measuring this bound form as a biomarker should be investigated.

The form in which these toxic metals occur in the diet is of importance in risk assessment and more total diet studies need to consider this issue. Biomarker measurement can provide useful evidence for enhanced uptake by susceptible groups.

# 1 General introduction

Food is one of the major routes by which inorganic contaminants enter the body. However, it is known that the absorption of lead, cadmium and other toxic metals can vary considerably depending on the form of the metal in the diet and the physiological status of the organism. There is concern that there may be susceptible groups in the population at special risk due to enhanced absorption of these metals; such groups might include those on unusual diets, those suffering from inadequate nutrition and the very elderly or very young.

The Ministry of Agriculture, Fisheries and Food (MAFF) identified this concern and commissioned the Institute for Environment and Health (IEH) to review the factors affecting the absorption of lead, cadmium, mercury and arsenic from the gastrointestinal tract.

A literature review, based on extensive literature searches, including the databases MEDLINE and EMBASE, was prepared by IEH staff and presented to an expert workshop, convened in January 1997 to evaluate the current state of knowledge on factors affecting the absorption of toxic metals from the diet. This report incorporates the initial literature review, updated in the light of discussions at the workshop, and also presents the overall conclusions from the expert workshop.

The review covers three areas:

- the factors affecting absorption of the four metals;
- the extent to which the chemical form of the metal in the diet affects its bioavailability; and
- the use of biomarkers to assess absorption of the metals from the diet.

For each of the four toxic metals the first and major section herein is concerned with factors affecting uptake. This includes:

- ❒ variations in and interactions between the normal physiological mechanisms involved in the absorption of essential and toxic metals;
- ❒ effects of dose and speciation of the metal;
- ❒ interactions with other nutrients (including vitamin deficiency states) and food components;
- ❒ effects of vegetarian and other unusual diets (including diets of specific ethnic subgroups);
- ❒ effects of pregnancy and lactation;
- ❒ variations in uptake during childhood and adolescence;
- ❒ the effects of old age;
- ❒ the effects of medication; and
- ❒ the effects of intestinal flora.

The second section, for each metal, describes the extent to which dietary studies have addressed the bioavailability of the various chemical forms of the metal in the diet. The usefulness of such studies for risk assessment is also discussed.

In the third section for each metal, on the use of biomarkers in assessing the absorption of toxic metals from the diet, the issues considered are:

- ❒ the general use of biomarkers in environmental and occupational medicine for assessing uptake of toxic metals;
- ❒ the usefulness of these markers in assessing dietary intake;
- ❒ the use and appropriateness of these markers in special risk groups;
- ❒ the use of non-invasive sampling methods including saliva, hair and teeth and *in vivo* physical methods such as X-ray fluorescence; and

- the importance of toxicokinetics in establishing appropriate sampling methods in susceptible groups.

The reviews for the four metals, lead, cadmium, mercury and arsenic, include information from animal and *in vitro* studies as well as volunteer and human population studies. It is clear that good quality human studies at dose levels relevant to current dietary levels are necessary for risk assessment. However, for many of the areas covered in this review there are no relevant studies, and the only available information is from animal studies or from populations that have been exposed to toxic or near toxic levels of the metals. Animal studies are often difficult to interpret and the doses used have often been too high to have any relevance to current UK human exposure levels. Differences in gut flora and physiology between humans and animals can affect the site of absorption and the chemical species being absorbed and hence throw doubt on the relevance of rodent studies to human risk. Earlier population studies may not be directly relevant to the current situation because of the fall in environmental exposure levels, notably lead exposure, over the last decade. All these factors must be considered when examining the studies reviewed in this report; and where they are of particular importance in interpreting the results, this is indicated in the text.

# 2 Lead

# 2.1 OCCURRENCE

Lead contamination occurs throughout terrestrial ecosystems but, except in areas with high lead containing rocks and soils, the predominant source of lead is from combustion of alkyl lead in petrol (WHO, 1995). The major sources of lead intake in the UK have been from air (lead dust from petrol), from drinking water and from food. These three sources have been reduced during the last decade and recent studies have shown a fall in blood lead concentrations by a factor of three since 1986 (Delves *et al.*, 1996; IEH, 1998). The highest geometric mean blood lead concentrations were found in men (3.6 μg/dl), while lower concentrations were found in women and children. The relative contributions of the three major sources of environmental lead to current blood lead concentrations are not known. However it appears that reduction of lead from petrol has been of major importance in the reduction of blood lead concentrations.

Information concerning both the total intake of lead from the diet in the UK and the occurrence of lead in different dietary components comes from the 1991 Total Diet Study (MAFF, 1994). This showed a decrease in total dietary lead intake in comparison with a similar study performed in 1988; this was due to the combination of a real decrease with an apparent decrease that arose from the use of lower detection limits in calculations. The mean upper bound intake of 0.028 mg/day found in the 1991 study was well within the provisional tolerable weekly intake for lead of 0.025 mg/kg (or 1.5 mg for a 60 kg adult) set by World Health Organization (WHO)/Food and Agriculture Organization (FAO) in 1993 (WHO, 1993).

# 2.2 FACTORS AFFECTING UPTAKE FROM THE INTESTINAL TRACT

## 2.2.1 MECHANISMS OF UPTAKE AND INTERACTIONS

The mechanisms of lead uptake from the human intestinal tract have been reviewed in detail by Fullmer (1992) and Sargent (1994). It is generally considered that lead passes across the intestinal mucosa by both passive and active transport, although the exact mechanisms are unclear (Fullmer, 1992). Passive transport probably occurs throughout the whole of the small intestine; it is dependent on a concentration gradient of ionised lead between the gut lumen and the plasma and may be linked to the extracellular movement of water *via* tight junctions (Sargent, 1994). Evidence indicates that lead is also actively transported across the mucosal surface. Metabolic inhibitors appear to suppress lead transport, suggesting the requirement of an energy supply, and some studies indicate the occurrence of a saturable process (Fullmer, 1992).

It appears that lead is actively transported by mucosal protein carriers that mediate calcium transport and that calcium can displace lead, although the interactions between lead and calcium metabolism are complex and not well understood. Calbindin-D, a member of the troponin C superfamily of calcium-binding proteins is probably involved (Fullmer, 1992; Sargent, 1994). A metabolite of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), appears to stimulate the production of calbindin-D, thus increasing lead and calcium absorption.

However, at a critical body lead burden, $1,25(OH)_2D$ synthesis is inhibited. Experimental studies in animals support this; good correlations between intestinal lead transport and calbindin-D levels have been observed when there is no additional lead provided in the diet (Fullmer, 1992).

Fullmer (1992) has summarised the important experimental evidence relating to the interactions between lead and calcium, and intestinal lead uptake and body lead retention.

- Dietary calcium deficiency is associated with an increase in the body burden of lead and the susceptibility to lead toxicity during chronic lead ingestion.

- Stimulation of the parathyroid and vitamin D endocrine system, which controls biosynthesis of $1,25(OH)_2D$ in the kidney, is associated with an increase in calcium and lead absorption when significant quantities of lead are not consumed.

Furthermore, high blood lead levels in children are associated with diminished calcium intake and diminished serum levels of $1,25(OH)_2D$ and, in experimental animals, chronic lead ingestion is also associated with reduced synthesis and circulating levels of $1,25(OH)_2D$.

Fullmer (1992) speculated that the relative importance of passive and active transport of lead across the intestinal mucosa depends on factors such as vitamin D status and calcium, phosphorus and lead intake levels. Other nutrients, including iron, zinc, and vitamins other than vitamin D, and also chelating agents influence intestinal lead uptake (described in Sections 2.2.3 and 2.2.7).

## 2.2.2 EFFECTS OF SPECIATION AND DOSE

### SPECIATION

There is little recent published information concerning the effect of lead speciation on intestinal uptake. In a recent review, Sargent (1994) considered that

higher acidity and smaller particle size result in increased lead solubility, which increases absorption. Some lead compounds are very much more soluble than others and this may affect their absorption. For example, dietary phosphorus inhibits the intestinal absorption of inorganic lead, probably through the binding of lead to form an insoluble complex (Sargent, 1994).

Cotter-Howells and Thornton (1991) found that the lead present in contaminated soil samples was largely unavailable in the human digestive tract. They examined garden soil and house dust samples in an old mining town in Derbyshire in 1988. All samples of garden soil (n = 42) had lead levels greater than 2000 μg/g and 50% of samples of household dust (n = 45) had lead levels exceeding 1000 μg/g. The mean level of lead on both hands of children aged between 1 and 2½ years was 13.1 μg. Overall the geometric mean blood lead level was 9.4 μg/dl (n = 10) for those children aged 1 to 8 years and 6.9 μg/dl (n = 3) for those aged 1 to 3 years, within the normal range in the UK at that time. Much of the lead in the soil was in the form of pyromorphite ($Pb_5(PO_4)_3Cl$), which is known to be of extremely low solubility. It was concluded that the low solubility could account for the low human bioavailability of the soil-lead in this study.

Heard and Chamberlain (1983) reported a study which investigated the uptake of $^{203}Pb$ among volunteers given radiolabelled lead chloride ($PbCl_2$) and lead sulphide (PbS). Although differences in uptake were found, they were not statistically significant. In a study by Rabinowitz *et al.* (1980), the uptake of three radioactive lead isotopes, prepared as a soluble nitrate salt, an insoluble sulphide and a complex with L-cysteine, was investigated in human volunteers; the lead samples were ingested either with food or in the fasting state. All three lead compounds were found to be taken up to the same extent.

Davis *et al.* (1992) found that lead, from contaminated soil at a mining site in Montana, USA, had low bioavailability when fed to laboratory rabbits. The major lead minerals present in the soil were anglesite (53%) and galena (24%).

## DOSE

There is little information about how the dose of dietary lead affects uptake and retention of lead in humans. The relative amount of lead from drinking water retained in the body certainly depends on dose (Sherlock *et al.*, 1982). A non-linear relationship was found between blood lead in women in Ayr and lead concentrations in the water in their kettles (water levels ranged from <10 μg/1 to >1500 μg/1). Balance studies show that retention varies considerably with age.

Metabolic balance studies have been carried out in young children (Alexander *et al*., 1973; Ziegler *et al.,* 1978), a group of adults (with no unusual occupational exposure; Thompson 1971) and a group of healthy elderly people (Bunker *et al.,* 1984) [the numbers of subjects in each study were small]. Alexander *et al.* (1973) found that in a group of eight healthy children (aged 3 months to 8½ years) the mean values for digestive tract uptake and retention were 53% and 18% of dietary intake respectively. Ziegler *et al*. (1978) carried out 89 metabolic balance studies in 12 normal infants (aged 14–746 days) and found that in 61 of the balance studies with daily intakes greater than 5 μg/kg/day, net absorption averaged 41.5% of the lead intake and net retention averaged 31.7% of the intake. Lead intake (range, 2–24 μg/kg/day) and lead retention were found to be positively correlated. However, among 'normal' adults, Thompson (1971) found that the average retention of lead was only 4% of the mean daily intake (n = 5), although in one individual there was net negative retention and in another retention was 12%. Finally, among elderly people there was an overall loss of lead from the body; the mean intake was 54.6 μg/day and the mean retention was –8.7 μg/day (i.e. a loss of 8.7 μg/day; Bunker *et al*., 1984). However, not all the studies took account of lead ingested from other sources, such as lead contamination of the hands or large airborne particles of lead ingested as a result of mucociliary action clearing them from the upper respiratory tract. Lead tracers have also been used to estimate the uptake (and subsequent distribution and elimination) of lead from the intestinal tract and lung (Chamberlain & Heard, 1981; Chamberlain, 1985). The size of the particles was found to be an important factor affecting fractional lung retention and the site of retention, which in turn was found to affect fractional uptake to blood. Presence or absence of food in the stomach affected fractional uptake of lead at this site (Chamberlain, 1985).

In a review by Fullmer (1992), it was concluded that animal studies indicate that when the body burden of lead reaches a critical level the efficiency of lead absorption decreases. Experiments in rats and chicks have shown that intestinal lead absorption is significantly inhibited by increasing levels of dietary lead. In one study 0.8% dietary lead completely inhibited absorption of lead in response to $1,25(OH_2)D$ from the gastrointestinal tract. It was considered that the effect was related specifically to inhibition of $1,25(OH)_2D$-mediated lead transport (Fullmer, 1992). Further details of the role of $1,25(OH)_2D$ in calcium homeostasis and the intestinal transport of lead are presented in Section 2.2.1.

## 2.2.3 OTHER NUTRIENTS

A number of nutrients and dietary components influence the uptake of lead from the intestinal tract. The interaction between lead and calcium absorption and the role of vitamin D in their transcellular transport is probably the best documented (see Section 2.2.1.). The role of other nutrients, including phosphorus, iron, other vitamins, phytic acid, methionine, fat and lactose, has been reviewed by several authors. DeMichele (1984) and Sargent (1994) have reviewed the relationship between nutrients and lead uptake and metabolism, in both animals and humans.

Animal studies have shown an inhibitory effect of dietary *phosphorus* on lead absorption (DeMichele, 1984; Sargent, 1994). Rats fed on phosphorus-deficient diets have been shown to have greater lead retention than phosphorus-replete controls; concomitant restriction of calcium intake caused an additive effect on lead retention. Other studies have also shown that increasing the levels of phosphorus and calcium in the diet reduces lead absorption. Studies in humans on the absorption of radiolabelled lead confirm the findings in animal models. Co-administration of phosphorus with calcium caused a greater reduction in lead absorption than when each was administered alone (Sargent 1994). Sargent (1994) concluded that lead probably forms an insoluble, poorly absorbed complex with phosphorus, however, DeMichele (1984) previously suggested that lead and phosphorus probably compete for shared absorption receptors. For the interaction to occur it is necessary for phosphorus to be present with the lead as it passes through the absorptive regions of the intestine.

Lead poisoning, especially in children, is often associated with *iron* deficiency. In a recent study, Hammad *et al.* (1996) investigated the relationship between dietary lead intake and blood lead levels in 299 children aged from 9 months to 5 years. Blood lead and serum ferritin levels were measured and information on nutritional status, socioeconomic status, medical history, and potential sources of lead exposure was collected. The blood lead levels ranged from 1–55 µg/dl (mean, 11.4 µg/dl) whilst serum iron (ferritin) ranged from 4–169 ng/ml. When multiple logistic regression analyses were used to adjust for covariates, a negative association ($p = 0.03$) was found between blood lead and dietary iron intake.

Ruff *et al.* (1996) investigated cognitive development in 42 2-year old, lead-poisoned (blood lead ranged from 25–55 µg/dl) children living in New York, USA, following intervention by combinations of chelation treatment, iron

supplementation, and steps to eliminate the sources of lead in the home environment. Cognitive improvement was strongly related to a decrease in blood lead in children who had been iron-sufficient at the beginning of the study period (mean ferritin, 23.4 ng/ml; n = 21), but not in those children who were iron-deficient (mean ferritin, 14.3 ng/ml) at the beginning of the study. The authors suggested that increased lead absorption due to iron-deficiency might, in part, account for this, although a number of other factors might also be important.

The effects of a lead smelter in Kosovo, Yugoslavia, on the blood lead levels of pregnant women in the surrounding district were investigated by Graziano *et al.* (1990). A number of factors were investigated including serum ferritin levels, cigarette smoking, alcohol and milk consumption. At midpregnancy women who had serum ferritin concentrations of less than 5 ng/ml had significantly higher blood lead levels than those with ferritin levels of 10 ng/ml or more.

Two studies on the effects of iron status and iron supplements on the retention of radiolabelled lead in humans were reported by Sargent (1994), but the results were considered to be controversial. Sargent (1994) concluded that at present there is no firm evidence that dietary iron intake has a direct effect on lead absorption mechanisms.

Interactions between lead and iron were discussed by Chowdhury and Chandra (1987) who concluded that animal experiments have demonstrated that iron deficiency leads to increased lead uptake from the digestive tract; details of the levels in these experiments, however, were not given. It has been suggested that iron and lead share a common carrier but it is not clear how this relates to a common calcium–lead carrier (Sargent, 1994).

Studies in rats have indicated that dietary *zinc* may also have a role in reducing lead absorption from the gastrointestinal tract. In one study, rats previously fed a zinc-deficient diet were fed zinc supplements, which resulted in reduction of lead absorption. In another study, a lower tissue content of lead was found in rats fed 500 mg/kg (ppm) zinc in the diet compared with those fed only 5 mg/kg (ppm; Chowdhury & Chandra, 1987).

DeMichele (1984) reported a study in which increased dietary zinc was found to reduce the accumulation of lead in the tissues of rats exposed to lead in the diet. Zinc was shown to reduce the lead-induced decrease of blood δ-aminolevulinic acid dehydratase (ALAD) activity. As a similar effect was not observed when lead was injected, it was concluded that the effect was probably due to decreased

uptake from the gastrointestinal tract. Flora and Tandon (1995) also reported evidence which suggests that lead-induced decrease in hepatic glutathione level, inhibition of blood ALAD activity and the increase in urinary excretion of aminolevulinic acid are reduced in the presence of methionine and zinc. As administration of zinc and methionine was more effective when given simultaneously with the lead exposure than after it, the effect was attributed to a decrease in lead absorption from the digestive tract.

Pace and Iannucci (1994) discussed the interactions between dietary vitamins, other than vitamin D, and lead absorption. However, few details of the studies cited were given. It was suggested that dietary *thiamin* ($B_1$), *pyridoxin* ($B_6$), and also *vitamin E* each reduce lead absorption in the intestinal tract. Some animal studies have indicated that *ascorbic acid* (vitamin C) also reduces intestinal absorption of lead, although it is also thought to act as a complementary chelator forming a poorly ionised, but soluble complex with lead. Thiamin, at least, is thought to interfere with lead absorption due to the formation of a compound between lead and thiamin or one of its metabolites. These vitamins have been investigated as 'adjuvants' to chelating agents used to treat lead intoxication (see Section 2.2.8; Pace & Iannucci, 1994; Flora & Tandon, 1995).

In a review of studies on lead and nutrition, DeMichele (1984) reported a study in rats that suggested dietary *fat* may influence lead absorption. Increasing the quantity of corn oil in the diet from 5% to 40% resulted in a 7–14-fold increase in the lead content of several tissues. In another study also reviewed by DeMichele (1984), the dietary addition of lecithin mixed with bile salts and choline increased lead uptake.

DeMichele (1984) noted that animal studies on the effect of milk on lead absorption are conflicting; this is not surprising as many constituents of milk such as calcium, vitamin D and phosphorus are thought to have the ability to reduce absorption, whilst, others, particularly *lactose*, may promote its absorption. In one study, in which rats were fed radiolabelled lead and also lactose in the diet, lead retention was significantly greater than when they were fed diets with either glucose, galactose or maltose in place of the lactose. Another study suggested that the facilitation of lead absorption by lactose was dependent on the age of the rats; the effect only occurred in rats 22–26 days postpartum. It was also found that the luminal concentration of lead must be below 100 ppm for the effect to take place, indicating the involvement of a saturable mineral transport system (DeMichele, 1984).

## 2.2.4 EFFECTS OF DIET

There is some evidence that the amount and type of food ingested affects lead absorption. On average, adult humans absorb about 10% of intestinal lead when food is also ingested; in the fasting state, however, absorption may increase to 35–50% of intestinal lead (Cohen & Roe, 1991; Sargent, 1994). Lower absorption of lead in the presence of food is due to the presence of lead chelators such as phytate and other nutrients, which either compete for carrier receptors or form insoluble complexes with lead (see Section 2.2.3).

In a group of 23 adults, James *et al.* (1985) investigated the uptake of $^{203}$Pb as lead acetate, taken halfway through ingestion of various food items and compared it with uptake in controls only drinking water. On the 12th hour of a 19 hour fast the volunteer subjects drank 100 ml of distilled water containing 0.27 μmol of lead acetate labelled with 0.1 MBq of $^{203}$Pb. Certain meals or food factors eaten by the test subjects were found to reduce absorption significantly compared with controls ($p<0.05$), namely, calcium phytate (1 g), EDTA (3 μmol), glucose, alcohol, breakfast (wheatflakes, bread and tea) and a liquid meal. An alcoholic drink taken on its own was found not to reduce lead absorption as much as a balanced meal. It has been suggested that heavy drinkers may have high lead blood levels, because they may replace meals with alcohol and thus are in a state to absorb lead readily. The authors concluded that the effect of a meal was due mainly to its content of calcium and phosphate salts, although lead uptake was probably further reduced by phytate.

Few studies have examined the influence of special diets on the uptake of lead in humans. Sherlock *et al.* (1985) investigated the blood lead concentration and lead intake in children of different ethnic origin in London. Three subgroups of children, vegetarian Asian, non-vegetarian Asian and Caucasian, were examined. Blood lead and serum 25-hydroxy vitamin D levels were significantly lower ($p<0.05$) in Asian children compared with Caucasian children, however, their total dietary lead intakes were not significantly different. When vegetarianism, hand washing before meals, and parental smoking habits were taken into account, blood lead levels were found not to be related to ethnic origin. Children who washed their hands regularly before eating had significantly lower blood lead levels ($p<0.05$), but vegetarianism was not significantly correlated to blood lead levels. Tarp and Hansen (1991) investigated blood lead levels in 309 adult Greenlanders. Smoking significantly affected the blood lead

levels regardless of diet, however, in non-smokers the consumption of locally produced food significantly affected blood lead levels.

Shaper *et al.* (1982) found an overall association between alcohol consumption and blood lead levels, in an investigation involving 7735 middle-aged men in 24 British towns. The association persisted after controlling for age, social class, body mass index, cigarette smoking, water lead levels and town of residence. Measurements of lead in different alcoholic beverages could not account for the differences, suggesting that elevated lead intake was not the major contributing factor. It was suggested that the differences may perhaps have been due to reduced excretion of lead due to alcohol-induced hepatic dysfunction.

## 2.2.5 PREGNANCY AND LACTATION

Lead is known to be transported across the placenta during pregnancy and correlations have been reported between maternal blood lead levels and developmental abnormalities. Pregnant women are considered to be especially susceptible to lead from occupational exposure. Wong *et al*. (1992) reviewed several studies which examined the relationship between maternal and infant blood lead levels. It was concluded that a maternal blood lead of 10–15 μg/dl may indicate grounds for concern. Although blood lead levels of pregnant women have been studied (Alexander & Delves, 1981; Lagerkvist *et al.,* 1993) there is little published information on the effect of pregnancy specifically on intestinal lead absorption.

Alexander and Delves (1981) compared the blood lead levels of two groups of pregnant and non-pregnant women, one living in an urban area of Newcastle-upon-Tyne and the other in a rural region of Northumberland. No significant difference was found between the pregnant groups and the non-pregnant controls (12.2 μg/dl *vs* 13.3 μg/dl; 0.59 μmol/l *vs* 0.64 μmol/l), although there was a significant decline in blood lead (13.3 to 10.6 μg/dl; 0.64 to 0.51 μmol/l; $p<0.01$) between 8 weeks gestation and full term in the pregnant women. A number of possible reasons for this decline were suggested, including changes in dietary or smoking habits or both and also the transfer of blood lead to fetal or placental tissues. In contrast, Lagerkvist *et al.* (1993) found significant increases in blood lead from week 32 of pregnancy to delivery in two groups of women; in one group located close to a metal smelter, mean blood lead levels increased from 2.9 μg/dl

to 3.5 µg/dl, and in another about 10–15 km distant from it, mean blood levels increased from 2.5 to 2.9 µg/dl. The authors suggested that this increase was due to the mobilisation of lead from bone.

Donald *et al.* (1986) compared the body retention of lead in pregnant laboratory mice given $^{203}$Pb (lead chloride) orally with that of non-pregnant mice. A solution of 2 ml $^{203}$Pb (activity 84.4 MBq) mixed with 18 ml of 0.13% lead acetate solution was administered to all adult mice following 7 days of exposure to 0.13% lead acetate in their drinking fluid. Intestinal absorption was estimated at 11, 14, 17, 20, 23 and 26 days of gestation. Those mice dosed on gestational day 17 had an estimated absorption significantly higher than the non-pregnant mice ($p<0.01$). Maldonado-Vega *et al.* (1996) used a rat model to investigate the effect of pregnancy and lactation on lead uptake from the intestinal tract and lead mobilisation and redistribution. Different schedules of lead exposure were used in three groups of rats. Drinking water containing 100 ppm lead acetate was given *ad libitum* to one group for 158 days before and during lactation, to a second group for 144 days before lactation, and to a third group for 14 days only during lactation. Results were compared with non-pregnant, lead exposed, matched control rats and non-exposed control rats. The study indicated that lactation increased the level of intestinal uptake of lead, the level of uptake by bone and mobilisation from bone. When animals were exposed to lead exclusively during lactation, intestinal absorption, specifically, was shown to be enhanced, with a 2.1-fold increase in blood lead in comparison with exposed, non-pregnant control animals.

## 2.2.6 SUSCEPTIBLE GROUPS: CHILDREN AND THE ELDERLY

According to Sargent (1994), studies in young animals and children have indicated that they absorb approximately 50% of ingested lead compared with 10% in adults. Coleman *et al.* (1983) reported one study where young rats absorbed almost 100% of the dose of lead administered and the level of absorption declined as the animals matured. Sargent (1994) suggested that this difference may be the result of a higher density of intestinal transport proteins during periods of high growth.

There is evidence that nutritional factors and fasting also affect the level of intestinal lead absorption in children and adults (see Section 2.2.3). A balanced diet and regular meals are therefore important in minimising lead absorption, particularly in children, as they tend to ingest lead on their hands as a result of greater hand to mouth activity during play (Sargent, 1994). Some children may also develop preferences to certain foods and have restricted diets (Bearer, 1995), which may increase their lead absorption.

There appears to be little information on absorption of lead in the elderly. Bunker *et al.* (1984) suggested that age-related changes in the gastrointestinal tract may alter the ability of the elderly to absorb some substances, and that low income, poor dentition, or loss of appetite may lead to a nutritionally inadequate diet. They investigated the intake and excretion of lead in 23 elderly people (11 men and 12 women ), living in the Southampton area, who appeared to be healthy, lived in their own homes, and selected their own diets. A negative balance between lead intake and excretion was found. Furthermore, there was no correlation between lead absorption and a variety of indices used to assess iron status. The authors concluded that in well-nourished elderly people there is no risk of accumulating lead at the dietary levels measured (average daily intake was 54.6 µg/day).

## 2.2.7 MEDICATION

Chelating agents, including soft tissue and bone lead mobilizers, are used in the therapy of lead poisoning (Coleman *et al.*, 1983; Flora & Tandon, 1995). Coleman *et al.* (1983) stated that some chelating agents, such as D-penicillamine, nitriloacetic acid, citric acid, sodium citrate, EDTA and DTPA, may increase absorption from the digestive tract. The authors suggested that the lipid solubility of the chelated lead may increase, and change its route of transport across the mucosa. Some dietary nutrients, particularly vitamins, have been suggested as useful adjuvants in chelation therapy; some of these may reduce intestinal absorption (see Section 2.2.3).

### 2.2.8 GASTROINTESTINAL MICROFLORA

No studies on the effects of micro-organisms in the intestinal tract on lead absorption were found.

## 2.3 DETERMINATION OF BIOAVAILABLE DIETARY INTAKE

Measurement of 'total' lead in food has been used in many studies designed to assess lead intake and the balance between absorption and excretion; details of these studies are presented in Sections 2.2.2 and 2.4.2. There is limited information concerning the effect of lead speciation on uptake within the gastrointestinal tract, and the relationship between lead speciation in food and uptake remains unclear (see Section 2.2.2). No studies involving the investigation of the lead species in dietary samples alongside a biomarker of lead exposure were found.

There appear to be no reported *in vivo* studies which have assessed the bioavailability of lead in dietary samples. A potential method for assessing bioaccessibility in food samples is illustrated by one study in which lead solid-phase speciation in smelter and mine waste materials was investigated under simulated *in vivo* gastric conditions (Gasser *et al.*, 1996). A sequential dissolution extraction scheme was used (extraction in $MgCl_2$, NaOH and EDTA) to

characterise the lead phases present. Release rates of lead associated with the waste material were measured with a stirred-flow reactor under simulated gastric conditions at a pH of 1–3 and at temperatures of 5–55°C. During the first 10 minutes, more than 50% of the total lead was released. Between 10 and 60 minutes, lead release was at slower rates, indicating that there are at least two different lead pools. Such a model does, however, assume that the transfer of lead from the gastrointestinal system to the blood is controlled by dissolved low molecular weight lead (II) species.

# 2.4 BIOMONITORING METHODS FOR DETERMINING UPTAKE FROM THE GASTROINTESTINAL TRACT

## 2.4.1 GENERAL OVERVIEW

The measurement of lead in blood has an established place as the major biomarker for lead exposure and uptake used in occupational and environmental medicine. Blood lead measurements are used under the 'Control of Lead at Work Regulations (1980)' to monitor worker exposure and have been used in the DH/DoE 1995 'Health Survey for England' to investigate exposure to environmental lead, 10 years after the major reduction of lead in petrol (Delves *et al.,* 1996). The measurement of blood lead is now a well established analytical technique and there are national and international quality assurance schemes.

There is also a considerable amount of data relating blood lead levels to clinical end-points, and more specifically to the development of childhood intelligence (WHO, 1995).

Other biomarkers of lead uptake have been used, especially those related to the inhibitory effects of lead on the haem biosynthesis pathway, for example, urinary ALAD, erythrocyte ALAD and zinc protoporphyrin. As the measurements of blood lead have become more reliable, these other biomarkers have been used less. A newer technique that has been used to measure bone lead is X-ray fluorescence (see Section 2.4.4). The biological monitoring of lead exposure has been reviewed by Tsuchiya (1986), Lauwerys and Hoet (1993) and Skerfving *et al.* (1993).

Studies have shown that blood lead measurements reflect the combined intake from airborne, dietary and water-borne sources and the relative contributions from these have been discussed in assessing the effects of reduction of lead in petrol (DoE, 1990). Further details of these biomarkers of lead exposure are presented in the following sections.

## 2.4.2 BLOOD LEAD

Both Lauwerys and Hoet (1993) and Tschuiya (1986) consider that blood lead is the best method for assessing recent lead exposure and lead burden in soft tissues. However, lead in blood does not necessarily correlate with total body burden; blood lead progressively reaches a plateau, whereas body burden (mainly in the form of bone lead) tends to increase throughout exposure. Skerving *et al.* (1993) recognised that blood lead measurements are straightforward for experienced laboratories. Various studies have examined blood lead levels in populations that may be considered to be susceptible to lead exposure or toxicity or both (e.g. children, pregnant women and the elderly).

A number of studies have investigated blood lead levels in children and the influence of diet or particular dietary components (Sherlock *et al.*, 1985; Sherlock & Quinn, 1986; Davies *et al.*, 1990; Hammad *et al.*, 1996; Lucas *et al.*, 1996). The study of Sherlock *et al.* (1985; see Section 2.2.4) used blood lead levels to investigate the influence of vegetarian and non-vegetarian diets on lead absorption in Asian and Caucasian children in London. Sherlock and Quinn (1986), in a study of 131 infants in Glasgow, found that blood lead concentrations

had non-linear (cube-root) relationships with water lead concentrations and dietary intakes of lead. Davies *et al.* (1990) investigated the blood lead levels in 97 2-year-old infants in Birmingham and found them to be significantly related to a combination of the amount of house dust lead and an overall rate of touching objects, to lead levels in water and to the parents' smoking habits. It was found that 63% of the lead intake originated from household dust (Davies, 1987). Two American studies on young children also showed associations between blood lead levels and dietary components. Hammad *et al.* (1996) found a negative association between blood lead and dietary iron intake (see Section 2.2.3). Lucas *et al.* (1996) found positive associations between blood lead levels and total calorific intake and also dietary fat.

Alexander and Delves (1981) carried out a cross-sectional study of blood lead levels in pregnant women living in an urban and also a rural area in north-east England. They found a significant decrease in blood lead throughout pregnancy and that women in rural areas had lower blood lead levels. In contrast, Lagerkvist *et al.* (1993) found a significant increase in blood lead levels during pregnancy among women living close to a smelter and also in a reference site, both in Sweden (see Section 2.2.5).

Bunker *et al.* (1984) investigated the balance between lead intake and excretion in 23 healthy elderly people (aged 69.7 to 85.5 years); blood lead levels were also measured. A negative net absorption of lead was found (mean = –2.5 μg/day). Absorption of lead correlated with whole blood level concentrations ($r = 0.44$, $p<0.05$). It was suggested that the negative balance may be due to loss of bone, with a subsequent mobilisation of lead. Muldoon *et al.* (1994) studied blood lead levels in 530 white women aged 65–87 years living in an urban area (Baltimore, n = 205) and a rural area (the Monogahela valley, Pennsylvania, n = 325). Urban residence, smoking, alcohol consumption, and years since menopause were positively associated with blood lead level, whereas body mass index, past history of breast feeding, current oestrogen replacement therapy, moderate physical activity, and calcium intake were inversely associated with blood lead level.

Results from the Health Survey for England 1995 and the ALSPAC study of children in the Avon region have shown a marked reduction in blood lead levels over the last 10 years (Delves *et al*, 1996; IEH, 1998). It is not clear how much of the decrease is due to changes in lead in food (solder in tin cans), lead in drinking water or lead released from petrol, nor how much residual blood lead levels are due to continuing background levels in the same media. Although blood lead levels are responsive to changes in intake in the short and long terms, blood lead

is only an adequate marker of dietary changes if the changes are sufficiently great over a short-term observation period. The use of other techniques, such as the change in isotopic ratios (see Section 2.4.3) following a volunteer feeding experiment, are required to distinguish dietary lead from airborne and water-borne sources.

## 2.4.3 ISOTOPIC METHODS

The ratio of $^{206}Pb$:$^{207}Pb$ often varies between different sources of exposure within a given location and this has been used to apportion the relative contribution of various environmental lead sources to body lead (Alexander *et al.,* 1993; Delves & Campbell, 1993). For this approach to be successful, it is necessary that the different sources of lead are isotopically distinct, and that increased body uptake is reflected by a measurable change in the isotopic composition of body lead (Delves, 1996).

Graziano *et al.* (1996) also used an isotopic method to assess the bioavailability of ingested lead. The ingested lead was derived from sherry stored in lead-crystal decanters made from Australian lead with a characteristic isotopic lead ratio. The change in isotopic lead ratios in the blood of volunteers after drinking sherry that had been stored in a lead-crystal decanter for 3 years compared with those seen when the subjects drank sherry from the original bottle revealed that on average 70% of the ingested lead was absorbed.

Levels of lead in teeth can be used effectively to assess long-term accumulation, from prenatal exposure to the time when they are shed, although levels will depend on sampling methods, tooth type, and resorption and tooth age at exfoliation (WHO, 1995). Gulson (1996) found significant differences between the isotopic lead compositions of incisal and cervical sections of deciduous teeth from children living in a mining and non-mining area of Australia. By comparing the isotopic ratio in shed deciduous teeth with that of dust and potable water samples, Alexander *et al.* (1993) were able to reveal different relative contributions of dust and potable water to the lead exposure of children living in geographically distinct areas.

## 2.4.4 X-RAY FLUORESCENCE

In a steady-state situation, bone contains about 90% of the total lead body burden. These long-term body stores of lead have been assessed by determining levels in bone using *in vivo* X-ray fluorescence. A $^{57}$cobalt or $^{109}$cadmium source is used to excite the lead, and characteristic K or L X-rays of lead are measured. While K X-rays measure lead located deep in the bone, L X-rays can be used to assess superficially located lead. The level of lead in bone is related to years of occupational or environmental exposure (Skerfving *et al.,* 1993). In occupationally exposed individuals, bone lead was shown by Skerfving *et al.* (1993) to correlate with integrated blood lead levels, measured over time. In retired workers who have little on-going exposure there is a correlation between bone and blood lead, as skeletal lead is an important endogenous source of exposure. The authors suggested that bone lead levels should provide a useful index of risk, particularly in the case of long-term exposure.

## 2.4.5 OTHER BIOMARKERS OF LEAD EXPOSURE

Several non-invasive methods of evaluating exposure have also been investigated, namely the measurement of lead in hair, nails, saliva and urine, however, contamination from ambient sources is problematic and makes the results difficult to interpret (Lauwerys & Hoet, 1993).

Chelating agents such as EDTA have been used to mobilise lead in soft tissues and trabecular bone, although it is considered that this method should be used as a diagnostic tool rather than a routine monitoring method.

A number of biomarkers of sub-clinical lead toxicity and susceptibility have also been investigated (Todd *et al.,* 1996). Polymorphism in the enzyme ALAD is thought to be associated with lead susceptibility. Studies on the occurrence of two common alleles, ALAD1 and ALAD2 and blood lead levels have shown that individuals who are homozygous or heterozygous for ALAD2 have significantly higher mean blood levels than those homozygous for ALAD1. These observations

support the hypothesis for the existence of a genetic basis for observed inter-individual differences in susceptibility to lead toxicity (Todd *et al.,* 1996).

## 2.4.6 TOXICOKINETICS

Studies in man indicate that the body burden of lead essentially falls into three compartments, blood and rapidly changing tissues, soft tissues and, finally, bone (Lauwerys & Hoet, 1993; Skerfving *et al.,* 1993; Philip & Gerson, 1994). Lead absorbed into the body rapidly forms a mobile pool which becomes associated with erythrocytes, leaving only between 1% and 5% free in the plasma. The half-life of this blood pool is about 35 days The second compartment is mainly composed of soft tissues where the biological half-life of lead is about 40 days. The third compartment, bone, has a lead half-life of about 20 years, according to Lauwerys and Hoet (1993); Philip and Gerson (1994) state that lead in bone has a residence time of up to 30 years. In the steady state, bone contains about 90% of the body lead burden. The bone compartment contains two distinct pools. One is inert and the other is labile and readily exchanges with blood or soft tissue. Evidence from retired workers indicates that skeletal lead may pose a risk of endogenous lead exposure.

## 2.4.7 DETERMINATION OF UPTAKE: CONCLUSIONS

Blood lead measurement is a well established marker for assessing total uptake into the body, irrespective of source. However, this lack of specificity may be a major limitation for its use in assessing dietary uptake at the low levels of exposure currently found in the UK. Nevertheless, blood lead measurements can be useful in assessing unusual exposures, such as lead contamination of herbal medicines or from local lead piping. The development of inductively coupled plasma mass-spectrometry now allows the use of isotopic techniques to study specific sources and routes of uptake. Such techniques may play a useful role in establishing the factors affecting dietary uptake in susceptible groups.

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# 3 Cadmium

# 3.1 OCCURRENCE

Cadmium has been released into air, land and water by mining and other industrial activities, and contaminated soil remains from past non-ferrous mining and smelting activities in various locations (WHO, 1992). The major non-occupational source of cadmium uptake is from tobacco smoking, and this can exceed absorption from the diet. Drinking water contributes little to total cadmium intake. High levels of cadmium have been detected in vegetables grown on contaminated land, and this has been associated with some increased body burden (Carruthers & Smith, 1979, Harvey *et al.*, 1979, Staessen *et al.*, 1994).

Daily dietary intake of cadmium for the UK is in the range of 10–20 µg/day. The Total Diet Study (MAFF, 1994) gave the mean upper bound dietary intake to be 18 µg/day of which 5% may be absorbed from the gastrointestinal tract (WHO, 1992). This intake is well below the 7 µg/kg provisional tolerable weekly intake (WHO, 1993). Smoking 20 cigarettes per day will result in inhalation of 2–4 µg of cadmium. Thus heavy smokers may easily absorb more cadmium from the inhalation pathway than from the diet in the UK (WHO, 1992).

The Total Diet Study data (MAFF, 1994) showed little change in average dietary intake of cadmium between 1988 and 1991. However, there is little information concerning trends in cadmium levels in the UK population. Among a group of civil servants in London, blood cadmium concentrations of 0.7 µg/l in non-smokers and 1.5 µg/l in smokers have been reported (Staessen *et al.*, 1990).

# 3.2 FACTORS AFFECTING UPTAKE FROM THE INTESTINAL TRACT

## 3.2.1 MECHANISMS OF UPTAKE AND INTERACTIONS

The main route of cadmium uptake in non-smokers with no industrial exposure is *via* the gastrointestinal tract. Gastrointestinal absorption is however, low. Limited observations in humans given radioactive cadmium indicate that the average absorption is about 5% (WHO, 1992).

Animal experiments suggest that cadmium is absorbed by a process of passive diffusion in the duodenum, jejunum and ileum. Sørensen *et al.* (1993), using low doses of isotopically labelled cadmium chloride ($^{109}CdCl_2$), showed that the major site for retention in the rat is the duodenum. Many mechanistic studies have investigated cadmium uptake using jejunal preparations. Kinetic studies indicate that cadmium absorption is a two step process in the rat. The first stage is uptake from the intestinal lumen across the brush border membrane into the mucosa cell, and the second stage is the transport from the mucosa into the blood stream and tissues (Foulkes, 1991). The latter stage is thought to be the slower and to regulate the absorption of cadmium. These studies indicate that the process of cadmium transport is neither energy dependant nor unidirectional. As yet there is no evidence from animal studies that specific carriers or simple thiols have a role in cadmium uptake (Foulkes, 1993; Ohta & Cherian, 1995).

Cadmium exists in the liver, kidneys and other tissues bound to metallothionein, a low molecular weight, sulphur-rich, metalloprotein. Friberg *et al.* (1974) and Webb (1975) proposed that metallothionein plays a role in the transport of cadmium from the liver to the kidneys. They suggested that the cadmium-metallothionein complex (Cd-MT) is released from the liver and transported to the kidneys, where it is freely filtered through the glomeruli, due to its small size, and is subsequently reabsorbed by the tubular cells. Chowdhury and Chandra (1987) reviewed metallothionein involvement in cadmium absorption, transport, storage and excretion, and in the interaction of cadmium with other cations. It is synthesised in response to exposure to metals such as cadmium, zinc and copper and also binds other metals, for example mercury, silver and tin. Cadmium bound to metallothionein and to other low molecular weight protein species may be transported across the membrane of renal proximal tubule cells; the Cd-MT complex will subsequently be degraded by proteases, generating free cadmium ions available for binding to cellular macromolecules (Waalkes & Goering, 1990).

Metallothionein appears to have a protective function against cadmium and other heavy metal toxicity. It has been proposed that at environmental and low occupational exposure levels, the cadmium is solely bound to metallothionein, but as accumulation increases there is 'spillage' of the metal to other proteins and toxicity occurs (Cherian, 1979).

In contrast, metallothionein may play a role in the development of nephrotoxicity by facilitating the accumulation of cadmium in the kidney as Cd-MT, which is preferentially transported to this site. Cadmium not bound to metallothionein does not enter the kidneys to the same extent (Cherian, 1979).

In *in vitro* studies, the uptake of Cd-MT by isolated intestinal brush border membranes was shown to be small compared with that of cadmium salts ($CdCl_2$; Ohta & Cherian, 1995), suggesting a different mechanism for the uptake of Cd-MT.

A recent study by Ohta and Cherian (1995) showed that iron and zinc affect the gastrointestinal absorption of both Cd-MT and $CdCl_2$.

Various studies reporting interactions between cadmium and zinc absorption and metabolism are summarised by Chowdhury and Chandra (1987). Although cadmium and zinc can interact in a number of ways, including at the enzymic level, it appears that when present in the diet interaction between these metals occurs at or in the mucosal cells responsible for their absorption. Zinc at $10^{-5}$ or

$10^{-3}$ mol/l is known to enhance cadmium absorption but absorption is depressed at higher zinc levels (Sahagian *et al.*, 1966). In a number of *in vitro* studies, cadmium has been shown to reduce the activity of several zinc dependent enzymes (Vallee & Ulmer, 1972). Vallee and Ulmer suggest that the toxicity of cadmium is at least partly due to competition between cadmium and zinc at cofactor sites in enzymes requiring zinc. Studies in rats have shown that induction of intestinal metallothionein synthesis by feeding of zinc salts caused a decrease in the deposition of cadmium in the liver when isolated perfused loops of intestine were incubated with cadmium (Ohta & Cherian, 1991). Furthermore, studies reported in a review by Fox (1979) showed that zinc deficiency could be exacerbated by the addition of cadmium to the diets of poults (2 mg/kg (ppm)), chicks (20 mg/kg (ppm)), and the drinking water of rats (3.4 µl/l (ppm)). In addition, young Japanese quail fed a zinc deficient diet had increased cadmium concentrations in the liver compared with quail receiving the normal amount of zinc in their diet.

Animal studies have produced valuable evidence for an interaction between levels of iron in the body and cadmium uptake (Fox, 1979; Friberg *et al.,* 1986; Jonnalagadda & Rao 1993; Ohta & Cherian, 1995). It appears that in iron deficiency, the intestinal iron uptake mechanisms are activated and cadmium competes with iron for absorptive sites at the mucosal brushborder membranes (Ohta & Cherian, 1995). Flanagan *et al.* (1978) showed that iron deficiency in mice produced marked increases in the cadmium content of the duodenal mucosa, in the transfer of cadmium through the intestinal mucosa into the body and in the proportion of cadmium that was deposited in the kidneys. In iron sufficient animals, ferritin absorbs cadmium in intestinal mucosa cells so strongly that metallothionein is not induced in these cells. However in iron-deficient animals with less ferritin to absorb the cadmium, metallothionein is induced, which binds the cadmium to the cells (Flanagan *et al.*, 1978).

Studies on human volunteers summarised by Chowdhury and Chandra (1987) showed that, at low levels of stored iron, absorption of labelled cadmium was increased, but at high levels of stored iron the amount of labelled cadmium was significantly reduced. More recently, Berglund *et al.* (1994), studying cadmium uptake in 57 female volunteers, showed that low serum ferritin (<30 µg/l) was highly correlated with raised blood cadmium concentrations.

# 3.2.2 EFFECTS OF SPECIATION AND DOSE

## SPECIATION

Relatively little is known about the effects of speciation of inorganic cadmium on intestinal absorption. Cadmium salts such as sulphide, carbonate or oxide are practically insoluble in water while the sulphate, nitrate and halogenates of cadmium are soluble (Jonnalagadda & Rao, 1993). Cadmium in meat and fish is bound to protein molecules such as metallothionein, but in plants cadmium appears to be bound to phytochelatins (Chaney, 1988). Some vegetables, (e.g. the soya bean) accumulate cadmium as low molecular weight (<10kDa) species (Page *et al.,* 1986). Cd-MT has been shown to survive *in vitro* simulated gastrointestinal digestion (Crews *et al.,* 1989).

In rodents, oral administration of Cd-MT resulted in increased accumulation in the kidney in contrast to the hepatic deposition of cadmium from $CdCl_2$ (Cherian & Shaikh, 1975; Tanaka *et al.,* 1975; Cherian, 1983; Ohta & Cherian, 1995). Intestinal uptake of Cd-MT was shown to be a slow process compared with $CdCl_2$ absorption using a rat intestinal loop preparation (Ohta & Cherian, 1991; Ohta & Cherian, 1995).

Although there is convincing evidence concerning the differential handling of inorganic and metallothionein bound cadmium in animals, there is an absence of detailed studies on the effects of cadmium speciation on intestinal absorption in humans (Hughes *et al.,* 1995).

There are, however, a few studies of individuals consuming cadmium in a specific protein-bound form other than metallothionein. One series of studies has involved members of an oyster fishing community in New Zealand who have an extremely high intake of cadmium (up to 300 µg per day) from their oyster consumption (Sharma *et al.,* 1983). Cadmium in these oysters is, to a great extent, bound to a metallothionein-like protein (Nordberg *et al.*, 1986). Sharma and colleagues (1983) have shown that increased blood and urine cadmium levels in these fishermen were not as high as was expected from the total amount of cadmium ingested. These findings contrast with those of Newton *et al.* (1984)

who showed, using intrinsically labelled crab meat, that the absorption of $^{115m}Cd$ from this meat in volunteers is approximately 2.7%, the same as the absorption of inorganic cadmium salts added to the diet.

## DOSE

All food, whether it is plant or animal based, contains cadmium, but the amount present may be greatly increased by environmental pollution (Sherlock, 1984). Daily intake of cadmium is in the range of 10–20 μg/day for the UK, Sweden and Belgium and 30–50 μg/day for Japan. At an absorption rate of 5%, daily uptake from food and water is in the range of 0.6–1.3 μg cadmium (WHO, 1992). Smoking 20 cigarettes a day will result in inhalation of 2–4 μg of cadmium. Thus heavy smokers may absorb more cadmium from the inhalation pathway than from the diet. Daily intakes of 150–250 μg have been reported for contaminated areas. At an absorption rate of 5% the daily uptake from the diet would be approximately 8–10 μg in these areas. Total daily uptake from contaminated land would be unlikely to exceed 20 μg (WHO, 1992).

Metabolic studies carried out in healthy elderly individuals between the ages of 70 and 85 years have yielded a mean cadmium intake of 8.6 μg/day with a daily retention of −1.7 μg/day, indicating an overall loss of cadmium from the body (Bunker *et al.,* 1984).

Although information is available concerning the doses of dietary cadmium that various populations are exposed to (see above), there appears to be no information on the effects of dose on the rate or extent of absorption in humans.

A study by Goon and Klaassen (1989), which measured the absorption of cadmium in isolated loops of rat intestine *in situ,* showed that absorption of cadmium is dose-independent at low dosages (<10 μg/kg) but dose-dependent at high doses (>10 μg/kg). Studies in rats using low oral doses of cadmium salts (1 and 10 μg/kg) have demonstrated that the concentration of cadmium accumulated by the kidney is substantially higher than that in the liver. However, as the dosage of cadmium increased, higher cadmium concentrations were observed in the liver compared with the kidney (Lehman & Klaassen, 1986). Thus it appears that the distribution of cadmium between liver and kidney is influenced by both the form and dose of the cadmium administered.

## 3.2.3 OTHER NUTRIENTS

The effects of iron and zinc on cadmium intake have been well documented and have already been discussed in Section 3.2.1. Other nutrients, including calcium and vitamin $D_3$, selenium, copper, manganese, phytic acid, and ascorbic acid, also play a role in cadmium uptake from the gastrointestinal tract.

In humans the interaction of cadmium and *calcium* was considered to be at least partly responsible for the Itai-itai disease in Japanese women (reviewed in Chowdhury & Chandra, 1987). The exposure of postmenopausal Japanese women to high levels of cadmium in the diet resulted in a severe form of osteomalacia. The classical features of the disease were reduced calcification of the skeleton, pain in the back and legs due to bone weakness and the development of pathological fractures. As postmenopausal women are likely to be calcium deficient and decreased calcium stores are associated with increased cadmium absorption (WHO, 1992; Fox, 1979), calcium was suggested to be the link between cadmium exposure and the osteomalacia. Increased amounts of cadmium deposited in the kidneys of the calcium deficient people was associated with an abnormal loss of calcium in the urine.

Cadmium has been shown to have an inhibitory effect on calcium absorption from the gastrointestinal tract, in quail (Richardson & Fox, 1974) and in rats (Gruden, 1977; Tsuruki *et al.,* 1978; Yuhas *et al.,* 1978). Both cadmium-induced physical damage to the gut wall and functional damage involving the vitamin-D receptor have been suggested to cause decreased calcium absorption. Inhibition of vitamin D stimulated calcium transport in rats *in vitro* by cadmium has been demonstrated by Tsuruki *et al.* (1978).

Cadmium and calcium have also been shown to interact in the bone. Cadmium deposited in the osteoid tissue has been reported to interfere with calcification, decalcification and bone remodelling. Bone strength and density have also been shown to decrease as a function of dietary cadmium and calcium levels (reviewed by Chowdhury & Chandra, 1987). Such studies support the proposal that calcium deficiency played a role in the development of Itai-itai disease. Cadmium has been suggested to alter calcium metabolism through vitamin D and the parathyroid hormone (see Chowdhury & Chandra, 1987). This has been proposed as an explanation of the induction of osteomalacia by cadmium. However no evidence

for the suppression of $1,25(OH)_2D_3$ production was found in monkeys administered cadmium for nine years (Kawashima *et al.,* 1988).

*Selenium* alters the absorption and tissue distribution of cadmium. Whanger (1979) showed that addition of 5 mg/kg (ppm) selenium to a diet containing 100 mg/kg (ppm) cadmium reduced cadmium levels in the liver and kidneys of rats but increased it in the testes in comparison with rats fed cadmium alone. Flegal *et al.* (1980) demonstrated decreased accumulation of cadmium in the liver, but not in the kidneys, of weanling pigs, following the addition of 3 mg/kg (ppm) selenium to a diet containing 50 mg/kg (ppm) cadmium. Cadmium itself has been shown to reduce selenium concentrations in the liver and blood; Mykkänen and Mutanen (1985) showed, in chicks, that cadmium decreased intestinal absorption and tissue deposition of selenium while increasing its retention in the intestinal tissue. Selenium has been demonstrated to increase the concentration of cadmium in the target organs, that is the testes, kidneys and liver, while still protecting them by diverting the cadmium from low molecular weight protein complexes to higher molecular weight protein complexes that contain selenium (Chen *et al.,* 1975).

Animal studies have shown that cadmium decreases liver and spleen *copper* concentrations and causes weight loss. Cadmium added to the diet of pregnant ewes results in low birthweight and growth rate during the neonatal period (Mills & Dalgarno, 1972). As with zinc, copper supplementation in excess of the normal requirement for sheep counteracted the effect of cadmium on growth. Another study with pregnant ewes indicated that placental transfer of copper is inhibited by cadmium (Bremner & Campbell, 1980). In his review, Fox (1979) discusses the effects of dietary nutrients in increasing or decreasing risks from cadmium toxicity. Studies reported showed that chicks fed a diet deficient in copper and iron had high levels of cadmium, which caused increased mortality, reduced growth, anaemia, atony and elongation of the gizzard. Copper supplements decreased mortality and increased the haematocrit of anaemic Japanese quail fed cadmium in the diet. A study doubling the required levels of zinc, manganese and copper in a soy diet of Japanese quail markedly decreased cadmium uptake. Interaction between cadmium and copper has not yet been demonstrated in humans.

Animal experiments have shown that supplemental *manganese* increases intestinal uptake of cadmium while cadmium appeared to increase renal and hepatic levels of manganese (Chowdhury & Chandra, 1987). There are no data to indicate an interaction between cadmium and manganese in humans.

*Phytic acid* is the major phosphorous storage constituent of most cereals, legumes, and oilseeds. Turecki and colleagues (1994) have shown that phytic acid decreases both the transport of cadmium across the intestinal wall in rats and the amount retained by the tissue, possibly by preventing the initial step of cadmium absorption, that is the binding of cadmium to the anionic sites on the mucosal surface and subsequent transport through the brush border membrane. The authors suggest that the probable explanation for this action is the formation of insoluble complexes involving phytic acid and calcium, with cadmium coprecipitated on these complexes.

*Ascorbic acid* has been shown to protect against anaemia and growth depression produced in young Japanese quail on a diet of 75 mg/kg (ppm) cadmium (reviewed by Fox, 1979). D-Isoascorbic acid has also been shown to protect against anaemia and growth depression and both forms protect against the poor bone mineralisation caused by cadmium. It appears that the primary effect of ascorbic acid is to improve iron absorption. Pathological changes produced by 75 mg/kg (ppm) cadmium in the diet of young Japanese quail were prevented or decreased by administering supplements of ascorbic acid. Furthermore, a combined supplement of 1% ascorbic acid and 400 mg/kg (ppm) iron as ferrous sulphate was remarkably effective against the toxic effects induced by a diet of 50 or 75 µg/kg cadmium in male and female rats (Fox, 1979).

## 3.2.4 EFFECTS OF DIET

Most foods contain relatively low levels of cadmium, but high concentrations are found in some mushrooms, liver, kidney and shellfish (Sherlock, 1984; WHO, 1992). Other foods such as cereals, rice and vegetables often have elevated levels of cadmium compared with dairy products, meat and fish (Sherlock, 1984; WHO 1992). It has been estimated that up to 75% of the 8.5 to 13.7 µg of dietary cadmium intake of Finnish children is provided by cereals, vegetables and milk products (Mykkänen *et al.,*1986).

Several studies have shown that vegetarians have lower concentrations of cadmium in the blood and urine than people on a normal mixed diet (Wing *et al.,* 1992; Srikumar *et al.,* 1992a,b, 1994; Berglund *et al.,* 1994). Berglund *et al.* (1994) examined the cadmium content in the blood and urine of two groups of women, on a high fibre, vegetarian diet or on a normal mixed diet. They found that women

on the high fibre diet had a greater intake of cadmium; daily intake of cadmium from food was 11 μg/day for the mixed diet compared with 16 μg/day for the high fibre diet. Although there was a tendency for higher concentrations of blood and urinary cadmium in the high fibre (high cadmium) group, the difference was not statistically different at the 5% level. The authors suggest fibre has an inhibitory effect on the gastrointestinal absorption of cadmium.

Vahter *et al.* (1996) examined the intake and uptake of cadmium in women on a mixed diet low in shellfish and women on a diet high in shellfish. The shellfish diets (median, 22.3 μg cadmium/day) contained twice as much cadmium as the mixed diets (median, 10.5 μg cadmium/day). However, there was no significant difference in blood or urinary cadmium between the two groups; the authors suggest this is due either to lower absorption of cadmium in the shellfish group than the mixed group or a difference in the kinetics of the absorbed calcium. Low serum ferritin levels were found in the mixed diet group and this could account for their increased absorption of cadmium (see Section 3.2.1). To investigate this, women with serum ferritin levels above 20 μg/l were compared in both groups. In these women higher dietary concentrations of cadmium in the shellfish group resulted in higher blood cadmium levels compared with those in the mixed diet population. The difference, however, was not in proportion to the difference in cadmium intake. Thus differences in the bioavailability and/or kinetics of dietary cadmium are apparently related to the type of diet.

A similar situation was observed among the members of a New Zealand oyster fishing community (Sharma *et al.,* 1983). Cadmium is, to a great extent, bound to a metallothionein-like protein and the members of the fishing community have an extremely high intake of cadmium (up to 300 μg per day) from their oyster consumption. Increased blood and urine cadmium levels were found, although levels were not as high as expected from the amount of cadmium ingested.

Consumption of food contaminated with cadmium has been shown to cause increased cadmium concentrations leading to health effects. Japanese families exposed to cadmium-contaminated food, mainly rice and water, had a total daily intake ten times that of most other countries of the world (Chowdhury & Chandra, 1987). A high prevalence of proteinuria was noted and in at least one area the exposure was high enough to cause severe bone disease, Itai-itai disease (already discussed in Section 3.2.3). For those people suffering from Itai-itai disease it was thought that low dietary intake of calcium, iron, and protein, with low levels of vitamin D, along with a high demand for calcium and vitamin D during pregnancy and lactation were contributing factors to cadmium poisoning.

Residents of Shipham, UK, were exposed to high levels of cadmium from vegetables grown in an old zinc-mining area (Carruthers & Smith, 1979; Harvey *et al.*, 1979). Cadmium and zinc are commonly found together in nature. Large quantities of cadmium were brought to the surface during zinc mining which resulted in highly contaminated soil. No significant biochemical changes or other disease manifestations were observed but in these residents the tissue cadmium levels were higher than normal (Carruthers & Smith, 1979, Harvey *et al.*, 1979). A 40-year follow up of residents who were living in Shipham in 1939 showed no significant effect on morbidity or mortality (Inskip *et al.*, 1982).

Smoking has a big impact on cadmium concentrations in the body. Smokers have higher blood and tissue cadmium concentrations than non-smokers and this has been demonstrated in many studies. Staessen *et al.* (1990) reported blood cadmium concentrations of 0.7 µg/l in non-smoking and 1.5 µg/l in smoking London civil servants. Smoking 20 cigarettes a day results in an uptake of 1–2 µg of cadmium compared with 0.6–1.3 µg, the uptake expected from food and water (WHO, 1992). Thus heavy smokers exposed to low levels of cadmium in their diet may absorb more cadmium from the inhalation pathway than from dietary sources.

## 3.2.5 PREGNANCY AND LACTATION

Studies have shown that pregnant mice and rats continually exposed to cadmium exhibit little increase in absorption during the time following conception through to day 15 of gestation but retain 2-fold more cadmium than non-pregnant animals during the whole pregnancy; this appears to be due to an increase in absorption just before delivery (Bhattacharyya, 1983). A similar situation was observed in women in Northern Sweden (Lagerkvist *et al.,* 1993) where blood cadmium levels significantly increased from week 32 to delivery.

Cadmium has been shown to accumulate in the placenta during pregnancy but transfer to the fetus is restricted (reviewed by Bhattacharyya, 1983). A study of placental transfer of cadmium in women living in Belgium showed maternal blood cadmium at delivery to be 1.2 µg/l but that of the placenta at term to be 9.3 µg/kg, almost 10-fold higher. However, cadmium in the blood of newborns was only 0.4 µg/l, one-third that of the mothers (Hubermont *et al.*, 1978). Cadmium levels were barely detectable in fetuses and in infants 0 to 1 month old

(Gross *et al.*, 1976). Similar results were obtained in animal studies. Both mice and rats show accumulation of cadmium in the placenta with low or negligible levels in the fetus (Bhattacharyya, 1983). These findings are however at variance with those reported by Kovar *et al.* (1984), who compared cadmium concentrations in human maternal and cord blood and suggested that the placenta does not prevent transfer of cadmium to the fetus.

Iron status in pregnant women also plays a role in cadmium accumulation. Wing *et al.* (1992) demonstrated that women who ate a low grain fibre diet and maintained a better iron status exhibited 45% less cadmium in their placentae than women who ate higher fibre diets and had low body iron stores.

Blood cadmium levels in smoking pregnant women were twice that of non-smoking pregnant women (Chowdhury & Chandra, 1987; Lagerkvist *et al.,* 1993). However blood cadmium levels in pregnant non-smoking women were shown to increase from week 32 to delivery but there was a significant decrease, especially from week 10 to 32 of pregnancy for smokers, possibly due to haemodilution (Lagerkvist *et al.,* 1993).

Animal studies summarised by Bhattacharyya (1983) show that during mid lactation, gastrointestinal absorption of cadmium in mice (and rats) was increased 2 to 3-fold. The increased cadmium content was deposited in maternal tissue mainly in the kidney, liver and mammary tissue. Very little of the cadmium absorbed was excreted into the urine or transferred to the pups. A study analysing the $^{109}CdCl_2$ concentrations in mammary tissue of lactating rats showed that cadmium accumulated in the mammary tissues with a long residence half-time, but the cadmium concentrations in the milk remained low (Lucis *et al.,* 1972). Studies on mice demonstrated that cadmium concentrations in milk throughout lactation were considerably lower than those in maternal blood (Bhattacharyya, 1983). Thus it would seem that transfer of cadmium to milk is restricted.

### 3.2.6 SUSCEPTIBLE GROUPS: CHILDREN AND THE ELDERLY

New born infants are almost free of cadmium, their total body burden being about 1 μg (Friberg *et al.,* 1986). However, it has been proposed that by 3 years of

age, children have accumulated almost one-third of their lifetime burden of cadmium (Fox, 1979). Animal studies reviewed by Bhattacharyya (1983) showed that in mice, between 4 to 23% of orally administered cadmium is absorbed during the time from 2 hours to 6 days of life. Most of this cadmium was retained in the gastrointestinal tract and subsequently slowly released into the faeces. Past this neonatal period young animals only absorbed from 0.59 to 1.3% of orally administered cadmium.

It has been suggested that ingested house dust or garden soil may be a source of cadmium in young children. A survey of metals in household dust yielded an average cadmium level of 6.9 mg/kg, suggesting that hand-to-mouth contact is a minor source of cadmium intake (0.7 μg cadmium daily; WHO 1992). However, in areas around point sources of the metal, hand to mouth exposure may be a significant source of cadmium. Levels of cadmium in household dust were found to be 193 μg/g in the vicinity of a small lead refinery in the UK (Muskett *et al.,* 1979). Thus the daily ingestion of 20 mg of this dust would result in the intake of approximately 4 μg of cadmium. A correlation has been found between cadmium intake from dust and the blood and urinary levels of cadmium in children in Belgium who were exposed to contaminated air (WHO 1992).

Cadmium body burden increases with age (reviewed by Friberg *et al.,* 1986; Chowdhury & Chandra, 1987; WHO 1992). Autopsy studies have shown that the concentration of cadmium increases in the liver with age; in the kidney cadmium concentration increases until the age of 50–60 years, after which it decreases (Chowdhury & Chandra, 1987). The authors suggest age-related functional and morphological changes in the renal cortex, lower levels of environmental pollutants in the past or the fact that food intake is decreased with age as possible explanations for this observation. Bunker *et al.* (1984) carried out a study on 23 healthy elderly volunteers on a self-selected diet. A negative balance was observed between absorption and excretion of cadmium, which the authors suggest may be due to tissue breakdown occurring with ageing, allowing previously accumulated cadmium to be released. In agreement with other studies a significant inverse correlation was found between body iron stores and cadmium absorption. The iron status of the subjects in the study was within the normal range, which may be partly responsible for the negative cadmium balance. This study suggests that healthy, well-nourished elderly people are not at risk of accumulating cadmium at normal dietary levels; the authors suggest, however, that in elderly people who are iron-deficient the cadmium balance might be positive.

### 3.2.7 MEDICATION

Sørensen *et al.* (1993) showed that addition of disulfiram to the diet of mice resulted in increased whole-body retention as well as increased intestinal deposition of cadmium. No other studies have been identified that report any effect of medication on cadmium uptake.

### 3.1.8 GASTROINTESTINAL MICROFLORA

No studies have been identified that address the effects of altered intestinal microflora on cadmium uptake.

## 3.3 DETERMINATION OF BIOAVAILABLE DIETARY INTAKE

The intake of dietary cadmium is usually assessed by measuring the total content of cadmium in the diet (Sherlock, 1984). However the bioavailability of cadmium is expected to vary depending on the form in which it occurs in food. Cadmium in meat and fish is bound to a large extent to metallothionein and is absorbed less effectively than inorganic cadmium (see Section 3.2.2). In plants, cadmium

appears to be bound to phytochelatins. Other than the experimental studies comparing the uptake of inorganic and metallothionein-bound cadmium, no reports have been found describing the effects of speciation on uptake. Similarly, reports comparing exposure to cadmium through the diet with individual or population exposure *via* other routes have depended upon the measurement of total cadmium in whole diet or in specific components rather than the measurement of different forms of dietary cadmium. A recent review of speciation of toxic metals in the diet (Hughes *et al.,* 1995) reports a lack of evidence on the effect of cadmium speciation on bioavailability.

Although phytic acid and fibre have been shown to affect cadmium absorption (see Section 3.2.3), it is not clear whether insoluble complexes pre-exist in the diet or are formed in the gastrointestinal tract. The overall effects of these complexes on cadmium bioavailability are also unclear.

In several studies of populations living on cadmium polluted land, increased levels of cadmium in locally grown vegetables have been investigated and compared with the body burdens of local inhabitants (Carruthers & Smith 1979; Harvey *et al.,* 1979; Staessen *et al.*, 1994). Individuals exposed to contaminated shellfish have been studied but with varying results (Sharma *et al.*, 1983, Vahter *et al.,* 1996). It appears that the measurement of different forms of dietary cadmium (i.e. free, complexed and those bound to animal and plant proteins) would allow more detailed assessments of bioavailability to be made.

# 3.4 BIOMONITORING METHODS FOR DETERMINING UPTAKE FROM THE GASTROINTESTINAL TRACT

## 3.4.1 GENERAL OVERVIEW

Measurement of cadmium in blood and urine has been explored extensively for assessing both occupational and environmental exposure (IEH, 1996). The relationships between blood and urine cadmium levels and body burden are complex and vary with the intensity and length of exposure. These relationships have been investigated in some detail following occupational exposure and recommendations have been made for suitable biological monitoring strategies and standards (Lauwreys & Hoet, 1993). However at exposure levels found outside the workplace the measurements of blood (and urine) cadmium are less useful (Berglund *et al.,* 1994, Staessen *et al.,* 1994). Berglund *et al.* (1994) noted that the metabolic models established for cadmium are mainly based on high-dose animal and human exposure situations and that these may not be valid for long-term low level exposures. Biomarkers of effect have also been useful in population studies (see below).

## 3.4.2 BLOOD CADMIUM

Several studies have shown that blood cadmium concentrations are influenced by diet (Watanabe *et al.,* 1985; Tarp & Hansen, 1991; Wing, 1992; Hovinga *et al.,* 1993, Berglund *et al.,* 1994) except where the limited bioavailability of cadmium from oysters and shellfish has been an issue (Sharma *et al.,* 1983; Vahter *et al.,* 1996). A study by Hovinga *et al.* (1993) measured blood cadmium to determine whether consumption of fish from the Great Lakes was an important predictor of cadmium uptake. Mean blood cadmium was significantly higher in fish eaters (0.6 μg/l) than in controls (0.41 μg/l). Vegetarians have a tendency for lower blood cadmium levels compared with people on mixed diets, except when the vegetarian diet is high in fibre (Wing *et al.,* 1992, Berglund *et al.,* 1994). Watanabe *et al.* (1985) indicated that, in comparison with people having a dietary cadmium intake of less than 20 μg/day, people with dietary cadmium uptake over 80 μg/day had higher blood cadmium levels .

Blood cadmium levels have been used to estimate cadmium uptake in pregnant women living beside or away from a metal smelter in Sweden (Lagerkvist *et al.,* 1993). No difference was found in blood cadmium levels between the two areas. However blood cadmium levels were shown to increase from week 32 to delivery in non-smoking women but to decrease in smoking women especially from week 10 to 32. Other studies have also shown that smoking contributes to blood cadmium concentrations (Staessen *et al.,* 1990*;* Tarp & Hansen, 1991; WHO, 1992; Hovinga *et al.,* 1993). Blood cadmium concentrations are considered to decrease after the age of 60 (WHO, 1992).

## 3.4.3 URINARY CADMIUM

At most environmental and low levels of occupational exposure continuing over some years, urinary cadmium levels increase in proportion to the amount of cadmium stored in the body, provided that renal cadmium binding sites are not saturated and cadmium-induced nephropathy has not yet occurred. In these situations there is a significant correlation between urinary cadmium concentration and cadmium levels in the kidney (WHO, 1992; Lauwerys & Hoet, 1993). Occupational exposure to high levels of cadmium results in saturation of

binding sites and causes the concentration in the renal cortex to plateau. At this point the cadmium cannot be retained in the kidney, is excreted into the urine at increasing concentrations and tends to reflect current exposure, (Mason *et al.*, 1988; Lauwreys & Hoet, 1993). WHO (1980) recommended that, following occupational exposure, the urinary concentration of cadmium should not exceed 10 μg/g creatinine and that improved control measures should be applied if a value exceeds 5 μg/g creatinine.

Urinary cadmium has been measured in many of the population studies and in dietary investigations that have reported blood cadmium measurements (see above). In their study of cadmium absorption in women, Berglund *et al.* (1994) found little difference between urinary cadmium levels in the low and high cadmium exposure groups, while Staessen *et al.* (1994) found significantly increased levels of urinary cadmium in individuals living close to smelters on contaminated ground.

Determination of metallothionein may also be used as a measure of urinary cadmium. Urinary cadmium levels are paralleled by metallothionein excretion (Lauwerys & Hoet, 1993). Metallothionein analysis presents an advantage over cadmium analysis in occupationally exposed subjects in that it is not subject to external contamination and has been shown to be a specific indicator of body burden. Metallothionein mRNA is currently being evaluated as an indicator of cadmium exposure (Stennard *et al.,* 1995).

## 3.4.4 FAECAL CADMIUM

Faecal cadmium may be used as an indicator of the daily amount ingested *via* food and water. Because only a small amount of cadmium is absorbed from the diet (5%; see Section 3.2.1 ) the faecal excretion represents the major fraction of the daily intake. Following a single oral dose of cadmium-polluted rice, almost 100% was eliminated in 3 days (Kjellstrom *et al.,* 1978). Faecal cadmium has been used in several studies to measure average daily intake *via* food in cadmium-polluted areas (Friberg *et al.,* 1986). Berglund *et al.* (1994) showed that cadmium content in the faeces in non-smoking women corresponded well with that in duplicate diets collected for the study.

## 3.4.5 OTHER BIOMARKERS OF CADMIUM EXPOSURE

Neutron activation analysis for measurement of cadmium in liver and kidney has been developed as a biomarker of long-term exposure and has been used successfully in several occupational studies (Armstrong *et al.,* 1992). However evaluation of tissue cadmium *in vivo* by neutron activation is not a routine procedure (Lauwerys & Hoet, 1993; IEH, 1996).

Hair cadmium is generally not considered to be a reliable indicator of either recent exposure or body burden as it is difficult to distinguish between endogenous cadmium and cadmium externally deposited on the hair. However some studies have used it as a biomarker for cadmium (Tarp & Hansen, 1991, Srikumar *et al.,* 1992a,b; 1994) and have shown differences in cadmium content of hair related to diet and smoking.

Other biological indicators that are used to evaluate exposure are the biological effect markers of renal dysfunction (Price *et al.,* 1996). These include serum β2-microglobulin and creatinine, urinary β2-microglobulin, urinary retinol-binding protein, lysozyme and other urinary proteins and enzymes. Urinary retinol-binding protein is now accepted as a sensitive indicator which is more practical than urinary β2-microglobulin due to its greater stability in urine (Chowdhury & Chandra, 1987). Roels *et al.* (1990) have proposed the use of decreased urinary kallikrein activity as an early index of increased cadmium uptake.

## 3.4.6 TOXICOKINETICS

Cadmium is accumulated in liver and kidney with a biological half-life of over 10 years. The average body burden of a normal individual is in the range of 5 to 40 mg and 50% of this burden is found in the liver and kidneys. In humans the three target organs are the lung, bone and kidney, although the kidney is generally accepted as the critical organ (Lauwerys & Hoet, 1993). The WHO review on cadmium (WHO, 1992) suggests that there are at least two compartments related to exposure to cadmium, one reflecting recent exposure with a half-life of 2–3 months and the other related to body burden with a half-life of many years.

### 3.4.7 DETERMINATION OF UPTAKE: CONCLUSIONS

The body burden of cadmium is accumulated over decades and the kinetics of this process are such that short-term changes in diet are not reflected in parallel changes in blood levels or urinary excretion. Urinary cadmium (and biomarkers of effect) do, however, have some use in assessing uptake from locally grown or collected produce in contaminated areas. It appears that faecal cadmium corresponds well to dietary intake and it has been suggested that it might be a more useful measure than duplicate diet sampling (Berglund *et al.,* 1994). Few studies have been found showing the usefulness of biomarkers in assessing the uptake of cadmium from the diet in vulnerable groups. Stable isotope techniques ($^{106}Cd$, $^{110}Cd$) have potential for measuring cadmium body burden levels, as methods for their determination continue to improve, while intrinsic labelling of natural foods with cadmium isotopes is a technique with considerable potential that needs further exploitation (Newton *et al.* 1984).

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# 4 Mercury

# 4.1 OCCURRENCE

Mercury use varies from very large scale (tonnes) in chloralkali plants to small amounts in mercury batteries and dental amalgams. Emission to air occurs during industrial processes, fossil fuel combustion, municipal waste incineration and non-ferrous metal production (WHO, 1991). Emitted mercury vapour is converted to soluble forms of mercury and deposited onto soil and into water. Exposure of the general population to inorganic mercury is mainly from dental amalgam and diet, although in most food stuffs mercury is below the level of detection (MAFF, 1994). The major dietary sources of mercury are fish and fish products, in which it occurs as methylmercury. The upper bound estimate of total mercury in the UK Total Diet Survey for 1991 was 2 μg/day; well within the FAO/WHO provisional tolerable weekly intake for methylmercury of 200 μg (WHO, 1987).

A Canadian study (Richardson *et al.,* 1995; see Section 4.3) reported total adult intake of mercury as 7.7 μg/day, of which 2.81 μg/day arose from dental amalgam and 1.82 μg/day from inorganic mercury (largely from foods other than fish).

The dietary intake of mercury was calculated for Finnish children between 3 and 18 years of age (Mykkänen *et al.,* 1986). Total mercury intake rose slightly with age, and ranged from 3.4 μg/day to 4.1 μg/day. The principal source of mercury was fish and fish products (37% of total intake), followed by milk and milk products (20%), fruit and berries (12%) and cereals (11%).

Several studies have investigated the release of mercury vapour from dental amalgam and the contribution of this source to total body burden (reviewed by WHO, 1991; Oskarsson *et al.,* 1996). Investigations of the effects of diet on inorganic mercury and methylmercury uptake at current dietary levels have often been confounded by the amount of mercury released from dental fillings.

In those not occupationally exposed, the concentration of mercury in urine is usually less than 5 μg/g creatinine and mercury in blood is usually below 1 μg/dl (Lauwerys & Hoet, 1993).

# 4.2 FACTORS AFFECTING UPTAKE FROM THE INTESTINAL TRACT

## 4.2.1 MECHANISMS OF UPTAKE AND INTERACTIONS

Exposure to dietary mercury and amalgam can include exposure to the metal, inorganic salts and organic species; the solubility, biotransformation and tissue distribution vary considerably between these forms. Although human exposure has mainly been to mercury vapour, mercury salts and methylmercury, poisoning epidemics have been caused by the misuse of grain dressed with ethylmercury and phenylmercury.

In the absence of evidence for active transport or facilitated diffusion, passive diffusion is the most likely process by which absorption occurs for most mercury species (see e.g. Rowland & Tozer, 1980). Water solubility and lipophilicity are major factors affecting absorption by passive diffusion. The gastrointestinal absorption of organomercurials is greater than the absorption of inorganic mercury compounds. Methylmercury is essentially completely absorbed. Gastrointestinal absorption of mercuric chloride is higher than that of mercurous chloride.

Human studies have shown that approximately 15% of mercuric nitrate and 95% methylmercury are absorbed (Miettinen, 1973).

Absorption of mercuric chloride administered intragastrically to female rats is dose-dependant (over the range 0.2–2.0 mg Hg/kg bw), with a greater proportion of higher doses (17.5 and 20 mg Hg/kg bw) being taken up (Piotrowski *et al.,* 1992). The higher uptake at higher dose levels was attributed to corrosion of the gastrointestinal tract lining. Studies in everted gut sacs and *in vivo* in rats, using *in situ* isolated perfused intestinal segments have indicated that inorganic mercuric compounds are absorbed by the jejunum (Foulkes & Bergman, 1993). Earlier studies had shown that absorption also takes place in other segments of the gastrointestinal tract (Sasser *et al*., 1973). The mechanisms by which inorganic mercury compounds are absorbed are not fully understood. However, mercurial compounds have a high affinity for sulphydryl groups, and passage through cells is influenced by the concentrations of extracellular, membrane and intracellular thiols. Foulkes and Bergman (1993) suggest that uptake of mercury into tissue involves a rapid and relatively temperature-insensitive influx into a pool readily accessible to extracellular chelating agents and that a second, more temperature-sensitive and more slowly exchanging pool exists which is relatively chelation-resistant. They suggest that surface charge and membrane fluidity are sufficient to account for the mercury uptake, and that there is no need to postulate the existence of specific transport mechanisms. Endo *et al.* (1991) found that metal chelating agents and cysteine decreased the uptake of mercuric chloride in rat small intestine *in situ* and *in vitro*. Clarkson (1993) reports that studies with both inorganic and methylmercury have revealed that mercury complexes formed with small molecular weight thiols (cysteine and glutathione) mimic endogenous thiol and thiol conjugates to such an extent that they are transported on their specific membrane carriers.

Many of the animal studies of the relative absorption of mercury salts and methylmercury have used mice rather than rats. Approximately 20% of orally administered mercuric chloride is absorbed by mice, as assessed by comparing oral and intraperitoneal administration of 1 or 5 μmol/kg bw (approximately 0.2 and 1.0 mg Hg/kg bw; Nielsen, 1992). Male mice retained more orally administered mercuric chloride than females. Inorganic mercuric mercury is excreted in bile in animal models, and may undergo enterohepatic cycling (Zalups & Barfuss, 1996).

Orally administered methylmercury is essentially completely absorbed by mice (Nielsen & Andersen, 1991a,b; Nielsen, 1992). An inverse relationship was found between administered dose (over the range 0.2–25 μmol/kg, approximately 0.04–5 mg Hg/kg bw) and the proportion retained in the body. Once absorbed, methylmercury is distributed to all tissues. Maximum levels in most tissues are

reached 4 days after dosing, but brain levels are maximal after approximately 6 days (WHO, 1990). Tissues contain varying quantities of inorganic mercuric material as well as organic mercury, and much of the mercury found in urine (73%) is inorganic mercuric material. Longer exposure times result in a larger proportion of inorganic mercury. The principal excretion product in urine and faeces is also inorganic mercuric material. Male mice absorb and excrete more of a single, orally administered dose of methylmercury in urine than females (Hirayama & Yatsutake 1986; Nielsen & Andersen, 1991a,b).There are also considerable strain differences in the excretion of methylmercury by mice (Doi, 1986; Nielsen & Andersen, 1991a,b).

Laboratory animals all excrete mercury in the bile, complexed with glutathione or other non-protein sulphydryl compounds. Following intravenous administration (5 μmol/kg bw; approximately 1mg Hg/kg bw) of methylmercuric chloride, rates of mercury excretion were significantly lower in guinea pigs and rabbits, in comparison with rats, mice and hamsters (Urano *et al.*, 1988).

## 4.2.2 EFFECTS OF SPECIATION

It is generally accepted that, in mammals, uptake of organic mercury by the gastrointestinal tract is essentially complete, and that less than 10% of inorganic mercury (as mercuric compounds from food) is taken up (reviewed by Chowdhury & Chandra, 1987; WHO, 1990; 1991; Cross *et al.*, 1995). Although ingestion of several grams of liquid (elemental) mercury results in elevated blood mercury levels, liquid mercury is poorly absorbed. Some incorporation into tissues and local tissue reaction may occur following accidental breakage of intestinal tubes, containers and thermometers containing mercury, and occasionally this has resulted in signs of systemic poisoning (WHO, 1991). Studies of environmental and dietary mercury exposure are confounded by the release of elemental mercury vapour from dental amalgam (Halbach, 1994).

The various species of mercury present in mammals are metabolically interconvertable (WHO, 1991). Under appropriate conditions metallic mercury vapour is oxidised to inorganic divalent (mercuric) mercury and divalent mercury is reduced to metallic mercury. Also inorganic mercury can be methylated and, on demethylation, methylmercury yields inorganic divalent mercury.

## 4.2.3 OTHER NUTRIENTS

Selenium, vitamin E and vitamin C (ascorbic acid) are claimed to be effective against mercury toxicity in animal studies (reviewed by Chowdray & Chandra, 1987; Pace & Ianucci, 1994). Selenite and compounds metabolised to selenite are considered to be the most effective protectors (Chowdhury & Chandra, 1987). Selenium may interact with intestinal mercury uptake in a manner analogous to its interaction with cadmium uptake. Mercury-induced mortality, growth suppression, renal tubular damage and excretion of mercury in rats are reduced if selenite is administered, but plasma mercury levels are increased.

Zorn and Smith (1990) found that mega doses of orally administered vitamin $B_{12}$ (with or without folic acid) or folic acid alone increased methylmercury concentrations in the liver of guinea pigs given mercuric chloride subcutaneously, apparently by influencing methylation of the mercuric chloride. Vitamin $B_{12}$ and vitamin C (in combination) raised brain methylmercury levels and vitamin C and folic acid (in combination) raised muscle methylmercury levels.

## 4.2.4 EFFECTS OF DIET

Much of the interest in the effects of non-typical diets has been due to the accumulation of methylmercury in fish and other seafood, and the major human disasters that have been associated with the intake of high levels of organic mercury from contaminated food. Locally caught fish were contaminated with high levels of methylmercury as a consequence of industrial discharge containing organically bound mercury into Minamata bay and on the Agano river. The bioconcentration of this organic mercury in the aquatic food chain resulted in the disasters which took place at Minamata and Niigata in Japan between 1951 and 1974 (Tsubaki & Irukayama, 1977). These contamination incidents and the high concentrations of organic mercury detected in seafood in other locations have resulted in a number of studies of local populations on fish diets (see below). Organic mercurials used as dressings on wheat intended for seed use have also entered the human food chain. An incident in Iraq (Bakir *et al.*, 1973; reviewed by WHO, 1976) resulted in an outbreak of severe mercury poisoning in 1971–1972, with at least 500 deaths, when wheat dressed with methylmercury was used for bread baking.

Fish or seafood consumption has been correlated with levels of mercury in hair in Japanese (Wakisaka *et al*., 1990), inhabitants of industrially non-polluted areas adjacent to the Adriatic Sea (Buzina *et al*., 1995), Sicilian fisher families (Valentino *et al.,* 1995) and Kuwaiti residents (Bou-Olayan & Al-Yakoob, 1994). Measurements of total mercury in scalp hair of pregnant and lactating women from a fishing community in Chile were significantly higher than those from similar women from an inland town, presumably because of increased fish consumption (Bruhn *et al*., 1994).

Mercury levels in hair samples have been examined in a group of 50 (28 male, 22 female) Swedish freshwater fish eaters (Oskarsson *et al*., 1990). Men had higher levels of mercury in their hair than women. Those eating fish (which had levels of mercury in their flesh of 0.4 mg/kg) from lakes Malaren and Vattern had higher levels of mercury in hair than those eating fish from lake Hjalmaren (mercury flesh levels approximately 0.2 mg/kg). The amount of fish eaten (grouped as more than or less than approximately 500 g/week) appeared to correlate with mercury content of hair. The authors claimed that an intake of approximately 0.3 mg/kg total mercury corresponded to a level of 6 mg/kg in hair at steady state and some 14% of the group exceeded this level.

Fish intake (as a surrogate for methylmercury exposure) has also been correlated with levels of mercury in blood in people from rural south east Sweden (Svensson *et al.,* 1992) and in groups of marine fishermen from the west coast and the east coast of Sweden (Svensson *et al*., 1995). While the blood of east coast fishermen contained twice as much mercury as a referent population, there was no correlation between fish consumption and urinary levels of mercury in the rural population; urinary levels were related to the number of amalgam fillings (i.e. elemental mercury exposure; Svensson *et al.,* 1992). Oskarsson *et al*. (1996) found that northern Swedish new mothers consuming one to two meals of fresh water fish per week during the 6 week period after delivery had significantly higher levels of total mercury in their blood compared with a group not consuming fish.

Brune *et al.* (1991) have extracted a series of reference values from the literature correlating whole blood levels of total mercury with amounts of fish consumed. Background blood mercury concentrations (no fish consumption) were given as 0.2 μg/dl. Blood mercury levels for low consumers (<2 fish meals/week), medium consumers (2–4 fish meals/week) and high consumers (>4 fish meals/week) were 0.48, 0.84 and 4.44 μg/dl. Data on the methylmercury content of the fish were not given. Svensson *et al.* (1992) found mercury levels of 1.8, 6.7 and 6.6 ng/g in whole blood for zero, two and three fish meals per week. Sakamoto *et al*. (1993) found

that levels of erythrocyte mercury correlated with fish consumption for a population from Kumamoto Prefecture, Japan.

Although there are numerous examples of diets high in methylmercury giving rise to increased blood and tissue levels in humans, there is little information concerning the effects of unusual dietary composition on uptake, especially at normal levels of mercury intake. This may reflect the rapid and almost complete absorption of methylmercury in the gastrointestinal tract. There is, however, experimental evidence that dietary modification can affect the retention of methylmercury in rodents. Mice fed pelleted diet, evaporated milk or a synthetic diet showed different rates of whole body mercury retention and faecal excretion after oral exposure to methylmercury (Rowland *et al*., 1984). Treatment with antibiotics to suppress gut flora reduced faecal excretion and the effect of dietary differences on the retention of mercury. In a further study designed to investigate the effects of varying dietary fibre types and concentrations, Rowland *et al*. (1986) showed that the incorporation of 30% bran decreased total mercury concentrations in the brain, blood and small intestine. The authors of these studies suggest that the dietary effects were, at least in part, mediated by a change in the intestinal flora and thus the extent of demethylation of methylmercury. Demethylation converts this form of rapidly absorbed mercury into inorganic mercury species that are much less easy to absorb.

Højbjerg *et al.* (1992) investigated the effects of varying levels and types of dietary fat on the retention of inorganic mercury and methylmercury following single oral doses (5 μmol/kg bw, approximately 1 mg/kg bw) to mice. There were changes in methylmercury retention with increasing percentage of dietary fat and there were differences between the fats used (coconut, soya and cod liver oils). Variation in the amount eaten and lack of control of energy levels in these studies make them difficult to interpret, but they do provide evidence that extremes of diet can effect methylmercury retention, although not necessarily absorption.

### 4.2.5 PREGNANCY AND LACTATION

There is evidence that psychomotor retardation may occur in human offspring as a consequence of high fish consumption (i.e. presumed methylmercury intake) by the mother, and that daily intakes by pregnant women at one quarter the minimum toxic daily intake for nonpregnant adults are toxic to the offspring (Clarkson,

1990). There are no data however, to suggest any differences in gastrointestinal absorption due to pregnancy. In Alaskan Eskimos (Galster, 1976), Greenland Inuit (Hansen *et al.*, 1990), Faroese (Grandjean *et al.*, 1992) and James Bay Cree Indians in Canada (Girard & Dumont, 1995), levels of mercury in maternal and cord blood increased with increasing maternal intake of marine food, a surrogate for methylmercury exposure. Mercury levels in fetal red blood cells were approximately two-fold those in maternal red blood cells (Glaster, 1976; Hansen *et al.,* 1990); the cord blood mercury consisted almost entirely of methylmercury. Skerfving (1988) found that fish eating and levels of mercury in maternal and cord blood were related, with a total blood mercury ratio of approximately 1.47:1 for cord blood:maternal blood. Although large inter-individual variations were found in another study by Suzuki and colleagues, cord blood to maternal blood ratios generally were somewhat similar, with a mean of 1.65 and a range 0.8–2.8 (reviewed by WHO, 1991); a slightly lower ratio (1.13) was obtained in the study on James Bay Cree Indians and cord blood levels also correlated with maternal hair levels (Girard & Dumont, 1995). Levels of mercury were also higher in placentae from mothers with higher intakes of seafood (Galster, 1976).

Both elemental mercury and methylmercury can pass through the rodent placenta into the fetus, and can pass into maternal milk (reviewed by WHO, 1990; Sundberg *et al.,* 1991; WHO, 1991; Sundberg & Oskarsson, 1992). Elemental mercury passes readily into the fetus but only low levels of inorganic mercuric mercury do so (Clarkson *et al.,* 1972). The amounts of methylmercury transported into the fetus in rats and pigs increases dramatically towards the end of pregnancy, and, in studies involving cross-suckling, placental transfer has been found to be more efficient than transfer in milk. It has been suggested that, in rat, methylmercury is transported across the placenta as its cysteine conjugate *via* the neutral amino acid carrier (Kajiwara *et al*., 1996).

High levels of mercury were reported in breast milk of mothers from Minamata and Iraq following major incidents involving mercury in seafood and dressed wheat seed (Amin-Zaki *et al*., 1976; Fujita & Takabatake, 1977). Mercury levels in breast milk were approximately 5% of those in blood in the Iraqi incident (Bakir *et al.,* 1973). High levels of mercury have also been found in breast milk when women are exposed to methylmercury as a result of normal dietary fish or marine food consumption (Galster, 1976; Skerfving, 1988). A recent study investigated the effect of maternal fish consumption (presumed methylmercury exposure) and amalgam fillings (elemental mercury exposure) on total and inorganic mercury levels in breast milk, blood and hair in 30 lactating women from northern Sweden who had relatively low levels of exposure (Oskarsson *et al.*, 1996). Inorganic and

total mercury levels in blood and milk were correlated with the number of amalgam fillings. No correlation was found between estimated intake of organic mercury from fish consumption and total, inorganic or organic mercury in milk. The data suggest that, firstly, at this level of exposure, inorganic mercury was transferred much more efficiently than organic mercury from blood to breast milk, and, secondly, dental amalgam was the main source of the mercury in the milk. There are few data, however, on the effects of lactation on the actual absorption of mercury species from the maternal gastrointestinal tract.

## 4.2.6 SUSCEPTIBLE GROUPS: CHILDREN AND THE ELDERLY

The dietary intake of mercury was studied in 176 children in Finland (Mykkänen *et al*., 1986). Total mercury intake rose only slowly (from 3.4 to 4.1 μg/day) with age (from 3–18 years). On a body weight basis this represents a decrease from 0.22 μg/kg/day at 3 years to 0.06 μg/kg/day at 18 years. Levels of mercury in urine in Japanese schoolchildren were correlated with fish consumption and dental amalgam fillings (Suzuki *et al*., 1993a).

Absorption of inorganic mercuric chloride was reported to be much higher in newborn rats than in adults (Kostial *et al*., 1978, 1983 in WHO, 1991). Some 38% of an oral dose was present 6 days after administration to newborn rats compared with 1% for adult rats. Retention of radioactivity 6 days after a single oral dose of radiolabelled mercuric chloride was approximately 50-fold higher in suckling rats (1 week old at dosing) than in the dams (Kostial *et al*., 1983).

In hamsters and guinea pigs, both inorganic mercuric mercury and methylmercury were transferred to offspring *via* mothers milk (Yoshida *et al*., 1994; Nordenhall *et al*., 1995). Suckling guinea pigs, mice and infant monkeys [species not specified] are probably incapable of excreting methylmercury, but the adult pattern of excretion establishes itself abruptly at the end of the suckling period (WHO, 1990, 1991; Yoshida *et al*., 1994). Biliary excretion of methylmercury is virtually absent until weaning. The ontogenic development of glutathione secretion in bile parallels this ability of the bile to secrete methylmercury, and microfloral ability to demethylate methylmercury is substantially less during suckling than later (WHO, 1990).

Although the relationship between dietary intake and urinary excretion of mercury in childhood has been studied there is a lack of information about absorption in this age group.

No studies concerning mercury absorption in the elderly were identified. Sakamoto *et al*., (1993) found that levels of methylmercury in red blood cells declined with age, even allowing for changes in fish consumption.

## 4.2.7 MEDICATION

No reports concerning potential influences of medicines or other non-nutrient chemicals on the absorption of inorganic or organic mercury were identified.

## 4.2.8 GASTROINTESTINAL MICROFLORA

Gut microflora are reported to both methylate and demethylate mercury. In *in vitro* studies, human oral and intestinal microflora have been shown to be capable of methylating mercury (Rowland *et al*., 1975; Heintze *et al*., 1983). Although earlier studies (Cross *et al*., 1978; Pan *et al.,* 1980) suggested that methylation occurred in humans exposed to elemental mercury, either during dentistry or when working in chloralkali battery factories, the more recent studies on dentists (Chang *et al.,* 1987) and chloralkali battery workers suggests that elemental mercury is not methylated *in vivo* (Barregård *et al.,* 1994). Insufficient quality control during analysis is thought to be responsible for the discrepancies which led to the earlier conclusions.

Gut microflora from rats demethylate methylmercury (Rowland *et al.,* 1975; 1978), thus permitting the excretion in faeces of the resulting inorganic mercuric mercury. Diet can influence the gut microflora, and hence the amount of demethylation taking place. The evidence for this comes both from studies in which rodents fed different diets were given antibiotics to suppress microflora, and also from *in vitro* studies using microflora (Rowland, 1981; Rowland *et al.,*

1984; 1986). The considerable inter-species differences in biliary flow, biliary excretion and enterohepatic circulation, as well as in the location and types micro-organisms present in the gut, suggest that this evidence from the rat is unlikely to be relevant to humans.

# 4.3 DETERMINATION OF BIOAVAILABLE DIETARY INTAKE

The differences in absorption, distribution and toxicity between elemental, inorganic and organic mercury are such that detailed dietary studies (speciation of dietary components) may be required to establish a full picture of bioavailable intake. A Canadian study (Richardson *et al.,* 1995) gave the total adult intake as 7.7 µg/day (0.11 µg/kg bw day), and an absorbed amount of 5.3 µg/day (0.076 µg/kg bw/day). Dental amalgam (elemental mercury) accounted for an intake of 2.81 µg/day and an uptake of 2.25 µg/day (36% of intake; 42% of absorbed mercury). Inorganic mercuric mercury was derived largely from foods other than fish, and accounted for an intake of 1.82 µg/day, but an uptake of only 0.18 µg/day. Fish consumption (organic mercury) accounted for 27% of the intake (40% of absorbed dose).

# 4.4 BIOMONITORING METHODS FOR DETERMINING UPTAKE FROM THE GASTROINTESTINAL TRACT

## 4.4.1 GENERAL OVERVIEW

The principal tissues used for measuring mercury uptake are blood and urine; hair, nail and saliva are also used to a lesser extent. Blood, urine and saliva are suitable media for measuring recent exposure to mercury vapour and inorganic salts. Monitoring of urinary levels is an established technique in widespread use in industry (Lauwerys & Hoet, 1993). Metallic and inorganic mercury distribute themselves equally between erythrocytes and plasma, while exposure to methylmercury produces higher levels in erythrocytes compared with plasma. It has been suggested that erythrocyte and plasma mercury can be used as indices of methylmercury and inorganic mercury uptake respectively (Svensson *et al.,* 1992). Methylmercury does not pass through the kidney, hence urine is not a suitable medium for monitoring organic mercury exposure.

Hair mercury reflects longer-term exposure to organic mercury, and is commonly used for measurements aimed at reflecting population exposure to methylmercury in fish (WHO, 1990). Hair levels are approximately 250-fold higher than whole blood levels (WHO, 1990). Although hair analysis is appropriate for measurement of environmental or dietary mercury exposure (reviewed by Katz & Katz, 1992), it is unsuitable as a medium for measuring industrial exposure to inorganic or

elemental mercury because of the possibilities for contamination of the samples through deposition (Lauwerys & Hoet, 1993). Longitudinal analysis of hair samples can identify the peak associated with time of acute poisoning (Suzuki *et al.,* 1993b), and is useful for recapitulation of levels present during pregnancy; hair samples from the segment corresponding to the pregnancy term taken at time of delivery and 6 months later have been found to be in close agreement (Cernichiari *et al.,* 1995a). There are, however, problems with the use of hair analysis to estimate individual exposure, due to inter-individual variability in uptake by hair.

Total and inorganic mercury have also been investigated in nail by Suzuki *et al*. (1989); levels were higher in fingernails than toenails. An attempt to use dentine as a biomarker of mercury exposure has been unsuccessful (Haller *et al*., 1993).

Reference values for total mercury levels in non-exposed populations are given by WHO (1990) as: whole blood 8 µg/l; hair 2 µg/g; urine 4 µg/l; and placenta 10 µg/g wet weight. Nixon *et al*. (1996) reported whole blood values of 0–8.4 µg/l for total mercury in a 'normal' population from the USA, and inorganic mercury values of 0–7.5 µg/l. Lauwerys and Hoet (1993) reported a value equivalent to 2 µg/l as a reference value in whole blood for non-fish eaters. Akesson *et al.* (1991) obtained a value of 16.9 nmol/l blood (approximately 3.4 µg/l) for 81 referents in a study in which mercury levels in the blood of dental personnel were compared with referents from Bleikinge county, Sweden. Nakagawa (1995) obtained reference values for mercury in hair in Japan of 2.23 µg/g (ppm), with averages of 2.98 for men and 2.02 for women (population aged between 11 and 82). This was considerably lower than values obtained in the early 1960s. In a study on mercury in pregnant Seychellois, mean and median levels of mercury in hair for each of the years between 1986 and 1989 were between 5.9 and 8.2 µg/g (ppm); 80% of the mercury was present in organic form (Cernichiari *et al*., 1995b). This population had a high consumption of fish, and 90% of the mercury in the fish was present as organic mercury. Amalgam tooth fillings are the main non-nutrient source of exposure to mercury, and account for background levels of blood and urinary mercury in the general population (Halbach, 1994).

## 4.4.2 TOXICOKINETICS

The major half-life of mercury in blood after industrial or experimental exposure to mercury vapour is about 3–4 days while the half-life for urinary mercury is about 40 days (reviewed by Gompertz, 1982). In humans, dietary methylmercury

is almost completely absorbed and distributed to all tissues within 4 days (reviewed by WHO, 1990). Maximum levels in brain are reached slightly more slowly, in about 5–6 days. Methylmercury in blood is almost entirely contained in the red blood cells (ratio blood:plasma of 20:1). The rate of excretion of mercury is directly proportional to body burden and can be described by a single half-life of approximately 50 days in fish-eating humans. Blood and hair half-lives (of 35–100 days, average 65 days) are similar, but there is greater variability in half-lives derived from hair samples (WHO, 1990).

The toxicokinetics of methylmercury have been examined in the cynomologus monkey, *Macaca fascicularis,* following single oral doses (Rice *et al.,* 1989). A two compartment model fitted the data. Absorption was essentially complete 6 hours after dosing and the half-life for the elimination ranged between 10 and 15 days. After repeated dosing on 3 days per week steady state was achieved at 92 days; this could not have been predicted satisfactorily from the single dose studies. Vahter *et al*. (1994) found that steady state levels for total mercury following daily oral doses occurred after 4 months for blood and 12–18 months for the occipital pole and thalamus of brain. Although methylmercury was eliminated from brain with a half-life of 35 days, inorganic mercury, presumably formed from demethylation in the brain, was retained for several years. Although disposition parameters for blood mercury following long-term oral administration of methylmercury were independent of dose up to 90 μg/kg bw/day, clearance was higher at 90 μg/kg/day in pregnant females than at lower doses (Stinson *et al*., 1989). Blood half-lives in neonatal offspring (nursery reared, apparently with formula milk without added mercury) were similar to those of their mothers.

## 4.4.3 DETERMINATION OF UPTAKE: CONCLUSIONS

The combined use of blood and urinary mercury measurements can give useful information concerning the relative contributions of organic and inorganic mercury from the diet and elemental mercury from dental amalgams. Methylmercury exposure is reflected in blood (erythrocyte) and hair levels whilst inorganic and amalgam mercury appears in the urine. Although hair analysis has not found a useful place in the assessment of occupational exposure, it has been used successfully in determining population exposure to organic mercury.

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# 5 Arsenic

# 5.1 OCCURRENCE

Arsenic is widely distributed in minerals and sediments (WHO, 1981). It is released to air and contaminates the environment surrounding smelters and power stations that burn high arsenic fuel. Non-occupational exposure is almost all *via* food and drinking water, however the form in which arsenic is ingested is of considerable toxicological importance. The high levels of arsenic that occur in fish are due to non-toxic organic arsenic species, the arsenobetaines.

MacIntosh *et al.* (1996) reported an average daily dietary intake of approximately 50 μg/day, derived from a food frequency questionnaire and residue data from the US Food and Drugs Administration Total Diet Study. Hughes *et al*. (1995) estimated total ingested inorganic arsenic in Canadian adults to be in the range 0.2–0.8 μg/kg bw per day, with the principal sources being food and drinking water. The MAFF Total Diet Study, in which the foods making the largest contribution (62%) to total arsenic were those in the 'fish' group, reported the mean upper bound intake to be 70 μg/day, well within internationally accepted safety limits (MAFF, 1994). Van Dokkum *et al.* (1989) found, from food basket studies, that 18 year old Dutch men consumed approximately 38 μg/day of arsenic; 52% of this consumption was obtained from fish, 12% from drinks and 8% from bread.

Mykkänen *et al.* (1986) have obtained estimates of dietary intake of arsenic in 1768 Finnish children. The average daily intake increased with age from 3–18 years, ranging from 28–41 μg/day, but on a bodyweight basis intake dropped with age from 1.8 μg/kg bw to 0.6 μg/kg bw. Chief sources of the arsenic were potatoes and vegetables (25%), milk and milk products (21%), cereals (16%), fish and fish products (15%) and fruit and berries (13%).

# 5.2 FACTORS AFFECTING UPTAKE FROM THE INTESTINAL TRACT

## 5.2.1 MECHANISMS OF UPTAKE AND INTERACTIONS

The main route of arsenic uptake in those not occupationally exposed, is from the diet. Experimental studies have shown the site of absorption is predominantly the small intestine in the cat and rat, with some absorption occurring in the colon; very little absorption occurs in the stomach (reviewed by HSE, 1986). Recent *in vivo* and perfusion studies on rat intestine have suggested that absorption of pentavalent arsenic is a saturable transport process, shared with phosphate, which may depend on sodium and hydrogen ion gradients (Gonzalez *et al.*, 1995). Elsenhans *et al.* (1987) showed that an increase in arsenic in the diet (up to 90 mg/kg (ppm)) led to a significant increase in tissue concentrations; this was linear for kidney and saturable for other tissues. Increasing dietary arsenic led to a decrease in the manganese content of the small intestine, a decrease in hepatic copper concentration and an increase in renal copper retention. Little is known, however, about the mechanisms of arsenic uptake or the effects of other nutrients on this process.

Absorption of soluble arsenic, as sodium arsenite (trivalent) or as sodium arsenate (pentavalent), is essentially complete in humans and animals, but absorption of the insoluble arsenic compounds is poor (reviewed by WHO, 1981; HSE, 1986). Inorganic arsenic is metabolised *in vivo* in humans and laboratory animals to monomethyl arsonic acid (minor product), dimethylarsenic acid

(cacodylic acid; major product) and trimethylarsine oxide (in trace amounts in animals; Buchet & Lauwerys, 1985; Yamauchi & Yamamura, 1985; HSE, 1986; Buchet & Lauwerys, 1987), which are excreted fairly rapidly, mainly in urine.

Arsenic is excreted in the bile (Gregus & Klaasen, 1986), and evidence from comparisons of biliary cannulated rats with intact rats suggests that some enterohepatic circulation may take place in this species, as some 24% of an administered dose of arsenic was found in the bile of cannulated animals within 2 hours of dosing, but only 6% was found in the faeces in the first 24 hours after dosing. Glutathione may be involved in this hepatobiliary elimination (Gyurasics *et al*., 1992) and may account for the variability in the amounts of urinary arsenic found over 24 hours after dosing in mice (Morel *et al.*, 1995; Telolahy *et al.*, 1995). However, similar studies in rabbit and dog revealed much lower biliary excretion. Observations on a subject acutely intoxicated with inorganic arsenic indicated that a significant portion of the ingested arsenic can be excreted in bile (reviewed by Lauwerys & Hoet, 1993).

The principal organic arsenic compound present in animal seafoods is arsenobetaine, followed by much smaller amounts of dimethylarsenate; only traces of other organic arsenic compounds are observed (Larsen *et al*., 1993; Branch *et al*., 1994; Le *et al*., 1994). Uptake of arsenobetaine by humans is relatively high, with 50–80% of the dose appearing in human urine within 2 days of ingestion and approximately 5% appearing in the faeces (WHO, 1981; Ishinishi *et al*., 1986). Recent studies confirm this high absorption (Arbouine & Wilson, 1992; Mürer *et al*., 1992; Buchet *et al*., 1994; Le *et al*., 1994). Studies in hamster also confirm that orally administered arsenobetaine is well absorbed, with about 90% of the arsenic being excreted in urine within 5 days (Yamauchi *et al*., 1986). Although arsenobetaine contributes to 'total arsenic' in the diet, due to its rapid absorption and excretion and low toxicity, it is usually considered separately from inorganic arsenic and the methylated compounds derived from inorganic arsenic during metabolism. Edible seaweeds contain arsenosugars as the main arsenic compounds present, this arsenic is also absorbed and excreted in urine in humans (Le *et al*., 1994).

## 5.2.2 EFFECTS OF SPECIATION

The high concentration of non-toxic arsenobetaine in fish and other seafood makes assessment of the intake of toxic arsenic species difficult. Distinction

between 'inorganic' and more complex organic species needs to be considered in any study of the mechanisms of arsenic absorption from the diet.

Intestinal uptake of inorganic arsenic depends on solubility. Both trivalent and pentavalent soluble arsenic compounds are well absorbed in humans (reviewed by WHO, 1981; HSE, 1986). However, insoluble arsenic selenide was poorly absorbed when self-administered by a single volunteer, and trace doses of radiolabelled arsenic acid were only absorbed to a limited extent (less than 30%) when administered to hamsters.

Oxidation of arsenite occurs readily in aqueous solutions, especially at low concentrations, and reduction of arsenate solutions occurs easily in the presence of bacteria or other biological material. The valency state of administered compound needs checking carefully to ensure the correct interpretation of feeding studies (HSE, 1986).

## 5.2.3 OTHER NUTRIENTS

Feeding experiments with rats have indicated interactions between inorganic arsenic and phosphate (Gonzalez, *et al.,* 1995) and effects of arsenic on tissue levels of copper and manganese (see Section 5.2.1; Elsenhans *et al.*, 1987). However no other reports of interactions with metals or the effects of macronutrients have been found.

## 5.2.4 EFFECTS OF DIET

The major interest in unusual diets has been due to the high concentrations of organoarsenic in seafood and the high intake of total arsenic by those on mainly seafood diets. Organic arsenic compounds in seafoods are readily absorbed from the gastrointestinal tract in humans and animals, with 50–80% of the dose appearing in the urine within two days (WHO, 1981; Ishinishi *et al.,* 1986; Arbouine & Wilson, 1992; Mürer *et al.*, 1992; Buchet *et al.*, 1994; Le *et al.*, 1994).

Although most arsenic in air, drinking water and soil is inorganic (Hughes *et al.*, 1995), both inorganic and organic arsenic are also present in a range of foodstuffs as well as seafood (see Section 5.3).

When high levels of arsenic (500 mg arsenic (as trioxide)/kg diet) were given orally to rats, dietary composition affected absorption (WHO, 1981). Reduced absorption was associated with milk-based diets, casein-supplemented cereal diets and cereal diets supplemented with specific amino acids. At lower arsenic levels (75 mg/kg diet) these interactions were not observed, suggesting they may have been associated with damage to the gastrointestinal mucosa.

## 5.2.5 PREGNANCY AND LACTATION

In humans, arsenic levels in the placenta, fetus and newborn increase with age (WHO, 1981, Ishinishi *et al.*, 1986). Cord blood levels of arsenic at parturition are approximately the same as maternal levels (WHO, 1981). Arsenic levels in the urine of pregnant women were similar to those from nonpregnant women from both the area around a Swedish smelter and a reference area; furthermore, levels differed little at 10, 32 and 39 weeks of pregnancy (Lagerkvist *et al.*, 1993).

Arsenic has been detected in newborn rats of dams given a diet containing 27 or 215 mg arsenic/kg diet [levels in newborn were not stated] (WHO, 1981). In studies in mice, orally administered inorganic arsenic reached the fetus when administered on day 18 of pregnancy; the proportion of methylated metabolites present in the fetus increased with time after the maternal dose (Hood *et al.*, 1988). Arsenic was found in embryonic tissue 24 or 96 hours after intravenous injection of pregnant hamsters with 20 mg/kg bw of radiolabelled arsenic (WHO, 1981). Dimethylarsenite passed through the placenta when administered intravenously to rats immediately prior to parturition, and fetal whole blood levels were comparable with maternal levels (WHO, 1981). Thus inorganic arsenic and its methylated metabolites would appear to be transferred freely to the fetus. However, there are few experimental data concerning the effects of pregnancy on the maternal uptake of arsenic from the gastrointestinal tract.

No reports concerning the effects of lactation on the uptake of dietary arsenic have been identified.

## 5.2.6 SUSCEPTIBLE GROUPS: CHILDREN AND THE ELDERLY

Levels of inorganic arsenic and its metabolites have been examined in five studies in children, all aimed at examining the consequences of environmental exposure to arsenic in polluted areas (Andren *et al*., 1988; Jensen *et al*., 1991; Diaz-Barriga *et al*., 1993; Trepka *et al*., 1996). In three studies a higher level of arsenic excretion was reported in children from exposed areas compared with those from control areas (Diaz-Barriga *et al.*, 1993; Bencko, 1995; Trepka *et al*., 1996). In the one study that considered diet, arsenic excretion was as strongly associated with fish consumption as with environmental exposure (Trepka *et al*., 1996). Urinary excretion of arsenic by children aged 3–10 years was approximately twice that of adults (Jensen *et al*., 1991). Urinary arsenic declines with age and boys have slightly, but statistically significantly, higher levels of arsenic in urine than girls (5.8 µg/g *versus* 4.2 µg/g creatinine; Andrén *et al*., 1988). No studies were identified that described the relative rates of dietary arsenic uptake in childhood compared with those in adults.

No studies on the absorption of arsenic in the elderly were identified.

## 5.2.7 MEDICATION

No studies concerning the effect of non-arsenic containing medicaments on arsenic uptake in humans were identified.

## 5.2.8 GASTROINTESTINAL MICROFLORA

No studies concerning the influence of gut microflora on arsenic uptake in humans were identified.

Rat caecal contents can reduce arsenate to arsenite under anaerobic conditions and methylate the arsenite formed to methylarsonic acid and dimethylarsenic acid *in vitro* (reviewed by HSE, 1986). However, no differences in metabolism of inorganic arsenic were found following either oral and intravenous administration of arsenic to rats or oral administration of arsenic to germ free or normal mice.

# 5.3 DETERMINATION OF BIOAVAILABLE DIETARY INTAKE

In dairy products, and in beef and pork, approximately 75% of the arsenic present is inorganic (Hughes *et al*., 1995). In animal seafoods arsenic is present principally as organic material, predominantly as arsenobetaine but with some dimethylarsenate (Mürer *et al*., 1992; Larsen *et al*., 1993; Branch *et al*., 1994; Buchet *et al*., 1994; Le *et al*., 1994). Edible seaweeds contain arsenosugars as their main arsenical material (Le *et al*., 1994). Organic arsenic in seafood is the predominant source of human arsenic uptake, and less than 10% of the arsenic in seafood is inorganic material (Friberg, 1988).

Limited information is available on the form of arsenic present in various foods, and few, if any, population studies of dietary intake have used speciation techniques to define the form of arsenic ingested.

# 5.4 BIOMONITORING METHODS FOR DETERMINING UPTAKE FROM THE GASTROINTESTINAL TRACT

## 5.4.1 GENERAL OVERVIEW

Urine, and to a much lesser extent, hair, nail and blood analyses have been used to measure arsenic uptake in the occupational setting. Dietary arsenic, especially that arising from fish and seafood consumption affects urinary levels of total arsenic and its metabolites. Reference levels of arsenic and its metabolites in urine have been reported to depend generally on diet, and dietary arsenic has been considered a confounder when measuring uptake following industrial exposure (Lauwerys & Hoet, 1993; Cornelis, 1996).

Inorganic arsenic and its methylated metabolites can be measured by atomic absorption spectroscopy following high performance liquid chromatography and hydride generation, and can be separated from arsenobetaines (Arbouine & Wilson, 1992).

There do not appear to be data available for current levels of arsenic biomarkers within the UK population, nor information concerning changes in such levels over time.

## 5.4.2 URINE

Measurements of arsenic in urine reflect recent exposure, that is over the previous few days (Ishinishi *et al*., 1986; Lauwerys & Hoet, 1993; Hakala & Pyy, 1995). Total arsenic (inorganic and organic), or inorganic arsenic, methylarsonic acid and dimethylarsenic acid can be measured separately. The sum of inorganic arsenic, methylarsonic acid and dimethylarsenic acid is now considered the most appropriate measure for industrial exposure. Lauwerys and Hoet (1993) report normal levels in adults of the sum of inorganic, methyl and dimethyl arsenic of less than 10 µg/l in Europe, 'somewhat higher' in the USA, and approximately 50 µg/l in Japan. Lagerkvist *et al*. (1993) report geometric mean values of inorganic arsenic plus metabolites of 0.08–0.12 µmol/l (approximately 70 µg/l) among pregnant Swedish women, and have found no differences either between those living near a smelter and those in a reference area, or between samples taken at different times (weeks 10, 32 and 39 of pregnancy). Ishinishi *et al*. (1986) report normal levels of total urinary arsenic, in the absence of seafood consumption, to be 5 µg/l.

Although fish consumption greatly affects total arsenic levels in urine (by up to 2000-fold), it has less effect on the levels of inorganic arsenic and its methylated metabolites (for which increases of 3–4-fold have been reported; Arbouine & Wilson, 1992; Mürer *et al*., 1992; Lauwerys & Hoet, 1993; Buchet *et al*., 1994).

## 5.4.3 HAIR AND NAIL

Organic arsenic from marine origin is not absorbed and thus hair and nail can only reflect intake of inorganic arsenic (Lauwerys & Hoet, 1993). Generally, it takes 1–2 days for hair to reach steady state levels of arsenic. Arsenic levels in hair correlate with time following treatment with arsenical drugs, but not with long-term exposure (up to 10 years) to high levels of inorganic arsenic from well water (Ishinishi *et al*., 1986). External contamination of hair with airborne arsenic is a confounder as it is not reliably removable (Lauwerys & Hoet, 1993; Ashraf *et al*., 1994).

Lauwerys and Hoet (1993) report normal values of arsenic in hair for non-occupationally exposed individuals to be less than 1–2 mg/kg; Ishinishi *et al.* (1986) reported levels less than 1 mg/kg (average 0.81 mg/kg; median 0.51 mg/kg) in 80% of one sample of 1000 people, and levels ranging from 0.02–8.17 mg/kg in another sample of 1250 people. A range of 0.25–60 mg/kg arsenic in hair was found in a third group of 110 people using well water with high arsenic levels (Ishinishi *et al.*, 1986).

## 5.4.4 BLOOD

Levels of arsenic in blood mainly reflect recent exposure (Lauwerys & Hoet, 1993). Background levels are 0.1–0.4 μg/dl, but blood levels can reach 5–6 μg/dl when arsenic contaminated water is consumed. No studies concerning measurements of arsenic metabolites in blood were identified. Ishinishi *et al.* (1986) suggest that blood is not a useful medium by which to measure exposure to arsenic.

## 5.4.5 TOXICOKINETICS

The toxicokinetic information available for arsenic mainly refers to industrial exposure to inorganic arsenic. It has been summarised by HSE (1986), Ishinishi *et al.* (1986) and Lauwerys and Hoet (1993). Absorbed arsenic is rapidly eliminated through the kidney, either unchanged or as mono- and dimethyl derivatives.

Approximately 25% of a single low oral dose of arsenite is excreted in urine within 24 hours and about 45–48% appears within 4 to 5 days. The overall half-life is approximately 30 hours, thus it takes about 5 days to achieve steady state. When continuous intake takes place, at steady state, some 60–70% of the intake appears in urine. Initially, inorganic arsenic predominates in the urine after a single dose of inorganic arsenic; overall, at least half of the excreted material is present as dimethylarsenite. Organoarsenicals from marine organisms are mainly excreted unchanged in urine (50–80% within 2 days; HSE, 1986; Ishinishi *et al.*, 1986; Lauwerys & Hoet, 1993).

### 5.4.6 DETERMINATION OF UPTAKE: CONCLUSIONS

Speciation techniques now allow the separation of inorganic arsenic species in urine from the non-toxic complex organoarsenicals derived from seafood and enough is known about the kinetics of absorption and excretion to understand the relationship between dietary intake and urinary biomarkers of arsenic uptake. Arsenic species have relatively short half-lives of absorption and excretion and, unlike the other toxic metals reviewed here, urinary levels reflect short-term changes in dietary intake. Analytical advances are such that, in dietary studies, measurement of total urinary arsenic should now be supplemented by measurement of individual species.

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# 6 Summary and conclusions

# 6.1 FACTORS AFFECTING UPTAKE FROM THE INTESTINAL TRACT

This section is organised to consider the issues that were addressed for each metal, issue by issue, rather than by metal as in the main text of the report.

## 6.1.1 MECHANISMS OF UPTAKE AND INTERACTIONS

### LEAD

Absorption occurs by active and passive transport and throughout the length of the small intestine. Complex relationships exist between lead and calcium transport. A metabolite of vitamin D, $1,25(OH)_2D$, is involved in the control of both calcium and lead uptake. Limited studies suggest that up to 10% of inorganic lead is absorbed from the diet in the adult and 30–40% is absorbed in children.

### CADMIUM

Absorption of cadmium salts is low (5%). They are absorbed in the small intestine, probably by passive diffusion. There is no evidence for specific cadmium carriers. Cadmium in food of animal origin may be present as Cd-MT and appears to be treated differently from inorganic cadmium as far as tissue distribution goes. Mechanisms for absorption of Cd-MT are thus of importance to the overall absorption of cadmium from the diet.

### MERCURY

Varying percentages of inorganic mercury are absorbed in different species — approximately 5% in humans, up to 20% in mice. The mechanisms for absorption of inorganic mercury salts are not clear although there is some evidence to suggest a role for sulphydryl peptides. Methylmercury needs to be considered separately: over 95% is absorbed. Enterohepatic circulation of methylmercury is of importance in establishing the extent and time course of absorption.

### ARSENIC

Absorption occurs mainly in the small intestine, and is essentially complete for soluble arsenic salts. Little is known about the mechanisms involved; recent studies suggest absorption is a saturable process and may be shared with phosphate. Enterohepatic circulation occurs in some species.

### CONCLUSIONS

Although in general the information on uptake and interactions is sparse for all four metals considered here, such information is not necessarily important for the interpretation of risks from dietary exposure. One exception is the role of Cd-MT, which may be critical to overall cadmium absorption from foods, and further studies on the absorption and factors affecting the absorption of this complex are required.

## 6.1.2 SPECIATION

### LEAD

Volunteer experiments with simple and fairly soluble inorganic lead compounds and complexes suggest that chemical form is unimportant for uptake, but low bioavailability has been shown for some complex salts found in lead-contaminated soil.

### CADMIUM

Absorption of cadmium salts appears to be related to extremes of solubility. The absorption of Cd-MT is slower than that of soluble $CdCl_2$ and other protein-bound forms of cadmium found in diet, and it has a different organ distribution.

### MERCURY

Low percentages of inorganic mercury are absorbed, but the absorption of organic (methyl) mercury is essentially complete.

### ARSENIC

The uptake of inorganic arsenic species depend on solubility. Both trivalent and pentavalent soluble species are well absorbed. Arsenic selenide is poorly absorbed. Absorption of complex organic arsenicals (e.g. arsenobetaine) is relatively high (50–80%).

### CONCLUSIONS

Although a knowledge about speciation is important for risk assessment, it is not a major issue with respect to lead in food, as the total levels of this metal in the diet are very low. It is of rather more importance for the other three metals considered here.

## 6.1.3 DOSE

### LEAD

The effects of dose on lead uptake from the diet in humans are difficult to find. At low doses, intake and retention appear to be correlated. However in animal studies, increased body burden is associated with decreased efficiency of absorption.

### CADMIUM

There is evidence available for the effects of increasing doses of dietary cadmium on body burden over time but not for the dose-effects related to individual salts or Cd-MT on the rate or extent of absorption in humans.

### MERCURY

Uptake of inorganic mercury is dose-dependant in the rat. Uptake of methylmercury is essentially complete in mice and humans and not dose-dependant.

### ARSENIC

An association between dietary levels of inorganic arsenic and tissue levels has been shown in a limited number of animal studies.

### CONCLUSIONS

Apart from elemental mercury and methylmercury, there are few data available to establish relationships between dose and amounts absorbed. However, such information is not essential for the assessment of absorption from the diet or risk assessment for the metals considered here at current levels of exposure found in the UK.

## 6.1.4 OTHER NUTRIENTS

### LEAD

Calcium and vitamin D status have complex effects on lead absorption in animals and humans. Dietary calcium deficiency is associated with an increase in the body burden of lead and susceptibility to lead toxicity. There is also evidence that phosphates, and zinc reduce lead absorption and some suggestion that lead

poisoning may be associated with iron deficiency. Large doses of other vitamins also reduce lead absorption, some act as chelators. Fats increase absorption. Milk also promotes lead absorption but it has been suggested that this is due to the effects of lactose.

## CADMIUM

Interaction of cadmium with calcium is involved in the pathogenesis of Itai-itai disease. The effects of iron and zinc are complex and vary with dose and experimental system. The effect of cadmium on calcium metabolism may be mediated by vitamin D. Animal studies indicate that increased cadmium leads to copper deficiency. Phytic acid decreases tissue absorption and retention of cadmium, and ascorbic acid protects against cadmium-induced toxicity.

## MERCURY

Administration of selenium has been shown to reduce mercury toxicity. Animal studies show that mega doses of $B_{12}$ and folate increase the methylation of inorganic mercury. Changing the fat and fibre content of the diet has been shown to affect the retention of methylmercury in mice, possibly by altering intestinal flora and hence the extent of demethylation of methylmercury.

## ARSENIC

Interaction with phosphate has been suggested based on animal experiments but information concerning other nutrients is not available.

## CONCLUSIONS

There are some important interactions between some of these four metals and other dietary components. Lead and cadmium absorption are both affected by calcium and iron intake and status. Studies of absorption of these toxic metals in calcium, iron and vitamin D deficiency states are therefore likely to be of importance in the assessment of dietary absorption among susceptible groups.

## 6.1.5 THE EFFECTS OF DIET

### LEAD

Few studies have examined the influence of varying dietary patterns on the uptake of lead in humans. Vegetarian diets do not appear to affect blood lead levels. However phytates and other nutrients can act as chelators, mainly reducing absorption.

### CADMIUM

Several studies show that vegetarians have lower concentrations of cadmium in blood and urine than people on a mixed diet. High blood cadmium levels occur in those eating diets based on contaminated shellfish.

### MERCURY

The majority of studies relate to unusual diets containing high levels of methylmercury (especially fish diets). However there is little information concerning the effects of unusual diets *per se* on the extent or rate of methylmercury absorption in humans.

### ARSENIC

There is little information concerning the effects of diet on actual absorption of inorganic or organic arsenic; organoarsenic in seafood is an important confounder. There is the suggestion that milk based diets reduce absorption of inorganic arsenic.

### CONCLUSION

Few data are available on the differential uptake of any of these four metals in association with different types of diet.

## 6.1.6 PREGNANCY AND LACTATION

### LEAD

Experiments in rats and mice have shown increased intestinal absorption during both pregnancy and lactation. Evidence in humans is contradictory and difficult to interpret due to the mobilisation of lead from bone.

### CADMIUM

The majority of studies in humans are concerned with changes in maternal blood, placental and cord blood cadmium. Accumulation occurs in the placenta with low transfer to the fetus. Iron status affects cadmium accumulation during pregnancy in humans. Little information is available concerning the effects of pregnancy on cadmium absorption from the gut of the mother. Animal studies show increased absorption during lactation but little transfer to milk.

### MERCURY

There are numerous studies of methylmercury transport and levels in women during pregnancy and lactation showing the ease with which it moves into the fetus. However, little has been published concerning the effects of pregnancy and lactation on absorption of inorganic or organic species from the gastrointestinal tract.

### ARSENIC

Inorganic arsenic and its methylated metabolites would appear to be transferred freely to the fetus, although there appears to be no information concerning the effects of pregnancy on the uptake of arsenic from the gastrointestinal tract.

### CONCLUSION

There is information available for all four metals concerning their concentrations in maternal blood during pregnancy and lactation, the placenta and cord blood and the effectiveness of transfer to the fetus and neonate in humans and animals. However there are few direct studies of the intestinal absorption of these metals during pregnancy and lactation.

## 6.1.7 CHILDREN AND THE ELDERLY

### LEAD

Studies in animals and young children show higher rates of absorption in childhood, possibly due to a higher density of intestinal transport proteins during periods of rapid growth.

A negative lead balance has been reported in the elderly.

### CADMIUM

A high proportion of the adult body burden is accumulated during childhood. High absorption rates occur in the neonatal period.

A negative balance between absorption and excretion has been reported in the elderly. However low iron stores may well be associated with increased absorption in this age group.

### MERCURY

Dietary intake has been studied in children from various populations. Urine levels of mercury correlate with fish intake and dental amalgam. Few studies appear to have investigated relative rates of gastrointestinal absorption in childhood.

No studies in the elderly have been identified.

### ARSENIC

No studies have been identified that describe the relative rates of arsenic uptake in children or the elderly in comparison with other adults.

### CONCLUSIONS

Although it is clear that lead and cadmium are more readily absorbed by young children than adults, more information about uptake of all four metals in childhood is required. Furthermore, little is known about the effects of iron deficiency, vitamin D levels (especially in Asian populations) and selenium levels on absorption of lead and cadmium, and further studies are required. The differential distribution of lead and mercury to the brain in children is also an important area that is poorly understood.

The few existing studies suggest negative balances for lead and cadmium in the elderly. The significance of this is not clear.

## 6.1.8 MEDICATION

Chelating agents may increase absorption of lead from the gastrointestinal tract.

No information has been identified for cadmium, mercury or arsenic.

### CONCLUSION

Little is known about the effects of medication on the absorption of any of these four metals. The effects of long-term medication, such as antibiotic therapy, calcium supplements, steroids and anti-inflammatory agents on toxic metal absorption are of concern.

## 6.1.9 GASTROINTESTINAL MICROFLORA

No information has been identified on lead or cadmium.

### MERCURY

The human gut microflora demethylates organic mercury permitting excretion of inorganic mercury. It is no longer thought, however, that human gut microflora are involved in methylation of inorganic mercury. Effects of unusual diets and antibiotics on methylmercury retention may be *via* changes in gut microflora.

### ARSENIC

There is evidence for methylation of inorganic arsenic by gut flora *in vitro*, but no evidence for a role of microflora *in vivo* in experiments with germ-free mice. No evidence in humans is available.

### CONCLUSION

The role of microflora is not of importance as far as toxic metal uptake or metabolism are concerned.

# 6.2 DETERMINATION OF BIOAVAILABLE DIETARY INTAKE

## LEAD

'Total' lead in food has been used as a measure of intake in most human studies. The relationship between lead speciation in food and uptake within the gastrointestinal tract remains unclear. No bioavailability studies comparing lead species in dietary samples with biomarkers of lead absorption have been identified.

## CADMIUM

The intake of dietary cadmium is usually assessed by measuring the total content of cadmium in the diet. However the bioavailability of cadmium is expected to vary depending on the form in which it occurs in food. Much of the cadmium in meat and fish is bound to metallothionein, which is absorbed less effectively than inorganic cadmium. Other than the experimental studies comparing the uptake of inorganic and metallothionein-bound cadmium, no reports describing the effects of speciation on uptake have been identified.

## MERCURY

The differences in absorption, distribution and toxicity between elemental, inorganic and organic mercury are such that detailed dietary studies (speciation of dietary components) may be required to establish a full picture of bioavailable intake. Few such studies are reported in the literature.

### ARSENIC

The high level of organic arsenic in seafoods is well established. However, limited information is available on the form of arsenic present in other foods and few population studies of dietary intake have used speciation techniques to define the form of arsenic being ingested.

### CONCLUSION

Measurements of 'total' metal in a particular food stuff or total diet sample should be considered a useful 'first screen'. It may then be important to identify particular metal species when the 'total' level is found to be high.

# 6. 3 BIOMONITORING METHODS FOR DETERMINING UPTAKE FROM THE GASTROINTESTINAL TRACT

### LEAD

Blood lead measurement is well established for assessing total uptake into the body, irrespective of source. However the inability to identify exposures from different sources may be a major limitation for its use in assessing dietary uptake at the low levels of exposure currently found in the UK. However, the ratio of

$^{206}Pb$:$^{207}Pb$ often varies between various sources of exposure within a given location, and this has been used to apportion the relative contribution of various environmental lead sources to body lead. The toxicokinetics of blood lead are such that measurement reflects integrated exposure over several weeks. Skeletal stores of lead reflect lifetime exposure and can be measured non-invasively by X-ray fluorescence.

## CADMIUM

The body burden of cadmium is accumulated over decades. The kinetics of this process are such that short-term changes in diet are not reflected in parallel changes in blood levels or urinary excretion. Both urinary cadmium and biomarkers of effect are, however, some use in assessing long-term uptake from locally grown or collected produce in contaminated areas.

## MERCURY

The combined use of blood and urinary mercury measurements can give useful information concerning the relative contributions of organic and inorganic mercury from the diet and elemental mercury from dental amalgams. Methylmercury exposure is reflected in blood (erythrocyte) and hair levels whilst inorganic and amalgam mercury uptake is reflected in urinary measurements. Although hair analysis has not found a useful place in the assessment of occupational exposure, it has been used successfully in determining population exposure to organic mercury.

## ARSENIC

Speciation techniques used for the measurement of urinary arsenic allow the separation of inorganic arsenic species from the non-toxic complex organoarsenicals derived from seafood. Enough is known about the kinetics of absorption and excretion of different species to understand the relationship between dietary intake and these urinary biomarkers of arsenic uptake. Arsenic species have relatively short half-lives of absorption and excretion and unlike the other toxic metals reviewed here, urinary levels reflect short-term changes in dietary intake. Analytical advances are such that measurement of total arsenic should be supplemented by measurement of individual species in dietary studies.

### CONCLUSIONS

Blood and urinary measurements of lead, cadmium and mercury are not suitable for determining short-term changes in uptake due to the relatively long half-lives of these metals in biological fluids. However the short half-lives of various arsenic species do allow short-term studies of uptake from different dietary sources. Further development of Cd-MT as an index of uptake of this species should be informative.

## 6.4 OVERALL CONCLUSIONS

Although numerous studies have compared dietary intake with various markers of absorption, there are several areas for which there is a surprising lack of information. This is especially notable where the effects of pregnancy and lactation on rates of absorption of the metals are concerned. Also much of the information in the literature comes from animal experiments at high dosages or from human studies of populations exposed to much higher levels those those currently occurring in the diet in the UK.

The differences between the toxicities of inorganic and organic forms of mercury and arsenic and the role of Cd-MT are of particular importance with respect to determinations of intake and uptake. However few population studies of dietary intake of these toxic metals have involved speciation studies. The measurement of the four metals in body fluids is well established for assessing their absorption and will give information concerning dietary uptake. However, the long half-lives of lead, cadmium and mercury in the body stores and fluids make such measures unsuitable for short-term dietary studies. Measurements of the relevant biomarkers are useful in assessing long-term uptake from contaminated land.

Based on the reviews and discussion presented herein, the major conclusions to be drawn are as follows.

- At the low levels at which lead, cadmium, mercury and arsenic currently occur in the diet, there is no general concern about population risk.

- Smoking can be the major source of cadmium in those not occupationally exposed.

- There is evidence that children can accumulate lead and cadmium at a proportionally faster rate than adults and this requires further investigation.

- The effect of vitamin D deficiency on lead absorption is of concern and should be investigated.

- The role of cadmium-metallothionein as a dietary constituent, as a determinant of organ distribution and as a biomarker needs further investigation.

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## Acknowledgments

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## IEH PUBLICATIONS

### IEH Reports

**Air Pollution and Health: Understanding the Uncertainties**
Report R1 published 1994

**Air Pollution and Respiratory Disease: UK Research Priorities**
Report R2 published 1994 (out of print)

**Natural and Man-Made Mineral Fibres: UK Research Priorities**
Report R3 published 1995

**Perinatal Developmental Neurotoxicity**
Report R4 published 1996

**The Use of Biomarkers in Environmental Exposure Assessment**
Report R5 published 1996

**Health Effects of Waste Combustion Products**
Report R7 published 1997

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Report R9 published 1998

**Non-auditory Effects of Noise**
Report R10 published 1997

### IEH Assessments

**Environmental Oestrogens: Consequences to Human Health and Wildlife**
Assessment A1 published 1995

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### Special Reports

**Understanding Asthma** published 1995

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**Health Effects of Ozone and Nitrogen Dioxide in an Integrated Assessment of Air Pollution** published 1997

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