

## The accumulation of potentially-toxic metals by grazing ruminants

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The main factors affecting the accumulation of potentially-toxic metals (PTM) by grazing animals are the presence of the metal, its concentration in herbage and at the soil surface, and the duration of exposure to the contaminated pasture and soil. In addition, the elapsed time between the contamination of the pasture and grazing, the quantity of soil ingested together with herbage, the mechanism of absorption of the metal into blood and the presence or absence of antagonistic metals can interact to influence the rate and extent of accumulation of heavy metals in edible body tissues. Models of the accumulation of metals by grazing animals may be used to determine the statutory limits of radionuclides and PTM in soils under grazed pastures. Meta-analysis of existing data, using a random-effects model, is a useful approach to understanding the factors affecting the accumulation of some metals, e.g. Cd. The target edible body tissues for the accumulation of most PTM are the liver and kidneys, with the exception of radiocaesium, which accumulates in muscle to a greater extent than in other tissues. The livers and kidneys of mature livestock that have been grazed on areas of pasture at the legal limit of contamination by Cd for more than one grazing season should be removed from the human food chain in order to reduce the risk of intake of Cd by the human population.

### Potentially-toxic metals: Grazing livestock: Contaminated pasture and soil: Accumulation in edible body tissues

The contamination of pastures and the accumulation of potentially-toxic metals (PTM) in grazing animals can occur on soils that are naturally rich in metals (for example, see Martin & Coughtrey, 1982), following accidental or anthropogenic events such as the fall-out of radiocaesium on grassland after the accident at the Chernobyl nuclear reactor (Howard & Beresford, 1994), or following the prolonged use of sewage sludge as a fertiliser (Wilkinson *et al.* 2001).

The definition of a 'heavy metal' is not clear. In many texts, metals with a density >4 or 5 g/l are considered as being heavy metals. However, this definition would include the lanthanides and actinides that, chemically, have distinct properties. The classification preferred by many researchers is that of Nieboer & Richardson (1980), in which elements with a density >5 g/l are grouped into three classes: A; B; borderline. Class A elements have a preference for ligands containing O (e.g. Mn, density 7.42 g/l). Class B elements show a preference to form ligands with N or S (e.g. Cd, density 8.65 g/l). Borderline elements are of intermediate nature between classes A and B (Table 1).

The chemical classification of 'heavy metals' into the three classes is important biologically. The characteristic of Class B elements to form ligands with N and/or S, includes metallothioneins (MT; Cousins, 1985; Lee *et al.* 1994), phytochelatin (Marschner, 1995), complexes with globular proteins (caeruloplasmin; Underwood & Suttle, 1999) and excretion in bile (e.g. Cd; Kiyozumi & Kojima, 1978). In grazing mammals the elements Cd and Pb tend to accumulate in the liver and kidneys, and in some cases (e.g. Pb) also in bone (Lee *et al.* 1999). Many elements interfere with essential enzyme reactions and/or organ function (see p. 273); hence their potential toxicity to the grazing animal and man. Of particular concern is Cd, because of the relatively low threshold of toxicity on the part of the animal to the presence of relatively low concentrations of this element in the body. The European Commission and national governments have recognised the potential toxicity of Cd and have set maximum limits for Cd in foods (Commission of the European Communities, 2001). The Commission of the European Communities (2002) has

**Abbreviations:** MT, metallothionein; OM, organic matter; PTM, potentially-toxic metals.

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**Table 1.** Classification of selected essential and non-essential metal ions into classes A and B and borderline (based on Nieboer & Richardson, 1980)\*

Class A	Borderline	Class B
Cs	Zn	Cd
Mn	Pb	Cu
Sr	Fe	Hg
	Cr	Ag
	Co	
	Ni	
	As	
	Sn	
	V	

\*For more detailed explanation, see Martin & Coughtrey (1982)

recently reviewed heavy metals in wastes and their potential risk to the human food chain.

The present review is principally concerned with the different possible routes of entry of PTM into the body of the grazing animal. Although radiocaesium is not a toxic metal, as defined in Table 1, it is included because of its importance in the human food chain.

### Contamination of grassland by potentially-toxic metals

Sources of PTM in soils have been reviewed extensively (for example, see Martin & Coughtrey, 1982; Alloway, 1995). The distribution of metals is not uniform through the soil profile, because of progressive weathering and disintegration of rocks, the incorporation of organic matter (OM) by biological processes and the deposition of regolith by aerial and fluvial processes. Leaching, erosion, biological and microbial processes modify the localisation of metals within the soil.

The lateral and horizontal mixing of the surface soil, especially during cultivation, are major processes by which the localisation of heavy metals within the top 0.25 m of the soil may be altered. Metals in cultivated soils are generally more homogeneously distributed in the top 0.25 m than in undisturbed soils. The concentration of metals in the top 0.075 m of the soil is of particular relevance because the roots of most grasses are in this region, and it is the surface soil that may be ingested along with herbage by the grazing ruminant (see p. 270). Contaminants may be deposited on the soil surface via aerial deposition (e.g. radionuclides, vehicle and industrial emissions), during flooding and by direct application to the ground (e.g. metals in fertilisers and in sewage sludge). The grazing animal can ingest the metals either by consuming herbage that is internally or externally contaminated (Aitken, 1997), or by consuming contaminated soil (see p. 271).

#### *Natural contamination of grassland*

In the UK there is considerable regional variation in PTM concentrations (McGrath & Loveland, 1992), with particularly high concentrations in soils derived from marine black shales in south west and north west England and the West Midlands. These 'teart' soils can contain high Mo

**Table 2.** Typical concentrations of potentially-toxic metals (mg/kg) in soils derived from various parent materials (Curtis *et al.* 1976)

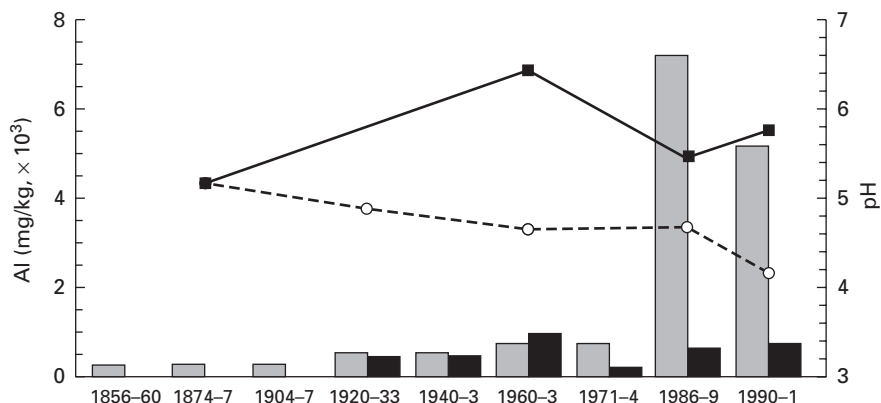
	Serpentine	Granite	Shale	Sandstone
Co	80	2	20	3
Cr	3000	5	200	30
Cu	20	10	10	10
Mn	3000	700	1000	200

concentrations, which reduce Cu availability in grazing stock. In carboniferous limestone areas where Pb is often mined, there are often fluvial-deposited sediments downstream, containing up to 6000 mg Pb/kg (Alloway, 1999). Soils derived from igneous rocks can contain high concentrations of PTM (Table 2). Leaching of metals from such soils may be variable, and the subsequent deposition of the leached minerals in sedimentary rock may lead to the accumulation of metals to potentially-toxic levels (Curtis *et al.* 1976).

The main factors affecting toxic metal uptake by plants are the chemical speciation of the mineral fraction of the soil, the soil OM content, and soil permeability and chemical properties (Alloway, 1999). The mineralogy of the soil (especially the clay mineral fraction and the cation-exchange capacity) affects the pattern of release and retention of cations in the soil, even though Hill *et al.* (1998*a,b*) did not find cation-exchange capacity to affect accumulation of PTM in lambs. Adsorption of PTM to ligands, e.g. iron, manganese and aluminium oxides, is pH dependent and conferred by the ability of the PTM to form hydroxy complexes, typically in the order Cd > Ni > Co > Zn >> Cu > Pb > Hg. Complex formation with OM is typically Pb > Cu > Fe = Al > Mn = Co > Zn (Alloway, 1999).

Soil chemical properties and soil permeability are closely related. The migration of air into the soil and leaching of soil water alter the oxidation–reduction conditions and soil solution pH. The balance of oxidation–reduction conditions can alter the PTM speciation, and under anoxic conditions microbial methylation of As, Hg, Sb, Se and Tl can convert these elements to more bioavailable and toxic forms (Lamy *et al.* 1993; Alloway, 1999). The combined effects of oxidation–reduction and change in soil pH on oxides of certain metals can lead to re-release or immobilisation of elements in the soil solution.

Soils derived from geological series of high metal concentration are the most likely to produce contaminated herbage. Thus, undisturbed soil profiles in metal-rich areas close to historical mining activity show elevated concentrations of metals in the uppermost part of the soil profile (Martin & Coughtrey, 1982; McGrath & Loveland, 1992), and are the most likely natural soils to be associated with supra-normal concentrations in plant tissues. Although the concentration of metals in plants generally reflects that in the soil, only in the case of Cd, Zn, Ag, Hg, Sn and Th does the range of metal concentration in plants reach equivalence with the median of that in the soil (Bowen, 1979). The range of concentrations of metals in plants is highly variable, reflecting, among other factors, the availability of the element, the presence or absence of competing ions and the composition and age of the plant community.



**Fig. 1.** Aluminium in hay from the Park Grass Experiment at Rothamsted, Herts. (■), Aluminium in hay, unlimed; (■), aluminium in hay, limed; (○- -○), pH, unlimed; (■-■), pH, limed. (From Blake *et al.* 1994.)

Soil acidification, historically the result of the depletion of the soil cation exchange capacity by rainfall, and more recently by the direct deposition of  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_2$  and  $\text{HNO}_3$  in rain following industrial activity, increases the availability of metals to the plant (Alloway, 1995). Thus, the UK statutory limits to the concentrations of Zn, Cu and Ni in soils under grass increase with soil pH (Statutory Instrument, 1989). Without the intermittent remediation of soil pH by the addition of lime, it is likely that the concentrations of PTM in herbage will increase, especially if the plant community is growing slowly. An example of progressive soil acidification enhancing metal availability is when reduction of soil pH to 4.5 at Rothamsted, Herts., UK (Fig. 1) caused the concentration of Al in hay to exceed the maximum tolerable limit for animal feed (National Research Council, 1980).

#### Accidental contamination of grassland

There are many examples of accidental contamination of grassland, e.g. by radionuclides, mine wastewater containing Cd, Zn, Cu, Pb and As, shotgun pellets (Pb, Sb, As and Ni), Zn and Cu in pylon 'drip', Cd in phosphatic fertilisers and a variety of metals in sewage sludge. However, few of the examples are linked directly to events leading to either toxicity of grazing livestock or to a reduction in animal performance (Commission of the European Communities, 2002).

**Radiocaesium.** Approximately  $1.9\text{--}5 \times 10^8$  Bq, comprising about thirty radionuclides, were released into the atmosphere from an explosion at the Chernobyl nuclear station in 1986. The most consequential radionuclides, from the point of view of the long-term contamination of land, were  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ , with half-lives (d) of  $1.1 \times 10^4$  and  $1.02 \times 10^4$  respectively (Savchenko, 1995). The migration of  $^{137}\text{Cs}$ ,  $^{134}\text{Cs}$  and  $^{131}\text{I}$  and their subsequent widespread deposition on land were the main causes of an increase in radioactivity in most European countries at this time (International Atomic Energy Authority, 1990).

Cs adsorbs strongly to clay particles in soil and consequently moves slowly through the soil profile, but it is also relatively unavailable to plants. In contrast,  $^{90}\text{Sr}$

binds weakly to soil and is readily available to plants (Winteringham, 1989; Savchenko, 1995). However, the soils of many of the upland areas of the UK affected by the fallout of radionuclides were of low pH and low in clay concentration and, therefore, did not immobilise Cs (Livens *et al.* 1991). The concentrations of  $^{134+137}\text{Cs}$  in the vegetation of north and north west areas of the UK 6 months after the Chernobyl accident were highly variable, probably reflecting the uneven pattern of rainfall after the accident rather than differences in soil type, and differences in herbage yield (for review, see Howard & Beresford, 1994).

**Contaminated natural fertilisers.** Phosphatic fertilisers contain  $\leq 280$  mg Cd/kg  $\text{P}_2\text{O}_5$  (Sumner & McLaughlan, 1996), leading to accidental contamination of surface layers of soil (Williams & David, 1973; Bramley, 1991) and accumulation in plant tissue (Lee *et al.* 1993). Phosphatic rocks of igneous origin usually contain less Cd than those of sedimentary origin ( $< 5$  mg Cd/kg  $\text{P}_2\text{O}_5$ ), but comprise only approximately 0.14 of world phosphate resources (Environmental Resources Management, 1999; Aldinger, 2002). Several EU member states have set limits to the concentration of Cd in fertilisers (Environmental Resources Management, 2001), and proposals to have a progressive reduction in the maximum concentration, ultimately to 20 mg Cd/kg  $\text{P}_2\text{O}_5$  by 2016 (Environmental Resources Management, 2001) are under consideration. Removal of Cd from rock phosphate is possible by co-crystallisation of  $\text{H}_3\text{PO}_4$  with  $\text{CaSO}_4$  (gypsum) or, less attractively, by calcination with  $\text{CaCl}_2$  at  $700\text{--}1100^\circ$  (Environmental Resources Management, 2001).

#### Anthropogenic contamination of grassland

**Mining.** Disturbed soils and spoil heaps from historical mining activity present a particular hazard to the grazing animal, since the concentrations of metals may be high in both topsoil and herbage. For example, Johnson & Eaton (1980) noted that the concentrations Cd, Zn and Pb were respectively three, two and five times higher in topsoil than in the subsoil around a derelict Pb-Zn mine. Pb is one of the most common causes of poisoning in farm animals (Livesey, 1994); some poisoning is a result of grazing

spoil heaps (Clegg, 1978). However, many incidents of Pb poisoning are the result of accidental exposure to point sources of Pb contamination of animal feed rather than chronic exposure (Livesey, 1994).

**Smelters and brickworks.** The uptake of Pb by plants is low (Wilson & Cline, 1966), but in areas where aerial deposition of Pb on to grassland has occurred, for example close to Pb smelters or roads (see p. 270), the Pb concentration on herbage may be elevated, especially in winter (Dorn *et al.* 1975). Martin & Coughtrey (1982), using data from Burkitt *et al.* (1972), reported increased Pb, Cd and Zn on perennial ryegrass (*Lolium perenne*) up to 10 km from a Pb–Zn smelter.

**Sewage sludge (biosolids).** The application of sewage sludge to grassland can result in elevated concentrations of PTM in the upper horizons of the soil (Davis *et al.* 1988). OM usually has a high cation-exchange capacity and strong absorptive capacity for PTM, which can be immobilised in soils rich in OM. However, the processes of OM degradation or chemical modification, for example when sewage sludge OM is added to soil, can alter the availability (Wilkinson *et al.* 2001, 2003).

Linear increases in Cd and Cu concentration in herbage, but not Pb, have been observed with increasing soil concentrations in a review by Stark *et al.* (1998). Under field-scale conditions Wilkinson *et al.* (2001) observed elevated concentrations of Cd, Cu and Pb in samples of unwashed herbage taken from grassland that had received repeated applications of sewage sludge over a 15-year period. The increase was greatest in the latter part of the grazing season. Some of the increases in PTM following application of sewage sludge were acknowledged to be derived from surface contamination.

**Road traffic.** Cars emit colloidal Pb and uncombusted Pb particulates containing tetra-alkyl lead, motor oil, Cd from tyres and Zn, Cu, Mn, Cr and other metals from wear of moving metallic parts in the car. The tetra-alkyl lead compounds (anti-knocking agents) are more toxic than Pb salts as they penetrate through epidermal cells in animals (Merian, 1991). Roadside Pb concentrations in soil and herbage are expected to decline following the removal of Pb anti-knock agents from petroleum in most of Europe by 2005, according to the Aarhus agreement (Anon, 1998). However, Pb in soil has a half-life of several decades, depending on the OM content (Fowler *et al.* 1995). Once deposited on the soil, the Pb is rapidly complexed with organic compounds and resides in the upper strata that contain the greatest OM content (Branford *et al.* 1998). Emissions of Pb worldwide have caused progressive increases in soil Pb concentrations, at least until the removal of Pb from petrol (Jones *et al.* 1991; Renberg *et al.* 2000). In many developing countries Pb continues to be used in fuel, which can result in Pb concentrations of 80–7000 mg/kg herbage DM on busy roads (Motto *et al.* 1970). Animals grazing near roads where vehicles are using leaded petrol develop increased Pb concentrations in their blood, due to uptake from both pasture and soil (Ward *et al.* 1978). Soil intakes as high as 400 g/d for grazing ewes (Healy, 1967) and 1.5 kg/d for cattle (Dewes, 1986) have been reported.

Pb has been found in the milk of cows grazing roadside areas (Pittia, 1992), in mule deer forage in the Rocky

Mountain National Park (Harrison & Dyer, 1984) and in hay and straw samples collected from roadsides (Swarup, 1993). Ward & Savage (1994) found substantial quantities of Pb in the blood, wool and hair samples from sheep and horses grazing near the London orbital motorway. Although Pb has a relatively short half-life in blood (about 4 weeks) regular consumption ensures elevated concentrations in grazing animals. However, Pb does not spread more than a few metres from a vehicular emission source, even in the direction of the prevailing wind, so the animals have to be very close to the road to become contaminated. Systems of grazing cattle, sheep and horses close to roadways are still common in developing countries, where Pb is often still added to petrol. Such animals are often tethered, reducing their opportunity to select unpolluted herbage.

The metal abrasion in vehicles during motion also releases Cd and Zn onto neighbouring pastureland, but not in sufficient concentrations to pose a risk to grazing animals. These metals are also considerably more mobile in soil than Pb. A form of emission of increasing importance is the low levels of Pt and Pd (<1 mg/kg fresh weight in soil and plants) derived from the catalytic after-burners of vehicular emissions (Hodge & Stallard, 1986).

#### **Ingestion of potentially-toxic metals by the grazing animal**

The localisation of PTM in plants is dependent on the ability of the plant to either exclude the metal at the root–soil interface, particularly in the presence of vesicular arbuscular mycorrhizae, or to prevent the movement of the metal within the plant. Exclusion at the root–shoot interface is generally related to the production by the plant of low-molecular-weight root exudates. These exudates are composed of sugars, amino acids, phenolic compounds and organic acids (Wiren *et al.* 1993). Typically, Mn, Fe and Al are sequestered within concretions around roots derived from an interaction with low-molecular-weight root exudates (Marschner, 1995). Mobilisation of PTM can also occur as a result of formation of complexes of Cd and Cu. These processes can have an effect on the uptake and movement of PTM in the root and shoot of the plant (Mench *et al.* 1988; Mench & Martin, 1991).

The presence of phytochelatins to detoxify certain metals in plants is important in plant physiological terms, but may not be an efficient mechanism for excluding PTM from uptake by the grazing ruminant. Even if the PTM is bound in a phytochelatin, the fate of the metal (which is present in the form of a glutamylcysteine compound in the rumen and small intestine) is not known, but it is likely that the complex is degraded rapidly by the rumen microbial population, thus releasing the bound PTM.

Cd and Zn disperse to most root and shoot tissues, but can be confined to the meristem. Pb is generally localised in the root, if it has been taken up, and Ni tends to be distributed throughout the plant (Marschner, 1995).

The mechanisms of adhesion of PTM to herbage are poorly understood (Chaney & Lloyd, 1979; Jones *et al.* 1979; Klessa & Desira-Buttegieg, 1992; Aitken, 1997, 1998). Cu, Zn and Fe are generally retained on herbage for periods greater than the UK statutory 21 d no-grazing period

after the application of sewage sludge (Statutory Instrument, 1989), even under periods of high rainfall (Aitken, 1997), but the fate of Cd, Ni and Pb is uncertain. The variability in retention of Pb is high ( $\leq 85\%$ ) dependent on herbage factors as well as the type and association of Pb in the sludge. The variability may be partially explained by the extent of PbS<sub>2</sub> formation and methylation of Pb in sludge digestion. Recontamination of the sward by rain splash and soil contamination of herbage is difficult to quantify (Beresford & Howard, 1991), but could affect the amount of sludge-derived solids adhered to the herbage.

### Accumulation by the grazing animal of potentially-toxic metals in body tissues

#### *Factors affecting uptake of elements by the animal*

**Selective grazing behaviour.** Cattle can detect lead acetate on pasture when it is present at concentrations of  $\geq 45$  mg Pb/kg DM (Strojan & Phillips, 2002). They avoid lead acetate if exposed to it for a long period, especially at high concentrations, probably due to feedback of adverse effects on digestion (Strojan & Phillips, 2002). However, cattle readily investigate novel objects in fields and can suffer acute toxicity following investigation of discarded Pb-containing batteries, pipes and other objects. If they only have access to herbage contaminated with Pb the intensity of their grazing declines, as they reduce the time that they spend grazing each day and their rate of biting the pasture. The latter may be an indication of greater selectivity during grazing, which is also suggested by the slower rate of walking of cattle while they graze Pb-contaminated pasture (Strojan & Phillips, 2002). However, exposure to Pb-contaminated pasture ameliorates the effects of Pb on cattle grazing behaviour, suggesting that either they learn to be more selective, or that they tolerate the contamination. The latter situation could occur with a gradual modification of rumen microflora so that Pb absorption decreases. However, there was no evidence of modification or an increase in herbage digestibility in sheep exposed to Pb-contaminated feed for 1, 4 or 7 weeks (Mohamed, 2002). A possible reason for the reduction in Pb aversion with experience is that cattle become accustomed to the taste. However, it is more plausible that the aversion originates from adverse effects of digestion. Cattle showed no obvious aversion to the taste of Pb on herbage when it was first presented to them in different concentrations (Strojan & Phillips, 2002). Taste aversions are more likely to develop for substances that have threatened the survival of grazing animals over a prolonged period.

**Antagonistic elements.** Antagonism may arise from direct competition for absorption sites in both the plant and animal, or from competition within the organism. Different mechanisms are responsible at different concentrations. For example, at low dietary concentrations Cd can directly compete with Cu for absorption sites, whereas at high concentrations the MT induction by Cd can bind Cu and render it unabsorbable (Gawthorne, 1987). A reduction in the Cu status of sheep has been observed with supplementary dietary Cd by Mills & Dalgarno (1972).

Cd absorption is most commonly affected by the Zn status of animals, and Cd has been demonstrated to increase Zn accumulation in sheep, perhaps by the stimulation of MT production (Doyle & Pfander, 1975; Grace *et al.* 1993; Lee *et al.* 1994). Conversely, Chiy *et al.* (1998) found that a high Zn intake reduces Cd absorption in sheep, suggesting competition between the two elements. Other researchers have found that Cd absorption in rats is independent of Zn status (Foulkes & Voner, 1981). A simple competitive mechanism is unlikely, partly because the absorption of Zn, but not Cd, is dependent on body status (Foulkes, 1984). The transport of Cd from the mucosa to the bloodstream is much less (approximately 1%) than that of essential metals such as Zn, where it may reach 50% (Foulkes, 1984). Cd absorbed into mucosal cells, but not transferred to the bloodstream, is bound to cell membranes (Taguchi & Suzuki, 1978) and returns to the gastrointestinal tract following the desquamation of these cells. By contrast, Zn is retained and may be released as required, depending on the body burden (Richards & Cousins, 1975).

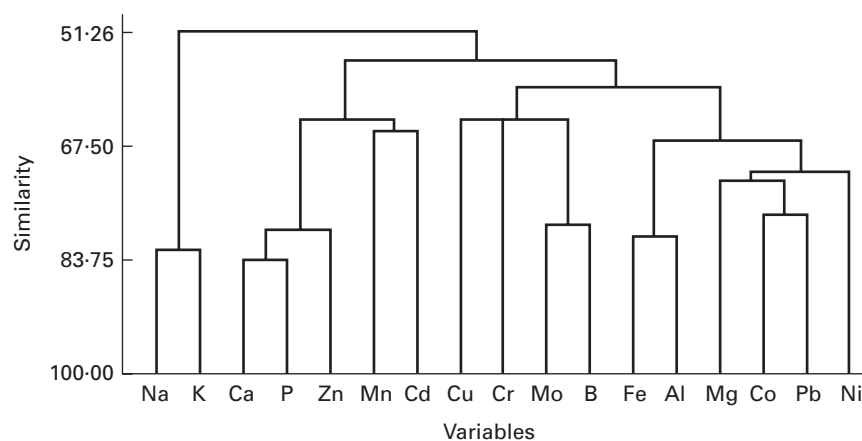
There is a negative effect of Cd in the small intestine on absorption of Fe, even at levels in the diet as low as 2.5 mg Cd/kg DM (for example, see Bremner, 1978). At high concentrations MT induce necrosis of the proximal intestinal absorptive cells, which affects uptake of minerals, such as Fe, for which the distal intestine cannot compensate (Valberg *et al.* 1977; Elsenhans *et al.* 1999). Pb is also commonly linked to Fe-deficiency-induced anaemia, although it is only rarely bound to MT. A reduction in haematopoiesis and inhibition of haem synthesis, with a decrease in  $\delta$ -aminolevulinic acid dehydrogenase, the enzyme that regulates haemoglobin production, has been observed in laboratory rodents. Pb poisoning of grazing mammals is most commonly acute following ingestion of Pb from discarded batteries, munitions or contaminated soil around areas where Pb was mined. It is not known whether livestock suffer anaemia following chronic Pb ingestion, such as near roadways.

As well as Fe, Pb directly interacts with Ca, P, Cu, Mn and Zn, all of which protect against Pb absorption. There are also likely to be further direct and secondary interactions, which are currently not well understood.

The similarities between metals in their absorption characteristics have been examined as a dendrogram (Fig. 2) for sheep given supplements of Cd and Zn (Chiy *et al.* 1998). Many of the elemental similarities can be predicted from the close correspondence in their atomic construction, e.g. Na and K, but other similarities derive from their competition for the anions, in particular sulphate.

#### *Mechanisms of absorption*

A range of potential sites for absorption of metals exist from the rumen to the small intestine, but in grazing ruminants all metals are subject to the overriding influences of the rumen microbial digestion. The extent of absorption is also dependent on the species of metal ingested, the age of the animal (young animals generally absorb a greater proportion of ingested metal than old animals; Kello & Kostial, 1977), other components of the feed (for review, see Rambeck,



**Fig. 2.** Dendrogram showing the similarity of elements in the mineral balance in sheep given supplements of cadmium and zinc. (From Chiy *et al.* 1998.)

1994), the pH of the medium (low pH dissociates ingested compounds) and the rate of passage through the gastrointestinal tract. Of these influences, age probably has the greatest effect, with young calves absorbing  $\leq 50\%$  of ingested Pb, compared with  $\leq 5\%$  by older cattle. Most metals are ingested in an organic form, typically bound to phytochelatin, which are absorbed to a greater extent than metals ingested in an inorganic form (Zachou *et al.* 1997). Chelating agents such as ascorbic acid readily form complexes with toxic metals, such as Cd, Cu and Hg, and protect against toxicity of these metals. An adequate protein status has the same effect on the absorption of Cd.

Ionophores, e.g. monensin, form lipid-soluble complexes with many metals, including Zn, which assists in their passage across membranes (Starnes *et al.* 1984; Snitsarev *et al.* 2001). They are often added to the diet of intensively-fed ruminants, but are only rarely offered to grazing animals.

Metal accumulation will only occur if homeostatic or homeorhetic mechanisms cannot maintain a constant body composition. The absorption of many essential metals is controlled by these mechanisms, e.g. Zn whose absorption can vary from  $< 10\%$  to  $> 80\%$  depending on the animal's status (Underwood & Suttle, 1999), but there is no reason why the mechanisms should have evolved for non-essential metals that were until recently rarely encountered in a sizeable quantity. Often these metals are characterised by low absorption rates, but they may utilise absorption mechanisms devised for other metals, e.g. Cd and Zn, Pb and Ca.

Cd is ingested either as Cd-MT or Cd-phytochelatin and may be partially digested in the gastrointestinal tract. The absorption of Cd takes place mainly in the proximal small intestine (Pigman *et al.* 1997), where it can damage the microvilli. Cd is initially absorbed by the intestinal mucosal cells and most of that absorbed eventually returns to the gastrointestinal tract following desquamation of the lumen cells, the rate of passage to the intestinal blood supply being extremely slow (Foulkes, 1984). In an experiment by Valberg *et al.* (1977) 300 g Cd/kg Cd intake was absorbed by the intestinal mucosa, but as little as 10 g/kg entered the body. Values for Cd absorption cited in the scientific

literature vary widely, from 30 g/kg to 200 g/kg (Doyle *et al.* 1974; Piscator, 1985; Fox, 1987), with a value of 50 g/kg being most commonly cited.

The uptake of Cd and Zn from the intestine is assisted by MT, which are low-molecular-weight cysteine-rich compounds present in moderate quantities in sheep (Henry *et al.* 1994). They compete with larger ligands, such as serum albumin (Foulkes & Voner, 1981) or those in fibre (Cherian, 1979), which render the metal unabsorbable, at least in single-stomached animals. Rumen bacteria can dissociate some large ligands, such as phytic acid that binds to Zn (Oberleas *et al.* 1966), and there is evidence for increased absorption of organically-bound Zn in ruminants (Spears, 1966). Dissociated Cd complexes with Mo and thiomolybdenates to form insoluble compounds (Smith & White, 1997) and bacterial MT, both of which have low absorption characteristics (Groten *et al.* 1992). Organically-bound Cd, chelated by the carboxyl and amino group of amino acids, is probably protected from rumen degradation in sheep (Lee *et al.* 1999). There is no clear evidence of increased absorption of other organically-bound heavy metals, e.g. Cu (Suttle, 1994), compared with inorganic forms, but in mice Turecki *et al.* (1998) found reduced absorption of Cd in yeast compared with CdCl.

The transport of Cd into the intestinal mucosal cells probably follows first-order kinetics, but may be assisted by low-molecular-weight ligands, in particular MT, although their presence is not obligatory for transport to take place (Foulkes, 1984). Saturation of the unassisted transport is probably reached at Cd concentrations of 0.01–1 mM (Koo *et al.* 1978; Foulkes, 1984). Many experiments have relied on measuring the Cd in target organs, principally the kidney, to estimate body retention. Estimates of the Cd-MT absorbed by the kidney vary from 41% (Cain & Griffiths, 1980) to 95% (Nordberg & Nordberg, 1975). The binding of Cd by MT exacerbates the toxic effects on the intestinal mucosal cells, particularly by disturbing mitochondrial function (Valberg *et al.* 1977).

Pb absorption is also affected by the species and age of animals, with some disagreement on the proportion of intake that is absorbed. As little as 1% was reported to be absorbed by sheep (Blaxter, 1950), but other estimates in young calves

are as high as 50 %. In short-term experiments absorption rates are often very high, as an extent of tissue saturation is reached, thereafter a steady-state is achieved, with much lower levels of retention following excretion in bile (Walker *et al.* 1996). As with many metals, proportionately more Pb is absorbed at high concentrations in feed as the sequestration of the Pb by chelating agents reaches saturation. There is no effective homeostatic protection against Pb absorption.

### Localisation of metal elements in the body

Localisation is focused on the body's mineral storage and detoxifying organs, usually the liver for short- to medium-term storage and detoxification, and the bones for long-term storage. The liver produces MT responsible for transporting Zn especially, but which will store considerable amounts of Cd.

Cd is present in the bloodstream as Cd–MT and Cd–albumin in plasma and Cd–MT in erythrocytes. Cd bound to albumin is removed from the bloodstream by the liver and bound to MT produced by the hepatocytes. During natural apoptosis of these cells or liver damage the Cd–MT is released into the bloodstream. Having a low molecular weight, Cd–MT is filtered by the kidney and re-absorbed in the proximal tubule. Here the complex is broken down by the lysosome system in the cells around the tubule lumen, and the Cd released will irreversibly damage tubular cells, causing interstitial fibrosis (Fowler, 1996), particularly when the detoxification system is overwhelmed. A similar condition has been described in human subjects (Fanconi syndrome; Marumo & Li, 1996). Mitochondrial activity is substantially reduced and the endoplasmic reticulum and nucleus can be damaged. Adverse effects on kidney function can be measured by the excretion of  $\beta$ 2-microglobulin. Cd is excreted in the urine (Kjellstrom & Nordberg, 1978), with a limited amount in bile and pancreatic juice (Prankel, 2002).

A meta-analysis of twenty-one experiments with sheep has recently been conducted which integrates previous findings in order to review existing legislation on permitted levels of Cd in animal feeds and organs (Prankel *et al.* 2001). Resulting predictions on Cd accumulation in sheep liver and kidneys are applicable to the broad set of exposure situations investigated in the individual studies. A random-effects model, weighted for inter- and intra-trial variability, found that the two major predictors of Cd concentration in liver and kidney were the duration of exposure to Cd and the Cd concentration in the feed. The presentation of organically-bound Cd, rather than inorganically-bound Cd, also affected accumulation. Current maximum-permitted levels of feed Cd were entered into the model to show the extent to which the model predicts that tissues will exceed fitness for human consumption (under current EU and UK legislation). Most Cd consumed by sheep is in the organic form (bound to plant protein), so the values derived by experiments using inorganically-bound Cd are less relevant. The maximum period of time for which sheep could be offered feed containing the legally-permitted maximum concentration of Cd before their kidney and liver exceeded maximum-permitted Cd concentrations was 130 and 175 d respectively. A sheep's lifespan can easily exceed 130 d

after weaning, and it is therefore not surprising that in national abattoir surveys sheep organs routinely exceed Cd limits. Potential measures to prevent increased risk to human health from dietary Cd of animal origin include prevention of the livers and kidneys of older sheep from contaminated regions entering the human food chain and strict implementation of current legislation. Reducing the animal's duration of exposure to high-Cd feed would be an expensive procedure. Lower limits on Cd concentration in sheep feed would enable the offal of older animals to be included, but would be difficult to impose as herbage that has been regularly fertilized with P or treated with sewage sludge already sometimes exceeds the legal limit. Routine removal of the liver and kidney of at least mature ewes from the human food chain is desirable in order to reduce human Cd intake.

In the laboratory rat the testis is one of the organs most affected by Cd, but Berry *et al.* (1999) were unable to show any adverse effects on semen quality of feeding rams 8 mg Cd/d for 60 d. This level would be typical of sheep grazing a polluted site. Cd also accumulates in bones, where it has been responsible for severe osteopaenia and fractures in human subjects (itai-itai disease in Japan). The level of Cd present in muscle, milk or other edible body fractions is so low that usually it cannot be detected by spectroscopy.

Pb is absorbed in the small intestine and transported in the erythrocytes, where it replaces Fe in several enzymes concerned with haemoglobin production. Pb has a half-life of about 30 d in blood, so it may be used in the diagnosis of chronic and acute toxicity, with a threshold for acute exposure of about 0.4  $\mu$ g/l. High levels may be recorded long after acute exposure during periods of bone mobilisation, e.g. during early lactation.

Pb is also sequestered by the liver, but unlike Cd little is bound to MT, and most Pb is concentrated into storage sites, or intranuclear inclusion bodies (Goyer & Wilson, 1975). It is ultimately stored in bones, particularly the long bones, although there are elevated concentrations in the kidney. The teeth and hair also contain elevated levels of Pb.

Secretion of Pb into milk is rare unless an acute dose is ingested, since there is an exponential relationship between Pb intake and output in milk. Pb levels in milk increase rapidly at blood concentrations of >0.2–0.3 mg Pb/kg (Oskarsson *et al.* 1992). Despite the protection of bovine milk from Pb contamination, it has sometimes contained more Pb than human milk (0.04 v. 0.01 mg Pb/l; Pinkerton *et al.* 1973). Cattle are likely to release bone Pb into milk at, and soon after, parturition (Galey *et al.* 1990).

A major risk to the human food chain is the accumulation of  $^{137}$ Cs in muscle tissue of ruminants grazing contaminated upland pastures (see pp. 269 and 270; Sansom, 1989; Beresford *et al.* 2000).

### Exposure and legal limits

Exposure is a function of the concentration of PTM in herbage and the duration of consumption. These two factors are not necessarily additive. A long exposure to a small dose of Cd, for example, would be unlikely to induce acute toxicity because of the ability of the liver to sequester Cd bound to MT. A short 'provocative' dose is likely to exceed the liver's

ability to sequester the metal and acute toxicity arises when the metal passes through the proximal tubule of the kidney.

The critical dose of Cd that will produce chronic toxicity in sheep is approximately 2.5 mg/kg body weight per d, with a dose >0.5 mg/kg body weight per d for 1 year likely to produce subclinical effects (Prankel, 2002). In the latter case it is recommended by Hapke & Abel (1977) that the liver and kidney are discarded in order to reduce Cd intake by human subjects. Accumulation in muscle only occurs after much higher doses (30 µg Cd/g feed), which are unlikely to occur even in the most polluted regions (Hapke & Abel, 1977).

The long half-life of Pb in the body, approximately one-third of the total lifespan, ensures that most absorbed Pb is accumulated rather than excreted. Maximum-permissible levels of Pb in soils have been established by the EU (Commission of the European Communities, 1991), and in some countries Codes of Good Practice reinforce measures to minimise contamination in soils (Ministry of Agriculture, Fisheries and Food, 1993). In animal feeds there is a legal limit set by the Feeding Stuffs Regulations in the UK for the Pb content of a complete diet feed (5 mg Pb/kg), with 10 mg Pb/kg allowed in raw material foods (Statutory Instrument, 1995). However, grass, lucerne (*Medicago sativa*) and clover (*Trifolium* spp.) meal may contain ≤40 mg Pb/kg, and phosphates may contain 30 mg Pb/kg, emphasising that some limits are set on the grounds of practicality rather than on objective criteria. A dose of 6 mg Pb/kg body weight per d fed for 60 d can be fatal to growing cattle (Hammond & Aronson, 1964), but adult cattle can tolerate up to twice this dose (Buck, 1970). The maximum-permissible level in the feed of cattle or sheep in the USA is 30 mg Pb/kg (National Research Council, 1980).

Many countries enforce limits of heavy metals in air, but these levels are mainly relevant to those working indoors in the metal industries, rather than animals (or human subjects) outside. Threshold limit values are defined for short (15 min) and long-term human exposure, and a limit that cannot be breached for any length of time. World Health Organization (1900) guidelines for Cd in air of 0.5–1 g/l are stricter than the Commission of the European Communities (2002) legal limit of 2 µg/l.

Concentrations of radiocaesium in upland pastures have declined progressively in the 22 years since the Chernobyl accident, as radioactivity decayed and the element moved down the soil profile and out of the root layer of the vegetation. Thus, the number of sheep in the UK under restricted movement because their muscle tissue contains more than the intervention concentration of 1000 Bq <sup>137</sup>Cs/kg fresh weight has decreased from 2 × 10<sup>6</sup> in 1986 to 200 000 in 2002 (UK Department for the Environment, Food and Rural Affairs, personal communication).

Ploughing of grassland, preceded by the incorporation of zeolite, has been found to decrease the level of radioactivity in subsequent crops by five- to sevenfold (Savchenko, 1995). However, in some unploughable upland peat soils the decrease in the concentration of Cs in herbage has been relatively slow and movement restrictions persist (UK Department for the Environment, Food and Rural Affairs, personal communication). Sheep grazing these areas are

monitored annually in May or June. If slaughter is desired, contaminated animals may be moved to uncontaminated lowland pastures. They are monitored again 3 months later and, if the concentration of <sup>137</sup>Cs in muscle tissue is below the current intervention limit, they may enter the food chain, subject to further monitoring at abattoirs.

Currently, in the UK the intervention concentration of <sup>137</sup>Cs in sheep muscle tissue is under review (UK Department for the Environment, Food and Rural Affairs, personal communication). The movement of sheep from affected areas to slaughter is no longer restricted in Northern Ireland (UK Department for the Environment, Food and Rural Affairs, personal communication).

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