ELSEVIER

The Science of the Total Environment 187 (1996) 167–183

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

Risk assessment of the threat of secondary poisoning by metals to predators of earthworms in the vicinity of a primary smelting works

David J. Spurgeon*, Stephen P. Hopkin

Ecotoxicology Research Group, School of Animal and Microbial Sciences, University of Reading, PO Box 228, Reading, RG6 6AJ, UK

Received 2 January 1996; accepted 22 March 1996

Abstract

An assessment was performed of the risk to predators of cadmium, copper, lead and zinc in the food chain soil — earthworm — vertebrate. To estimate risk, the scheme proposed by Romijn et al. (1993, 1994) was used. The procedure compares mean bioconcentration factors (BCFs) (concentration in earthworms divided by concentration in soil) for the pollutants in prey with a predator sensitivity value. This allows maximum permissible concentrations (MPCs) to be determined for each metal. For the study, BCFs of the four metals were recorded in the tissues of earthworms collected from a number of sites located around a primary smelting works where soils are heavily contaminated with cadmium, copper, lead and zinc. Predator sensitivity was estimated using literature toxicity data as a hazardous concentration for 5% of species (HC5) by the technique of Aldenberg and Slob (1993). Comparison of field BCFs and literature $HC5_{predator}$ values gave MPCs for metals in soil of 0.017 μg Cd g^{-1}, 18.9 μg Cu g^{-1}, 30.4 μg Pb g^{-1} and 36.1 μg Zn g^{-1} which would theoretically protect 95% of predators from poisoning. A comparison of the calculated MPCs for cadmium, copper, lead and zinc with the concentrations of these metal found in a range of field soils indicates that the MPCs are almost always exceeded, even in uncontaminated agricultural soils. Possible causes for the over-assessment of the risk of secondary poisoning are discussed and an alternative strategy based on the use of critical target organ levels is outlined.

Keywords: BCF; HC5; Maximum permissible concentrations; Food-chain; Kidney

1. Introduction

Secondary poisoning occurs when predators are exposed to physiologically damaging concentrations of pollutants via their food. Since earthworms form an important component of the diet of many species, they represent a pathway for the movement of pollutants through food chains. Indeed, cases of secondary poisoning due to the consumption of contaminated worms, have been found for DDT, Dieldrin, and various organophosphates (Cooke et al., 1992). Of the risk

* Corresponding author.

0048-9697/96/$15.00
PII S0048-9697(96)05132-7

assessment procedures currently in use, or under development, only the scheme developed by the German Federal Environmental and State Working group considers secondary poisoning (Van Straalen et al., 1994). Comprehensive environmental protection demands that predators, as well as primary consumers, should be protected. Thus, there is a requirement to extend the scope of current risk assessment methodology to assess the probability of ingestion of critical doses by predators.

The aims of the current paper are to determine if the accumulation of metals by earthworms may endanger predators and to look at the ways in which this risk can be assessed. To carry out a fully integrated assessment of the threat posed for predators by accumulated pollutants, five factors should be considered: (1) the concentrations of pollutants in worms; (2) the pollutant doses causing effects on predators; (3) the effects of any behavioural changes of earthworms on predator diets; (4) the proportion of worms in the diet; (5) selection against contaminated worms due to taste (Cooke et al., 1992). No current risk assessment process considers all the above parameters. However, simpler risk assessment procedures do exist. Romijn et al. (1993, 1994) proposed a procedure that considers the relationship between prey and soil contaminant levels (expressed as a bioconcentration factor for prey/soil concentrations) and the metal doses causing sublethal effects on predators using the algorithm:

$$MPC = HC5_{predator}/BCF \quad (1)$$

where:

MPC, maximum permissible risk concentration for the pollutant in soil; $HC5_{predator}$, the hazardous concentration for 5% of predator species (Aldenberg and Slob, 1993); BCF, mean bioconcentration factor for the pollutant in the tissues of prey animals (i.e. conc. in animal/conc. in soil or diet).

Romijn et al. (1993a,b) determined MPCs for a range of chemicals, in both aquatic and terrestrial food chains. The calculated MPCs were compared to HC5s for the direct effects of the pollutants on primary consumers. From these comparisons, it was concluded that there were a number of pollutants for which predators were at risk from lower soil concentrations than were primary consumers. In particular it was found that there may be secondary risks for methyl mercury and PCB153 in aquatic systems and cadmium and methyl mercury in terrestrial food chains. Shore and Douben (1994) also concluded that there is potential risk to some predators due to secondary poisoning resulting from metal accumulation in prey species. They noted that for some species of terrestrial small mammals exposed to increased cadmium from food, the levels of metal in the kidney exceeded concentrations found to cause renal dysfunction and histopathological changes in laboratory exposed animals (Samarawickrama, 1979; Chmielnicka et al., 1989).

The work of Romijn et al. (1993, 1994) and Shore and Douben (1994) indicate that metals may have important secondary effects on vertebrates. Despite this, there is currently a lack of knowledge of the potential effects of metals such as copper, lead and zinc on vertebrates and some uncertainty as to the extent of these risks to populations at contaminated field sites. Thus in the current paper, the threat of accumulated cadmium, copper, lead and zinc in earthworms collected from sites in the region around a smelting works is compared to vertebrate toxicity data from the literature to determine the risk posed by aerial deposition from the smelter for predators consuming contaminated prey.

2. Materials and methods

To judge the risk of accumulated metals to predators, it was decided to focus on the food chain: soil — earthworms — mammalian/avian predators. For the assessment, the scheme of Romijn et al. (1994) was used. Thus, it was necessary to obtain data detailing the relationships between soil and earthworm metal levels and the metal doses that may cause sub-lethal effects on predators.

To resolve the relationship between worm and soil metal levels and to determine the mean BCF values required for the risk calculation, metal levels were measured in earthworms and soils collected during a survey of earthworm populations conducted at Avonmouth in south-west England. This area is known to be subject to high aerial inputs of metals from a primary lead-zinc-cadmium smelting works located in the region, with the result that there is a gradient of increasing cadmium, copper, lead and zinc concentrations with proximity to the factory (Coy, 1984; Martin and Bullock, 1994; Vale and Harrison, 1994).

A total of 22 sites situated over a range of distances from the smelter were visited during the study. At each site, four 0.25 × 0.25 m quadrats were dug. All earthworms were sorted from the soil, identified to species, starved for 72 h to remove any soil present in the gut and stored in plastic tubes at −20°C. For metal analysis, whole worms were digested in nitric acid using the method described by Hopkin (1989) and were analysed for cadmium, copper, lead and zinc by flame (Varian Spectra AA-30) atomic absorption spectrophotometry. To determine BCFs, it was also necessary to analyse soils from the sites at which the worms were sampled. Thus, the cadmium, copper lead and zinc contents of nitric acid digests for all soils were determined. During both soil and animal analyses, standard reference materials (tomato leaf and bovine liver from the National Bureau of Standards, Washington, lobster hepatopancreas from the National Research Council, Canada, and calcareous loam soil from the Community Bureau of Reference, Brussels) were used as recommended by Hopkin (1989). In all cases values obtained for these materials were within 10% of certified values.

In addition to the calculation of BCFs for earthworms at each site and a mean value for each metal, soil-worm metal relations were also plotted by linear regression of $\log_{10}$ transformed values against $\log_{10}$ soil metal concentration. For all four metals, log transformations were applied to normalise the data, since raw data are positively skewed (Morgan and Morgan, 1988). The 95% confidence intervals (CI) for the regression were calculated based on the prediction intervals for the mean soil metal concentration, thus giving straight line CIs. The regression parameters for individual species were calculated using values from sites at which at least three individuals were collected.

The pH and organic matter content of soils at the field sites used in this study have already been measured as part of a previous study conducted by Spurgeon and Hopkin (in press). The soils in the Avonmouth area are predominantly clay loams with a neutral pH and a high organic matter content. The pH of surface soils at the 22 sites ranged from 7.32 at Site 21 (farthest from the smelter) to 5.56 at Site 2, although this was the only site in the region with a pH below 6.5. The lower pH at Site 2 (the closest site to the smelter) is probably the result of acid deposition from the sulphuric acid plant that adjoins the factory (Martin and Bullock, 1994). Site soils generally had a high organic matter content with loss on ignition of 15–29.9%. No relationship was found between the percentage organic matter and distance from the smelter.

Direct measurements of the toxicity of metals to predators, were not feasible, due to legal difficulties, expense and long term nature of such work. Therefore, for this study, predator sensitivity was assessed by collecting toxicity values from the literature. A preliminary review of the toxicity data available for the four metals, showed that results for earthworm predators are rarely available. Consequently, as in the study of Romijn et al. (1993, 1994), data for all mammal and bird species were considered. This approach may pose problems for the risk assessment, since variations within the tested species may not represent those for earthworm predators. However, the use of these additional data was considered valid, since it would increase the reliability of the $HC5_{predator}$ as the uncertainty factor applied would be reduced (Van Straalen, 1993).

To calculate $HC5_{predator}$ values for each metal, LC_{50}s and mortality, growth and reproduction no observed effect concentrations (NOECs) were used. Reproduction values were taken from studies on spermatogenesis, fertility, pregnancy rate, egg fertility, hatchability and chick survival, as well as those measuring numbers of eggs and young. All values are given based on the concentration of metal in the supplied food. For the selection of suitable values, the quality control procedures suggested by Romijn et al. (1994) were used. If for a given test, a parameter was reduced by more than 20% at the lowest dose tested, the NOEC was calculated using a safety factor of two on that dose. For studies in which no effect was found at the highest test concentrations, this value was used as a NOEC, although values calculated in this way were rejected if suitable data for the same species were available. For short-term studies (< 1 month), a safety factor of 10 was applied to allow for uncertainties in the reliability of the NOEC. A mean NOEC value was calculated if more than one NOEC was found for any parameter for a given species.

For the determination of the $HC5_{predator}$ values, the technique described by Aldenberg and Slob (1993) was applied when six or more NOECs were available. This technique permits 50% and 95% CIs for the $HC5_{predator}$ to be calculated. If there were insufficient NOEC data to apply the distribution based model, the $HC5_{predator}$ value was calculated using the safety factor scheme proposed by the US EPA (Stephan et al., 1985). If three or more LC_{50} values were found, the factor used was 100. If NOEC data were available, a safety factor of 10 was applied to the lowest value. For data calculated in this way, 50% and 95% CIs could not be assessed. After calculation of mean BCFs and the $HC5_{predator}$, MPCs for each metal were calculated using Eq. 1. For the assessment of MPCs, the 50% CI was used when the HC5 was calculated using the distribution model as recommended by Romijn et al. (1993, 1994).

Romijn et al. (1994) outlined some considerations for the application of the secondary poisoning algorithm in terrestrial ecosystems. Because pollutant accumulation in earthworms is a two stage process dependent on soil and earthworm properties, BCFs can vary considerably for worms collected from different soils. In particular, changes in pH and percentage organic matter content are important in determining the body burdens of earthworms at a given soil metal level. Since the current paper describes the results of a site specific study, it is important to note that the MPC generated are not general values, but relate to the soil condition prevalent at Avonmouth.

3. Results

3.1. Calculation of earthworm BCFs

Soil metal levels were highest at sites close to the smelter (Sites 1 and 4), and declined exponentially with distance from the factory [see Martin and Bullock (1994), Hopkin (1989) and Hopkin et al. (1986) for full details of the spatial distribution of metals at Avonmouth]. Earthworm population sampling indicated that all worms were absent from six sites close to the factory at which metal concentrations were high (Spurgeon, in press). Consequently, for this study mean earthworm metal concentrations for regression calculations and BCF determination could be assessed for a total of 16 sites (Table 1). For the calculation of regression parameters for individual species, sufficient worms were available to calculate mean metal contents for *Lumbricus terrestris* and *Lumbricus rubellus* at 10 sites, for *Lumbricus castaneus* at eight sites, for *Allolobophora chlorotica* at 11 sites, for *Aporrectodea rosea* at nine sites and for *Aporrectodea caliginosa* at six sites.

For all four metals, the relationships between log transformed soil and worm concentrations were linear (Fig. 1a–d). For cadmium, worm metal levels were above soil values at all sites visited, although BCFs were lower at sites close to the smelter (Fig. 1a). For example, at Site 22, the cadmium BCF was 115, but was reduced to 2.6 at Site 6 (the most contaminated site at which worms were found) (Table 1). Worm tissue concentrations of copper, lead and zinc were also higher at the most contaminated sites for (Fig. 1b–d). At low zinc and copper levels, BCFs above one were found (Table 1); however, as soil concentrations increased to levels above 135 μg Cu g^{-1} and 1000 μg Zn g^{-1}, earthworm copper and zinc BCFs decreased to below one (Fig. 1b,d). A similar relationship between soil and worm zinc was found by Morgan and Morgan (1988), although the values above which BCFs fell below unity were <900 μg Zn g^{-1} and <550 μg Zn g^{-1} for *Lumbricus rubellus* and *Dendrobaena rubidus*, respectively. Earthworm lead concentrations were below soil values for all sites except for Site 16 where the worm level marginally exceeded that in soil (Fig. 1c, Table 1). This agrees with the results of Morgan and Morgan (1988).

Comparisons of the regression slope parameters for each metal indicated that the highest rise in earthworm burden in proportion to soil concentration was for lead (Fig. 1c). Worm cadmium and

Table 1
Bioconcentration factor (BCFs) for earthworms collected from 16 sites in the Avonmouth region

	Distance from smelter (km)	Cadmium worm/soil BCF	Copper worm/soil BCF	Lead worm/soil BCF	Zinc worm/soil BCF
Site 6	1.3	2.59	0.28	0.26	0.40
Site 7	1.3	6.11	0.26	0.26	0.58
Site 9	1.7	3.81	0.18	0.08	0.33
Site 10	1.8	6.53	0.31	0.15	0.44
Site 11	1.8	5.99	0.67	0.16	1.43
Site 12	2.7	4.86	0.23	0.08	0.63
Site 13	2.9	23	0.56	0.06	1.16
Site 14	3.1	10.2	0.49	0.24	1.02
Site 15	4.6	11.4	0.42	0.24	0.60
Site 16	5.1	22.8	2.11	1.25	3.69
Site 17	5.2	64.3	0.51	0.19	0.81
Site 18	5.8	13.2	0.36	0.18	2.18
Site 19	6.5	19.8	0.89	0.22	1.99
Site 20	8.7	9.26	0.25	0.12	0.88
Site 21	9.7	32.6	1.24	0.25	1.83
Site 22	110	115	2.40	0.45	13.3
Mean BCF		23.43	0.74	0.28	2.08

Sites numbers correspond to those used in an earthworm population survey conducted in the region by Spurgeon (in press). All BCFs are calculated by dividing the earthworm metal concentration for all species (expressed in $\mu g\ g^{-1}$) by the topsoil (0- to 2-cm layer) metal values (nitric acid digests, dry wt. basis). The mean worm/soil BCF used as input for secondary risk assessment is also given.

copper levels increased less strongly as soil metal increased (Fig. 1a,b), while worm zinc was only marginally affected by a rise in soil metal level (Fig. 1d). The fact that regression slopes for all metals are below one indicates that there is an inverse relationship between BCFs for earthworms and the concentration of metal in soils. BCFs are lowest at the most contaminated sites (Table 1). Since toxic effects on predators would be expected only at the sites where metals levels are highest and thus BCFs lowest, the use of the mean BCF value may result in a slight over-assessment of risk. For example the mean cadmium BCF of 23.43 exceeds the BCFs found at Sites 6–12 by at least a factor of three (Table 1).

If log worm metal concentrations are plotted against log soil values for each species, it is clear that the trend for burdens to increase with rising soil level is consistent for all worms (Fig. 2a–d). No clear evidence indicating species dependent accumulation was found, although at the most contaminated sites, *Lumbricus terrestris* frequently had the highest metal concentrations, while those for *Aporrectodea rosea* and *Aporrectodea caliginosa* were often low (Fig. 2a–d). However, during the population survey, it was noted that these two species were frequently absent from sites close to the smelter where *Lumbricus terrestris*, *Lumbricus rubellus* and *Lumbricus castaneus* persisted (Spurgeon, in press). Thus, the absence of these species at the most contaminated sites may affect the form of the regression.

As no clear evidence for species dependent regression was found, it was decided to conduct the assessment of secondary poisoning risk by pooling the BCF data. Calculation of mean BCFs from values for each of the 16 sites from which worms were collected, gave the highest value for cadmium, with a mean value of 23.4. The mean BCF for zinc also exceeded unity and was 2.08, while the values for copper and lead were both below one being 0.74 and 0.28, respectively (Table 1). These values were used as input to the model for calculating secondary risk MPCs.

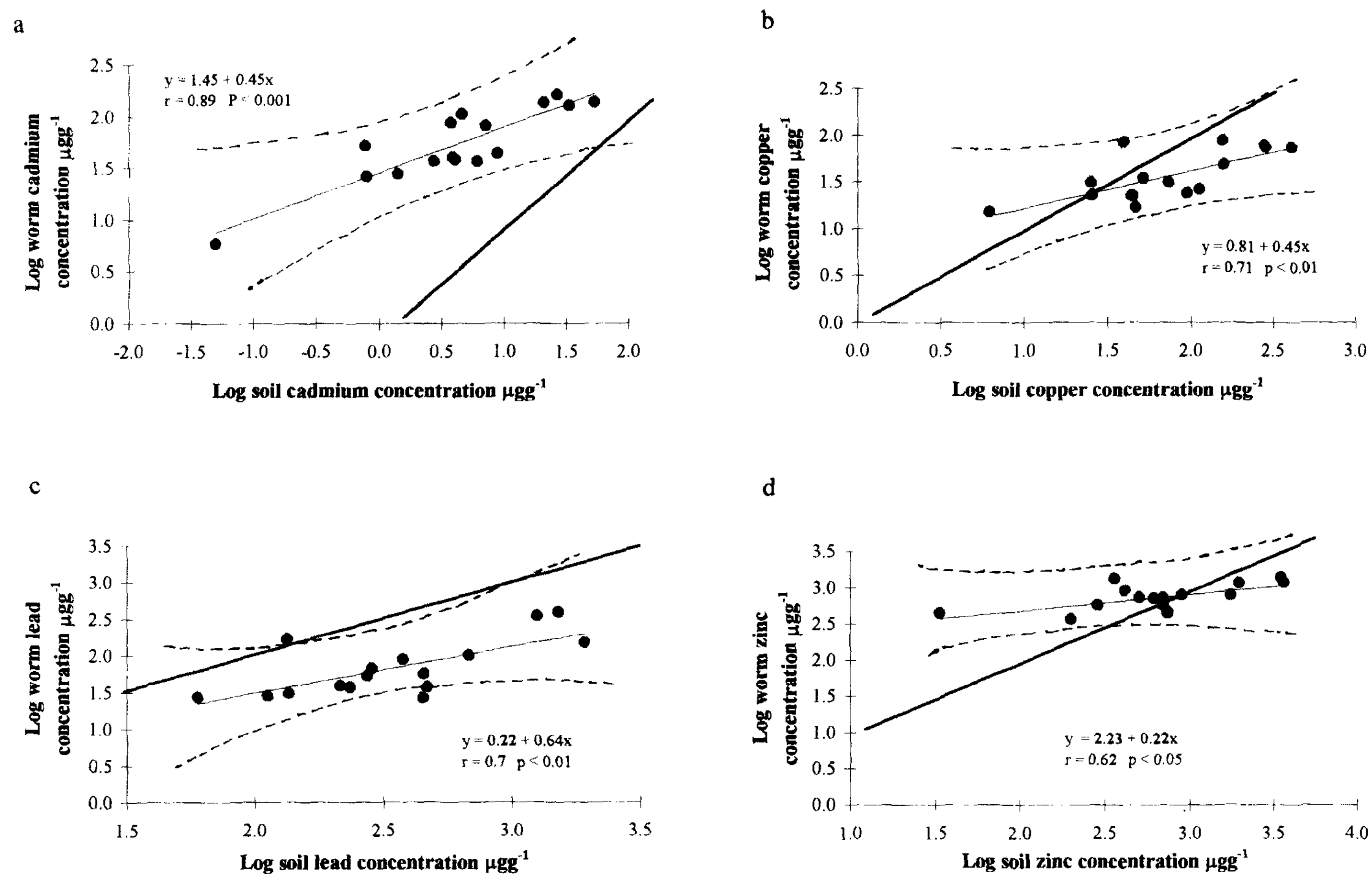

Fig. 1a,b. Relationships between $\log_{10}$ worm and $\log_{10}$ soil cadmium (a), copper (b), lead (c) and zinc (d) concentrations for earthworms collected from 16 sites at Avonmouth. Worm values are derived from the mean content for all animals collected from four 0.25 × 0.25 m quadrats. Dashed lines indicate 95% confidence intervals for the regression. The solid dark line represents a BCF of one (i.e. worm metal level = soil metal level).

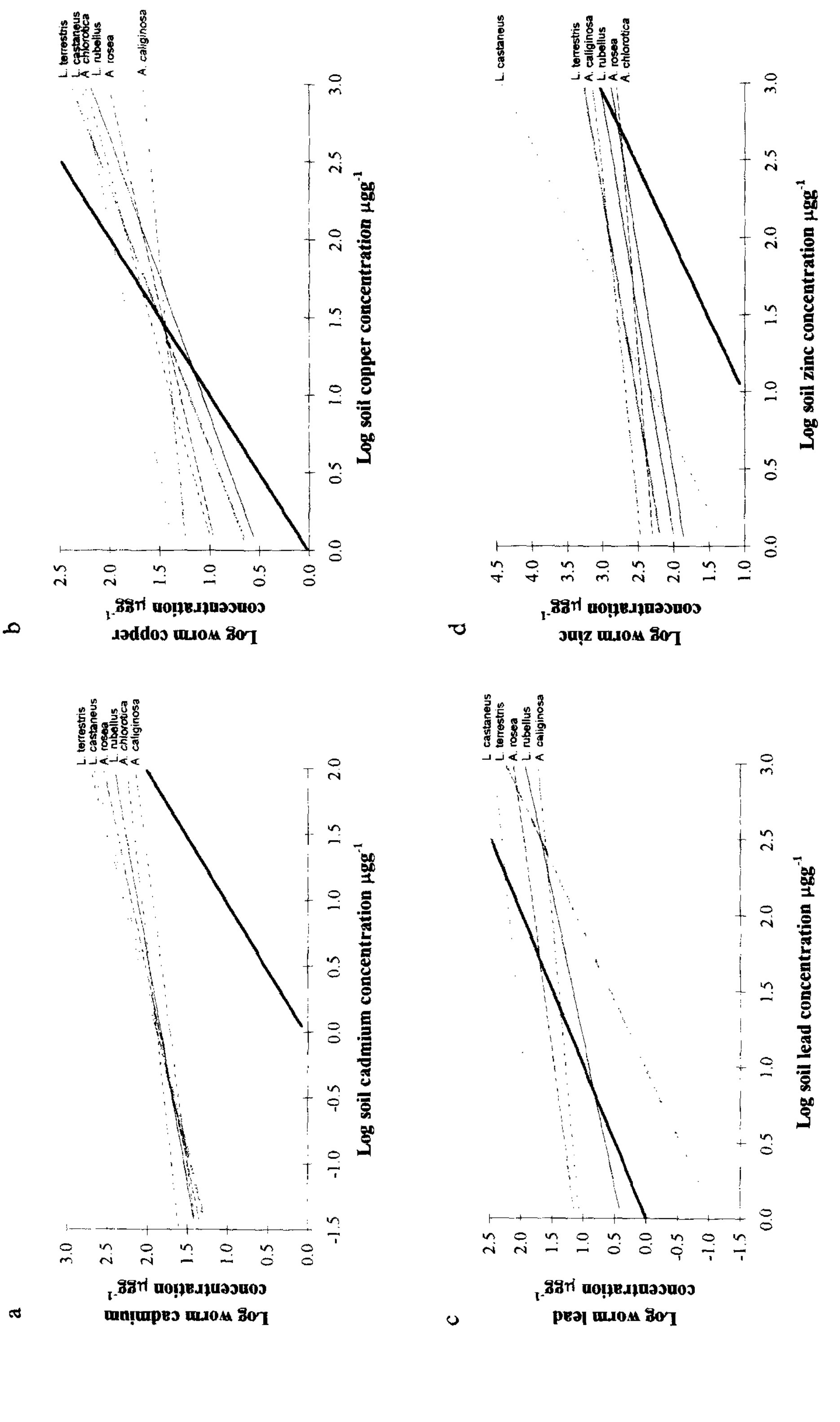

Fig. 2a,b. Regression lines of the relationship between log worm and soil cadmium (a), copper (b), lead (c) and zinc (d) for six species of earthworms collected from 16 sites at Avonmouth (except for lead in *A. chlorotica* where samples were lost). The solid dark line represents a BCF of one.

3.2. Estimation of $HC5_{predator}$ values and the calculation of MPCs

Data collected for the chronic toxicity of cadmium to birds and mammal are given in Table 2. A total of 10 NOEC values were found. From these toxicity results, 50% and 95% CIs for the $HC5_{predator}$ value could be calculated for birds and mammals as well as the combined data (Table 3). The $HC5_{predator}$ values calculated for cadmium indicate that birds may be more sensitive than mammals, although the lower value is also due in part to the larger safety factor required for bird calculation due to the lower number of available values. For the calculation of MPCs, $HC5_{predator}$ values from the combined data were used. Calculation of MPCs from $HC5_{predator}$ values by comparison with mean BCFs indicated that the value for 50% certainty of protection of 95% of species was 0.017 μg Cd g^{-1} in the diet.

From the data collected, it is clear that copper is less toxic for predators than cadmium (Table 4). This is not surprising, since copper is an essential element in vertebrate nutrition (Scheinberg, 1991) and as such, is added to the diet of some species (e.g. domestic swine, poultry) to increase growth rates in copper deficient areas (Cavanagh and Judson, 1994). The quantity of toxicity data available for copper was lower than for cadmium with only four NOEC values found. Insufficient data were therefore available to calculate an HC5 from the distribution model of Aldenberg and Slob (1993) and the EPA safety factors technique of Stephan et al. (1985) had to be applied. The $HC5_{predator}$ value calculated in this way for the combined bird and mammal data was 14 μg Cu g^{-1}. Using this value and the mean worm/soil BCF, an MPC value for the protection of predators from copper intoxication of 18.9 μg Cu g^{-1} was calculated.

Sufficient data were available for lead to calculate 50% and 95% CI $HC5_{predator}$ values, using the distribution based model (Table 5). The collected data indicated that the toxicity of lead to predators is lower than for both cadmium and

Table 2
Oral and dietary toxicity values for birds and mammals exposed to increased concentrations of cadmium in laboratory toxicity tests (Romijn et al., 1993b)

Parameter	Species	Reported value	Converted value	Reference
Birds				
Growth NOEC	*Meleagris gallopava*	2	0.2	Supplee (1961)
Reproduction NOEC	*Anas platyrhynchos*	1.6	1.6	White et al. (1978)
Reproduction NOEC	*Gallus domesticus*	12	12	Leach et al. (1979)
Growth NOEC	*Coturnix c. japonica*	75	38	Richardson et al. (1974)
Mammals				
Growth NOEC	*Macaca mulatta*		16.5	Mean value
		3	3	Nomiyama et al. (1987)
		30	30	Masaoka et al. (1994)
Reproduction NOEC	*Mus musculus*	7	7	Zenick et al. (1982)
Growth NOEC	*Ovis amon aries*	15	15	Doyle et al. (1974)
Growth NOEC	*Rattus norvegicus*		16.7	Mean value
		10	10	Loeser (1980)
		25	25	Prigge (1978)
		30	15	Groten et al. (1991)
Growth NOEC	*Bos primigenius taurus*	40	40	Powell et al. (1964)
Growth NOEC	*Oryctolargus cuniculus*	1	1	Peter et al. (1995)
Growth NOEC	*Sus scrofa domesticus*		45	Mean value
		40	40	Krajnc et al. (1986)
		50	50	Cousins et al. (1973)

Table 3
HC5 values with 50% and 95% confidence intervals where calculable from the bird, mammal and combined toxicity data given in Tables 2, 4, 5 and 6

	Data used for calculation	Cadmium	Copper	Lead	Zinc
HC5 50% confidence interval	Birds	0.04	—	1.7	—
	Mammals	1.29	—	197	—
	Combined	0.4	—	8.5	—
HC5 95% confidence interval	Birds	0.00001	—	0.006	—
	Mammals	0.13	—	10.27	—
	Combined	0.05	—	0.45	—
HC5 safety factor calculated	Birds	—	14	—	—
	Mammals	—	24.2	—	75
	Combined	—	14	—	75
Mean soil/worm BCF	All species	23.4	0.74	0.28	2.08
MPC soil concentration ($\mu g\ g^{-1}$)	BCF/HC5	0.017	18.9	30.4	36.1

The HC5s have been calculated from the method of Aldenberg and Slob (1993) (both 50 and 95% confidence values given) when five or more NOEC vales were available, and the safety factor of Stephan et al. (1985) for less than five values. Mean worm/soil BCFs are calculated for worms collected from a range of sites in the Avonmouth region. MPCs are calculated using the algorithm proposed by Romijn et al. (1993b) given in Eq. 1.

copper, although a number of cases of accidental lead poisoning of livestock exposed to lead based paints are recorded in the literature (Clayton and Clayton, 1981; Ewers and Schlipköter, 1991). From the values collected, combined bird and mammal 50% and 95% CI $HC5_{predator}$ values of 8.5 and 0.45 μg Pb g^{-1} were calculated. The 50% CI for the $HC5_{predator}$ was compared to the soil-

Table 4
Oral and dietary toxicity values for birds and mammals exposed to increased concentrations of copper in laboratory toxicity tests

Parameter	Species	Reported value	Converted value	Reference
Birds				
Growth NOEC	*Gallus domesticus*		140	Mean value
		400	40	Funk and Baker (1991)
		480	240	Leach et al. (1990)
Mammals				
Mortality LD_{50}	*Rattus norvegicus*		242	Mean value
		66	66	Clayton and Clayton (1981)
		417	417	Clayton and Clayton (1981)
		233	233	Clayton and Clayton (1981)
Growth NOEC	*Mus muscullus*	4000	4000	Herbert et al. (1993)
Growth NOEC	*Rattus norvegicus*		1025	Mean value
		2000	2000	Herbert et al. (1993)
		50	50	Clayton and Clayton (1981)

Table 5
Oral and dietary toxicity values for birds and mammals exposed to increased concentrations of lead in laboratory toxicity tests

Parameter	Species	Reported value	Converted value	Reference
Birds				
Reproduction NOEC	*Anas platyrhynchos*	100	100	Haegele et al. (1974)
Reproduction NOEC	*Coturnix coturnix japonica*	100	10	Morgan et al. (1975)
Reproduction NOEC	*Colinus virginianus*	1500	1500	Damron and Wilson (1975)
Growth NOEC	*Falco sparveius*	125	12.5	Pattee (1984)
Growth NOEC	*Gallus domesticus*		388	Mean value
		100	100	Damron et al. (1969)
		1200	1200	Wittman et al. (1994)
		500	250	Donaldson and McGowan (1989)
		1	1	Bakalli et al. (1995)
Mammals				
Reproduction NOEC	*Rattus norvegicus*		893	Mean value
		670	670	Clayton and Clayton (1981)
		1340	1340	Azar et al. (1972)
		1340	134	Leonard et al. (1972)
Reproduction NOEC	*Cavia porcellus*		2500	Mean value
		2500	2500	Jacquet et al. (1976)
		2500	2500	Jacquet et al. (1976)
Reproduction NOEC	*Mus musculus*	6670	6670	Leonard et al. (1972)
Growth NOEC	*Mus musculus*	3340	334	Jacquet and Gerber (1974)
Growth NOEC	*Rattus norvegicus*	2000	2000	Liao et al. (1995)

worm mean lead BCF to give MPCs of 30.4 μg Pb g^{-1}.

Data collected from the literature suggest that zinc toxicity is intermediate between that of copper and lead (Table 6), although irritant effects on the alimentary tract may occur on ingestion of some zinc salts (Ohnesorge and Wilhelm, 1991). As for copper, zinc is an essential element in vertebrate nutrition and thus not perceived as a major threat to vertebrates (Ohnesorge and Wilhelm, 1991), although zinc toxicosis at high doses has been observed in animals ingesting zinc based metal artifacts, particularly coins. Insufficient NOEC data were available for the calculation of a distribution HC5. Consequently the EPA safety factor technique was used to calculate an $HC5_{predator}$ of 75

Table 6
Oral and dietary toxicity values for birds and mammals exposed to increased concentrations of zinc in laboratory toxicity tests

Parameter	Species	Reported value	Converted value	Reference
Mammals				
Mortality LD_{50}	*Cavia porcellus*	730	73	Clayton and Clayton (1981)
Mortality NOEC	*Felix catus*	780	780	Clayton and Clayton (1981)
Reproduction NOEC	*Rattus norvegicus*		750	Mean value
		1000	1000	Clayton and Clayton (1981)
		5000	500	Ketchenson et al. (1969)
Reproduction	*Ovis amon aries*	750	750	Campbell and Mills (1979)
Growth NOEC	*Rattus norvegicus*	5000	5000	Clayton and Clayton (1981)

μg Zn g^{-1}. This value was used to generate an MPC of 36.1 μg Zn g^{-1}. A summary of BCF and HC5 data used for the calculation of secondary poisoning MPC for the four studied metals is given in Table 3.

4. Discussion

4.1. BCFs for metals in worms

The highest Biological Concentration Factors (BCFs) for cadmium in worms were found at the least contaminated sites. BCFs fell as soil metal levels increased (Fig. 1a), although they were always above one. Zinc BCFs were above one at low soil concentrations (Fig. 1b, Table 1), but decreased below unity at higher soil levels (Fig. 1d). A similar accumulation pattern was also found for copper, although the concentrations at which BCFs decreased below one were lower (Fig. 1b). Lead was usually assimilated at levels below those present in soil (Fig. 1c). Calculation of worm/soil BCF gave highest values for cadmium (23.4), followed by zinc (2.08), copper (0.74) and finally lead (0.28). These BCF values are in agreement with previous work comparing earthworm burdens with soil metal values across a range of sites (Ma, 1982; Beyer et al., 1982; Ireland, 1983; Morgan and Morgan, 1988; Bengtsson and Tranvik, 1989; Beyer and Stafford, 1993).

Janssen et al. (1991) investigated the accumulation and excretion patterns for seven metals in terrestrial arthropods. Results indicated that excretion rates depended on the metal to which the animals were exposed. Essential elements such as copper and zinc were generally found to be regulated i.e. levels did not increase at high soil concentrations, whereas for non-essential metals such as cadmium and lead, body burden was more dependent on external metal concentration (Janssen and Horgervorst, 1993). The results of our study indicate that these patterns of accumulation also apply for earthworms. Regression slopes for copper and zinc are below those for cadmium and lead (Fig. 1a–d), suggesting that there is stronger regulation of these metals (Morgan and Morgan, 1988).

Differences in the physiological mechanisms for the accumulation and elimination of essential and non-essential elements have been demonstrated by Van Gestel et al. (1993). Earthworms were exposed to soils contaminated with cadmium and zinc (as the chloride salt) for 3 weeks. Both metals were assimilated at the highest concentrations. However, after a 3-week elimination period in clean soil, zinc concentrations in the worms were reduced to levels similar to those in unexposed worms, while cadmium remained high. Thus, the essential element zinc was rapidly eliminated while cadmium was retained.

Clearly-defined differences in species-specific assimilation were not found for any of the metals studied (Fig. 2a–d). This finding is in contrast to the studies of Morgan and Morgan (1988) and Morgan and Morris (1982) who found consistent species-specific variations in the metal burdens of *Lumbricus rubellus* and *Dendrobaena rubidus* at sites contaminated by mine spoil. In these studies higher zinc and cadmium and lower lead concentrations were found for *Lumbricus rubellus*. Differences in metal concentrations between earthworm species have also been found by Ash and Lee (1980), Morgan et al. (1986) and Wright and Stringer (1980). The absence of large inter-specific differences for metal concentrations in the current study, indicate that it is not necessary to use species specific BCFs for assessing secondary poisoning risk for predators of earthworms at Avonmouth.

As an input for risk assessment, the use of BCFs measured directly from worms collected in the field allows the exposure to metals in the diet of predators at Avonmouth to be estimated with some confidence. However it is important to note that the values calculated in this study cannot always be extrapolated directly to sites contaminated by metal derived from smelters and other sources. Earthworm BCFs for metals are dependent on soil properties (Bengtsson and Tranvik, 1989; Romijn et al., 1994). In particular, pH and the organic matter content of soil can have an important influence on the availability and hence the BCFs for metals in earthworms (Ma, 1982; Morgan and Morgan, 1988; Spurgeon and Hopkin, 1996). BCFs tend to be highest in soils with a low pH and low organic matter content (Van Gestel, 1992).

The use of the BCF from worms at Avonmouth for proposing a general MPC will tend to underestimate exposure for contaminated sites with a low pH and organic matter content, since soils at Avonmouth are close to neutral pH (5.56–7.32) and have a high percentage loss on ignition (15–29.9%). The neutral pH and high organic matter content of Avonmouth soils result in a relatively low availability of metals in the region. Thus, despite the high concentrations of metals present at the study sites closest to the smelter (312 μg Cd g^{-1}, 2610 μg Cu g^{-1}, 15 600 μg Pb g^{-1} and 32 900 μg Zn g^{-1} at Site 1), the concentrations of these elements present in the soluble fraction are relatively low. For example, of the zinc present in Site 1 soil, only 0.18% (57.5 μg Zn g^{-1}) was present in the water soluble fraction (Spurgeon and Hopkin, 1996).

4.2. The 'ecological relevance' of the detailed risk assessment technique

A risk assessment procedure that correctly describes both predator sensitivity and exposure will produce ecologically relevant values. However, comparison of the MPC concentrations calculated in this paper with concentrations of metals in field soils indicates that the derived values are unlikely to describe risk to predators correctly. The values are too low. For example, the cadmium, copper and zinc MPCs are below the levels of these metals found in uncontaminated soil (Ohnesorge and Wilhelm, 1991; Scheinberg, 1991; Stoeppler, 1991), while those for lead only marginally exceed uncontaminated values (Ewers and Schlipköter, 1991). To examine how this overestimation of toxicity occurs, the assumptions made for risk predictions will be considered.

As outlined earlier, the determination of worm/soil BCFs using individuals sampled from a site such as Avonmouth, should ensure that the metal concentrations in the diets of predators at each site around the factory are adequately assessed. The use of native worms rather than the introduction of clean worms to soils in the laboratory and the long-term nature of contamination should ensure that worm metal burdens are at equilibrium with soil values. A major problems is that the use of a mean BCF value may over-assess exposure at the more contaminated sites, since BCF at these sites are lower than the mean value. However, Romijn et al. (1994) considered the use of a constant BCF as an 'acceptable shortcoming of the model'. Correctly predicting dietary metal concentration does not guarantee an accurate estimate of exposure for critical tissues, since metals in prey may not be completely taken-up by predators (Morgan et al., 1986). For example, Friberg et al. (1974) proposed a model for assessing the risk of dietary cadmium for humans in which it was assumed that only 5% of ingested cadmium was absorbed.

A major problem with the risk assessment procedure is the scarcity of the data available for estimating $HC5_{predator}$ values. Sufficient data were available to calculate $HC5_{predator}$ values using the distribution based model for cadmium and lead, while for copper and zinc the safety factor method described by Stephan et al. (1985) was used. The safety factor method normally gives values below those calculated from the distribution method (Romijn et al., 1993), although this is desirable, as the quantity of data and thus, the certainty of the prediction is reduced. The predictions made in this way will, by their nature, be conservative. For example in the case of zinc, this is so because the value of 75 μg Zn g^{-1} calculated in this study is well below the levels of zinc found in worms even at the most uncontaminated site (Fig. 1d).

A further problem associated with assessing toxicity for predators from literature data is that the tests, from which vertebrate NOECs were obtained, are not designed to accurately assess toxicity. Studies are usually designed for examining the biochemical and physiological fate of absorbed metals. Additionally, many of the laboratory toxicity experiments may overestimate the effects of metals on vertebrates. Studies of the toxicity of metals in terrestrial invertebrates have indicated that metal toxicity can be influenced by the form of the chemical to which the test animals are exposed (Hopkin and Hames, 1994; Spurgeon et al., 1994; Spurgeon and Hopkin, 1995; Smit and Van Gestel, in press). In the majority of the vertebrate tests, the metals were added to the diet as soluble salts, usually chlorides, nitrates or oxides. The addition of metals to diet in this form almost certainly results in higher metal availability for the test animals, thus leading to an over-assessment of toxicity (Groten et al., 1990).

The use of surrogate species when assessing the sensitivity of earthworm predators introduces further inaccuracies in the assessment protocol, although the use of toxicity values from unrepresentative species could not be avoided due to the lack of data for worm predators. Sensitivity of vertebrates to toxicants is often found to differ between species due to differences in physiological mechanisms available for detoxification (Walker, 1993). For example, Shore (1995) and Shore and Douben (1994) have suggested that species such as shrews, that are liable to high cadmium exposure due to their carnivorous diet, may be less susceptible to cadmium intoxication than herbivorous species such as *Microtus*. This reduced sensitivity for cadmium is probably due to the development of more efficient detoxification systems using metallothionein proteins (Shore and Douben, 1994).

The increased sensitivity of surrogate species in laboratory tests is supported by results from this study and the work of Read and Martin (1993). To illustrate, mean soil cadmium level was 0.05 μg Cd g^{-1} at Site 22, and mean earthworm tissue cadmium content was 5.8 μg Cd g^{-1} at this site. This cadmium concentration is above the NOEC calculated for three of the tested species (Table 2). However, in the study of Read and Martin (1993), no evidence of a decline in small mammal populations in a number of woods in the Avonmouth area was found even at their most contaminated site (Haw wood, approximately 2 km from the factory). Worms collected from close to this site during the present study contained 43.3 μg Cd g^{-1}, above all but one of the vertebrate cadmium NOECs (Table 2). Thus, small mammals in the field appear unaffected by cadmium concentration in worms that exceed the laboratory mammalian NOEC by up to a factor of 14.

4.3. Alternative strategies for estimating the risk of accumulated pollutants for predators

The use of the simple algorithm described by Romijn et al. (1994) does not appear to correctly describe the risk posed by metals accumulated in the tissues of earthworms for their predators. The primary reason for this appears to be the scarcity and inconsistent nature of the toxicity data available for vertebrates. No data are available for earthworm predators, while the toxicity values obtained for surrogate species, usually exposed to via the addition of a soluble salt to diet, are not representative of those for predators in the field. Clearly there is a requirement to refine current strategies for categorising risks for predators consuming contaminated prey if realistic MPCs are to be determined. It is not within the scope of the current paper to determine a fully integrated model for the categorisation of the risk assessment of secondary poisoning. However, suggestions of the factors that should be considered within a risk assessment procedure can be made.

The use of data from surrogate species exposed to soluble metal salts present in the diet does not appear to offer a viable alternative to the use of data from earthworm predators exposed via the consumption of contaminated food in the field. However, to conduct a range of toxicity tests in the laboratory for earthworm predators such as badgers, foxes, moles and shrews with a range of pollutants presents serious financial and ethical considerations. An alternative may be to base a risk assessment on metal levels in the critical tissues of earthworm predators to indicate whether there is any effect of metal on exposed species. Renal toxicity threshold concentrations could form the basis of such a risk assessment, since the metal concentrations causing histopathological effects on organs will relate directly to actual exposure.

Raised liver and kidney metal concentrations have been found in a range of small mammal species including the shrews *Sorex araneus* and *Sorex minutus* the mole *Talpa europea* and the field vole *Microtus agrestis* at contaminated sites (Alberici et al., 1989; Hunter et al., 1987, 1989; Ma, 1987, 1989; Ma et al., 1991; Pankakoski et al., 1993; Read and Martin, 1993). Shore (1995) noted a clear relationship between log soil cadmium and lead concentrations and the levels of these metals present in the kidneys and livers of small mammals. It was concluded that the levels of metals in the tissues most likely to be subject to accumulation could be used as a measure of the exposure of small mammal species.

Data for the concentrations of cadmium and lead that cause kidney damage have been determined from histological studies on surrogate species (Shore and Douben, 1994). Kidney metal concentrations were considered since this organ is regarded as the critical target site for chronic cadmium toxicity (Friberg et al., 1974). Nicholson et al. (1983) and Goyer et al. (1970) found that concentrations of 119 μg Cd g^{-1} and 120 μg Pb g^{-1} wet weight kidney caused renal dysfuction and histopathological changes in vertebrates, while a WHO group concluded that the critical human kidney cadmium level lies between 100 and 300 μg Cd g^{-1}, with 200 μg Cd g^{-1} as the most likely estimate (WHO, 1992). These kidney intoxication levels, coupled with data detailing dietary uptake, physiological and tissue distribution and excretion of metals offer the chance to model the potential for nephrotoxic effects and relate them to actual dietary exposure.

Models for predicting the accumulation of cadmium in the kidneys of exposed organisms have already been developed for toxicological studies of dietary cadmium ingestion. The method of Friberg et al. (1974) uses exposure data determined as the cadmium concentration in the diet, daily food intake and the period of exposure, coupled with toxicological and biological knowledge such as the tissue distribution of cadmium, absorbed and excretion rates, and mean kidney weight to predict the concentrations of cadmium in kidney cortex.

Many of the parameters outlined in the model of Friberg et al. (1974) are included in the BIOMAG model. Gorree et al. (1995) used this model to estimate cadmium concentrations in the kidney of a range of predators in an area contaminated by the Budel smelting works in The Netherlands. Validation of the model by comparing measured kidney concentrations to predictions indicated that there is a tendency for the model to overestimate kidney levels. This was attributed to the fact that values used for physiological parameters such as excretion and uptake were identical for all species, although they may vary considerably. More accurate values are required for use in the model if accurate species-specific predictions are to be made.

Monitoring potential effects of dietary metal exposure for predators by comparisons with proposed critical organ concentrations has already been undertaken by Ma (1987) and Shore and Douben (1994). In the work of Ma (1987), a clear link was noted between metal concentrations in the organs of *Talpa europea*, levels in earthworms and the pH of metal contaminated soils. At sites contaminated by smelter emissions, earthworms were found to have increased metal burdens. The kidney cadmium and lead concentrations of moles present at these contaminated sites were 224 μg Cd g^{-1} and 338 μg Pb g^{-1} dry weight, respectively. Thus, the concentrations of cadmium and lead in mole kidneys exceeded the proposed diagnostic kidney intoxication levels of 119 μg Cd g^{-1} and 120 μg Pb g^{-1} suggesting deleterious effects due to these metals. The use of physiological and kinetic data for the exposure, uptake and effects of metals in the critical tissues of mammals therefore appears to offer potential for both the risk assessment and monitoring of the effects of pollutants on predators in the field.

Earthworms clearly represent an important pathway for the movement of pollutants through terrestrial food-chains due to their potential for accumulation and bioconcentration of pollutants and their density, biomass and importance in the diets of predators. However, although exposure of predators in the field can be estimated with some accuracy by the direct measurement of earthworm pollutant burdens, it is impossible to estimate the range of sensitivity for predators. Consequently, the use of a simple algorithm comparing the sensitivity of predators to their potential exposure via the tissues of earthworms does not appear to be sufficient to determine the concentrations of pollutants in soils that are safe for predators. The use of critical organ concentrations appears to offer an alternative.

Acknowledgement

This work was supported by a research grant from the Leverhulme Trust.

References

Alberici, T.M., W.E. Sopper, G.L. Storm and R.H. Yahner, 1989. Trace metals in soil vegetation and voles from mine land treated with sewage sludge. J. Environ. Qual., 18: 115–120.

Aldenberg, T. and W. Slob, 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotoxicol. Environ. Saf., 25: 48–63.

Ash, C.P. and D.L. Lee, 1980. Lead, cadmium, copper and iron in earthworms from roadside sites. Environ. Pollut. (Ser. A.), 22: 59–67.

Azar, A., H.J. Trochimoicz and M.E. Maxfield, 1972. Review of lead studies in animals carried out at the Maskell laboratory: two year feeding study and response to haemmorhagic study. In: Proceedings of the International Symposium on the Environmental Health Aspects of Lead, Amsterdam, pp. 199–209.

Bakalli, R.I., G.M. Pesti and W.L. Ragland, 1995. The magnitude of lead toxicity in broiler-chickens. Vet. Hum. Toxicol., 37: 15–19.

Bengtsson, G. and L. Tranvik, 1989. Critical metal concentrations for forest soil invertebrates. Water Air Soil Pollut., 47: 381–417.

Beyer, W.N. and C. Stafford, 1993. Survey and evaluation of contaminants in earthworms and in soils derived from dredged material at confined disposal facilities in the Great Lakes region. Environ. Monit. Assess., 24: 151–165.

Beyer, W.N., R.L. Chaney and B.M. Mulhern, 1982. Heavy metal concentrations in earthworms from soil amended with sewage sludge. J. Environ. Qual., 11: 381–385.

Campbell, G.L. and R.M. Mills, 1979. The toxicity of zinc to pregnant sheep. Environ. Res., 26: 1–13.

Cavanagh, N.A. and G.J. Judson, 1994. Copper-oxide powder as a supplement for sheep. J. Trace Elem. Electrolytes Health Dis., 8: 183–188.

Chmielnicka, J., T. Halatek and U. Jedlinska, 1989. Correlation of cadmium-induced nephropathy and the metabolism of endogenous copper and zinc in rats. Ecotoxicol. Environ. Saf., 18: 268–276.

Clayton, G.D. and F.E. Clayton, 1981. Patty's Industrial Hygiene and Toxicology. Wiley, Chichester, UK.

Cooke, A.S., P.W. Greig-Smith and S.A. Jones, 1992. Consequences for vertebrate wildlife of toxic residues in earthworm prey. In: H. Becker et al. (Eds), Ecotoxicology of Earthworms. Intercept Ltd., Hants, UK, pp. 139–155.

Cousins, R.J., A.K. Barber and J.R. Trout, 1973. Cadmium toxicity in growing swine. J. Nutr., 103: 964–972.

Coy, C.M., 1984. Control of dust and fumes at a primary zinc and lead smelter. Chemistry in Britain, May, 1984: 418–420.

Damron, B.L., C.F. Simpson and R.H. Harms, 1969. The effect of feeding various levels of lead on the performance of broilers. Poult. Sci., 48: 1507–1509.

Damron, B.L. and H.R. Wilson, 1975. Lead toxicity of bobwhite quail. Bull. Environ. Contam. Toxicol., 14: 489–496.

Donaldson, W.E. and C. McGowan, 1989. Lead toxicity in chickens — interactions with toxic dietary levels of selenium. Biol. Trace Elem. Res., 20: 127–133.

Doyle, J.J., W.H. Pfander, S.E. Grebing and J.O. Pierce, 1974. Effect of dietary cadmium on growth, cadmium absorption and cadmium tissue levels in growing lambs. J. Nutr., 104: 160–166.

Ewers, U. and H.W. Schlipköter, 1991. Lead. In: E. Merian (Ed.), Metals and their Compounds in the Environment. VCH, Weinheim, pp. 971–1014.

Friberg, L., M. Piscator, G.F. Nordberg and T. Kjellström, 1974. Cadmium in the Environment. CRC Press, Cleveland, OH.

Funk, M.A. and D.H. Baker, 1991. Toxicity and tissue accumulation of copper in chicks fed casein. J. Anim. Sci., 69: 4505–4511.

Gorree, M., W.L.M. Tamis, T.P. Traas and M.A. Elbers, 1995. BIOMAG: a model for biomagnification in terrestrial food chains. The case of cadmium in the Kempen, The Netherlands. Sci. Total. Environ., 168: 215–223.

Goyer, R.A., D.L. Leonard, J.F. Moore, B. Rhyne and M. Krigman, 1970. Lead dosage and the role of the intranuclear inclusion body. Arch. Environ. Health, 20: 705–711.

Groten, J.P., E.J. Sinkeldam, J.B. Luten and P.J. Van Bladeren, 1990. Comparison of the toxicity of inorganic and liver incorporated cadmium — A 4-week feeding study in rats. Food Chem. Toxicol., 28: 435–441.

Groten, J.P., E.J. Sinkeldam, T. Muys, J.B. Luten and P.J. Van Bladeren, 1991. Food Chem. Toxicol., 29: 249–258.

Haegele, M.A., R.K. Tucker and R.H. Hudson, 1974. Effects of dietary mercury and lead on eggshell thickness in mallards. Bull. Environ. Contam. Toxicol., 11: 5–11.

Herbert, C.D., M.R. Elwell, G.S. Travlos, C.J. Fitz and J.R. Bucher, 1993. Subchronic toxicity of cupric sulfate administered in drinking-water and feed to rats and mice. Fund. Appl. Toxicol., 21: 461–475.

Hopkin, S.P., 1989. Ecophysiology of Metals in Terrestrial Invertebrates. Elsevier Applied Science, Barking, UK.

Hopkin, S.P. and C.A.C. Hames, 1994. Zinc, among a 'cocktail' of metal pollutants, is responsible for the absence of the terrestrial isopod *Porcellio scaber* from the vicinity of a primary smelting works. Ecotoxicology, 2: 68–78.

Hopkin, S.P., G.N. Hardisty and M.H. Martin, 1986. The woodlouse *Porcellio scaber* as a 'biological indicator' of zinc, cadmium, lead and copper pollution. Environ. Pollut., 11B: 271–290.

Hunter, B.A., M.S. Johnson and D.J. Thompson, 1987. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. 3. Small mammals. J. Appl. Ecol., 24: 601–614.

Hunter, B.A., M.S. Johnson and D.J. Thompson, 1989. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. 4. Tissue distribution and age accumulation in small mammals. J. Appl. Ecol., 29: 89–99.

Ireland, M.P., 1983. Heavy metal uptake and tissue distribution in earthworms. In: J.E. Satchell (Ed.), Earthworm Ecology. Chapman and Hall, London, pp. 247–265.

Jacquet, P. and G.B. Gerber, 1979. Teratogenic effects of lead in the mouse. Biomedicine, 30: 223–229.

Jacquet, P., A. Leonard and G.B. Gerber, 1976. Action of lead on the early divisions of the mouse embryo. Toxicology, 6: 129–132.

Janssen, M.P.M. and R.F. Hogervorst, 1993. Metal accumulation in soil arthropods in relation to micro-nutrients. Environ. Pollut., 79: 181–189.

Janssen, M.P.M., A Bruins, T.H. De Vries and N.M. Van Straalen, 1991. Comparison of cadmium kinetics in four soil arthropod species. Arch. Environ. Contam. Toxicol., 20: 305–312.

Ketchenson, M.R., G.P. Barron and D.H. Cox, 1969. Relationship of maternal dietary zinc during gestation and lactation development and zinc, iron and copper content of the postnatal rat. J. Nutr., 98: 303–311.

Krajnc, E.I., P.L. Schuller, J.M. Garbis-Berkvens, P.L.M. Berende, P.W. Helleman, F.X.R. Van Leeuwen and M.A.M. Franken, 1986. Staeling en toxiciteit van cadmium bij varkens. Internal Report RIVM No., 617716 001, Bilthoven, The Netherlands.

Leach, R.M., K. Wei-Li Wang and D.E. Baker, 1979. Cadmium and the food chain: the effects of dietary cadmium on tissue composition in chicks and laying hens. J. Nutr., 109, 437–443.

Leach, R.M., C.I. Rosenblum, M.J. Amman and J. Burdette, 1990. Broiler chicks fed low calcium diets., 2. Increased sensitivity to copper toxicity. Poult. Sci., 69: 1905–1910.

Leonard, A., B. Linden and G.B. Gerber, 1972. Etude chez la souris des effets genetiques et cytogenetiques d'une contatmination par le plomb. In: Proceedings of the International Symposium on the Environmental Health Aspects of Lead, Amsterdam, pp. 303–309.

Liao, J.W., S.F. Tsai and S.C. Wang, 1995. Sub-acute toxicity of lead administered by feeding in rats. J. Chin. Vet. Soc., 20: 338–347.

Loeser, E., 1980. A 2-year oral carcinogenicity study with cadmium in rats. Cancer Lett., 9: 191–198.

Ma, W.C., 1982. The influence of soil properties and worm related factors on the concentration of heavy metals in earthworms. Pedobiologia, 24: 109–119.

Ma, W.C., 1987. Heavy metal accumulation in the mole, *Talpa europea* and earthworms as an indicator of metal availability in terrestrial environments. Bull. Environ. Contam. Toxicol., 39: 933–938.

Ma, W.C., 1989. Effects of soil pollution with metallic lead pellets on lead bioaccumulation and organ/body weight alterations in small mammals. Arch. Environ. Contam. Toxicol., 18: 617–622.

Ma, W.C., W. Denneman and J. Faber, 1991. Hazardous exposure of ground-living small mammals to cadmium and lead in contaminated terrestrial ecosystems. Arch. Environ. Contam. Toxicol., 20: 266–270.

Martin, M.H. and R.J. Bullock, 1994. The impact and fate of heavy metals in an oak woodland ecosystem. In: S.M. Ross (Ed.), Toxic Metals in Soil-plant Systems. Wiley, Chichester, UK, pp. 327–365.

Masaoka, T., F. Akahori, S. Arai, K. Nomiyama, H. Nomiyama, K. Kobayashi, Y. Nomura and T. Suzuki, 1994. A 9-year chronic toxicity study of cadmium ingestion in monkeys. 1. Effects of dietary-cadmium on the general health of monkeys. Vet. Hum. Toxicol., 36: 189–194.

Morgan, A.J. and B. Morris, 1982. The accumulation and intracellular compartmentation of cadmium, lead, zinc and calcium in two earthworm species (*Dendrobaena rubida* and *Lumbricus rubellus*) living in highly contaminated soil. Histochemistry, 75: 269–285.

Morgan, A.J., B. Morris, N. James, J.E. Morgan and K. Leyshon, 1986. Heavy metals in terrestrial macroinvertebrates: species differences within and between trophic levels. Chem. Ecol., 2: 319–334.

Morgan, G.W., F.W. Edens, P. Thaxton and C.R. Parkhurst, 1975. Toxicity of dietary lead in Japanese quail. Poult. Sci., 54: 1636–1642.

Morgan, J.E. and A.J. Morgan, 1988. Earthworms as biological monitors of cadmium, copper, lead and zinc in metalliferous soils. Environ. Pollut., 54: 123–138.

Nicholson, J.K., M.D. Kendall and D. Osborn, 1983. Cadmium and nephrotoxicity. Nature, 304: 633–635.

Nomiyama, K., F. Akahori, F. Noiyama, T. Masaoka, S. Arai, Y. Nomura, M. Yotoriyama, K. Kobayashi, T. Suzuki, H. Kawashima and A. Onozawa, 1987. Dose-effect and Dose-response Relationships in Rhesus Monkeys after Administration of Cadmium Containing Diet for 9 Years. Environ. Health, Report No., 53. Japan Public Health Association, Tokyo, Japan.

Ohnesorge, F.K. and M. Wilhelm, 1991. Zinc. In: E. Merian (Ed.), Metals and their Compounds in the Environment. VCH, Weinheim, pp. 1309–1342.

Pankakoski, E., H. Hyvarinen, M. Jalkanan and I. Koivisto, 1993. Accumulation of heavy metals in the mole in Finland. Environ. Pollut., 80: 9–16.

Pattee, O.H., 1984. Eggshell thickness and reproduction in American kestrels exposed to chronic dietary lead. Arch. Environ. Contam. Toxicol., 13: 29–34.

Peter, M., U. Vladimir and T. Robert, 1995. Distribution of cadmium and its influence on the growth of offspring after an experimental application to female rabbits. J. Environ. Sci. Health A, 30: 51–62.

Powell, G.W., W.J. Miller, J.D. Morton and C.M. Clifton, 1964. Influence of dietary cadmium on development of young chickens. Poult. Sci., 53: 2026–2029.

Prigge, E., 1978. Early signs of oral and inhalative cadmium in rats. Arch. Toxicol., 40: 231–247.

Read, H.J. and M.H. Martin, 1993. The effects of heavy metals on populations of small mammals from woodlands in Avon (England); with particular emphasis on metal concentrations in *Sorex araneus* L. and *Sorex minutus* L. Chemosphere, 27: 2197–2211.

Richardson, M.E., M.R. Spivey-Fox and B.E. Fry, 1974. Pathological changes produced in Japanese quail by ingestion of cadmium. J. Nutr., 104: 323–338.

Romijn, C.A.F.M., R. Luttik, D.V.D. Meent, W. Sloof and J. Canton, 1993a. Presentation of a general algorithm to include effect assessment on secondary poisoning in the derivation of environmental quality criteria. 1. Aquatic food chains. Ecotoxicol. Environ. Saf., 26: 61–83.

Romijn, C.A.F.M., R. Luttik and J. Canton, 1994. Presentation of a general algorithm to include effect assessment on secondary poisoning in the derivation of environmental quality criteria. 2. Terrestrial food chains. Ecotoxicol. Environ. Saf., 27: 107–127.

Romijn, C.A.F.M., R. Luttik and J. Canton, 1994. Presentation of a general algorithm to include effect assessment on secondary poisoning in the derivation of environmental quality criteria. 2. Terrestrial food chains. Ecotoxicol. Environ. Saf., 27: 107–127.

Samarawickrama, G.P., 1979. Biological effects of cadmium in mammals. In: W. Webb (Ed.), The Chemistry, Biochemistry and Biology of Cadmium. Elsevier, Amsterdam, pp. 341–421.

Scheinberg, H., 1991. Copper. In: E. Merian (Ed.), Metals and their Compounds in the Environment. VCH, Weinheim, pp. 893–908.

Shore, R.F., 1995. Predicting cadmium, lead and fluoride levels in small mammals from soil residues and by species-species extrapolation. Environ. Pollut., 88: 333–340.

Shore, R.F. and P.E.T. Douben, 1994. The ecotoxicological significance of cadmium intake and residues in terrestrial small mammals. Ecotoxicol. Environ. Saf., 29: 101–112.

Smit, C.E. and C.A.M. Van Gestel (in press). Comparison of the toxicity of zinc for the springtail *Folsomia candida* in artificially contaminated and polluted field soils. Appl. Soil Ecol., in press.

Spurgeon, D.J. and S.P. Hopkin, 1996. Effects of variations in the organic matter content and pH of soils on the availability and toxicity of zinc to the earthworm *Eisenia fetida*. Pedobiologia, 40: 80–96.

Spurgeon, D.J. and S.P. Hopkin (in press). The effects of metal contamination on earthworm populations around a smelting works — quantifying species effects. Appl. Soil Ecol., in press.

Spurgeon, D.J. and S.P. Hopkin, 1995. Extrapolation of the laboratory-based OECD earthworm test to metal-contaminated field sites. Ecotoxicology, 4: 190–205.

Spurgeon, D.J., S.P. Hopkin and D.T. Jones, 1994. Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): Assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. Environ. Pollut. (Ser. A), 84: 123–130.

Spurgeon, D.J. (in press). Extrapolation of laboratory toxicity results to the field — A case study using the OECD artificial soil earthworm toxicity test. In: N.M. Van Straalen and H. Løkke (Eds), Ecological Principles for Risk Assessment of Contaminants in Soil. Chapman and Hall, London.

Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs, 1985. Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. PB85-227049. US Environmental Protection Agency, National Technical Information Service, Springfield, VA.

Stoeppler, M., 1991. Cadmium. In: E. Merian (Ed.), Metals and their Compounds in the Environment. VCH, Weinheim, pp. 804–851.

Supplee, W.C., 1961. Production of zinc deficiency in turkey poults by dietary cadmium. Poult. Sci., 40: 827–828.

Vale, J.A. and J. Harrison, 1994. Aerial inputs of pollutants to the Severn estuary. Biol. J. Linn. Soc., 51: 45–54.

Van Gestel, C.A.M., 1992. The influence of soil characteristics on the toxicity of chemicals for earthworms: a review. In: H. Becker et al. (Eds), Ecotoxicology of Earthworms. Intercept Ltd., Hants, UK, pp. 44–54.

Van Gestel, C.A.M., E.M. Dirven-Van Breemen and R. Baerselman, 1993. Accumulation and elimination of cadmium, copper, chromium and zinc and effects on growth and reproduction in *Eisenia andrei* (Oligochaeta, Annelida). Sci. Total. Environ. Suppl., Part 1: 585–597.

Van Straalen N.M., 1993. An ecotoxicologist in politics. Oikos, 62: 255–256.

Van Straalen, N.M., P. Leeuwangh and P.B.M. Stortelder, 1994. Progressing limits for soil ecotoxicological risk assessment. In: M.H. Donker, H. Eijsackers and F. Heimbach (Eds), Ecotoxicology of Soil Organisms. Lewis, Chelsea, MI, pp. 397–409.

Walker, C.H., 1993. Birds. In: P. Calow (Ed.), Handbook of Ecotoxicology, Vol. 1. Blackwell, Oxford, pp. 326–338.

White, D.H., M.T. Finley and J.F. Ferrell, 1978. Histopathologic effects of dietary cadmium on kidneys and testes of mallard ducks. J. Toxicol. Environ. Health, 4: 551–558.

Wittman, M., F.X. Roth and M. Kirchgessner, 1994. Self-selection of lead supplemented diets by chicks. 1. Effects of lead on performance of chicks. Arch. Geflugekunde, 58: 38–45.

World Health Organisation, 1992. Environmental Health Criteria, 134: Cadmium. WHO, Geneva.

Wright, M.A. and A. Stringer, 1980. Lead, zinc and cadmium content of earthworms from pasture in the vicinity of an industrial smelting complex. Environ. Pollut. (Ser. A), 23: 313–321.

Zenick, H., L. Hastings, M. Goldsmith and R.J. Niewenhuis, 1982. Chronic cadmium exposure: relation to male toxicity and subsequent fetal outcome. J. Toxicol. Environ. Health, 9: 377–387.