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Development and Validation of Bioaccumulation Models for Earthworms

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PREFACE

While considerable research has been conducted on contaminant transfer from soil to earthworms, most studies focus on only a single location, and external validation of transfer models has not been performed. The purpose of this document, then, was to develop a database of soil and tissue concentrations for 9 inorganic and 2 organic chemicals based on data from 31 studies from 11 countries and 5 states. This information will form a critical component in many ecological risk assessments performed on the Oak Ridge Reservation. Related plant and small mammal data are presented in companion reports ES/ER/TM-218 and ES/ER/TM-219, respectively.

This work was performed under Work Breakdown Structure 1.4.12.2.3.04.05.02, Activity Data Sheet 8300 (CCADS-8323).

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ABBREVIATIONS

CEC	cation exchange capacity
DTPA	diethylenetriaminepentaacetic acid
GI	gastrointestinal
OM	organic matter
PCBs	polychlorinated biphenyls
PD	proportional deviation
TCDD	tetrachlorodibenzo-p-dioxin
UFs	uptake factors
UPL	upper prediction limit

EXECUTIVE SUMMARY

Estimation of contaminant concentrations in earthworms is a critical component in many ecological risk assessments. Without site-specific data, literature-derived uptake factors (UFs) or models are frequently used. While considerable research has been conducted on contaminant transfer from soil to earthworms, most studies focus on only a single location. External validation of transfer models has not been performed.

We developed a database of soil and tissue concentrations for 9 inorganic (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Zn) and 2 organic [polychlorinated biphenyls (PCBs) and tetrachlorodibenzo-p-dioxin (TCDD)] chemicals based on data from 32 studies from 11 countries and 5 states. Only studies that presented "total" concentrations in depurated earthworms were included. UFs— earthworm concentration/soil concentration— and regression models of natural-log-transformed concentrations of each analyte in soil and earthworms were developed. Multiple regression models incorporating soil pH and log-transformed soil Ca were also developed. Models were developed using data from 26 studies and then were applied to the data from the remaining 6 studies. Estimated and observed earthworm concentrations were compared using nonparametric Wilcoxon signed-rank tests. Relative accuracy and quality of different estimation methods were evaluated by calculating the proportional deviation ([measured - estimate]/measured) of the estimate from the measured value and the percentage of estimates that exceeded measured values.

With the exception of Cr, significant, single-variable (e.g., soil concentration) regression models were fit for each analyte. Inclusion of soil Ca improved model fits for Cd and Pb. Soil pH only marginally improved model fits. The best general estimates of chemical concentrations in earthworms were generated by simple ln-ln regression models for As, Cd, Cu, Hg, Mn, Pb, Zn, and PCBs. No method accurately estimated Cr or Ni in earthworms. The best conservative estimates of chemical concentrations in earthworms were generated by the upper 95% prediction limit for the simple ln-ln regression models for Cd, Cu, Hg, Zn, and PCBs. The 90th percentile UFs generated the best conservative estimates for As, Cr, and Pb. While multivariate regression models including pH generated better estimates for a few analytes, in general, the predictive utility gained by incorporating environmental variables was marginal.

Because the available data indicate that bioaccumulation by earthworms is non-linear, decreasing as soil concentration increases, and UFs implicitly assume that accumulation is linear and constant across all soil concentrations, the use of log-linear regression models to estimate earthworm bioaccumulation is recommended. For applications where conservative estimates are desired, the upper 95% prediction limit on the simple regression is recommended.

1. INTRODUCTION

Estimation of the risks that soil contamination presents to vermivorous (e.g., earthworm-eating) wildlife requires measuring the contaminant concentration in earthworms. These data may be acquired either by direct measurement or estimation. Direct measurement consists of collecting and analyzing contaminant concentrations in earthworms from contaminated sites. Because this approach provides information on the actual contaminant loading in on-site earthworms, direct measurement contributes the least uncertainty to exposure estimates and is therefore the preferred approach. However, for various reasons (incompatible sampling schedule; insufficient time, personnel, or finances to support field sampling; etc.), direct measurement may not be feasible. When direct measurement of contaminants is not possible, estimation is the only alternative.

Contaminant loads in earthworms may be estimated using uptake factors (UFs) or empirically derived regression models. UFs, the ratios of contaminant concentrations in earthworms to those in soil, are the simplest method for estimating contaminant loads in earthworms. In practice, if the contaminant concentration in soil is known (likely in almost all retrospective ecological risk assessments), the concentration in earthworms may be estimated by multiplying the soil concentration by the UF. The use of UFs depends on the assumption that the concentration of chemicals in organisms is a linear, no-threshold function of concentrations in soil. This is expected to be the case for xenobiotic chemicals like polychlorinated biphenyls (PCBs) that are passively accumulated and not metabolized to any significant extent. It will not be the case if the chemical in question is well-regulated by the organism, either because it is an essential nutrient or because it is a toxicant with effective inducible mechanisms for metabolism or excretion. Such regulated chemicals will, within the effective concentration range for the mechanism, have nearly constant concentrations in earthworms regardless of soil concentrations, except at deficient concentrations.

Various complex patterns are also possible due to lack of induction at low concentrations, saturation kinetics at high concentrations, toxicity at high concentrations, or other processes. Despite these situations that lead to violation of the assumptions, UFs are commonly used in risk assessments. Published sources of earthworm UFs are summarized in Table 1.

Regression models are another approach to estimating contaminant concentrations in earthworms. These models are generally simple linear or log-linear regressions of the soil contaminant concentration on the earthworm concentration. Soil pH, soil Ca concentration, percent organic matter in soil, etc., may also be included in the models as predictive parameters (e.g., Beyer et al. 1987, Corp and Morgan 1991). Published sources of earthworm uptake models are summarized in Table 1.

While there has been considerable research concerning the uptake of soil contaminants by earthworms, most studies use data from a limited number of locations and focus on a limited number of analytes. In addition, no studies have attempted to validate the accuracy of UFs or models in predicting contaminant concentrations in earthworms at other locations. The purpose of this report was to assemble a database of soil and earthworm contaminant concentration data from published literature for a wide range of contaminants, develop UFs and other bioaccumulation models from these data, and then evaluate the accuracy of the estimates using independent data that was not included in the model development. The validation step allows the reliability of the models to be determined.

Table 1. Summary of sources of soil-earthworm UFs and uptake models

Study Location	Analytes with UFs	Analytes with Models	Reference
Pennsylvania, USA	Cd, Cu, Pb, Ni, and Zn	Cd	Beyer et al. 1982
Maryland, USA		Pb, Cu, Cd, and Se	Beyer et al. 1987
Finland	Al, Cd, Cu, Fe, Hg, Mn, V, and Zn		Braunschweiler 1996
Wales, Great Britain	Pb	Ca, Cd, Cu, Pb, and Zn	Corp and Morgan 1991
Warsaw, Poland	Cd, Cu, Pb, Zn		Czarnowska and Jopkiewicz 1978
Germany	Cd, Pb, and Zn		Emmerling et al. 1997
Denmark		Se	Nielsen and Gissel-Nielsen 1975
Netherlands	Cd, Cu, Mn, Ni, Pb, and Zn		Hendriks et al. 1995
Netherlands	Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn	Pb and Zn	Ma 1982
Netherlands		Cd, Cu, Pb, and Zn	Ma et al. 1983
Seveso, Italy	TCDD		Martinucci et al. 1983
Models fit to data from multiple locations.		Cd, Cu, Ni, Pb, and Zn	Neuhauser et al. 1995
Montana, USA	As, Cd, Cu, and Zn		Pascoe et al. 1996
Illinois, USA	Cd, Cr, Cu, Ni, and Pb		Pietz et al. 1984
Reading, Great Britain	Cd, Cu, Pb, and Zn	Cd, Cu, Pb, and Zn	Spurgeon and Hopkins 1996
Tennessee, USA	Cd, Pb, Zn		Van Hook 1974

TCDD = tetrachlorodibenzo-p-dioxin

In this report, both UFs and regression models were developed and tested, because, while regression models are most likely to consistently provide the best estimate of earthworm body burdens, UFs are required by some regulatory agencies. In addition, when no regression model fits the uptake data well, a conservative UF may be employed in screening assessments to determine whether site-specific studies are needed. The models presented in this report will facilitate the more accurate estimation of contaminant exposure experienced by earthworm-consuming wildlife on the Oak Ridge Reservation (ORR) and at other contaminated sites. Additional models for estimating contaminant bioaccumulation by sediment biota, plants, and small mammals are presented in Jones et al. (1998), Efroymson et al. (1998), and Sample et al. (1998).

2. MATERIALS AND METHODS

2.1 DATABASE DEVELOPMENT

A literature search was performed for studies that reported chemical concentrations in co-located earthworm and soil samples. To ensure relevancy to field situations, only field studies in which resident earthworms were collected were considered. All earthworm tissue burdens were therefore assumed to be at equilibrium with soil concentrations. Because soil residues in the earthworm gastrointestinal (GI) tract may be highly variable and therefore may significantly bias body burden measurements, only depurated earthworms were included. Samples in which the GI tract had been dissected or manually flushed were also considered suitable.

To ensure comparability of data, only "total" chemical analyses of both soil and earthworms (e.g., resulting from extractions of metals using concentrated acids) were included. Data resulting from diethylenetriaminepentaacetic acid (DTPA), acetic acid, and other mild extraction methods were excluded. The mean (or composite) chemical concentration in soil and earthworms reported for each sampling location evaluated in each study was considered an observation. If data for multiple earthworm species were reported at a site, each was considered a separate observation. Soil and earthworm data in the database were reported as mg/kg dry weight. If studies reported earthworms in terms of wet weight concentrations, dry weight concentrations were estimated assuming a 84% water content (EPA 1993). Data concerning earthworm species, soil pH, % organic matter (OM), cation exchange capacity (CEC), soil texture, and soil Ca concentration (mg/kg dry wt) were included in the database whenever reported. Summaries of the analytical methods and data presented for each study included in the database are presented in Appendix A. The earthworm bioaccumulation database is presented in Appendix B.

2.2. MODEL DEVELOPMENT AND VALIDATION

The earthworm bioaccumulation database was segregated into two groups. Twenty-six studies were assigned to the "model" dataset and were used for model development. The remaining six studies were designated the 'validation' dataset and were employed to test the accuracy and predictive utility of the UFs and bioaccumulation models. Segregation of studies into model and validation datasets was arbitrary and based on the sequence of when copies of the studies were acquired (i.e., the final six studies obtained were used for the validation dataset). Because sampling and analytical variability and environmental characteristics are likely to be correlated among data from the same study, it was assumed that data from wholly independent studies (e.g., studies from which no data were included in the model development) would be unbiased and would provide a better test of the UFs and models than would randomly selected observations extracted from the total dataset.

UFs, (contaminant concentration in earthworms/contaminant concentration in soil), were calculated for each observation and analyte in the model dataset. Summary statistics were generated for each analyte. The Shapiro-Wilk test (PROC UNIVARIATE; SAS Inst. Inc. 1988a) was applied to the untransformed and natural-log transformed UFs for each analyte to determine whether the distribution of the UFs was normal or log-normal, respectively.

To evaluate if there was a linear relationship between the contaminant concentration in soil and that in earthworms, simple and multiple regressions were performed using SAS PROC REG (SAS Inst. Inc. 1988b). Contaminant concentrations in both soil and earthworms were natural-log (ln)

transformed prior to regression analyses. Because data concerning the number of individuals included in composites or means were not available for all observations, no weighting of observations was applied. Simple linear regression models of ln-earthworm concentration on ln-soil concentration were developed for each analyte. Multiple regression models incorporating soil pH and ln soil Ca concentration, singly and combined, were developed for each analyte for which adequate data were available.

UFs and regression models developed from the "model" dataset were applied to the soil concentration data in the "validation" dataset, and estimated contaminant concentrations in earthworms were generated. To evaluate the appropriateness and accuracy of various methods for generating estimates for general application, estimated concentrations in earthworms were generated using the median UF, simple and multiple regression models developed in the current study, and selected published bioaccumulation models (Table 2). Because conservative estimates are needed for some purposes (e.g., screening assessments), estimates were also generated using the 90th percentile UF and the upper 95% prediction limit (95% UPL) for the simple regression model from this study. The 95% UPL was calculated according to Dowdy and Wearden (1983).

Table 2. Selected earthworm bioaccumulation models from the literature

Analyte	Model	Reference
Cd	log (worm)[a] = 0.66 log (soil)[a] + 1.21	Neuhauser et al. 1995
Cu	log (worm) = 0.57 log (soil) + 0.39	Neuhauser et al. 1995
Pb	log (worm) = 0.74 log (soil) + 0.05	Neuhauser et al. 1995
Ni	log (worm) = 0.98 log (soil) + 0.67	Neuhauser et al. 1995
Zn	log (worm) = 0.27 log (soil) + 2.09	Neuhauser et al. 1995
Se	log (worm) = 1.07 + (soil Ca)[b]	Beyer et al. 1987
Se	worm = 8.7 (soil) + 6.4	Nielsen and Gissel-Nielsen 1975

[a] All concentrations expressed as mg/kg dry weight, unless otherwise noted.
[b] Soil Ca expressed as meq/100 g.

For each analyte and estimation method (e.g., UF, models from this study, published models, etc.), differences between estimated and measured concentrations in validation earthworms were evaluated using Wilcoxon signed-rank tests (PROC UNIVARIATE; SAS Inst. Inc. 1988a). Differences were considered significant if $p(H_0=0) \leq 0.05$. Relative accuracy and quality of different estimation methods were evaluated by calculating the proportional deviation of the estimate from the measured value:

$$PD = (M_i - E_i) / M_i$$

where

PD = proportional deviation
M_i = measured concentration for chemical in earthworm at soil concentration (I)
E_i = estimated concentration for chemical in earthworm at soil concentration (I)

Negative values for PD indicate overestimation while positive PD values indicate underestimation. The percentage of estimated values that exceeded their corresponding measured value was also

tabulated for each chemical and estimation method. Relative quality of general estimation methods was evaluated by the following criteria:

1. median PD closest to 0 (indicates estimates center around measured values),
2. PD with narrowest range (indicates relative accuracy of method),
3. percentage overestimation closest to 50% (indicates estimates center around measured values), and
4. difference between estimated and measured values not significantly different as determined by Wilcoxon signed-rank tests.

Relative quality of conservative estimation methods was evaluated by

1. smallest, negative median PD value (indicates method overestimates while minimizing the degree of overestimation) and
2. PD with narrowest range (to minimize the degree of overestimation);

In addition to the use of PD values, a graphical evaluation of measured versus estimated concentrations in earthworms was performed by plotting the earthworm concentrations against the corresponding measured soil concentration.

Linear regressions of the natural-log transformed earthworm and soil "validation" data were performed and compared to simple models (i.e., soil concentration only) developed from the "model" dataset using the F-test procedure for comparing regression lines outlined in Draper and Smith (1981). Differences were considered significant if $p \leq 0.05$.

Following validation analyses, the "model" and "validation" datasets were pooled, and UFs and simple and multiple regression models were recalculated. These results were reported as the final UF or model.

Data for additional analytes were present in the "validation" dataset that were unrepresented in the "model" dataset. UFs were generated and summary statistics and distributions were determined for these analytes. Because these data represent only a single study, the remedial investigation for the Bear Creek Valley on the ORR, regression models were not fit to these data. These data are presented in Appendix C.

3. RESULTS

3.1 MODELING RESULTS

A total of 32 studies were identified that contained data suitable for inclusion in the earthworm bioaccumulation database (Appendix A). The "model" portion of the database consisted of 26 studies, representing 11 countries and 5 states. Data from the remaining 6 studies, representing Spain, Great Britain and the United States (Tennessee), were retained for validation purposes. Scatterplots of the observations from the "model" and "validation" datasets are presented in Figs. 1 through 6.

UFs and simple regression models were developed for nine inorganic and two organic chemicals (Tables 3 and 4). The number of studies available for each analyte ranged from a minimum of two studies for Mn, PCBs, and tetrachlorodibenzo-p-dioxin (TCDD) to a maximum of 17 studies for Cd (Table 3). With the exception of As and Ni, the distribution of all UFs was best described by the lognormal distribution (Table 3); As and Ni were best fit by a normal distribution. Median UFs for 6 chemicals (As, Cr, Cu, Mn, Ni, and Pb) were <1, indicating no biomagnification (Table 3). Median UFs>1 were observed for the remaining 5 chemicals (Cd, Hg, Zn, PCB, and TCDD; Table 3). [**Note:** the mean and standard deviation of the natural-log-transformed UFs are presented as parameters for describing the UF distributions for those analytes best fit by a lognormal distribution. While the untransformed UFs are best fit by a lognormal distribution, the natural-log-transformed UFs are normally distributed. These parameters may be used in two ways. They may be applied to normal distribution functions in Monte Carlo simulation software, however the output from the Monte Carlo sampling from this distribution must be back-transformed (e.g., e^y, where y=sampling result). Alternatively, they may be incorporated into the LOGNORM2 function in the @RISK Monte Carlo simulation software (Palisades Corp. 1994) or equivalent functions in other software. Use of the LOGNORM2 function requires no back-transformation. Comparable results are obtained using either approach.]

Regression of ln (earthworm) on ln (soil) produced significant model fits for all chemicals except Cr (Table 4). With the exception of Ni, slopes of all significant regression models were positive (Table 4; Figs. 1 through 6). Intercepts differed significantly from 0 for all chemicals except Hg, Mn, and Pb (Table 4). r^2 values for the significant models ranged from 0.22 (Cu) to 0.93 (PCB and TCDD).

Additional descriptive variables (e.g., pH, soil Ca concentration) were not available for all observations included in the "model" dataset; addition of these variables resulted in decreases in sample sizes. Consequently, the simple and multiple regression models are not directly comparable. While inclusion of soil pH in the regression model resulted in significant model fits for six chemicals (Table 5), only for Ni, Pb, and Zn did pH contribute significantly to the model fit. In the case of Ni, pH contributed significantly to the model fit while soil Ni did not (Table 5). Correlation analysis indicated no correlation between that soil Ni and soil pH ($r=0.31$, $p=0.3$).

Inclusion of ln soil Ca resulted in significant model fits for five chemicals (Table 6), however, only for Cd and Pb did soil Ca contribute significantly to model fit. When both soil Ca and soil pH were included in the model, significant fits were obtained for Cd, Cu, Pb, and Zn, albeit with dramatically reduced sample sizes (Table 7). For each chemical only two of the three dependent variables included contributed significantly; soil and pH for Cu, Pb, and Zn and soil and soil Ca for Cd (Table 7).

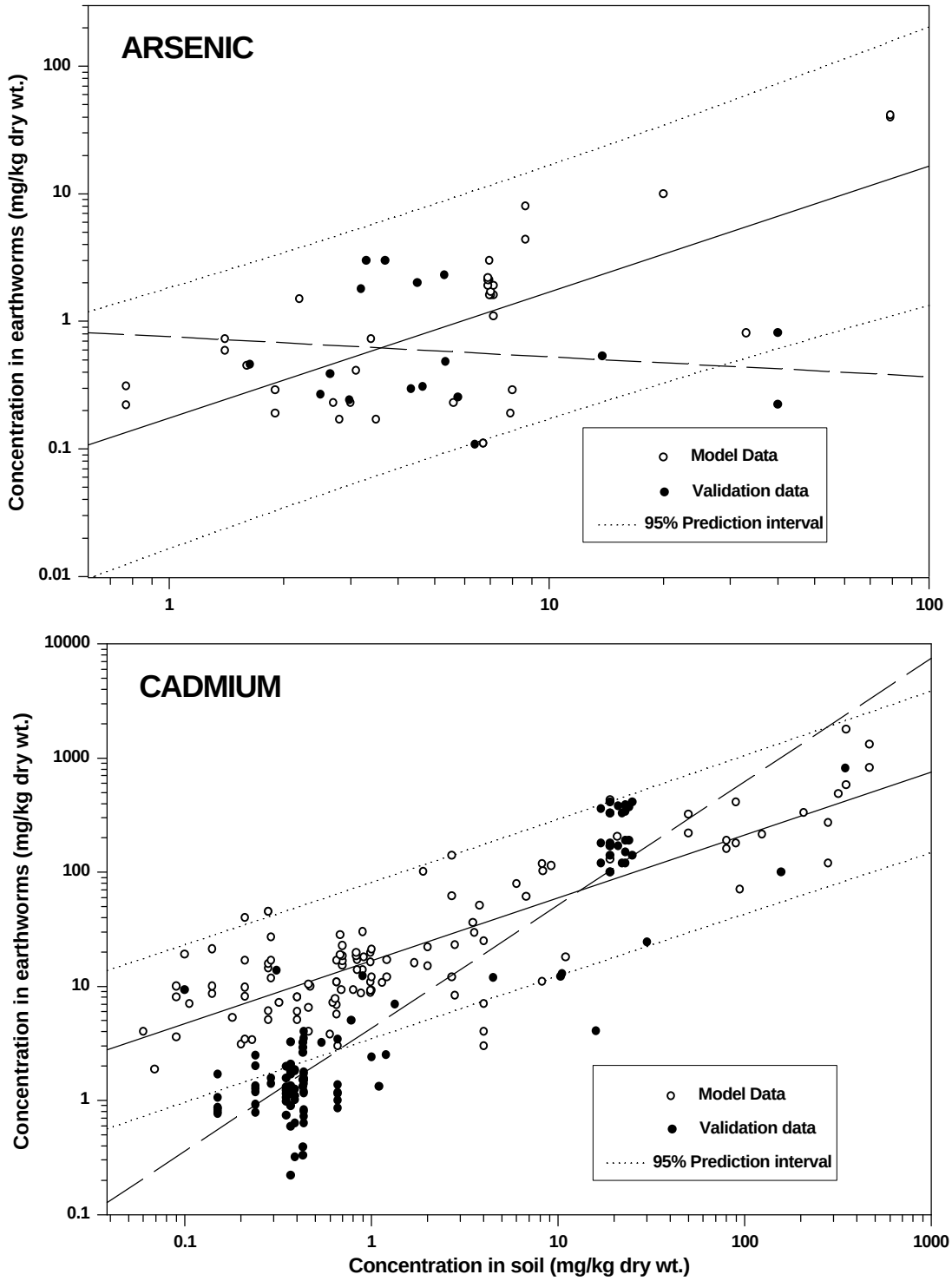

Fig. 1. Scatterplot of model and validation As and Cd data. Lines represent simple linear regression models of natural-log-transformed data for both model (solid) and validation (dashed) datasets. Dotted lines represent 95% prediction interval for model data. Regression models for model and validation datasets differed significantly ($p<0.0001$) for both As and Cd.

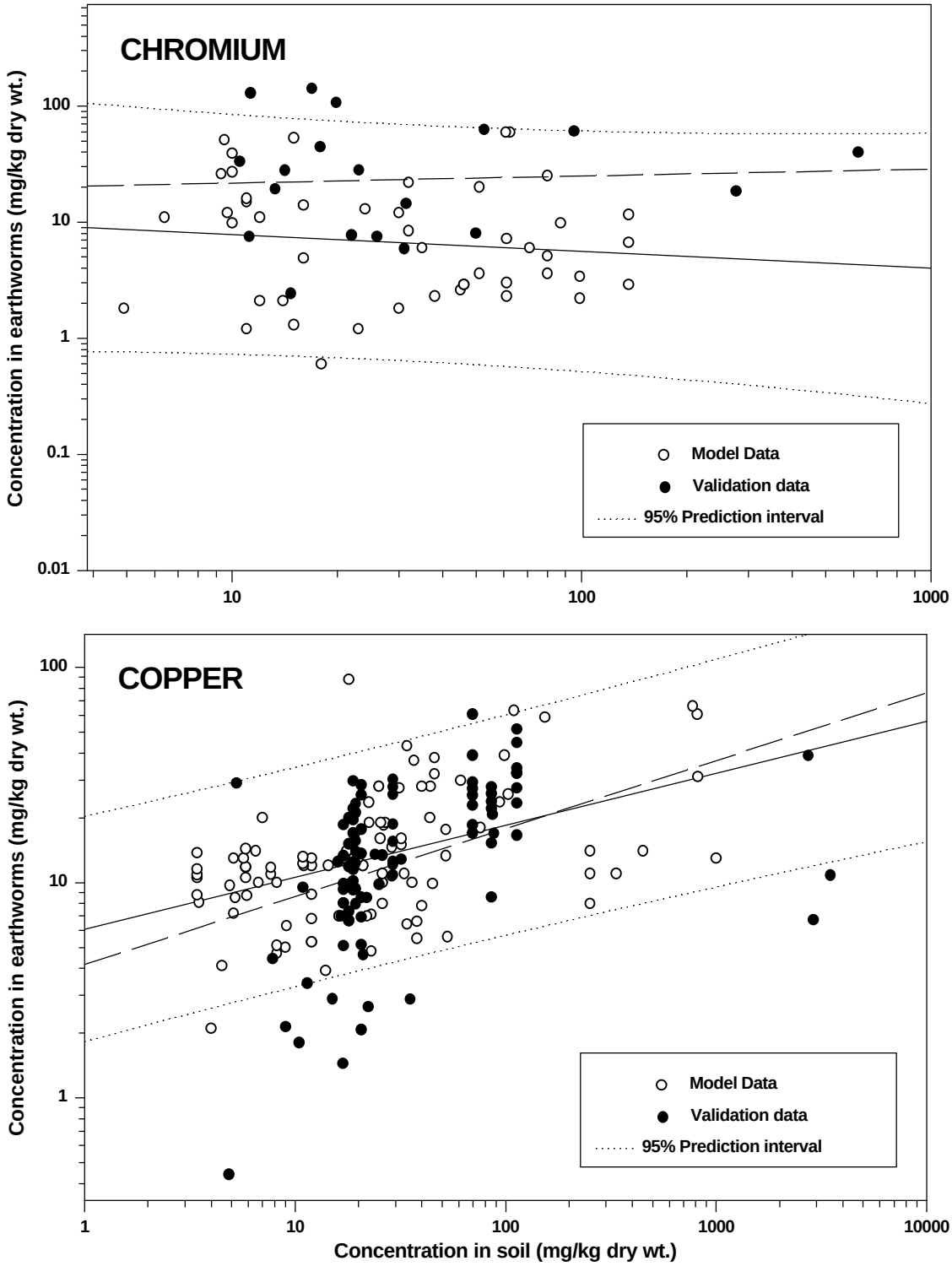

Fig. 2. Scatterplot of model and validation Cr and Cu data. Lines represent simple linear regression models of natural-log-transformed data for both model (solid) and validation (dashed) datasets. Dotted lines represent 95% prediction interval for model data. Regression models for model and validation datasets differed significantly ($p<0.001$) for Cr; models did not differ ($p=0.28$) for Cu.

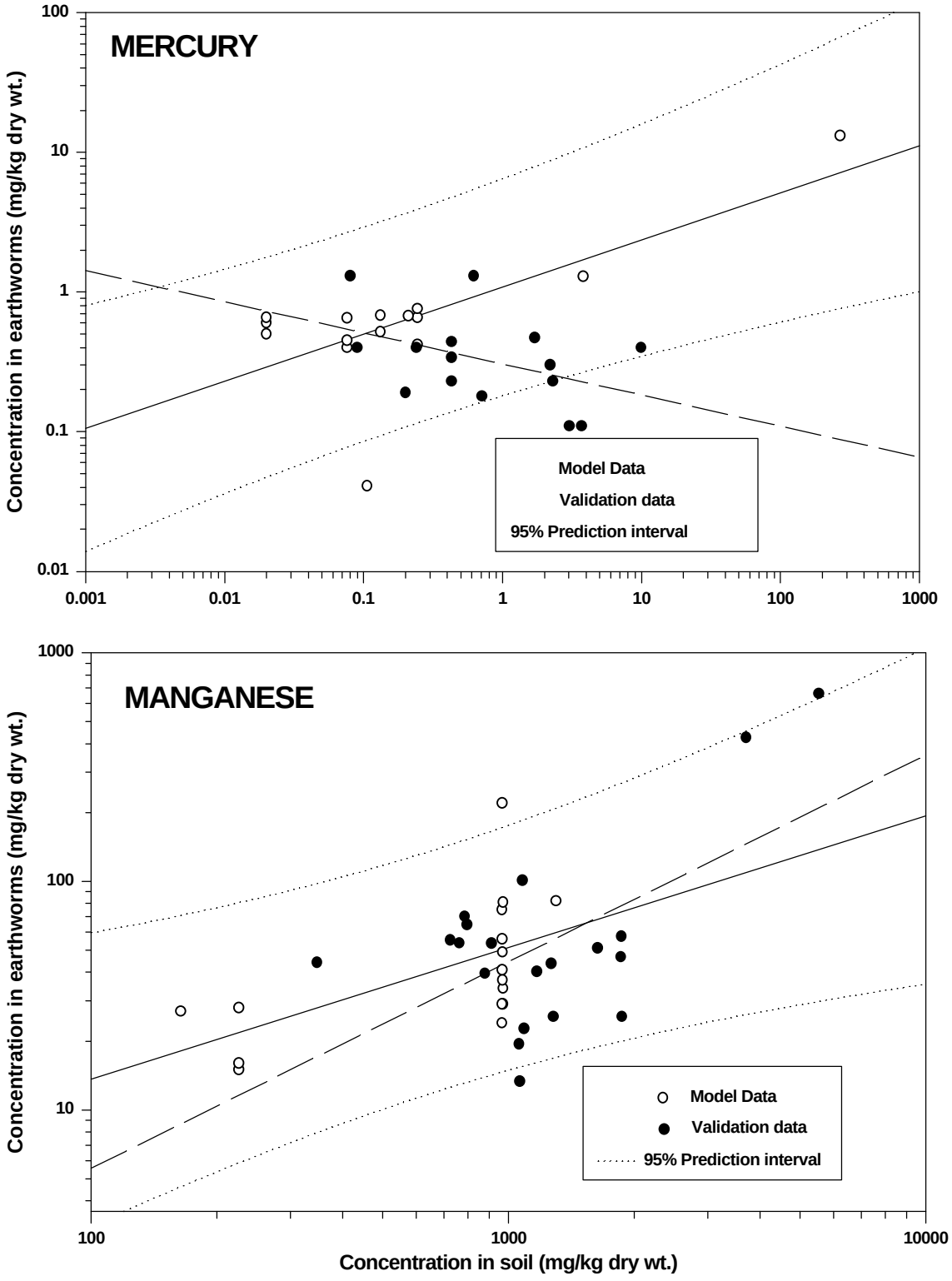

Fig. 3. Scatterplot of model and validation Hg and Mn data. Lines represent simple linear regression models of natural-log-transformed data for both model (solid) and validation (dashed) datasets. Dotted lines represent 95% prediction interval for model data. Regression models for model and validation datasets differed significantly ($p<0.001$) for Hg; models did not differ ($p=0.54$) for Mn.

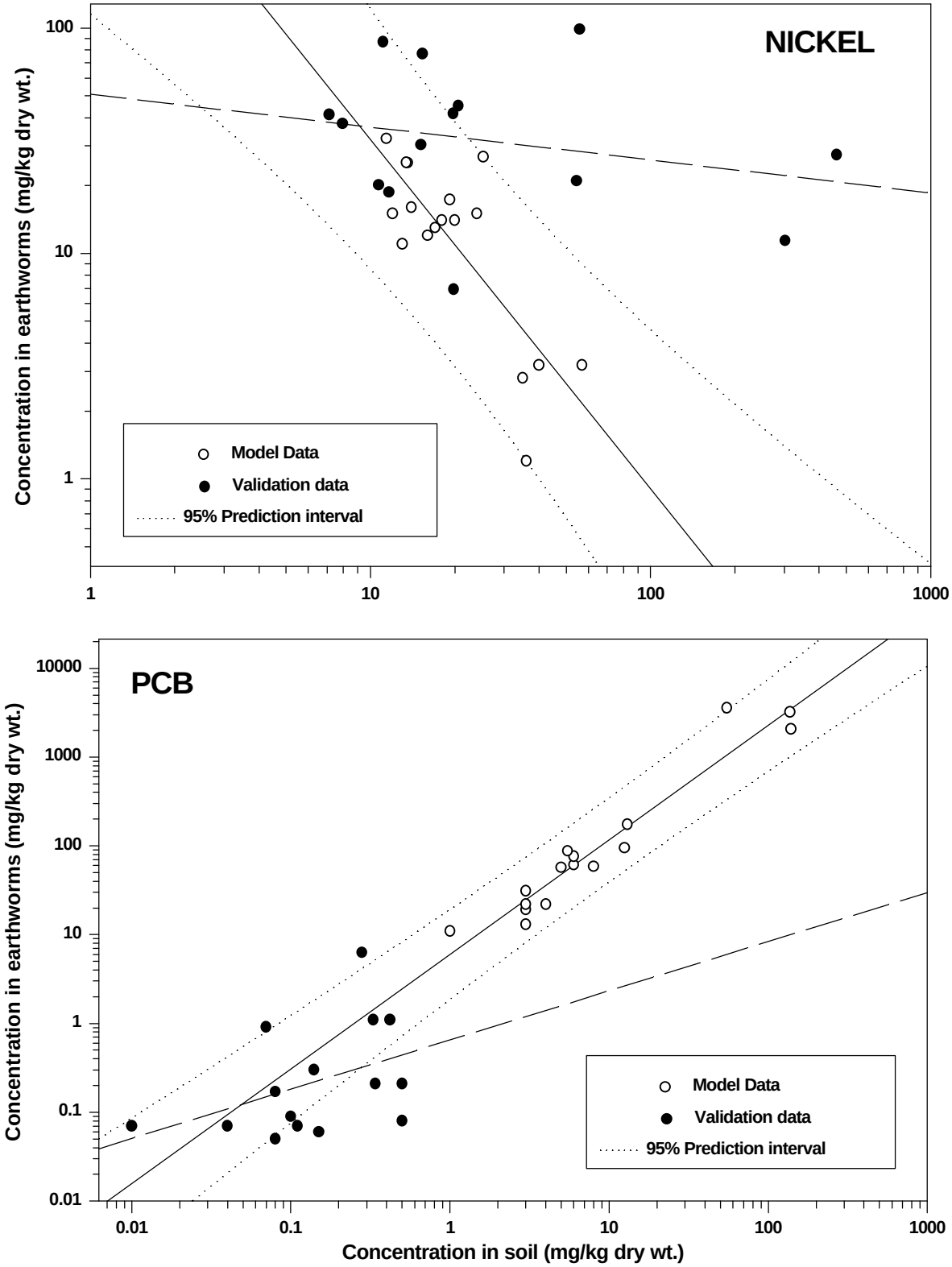

Fig. 4. Scatterplot of model and validation Ni and PCB data. Lines represent simple linear regression models of natural-log-transformed data for both model (solid) and validation (dashed) datasets. Dotted lines represent 95% prediction interval for model data. Regression models for model and validation datasets differed significantly ($p<0.01$) for both Ni and PCB.

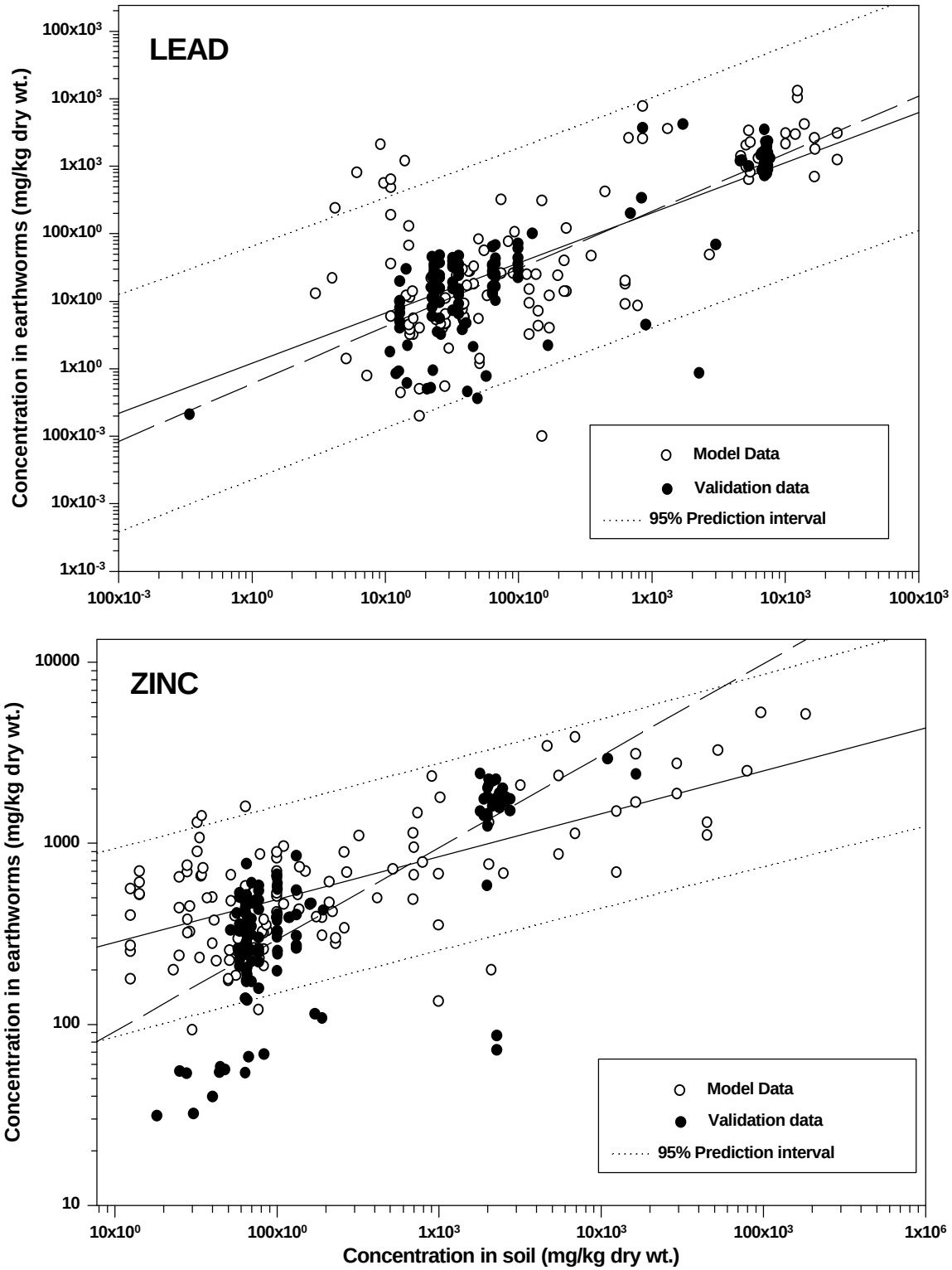

Fig. 5. Scatterplot of model and validation Pb and Zn data. Lines represent simple linear regression models of natural-log-transformed data for both model (solid) and validation (dashed) datasets. Dotted lines represent 95% prediction interval for model data. Regression models for model and validation datasets differed significantly ($p<0.0001$) for Zn; models did not differ ($p=0.49$) for Pb.

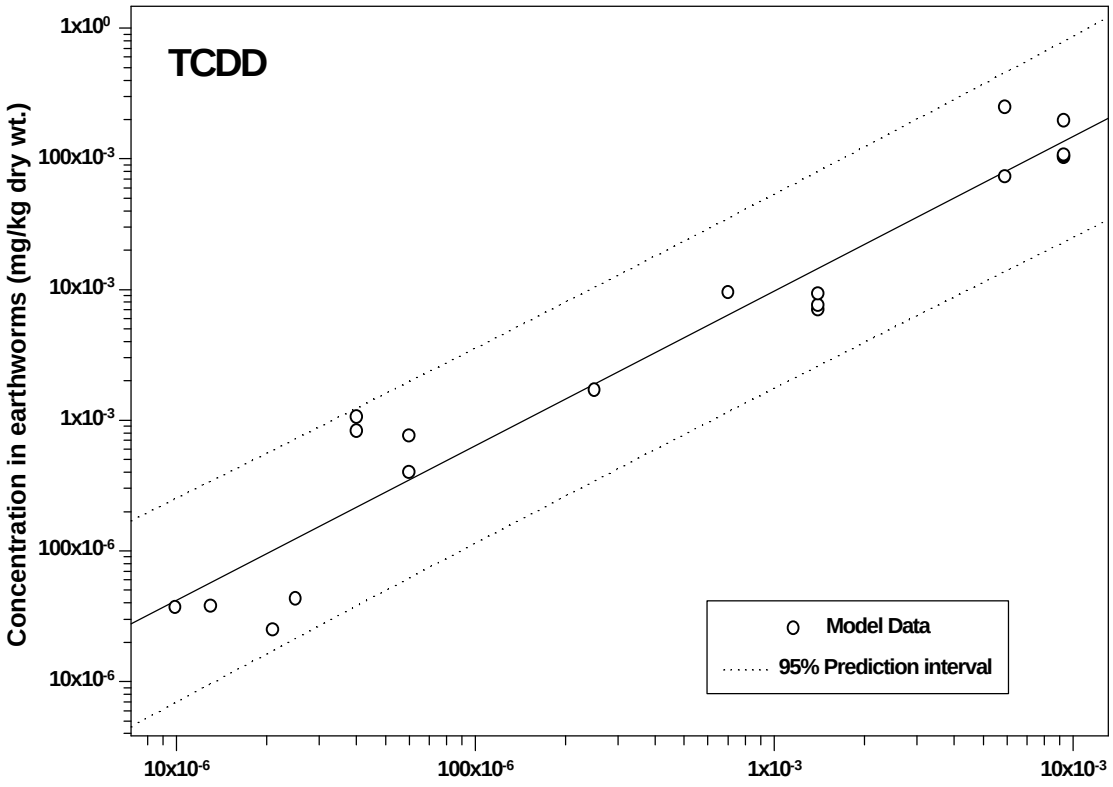

Fig. 6. Scatterplot of model data for TCDD. Line represents simple linear regression model of natural-log-transformed data. Validation data were unavailable for this chemical.

Table 3. Summary statistics for literature-derived soil-to-earthworm UFs

Analyte	N (Studies)	N (Observations)	Mean	Standard Deviation	Minimum	Median	90th Percentile	Maximum	Mean of Natural Log-transformed values	Standard Deviation of Natural Log-transformed values	Distribution
As	3	36	0.2656	0.2116	0.0164	0.2361	0.5214	0.9250			normal
Cd	17	114	27.1682	37.5895	0.4286	14.2603	66.0377	190.0000	2.58768	1.28036	lognormal
Cr	5	48	0.7080	1.1496	0.0212	0.1607	2.7000	5.3680	-1.48636	1.5555	lognormal
Cu	13	103	0.9283	0.9135	0.0130	0.6364	2.2807	4.8890	-0.57464	1.14691	lognormal
Hg	4	15	8.5537	11.0986	0.0488	3.9334	30.0000	33.0000	1.16596	1.77202	lognormal
Mn	2	16	0.0742	0.0551	0.0249	0.0605	0.1646	0.2280	-2.80288	0.62809	lognormal
Ni	3	17	0.9200	0.7418	0.0333	0.7778	1.8881	2.8330			normal
Pb	15	119	6.3297	26.7336	0.0007	0.2250	4.3243	228.2610	-1.10093	2.05196	lognormal
Zn	15	123	8.2364	11.0731	0.0247	3.7816	25.0000	49.5100	1.03218	1.83458	lognormal
PCB	2	16	14.1790	14.4186	4.3333	10.6667	23.4945	65.2270	2.40307	0.64066	lognormal
TCDD	2	19	11.7404	9.8083	1.1905	11.0108	22.2290	42.0680	2.1132	0.8918	lognormal

Table 4. Results of regression of ln (earthworm) on ln (soil)

	N	B0±SE	B1±SE	r^2	P model fit
As	36	-1.747±0.3542^{c}	0.9884±0.1804^{c}	0.47	0.0001
Cd	114	2.8216±0.0766^{c}	0.5512±0.03343^{c}	0.71	0.0001
Cr	48	2.3957±0.653^{c}	-0.146±0.1863^{NS}	0.01	0.44
Cu	103	1.8059±0.1528^{c}	0.2414±0.04503^{c}	0.22	0.0001
Hg	15	0.0781±0.2594^{NS}	0.3369±0.0915^{b}	0.51	0.0028
Mn	16	-0.043±1.3719^{NS}	0.5759±0.2096^{a}	0.35	0.016
Ni	17	7.033±0.9409^{c}	-1.548±0.3097^{c}	0.62	0.0002
Pb	119	0.0752±0.4153^{NS}	0.7612±0.07586^{c}	0.46	0.0001
Zn	123	5.0981±0.1384^{c}	0.2373±0.0239^{c}	0.45	0.0001
PCB	16	1.7903±0.2358^{c}	1.2909±0.09404^{c}	0.93	0.0001
TCDD	19	3.533±0.810^{c}	1.182±0.074^{c}	0.94	0.0001

model: ln(earthworm)=B0+B1(ln[soil])
^{NS} not significant: p>0.05.
^{a} p<0.05.
^{b} p<0.01.
^{c} p<0.001.

Table 5. Results of regression of ln (earthworm) on ln (soil) and pH

Analyte	N	B0±SE	B1±SE	B2±SE	r^2	P model fit
As	36	0.341±1.245^{NS}	1.0908±0.1847^{c}	-0.41611±0.2384^{NS}	0.51	0.0001
Cd	75	3.84±0.5653^{c}	0.5482±0.04668^{c}	-0.15294±0.09318^{NS}	0.67	0.0001
Cu	83	2.087±0.384^{c}	0.2894±0.0517^{c}	-0.07384±0.069^{NS}	0.29	0.0001
Ni	13	2.862±0.6393^{b}	-0.4625±0.2333^{NS}	0.2074±0.0418^{c}	0.72	0.0018
Pb	80	5.233±1.2657^{c}	0.7253±0.1122^{c}	-0.82195±0.2299^{c}	0.36	0.0001
Zn	86	4.453±0.3485^{c}	0.234±0.02958^{c}	0.12845±0.05867^{a}	0.49	0.0001

model: ln(earthworm)=B0+B1(ln[soil])+B2(pH)
^{NS} not significant: p>0.05.
^{a} p<0.05.
^{b} p<0.01.
^{c} p<0.001.

Table 6. Results of regression of ln (earthworm) on ln (soil) and ln (soil Ca)

Analyte	N	B0±SE	B1±SE	B2±SE	r^2	P model fit
Cd	56	5.8213±0.5896^{c}	0.8232±0.07248^{c}	-0.39236±0.0814^{c}	0.81	0.0001
Cu	32	1.8809±0.3383^{c}	0.1054±0.1002^{NS}	0.07289±0.0758^{NS}	0.36	0.0016
Mn	16	3.5354±3.7592^{NS}	0.3611±0.2966^{NS}	-0.24575±0.2404^{NS}	0.4	0.04
Pb	39	0.5998±0.9629^{NS}	1.2419±0.1174^{c}	-0.42872±0.1034^{c}	0.76	0.0001
Zn	54	5.8957±0.3803^{c}	0.2797±0.0648^{c}	-0.10903±0.0836^{NS}	0.5	0.0001

model: ln(earthworm)=B0+B1(ln[soil])+B2(ln[soil Ca])
^{NS} not significant: p>0.05.
^{a} p<0.05.
^{b} p<0.01.
^{c} p<0.001.

Table 7. Results of regression of ln (earthworm) on ln (soil), ln (soil Ca), and pH

Analyte	N	B0±SE	B1±SE	B2±SE	B3±SE	r^2	P model fit
Cd	29	5.29±1.0326[c]	0.89±0.1196[c]	-0.5095±0.1462[b]	0.22896±0.1411[NS]	0.88	0.0001
Cu	27	2.4±0.3263[c]	0.29±0.05869[c]	0.0512±0.04412[NS]	-0.12232±0.0581[a]	0.86	0.0001
Pb	12	3.61±1.0152[b]	0.9±0.1402[c]	0.1191±0.2115[NS]	-0.82338±0.3315[a]	0.88	0.0005
Zn	29	4.16±0.74[c]	0.41±0.1289[b]	-0.2986±0.2083[NS]	0.42723±0.2067[a]	0.75	0.0001

model: ln(earthworm)=B0+B1(ln[soil])+B2(ln[soil Ca])+B3(pH)
[NS] not significant: $p>0.05$.
[a] $p<0.05$.
[b] $p<0.01$.
[c] $p<0.001$.

3.2 VALIDATION RESULTS

Data for model validation were available for all nine organic chemicals and for PCBs. No validation data were available for TCDD. While UFs and models for Se were not developed as part of the current study, data were available to validate two published Se uptake models.

Comparison of simple regression models for the "model" and "validation" data indicated that the models differed significantly ($p<0.01$) for 7 of 10 chemicals: As and Cd (Fig. 1), Cr (Fig. 2), Hg (Fig. 3), Ni and PCB (Fig. 4), and Zn (Fig. 5). No significant differences ($p \geq 0.28$) between "model" and "validation" regressions were observed for Cu (Fig. 2), Mn (Fig. 3), and Pb (Fig. 5).

Based on the full validation dataset, significant differences between measured and estimated concentrations were observed for 7 of 10 chemicals for the median UF, 4 of 9 chemicals for the simple regression model, and 4 of 5 chemicals for the models from Neuhauser et al. (1995; Table 8). The median UF and simple regression models overestimated concentrations for more than 50% of observations for 7 of 10 and 7 of 9 chemicals, respectively (Table 8). In contrast, models from Neuhauser et al. (1995) underestimated concentrations for >50% of observations for 5 of 5 chemicals (Table 8). For all three estimation methods, median proportion deviations of estimated values from measured values ranged from a minimum of -0.005 for the simple regression model for Cu to a maximum of -6.35 for the median UF for Hg (Table 8). Using the selection criteria outlined previously, the best estimates for As, Cu, Hg, Mn, PCB, Pb, and Zn were produced using the simple regression model, for Cd using the model from Neuhauser et al. (1995), and for Cr using the median UF (Table 8). Graphical presentations of measured an estimated concentrations of analytes in earthworm for given soil concentrations are presented in Figs. 7 through 17.

Because soil pH and soil Ca were reported for few observations in the "validation" dataset, fewer data were available for validation of the multiple regression models that include these variables. Using this reduced dataset, significant differences between measured and estimated concentrations were observed for 4 of 6, 1 of 6, 2 of 6, 4 of 4, and 4 of 4 chemicals for the median UF, simple regression, multiple regression w/pH, multiple regression with Ca, and the multiple regression with both pH and Ca, respectively (Table 9). Of the five estimation methods, the multiple regression model that included pH generated estimates with the smallest median and range PD values for As, Cd, and Cu, while the simple regression model generated estimates with the smallest median and range PD values for Pb and Zn (Table 9). Comparison of estimates using the Wilcoxon signed-rank test indicated that while

estimates from the simple regression and the regression with pH differed significantly for As ($p=0.002$), estimates did not differ significantly for Cd, Cu, Pb, or Zn ($p>0.05$).

Ni concentrations in earthworms were not estimated well by any method (Fig. 13). Using the whole validation dataset, Ni estimates generated by the median UF and by the model from Neuhauser et al. (1995) model did not differ significantly from measured values, while those based on the simple regression model did (Table 8). Using the restricted validation dataset, significant differences between estimated and measured values were observed for the median UF and the multiple regression including pH; in contrast, no differences were observed for estimates from the simple regression model (Table 9). When viewed graphically, it is clear that estimates from the median UF, simple regression and the Neuhauser et al. (1995) regression models do not reflect the measured data (Fig. 13). In contrast, while the multiple regression model that included pH underestimated most observations, the estimates generated by this model more closely reflect measured values than those from any other method.

Table 8. Comparison of quality of general estimation methods as determined by the proportional deviation (PD) of the estimated values from measured values. PD = (measured - estimate)/measured. Negative PD values indicate overestimates while positive PD values indicate underestimates

		Median UF		Simple regression model		Regression model from Neuhauser et al. 1995	
Analyte	N	Median PD (range)	% Over Estimated	Median PD (range)	% Over Estimated	Median PD (range)	% Over Estimated
As	17	-1.62^{NS} (-41.35 to 0.74)	65	-0.89^{NS} (-28.96 to 0.81)	65		
Cd	112	-1.98^{c} (-55.34 to 0.85)	88	-4.73^{NS} (-43.15 to 0.79)	74	-0.06^{a} (-6.91 to 0.94)	40
Cr	19	0.76^{b} (-1.49 to 0.99)	16				
Cu	94	-0.51^{c} (-273.62 to 0.88)	71	-0.005^{NS} (-19.25 to 0.72)	51	0.22^{c} (-19.68 to 0.87)	29
Hg	15	-6.35^{c} (-131.31 to 0.76)	87	-2.31^{b} (-14.28 to 0.64)	87		
Mn	20	-0.55^{NS} (-3.85 to 0.52)	60	-0.24^{NS} (-2.99 to 0.79)	55		
Ni	14	0.60^{NS} (-19.60 to 0.90)	29	0.71^{a} (-0.60 to 1.00)	36	0.75^{NS} (-11.09 to 0.94)	29
PCB	16	-5.10^{b} (-65.67 to 0.53)	88	-0.78^{a} (-29.61 to 0.82)	81		
Pb	126	0.29^{a} (-590.28 to 0.95)	37	0.19^{c} (-446.97 to 0.95)	40	0.28^{c} (-369.90 to 0.96)	33
Zn	121	-0.32^{c} (-118.39 to 0.68)	65	-0.28^{NS} (-13.25 to 0.60)	60	0.93^{c} (0.09 to 0.97)	0

^{NS} Estimate not significantly different from measured as determined by Wilcoxon signed-rank test.
^{a} Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p<0.05$.
^{b} Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p<0.01$.
^{c} Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p<0.001$.

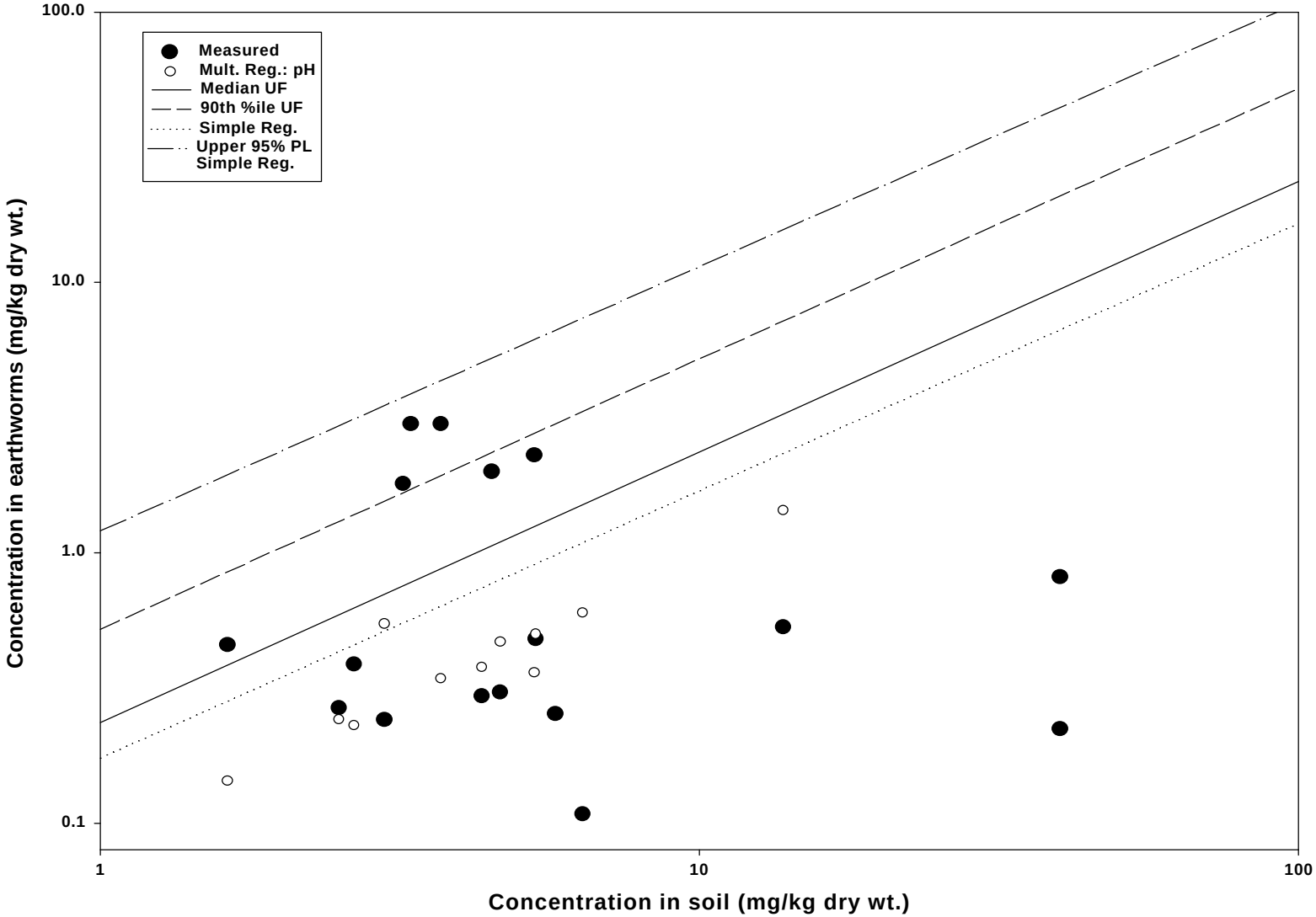

Fig. 7. Comparison of measured As concentrations in earthworms from the validation dataset to estimated As concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured As concentrations in earthworms and estimates generated by the multiple regression model that included soil pH are represented as point values. Estimates of As concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression model from this study are represented as lines.

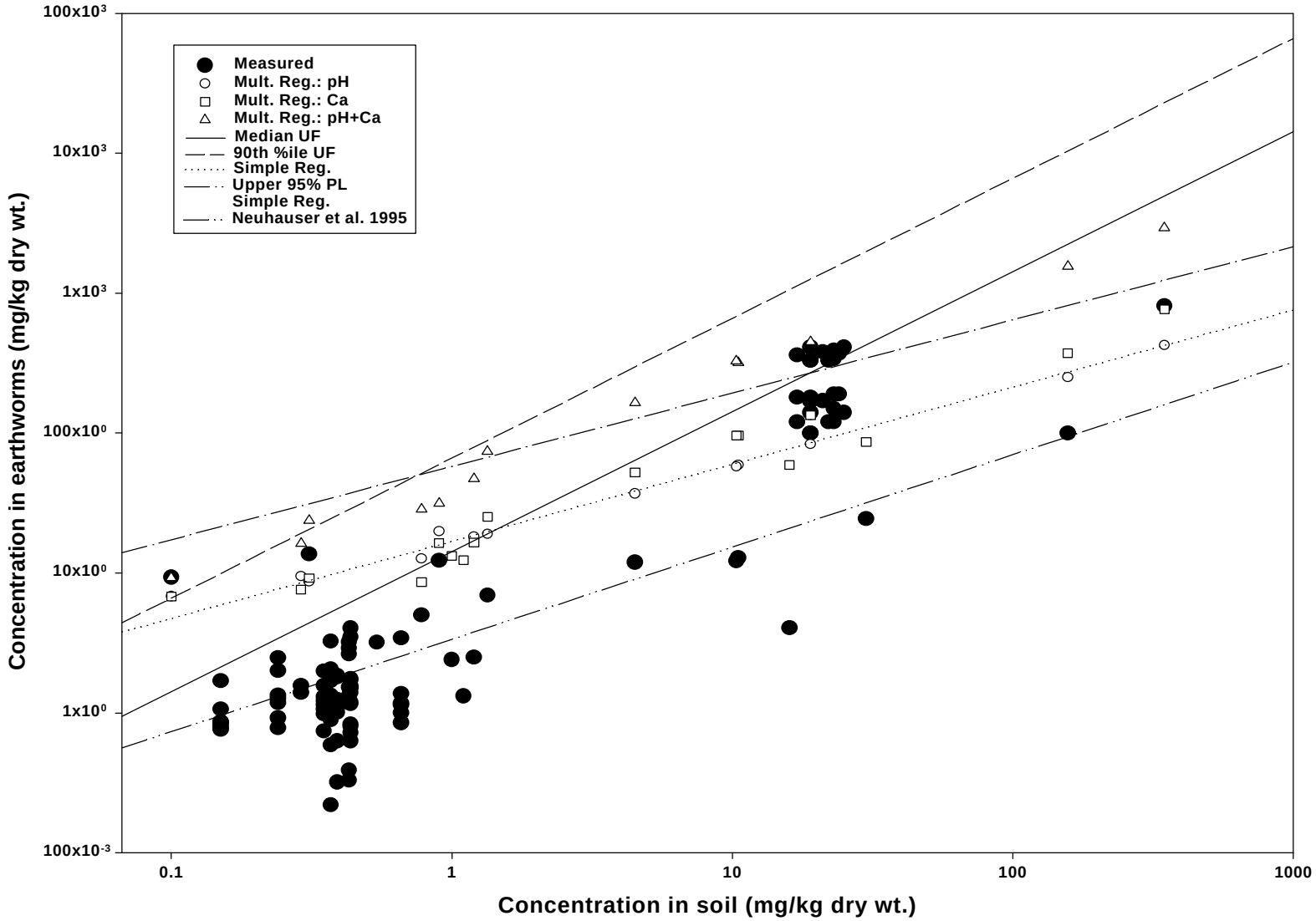

Fig. 8. Comparison of measured Cd concentrations in earthworms from the validation dataset to estimated Cd concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured Cd concentrations in earthworms and estimates generated by the multiple regression models that included soil pH, soil Ca, and both pH and Ca, and the regression model from Beyer et al. (1987) are represented as point values. Estimates of Cd concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression models from this study and from Neuhauser et al. (1995) are represented as lines.

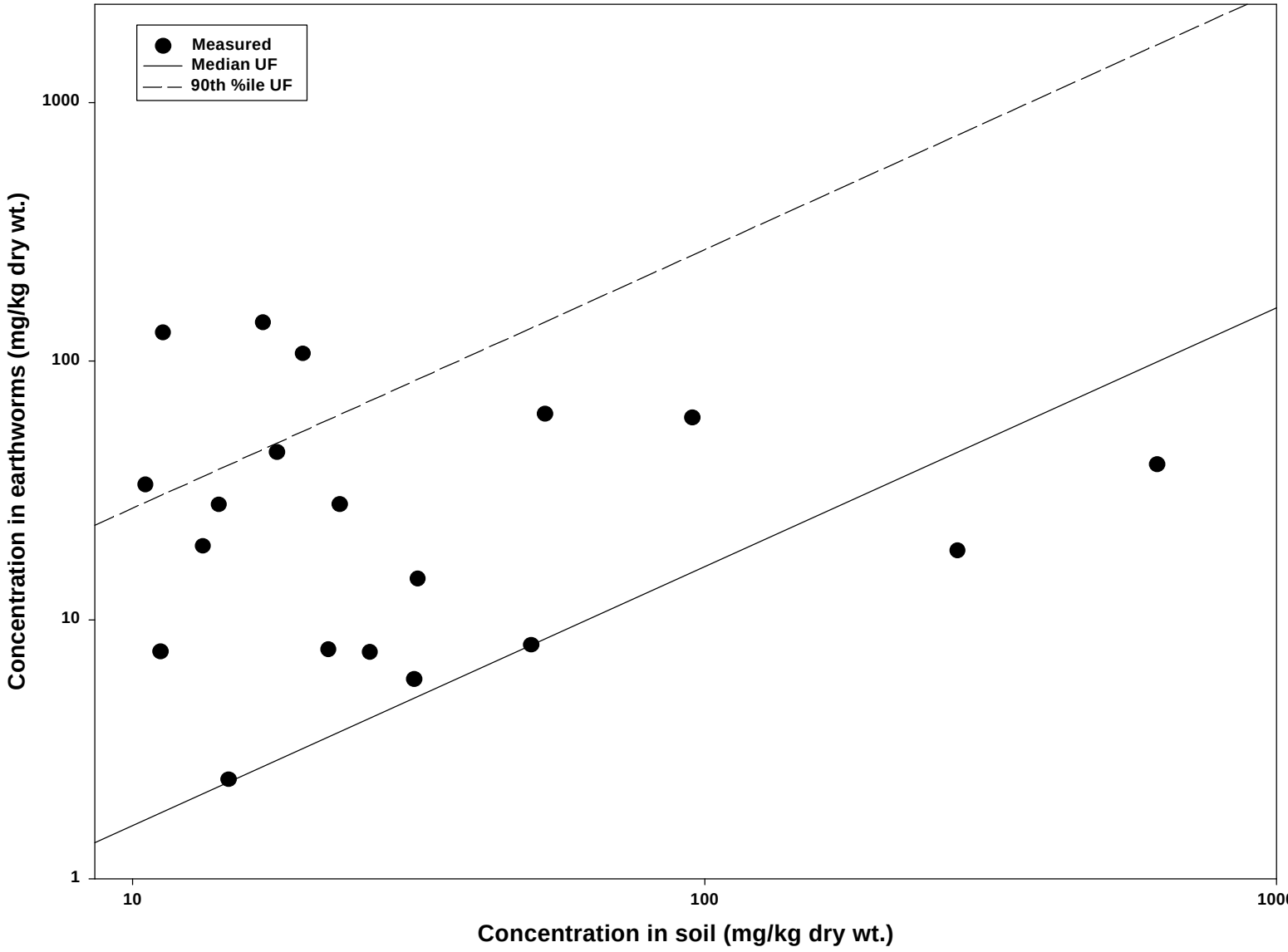

Fig. 9. Comparison of measured Cr concentrations in earthworms from the validation dataset to estimated Cr concentrations in earthworms. Estimates were generated by applying UFs to the measured soil concentration from the validation dataset. Measured Cr concentrations in earthworms are represented as point values. Estimates of Cr concentrations in earthworms generated by the median and 90th percentile UFs are represented as lines.

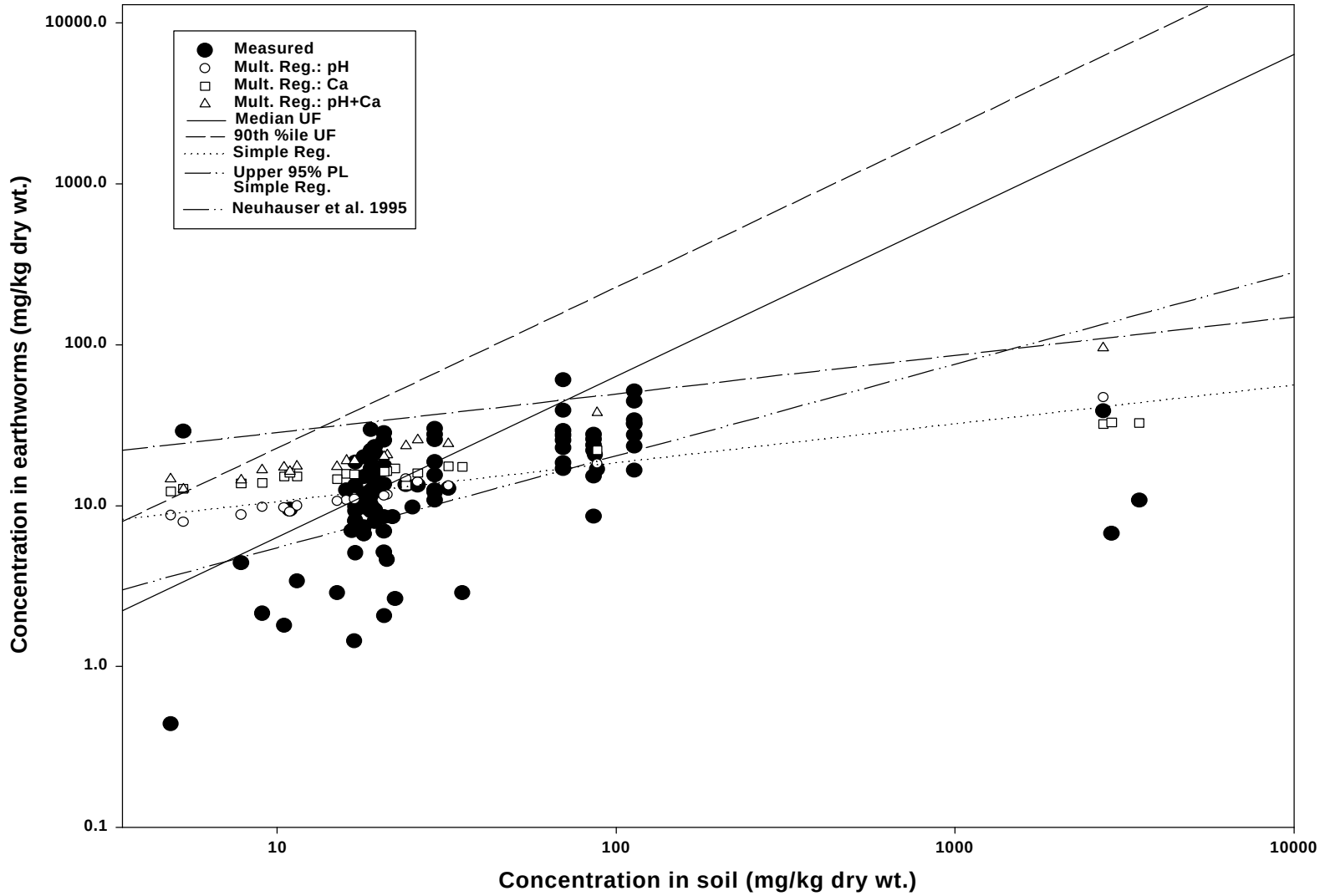

Fig. 10. Comparison of measured Cu concentrations in earthworms from the validation dataset to estimated Cu concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured Cu concentrations in earthworms and estimates generated by the multiple regression models that included soil pH, soil Ca, and both pH and Ca are represented as point values. Estimates of Cu concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression models from this study and from Neuhauser et al. (1995) are represented as lines.

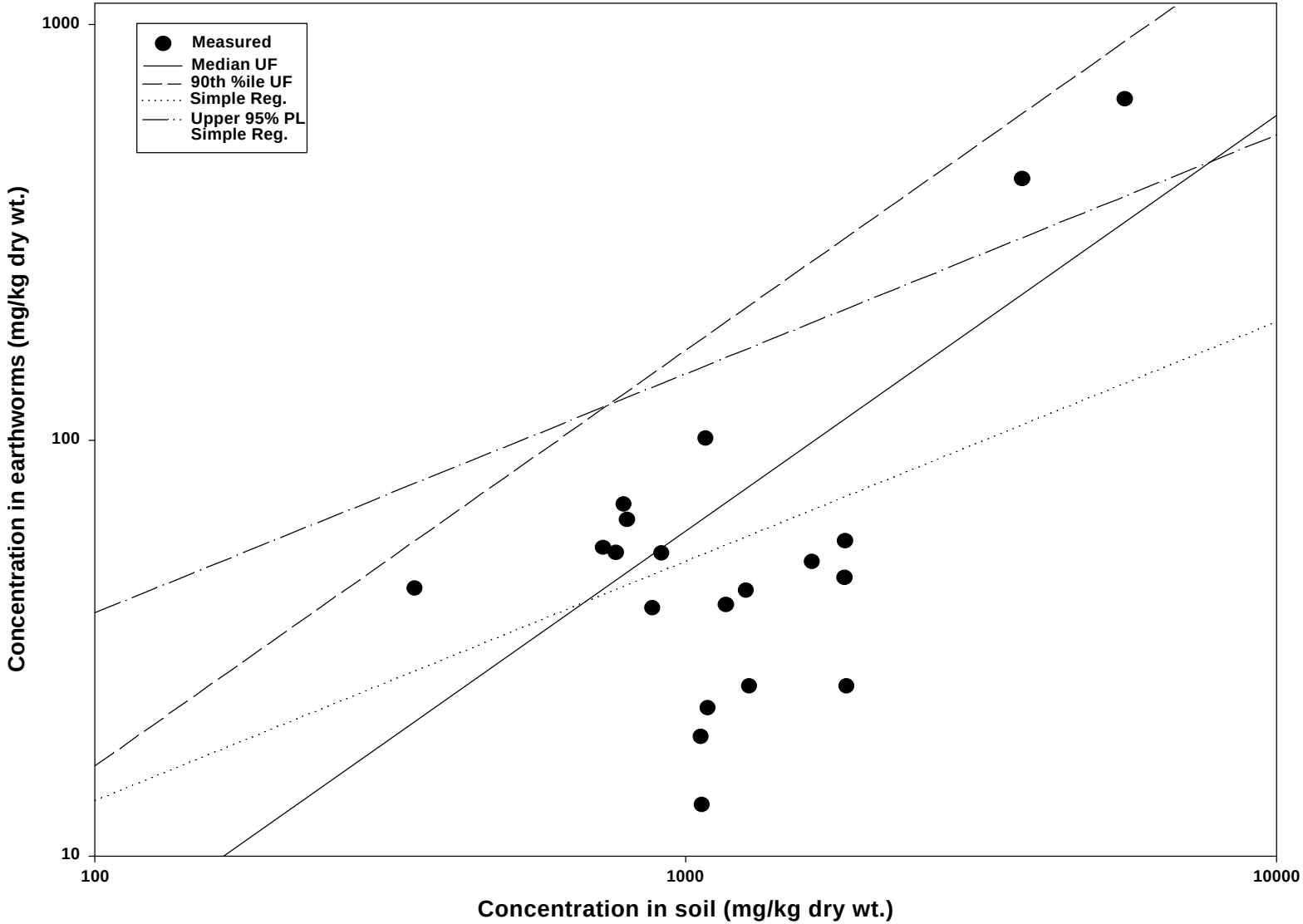

Fig. 11. Comparison of measured Hg concentrations in earthworms from the validation dataset to estimated Hg concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured Hg concentrations in earthworms are represented as point values. Estimates of Hg concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression model from this study are represented as lines.

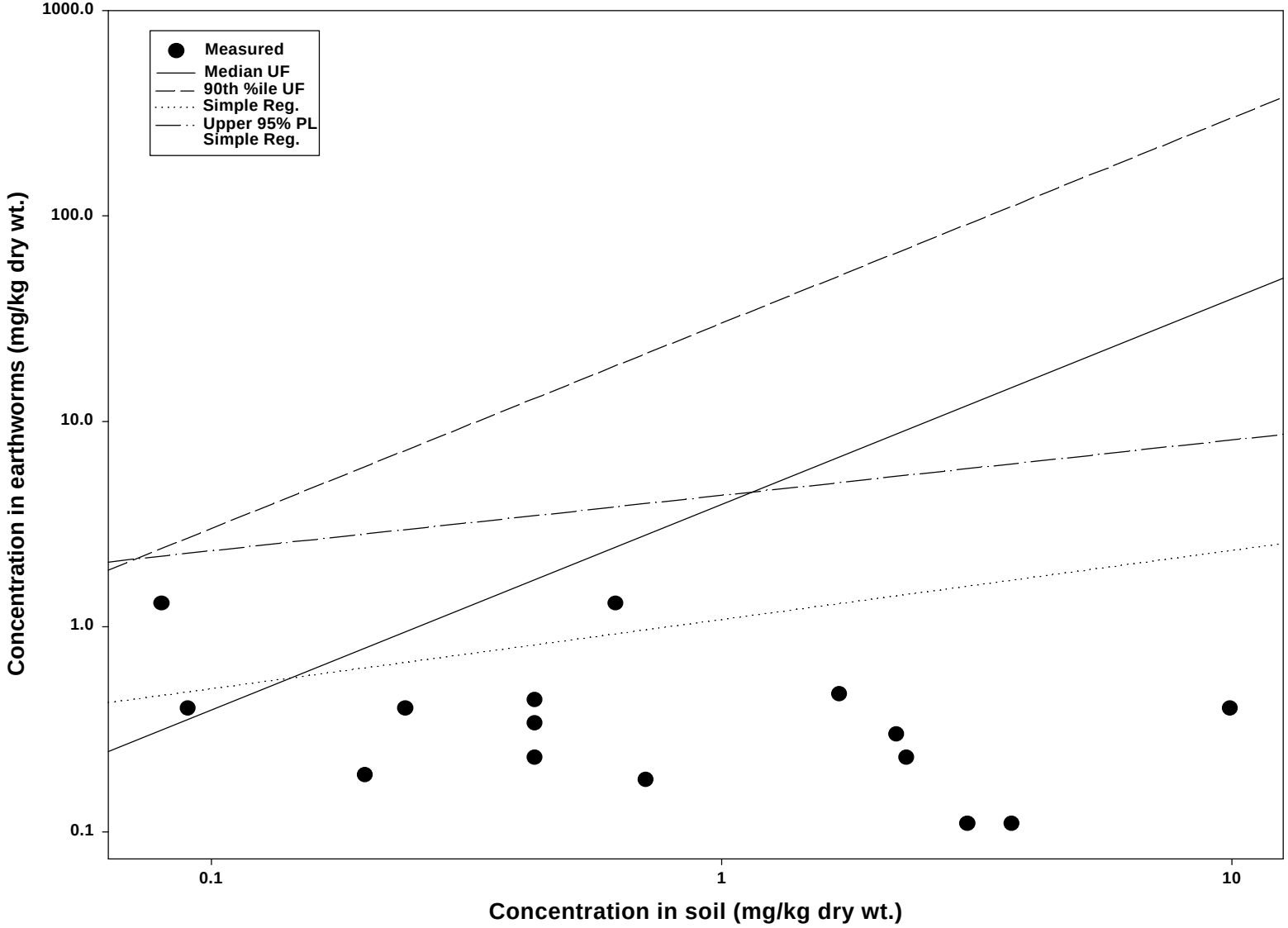

Fig. 12. Comparison of measured Mn concentrations in earthworms from the validation dataset to estimated Mn concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured Mn concentrations in earthworms are represented as point values. Estimates of Mn concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression model from this study are represented as lines.

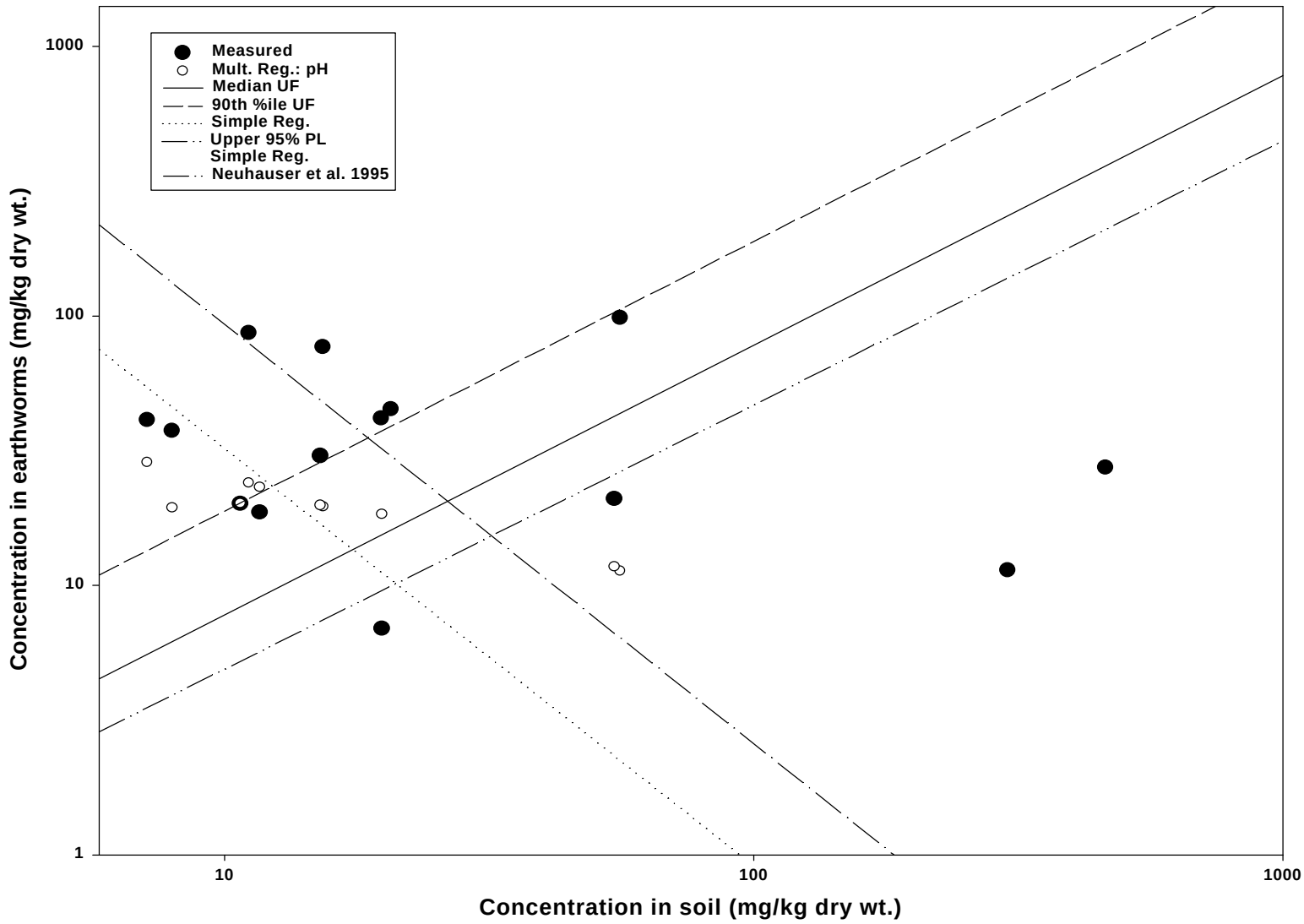

Fig. 13. Comparison of measured Ni concentrations in earthworms from the validation dataset to estimated Ni concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured Ni concentrations in earthworms and estimates generated by the multiple regression model that included soil pH are represented as point values. Estimates of Ni concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression models from this study and from Neuhauser et al. (1995) are represented as lines.

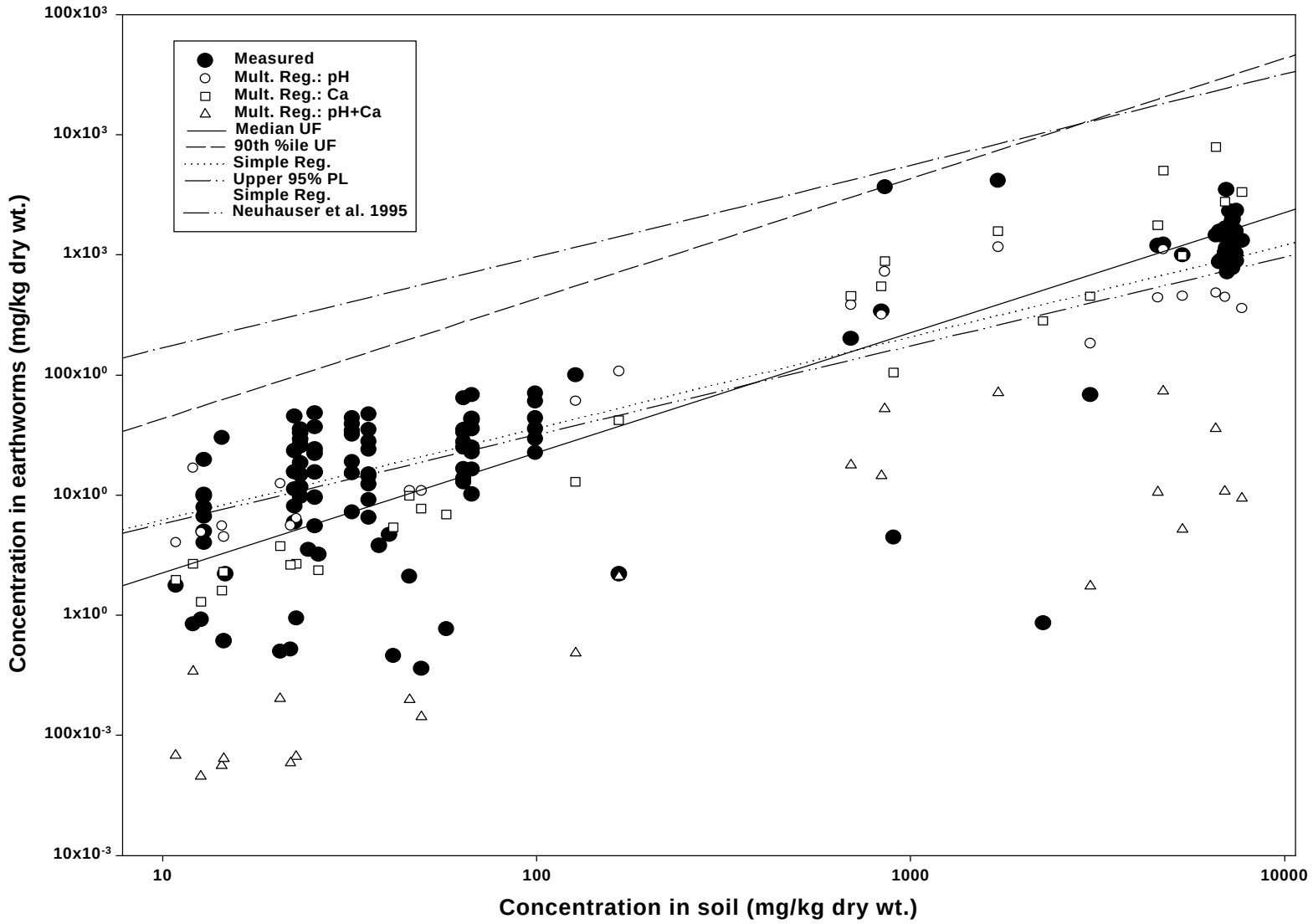

Fig. 14. Comparison of measured Pb concentrations in earthworms from the validation dataset to estimated Pb concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured Pb concentrations in earthworms and estimates generated by the multiple regression models that included soil pH, soil Ca, and both pH and Ca are represented as point values. Estimates of Cu concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression models from this study and from Neuhauser et al. (1995) are represented as lines.

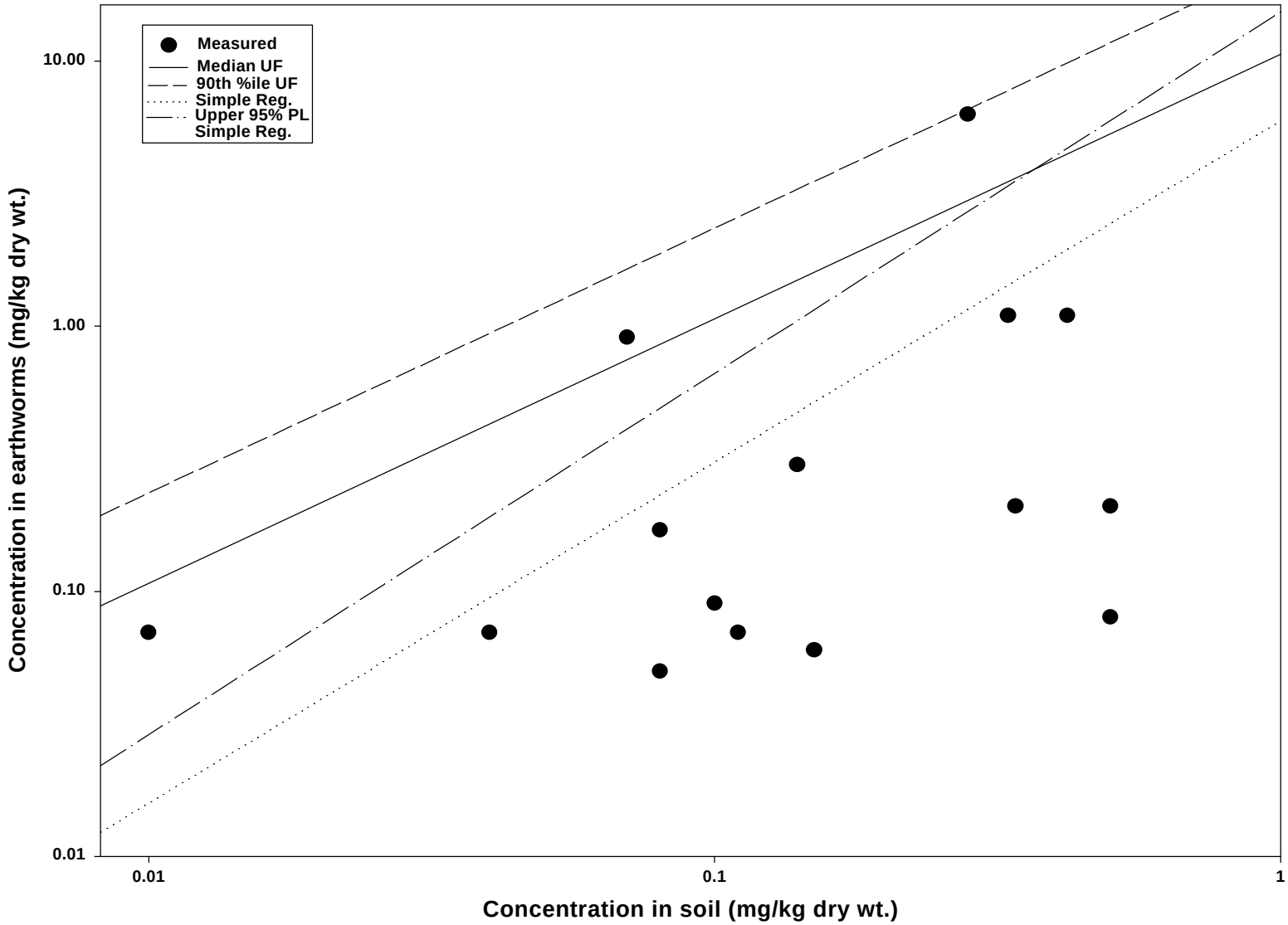

Fig. 15. Comparison of measured PCB concentrations in earthworms from the validation dataset to estimated PCB concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured PCB concentrations in earthworms are represented as point values. Estimates of PCB concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression model from this study are represented as lines.

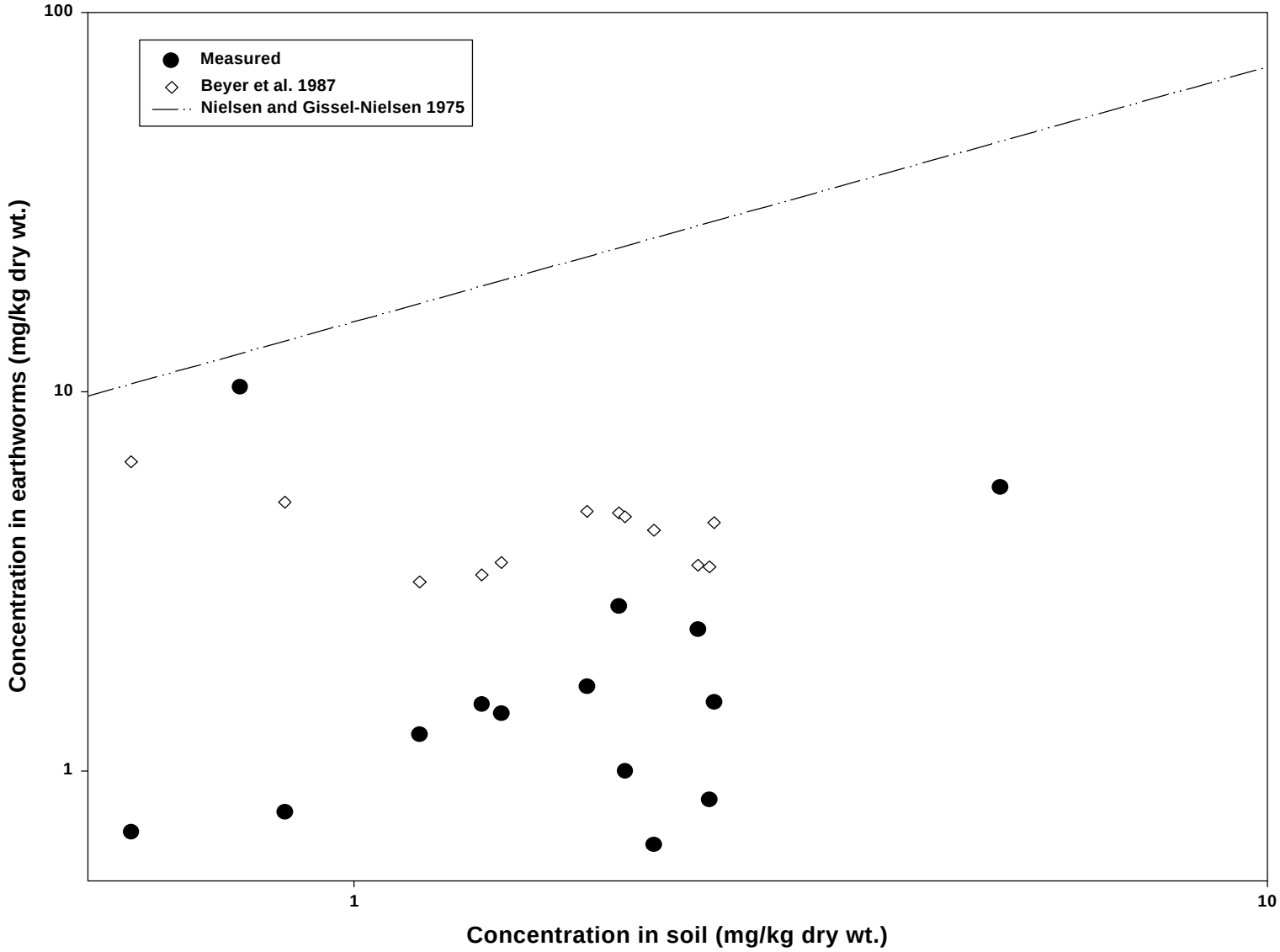

Fig. 16. Comparison of measured Se concentrations in earthworms from the validation dataset to estimated Se concentrations in earthworms. Estimates were generated by applying literature derived models to the measured soil concentration from the validation dataset. Measured Se concentrations in earthworms and estimates generated by the regression model from Beyer et al. (1987) are represented as point values. Estimates of Se concentrations in earthworms generated by the regression model from Nielsen and Gissel Nielsen (1975) are represented as lines.

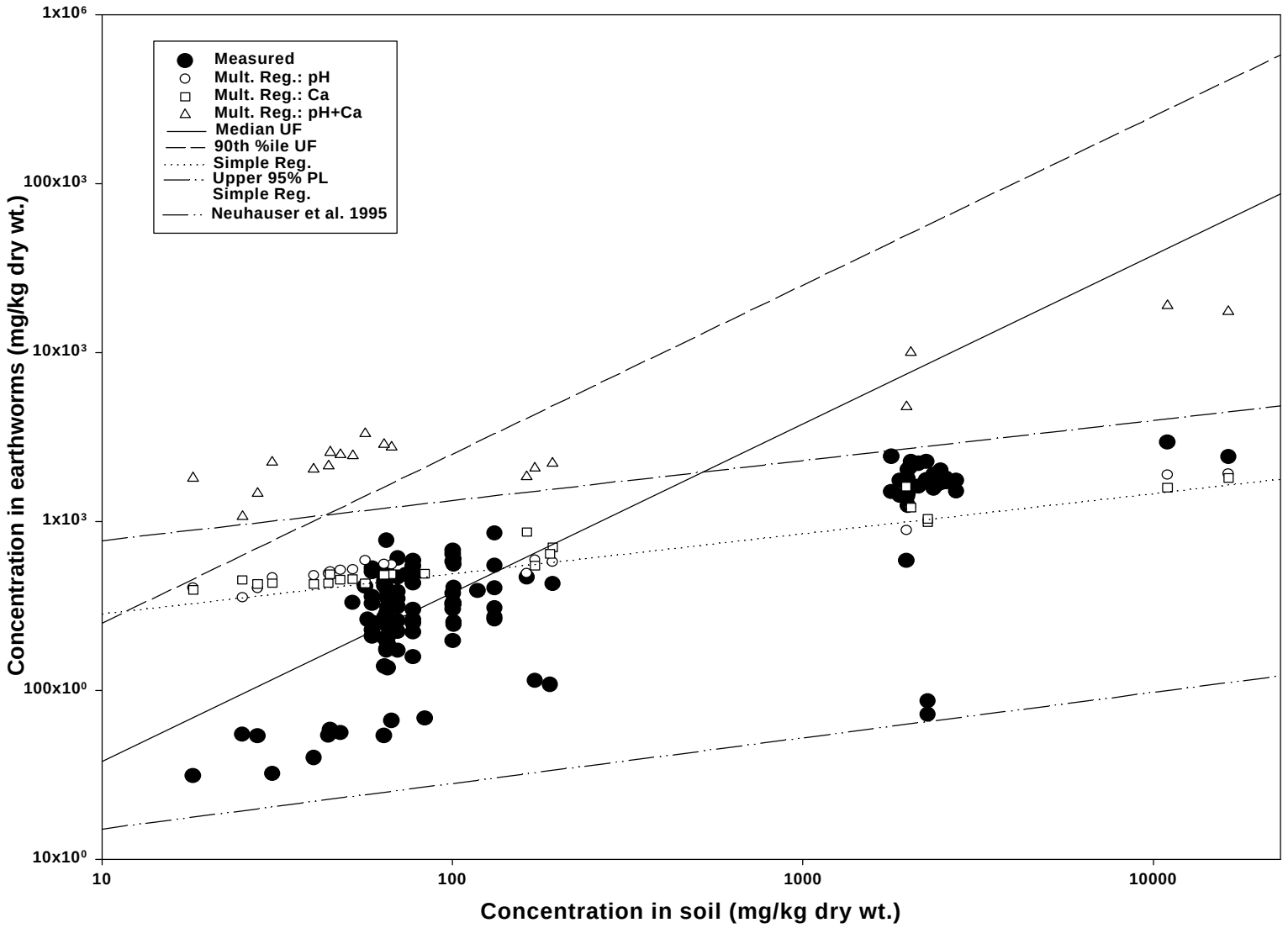

Fig. 17. Comparison of measured Zn concentrations in earthworms from the validation dataset to estimated Zn concentrations in earthworms. Estimates were generated by applying UFs or models to the measured soil concentration from the validation dataset. Measured Zn concentrations in earthworms and estimates generated by the multiple regression models that included soil pH, soil Ca, and both pH and Ca are represented as point values. Estimates of Zn concentrations in earthworms generated by the median UF, 90th percentile UF and simple regression models from this study and from Neuhauser et al. (1995) are represented as lines.

Table 9. Comparison of quality of general estimation methods as determined by the proportional deviation (PD) of the estimated values from measured values. PD = (measured-estimate)/measured. Negative PD values indicate overestimates while positive PD values indicate underestimates. Only observations from the "validation" dataset for which soil pH and soil Ca measurements were available were included

		Median UF		Simple regression model		Regression model w/ pH		Regression model w/ Ca		Regression model w/ pH and Ca	
Analyte	n	Median PD (range)	% Over Estimated	Median PD (range)	% Over Estimated	Median PD (range)	% Over Estimated	Median PD (range)	% Over Estimated	Median PD (range)	% Over Estimated
As	11	-1.62[NS] (-12.95 to 0.71)	73	-0.89[NS] (-9.08 to 0.79)	73	-0.04[NS] (-4.57 to 0.89)	56				
Cd	13	-1.76[b] (-21.39 to 0.85)	85	-1.85[NS] (-6.43 to 0.50)	69	-1.52[NS] (-6.25 to 0.51)	69	-2.65[a] (-6.89 to 0.33)	69	-9.35[c] (-25.93 to 0.01)	92
Cu	17	-1.69[b] (-43.82 to 0.88)	82	-1.26[b] (-19.25 to 0.69)	76	-0.99[b] (-18.80 to 0.73)	82	-2.12[c] (-26.89 to 0.56)	82	-2.27[c] (-32.26 to 0.56)	94
Ni	10	0.60[b] (-1.23 to 0.90)	20	0.11[NS] (-0.60 to 0.98)	50	0.39[a] (-1.65 to 0.89)	30				
Pb	23	-0.38[NS] (-29.71 to 0.95)	65	0.13[NS] (-57.05 to 0.95)	48	-0.91[NS] (-47.74 to 0.81)	52	-1.65[a] (-20.50 to 0.95)	78	0.96[c] (0.05 to 1.00)	0
Zn	19	-2.23[b] (-24.67 to 0.48)	89	-5.41[NS] (-10.49 to 0.56)	84	-5.43[NS] (-13.46 to 0.46)	84	-6.35[a] (-12.42 to 0.47)	84	-18.51[c] (-68.66 to -2.94)	100

[NS] Estimate not significantly different from measured as determined by Wilcoxon signed-rank test.
[a] Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p < 0.05$.
[b] Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p < 0.01$.
[c] Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p < 0.001$.

No models were developed for Se as part of this study, but two were identified in the literature (Beyer et al. 1987, Nielsen and Gissel-Nielsen 1975). While both models significantly ($p>0.001$) overestimated 100% of measured values, the closest estimates were generated using the model from Beyer et al. (1987; Fig. 16). Median (range) PD for estimates generated by the models from Beyer et al. (1987) and Nielsen and Gissel-Nielsen (1975) were -1.71 (-5.76 to -0.48) and -12.36 (-37.95 to -7.59), respectively.

Among conservative estimation methods, both the 90th percentile UF and the 95% UPL significantly overestimated concentrations in earthworms for all analytes except Ni (Table 10). [**Note:** methods and parameters for calculating the 95% UPL are presented in Appendix D]. The 95% UPL produced the best, conservative estimate (i.e., smallest negative median and range PD) for Cd, Cu, Hg, PCB, and Zn with percent overestimates ranging from 81% to 100% (Table 10). The best conservative estimates for concentrations of As and Pb in earthworms were generated by the 90th percentile UF. Neither method produced a good conservative estimate for Ni; percent overestimation did not exceed 50% for either method (Table 10). Because a regression model could not be fit for Cr, only the 90th percentile UF was available for this analyte.

3.3 FINAL UFs AND MODELS

Final UFs and regression models, incorporating data from both the "model" and "validation" datasets, were calculated for all analytes. UFs based on the combined dataset were, in general, similar to those based only on the "model" dataset (Table 11). UFs for all analytes, except As and Ni, were lognormally distributed. Median UFs for As, Cr, Cu, Mn, Pb, and Se were <1 while those for Cd, Hg, Zn, PCB, and TCDD were >1 (Table 11). However, for Ni, the median UF increased from 0.78 to 1.058 in the combined dataset.

With the exception of Ni and Hg, results of simple regression analyses differed little between the "model" and combined datasets. For Ni and Hg, significant model fits that had been obtained using the "model" dataset were not obtained with the combined dataset (Table 12). For Se, using all 14 available observations did not result in a significant fit (Table 12). However, by excluding a single outlying observation (see Fig. 16), a significant model fit was obtained (Table 12).

Among models that included soil pH, pH dropped out as a significant variable for Zn while entering as a contributor for Cd; for Ni the model fit was no longer significant (Table 13). For models that include soil Ca, soil Ca entered as a significant variable for Zn, while the chemical concentration in soil was significant for Cu and Mn (Table 14). In the multiple regression models that included both soil pH and Ca, soil pH and intercept dropped out as significant parameters for Cu and Pb, respectively, while soil Ca entered as significant for Zn (Table 15). In virtually all cases, r^2 values declined with the inclusion of the "validation" data in both the simple linear and multiple regression models.

In addition to the analytes represented in both the "model" and "validation" datasets, another 20 analytes were represented in the "validation" dataset but not in the "model" dataset. Summary statistics for UFs for these analytes and scatterplots for chemicals with 5 or more observations are presented in Appendix C, Table C-1 and Figs C-1 to C-4.

Table 10. Comparison of quality of conservative estimation methods as determined by the proportional deviation (PD) of the estimated values from measured values.

PD = (measured-estimate)/measured. Negative PD values indicate overestimates while positive PD values indicate underestimates.

		90th Percentile UF		Upper 95% Prediction Limit for Simple regression model	
Analyte	N	Median PD (range)	% Over Estimated	Median PD (range)	% Over Estimated
As	17	-4.78[a] (-92.53 to 0.43)	82	-11.95[c] (-195.78 to -0.29)	100
Cd	112	-12.80[c] (-259.89 to 0.29)	99	-19.39[c] (-155.63 to 0.33)	90
Cr	19	-3.02[a] (-40.89 to 0.76)	79		
Cu	94	-4.43[c] (-983.23 to 0.58)	99	-1.69[c] (-53.16 to 0.25)	97
Hg	15	-55.09[c] (-1008.09 to -0.85)	100	-13.81[c] (-54.82 to -0.56)	100
Mn	20	-3.22[c] (-12.18 to -0.30)	100	-2.50[b] (-10.27 to 0.42)	90
Ni	14	0.025[NS] (-49.02 to 0.76)	50	0.16[NS] (-3.64 to 0.99)	43
PCB	16	-12.43[c] (-145.84 to -0.0442)	100	-3.23[a] (-72.33 to 0.61)	81
Pb	126	-12.57[c] (-11362 to -0.006)	100	-20.74 [c] (-12021 to -0.36)	100
Zn	121	-7.71[c] (-788.29 to -1.10)	100	-2.49[c] (-37.65 to -0.09)	100

[NS] Estimate not significantly different from measured as determined by Wilcoxon signed-rank test.

[a] Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p < 0.05$.

[b] Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p < 0.01$.

[c] Estimate significantly different from measured as determined by Wilcoxon signed-rank test; $p < 0.001$.

Table 11. Summary statistics for literature-derived soil-earthworm UFs following inclusion of validation data

Analyte	N (Studies)	N (Observations)	Mean	Standard Deviation	Minimum	Median	90th Percentile	Maximum	Mean of Natural Log-transformed values	Standard Deviation of Natural Log-transformed values	Distribution
As	4	53	0.258	0.236	0.006	0.224	0.523	0.925	-1.913	1.232	normal
Cd	21	226	17.105	29.389	0.253	7.708	40.690	190.000	2.036	1.245	lognormal
Cr	6	67	1.099	1.987	0.021	0.306	3.162	11.416	-1.139	1.637	lognormal
Cu	16	197	0.754	0.804	0.002	0.515	1.531	5.492	-0.759	1.130	lognormal
Hg	5	30	5.231	8.896	0.030	1.693	20.625	33.000	0.171	2.044	lognormal
Mn	3	36	0.064	0.047	0.012	0.054	0.124	0.228	-2.986	0.708	lognormal
Ni	4	31	1.656	1.850	0.033	1.059	4.730	7.802	-0.251	1.515	normal
Pb	20	245	3.342	18.822	0.000	0.266	1.522	228.261	-1.181	1.723	lognormal
Se	1	14	1.798	3.325	0.300	0.985	1.340	13.733	-0.018	0.859	lognormal
Zn	20	244	5.766	8.415	0.025	3.201	12.885	49.510	0.909	1.501	lognormal
PCB	3	32	8.909	12.118	0.000	6.667	15.909	65.227	1.458	1.440	lognormal
TCDD	2	19	11.740	9.808	1.191	11.011	22.229	42.068	2.113	0.892	lognormal

Table 12. Results of regression of ln (earthworm) on ln (soil) following inclusion of validation data

	N	B0±SE	B1±SE	R^2	P model fit
As	53	-1.421±0.327^{c}	0.706±0.169^{c}	0.26	0.0001
Cd	226	2.114±0.079^{c}	0.795±0.037^{c}	0.67	0.0001
Cr	67	2.481±0.581^{c}	-0.067±0.165^{NS}	0.0026	0.68
Cu	197	1.675±0.141^{c}	0.264±0.040^{c}	0.18	0.0001
Hg	30	-0.684±0.198^{b}	0.118±0.089^{NS}	0.06	0.19
Mn	36	-0.809±1.121^{NS}	0.682±0.163^{c}	0.34	0.0002
Ni	31	3.677±0.635^{c}	-0.260±0.196^{NS}	0.06	0.19
Pb	245	-0.218±0.245^{NS}	0.807±0.044^{c}	0.58	0.0001
Se (w/ outlier)	14	0.346±0.291^{NS}	0.253±0.397^{NS}	0.03	0.53
Se (w/o outlier)	13	-0.075±0.194^{NS}	0.733±0.256^{a}	0.43	0.016
Zn	244	4.449±0.132^{c}	0.328±0.024^{c}	0.45	0.0001
PCB	31	1.410±0.210^{c}	1.361±0.088^{c}	0.89	0.0001
TCDD	19	3.533±0.810^{c}	1.182± 0.074^{c}	0.94	0.0001

model: ln(earthworm)=B0+B1(ln[soil])
^{NS} Not Significant: p>0.05.
^{a} p<0.05.
^{b} p<0.01.
^{c} p<0.001.

Table 13. Results of regression of ln (earthworm) on ln (soil)and pH following inclusion of validation data

Analyte	N	B0±SE	B1±SE	B2±SE	R^2	P model fit
As	47	-0.185±0.996^{NS}	0.993±0.171^{c}	-0.291±0.173^{NS}	0.43	0.0001
Cd	87	4.249±0.580^{c}	0.553±0.045^{c}	-0.237±0.095^{a}	0.64	0.0001
Cu	100	2.262±0.447^{c}	0.337±0.0580^{c}	-0.149±0.078^{NS}	0.26	0.0001
Ni	23	2.014±0.999^{NS}	-0.118±0.289^{NS}	0.229±0.126^{NS}	0.14	0.23
Pb	103	5.459±1.129^{c}	0.841±0.086^{c}	-0.975±0.194^{c}	0.51	0.0001
Zn	105	4.618±0.482^{c}	0.316±0.039^{c}	-0.006±0.079^{NS}	0.39	0.0001

model: ln(earthworm)=B0+B1(ln[soil])+B2(pH)
^{NS} Not Significant: p>0.05.
^{a} p<0.05.
^{b} p<0.01.
^{c} p<0.001.

Table 14. Results of regression of ln (earthworm) on ln (soil) and ln (soil Ca) following inclusion of validation data

Analyte	N	B0±SE	B1±SE	B2±SE	R^2	P model fit
Cd	73	6.154±0.707[c]	0.878±0.082[c]	-0.474±0.096[c]	0.72	0.0001
Cu	53	1.998±0.605[b]	0.248±0.120[a]	-0.062±0.111[NS]	0.13	0.027
Mn	32	1.139±1.793[NS]	0.528±0.179[b]	-0.113±0.117[NS]	0.3	0.006
Pb	67	-0.120±0.855[NS]	1.324±0.087[c]	-0.461±0.104[c]	0.79	0.0001
Zn	77	6.001±0.510[c]	0.532±0.073[c]	-0.359±0.097[c]	0.5	0.0001

model: ln(earthworm)=B0+B1(ln[soil])+B2(ln[soil Ca])
[NS] Not Significant: p>0.05.
[a] p<0.05.
[b] p<0.01.
[c] p<0.001.

Table 15. Results of regression of ln (earthworm) on ln (soil), ln (soil Ca), and pH following inclusion of validation data

Analyte	N	B0±SE	B1±SE	B2±SE	B3±SE	R^2	P model fit
Cd	42	6.435±1.316[c]	0.860±0.131[c]	-0.477±0.175[b]	-0.032±0.179[NS]	0.74	0.0001
Cu	44	2.022±1.011[NS]	0.498±0.154[b]	-0.128±0.135[NS]	-0.021±0.187[NS]	0.31	0.002
Pb	35	2.453±1.414[NS]	1.176±0.097[c]	0.055±0.173[NS]	-0.934±0.300[b]	0.88	0.0001
Zn	48	3.206±1.027[b]	0.814±0.114[b]	-0.865±0.190[c]	0.832±0.237[b]	0.63	0.0001

model: ln(earthworm)=B0+B1(ln[soil])+B2(ln[soil Ca])+B3(pH)
[NS] Not Significant: p>0.05.
[a] p<0.05.
[b] p<0.01.
[c] p<0.001.

4. DISCUSSION

In this study, we used published data to develop UFs and regression models to describe the bioaccumulation of 9 inorganic and 2 organic chemicals by earthworms from soil. Our UFs are comparable to other, independent UFs reported in the literature; mean UFs for 11 chemicals fell generally within the range of UFs developed in this study (Table 16). In some cases mean literature UFs are virtually identical to those derived in this study (e.g., Cd, Cu, Fe; Table 16). Mean UFs that fell outside the range derived in our study included Mn and V reported in Braunschweiler (1996) and Fe reported in Ma (1982; Table 16). It should be noted that the data available for UF development for these analytes in our study was limited. In the cases of Fe and V, data were restricted to a single study from one location, the ORR in Tennessee. Lack of comparability may relate to the fact that UFs were based on non-overlapping ranges of soil concentrations. For example, the range of Fe concentrations considered by Ma (1982) was <5,000 to ~50,000 mg/kg, while that in our study was 200 to 1800 mg/kg.

Table 16. Comparison of mean UFs reported in literature[a] to those from this study

	Reference						UFs from this study	
Analyte	Braunschweiler 1996	Emmerling et al. 1997	Pascoe et al. 1996	Hendriks et al. 1995	Spurgeon and Hopkins 1996	Ma 1982	Mean	Range
Al	0.011						0.053	0.008 - 0.20
As			0.162				0.258	0.006 - 0.93
Cd	9.7	36.5	3.78	9.6	23.43	42.2	17.12	0.253 - 190
Cr						0.072	1.1	0.02 - 11.4
Cu	0.76		0.132	0.31	0.74	0.867	0.75	0.002 - 5.2
Fe	0.038					0.203	0.038	0.006 - 0.1
Hg	1.2						5.23	0.03 - 33
Mn	0.29			0.11		0.177	0.064	0.012 - 0.23
Pb		0.38		0.089	0.28	0.932	3.34	0 - 228
Ni				0.13		0.346	1.66	0.033 - 7.8
V	0.12						0.039	0 - 0.088
Zn	3.4	4.8	0.544	1.8	2.08	16.117	5.77	0.025 - 49.5

[a] These studies were not used for model development because no raw data were presented. Only mean UFs were reported by the authors.

Regression models developed in our study are also comparable to others reported in the literature. Slopes from log-regression models for five chemicals obtained from four published studies generally fell within, or just outside of the 95% confidence limits for the slopes for simple regression models from our study (Table 17). The only exception to this rule is Ni, which is discussed in more detail in Table 17.

Table 17. Comparison of slopes from log-regression models from the literature to those from this study

Analyte	Spurgeon and Hopkin 1996	Corp and Morgan 1991	Ma et al. 1983	Neuhauser et al. 1995	This study		
					slope±SE	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Cd	0.45	0.55	0.49	0.66	0.55±0.03	0.484	0.616
Cu	0.45	0.29	0.34	0.57	0.26±0.04	0.182	0.338
Ni				0.98	-1.58±0.31	-2.188	-0.972
Pb	0.64	0.64	0.99	0.74	0.8±0.04	0.714	0.886
Zn	0.22	0.29	0.24	0.27	0.24±0.02	0.193	0.287

Following model development, we used independent data, derived from the ORR and from published studies, to validate UFs and models produced in our study and by other researchers. Chemical concentrations in earthworms were best estimated by simple ln-ln regression models for 8 of 10 analytes (As, Cd, Cu, Hg, Mn, Pb, Zn, and PCB) for which models were developed and validation data were available. (It should be noted that the model that best estimated Cd concentrations in earthworms was from Neuhauser et al. (1995) and not the current study.) Exceptions were Cr and Ni, for which no estimation method worked well.

The observation that simple ln-ln regression models best fit the data indicates that the bioaccumulation of contaminants by earthworms decreases as soil concentrations increase. Similar conclusions are reported by Neuhauser et al. (1995). Mechanisms for this decrease in accumulation may include an increase in elimination rate as soil concentration increases or toxicity. Terhivuo et al. (1994) observed higher uptake rates for Pb among earthworms from uncontaminated sites than was observed for Pb-contaminated soils adjacent to a smelter. They suggest that while earthworms from uncontaminated areas are unable to regulate Pb uptake, earthworms residing in contaminated soils "acclimatize" and develop mechanisms to regulate Pb. Fordham and Wilber (1992) observed that bioaccumulation was lower while mortality was higher among earthworms in sewage-sludge-amended soils with increasing concentrations of Cd and Pb. An increase in toxicity at higher soil concentrations was suggested as a possible explanation for the decrease in accumulation.

In comparison to ln-ln regression models, the assumption implicit with UFs, that the rate of uptake is constant across all soil concentrations, is clearly not supported by the validation data. The results of our analysis argue for a shift away from the use of simplistic UFs and toward the application of more appropriate, biologically relevant models of bioaccumulation.

In a recent study, Abdul Rada and Bouche (1995) sampled and analyzed Cd, Cu, Ni, Pb, and Zn content in soil and earthworms from 186 locations across France. Although they used data similar to the published data we used (e.g., co-located soil and earthworm samples, strong acid extractions, and total chemical analysis), Abdul Rada and Bouche (1995) concluded that despite some correlation between soil analyses and chemical content in earthworms, contaminant levels in earthworms could not be predicted from concentrations in soil. The primary reason for this conclusion could be the lack of an adequately large range of soil concentrations. For example, in their study, maximum Cd and Pb concentrations were 8 and 9000 mg/kg, respectively. In contrast, our models for Cd and Pb were based on data with maximum soil concentrations of 467 and 24550 mg/kg, respectively. Development of models based on broader ranges of soil concentrations allows patterns of uptake to be observed that may not be evident across more narrow ranges of soil concentrations.

Other researchers have found that other soil parameters such as soil pH and soil Ca concentration may influence uptake of certain chemicals by earthworms. For example, Beyer et al. (1987) observed that, in addition to soil organic matter and Mg content, Pb uptake was inversely correlated to soil pH. Corp and Morgan (1991) also report an inverse relation between Pb uptake and pH. Pb uptake has also been found to be inversely correlated to soil Ca (Morris and Morgan 1986, Morgan and Morgan 1991). Ma (1987) observed that bioaccumulation of both Pb and Cd increased at lower pH. As would be expected, because data from these studies were included in our database, we obtained similar results.

However, while inclusion of these additional variables may help describe uptake of contaminants by earthworms, we found that their utility in predicting concentrations was marginal. Models that included soil Ca or both soil Ca and pH produced poorer estimates than the simple regression models. While models that included pH produced better estimates for As, Cd, and Cu, only for As was there a significant difference between simple and multiple regression estimates. Due to a limited number of observations in the "validation" dataset that possessed all the needed measurements, conclusions concerning the utility or lack thereof for these multiple regression models, however, must be viewed with caution. Additional validation of these models, using data representing more locations and soil conditions, is needed.

Available data indicate that Cr concentrations in earthworms are poorly predicted by soil Cr concentrations. The bioaccumulation of Cr is highly dependent on chemical species; Cr+6 is more bioavailable than Cr +3 (Eisler 1986). Because information concerning Cr species in soil was not available for any study considered and therefore was not included in models or UFs, the lack of fit we observed may be related to differences in Cr speciation in soil. Additional research focusing on the potential influence of Cr speciation on bioavailability and bioaccumulation in earthworms is needed.

Prediction of Ni concentrations in earthworms was also problematic. Available data are contradictory. For example, field data collected by Neuhauser et al. (1995) indicated a significant, positive relationship between soil Ni and concentrations in earthworm tissues. In contrast, no correlation between soil Ni and concentrations in earthworm tissues was observed by Abdul Rada and Bouche (1995) and Beyer et al. (1982), while combined data from other studies indicated a negative relationship (Neuhauser et al. 1995, this study). Neuhauser et al. (1995) attributes the lack of agreement among studies to the narrow ranges of soil Ni concentrations considered (most, including this one, are based on soil Ni of $\leq$60 mg/kg) or variability in depuration times. Our results also suggest that soil pH may influence Ni accumulation by earthworms. Despite producing estimates that differed significantly from measured values (Table 9), the distribution of estimates from the multiple regression model that included pH more closely reflect the distribution of measured values than estimates generated by any other method (Fig. 13). Additional research is needed to investigate the influence of soil pH on bioaccumulation of Ni by earthworms.

The models and UFs we developed are very generalized, representing multiple earthworm species, seasons, and soil types and characteristics. Contaminant uptake has been shown to differ by earthworm species (Morgan and Morgan 1993, Terhivuo et al. 1994, Spurgeon and Hopkins 1996). Contaminant uptake may also differ seasonally. Morgan and Morgan (1993) report Cd and Zn concentrations to be lower during diapause than in "active" worms. Bengtsson and Rundgren (1992) observed lower uptake rates for Pb during winter. Similar seasonal variation in contaminant concentration in earthworm tissues is reported by Braunsweiler (1996). As a consequence of the generalized nature of the UFs and

models, uncertainty associated with estimates generated for a given location may be high. Uncertainty associated with estimated concentrations in earthworms could be reduced if future models are developed to incorporate these sources of variation.

Co-occurring chemicals in soil may also influence bioaccumulation. For example, Beyer et al. (1982) observed that while soil Cd accounted for 87% of the variability of Cd in earthworms, inclusion of Zn in the model significantly improved the model fit and accounted for an additional 5% of variability. Soil Zn was negatively correlated with Cd in worms. Other regression models developed by Beyer et al. (1987) indicate that Cu concentrations in earthworms are a function of decreasing soil Ca and increasing soil K, and that Se concentrations in earthworms are a function of increasing Ca in soil. In neither case did Cu or Se contribute significantly to explaining bioaccumulation. Because multiple analytes are present at most contaminated sites, uncertainty may be reduced by incorporating multiple analytes in bioaccumulation models.

5. RECOMMENDATIONS

In the context of an ecological risk assessment, the best data to use to estimate bioaccumulation of contaminants in soil by earthworms will always be site-specific data. Ideally, earthworms should be collected from multiple areas within the contaminated site and from reference areas (preferably at locations where soil samples are also collected), and analyses for contaminants of concern in earthworm tissue should be performed. In the absence of site-specific data, UFs or models should be used.

Because the available data indicate that bioaccumulation by earthworms is non-linear, decreasing as soil concentration increases, and UFs implicitly assume that accumulation is linear and constant across all soil concentrations, the use of log-linear regression models to estimate earthworm bioaccumulation is recommended. For applications where conservative estimates are desired, such as screening ecological risk assessments, the 95% UPL for the simple regression is recommended. Methods and parameters for calculating the 95% UPL are presented in Appendix D. In general, because they are based on a larger, more robust dataset, the regression models and UFs from the combined datasets (Tables 11 through 15) should be used. Due to the uncertainties associated with the models, it is highly recommended that users perform uncertainty analyses. It should be noted that, because the models incorporate data from multiple sites and species, as well as multiple studies, these calculated uncertainties would represent variance among sites and combinations of species and not simply lack of knowledge (i.e., true uncertainty). Contaminant-specific recommendations and justifications are outlined in Table 18.

Table 18. Recommended application of bioaccumulation models. All recommendations are from the combined validation dataset unless otherwise noted

Analyte	For general estimates	For conservative estimates
As	simple regression or multiple regression that includes pH	90th percentile UF
Cd	simple regression or model from Neuhauser et al. (1995)	95% UPL
Cr	None[a]	90th percentile UF
Cu	simple regression	95% UPL
Hg	simple regression based on model data [b]	95% UPL
Mn	simple regression	95% UPL
Ni	None [a]	90th percentile UF
Pb	simple regression	90th percentile UF
Se	simple regression with outlyer removed [c]	95% UPL
Zn	simple regression	95% UPL
PCB	simple regression	95% UPL
TCDD	simple regression	95% UPL

Table 18. (cont.)

Analyte	For general estimates	For conservative estimates
Analytes in Appendix C	median UF [d]	90th percentile UF[d]

[a] None recommended because no method accurately predicted bioaccumulation.
[b] Recommended because addition of validation data resulted in non-significant model.
[c] Recommended because significant model fit was obtained with outlying value removed.
[d] Recommended because regression analyses were not performed.

6. REFERENCES

Abdul Rada, A. M. M., and M. B. Bouche. 1995. "Earthworm contribution to ecotoxicological assessments." *Acta Zool. Fennica.* 196: 307-310

Andersen, C. 1979. "Cadmium, lead, and calcium content, number and biomass, in earthworms (Lumbricidae) from sewage sludge treated soil." *Pedobiologia* 19: 309-319.

Andersen, C., and J. Laursen. 1982. "Distribution of heavy metals in *Lumbricus terrestris, Aporrectodea longa*, and *A. rosea* measured by atomic absorption and x-ray fluorescence spectrometry." *Pedobiologia* 24: 347-356.

Beyer, W. N., and E. J. Cromartie. 1987. "A survey of Pb, Cu, Zn, Cd, Cr, As, and Se in earthworms and soil from diverse sites." *Environ. Monit. Assess*. 8: 27-36.

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

Beyer, W. N., O. H. Pattee, L. Sileo, D. J. Hoffman, and B. M. Mulhern. 1985. "Metal contamination in wildlife living near two zinc smelters." *Environ. Pollut.* (Series A) 38:63-86.

Beyer, W. N., G. Hensler, and J. Moore. 1987. "Relation of pH and other soil variables to concentrations of Pb, Cu, Zn, and Se in earthworms." *Pedobiologia.* 30: 167-172.

Bengtsson, G., and S. Rundgren. 1992. "Seasonal variation of lead uptake in the earthworm *Lumbricus terrestris* and the influence of soil liming and acidification." *Arch. Environ. Contam. Toxicol.* 23: 198-205.

Braunschweiler, H. 1996. "Seasonal variation in the content of metals in the earthworm *Dendrobaena octaedra* (Sav.) In Finnish forest soils." *Acta Zool. Fenn.* 196: 314-317

Bull, K. R., R. D. Roberts, M. J. Inskip, G. T. Goodman. 1977. "Mercury concentrations in soil, grass, earthworms and small mammals near an industrial emission source." *Environ. Poll.* 12: 135-140.

Carter, A. 1983. "Cadmium, copper, and zinc in soil animals and their food in a red clover system." *Can. J. Zool.* 61: 2751-2757.

Corp, N. and A. J. Morgan. 1991. "Accumulation of heavy metals from polluted soils by the earthworm, *Lumbricus rubellus*: can laboratory exposure of "control" worms reduce biomonitoring problems?" *Environ. Pollut.* 74: 39-52.

Czarnowska, K., and K. Jopkiewicz. 1978. "Heavy metals in earthworms as an index of soil contamination." *Polish J. Soil Sci.* 11: 57-62.

Diercxsens, P. And D. de Weck, N. Borsinger, B. Rosset, and J. Tarradellas. 1985. "Earthworm contamination by PCBs and heavy metals." *Chemosphere*. 14: 511-522.

Dowdy, S., and S. Wearden. 1983. *Statistics for Research.* John Wiley and Sons, New York.

Draper, N., and H. Smith. 1981. *Applied Regression Analysis.* John Wiley and Sons. New York. 709 pp.

Efroymson, R., B. L. Jackson, D. S. Jones, B. E. Sample, G. W. Suter II and C. J. E. Welsh. 1996. *Waste Area Grouping 2 Phase I Task Data Report and White Oak Creek Watershed Screening Ecological Risk Assessment*. ORNL/ER-366. Oak Ridge National Laboratory, Oak Ridge, TN.

Efroymson, R., B. E. Sample, G. W. Suter, II, and T. L. Ashwood. 1997. *Soil-Plant Contaminant Uptake Factors: Review and Recommendations for the Oak Ridge Reservation.* ES/ER/TM-198. Oak Ridge National Laboratory, Oak Ridge, TN.

Eisler, R. 1986. "Chromium hazards to fish, wildlife, and invertebrates: a synoptic review." *U.S. Fish and Wildlife Serv. Biol. Rept.* 85(1.6). 60 pp.

Emmerling, C., K. Krause, and D. Schroder. 1997. "The use of earthworms in monitoring soil pollution by heavy metals." *Z. Pflanzenernahr. Bodenk.* 160: 33-39.

EPA (U. S. Environmental Protection Agency). 1993. "Wildlife Exposure Factors Handbook." Volume I. Office of Research and Development, Washington, D.C. EPA/600/R-93/187a.

ERT. 1987. *Land treatment effects on wildlife populations in red pine plantations: Final Report*. Prepared for Nekoosa Papers, Inc. 160 pp.

Fischer, E., and L. Koszorus. 1992. "Sublethal effects, accumulation capacities and elimination rates of As, Hg, and Se in the manure room, *Eisenia fetida* (Oligochaeta, Lumbricidae)." *Pedobiologia* 36: 172-178.

Fordham, C. L., and C. G. Wilber. 1992. "Effects of composted sewage sludge on the earthworm *Lumbricus terrestris*." pp. 238-244. In: *Ecotoxicology of Earthworms,* ed. P.W. Grieg-Smith, H. Becker, P.J. Edwards, and R.Heimbach. Intercept, LTD., Andover, UK.

Gish, C. D., and R. E. Christensen. 1973. "Cadmium, nickel, lead, and zinc in earthworms from roadside soil." *Environ. Sci. Technol.* 7: 1060-1062.

Helmke, P. A., W. P. Robarge, R. L. Korotev, and P. J. Schomberg. 1979. "Effects of soil-applied sewage sludge on concentrations of elements in earthworms." *J. Environ. Qual.* 8: 322-327.

Hendriks, A. J., W.-C. Ma, J. J. Brouns, E. M. de Ruiter-Dijkman, and R. Gast. 1995. "Modelling and monitoring organochlorine and heavy metal accumulation in soils, earthworms, and shrews in Rhine-Delta floodplains." *Arch. Environ. Contam. Toxicol.* 29: 115-127.

Ireland, M. P. 1975. "Metal content of *Dendrobaena rubida* (Oligochaeta) in a base metal mining area." *Oikos* 26: 74-79.

Ireland, M. P. 1979. "Metal accumulation by the earthworms *Lumbricus rubellus, Dendrobaena venata,* and *Eiseniella tetraedra* living in heavy metal polluted sites." *Environ. Poll.* 19: 201-206.

Jones, D. S., R. A. Efroymson, C. Hadden, B. L. Jackson, B. E. Sample, G. W. Suter, II, and C. J. E. Welsh 1996. *Report on the Remedial Investigation of Bear Creek Valley at the Oak Ridge Y-12 Plant, Oak Ridge, Tennessee: Vol. 6 Appendix G - Baseline Ecological Risk Assessment Report and Vol. 1 Chapter 6 - Baseline Ecological Risk Assessment*. DOE/OR/01-1455/V1 and V6&D1. Oak Ridge National Laboratory, Oak Ridge, TN.

Jones, D. S., B. E. Sample, and G. W. Suter, II. 1997. *Biota-sediment accumulation factors for invertebrates: review and recommendations for the Oak Ridge Reservation*. ES/ER/TM-214. Oak Ridge National Laboratory, Oak Ridge, TN.

Kreis, B., P. Edwards, G. Cuendet, and J. Tarrdellas. 1987. "The dynamics of PCBs between earthworm populations and agricultural soils." *Pedobiologia* 30: 379-388.

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 euorpa*, and earthworms as an indicator of metal bioavailability in terrestrial environments." *Bull. Environ. Contam. Toxicol.* 39: 933-938.

Ma, W., T. Edelman, I. van Beersum, and T. Jans. 1983. "Uptake of cadmium, zinc, lead, and copper by earthworms near a zinc-smelting complex: influence of soil pH and organic matter." *Bull. Environ. Contam. Toxicol.* 30: 424-427.

Marino, F., A. Ligero, and D. J. Diaz Cosin. 1992. "Heavy metals and earthworms on the border of a road next to Santiago (Galicia, Northwest of Spain). Initial results." *Soil Biol. Biochem.* 24: 1705-1709.

Martinucci, G. B., P. Crespi, P. Omodeo, G. Osella, and G. Traldi. 1983. *Earthworms and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) in Seveso*. pp 275-283 in J. E. Satchell, ed. Earthworm Ecology. Chapman and Hall, NY. 495 pp.

Morgan, J. E., and A. J. Morgan. 1990. "The distribution of cadmium, copper, lead, zinc, and calcium in the tissues of the earthworm *Lumbricus rubellus* sampled from one uncontaminated and four polluted soils." *Oecologia.* 84: 559-566.

Morgan, J. E., and A. J. Morgan. 1991. "Differences in the accumulated metal concentrations in two epigeic earthworm species (*Lumbricus rubellus* and *Dendrodrilus rubidus*) living in contaminated soils." *Bull. Environ. Contam. Toxicol.* 47: 296-301.

Morgan, J. E., and A. J. Morgan. 1993. "Seasonal changes in the tissue-metal (Cd, Zn, and Pb) concentrations in two ecophysiologically dissimilar earthworm species: pollution-monitoring implications." *Environ. Pollut.* 82: 1-7.

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.

Morris, B., and A. J. Morgan. 1986. "Calcium-lead interactions in earthworms: observations on *Lumbricus terrestris* L. sampled from a calcareous abandoned lead mine site." *Bull. Environ. Contam. Toxicol.* 37: 226-233.

Neuhauser, E. F., Z. V. Cukic, M. R. Malecki, R. C. Loehr, and P. R. Durkin. 1995. "Bioconcentration and biokinetics of heavy metals in the earthworm." *Environ. Poll.* 89: 293-301.

Nielsen, M. G., and G. Gissel-Nielsen. 1975. "Selenium in soil animal relationships." *Pedobiologia* 15: 65-67.

Palisades Corp. 1994. @RISK: *Risk analysis for spreadsheets.* Palisade Corporation, Newfield, NY. 302 pp.

Pascoe, G. A., R. J. Blanchet, and G. Linder. 1996. "Food chain analysis of exposures and risks to wildlife at a metals-contaminated wetland." *Arch. Environ. Contam. Toxicol.* 30: 306-318.

Pietz, R. I., J. R. Peterson, J. E. Prater, and D. R. Zenz. 1984. "Metal concentrations in earthworms from sewage sludge amended soils at a strip mine reclamation site." *J. Environ. Qual.* 13: 651-654.

Pizl, V., and G. Josens. 1995. "Earthworm communities along a gradient of urbanization." *Environ. Poll.* 90:7-14.

Sample, B. E., J. Beauchamp, R. Efroymson, G. W. Suter, II, and T.L. Ashwood. 1997. *Development and Validation of Literature-based Bioaccumulation Models for Small Mammals.* ES/ER/TM-219. Oak Ridge National Laboratory, Oak Ridge, TN.

SAS Inst. Inc. 1988a. *SAS Procedures Guide, Release 6.03 Edition.* SAS Institute Inc., Cary, NC. 441 pp.

SAS Inst. Inc. 1988b. *SAS/STAT User's Guide, Release 6.03 Edition*. SAS Institute Inc., Cary, NC 1028 pp.

Spurgeon, D. J., and S. P. Hopkins. 1996. "Risk assessment of the threat of secondary poisoning by metals to predators of earthworms in the vicinity of a primary smelting works." *Sci. Total. Environ.* 187: 167-183.

Talmage, S. S., and B. T. Walton. 1993. "Food chain transfer and potential renal toxicity of mercury to small mammals at a contaminated terrestrial field site." *Ecotoxicology* 2: 243-256.

Terhivuo, J., E. Pankakoski, H. Hyvarinen, and I. Koivisto. 1994. "Pb uptake by ecologically dissimilar earthworm (Lumbricidae) species near a lead smelter in south Finland." *Environ. Pollut.* 85: 87-96.

van Hook, R. I. 1974. "Cadmium, lead, and zinc distributions between earthworms and soils: potentials for biological accumulation." *Bull. Environ. Contam. Toxicol.* 12:509-512.

van Rhee, J. A. 1977. "Effects of soil pollution on earthworms." *Pedobiologia* 17: 201-208.

Yeates, G. W., V. A. Orchard, T. W. Speir, J. L. Hunt, and M. C. C. Hermans. 1994. “Impact of pasture contamination by copper, chromium, and arsenic timber preservative on soil biological activity.” *Biol. Fert. Soils.* 18: 200-208.