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TESTING OF METAL BIOACCUMULATION MODELS WITH MEASURED BODY BURDENS IN MICE

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Abstract—Estimates of chemical accumulation in prey organisms can contribute considerable uncertainty to predictive ecological risk assessments. Comparing body burdens calculated in food web models with measured tissue concentrations provides essential information about the expected accuracy of risk indices. Estimates of arsenic, cadmium, copper, lead, and nickel body burdens in house mice (*Mus musculus*) inhabiting a seasonal wetland were generated with two small mammal bioaccumulation models. Published soil-to-small mammal bioaccumulation regression models produced accurate estimates of arsenic and lead body burdens but failed to adequately predict copper and nickel levels in mice. Incorporating conservative prediction intervals in the regression models shows potential for successful applications in screening-level risk assessments. A simple mechanistic cumulative ingestion bioaccumulation model overpredicted lead levels in mice generally by less than one order of magnitude but greatly overpredicted concentrations of arsenic, copper, and nickel. Better estimates of absorption and elimination of ingested metals and knowledge of specific arthropod taxa in house mouse diets are likely to improve the accuracy of the cumulative ingestion model. Applying Monte Carlo simulations to the soil–small mammal regression models generated probabilistic estimates of body burdens that were consistent with deterministic results. However, deterministic minimum and maximum predictions of the ingestion model were excessively conservative (widely spaced) relative to lower and upper probabilistic percentiles. Metal levels predicted in individual mice on the basis of mouse-specific parameter values and exposures were not significantly more accurate than bioaccumulation predictions for the sitewide population.

Keywords—Bioaccumulation model Metals Assimilation Small mammals Ecological risk assessment

INTRODUCTION

Ecological risk assessments evaluate the probability of adverse impacts to organisms posed by contaminants in the environment. To analyze risks to wildlife receptors, toxicological data typically are compared to estimated rates of chemical intake. Among the information required to predict exposures are chemical concentrations in food items ingested by the species of concern, which may be either empirically measured or modeled. Tissue analyses can be costly and are destructive to sensitive species, and certain animals may be difficult to collect, which can result in small sample sizes and data gaps. Consequently, predictive bioaccumulation models are the primary tools used to estimate dietary exposure concentrations in screening-level ecological risk assessments.

Models used to predict bioaccumulation in food webs include empirical models such as regressions or bioaccumulation factors (BAFs) derived from measured concentrations in organisms and food or environmental media [1–5]. More complex models are also used in which chemical exposure processes, assimilation in tissues, or trophic transfer are represented mechanistically [6–10].

Since important remediation and land use decisions regarding hazardous waste sites can be based in part on predictive exposure models, it is critical to test results against observed tissue concentrations to examine model accuracy. Validation of terrestrial wildlife bioaccumulation models has met with varied success. Alsop et al. [9] predicted concentrations of lead, zinc, and dioxin in deer mice (*Peromyscus manic-*

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ulatus) with an ingestion-based bioaccumulation model. The model underestimated metal concentrations in mice at low soil concentrations, overestimated metal body burdens at high soil concentrations, and underestimated body burdens of dioxin across the range of observed soil concentrations [9]. An assumption that body burden was numerically equal to the lifetime average daily dose of a chemical may have contributed to the prediction errors. Pascoe et al. [8] calculated body burdens of five metals in deer mice and meadow voles (*Microtus pennsylvanicus*) with an ingestion model and found that tissue concentrations were substantially overestimated when the balance of absorption and elimination (assimilation efficiency) of metals was assumed to be 10% of the amount ingested. By assuming that the differences between measured and modeled concentrations of metals were entirely due to errors in the assimilation efficiency factor applied, Pascoe et al. [8] estimated the true assimilation of metals at only 0.0002 to 0.1% of total intake. However, uncertainty in other variables, such as animal age, feeding rate, and diet variability, should also be considered as sources of potential error in such models.

Menzie et al. [11] modeled DDT residues in birds and small mammals with tissue:food bioaccumulation factors, but predictions were plagued by large uncertainties, and validation was limited by small sample sizes. However, dieldrin levels in birds and mammals were adequately predicted by a model that incorporated distributions of tissue:soil BAFs derived from site-specific data [12]. Likewise, estimates of mercury and polychlorinated biphenyl residues in great blue heron (*Ardea herodias*) eggs made with a stochastic food web model were in reasonable agreement with concentrations measured in a limited number of egg samples [7]. Gorree et al. [13]

successfully estimated cadmium levels in kidneys of roe deer (*Capreolus capreolus*) using a stochastic food chain model based on BAFs, but concentrations calculated for barn owls (*Tyto alba*) were excessive.

In addition to testing the accuracy of bioaccumulation models, it is useful to examine predictions in the context of variability and uncertainty in parameter values, a task most commonly accomplished through Monte Carlo simulation [7]. Other advantages of Monte Carlo analysis are presentation of model estimates as probability distributions across a population and ranking of input variables by their relative effects on model output through sensitivity analysis [14]. When both probabilistic and deterministic estimates of tissue concentration or risk are generated from alternate versions of a model, results may be compared to determine the relevance of traditional deterministic estimates [15].

Spatial scales over which bioaccumulation, exposure, or risk models are applicable vary from sitewide analyses with single distributions of chemical concentrations to landscape-level studies that integrate heterogeneously distributed concentrations within the home ranges of a wide-ranging receptor species [16] or across a population [17]. On a finer spatial scale, measuring contaminant concentrations and other variables within home ranges that are small relative to a site enable tissue burdens, exposures, or risks to be estimated for individual animals. This individual approach cannot misrepresent such exposures or risks except for the effects of uncertainty and variability in the model itself since predictions are made for individuals with home ranges and other attributes (body weight and food concentrations) that are known with high confidence. The ability of sitewide calculations to represent the local population can therefore be confirmed by comparing predicted distributions of body burdens to estimates for individual animals. The question of whether bioaccumulation models differ in accuracy between individual animal and sitewide predictions, based on comparisons with sampled tissue concentrations, also warrants study.

This study was conducted to test predictive models of metal bioaccumulation in mice against empirical, site-specific data. We had three objectives: estimate whole-body concentrations of arsenic, cadmium, copper, lead, and nickel in house mice (*Mus musculus*) with two types of small mammal bioaccumulation models and evaluate the predictive ability of each model through comparisons with measured carcass concentrations; determine whether predictions of body burdens in individual mice with known home ranges are more accurate than sitewide estimates; and produce Monte Carlo–simulated predictions of whole-body concentrations with both models and relate probabilistic distributions of estimates to deterministic estimates.

The first model type utilized was a collection of bioaccumulation regression equations developed by Sample et al. [4] that relate body burdens of small mammals to chemical concentrations in ambient soils. The regression models have not been widely tested against independent data.

The second model applied was an ingestion dose-based bioaccumulation model that is similar to other simple mechanistic exposure models used in risk assessments and reported in the literature (e.g., [8,10]). In the present study, this ingestion model was parameterized by measured residues of metals in soils and food. Included in the model is a gastrointestinal absorption–elimination (AE) factor that represents assimilation of ingested metals in the animal.

MATERIALS AND METHODS

Body burdens of metals were modeled for house mice residing at a seasonal brackish wetland described in Torres and Johnson [18]. The bioaccumulation models were assigned input concentrations of arsenic, cadmium, copper, lead, and nickel measured in soils and sediments (collectively termed soils) and/or dietary items at the site. Model estimates of metal concentrations in house mice were then compared with measured residues in the mice.

Three sets of body burdens were calculated for house mice: deterministic individual predictions, deterministic sitewide predictions, and probabilistic sitewide predictions. Individual mouse predictions were based on exposure estimates of individual mice in their respective home ranges, while sitewide predictions were made for the mouse population across the entire site. To represent the uncertainty and/or variability of tissue concentrations in individual mice and within the population, we calculated minimum, mean, and maximum deterministic predictions (see the following discussion). The mean concentration was defined for individual-level estimates as the expected body burden of particular mice. Probabilistic sitewide estimates were displayed graphically along with the 5th-percentile, median, and 95th-percentile output values.

Soil–small mammal bioaccumulation regression models

Simple linear regression models developed by Sample et al. [4] relate chemical concentrations in small mammals (dependent variable) to concentrations in ambient soils (independent variable). The regression equations were developed from colocated soil and small mammal whole-body or carcass concentrations from a variety of studies. Sample et al. [4] considered trophic group, significance of model fit, and validation status in recommending specific models to predict body burdens. Models of cadmium, copper, and nickel used in this study for the omnivorous house mouse have been validated with independent data sets by Sample et al. [4] (Table 1).

Mice were live trapped as described in Torres and Johnson [18] to identify long-term residents of the site and characterize exposures of house mice in their home ranges. Thirteen mice were captured for metals analysis to test sitewide bioaccumulation predictions; 11 individuals were used to generate individual-level predictions. Methods of soil collection and metals analysis are provided in Torres and Johnson [18].

For deterministic sitewide predictions, minimum, mean, and maximum body burdens of house mice were estimated from the regression models on the basis of soil concentrations measured across the entire site (Table 2). The minimum body burden was calculated as the lower 95% prediction limit of the tissue level predicted by the minimum soil concentration. Similarly, the maximum body burden was represented as the upper 95% prediction limit of the tissue level predicted by the maximum soil concentration. Statistics for prediction intervals used with the regressions were provided by Sample et al. [4]. The mean sitewide body burden was calculated directly from the mean soil concentration. Deterministic predictions of body burdens in the 11 individual mice were made similarly to sitewide estimates but were based on soil concentrations tabulated within each home range.

Probabilistic distributions of tissue concentrations were generated from Monte Carlo simulations of the regression models. Sitewide metal concentrations in soil were fitted to continuous distributions (Table 2) using BestFit™ (Palisade, New York, NY, USA). Distributions were selected on the basis

Table 1. Small mammal bioaccumulation regression models[a]

Metal	Source data set		Regression model statistics			
	n	Trophic group(s)[b]	b_0	b_1	r^2	p (model)
Arsenic	37	O	−4.5796	0.7354	0.41	<0.0001
Cadmium	33	O	−1.5383	0.5660	0.63	<0.0001
Copper	28	O	1.4592	0.2681	0.48	<0.0001
Lead	138	A	0.0761	0.4422	0.37	<0.0001
Nickel	36	A	−0.2462	0.4658	0.55	<0.0001

[a] Regression models of small mammal whole-body and carcass concentrations on soil concentrations developed by Sample et al. [4]: ln(small mammal concn., mg/kg dry wt) = $b_0 + b_1$[ln(soil concn., mg/kg dry wt)].
[b] O = omnivores; A = all trophic groups (herbivores, omnivores, and insectivores).

of the Kolmogorov–Smirnov and Anderson–Darling goodness-of-fit tests. Each Monte Carlo simulation consisted of 10,000 iterations; in each iteration, body burdens were generated in two steps. First, a soil concentration was sampled from the distribution of soil concentrations (Table 2) with the Latin hypercube method. Second, a body burden was sampled from the distribution of body burdens predicted at that soil concentration by the regression model. Predicted body burdens corresponding to a given soil concentration were assumed to be normally distributed with a variance computed from regression model statistics [4]. Thus, both variability in soil levels and uncertainty and variability in the model were incorporated into simulated body burdens. To facilitate comparison with measured values, dry-weight predictions of both the deterministic and the probabilistic models were converted to wet weight assuming a 68% water content in small mammals [19].

Cumulative ingestion bioaccumulation model

The second model used is based on the assimilation of metals from ingested food and soils over the estimated lifetimes of house mice. Exposure to metals in drinking water was not represented in the model since no standing water existed at the site during the period for which exposures were calculated.

The cumulative ingestion bioaccumulation model is represented with the following equation:

$$\mathrm{WBC} = \{[(C_F \times CF_F \times IR_F) + (C_S \times CF_S \times IR_S)] \times (A \times CF_A) \times AE\}/(BW \times CF_{BW}) \quad (1)$$

where WBC = whole-body concentration (mg/kg body weight, wet wt), C_F = concentration of metal in food (mg/kg), CF_F = food concentration unit conversion factor (0.001 kg/g), IR_F = intake rate of food (g/d), C_S = concentration of metal in soil (mg/kg), CF_S = soil concentration unit conversion factor (0.001 kg/g), IR_S = intake rate of soil (g/d), A = estimated final age (weeks), CF_A = age unit conversion factor (7 d/week), AE = gastrointestinal absorption–elimination factor, BW = body weight (g), and CF_{BW} = body weight unit conversion factor (0.001 kg/g). This model follows the general framework of the small mammal body burden model in Pascoe et al. [8]. Minimum, mean, or maximum values of each parameter were grouped with the corresponding values of other model parameters to produce the three deterministic sitewide or individual estimates. Monte Carlo simulations of the ingestion model were performed with 10,000 iterations, and distributions of parameter values were sampled with the Latin hypercube method.

To identify local foods consumed by house mice, dietary analyses for eight mice were conducted on fecal samples collected from traps. Based on fecal analysis, the diet used for bioaccumulation modeling consisted of 80.3% *Scirpus robustus* seeds and 19.7% arthropods [18]. No breakdown of arthropod parts by taxa was made. Seeds of *S. robustus* in wetland areas and of *Hordeum marinum* in the upland portion of the site were collected as described in Torres and Johnson [18]. *Armadillidium vulgare* (pillbug, Isopoda), *Phidippus* sp. (jumping spider, Arachnida), and *Araneus* sp. (Arachnida) were collected to represent arthropods at the site.

Food concentrations (C_F) of metals were measured in seeds and arthropods (Table 2; see Torres and Johnson [18] for analytical methods). Minimum, mean, and maximum seed concentrations in each home range were applied for individual mouse calculations; these fell within the ranges given in Table 2. In sitewide deterministic calculations of WBC, minimum, mean, and maximum concentrations measured in *S. robustus* seeds among all locations were used. Lead was the only metal analyzed for in *H. marinum* seeds because of limited sample mass; lead concentrations in both seed types were averaged and weighted by the relative proportions of each habitat type (wetland or upland) at the site. In probabilistic simulations, *S. robustus* and *H. marinum* seed concentrations were randomly sampled from the pool of measured values (bootstrapped) because of the limited number of samples. Each arthropod taxon was analyzed in a composite sample, yielding a single concentration of each metal. Consequently, the same, fixed-point estimates of each metal concentration were applied for individual, deterministic sitewide, and probabilistic sitewide calculations (Table 2). Metal concentrations in soils consumed incidentally by house mice (C_S) were identical to those used in the small mammal bioaccumulation regression model (Table 2).

Values of exposure variables other than metal concentrations are shown in Table 3. Variable values used for individual mouse modeling were derived equivalently to and fell within the ranges of values applied for the sitewide population. Food intake rates (IR_F) of house mice were estimated from field metabolic rate (FMR) [20] and metabolizable energies (ME) of foods consumed (see the following discussion). The FMRs (kcal/d) were calculated from an allometric log(FMR)-log(body weight) regression model developed for free-living rodents [20]. Uncertainty in deterministic estimates of FMR was represented with regression model prediction intervals [20]. In Monte Carlo simulations, a body weight was first selected from the fitted distribution of body weights. An FMR was then sampled from a normal distribution of FMRs associated with that body weight in the regression model on the

Table 2. Metal concentrations in soils and food items used for deterministic and probabilistic sitewide modeling of house mouse body burdens

Component	Concentration (mg/kg dry wt)				
	Arsenic	Cadmium	Copper	Lead	Nickel
Deterministic models					
Soil (n = 40)[a]	4 (10, 15)	1.4 (1.8, 2.2)	70 (236, 1,510)	38 (107, 413)	90 (122, 219)
Plant seeds (n = 11 or 12)[a,b]	0.015 (0.030, 0.064)	0.3 (0.3, 0.3)	9.8 (12.7, 16.3)	0.015 (0.093, 0.376)[c]	0.3 (1.0, 1.9)
Arthropods[d]	1.04	4.4	757	1.80	4.6[e]
Probabilistic models[f]					
Soil[g]	Normal (10.35, 2.94)	Logistic (1.75, 0.114)	Pareto (1.28, 70)	Pearson 5 (3.34, 250)	Log-logistic (90, 28.70, 3.94)
Arthropods[d]	1.04	4.4	757	1.80	4.6[e]

[a] Values are mean (minimum, maximum).
[b] *Scirpus robustus* seeds; one-half of the detection limits were applied for undetected levels of cadmium, lead, and nickel.
[c] Lead concentrations (n = 13) include composite analyses of *Hordeum marinum* seeds.
[d] Averages of composite samples of three taxa. Wet-weight concentrations were converted to dry weight assuming a 65% water content in arthropods.
[e] One-half of the detection limit was applied for undetected levels of nickel in *Araneus* sp.
[f] Stochastic values of seed concentrations were generated by random sampling of measured concentrations (bootstrapping).
[g] Fitted distributions (parameters in parentheses).

basis of the variance in predicted values calculated from regression statistics in Nagy [20].

The ME content of a food is the gross energy content minus energy lost in feces and urine, or the energy that is metabolizable for production and maintenance of an animal. Energy assimilation efficiency is the fraction of the gross energy that is metabolizable, specific to a food type and consumer. Point values of ME contents of foods consumed by mice were estimated from gross energy contents multiplied by assimilation efficiency factors (Table 3). Assimilation efficiency factors for seeds and arthropods were based, respectively, on values for seeds and nuts consumed by mice and voles, and for insects eaten by small mammals [19]. For Monte Carlo simulations, probability distributions of seed ME contents were assumed to be normal and were calculated from means and standard deviations of gross energy and assimilation efficiency (Table 3). Because insufficient data were available to characterize distributions of gross energy in arthropods, a point estimate of arthropod ME content was applied.

In general, food intake rates (IR_F) were calculated as follows:

$$IR_F \text{ (g/day)} = \frac{\text{FMR (kcal/day)}}{\text{ME (kcal/g)}} \quad (2)$$

Separate intake rates were calculated for the two food categories in the house mouse diet. Seed intake rate (IR_{seed}) was represented according to the equation

$$IR_{seed} \text{ (g/day)} = \{\text{FMR (kcal/day)} - [\text{arthropod intake (g/day)} \times \text{arthropod ME (kcal/g)}]\} \div [\text{seed ME (kcal/g)}] \quad (3)$$

Arthropod intake may be expressed as seed intake divided by 4.076 (based on the dietary ratio of seeds to arthropods), so Equation 3 becomes the following:

$$IR_{seed} \text{ (g/day)} = \frac{[\text{FMR (kcal/day)}]}{\left[\text{seed ME (kcal/g)} + \frac{\text{arthropod ME (kcal/g)}}{4.076}\right]} \quad (4)$$

For each deterministic calculation or Monte Carlo iteration, arthropod intake rate was then calculated from the seed intake rate using the seed:arthropod dietary ratio (4.076:1).

The amount of soil ingested incidentally by mice was derived from an estimated dry-weight percentage of soil in diet (2.2%), which was based on levels of soil in the diets of white-footed mice (*Peromyscus leucopus*) and meadow voles (*M. pennsylvanicus*) [21]. Soil intake rate (IR_S) was calculated as 0.022 multiplied by the food intake rate of house mice.

Final ages (Table 3) were estimated for individuals first captured through the 12th trapping week and trapped for more than one week (n = 45). Ages were estimated on the basis of body weight at first capture and the time between first and last captures. Ages were fit to a distribution using the same procedure applied for soil concentrations (see the previous discussion).

The gastrointestinal AE factor is defined as the fraction of a total metal dose that is assimilated following absorption, metabolism, and excretion, a definition similar to that for assimilation efficiency in Penry [22]. This parameter was based

Table 3. Exposure parameter values used in the cumulative ingestion bioaccumulation model

Parameter[a]	Sitewide deterministic parameter values			Probabilistic distribution/point estimate	Source
	Minimum	Mean	Maximum		
Body weight (g fresh wt)	9.0	15.1	25.0	Weibull (4.86, 16.44)	Measured
Age (weeks)	6.0	16.1	31.0	Gamma (7.58, 2.13)	Measured/estimated
FMR (kcal/g/d)	0.244[b]	0.658[c]	1.857[d]	Normal (expected [log FMR], 0.061)[e]	[20]
S. robustus and grass seeds					
Gross energy (kcal/g dry wt)	—	5.07[f]	—	5.07[f]	[37]
Assimilation efficiency factor	—	0.85	—	0.85	[19]
Metabolizable energy (kcal/g dry wt)	3.29[g]	4.30	5.32[g]	Normal (4.30, 1.01)[h]	Calculated[i]
Arthropods					
Gross energy (kcal/g dry wt)	5.36[j]	5.52[j]	5.90[j]	5.52	[19, 37]
Assimilation efficiency factor	0.82[k]	0.87[k]	0.92[k]	0.87	[19]
Metabolizable energy (kcal/g dry wt)	4.40	4.80	5.43	4.80	Calculated[k]
GI absorption–elimination factor					
Arsenic	—	0.09[l]	—	0.09[l]	[23]
Cadmium	—	0.032	—	0.032	[38]
Copper	—	0.28[l]	—	0.28[l]	[39]
Lead	—	0.1	—	0.1	[40]
Nickel	—	0.1	—	0.1	[41]

[a] FMR = field metabolic rate. GI = gastrointestinal.
[b] 95% lower prediction limit of FMR for maximum body weight.
[c] Expected FMR for mean body weight.
[d] 95% upper prediction limit of FMR for minimum body weight.
[e] Distribution of predicted FMRs assumed normal; variance of predicted values calculated from regression statistics in Nagy [20].
[f] Mean gross energy content of seeds (n = 57 species).
[g] Calculated as mean ± 1 standard deviation (SD) (calculated from SDs of gross energy and assimilation efficiency).
[h] Distribution of calculated metabolizable energy (ME) assumed normal; SD of ME calculated from SDs of gross energy and assimilation efficiency.
[i] Metabolizable energy = gross energy × assimilation efficiency.
[j] Lowest mean, central mean, and highest mean of data from cited sources.
[k] Mean ± 1 SD.
[l] Calculated from data presented in source.

on the variable used in the Pascoe et al. [8] model of metal bioaccumulation in small mammals. Absorption and elimination of metals were assumed to be constant over time and for all ingested food and soil sources of metals. Metal-specific, fixed-point AE factors based on mammalian studies were applied for both deterministic and probabilistic calculations (Table 3), as insufficient data were found to develop ranges or distributions. The AE factors applied for arsenic and copper were derived from studies in which both absorption and retention/elimination were measured, but the AE factors for cadmium, lead, and nickel were based on absorption fractions only. As a result, AE factors for lead and nickel may overestimate assimilation, but omission of cadmium excretion is not expected to be critical because this metal is excreted very slowly once absorbed [23].

Body weights of mice were measured in the field. Weights for individual modeling were time-weighted averaged over the period each mouse was trapped (n = 11 individuals, mean of 13.7 weeks). Weights for sitewide analyses were pooled from all mouse captures (n = 399), from which the fifth-percentile, mean, and maximum body weights were applied as fixed-point estimates (Table 3). Fitted distributions of body weights (Table 3) were applied in Monte Carlo simulations.

Model evaluation

Modeled whole-body concentrations in house mice were compared with measured carcass levels to evaluate the predictive ability of each model. Carcasses were prepared and analyzed for metals as described in Torres and Johnson [18]. Deterministic sitewide predictions were tested against metal concentrations in carcasses of all 13 individuals. Individual mouse predictions were compared with levels observed in each of the 11 individuals whose home range–specific exposures were estimated.

Estimated and observed tissue concentrations were compared with both proportional deviations and statistical tests. Proportional deviations were calculated as follows:

$$\text{Proportional deviation} = \frac{\text{measured concentration} - \text{estimated mean concentration}}{\text{measured concentration}} \quad (5)$$

Proportional deviations and statistical comparisons were based on mean model predictions. The degree to which measured concentrations overlapped ranges of deterministic estimates (minimum to maximum) was also evaluated.

The significance of differences between individual estimates ($n \leq 11$) and measured concentrations were evaluated with two-tailed, paired, nonparametric tests. When differences or transformed differences between paired values were distributed symmetrically, the Wilcoxon signed-rank test was used [24]. Although this test assesses differences on the basis of whether one data set is consistently higher or lower than the other, the relative magnitudes of pair differences are incorporated into the rank calculation. If paired differences were not distributed symmetrically, the similar but slightly less powerful sign test [25] was applied. Cadmium and nickel predic-

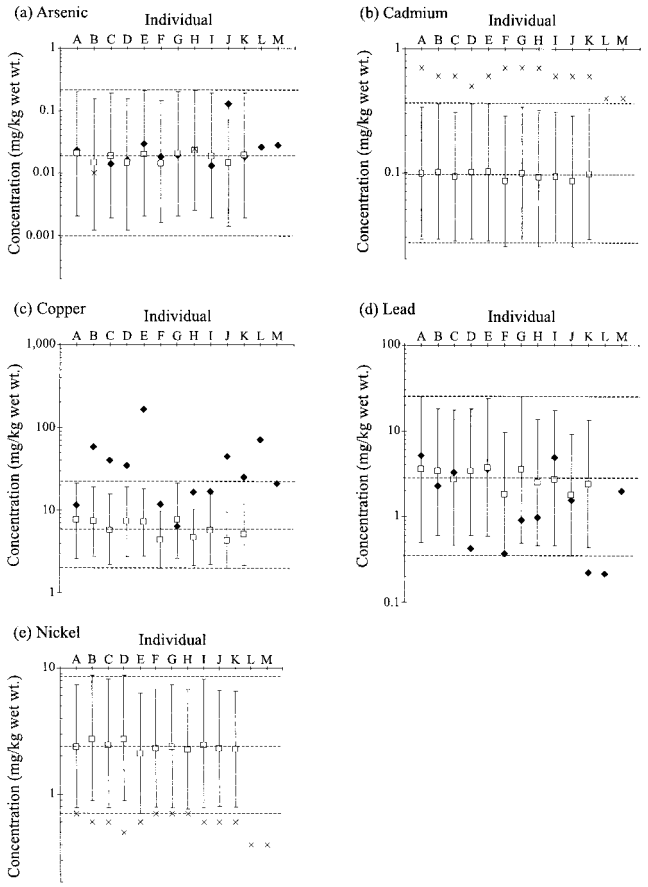

Fig. 1. Comparison of measured carcass concentrations and predicted body burdens of metals in individual and sitewide house mice generated with the deterministic bioaccumulation regression models. High–low bars represent estimated maximum and minimum concentrations in each mouse. Middle, high, and low dashed horizontal lines represent mean, maximum, and minimum predictions, respectively, of concentrations in sitewide mice. ♦ = measured concentration, □ = predicted individual concentration, × = undetected (detection limit).

tions were not validated because these metals were undetected in carcasses. Arsenic predictions for two individuals were excluded from tests because arsenic was not detected in their carcasses.

To determine whether individual estimates of body burdens were more accurate than sitewide estimates, sign or Wilcoxon signed-ranked tests were applied. These tests were performed on absolute values of differences between predicted mean concentrations and measured concentrations ($n \leq 11$) for individuals versus those for sitewide predictions. As with the previously described tests of model accuracy, individual and sitewide predictions of cadmium and nickel levels could not be evaluated statistically, and two individuals were excluded from arsenic analyses.

RESULTS

Soil–small mammal bioaccumulation regression models

Deterministic estimates of whole-body arsenic concentrations in house mice made with the bioaccumulation regression models [4] accurately predicted concentrations measured in carcasses (mean of 0.027 mg/kg wet wt, $n = 13$, calculated with two nondetects replaced by one-half of the detection limit) (Fig. 1a). No significant difference was observed between measured and estimated arsenic concentrations in individuals based on signs of their differences (sign test; $B = 5$, $n = 9$, $p > 0.05$) (Table 4). The magnitudes of differences were also small, proportional deviations of estimated values from measured levels were low (Table 4), and all detected carcass concentrations fell within the ranges of sitewide and individual estimates (Fig. 1a).

The accuracy of modeled concentrations of cadmium in mice could not be evaluated because this metal was undetected in all carcasses (mean detection limit of 0.6 mg/kg, $n = 13$; Fig. 1b).

Copper concentrations modeled in individuals were consistently lower (Wilcoxon signed-rank test; $T = 7$, $n = 11$, $p = 0.02$; Table 4) than measured concentrations in carcasses (mean of 40.1 mg/kg, $n = 13$) (Fig. 1c). Although proportional deviations of estimated values from measured levels were low (Table 4), less than half the measured levels in carcasses fell within the ranges of sitewide estimates (Fig. 1c).

Lead concentrations in house mouse carcasses (mean of 1.98 mg/kg, $n = 13$) were successfully predicted by the lead regression model. Estimated and measured values were not significantly different (Wilcoxon signed-rank test; $T = 13$, $n = 11$, $p = 0.1$; Table 4). Eleven of 13 measured concentrations were within one order of magnitude of the predicted mean sitewide concentration, and large portions of measured concentrations fell within the ranges of individual and sitewide predictions (Table 4 and Fig. 1d).

Nickel was undetected in all carcasses, but, on the basis of detection limits (mean of 0.6 mg/kg, $n = 13$), it appears that the model overpredicted nickel concentrations in mice (Fig. 1e).

Monte Carlo simulations of the bioaccumulation regression models produced skewed distributions of predicted body burdens, with most individuals expected to have low or moderate tissue levels of metals and fewer mice projected to exhibit high levels. The relative skewness and spread in modeled output varied among metals; distributions of lead concentrations were among the more skewed, with a long tail at higher concentrations (Fig. 2). Probabilistic distributions of body burdens were generally consistent with deterministic sitewide estimates. Median simulated concentrations approximated mean values predicted by deterministic calculations, as shown in the lead ouput (Fig. 2). However, deterministic minimum and maximum predictions for all metals were more widely spaced, or conservative, than the 5th- and 95th-percentile values of Monte Carlo output (e.g., Fig. 2). Probabilistic estimates of metal body burdens were relatively insensitive to soil concentration (the only input variable in the regression models). Rank correlation coefficients of model output to soil concentration were low, ranging from 0.08 for nickel to 0.35 for copper (Table 5).

No significant differences in accuracy were detected between individual and sitewide deterministic predictions for arsenic (Wilcoxon signed-rank test; $T = 12$, $n = 8$, $p > 0.5$), copper (Wilcoxon signed-rank test; $T = 31$, $n = 11$, $p > 0.5$), or lead (Wilcoxon signed-rank test; $T = 23$, $n = 11$, $p > 0.2$) on the basis of the absolute values of deviations between predicted and measured values (Table 4). However, mean proportional deviations of modeled concentrations from measured values were slightly lower for individual than for sitewide estimates of arsenic, copper, and lead levels (Table 4).

Table 4. Accuracy of deterministic model predictions of metal concentrations in house mice

Metal/model	Sitewide mean PD[a]	Individual mouse estimates		
		Mean PD[a]	Measured concentration relative to estimated mean concentration[b]	Accuracy of sitewide relative to individual estimates[c]
Arsenic				
Regression	0.13	0.07	NS (S)	NS (W)
Cumulative ingestion	−33.5	−42.1	<** (W)	NS (W)
Cadmium[d]	—	—	—	—
Copper				
Regression	0.70	0.67	>* (W)	NS (W)
Cumulative ingestion	−37.8	−51.5	<** (W)	<* (W)
Lead				
Regression	−2.97	−2.27	NS (W)	NS (W)
Cumulative ingestion	−6.10	−6.18	<** (W)	NS (W)
Nickel[d]				
Regression	—	—	<[e]	—
Cumulative ingestion	—	—	<[e]	—

[a] PD = proportional deviation; PD = (measured concn. − estimated mean concn.)/measured concn.
[b] Paired test for differences between measured and estimated concentrations (n = 9 or 11) based signs of differences. S = Sign test; W = Wilcoxon signed-rank test; < = measured values significantly less than estimated values; > = measured values significantly greater than estimated values; NS = no significant difference ($p > 0.05$); * = $p < 0.05$; ** = $p < 0.01$.
[c] Paired test for differences in accuracy of sitewide versus individual estimates based on signs of differences between estimated and measured values. W = Wilcoxon signed-rank test; < = differences between measured and estimated values significantly less for sitewide estimates than for individuals; NS = no significant difference ($p > 0.05$); * = $p < 0.05$.
[d] Cadmium and nickel estimates could not be evaluated by most criteria because these metals were undetected in carcasses.
[e] Qualitative conclusion based on detection limits for undetected nickel concentrations.

Cumulative ingestion bioaccumulation model

Whole-body concentrations in house mice generated with the cumulative ingestion model were clearly higher than observed carcass levels of four of the five metals considered (Fig. 3). Estimated arsenic levels in individuals were consistently higher than measured values in carcasses (Wilcoxon signed-rank test; $T = 0$, $n = 9$, $p = 0.005$; Table 4). Proportional deviations of measured levels from estimated values in mice were high (Table 4), and only one measured concentration fell within the calculated range of arsenic levels (Fig. 3a).

Because cadmium was undetected in all mice, model predictions of cadmium body burdens could not be evaluated for accuracy (Fig. 3b).

Copper concentrations estimated for individuals consistently exceeded measured values (Wilcoxon signed-rank test; $T = 0$, $n = 11$, $p = 0.001$; Table 4). As with arsenic, the magnitudes of the differences were also substantial, indicated by the high proportional deviations between measured levels and estimates and by the observation that only a single measured carcass concentration was within the range of sitewide estimates (Fig. 3c).

Modeled levels of lead in mice generally exceeded measured concentrations, though by a lesser degree than was the case with arsenic and copper (Fig. 3d). Individual estimates were consistently greater than observed body burdens (Wilcoxon signed-rank test; $T = 1$, $n = 11$, $0.001 < p < 0.005$; Table 4). However, the majority of measured carcass concentrations fell within the range of sitewide estimates (Fig. 3d).

Nickel was undetected in all carcasses, but individual and sitewide model predictions appeared to exceed nickel concentrations in mice on the basis of detection limits (Fig. 3e).

Like the regression models, Monte Carlo simulations of the cumulative ingestion model produced skewed distributions of body burdens (e.g., output for lead in Fig. 4). Most of the simulated concentrations of metals well exceeded detected carcass concentrations, which was consistent with deterministic model results. Median tissue levels generated in Monte Carlo simulations were close to deterministic mean estimates (e.g., Fig. 4). As with predictions of the regression models, deterministic minimum and maximum estimates of the ingestion model were more widely spaced than the probabilistic 5th- and 95th-percentile benchmarks (e.g., Fig. 4).

The cumulative ingestion model incorporated six stochastic variables in the calculation of body burdens. Simulated body burdens were most sensitive to log(FMR), age, metabolizable energy content of seeds, and body weight (Table 5). Soil concentration exerted an important influence on lead and arsenic levels in mice (Table 5). Model sensitivities to concentrations in arthropods, metabolizable energy content of arthropods, diet composition, and AE factor could not be calculated because these parameters were incorporated as fixed-point values in the ingestion model.

The proportional contributions of ingested soil, seeds, and arthropods to the total modeled intake of each metal by house mice were calculated to illustrate the relative importance of each source based on assumptions and parameter values associated with the mean scenario of the deterministic model (Table 6). Arthropods and incidentally ingested soil appear to be significant sources of ingested metals, while a relatively minor fraction of ingested metals originate in seeds (Table 6).

Minimum and maximum sitewide predictions exhibited a substantially greater spread in concentrations than did individual predictions (Fig. 3). As a result, ranges of sitewide estimates more frequently coincided with measured carcass levels than did ranges of individual estimates (Table 4 and Fig. 3). Individual and sitewide concentrations generated by the ingestion model did not significantly differ in accuracy in predicting carcass levels of arsenic (Wilcoxon signed-rank test;

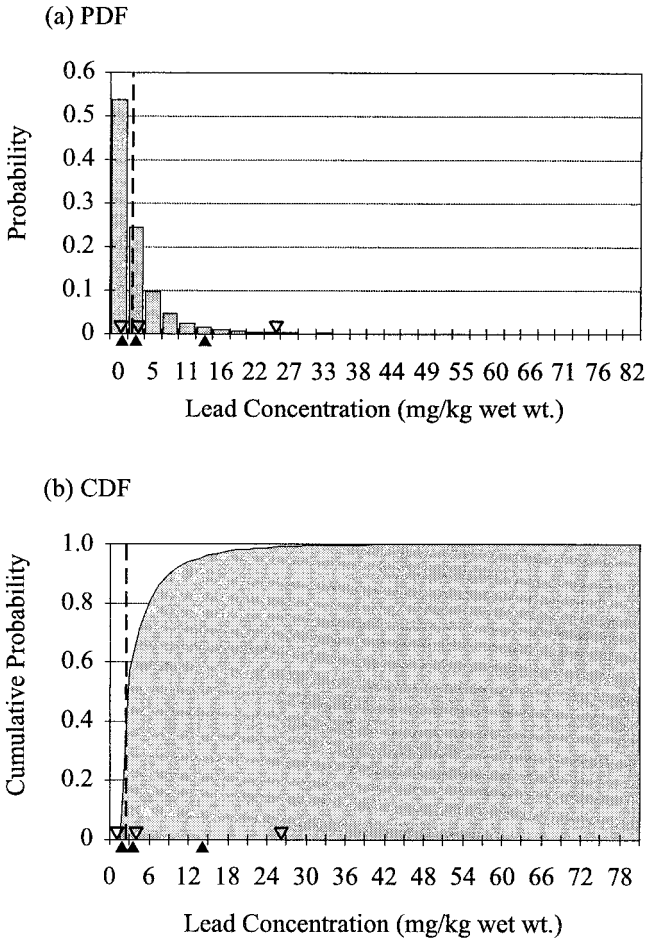

Fig. 2. (**a**) Probability density function (PDF) and (**b**) cumulative distribution function (CDF) of lead body burdens in house mice simulated with the bioaccumulation regression model. ▲ = 5th-, 50th-, and 95th-percentile predictions; ∇ = deterministic minimum, mean, and maximum predictions; dashed line = mean carcass concentration measured, 1.984 mg/kg.

$T = 8$, $n = 9$, $p > 0.1$) or lead (Wilcoxon signed-rank test; $T = 12$, $n = 11$, $p > 0.05$; Table 4). In contrast, the sitewide estimate of copper bioaccumulation was consistently more accurate than individual estimates of copper levels (Wilcoxon signed-rank test; $T = 7$, $n = 11$, $p < 0.05$). Qualitatively, sitewide estimates exhibited slightly lower mean proportional deviations from observed concentrations of arsenic, copper, and lead compared with those of individual estimates (Table 4).

DISCUSSION

The majority of the data incorporated in the soil–small mammal regression models are whole-body concentrations [4]; the cumulative ingestion model likewise calculates whole-body tissue burdens. Certain tissues (pelage, skin, digestive tracts, feet, and tails) were removed from analyzed carcasses to exclude unassimilated metals in ingested foods and limit potential metal contamination from particles on external hair and skin [18]. It is plausible that these exclusions may have contributed to any differences identified between modeled and empirical results. Nevertheless, evidence exists that differences between carcass and whole-body concentrations can be minor. For example, Beyer et al. [26] found very similar levels of cadmium, copper, lead, and zinc in carcasses and whole bodies of small mammals collected near zinc smelters. If removal of tissues in this study caused carcass concentrations to deviate from whole-body concentrations, the metal most likely affected is arsenic, which accumulates substantially in hair and nails.

Soil–small mammal bioaccumulation regression models

The metal-specific regression models developed by Sample et al. [4] (Table 1) produced accurate estimates of arsenic and lead concentrations in whole bodies of house mice (Table 4 and Figs. 1a and d). Although successfully tested in the present study, neither model was previously validated with a larger set of independent data [4]. The copper bioaccumulation model yielded predictions that were generally within one order of magnitude of measured body burdens in this study but tended to underpredict them (Table 4 and Fig. 1c). In contrast, the nickel model appeared to overpredict nickel concentrations in house mice based on the detection limits for undetected concentrations (Fig. 1e). Both the copper and the nickel models were independently validated by Sample et al. [4]. The differing success by which the regression models predicted body burdens of each metal in this study and by which the models were previously validated underscore the variable accuracy that the models are likely to exhibit when applied in distinct cases. The predictive abilities of the models depend on how closely a variety of factors at a particular site coincides with those represented in the original data, including the range of soil concentrations, chemical speciation, soil characteristics,

Table 5. Sensitivities of probabilistic bioaccumulation models to input parameters

	Rank correlation coefficient of output[b] to input parameter				
Model/input parameter[a]	Arsenic	Cadmium	Copper	Lead	Nickel
Small mammal bioaccumulation regression models					
Soil concentration (mg/kg dry wt)	0.12	0.09	0.35	0.23	0.08
Cumulative ingestion bioaccumulation model					
Log (FMR, kcal/d)	0.59	0.61	0.61	0.48	0.59
Age (weeks)	0.55	0.57	0.56	0.45	0.56
Metabolizable energy of seeds (kcal/g)	−0.27	−0.28	−0.28	−0.22	−0.28
Soil concentration (mg/kg dry wt)	0.22	−0.010	0.04	0.59	0.10
Body weight (g)	−0.17	−0.17	−0.17	−0.14	−0.17
Seed concentration (mg/kg dry wt)	0.04	—	0.01	0.04	0.17

[a] FMR = field metabolic rate.
[b] Monte Carlo–simulated body burdens in house mice (mg/kg fresh wt).

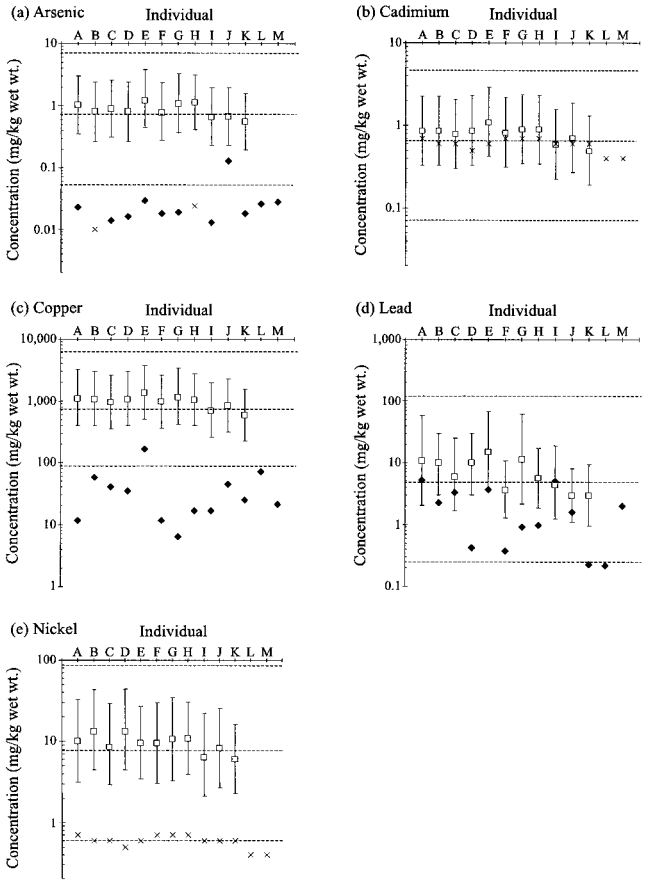

Fig. 3. Comparison of measured carcass concentrations and predicted body burdens of metals in individual and sitewide house mice generated with the deterministic cumulative ingestion bioaccumulation model. High–low bars represent predicated maximum and minimum concentrations in each mouse. Middle, high, and low dashed horizontal lines represent mean, maximum, and minimum predictions, respectively, of concentrations in sitewide mice. ♦ = measured concentration, □ = predicted individual concentration, × = undetected (detection limit).

and the small mammals of interest along with their respective ages, sexes, diets, life histories, and behaviors.

Numerical ranges between minimum and maximum predictions were broad (Fig. 1), reflecting variability and uncertainty inherent in the models as well as variability in input soil concentrations at the site or within individual home ranges. These wide prediction ranges are limited in precision, but applying such conservative ranges in calculated body burdens may be useful for modeling exposures to predators in screening-level ecological risk assessments. The relatively large ranges of estimated tissue concentrations appear appropriate for copper and lead, whose concentrations in carcasses were variable (Figs. 1c and d).

In general, the magnitude of spread of the Monte Carlo–simulated distributions was determined largely by stochasticized statistical variances in the regression models and to a lesser extent by variation in soil concentrations at the site. The considerable variability and uncertainty associated with the regression models was evident from the low sensitivities of model output to soil concentration (Table 5).

Cumulative ingestion bioaccumulation model

The cumulative ingestion bioaccumulation model substantially overestimated arsenic and copper concentrations in house mouse carcasses (Figs. 3a and c) and appeared to overestimate nickel concentrations based on detection limits for undetected values (Fig. 3e; see Table 4). Maximum estimates for all metals were unrealistically high for body burdens of small mammals, as were mean levels predicted for arsenic, copper, and nickel. Although lead concentrations in carcasses were consistently overestimated, most measured values were within one order of magnitude of the mean sitewide prediction (Table 4 and Fig. 3d).

Table 6. Fractions of total metal intake by house mice originating from each source in the cumulative ingestion bioaccumulation model

Metal	Fraction of total metal intake[a]		
	Soil (2%)[b]	Seeds (79%)[b]	Arthropods (19%)[b]
Arsenic	0.51	0.05	0.44
Cadmium	0.03	0.23	0.74
Copper	0.03	0.06	0.91
Lead	0.85	0.03	0.12
Nickel	0.63	0.18	0.19

[a] Total metal intake (mg/d) calculated from mean parameter values.
[b] Dry-weight percentage in diet.

Error in the cumulative ingestion model can extend from model structure or parameter values. Sources of error in model structure may include simplifications such as time-averaged body weights and doses of ingested metals. In reality, body weights and chemical exposures of a small mammal vary over time (from gestation to postnatal and juvenile development to adulthood) and among seasons during an individual's lifetime as food habits, caloric intake, and growth rates change. Modeled food ingestion rates, based in part on the FMR, should approximate food intake of nonbreeding adult mice but may underestimate that of growing individuals and pregnant and lactating females [27]. A large portion of the body burden of some metals may be assimilated during gestation. Arsenic, cadmium, lead, and nickel have been shown to transfer across the placenta to the fetal circulatory system [28], and copper levels are higher in the livers of newborn mammals than in those of adults [29]. Another simplification of model structure is the assumed constant assimilation of ingested metals, expressed as an AE factor.

Prediction error of the ingestion model is also likely to originate from the values of certain parameters, particularly diet composition and the AE factor. Seasonal variability in the local house mouse diet was not accounted for in the model; the summer and fall diet, consisting primarily of *S. robustus* seeds and arthropods, was assumed to contribute to the majority of the lifetime dietary exposures to metals of individuals sacrificed in late fall. Additional uncertainty extends from lack of knowledge of specific arthropod taxa consumed by mice. Metal concentrations in the three species of isopods and arachnids (Table 2) are expected to represent conservative upper bounds for levels in arthropods at the site. Unlike concentrations in seeds and soils, metal concentrations in arthropods were represented as single, fixed-point estimates that did not account for their variability in these prey.

The most uncertain variable in the cumulative ingestion model is the AE factor. Since point estimates of gastrointestinal absorption and elimination of each metal were applied (Table 3), no uncertainty or variability in this parameter was accounted for in bioaccumulation predictions. Gastrointestinal bioavailability of metals from ingested food and soil is known

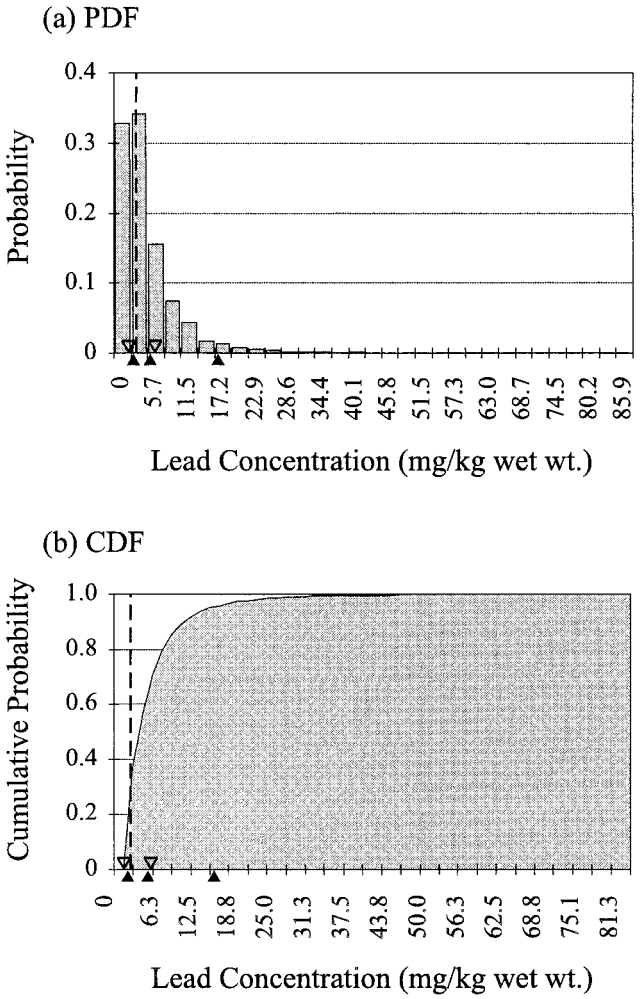

Fig. 4. (**a**) Probability density function (PDF) and (**b**) cumulative distribution function (CDF) of lead body burdens in house mice simulated with the cumulative ingestion bioaccumulation model. ▲ = 5th-, 50th-, and 95th-percentile predictions; ∇ = deterministic minimum and mean predictions (maximum of 117 mg/kg extends beyond scale of graph); dashed line = mean carcass concentration measured, 1.984 mg/kg.

to vary with respect to chemical form, aqueous solubility, age, and nutritional condition of an animal [30,31]. The AE factors used in this study are additionally uncertain because they were generally derived from studies of ingested metal salts and based on short-term retention and excretion experiments. To apply more realistic values of gastrointestinal absorption and assimilation in wildlife, more relevant bioavailability data from experiments with metal molecules contained in a variety of soil and food matrices are needed (e.g., [32,33]).

To prioritize which parameters could most benefit from future refinements of the ingestion model, sensitivities of model output to the stochastic parameters were calculated. Input parameters contributing most strongly to whole-body concentrations were log(FMR), age, and metabolizable energy of seeds (Table 5). Measuring metabolic rates of house mice in the field and metabolizable energy of seeds and arthropods could reduce model error, but other parameters whose model sensitivities were not calculated are more uncertain (e.g., AE factor and arthropod taxa in diet).

Based on calculated fractions of metal intake from ingested items, house mice generally received most of their exposures from soil and arthropods (Table 6). This suggests that identifying specific arthropod taxa in the local house mouse diet and including variability in arthropod concentrations in the model would be critical to improving bioaccumulation estimates. Since incidentally ingested soil appears to contribute significant metal doses to mice, a need also exists to examine the gastrointestinal absorption of metals bound to soils. While a constant AE factor was applied in the model, it is more likely that absorption of metals from plant and animal tissues differs from absorption of metals on soil particles. The considerable variability in gastrointestinal bioavailability of metals from ingested soil [34,35] should also be accounted for in exposure models.

Distributions of predicted body burdens in the Monte Carlo simulations are products of combined uncertainty and variability in parameter values. Since these factors were not explicitly distinguished, output distributions of the ingestion model are approximations of the expected tissue concentrations in house mice. Mean deterministic and median probabilistic predictions were similar in magnitude, but minimum and maximum deterministic values were much more widely spaced than the 5th- and 95th-, and even 1st- and 99th- (not shown), percentile probabilistic predictions (e.g., Fig. 4). This resulted from grouping extreme (minimum and maximum) values of multiple exposure variables in deterministic calculations, which usually leads to compounded conservatism in exposure and risk estimates [36]. Probabilistic 5th- and 95th-percentile estimates yielded slightly more relevant bounds of bioaccumulation in mice since parameters were sampled independently in the Monte Carlo simulations.

Individual and sitewide bioaccumulation estimates

Predictions of bioaccumulation in individual mice with the regression models were slightly, but not significantly, more accurate than sitewide mean predictions (Table 4). Despite direct measurements of exposure concentrations and parameter values for individuals, sitewide mean estimates of the ingestion model were qualitively slightly more accurate than individual mouse estimates (Table 4 and Figs. 3a, c, and d), but only because older individuals were preferentially sampled, resulting in more highly overpredicted body burdens in those mice. Also, the absence of home range–specific arthropod concentrations in the ingestion model may have limited the accuracy of individual predictions.

Individual-level, spatially explicit modeling of bioaccumulation in small mammals requires extensive live trapping and media sampling. Individuals may acquire substantial portions of their body burdens of contaminants during gestational and postnatal development in maternal home ranges prior to entering the trappable population and dispersing. Consequently, adequately parameterizing such models is difficult. Only some individuals trapped in this study utilized compact home ranges deemed suitable for individual modeling, while others were transient or ranged across variable portions of the site. Since sitewide estimates of both models employed in this study were representative of collective individual estimates and sitewide predictions were not significantly less accurate than individual predictions, the sitewide approach to modeling bioaccumulation is preferred.

Recommendations for exposure modeling in ecological risk assessments

While mapping spatial use patterns of individuals may not justify the effort of extensive live trapping, trapping can de-

termine which individuals are transients and which are long-term residents of a site and thus more appropriate for tissue analysis sampling. Not all areas of a site are used by small mammals because of disturbance or poor habitat quality, which typically coincide with the most contaminated areas. Trapping can therefore help identify areas that are relevant for sampling media and biota for inclusion in food web modeling. Trapping also facilitates collecting feces or stomach contents of small mammals for diet analysis. Site-specific diet analyses are critical to improve bioaccumulation and exposure estimates since much wildlife exposure modeling is plagued by lack of information about local food webs [10]. Diet analyses are especially critical for species whose diets are variable or opportunistic.

Predictive bioaccumulation and exposure models can introduce considerable uncertainty into screening-level ecological risk assessments, prompting the need for model validation through tissue sampling. The soil–small mammal bioaccumulation regression models developed by Sample et al. [4] can adequately predict whole-body concentrations of chemicals in small mammals, as illustrated by arsenic and lead in this study. The accuracy of these models varies among metals and among sites at which they are applied, but use of prediction intervals can help ensure conservative estimates for screening-level assessments. Modifying the models by incorporating additional regression parameters, such as soil properties and differentiating between metal species in soil, could reduce prediction error.

The cumulative ingestion bioaccumulation model as parameterized in this study produced excessive estimates of body burdens of most metals in house mice. Which parts of the model contributed most to prediction error are unknown, but certain refinements would increase the realism of the model. Diet composition, body weight, and field metabolic rate can be represented as factors that change over seasons and an individual's lifetime. Also, elements of mechanistic toxicokinetic models (e.g., absorption, metabolism, distribution, and elimination kinetics) may be applied in place of constant AE factors. Along with the these improvements to the structure of wildlife exposure models, more experimental data on gastrointestinal absorption and assimilation of chemicals from various soil and food matrices (e.g., plant parts, soft animal tissues, exoskeletons, bone, scales, hair, and feathers) are needed. Simpler statistic-based models, such as empirical regressions or BAFs, may generate more accurate predictions of chemical doses and bioaccumulation in many cases and are easier to parameterize. However, mechanistic models can be promising tools for increasing the understanding of contaminants in wildlife as they are tested and refined.

SUMMARY

Soil–small mammal bioaccumulation regression models developed by Sample et al. [4] produced accurate estimates of arsenic and lead body burdens in house mice but did not adequately predict copper or nickel concentrations in mice. A mechanistic ingestion-based model of bioaccumulation overpredicted most concentrations of lead by less than one order of magnitude but substantially overpredicted levels of arsenic, copper, and nickel. Monte Carlo simulations of the regression models generated probabilistic distributions of body burdens that were consistent with deterministic results. However, deterministic minimum and maximum predictions of the ingestion model were excessively conservative relative to lower and upper probabilistic percentiles. Predictions of metal concentrations in individual mice were not significantly more accurate than sitewide predictions and failed to justify the additional effort of individual-level modeling.

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