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Bioavailability of Cadmium in Food and Water: A Case Study on the Derivation of Relative Bioavailability Factors for Inorganics and Their Relevance to the Reference Dose[1]

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Published studies in which rats were exposed to $CdCl_2$ in standard chow or drinking water were analyzed to compare the relative bioavailability of cadmium from the two media. Relative bioavailability was assessed from estimates of the rate of accumulation of cadmium in kidney cortex or liver. Data were grouped into tiers based on study design and reporting of data: Tier 1, identical experimental protocols and dosage can be estimated; Tier 2, very similar or identical protocols and dosage can be estimated; Tier 3, protocols may differ and dosage can be estimated; and Tier 4, protocols may differ and dosages cannot be estimated (but concentration of cadmium in food or water is reported). Tiers were nested, such that Tier 4 contained all relevant studies; Tier 3 included data sets from Tiers 1 and 2; and Tier 2 included the data set from Tier 1. Data within Tiers 1, 2, and 3 were subjected to a linear regression analysis with dosage as the independent variable and tissue accumulation rate as the dependent variable to determine whether bioavailability of cadmium was significantly different based on medium of administration. The results of this analysis show the following: (1) In rats receiving food and drinking water *ad libitum*, the bioavailability of cadmium in drinking water is not significantly different ($P > 0.05$) from the bioavailability of cadmium in food when dosages are less than 4 mg/kg body wt/day. (2) Cadmium decreases food and water consumption; therefore, assessments of relative bioavailability should be made based on actual dosage rather than exposure levels. (3) Diet composition and status of the gastrointestinal tract are probably a more important determinant of the bioavailability of cadmium than is the exposure medium. (4) Studies of the effect of total diet composition on bioavailability of cadmium may be more relevant than are studies of the effect of the exposure medium. It is concluded from this analysis that the bioavailability of cadmium in food is not different from that in water when diet is provided *ad libitum*. Therefore, we recommend that distinct RfDs for cadmium in food and drinking water should not be based on the assumption that the bioavailability of cadmium in drinking water is greater than that of cadmium in food.

INTRODUCTION

A reference dose (RfD) is defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure (mg/kg/day) to the human population

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(including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime" (U.S. EPA, 1994). RfDs that are verified and on the U.S. EPA Integrated Risk Information System (IRIS) (U.S. EPA, 1994) represent a consensus within the U.S. EPA regarding the risk to human health posed by exposure to specific chemicals. RfDs are used in risk characterizations of hazardous waste sites where human exposure to chemicals may occur from ingesting contaminated food, water, or soil (U.S. EPA, 1989).

The RfD for a chemical can be specific for an exposure medium if there is sufficient evidence to define a unique dose–response relationship for the chemical in that medium. Separate RfDs for cadmium in food and drinking water have been derived based on the assumption that the bioavailability of cadmium in water is greater than that from food by a factor of 2 (i.e., 5% vs 2.5%, respectively) (U.S. EPA, 1994). Separate RfDs for manganese in food and drinking water have also been derived (U.S. EPA, 1994). Distinct RfDs for nickel in food and drinking water also may be considered based on the apparent greater bioavailability of nickel in drinking water compared to that in food (Sunderman *et al.*, 1989).

The application of relative absorption factors to adjust intakes used in determining hazard quotients and cancer risks in risk characterizations is discussed in the Risk Assessment Guidelines for Superfund (RAGS) (U.S. EPA, 1989, Appendix A). For the hazard quotient (HQ), the adjustment takes the form

$$HQ = I/RfD$$

$$HQ_{ADJ} = I \cdot RAF/RfD,$$

where HQ_{ADJ} is the adjusted hazard quotient, RAF is the relative absorption factor, and I is the oral intake (mg/kg/day). In this context, the RAF is defined as the ratio of the absorption of a chemical in "the medium of concern" to that of the same chemical in the exposure medium used in the studies that formed the basis of the RfD. Similarly, in estimating cancer risk (CR)

$$CR = I \cdot SF$$

$$CR_{ADJ} = I \cdot RAF \cdot SF,$$

where SF is the cancer risk slope factor. The implication is that an RfD or slope factor based on studies of one exposure medium (e.g., food) may not be directly applicable to estimating the hazard quotient or cancer risk for an exposure through a different medium (e.g., drinking water or soil) if the bioavailability of chemicals in these media are dissimilar.

The verification of media-specific RfDs for cadmium based on relative bioavailability and the discussion in RAGS of bioavailability adjustments of intake in determining hazard quotients and cancer risks may be indicative of a wider application of either or both approaches in the future. It is, therefore, appropriate to consider in some depth the concept of relative bioavailability from the perspective of both methodological issues inherent to the derivation of quantitative expressions of relative bioavailability and the application of these expressions to risk assessment. This study was conducted to explore one dimension of this problem: What constitutes adequate evidence to support the derivation of an estimate of relative bioavailability?

A case study was performed in which experimental data on the bioavailability of cadmium were evaluated in an attempt to derive estimates of the relative bioavailability

of cadmium in food compared to drinking water. Cadmium was selected for the case study because of the abundance of published data on the subject, which would allow an exploration of the impact of data quality and methodology on the estimation of relative bioavailability. Data on cadmium were examined with the objective of exploring approaches to the derivation of media-specific bioavailability factors for inorganics that might facilitate consistent integration of bioavailability information in risk assessment. The results of this study suggest that, in rats provided food and water *ad libitum*, bioavailability of cadmium in food and drinking water is not significantly different at dosages less than 4 mg/kg body wt/day. The implications of this finding on the RfDs for cadmium and on the concept of media-specific RfDs in general are discussed.

METHODS

Data Collection

Computer literature searches were conducted in 1991 of the following on-line bibliographic data bases: HSDB, RTECS, TSCATS, and TOXLINE (for 1986–1991). Reviews were used to identify earlier relevant literature (ATSDR, 1992; U.S. EPA, 1980, 1981, 1986, 1988; Friberg *et al.*, 1974, 1985; Kjellström and Nordberg, 1978; Friberg, 1984; Foulkes, 1986; Fox, 1983; Tsuchiya, 1978). References were tree-searched to identify additional pertinent literature.

Definition of Relative Bioavailability

Bioavailability can be defined as the fraction (F) of the oral dose that enters the systemic circulation (Gibaldi and Perrier, 1982). Assuming a bioavailability of an intravenous dose of 1, F for an oral dose (F_{oral}) is defined

$$F_{oral} = (D_{iv} \cdot AUC_{oral})/(D_{oral} \cdot AUC_{iv}), \quad (1)$$

where

D_{iv} = intravenous dose
D_{oral} = oral dose
AUC_{oral} = area under plasma (or blood) concentration vs time curve for oral dose
AUC_{iv} = area under plasma (or blood) concentration vs time curve for intravenous dose.

Similarly, relative bioavailability of the chemical in food and water ($F_{f/w}$) are defined as:

$$F_{f/w} = F_{food}/F_{water}. \quad (2)$$

The above definitions of bioavailability (Eq. (1)) could not be applied to cadmium because there are insufficient kinetic data to support estimates of AUC. Two indices of $F_{f/w}$ were evaluated in this study: the rate of accumulation of cadmium in the renal cortex and the rate of accumulation of cadmium in the liver. The concept of using measurements of accumulation or concentration in specific tissues as an index of relative bioavailability is not new and has been applied to other inorganics. For example,

the concentration of lead in blood has been used as an index of relative bioavailability of lead (Mushak, 1991). This approach is valid providing that the amount or concentration of the compound in the selected tissue is linearly related to whole body burden. This appears to be true for cadmium in renal cortex and liver, which contain most of the cadmium body burden (Friberg *et al.*, 1985).

Assessment of Rates of Accumulation of Cadmium in Tissue

Relative bioavailability of cadmium was assessed by comparing the rates of accumulation of cadmium in renal cortex and whole liver (μg/g tissue wet wt/day) across dosages (mg/kg/day) resulting from exposure of rats to cadmium in rat chow or drinking water. The data on laboratory rats were selected for analysis because the rat has been used extensively as an experimental model for cadmium-induced nephrotoxicity (Friberg *et al.*, 1985). Rates of accumulation rather than total tissue burdens were used to assess bioavailability because most of the published studies reported concentrations of cadmium in tissues (e.g., μg/g) rather than cadmium burdens and did not report organ weights. Furthermore, data on body weights were often inadequate to support reliable estimates of organ weights.

Several assumptions were made in applying the above approach. In rats exposed daily to cadmium at any one dose level, concentrations of cadmium in the renal cortex and liver were assumed to increase at a constant rate over time until concentrations in the renal cortex reached ≈200 μg cadmium/g wet wt. This is supported by studies of Bernard *et al.* (1983) and Kajikawa *et al.* (1981), in which, during chronic exposure to cadmium, concentrations in the renal cortex increased approximately linearly over time until the concentration reached 200–250 μg/g wet wt and then decreased steadily over the remaining exposure period. To validate this assumption for the data sets used in this analysis, data from each study in which concentrations of cadmium in the renal cortex and liver were measured at more than two exposure durations were subjected to a linear regression analysis of concentration of cadmium in tissue against exposure duration. Values of r^2 were ≥0.80 in 98 and 80% of the data sets of cadmium in the renal cortex and liver, respectively, that were obtained from studies in which the concentration of cadmium in the renal cortex did not exceed 200 μg/g wet wt. Therefore, the analysis was limited to exposure durations and dosages in which concentrations of cadmium in the renal cortex were <200 μg/g wet wt.

The rates of accumulation of cadmium (μg cadmium/g wet wt/day) were estimated by dividing the concentration (μg cadmium/g wet wt) by the exposure duration in days. In some studies, the concentration of cadmium in the entire kidney, rather than in the renal cortex, was reported; in these cases, the concentration of cadmium in the renal cortex was estimated as 1.25 times the concentration of cadmium in the whole kidney (Friberg *et al.*, 1985). The concentration of cadmium throughout the liver was assumed to be uniform. Some studies reported concentrations of cadmium in dry tissue; these values were converted to concentrations of cadmium in wet tissue by dividing concentrations of cadmium in dry renal cortex by 5 and concentrations of cadmium in dry liver by 3 (Zalups *et al.*, 1987).

Estimation of Dosage from Exposure Level

Most studies reported exposure levels (e.g., ppm), rather than the dosage (e.g., mg/kg/day). To capture as many data as possible in the analysis, dosages were estimated

if adequate supporting data were reported. In general, daily water consumption in rats exceeds food consumption by a factor of 1.4 in young rats and 1.6–1.7 in adult rats (U.S. EPA, 1987). Therefore, if no other variables are considered, dosages from water can be expected to exceed dosages from food at the same exposure level. However, cadmium in drinking water decreases water consumption in rats (Baranski and Sitarek, 1987; Borzelleca *et al.*, 1989; Decker *et al.*, 1958; Fingerle *et al.*, 1982; Fowler *et al.*, 1975; Kotsonis and Klaassen, 1978; Mangler *et al.*, 1988; Sorrell and Graziano, 1990; Stacey *et al.*, 1988; Zenick *et al.*, 1982) and, similarly, cadmium in rat chow decreases food consumption (Groton *et al.*, 1991; Itokawa *et al.*, 1974; Machemer and Lorke, 1981; Nogawa *et al.*, 1981; Pond and Walker, 1975; Sporn *et al.*, 1970; Sugawara and Sugawara, 1974). The effect of cadmium on water and food consumption in rats is illustrated in Fig. 1. At a concentration of 50 ppm cadmium in drinking water, water consumption was decreased by approximately 40%, whereas at a concentration of 50 ppm cadmium in rat chow, food consumption was decreased by approximately 5%. At an exposure level of 10 ppm, water and food consumption were decreased by approximately 10 and 1%, respectively. To statistically compare the exposure-level-related decrease in food and water consumption, dose–response data for each medium were fitted to three models: linear, exponential, and reciprocal (data not shown). For each of the regression models, the predicted value for water consumption at 50 ppm cadmium was significantly smaller than that of food (i.e., the 95% confidence intervals for the regression models did not overlap). However, for concentrations <10 ppm the decrease in water consumption was not significantly different from that of food. This suggests that at concentrations of cadmium <10 ppm, food and water consumption decrease to a similar extent, but at higher concentrations the decrease in water consumption may exceed the decrease in food consumption. Therefore, the ratio of dosage from drinking water to that from rat chow is not constant across exposure level.

Dosages were estimated from reported concentrations of cadmium in food or drinking water and reported or estimated body weights and water or food consumption. Where only initial and final body weights were reported, average body weights were estimated as the initial body weight plus half the body weight gain. Where only initial body weights or initial ages of rats were reported, average body weights were estimated using reference growth curves for specific strains and sexes of rats (U.S. EPA, 1987). With two exceptions, dosage was not estimated if initial body weights or ages were not reported or if food or water consumption were not reported. The exceptions were two dietary studies that reported body weight data and that food consumption was similar in control and exposed rats; however, food consumption data were not provided (Loeser and Lorke, 1977; Maji and Yoshida, 1974). Dosages in these studies were calculated using the body weight data provided in studies and reference values for food consumption in rats (U.S. EPA, 1987).

Data Tiers

The entire data set was divided into four tiers, based on similarity of study design and reporting of data. The tiers represent a range of data profiles that might be encountered in an assessment of bioavailability of any chemical. The tiers were nested, such that Tier 4 includes all studies in Tiers 1, 2, and 3; Tier 3 includes studies in Tier 1 and 2; and Tier 2 includes the study in Tier 1.

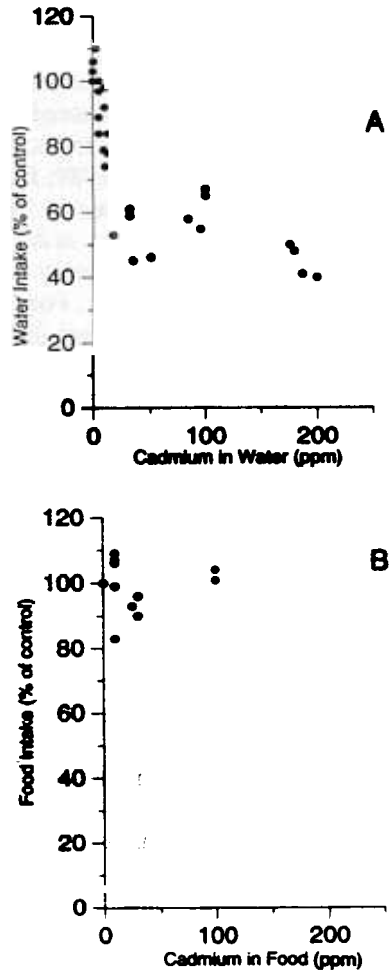

FIG. 1. Effects of exposure to cadmium in drinking water or food on intake of drinking water (A) or food (B), respectively. Controls were not exposed to cadmium. The effect of cadmium in drinking water on water intake is greater than the effect of cadmium in food on food intake. Data on drinking water intake are from Baranski and Sitarek (1987), Borzelleca *et al.* (1989), Decker *et al.* (1958), Fingerle *et al.* (1982), Fowler *et al.* (1975), Kotsonis and Klaassen (1978), Mangler *et al.* (1988), Sorrell and Graziano (1990), Stacey *et al.* (1988), and Zenick *et al.* (1982). Data on food intake are from Groton *et al.* (1991), Itokawa *et al.* (1974), Machemer and Lorke (1981), Pond and Walker (1975), Sporn *et al.* (1970), and Sugawara and Sugawara (1974).

Tier 4 includes all of the studies used in this analysis and consisted of data from studies in which rats were exposed to cadmium in rat chow or drinking water, from which an exposure level (ppm) was reported. Studies in Tier 4 varied considerably with respect to strain, body weight, age, and sex of rats, exposure level, and duration of administration.

Tier 3 consists of data from studies in which rats were exposed to cadmium in rat chow or drinking water, from which dosage (mg/kg/day) was reported or could be accurately calculated. No attempt was made to match experimental designs; therefore,

the studies varied considerably with respect to rat strain, body weight, age, and sex of rats, dose level, and duration of administration.

Tier 2 consists of data from studies in which rats were exposed to cadmium in rat chow or drinking water using identical or similar protocols and for which dosage was reported or could be accurately calculated. The data were organized into data groups, within which experimental protocols were closely matched with respect to strain, sex, initial and final age, and initial and final body weights of the rats; duration of treatment; and dosage.

Tier 1 consists of data from studies in which identical exposure and analytical protocols were used to compare animals exposed to cadmium in rat chow or drinking water and for which dosage was reported or could be accurately calculated. Tier 1 is considered to contain the most useful data for determining relative bioavailability, and is also usually the most scarce type of data.

Statistical Analysis of the Data

Data from Tiers 1, 2, and 3 were subjected to linear regression analysis to determine whether the rates of accumulation of cadmium in the liver and renal cortex, as a function of dosage, were significantly different ($P < 0.05$), based on medium of administration. The slope of the regression lines relating tissue accumulation and dosage in food (m_f) and water (m_w) were used as indices of bioavailability of cadmium in each medium; thus, $F_{f/w}$ was defined as the ratio of the slopes (m_f/m_w):

$$F_{f/w} = m_f/m_w. \tag{3}$$

The regression model that was used to estimate m_f and m_w is as follows (Mendenhall, 1968)

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2, \tag{4}$$

where

Y = rate of accumulation of cadmium in tissue (μg/g wet wt/day)
X_1 = 0 if the exposure medium is food, or 1 if the medium is water
X_2 = dosage (mg/kg body wt/day).

If the medium of exposure is food (i.e., $X_1 = 0$), Eq. (4) reduces to

$$Y = \beta_0 + \beta_2 X_2 \tag{5}$$

and β_2 is the slope of the line relating tissue accumulation rate and dosage in food (m_f). For exposures from water (i.e., $X_1 = 1$):

$$Y = \beta_0 + \beta_1 + (\beta_2 + \beta_3) \cdot X_2. \tag{6}$$

and $\beta_2 + \beta_3$ is the slope of the line relating tissue accumulation rate and dosage from water (m_w). Thus, β_3 is the difference between the slopes for water and food ($m_w - m_f$) and the ratio $\beta_2/(\beta_2 + \beta_3)$ is the ratio of the slopes, m_f/m_w, or $F_{f/w}$.

Two null hypotheses were tested. The first compared $F_{f/w}$ with 1 (i.e., H_0:$F_{f/w} = 1$ vs H_1:$F_{f/w} \neq 1$). The null hypothesis in this case is equivalent to H_0:$m_f/m_w = 1$, which can be expressed in terms of model parameters as H_0:$\beta_3 = 0$. The second hypothesis compared $F_{f/w}$ with 0.5 (i.e., H_0:$F_{f/w} = 0.5$ vs H_1:$F_{f/w} > 0.5$); the assumption used in

the derivation of the chronic oral RfDs for cadmium in food and drinking water was that $F_{f/w} = 0.5$ (U.S. EPA, 1994). The null hypothesis in this case is equivalent to $H_0: m_f/m_w = 0.5$, which can be expressed in terms of model parameters as $H_0: \beta_2 = \beta_3$. The null hypotheses were tested by a t statistic (Mendenhall, 1968) and were rejected if the P value was less than 0.05. The statistical analyses and data plots were developed with Statgraphics (Version 5.0) (STSC, 1991).

RESULTS

Data Profile

Forty-four studies were identified in which rats were exposed subchronically or chronically (up to 644 days) to cadmium in rat chow or drinking water, and cadmium concentrations in the liver and/or kidney were measured. Only data from studies in which rats were fed standard rat chow were used in the analysis; this included control groups (e.g., cadmium given in standard rat chow) from several studies in which the effects of dietary composition on cadmium uptake were studied (e.g., cadmium given in low-iron rat chow). One study that exposed rats to cadmium in pig liver was not included in the analysis (Groten *et al.*, 1990). Because the chemical form of cadmium may influence bioavailability, only studies using cadmium chloride ($CdCl_2$) were used in the analysis; studies of cadmium acetate (Carmignani and Boscolo, 1984; Kanisawa and Schroeder, 1969; Yuhas *et al.*, 1979) and of cadmium oxide (Weigel *et al.*, 1984) were excluded. Studies in which concentrations of cadmium in the renal cortex exceeded 200 μg/g wet wt (Abe *et al.*, 1972; Sugawara and Sugawara, 1974), a study of hypertension-sensitive rats (Ohanian *et al.*, 1978), and a study that reported unusually high mortality rates (Nogawa *et al.*, 1981) were also excluded from the analysis. The remaining 35 studies were used in the analysis, of which 16 studies provided enough information to estimate dosage. Studies used in the analysis are listed in Table 1.

Tier 1 Data

Tier 1 data were obtained from one study in which rats of the same strain, sex, age, and weight were exposed to $CdCl_2$ in rat chow or drinking water under otherwise identical exposure conditions, and identical analytical procedures were used to quantify uptake of cadmium into tissue (Buhler *et al.*, 1981). Fifty-five groups of rats (two per sex per group) were exposed to five different exposure levels of cadmium in the diet or six different exposure levels of cadmium in drinking water; rats were sacrificed after 1, 2, 4, 8, or 12 weeks of treatment for measurement of cadmium in liver and renal cortex. Thus, 55 estimates of the rates of accumulation of cadmium in the liver and renal cortex were reported. Male and female Wistar rats were provided water *ad libitum* containing 0.003–1.00 ppm $^{109}CdCl_2$ and rat chow to which no cadmium was added or rat chow containing 0.001–1.00 ppm $^{109}CdCl_2$ and drinking water to which no cadmium was added. Concentrations of cadmium in liver and kidney were determined after 1, 2, 4, 8, or 12 weeks of exposure. Estimates of fractional absorption (body burden/cadmium intake) associated with each measurement of cadmium in tissue were reported, allowing estimates of dosages as follows: dosage (mg/kg/day) = body burden/fractional absorption/reference body weight of Wistar rats (U.S. EPA, 1987)/

TABLE I

SUMMARY OF STUDIES ON CADMIUM BIOAVAILABILITY INCLUDED IN THE ANALYSIS

Study	Rat strain/sex	Media	Duration (days)	Tier(s)
Aughey *et al.*, 1981	Male Wistar	Water	7, 14, 28, 56, 84	4
Aughey *et al.*, 1984	Male Wistar	Water	21, 28, 42, 56, 70, 84, 168	4
Bernard *et al.*, 1980	Female Sprague–Dawley	Water	31, 70, 92, 122, 153, 183, 214, 336	4
Bernard *et al.*, 1981	Female Sprague–Dawley	Water	31, 70, 92, 122, 153, 183, 244	4
Bernard *et al.*, 1983	Female Sprague–Dawley	Water	70, 122, 183, 244, 305	4
Bernard and Lauwerys, 1981	Female Sprague–Dawley	Water	122	4
Buhler *et al.*, 1981	Male and female Wistar	Water or food	7, 14, 28, 56, 84	1–4
Cousins *et al.*, 1977	Male Sprague–Dawley	Food	98	2–4
Decker *et al.*, 1958	Male and female Sprague–Dawley	Water	183, 365	3–4
Eakin *et al.*, 1980	Male OSU brown rats	Food	28, 56, 84, 112	4
Fingerle *et al.*, 1982	Male and female Sprague–Dawley	Water	574, 644	3–4
Fowler *et al.*, 1975	Male Charles River	Water	42, 84	3–4
Groten *et al.*, 1991	Male Wistar	Food	28, 56	2–4
Itokawa *et al.*, 1974	Male Wistar	Water	120	2–4
Jamall *et al.*, 1989	Male Sprague–Dawley	Water	49	2–4
Kajikawa *et al.*, 1981	Male Wistar	Water	28, 112, 168, 224, 280	4
Kawamura *et al.*, 1978	Female Wistar	Water	90	4
Kotsonis and Klaassen, 1978	Male Sprague–Dawley	Water	21,42,84,168	2–4
Larsson and Piscator, 1971	Female Sprague–Dawley	Water	28, 56	
Loeser and Lorke, 1977	Male and female Wistar	Food	28, 56, 84	2–4
Maji and Yoshida, 1974	Male and female Wistar	Food		3–4
Mangler *et al.*, 1988	Female Sprague–Dawley	Water	183, 365, 549	2–4
Nation *et al.*, 1984	Male Sprague–Dawley	Food	57	
Pribble and Weswig, 1973	Male and female brown rats	Water or food	549	
Prigge *et al.*, 1977	Male Wistar	Water	52	4
Prigge, 1978	Female Wistar	Water	90	4
Rosenberg and Kappas, 1991	Male Sprague–Dawley	Water	5	3–4
Sakata *et al.*, 1988	Male Wistar	Water	12, 26, 50, 100	4
Shaikh *et al.*, 1989	Male Wistar	Water	730	4
Tewari *et al.*, 1986	Male albino ITRC	Food	15, 30, 45, 60	4
Uthe and Chou, 1980	Female Sprague–Dawley	Food	90	2–4
Viau *et al.*, 1984	Female Sprague–Dawley	Water	365	4
Washko and Cousins, 1975	Male Sprague–Dawley	Water	56	2–4
Zenick *et al.*, 1982	Male Sprague–Dawley	Water	75	2–4

duration of exposure. The estimated dosages ranged from 0.00008 to 0.147 mg cadmium/kg/day from drinking water and 0.00009 to 0.124 mg cadmium/kg/day from rat chow.

Figure 2 shows plots of the rates of accumulation of cadmium in tissue against dosage in food and drinking water. Over the entire dosage range examined (<0.14 mg/kg/day), the estimates of $F_{f/w}$ given by the ratio of the slopes (m_f/m_w) were 1.00, based on renal cortex, and 1.19, based on liver cadmium; both were not significantly different from 1 ($P = 0.974$, renal cortex; $P = 0.420$, liver) but were significantly greater than 0.5 ($P < 0.005$) (Table 2). The sensitivity of the estimate of $F_{f/w}$ to dosage was examined by analyzing lower dosage ranges. The estimates of $F_{f/w}$ were not significantly different from 1 for dosage ranges <0.1 and <0.01 mg/kg/day (Table 3). Dosage ranges lower than <0.01 were not analyzed because of insufficient numbers of observations (e.g., $N = 5$ at dosage range <0.001 mg/kg/day).

Tier 2 Data

Tier 2 data were obtained from 11 studies (including Buhler *et al.*, 1981) (Table 1). The data were organized into 31 data groups; within each group, experimental protocols were closely matched with respect to strain, sex, initial and final age, and initial and final body weights of the rats, duration of treatment, and dosage. The 31 data groups yielded a total of 64 estimates of the rate of accumulation of cadmium in the liver and 67 estimates of the rate of accumulation of cadmium in the renal cortex (Fig. 3). Over the entire dosage range examined in these studies (0.00008–3.89 mg cadmium/kg/day), the estimate of $F_{f/w}$ given by the ratio of the slopes (m_f/m_w) was 1.08, based on renal cortex cadmium, and 0.92, based on liver cadmium. The estimates of $F_{f/w}$ were not significantly different from 1 ($P = 0.642$, renal cortex; $P = 0.739$, liver), but were significantly greater than 0.5 ($P < 0.005$, renal cortex; $P < 0.025$, liver) (Table 2).

Tier 3 Data

Tier 3 data were obtained from 16 studies (including those in Tiers 1 and 2) (Table 1). These studies provided 152 and 160 estimates of the rate of accumulation of cadmium in the liver and renal cortex, respectively (Fig. 4). The studies varied with respect to strain, body weight, age and sex of rats, dose level, and duration of administration. Over the dose entire range examined in these studies (0.00008–13.2 mg/kg/day), the estimate of $F_{f/w}$ given by the ratio of the slopes (m_f/m_w) was 1.56 for renal cortex and 1.24 for liver (Table 2). The estimate of $F_{f/w}$ based on renal cortex cadmium was significantly different from 1 ($P < 0.001$); the estimate based on liver cadmium was not significantly different from 1 ($P = 0.062$). Both estimates were significantly greater than 0.5 (<0.005).

The sensitivity of the estimate of $F_{f/w}$ to dosage was examined by analyzing lower dosage ranges (Table 4). Compression of the high end of the dosage range to the same range as Tier 2 (<4 mg/kg/day) decreased the estimate of $F_{f/w}$ from 1.56 to 1.16; the latter was not significantly different from 1 ($P = 0.298$). This is consistent with the results from the analysis of the Tier 2 data, even though the number of observations within this dosage range in Tier 3 (113, renal cortex; 110, liver) was considerably

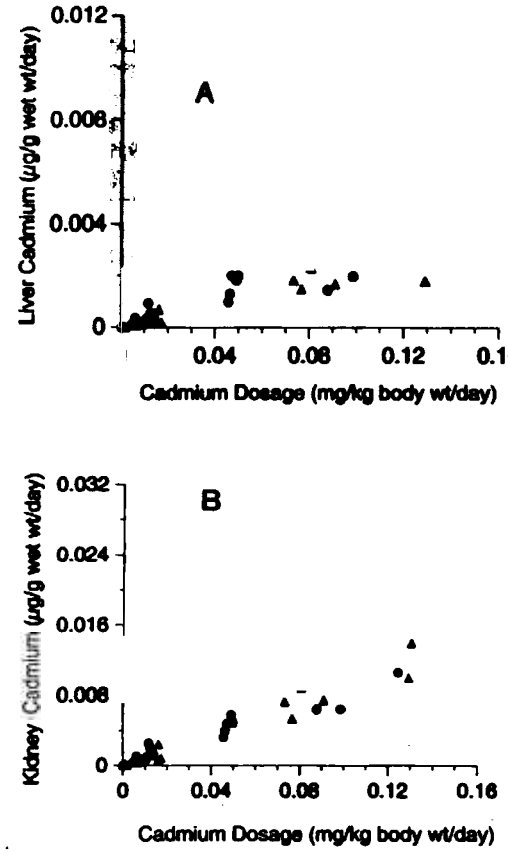

FIG. 2. Rate of accumulation of cadmium (mg Cd/g wet wt tissue/day) in the liver (A) and kidney cortex (B) of rats vs cadmium dosage in food (●) or drinking water (▲) for the Tier 1 data set. The regression slopes for food and drinking water are not significantly different ($P > 0.05$, see Table 2). Data were obtained from Buhler *et al.* (1981) and include 55 estimates of rates of accumulation over a dosage range of 0.0008–0.147 mg/kg/day and exposure durations of 1–12 weeks.

greater than in Tier 2 (67, renal cortex; 64, liver). The estimates of $F_{f/w}$ varied considerably as the dosage range was further compressed and were significantly different from 1 for the dosage range <1 mg/kg/day (<0.001), but were not significantly different from 1 for the dosage ranges <0.1 and <0.01 mg/kg/day. The latter two ranges were within the dosage range of the Tier 1 data and are consistent with the results of the analysis of the Tier 1 data.

Tier 4 Data

Tier 4 data consisted of 35 studies (including those in Tiers 1, 2, and 3) providing 282 and 301 estimates of the rate of accumulation of cadmium in the liver and renal cortex, respectively (Table 1). The studies varied with respect to strain, body weight, age and sex of rats, exposure level, and duration of administration. Included in Tier 4 were studies in which dosages were not reported and could not be estimated; therefore,

TABLE 2

SUMMARY OF ESTIMATES OF RELATIVE BIOAVAILABILITY ($F_{f/w}$) OF CADMIUM FOR TIERS 1, 2, AND 3

Tier	Tissue	m_w[a] (95% CL) (N)	m_f[a] (95% CL) (N)	$m_w - m_f$ (95% CL)	$F_{f/w}$[b] (m_f/m_w)	P: H_0: $F_{f/w} = 1$ H_1: $F_{f/w} \neq 1$	P: H_0: $F_{f/w} = 0.5$ H_1: $F_{f/w} > 0.5$
	Kidney	0.126 (0.108–0.145) (30)	0.126 (0.083–0.168) (25)	0.001 (−0.041–0.042)	1.00	0.974	<0.005
2	Kidney	0.096 (0.73–0.118) (35)	0.104 (0.086–0.122) (32)	−0.008 (−0.044–0.027)	.08	0.642	<0.005
3	Kidney	0.061 (0.51–0.071) (69)	0.095 (0.088–0.101) (55)	−0.034 (−0.047–−0.020)	1.56	<0.001	<0.005
	Liver	0.037 (0.029–0.044) (30)	0.044 (0.025–0.064) (25)	−0.007 (−0.026–0.011)	.19	0.420	<0.005
2	Liver	0.064 (0.044–0.083) (32)	0.059 (0.045–0.074) (32)	0.004 (−0.021–0.029)	0.92	0.739	<0.025
3	Liver	0.067 (0.051–0.083 (64)	0.083 (0.078–0.089) (55)	−0.016 (−0.033–0.001)	.24	0.062	<0.005

[a] m_w and m_f refer to the slopes the regression lines relating the rate of accumulation of cadmium in tissue (μg/g wet wt/day) to cadmium dosage (mg/kg/day).

[b] The ratio of the slopes (m_f/m_w) is an estimate of relative bioavailability ($F_{f/w}$).

rates of accumulation can be compared across concentrations of cadmium (range, 0.001–200 ppm), but not across dosages (Fig. 5). A linear regression analysis to estimate $F_{f/w}$ was not attempted with the Tier 4 data because cadmium affects food and water consumption to different degrees (Fig. 1) and, therefore, the food/water dosage ratio and slope ratio would not be expected to remain constant as cadmium concentrations in the two media increase over the range 0.001–200 ppm.

DISCUSSION

This analysis of published studies compared the bioavailability of cadmium administered to rats in rat chow or in drinking water. Rates of accumulation of cadmium in the renal cortex or liver were used as indices of bioavailability to estimate relative bioavailability ($F_{f/w}$). The data were grouped into four nested tiers in an attempt to examine the effect of study design and reporting of data on the assessment of $F_{f/w}$. The results of this analysis indicate that bioavailability of cadmium in food (rat chow) is not significantly different from bioavailability of cadmium in drinking water when rats are maintained on food and water *ad libitum* and exposed to cadmium dosages below 4 mg/kg body wt/day. This is supported by the analyses of the data in Tiers 1

TABLE 3

SUMMARY OF ESTIMATES OF RELATIVE BIOAVAILABILITY ($F_{f/w}$) OF CADMIUM FROM TIER 1 DATA

Dosage[a] (mg/kg/day)	Tissue	m_w[b] (95% CL) (N)	m_f[b] (95% CL) (N)	$m_w - m_f$ (95% CL)	$F_{f/w}$[c] (m_f/m_w)	P H_0: $F_{f/w} = 1$ H_1: $F_{f/w} \neq 1$
<0.15	Kidney	0.126 (0.108–0.145) (30)	0.126 (0.083–0.168) (25)	0.001 (−0.041–0.042)	1.00	0.974
<0.1	Kidney	0.097 (0.084–0.109) (25)	0.122 (0.0.66–0.177) (23)	−0.008 (−0.078–0.028)	1.26	
<0.01	Kidney	0.080 (0.051–0.109) (15)	0.108 (0.066–0.150) (11)	−0.028 (−0.074–−0.018)	1.35	
<0.15	Liver	0.037 (0.029–0.044) (30)	0.044 (0.025–0.064) (25)	−0.007 (−0.026–0.011)	1.19	
<0.	Liver	0.025 (0.021–0.030) (25)	0.045 (0.018–0.071) (23)	−0.019 (−0.044–0.005)	1.80	0.129
<0.01	Liver	0.024 (0.015–0.033) (15)	0.037 (0.021–0.053) (11)	−0.013 (−0.029–0.003)	1.54	

[a] The dosage range was 0.00008–0.147 mg cadmium/kg/day.

[b] m_w and m_f refer to the slopes the regression lines relating the rate of accumulation of cadmium in tissue (μg/g wet (wt/day) to cadmium dosage (mg/kg/day).

[c] The ratio of the slopes (m_f/m_w) is an estimate of relative bioavailability ($F_{f/w}$).

and 2 (Table 2). Estimates of $F_{f/w}$ derived from Tier 1 were 1.00 when based on renal cortex cadmium and 1.19 when based on liver cadmium. When the analysis was constrained to lower dosage ranges within Tier 1, the estimates of $F_{f/w}$ were higher, 1.26–1.35, based on renal cortex cadmium, and 1.54–1.80, based on liver cadmium. Although this suggests a possible trend toward higher bioavailability from food than drinking water at lower dosages, none of the estimates from Tier 1 were significantly different from 1 ($P < 0.05$). These results provide a basis for evaluating the validity of the hypothesis that $F_{f/w} = 0.5$, the estimate of relative bioavailability that was used in the derivation of the chronic oral RfDs for cadmium in food and drinking water (U.S. EPA, 1994). Our analysis does not support the U.S. EPA estimate of $F_{f/w} = 0.5$ and indicates that $F_{f/w}$ is closer to 1.0.

A significant difference between the bioavailability of cadmium in food and drinking water was detected in the analysis of the Tier 3 data when dosages ≥4 mg/kg/day were included in the analysis, but not when the range included all dosages that were <4 or <0.1 mg/kg/day (Table 4). The lower dosage range is comprised largely of Tier 1 and Tier 2 data; therefore, the absence of a significant difference in bioavailability is consistent with the results of the Tier 1 and 2 analyses. The differences in bioavailability that were evident when the higher dosages were included in the analysis may reflect an effect of dosage on bioavailability or an effect of one or more data quality variables on the estimate of $F_{f/w}$. The studies included in Tier 3 varied considerably with respect

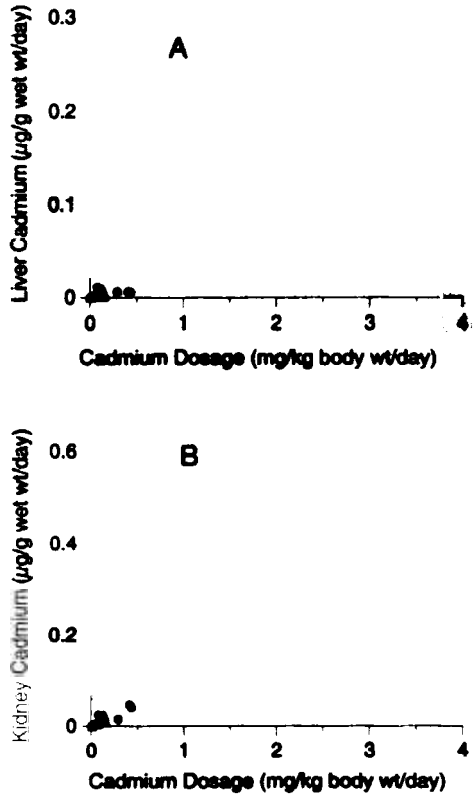

FIG. 3. Rate of accumulation of cadmium (mg Cd/g wet wt tissue/day) in the liver (A) and kidney cortex (B) of rats vs cadmium dosage in food (●) or drinking water (▲) for the Tier 2 data set. The regression slopes for food and drinking water are not significantly different ($P > 0.05$, see Table 2). Data were obtained from 11 studies (see Table 1). The data were organized into 31 data groups: within each group, experimental protocols were closely matched with respect to strain, sex, initial and final age, initial and final body weights of the rats, duration of treatment, and dosage. The 31 data groups yielded a total of 64 estimates of the rate of accumulation of cadmium in the liver and 67 estimates of the rate of accumulation of cadmium in the renal cortex over a dosage range of 0.00008–3.89 mg Cd/kg/day.

to rat strain, body weight, age, and sex of rats, dose level, and duration of administration. These variables would be expected to have a greater impact at the higher end of the dosage range because most of the studies that were unique to Tier 3 (i.e., that were not contained in Tiers 1 and 2) were studies of dosages exceeding 0.1 mg/kg body wt/day. Although an effect of dosage per se cannot be ruled out, an effect of uncontrolled data quality variables is consistent with the instability of the estimates of $F_{f/w}$ as the dosage range was compressed and the data quality was increased. For example, estimates of $F_{f/w}$ based on renal cortex cadmium were 1.56, 1.16, 0.34, 0.60, and 1.35 for the dosage ranges <14, <4, <1, <0.1, and <0.01 mg/kg body wt/day, respectively. The range of estimates based on liver cadmium was similarly wide and unrelated to dosage (0.58–1.54). If relative bioavailability varied only with dosage, one would expect a more consistent dosage-related trend in the value of $F_{f/w}$; this is not evident from the analysis of the Tier 3 data.

One objective of this study was to attempt to understand the cadmium data base from the perspective of data quality factors that might impact an assessment of bio-

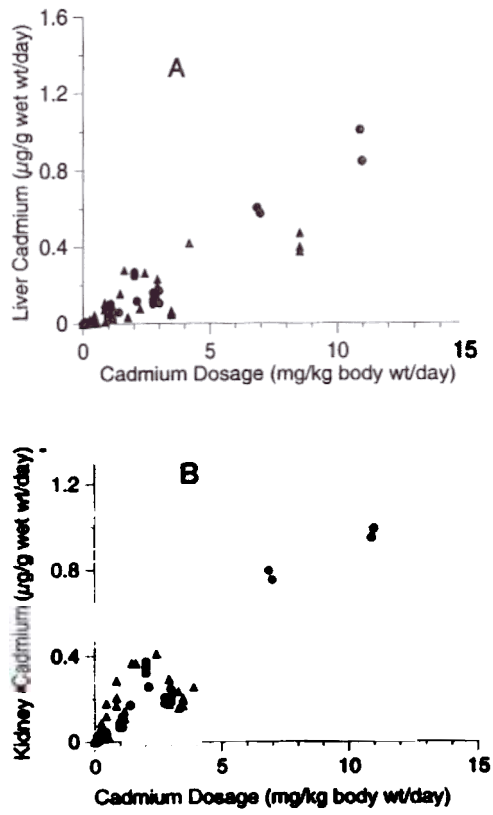

FIG. 4. Rate of accumulation of cadmium in the liver (A) and kidney cortex (B) of rats vs cadmium dosage in food (●) or drinking water (▲) for the Tier 3 data set. The regression slopes for accumulation in renal cortex from food and drinking water are not significantly different ($P > 0.05$). The regression slopes for accumulation in liver are significantly different ($P = 0.06$) (see Table 2). Data were obtained from 16 studies (see Table 1) and included 152 and 160 estimates of the rate of accumulation of cadmium in the liver and renal cortex, respectively, over a dosage range of 0.00008–13.2 mg/kg/day. Unlike the Tier 2 data set (Fig. 3), the Tier 3 data were not grouped based on experimental design criteria.

availability of inorganics in general. As one might expect, the relatively large data base of published studies on cadmium includes a wide range of study designs and reports that vary considerably with respect to experimental detail and types of data reported. Nearly all of the relevant studies reported cadmium exposure (ppm), rather than dosage (e.g., mg/kg/day), thereby necessitating estimation of dosage for inclusion of the study in the analysis. This introduced an additional element of uncertainty into the analysis when data on either individual animal body weights or food and water consumption were not provided. Data on food and water consumption are particularly important for estimating cadmium dosages because both are decreased by cadmium exposure (Fig. 1). Reliable estimates of cadmium dosages cannot be obtained when data on food and water consumption are not provided; therefore, such studies were excluded from Tiers 1 to 3.

The observation that bioavailability of cadmium in rat chow or drinking water is not significantly different at dosages below 4 mg/kg/day does not exclude the

TABLE 4

SUMMARY OF ESTIMATES OF RELATIVE BIOAVAILABILITY (F_{0w}) OF CADMIUM FROM TIER 3 DATA

Dosage[a] (mg/kg/day)	Tissue	m_w[b] (95% CL) (N)	m_f[b] (95% CL) (N)	$m_w - m_f$ (95% CL)	F_{0w}[c] (m_f/m_w)	P H_0: $F_{0w} = 1$ H_1: $F_{0w} \neq 1$
<14	Kidney	0.061 (0.051–0.071) (69)	0.095 (0.088–0.101) (55)	−0.034 (−0.047–−0.020)	1.56	<0.001
<4	Kidney	0.081 (0.064–0.099) (62)	0.094 (0.079–0.109) (51)	−0.013 (−0.038–0.012)	1.16	0.298
<1	Kidney	0.240 (0.201–0.280) (44)	0.082 (0.074–0.090) (36)	0.159 (0.118–0.200)	0.34	<0.001
<0.1	Kidney	0.143 (0.068–0.218 (28)	0.086 (0.036–0.137) (23)	0.057 (−0.031–0.144)	0.60	0.199
<0.01	Kidney	0.080 (0.051–0.109) (15)	0.108 (0.066–0.150) (11)	−0.028 (−0.074–0.018)	1.35	0.223
<14	Liver	0.067 (0.051–0.083) (64)	0.083 (0.078–0.089) (55)	−0.016 (−0.033–0.001)	1.24	0.062
<4	Liver	0.046 (0.033–0.058) (59)	0.063 (0.051–0.063) (51)	−0.018 (−0.035–0.000)	1.37	0.052
<1	Liver	0.089 (0.072–0.106) (44)	0.051 (0.043–0.058) (36)	0.038 (0.018–0.059)	0.58	
<0.1	Liver	0.032 (0.021–0.043) (28)	0.032 (0.009–0.055) (23)	0.000 (−0.025–0.025)	1.00	
<0.01	Liver	0.024 (0.015–0.033 (15)	0.037 (0.021–0.053) (11)	−0.013 (−0.029–0.003)	1.54	

[a] The dosage range was 0.00008–13.2 mg/kg/day.

[b] m_w and m_f refer to the slopes the regression lines relating the rate of accumulation of cadmium in tissue (μg/g wet wt/day) to cadmium dosage (mg/kg/day).

[c] The ratio of the slopes (m_f/m_w) is an estimate of relative bioavailability (F_{0w}).

possibility of an influence of diet on cadmium bioavailability nor is it inconsistent with data that have demonstrated effects of diet composition on bioavailability of cadmium in the rat and other species. There is extensive evidence that certain dietary components can influence the absorption of cadmium from the gastrointestinal tract. Those that have been reported to decrease absorption include high protein, calcium, iron, zinc, and fiber (Andersen *et al.*, 1992; Friberg *et al.*, 1985). That a given dietary factor decreases bioavailability under specific experimental conditions does not necessarily imply that bioavailability will be less when chronic exposure is from the diet than when chronic exposure is from drinking water.

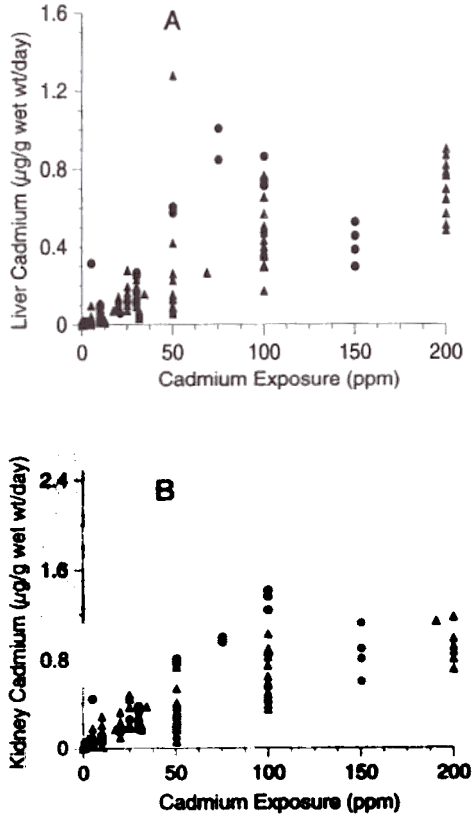

FIG. 5. Rate of accumulation of cadmium in the liver (A) and kidney cortex (B) of rats vs cadmium exposure (ppm) in food (●) or drinking water (▲) for the Tier 4 data set. Data were obtained from 35 studies (see Table 1) and included 282 and 301 estimates of the rate of accumulation of cadmium in the liver and renal cortex, respectively. Included were studies in which dosages were not reported and could not be estimated; therefore, rates of accumulation are compared across concentrations of cadmium (ppm), but not across dosages (mg/kg/day). A linear regression analysis was not attempted because cadmium affects food and water consumption to different degrees (see Fig. 1), and, therefore, the food/water dosage ratio and slope ratio would not be expected to remain constant as cadmium concentrations in the two media increase over the exposure range 0.001–200 ppm.

The classic study of James *et al.* (1985) demonstrated the importance of feeding behavior and timing of exposure on the effect of diet on bioavailability of lead. This study demonstrated that the bioavailability of lead ingested in water (as reflected by the concentration of lead in blood) was decreased by food intake; however, the magnitude of the effect varied inversely with the latency between meals and the ingestion of lead. These results suggest that the contents of the gastrointestinal tract at the time of exposure is an important factor in determining bioavailability of an ingested inorganic and is perhaps more important than the exposure medium in which the inorganic is contained.

Support for application of this concept to cadmium is provided by the studies of Andersen *et al.* (1992), Engström and Nordberg (1978), Kello and Kostial (1977), and Rabar and Kostial (1981). In each of these studies, the medium of administration of

cadmium remained the same for each group of animals (water by gavage), but the *ad libitum* food varied in composition among the different groups. The animals were fed different types of food for days to weeks prior to, and after, administration of a single gavage dose of radiolabeled $CdCl_2$ in water. The bioavailability of the cadmium was determined by measuring whole-body burden after most of the unabsorbed cadmium was excreted in the feces. Andersen *et al.* (1992) reported that retention (estimated 10 days after dosing) of cadmium in mice decreased with increased fiber content of the food and that mice fed standard mouse or rabbit pellets retained a much smaller percentage of the initial dose (0.5–0.8%) than that observed in the other groups fed semisynthetic diets with varying fat, protein, and fiber content (2.2–4.2%). Rabar and Kostial (1981) reported retention (estimated 6 days after dosing) of cadmium as 1.8, 8.3, 7.3, and 7.8% of the initial dose in rats fed rat chow, "tinned luncheon meat," whole wheat bread, or milk, respectively. Two studies have reported higher retention of cadmium (estimated 14 and 28 days after dosing) after a single gavage dose of radiolabeled $CdCl_2$ in water in adult mice fed milk (5.6 and $\approx$4.0%) than that in adult mice fed standard rat chow (0.3 and $\approx$1.5%) (Kello and Kostial, 1977; Engström and Nordberg, 1978). In all four of the above studies, the medium of administration for all groups was water, while the type of food ingested was varied among the different groups. The results of these studies suggest that the uptake of cadmium from water may be determined more by the nature of the total diet than by the medium of consumption. In addition, absorption of cadmium may vary greatly among different types of diets, including relatively low absorption from animal chow, compared to that of foods normally consumed by humans.

It follows that exposure and feeding protocols will be important variables in any experimental assessment of bioavailability factors that are intended to support extrapolations to human chronic exposure scenarios. In chronic exposure protocols in which animals are provided food and water *ad libitum*, the test inorganic will mix with components of the diet in the gastrointestinal tract regardless of whether it is ingested in the drinking water or diet; therefore, in studies in which similar diets are administered, it might be anticipated that bioavailability of the test inorganic in two media will be similar. However, in acute exposure protocols, relative bioavailability may vary with timing of exposure to the test inorganic in drinking water (or gavage) in relation to feeding schedules. Given the above considerations, it is not surprising that differences in bioavailability of cadmium could not be detected in rats exposed subchronically or chronically to similar diets with cadmium in the diet or drinking water.

These observations are relevant to the cadmium RfDs and the use of relative bioavailability factors in risk characterization in general. Human exposure to cadmium (and other inorganics) in food and water resembles the chronic *ad libitum* conditions of the studies included in this analysis. The human stomach often contains ingesta, and the human small intestine essentially always contains ingesta. Because cadmium absorption occurs primarily in the small intestine (Friberg *et al.*, 1985), it is likely that cadmium in food and water ingested by humans mixes prior to and during absorption. Therefore, differences in bioavailability of cadmium, and perhaps other inorganics in drinking water and food, may not occur in most individuals or human populations. Studies of humans do not support a difference between the bioavailability of cadmium in drinking water and food. The average values of percentage absorption that have been reported range from approximately 1.5 to 10.0%; large variations are observed

among individuals, with no obvious differences in relative absorption for cadmium administered in food or water (Ellis *et al.*, 1979; Flanagan *et al.*, 1978; Kitamura, 1972; Koizumi, 1975; McLellan *et al.*, 1978; Newton *et al.*, 1984; Rahola *et al.*, 1972; Shaikh and Smith, 1980; Yamagata *et al.*, 1974). However, problems inherent to measuring whole-body burden of cadmium in humans may obscure the detection of differences in bioavailability of cadmium in food and drinking water.

We conclude from this analysis that the bioavailability of cadmium in food is not measurably different from that in water when rats are fed *ad libitum*. In absence of data to the contrary, it is reasonable to extrapolate conclusions to humans, who normally ingest food *ad libitum*. Therefore, we recommend that distinct RfDs for cadmium in food and drinking water should not be based on the assumption that the bioavailability of cadmium in drinking water is greater than that of cadmium in food. It would be inappropriate to extrapolate results obtained from this analysis to other inorganics. The kinetics, site, and mechanisms of absorption may be important factors in determining the extent to which bioavailability of a given inorganic will be affected by exposure medium. Furthermore, the quality of the data base on which assessments of relative bioavailability must be based will vary considerably for different inorganics; this will affect the uncertainty of the assessments. In this latter regard, it will be important to establish data quality and reporting criteria for both the derivation of relative bioavailability factors and the design of experimental studies that will support future assessments of relative bioavailability.

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