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Bioaccumulation of Cadmium from Durum Wheat Diets in the Livers and Kidneys of Mice

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The bioaccumulation of cadmium in humans is of concern as it has a long half-life in the human body, and chronic exposure has been linked to kidney dysfunction (Wilks *et al.* 1990) and prostate tumours (Wagner 1993; Ekman 1999). Occupational exposure, diet, smoking, and drinking water are the key pathways by which Cd is taken up by people. The potential for transfer of Cd to humans from diet is of particular interest, as Cd concentrations in agricultural soils can be elevated due to their amendment with applications of phosphate fertilizers, animal manures, and sewage sludge, as well as, long-range transport of anthropogenic emissions containing metals. Agricultural soils can also be elevated in Cd as a result of their geological origin, as seen with the black Prairie soils of Canada and the United States of America (Garrett 1994). Whatever the source, elevated Cd in agricultural soils has led to grain and oil seed products with Cd concentrations that are in excess of the WHO provisional maximum allowable concentration in foodstuffs (0.1 mg/kg, WHO 1989). These elevated Cd concentrations have become a non-tariff trade barrier to the export of this wheat, despite the lack of information about the bioavailability of grain Cd to the human digestive system.

The long-term goal of this research is to determine the influence of Cd speciation in foods on the accumulation of Cd in mammalian organs, and thus contribute to the estimation of risk to human health from dietary intake of Cd. The objective of this study was to compare bioaccumulation of plant-incorporated and soluble salt Cd from durum grain diets in liver and kidney tissues of mice.

MATERIALS AND METHODS

There were three experimental diets, each with multiple concentrations of Cd: hydroponically-grown grain containing Cd accumulated from $Cd(NO_3)_2 \cdot 4\,H_2O$ provided to the roots in the nutrient solution ('incorporated'); field grown grain amended with $Cd(NO_3)_2 \cdot 4H_2O$ dissolved in high purity water and added to the wheat grain before feeding ('amended'); and, the same field-grown grain, plus $Cd(NO_3)_2 \cdot 4H_2O$ dissolved in high purity water administered to the mice separately from the grain diet by gavage ('gavage'). Each of these three diets had four Cd concentrations; a fourth diet consisted of five replicates of field-grown grain alone

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Since the two $Cd(NO_3)_2 \cdot 4H_2O$ augmented diets used field grown grain which contained small quantities of plant-incorporated Cd, the doses and tissue accumulations from these diets were the sums of these two sources of Cd. So, the concentration of plant-incorporated Cd and corresponding tissue concentrations of Cd observed for this fourth diet were subtracted from the doses and accumulations observed for the gavage and amended diets. The contribution of the field grown grain to the doses of the amended and gavage treatments was unique for each dose because of variation in the quantity of grain eaten by the cage of mice, and ranged from 30% of the lowest dose to just 7% of the highest dose. The adjusted data for the gavage and amended diets therefore represent the doses of Cd and accumulations attributable to the $Cd(NO_3)_2 \cdot 4H_2O$. The incorporated grain was produced by planting germinated seeds of *Triticum turgidum* in rock wool plugs, which were inserted into Styrofoam disks floating on the tops of nursery plant pots filled with complete plant nutrient solution (Chan, 1996). The solution was constantly recirculated among the other pots of the same treatment, and a reservoir; the solution in the reservoir was partially replaced every two days, and completely replaced every two weeks. The solutions for the four treatments contained $Cd(NO_3)_2 \cdot 4H_2O$ (0.05, 0.5, 5.0 and 50.0 $\mu g \cdot L^{-1}$) in which the plants were grown from seedling to the grain-drying stage of development. Once the plants were dry, the ripe grain was collected, the husks were removed, and Cd concentration determined for each treatment using a Graphite Furnace Atomic Absorption Spectrometer (GF-AAS). These concentrations of plant-incorporated Cd became the four target concentrations for the gavage and amended diets. The grain was coarsely ground in a coffee grinder to produce crumbles ranging in size from 10-50% of the original grain kernel. The crumbles for the four Cd-amended diets were wetted with equal volumes (different concentrations) of $Cd(NO_3)_2 \cdot 4H_2O$ dissolved in high purity water; the grain was mixed and allowed to air dry before feeding. For the incorporated and gavage diets, the grain crumbles were amended with the same volume of high purity water (without the $Cd(NO_3)_2 \cdot 4H_2O$) and allowed to air dry.

The animals used in the study were female CD1 mice (Charles River Laboratories, Montreal, QC, Canada). The mice weighed between 22 and 24 grams at delivery and were housed 3 per cage in stainless steel wire bottomed hanging cages (24.5 cm X 18 cm X 18 cm) in the Central Animal Facility at the University of Guelph. The Canadian Council on Animal Care requires multiple animals per cage, to ensure good mental health of the animals. Females were thus chosen for this study, as they exhibit less aggressive behaviour in group housing than do males. The animals were randomly assigned to the treatment groups and allowed one week to acclimatize to their surroundings before the experiment was started. During the acclimation period the diet consisted of durum wheat and water *ad libitum.* During the feeding study, they were given Cd-free water to drink and one of the durum wheat diets, containing known amounts of Cd, *ad libitum;* the gavage treatment for the animals receiving the Cd nitrate solution was administered daily. Grain consumption was determined every two days for each

treatment and total food intake for each cage was calculated at the end of the experiment. On day eleven of the study the mice were weighed, euthanised using CO_2, and the livers and kidney tissues collected and weighed. The liver and kidneys of each animal were then stored at -80°C until analysis for Cd. Mouse tissues and a homogenous sample of each wheat diet were hot-acid digested by the closed Teflon vessel method of Topper and Kotuby-Amacher (1990). A weighed sample of liver or kidney tissue (up to 2.0g fresh weight, if available) was placed in a Teflon digestion vessel with 10 mL trace metal grade HNO_3. The digestion was carried out unsealed at room temperature for 5 hours, and then the vessels were sealed and placed in an oven at 110°C overnight. NIST Standard Reference Material #1570a (US Department of Commerce, National Institute of Standards and Technology, Gaithersberg, MD) was digested with each run. It contained Cd at a concentration of 2.89±0.07 $\mu g \cdot g^{-1}$; this value was achieved within 10% each time. The Cd concentration in each tissue digest was determined by GF-AAS. Acid blanks were digested with every group of tissues, and analysed for Cd content; tissue digests were diluted as necessary if the concentration of Cd exceeded the upper limit of detection for the GF-AAS. The total dose of Cd consumed during the feeding trial by each cage of three animals was calculated from the amount of grain consumed and the concentration. Since each experimental unit consisted of three mice for whom only total dietary intake of Cd was calculable, and each experimental unit had a unique combination of food consumption and Cd concentration, there was no true replication of any dose. Each liver and kidney tissue Cd concentration as presented (Table 1) is the mean for the three animals in each experimental unit; standard error of the mean value for liver Cd concentration is also presented, as these organs were analysed separately for each of the three mice in the cage. Standard errors for kidney Cd concentration are not presented; these organs were so small that they had to be combined for Cd analysis because of the instrument's level of detection. Because of the lack of true replication, individual doses of the diets could not be compared with great statistical power. Instead, the diets were compared by calculating the dose response relationship for each diet and tissue, and then comparing pairs of these relationships within tissues using linear orthogonol contrasts (Draper and Smith 1981). The tissue concentrations were tested for normal distribution of residuals, and homogenous error sums of squares among treatments; in all cases, transformation to natural logarithms before generating the dose response relationships was required in order to satisfy Wilk's criterion for normality, as the effect of diet on the tissue Cd concentrations was proportional. Bioaccumulation indices for liver and kidney were determined by calculating the ratio between tissue concentration and dose (g tissue $Cd \cdot g^{-1}$ Cd dose). The arithmetic values for this variable satisfied Wilk's criterion for normality, so they were not transformed; the kidney and liver bioaccumulation indices were compared between diets by t-tests.

RESULTS AND DISCUSSION

There appeared to be no adverse effect of the feeding regimes on the health of the

mice. The food intake and final body weights, for the various treatments, did not suggest any treatment effect (Table 1); liver and kidney fresh weights at the end of the study were not related to diet (data not shown).

Visual comparison of the bioaccumulation data for each diet and dose indicate that for similar doses, administration of $Cd(NO_3)_2 \cdot 4H_2O$ (a soluble inorganic salt) by gavage resulted in greater Cd accumulation in liver and kidney than when the $Cd(NO_3)_2 \cdot 4H_2O$ was added to the diet, or when Cd was plant-incorporated into grain (Table 1). The data also demonstrate that kidney tissue accumulates a

Table 1. Total dose of Cd ($\mu g \cdot g^{-1}$ body weight), consumption of grain ($g \cdot mouse^{-1}$) final body weight ($g \cdot mouse^{-1}$), and tissue Cd concentrations ($ng \cdot g^{-1}$).

Diet	Cd Dose $\bar{x}$	Grain Consumed $\bar{x}$	Body Weight $\bar{x}$	Kidney $\bar{x}$	Liver $\bar{x}$ (s.e.m.)
Incorporated	3.37	86.6	26.5	229	96.9 (6.83)
	0.607	79.9	23.9	49.5	18.4 (2.58)
	0.534	82.9	24.7	42.2	16.6 (0.96)
	0.199	67.3	23.9	22.3	8.89 (1.03)
Amended	2.03	70	25.5	226	111 (33.61)
	0.472	85.4	23.9	9.32	9.44 (2.11)
	0.314	87.2	25.4	34.9	15.5 (4.73)
	0.185	80.7	25.1	32.4	10.8 (1.0)
Gavage	4.98	79.2	24.6	1520	1410 (155)
	0.852	98.3	25.7	306	196 (57.8)
	0.751	64.6	24.6	189	127 (22 5)
	0.498	92.5	25.1	130	48 (1.73)

greater Cd concentration from a given dose than does liver tissue, although the liver accumulated a greater mass of Cd than the kidney (data not shown). The tissue concentrations in both liver and kidney by and large demonstrated the expected relationship between dose and accumulation, in that as dose increased, so did accumulation (Table 1). The exceptions to this generalization are the tissue concentrations resulting from the amended diet at the 0.472 $\mu g \cdot g^{-1}$ final body weight dose. The Cd concentrations found in kidney (0.932×10^{-2} µg $Cd \cdot g^{-1}$ fresh weight) and liver (0.944 µg $Cd \cdot g^{-1}$ fresh weight) are very low relative to the tissue concentrations realized from similar or even lower doses of the same diet.

Examination of experimental records revealed no reason for this anomalous finding. The amended doses were likely the ones with the most variability among subsamples of feed, so perhaps the feed sample which was analysed for Cd was not representative of the entire diet. However, the Cd concentration in the feed as determined by analysis is consistent with the amount of Cd which was added to the grain as $Cd(NO_3)_2 \cdot 4H_2O$. The tissue Cd analyses from the three separate mice that constitute the mean for that treatment were quite similar, so it is unlikely that the tissue concentration is not representative of the true tissue concentrations. For these reasons, the anomalous data were included in all statistical analyses,

The regression relationship for each of the diets and tissues was linear, most with a high r^2 value (Table 2). The lower r^2 values for the amended diet reflect the

Table 2. Regression relationships between *ln* tissue Cd concentration and *Zn* Cd dose, r^2 values and *F* test for similarity of equations.

Organ	Diet	Equation	r^2	F^*
Kidney	gavage	*ln* [Cd] = -1.34 + 1.086 (*ln* dose)	0.97	a
	amended	*ln* [Cd] = -2.60 + 0.878 (*ln* dose)	0.47	a
	incorporated	*ln* [Cd] = -2.54 + 0.837 (*ln* dose)	0.99	a
Liver	gavage	*ln* [Cd] = -1.90 + 1.43 (*ln* dose)	0.98	b
	amended	*ln* [Cd] = -3.16 + 1.01 (*ln* dose)	0.81	c
	incorporated	*ln* [Cd] = -3.48 + 0.845 (*ln* dose)	0.99	c

* equations with the same letter were not different from each other.

anomalous data for liver and kidney Cd concentration observed at the 0.472 $\mu g \cdot g^{-1}$ final body weight dose (Table 2); otherwise, the linear regression relationships account for a very high proportion of the variability in the dependent variable. For kidney tissue Cd concentration, the slopes for gavage and incorporated diets were greater than zero ($P \leq 0.05$); the slope for the amended diet was not different from zero. For liver tissue Cd concentration, all slopes were greater than zero ($P \leq 0.001$). The whole regression relationships for pairs of diets were compared using an F-test of the ratio of contrast MS/error MS, with 2,6 degrees of freedom. For liver, the regression relationships for the amended and incorporated diets were different from the gavage diet ($P \leq 0.05$); for kidney, however, the whole relationships for amended and incorporated diets were different from gavage only a t$P=0.13$.

Comparison of the bioaccumulation indices (tissue Cd accumulation/Cd dose) for the diets suggests similar differences between the diets as indicated by the regression relationships (Table 3). The liver and kidney bioaccumulation indices for the amended and incorporated diets were not different from each other ($P \geq$

0.05) but the bioaccumulation indices for the amended and incorporated diets were different from the bioaccumulation indices for the gavage diet ($P \leq 0.05$)

Table 3. Bioaccumulation indices: tissue [Cd] ($\mu g.g^{-1}$ organ) / dose ($\mu g.g^{-1}$ body)

Organ	Gavage $\bar{x}$ (s.e.m.)	Amended $\bar{x}$ (s.e.m.)	Incorporated $\bar{x}$ (s.e.m.)
Kidney	0.265 a* (.023)	0.129 b (.030)	0.0836 b (.009)
Liver	0.158 a (.027)	0.0399 b (.007)	0.0324 b (.003)

*treatments with different letters within rows were significantly different ($P \leq 0.05$)

(Table 3). The effect of diet on kidney accumulation of Cd, which was not evident in comparison of the whole regression relationships, is because of the increased power (degrees of freedom) in the comparison of bioaccumulation indices. The bioaccumulation indices also suggest that the kidney accumulated more Cd per unit of dietary dose than the liver; at first glance, this appears to disagree with the findings of higher accumulation in liver versus kidney observed by Shaikh *et al.* (1993) in mice and by Ando *et al.* (1998) in rats. However, Ando *et al.* (1998) also demonstrated that at low doses, kidney accumulation was favoured over liver, and it was at the higher doses that liver concentrations were consistently higher, closely related to the differences in metallothionein concentrations. The fact that Shaikh *et al.* (1993) administered the Cd by subcutaneous injection would also be expected to increase bioavailability and the relative dosage compared to our study. Our data demonstrate that plant incorporated Cd in a whole wheat grain diet is similarly bioavailable as Cd in a soluble inorganic salt supplied with the grain diet. The enhanced bioavailability of the soluble inorganic Cd salt delivered by gavage may be due to the timing of the gavage treatments, which coincided with the end of the animals' sleep period, a time when their stomachs are likely quite empty.

Our data can be looked at in the context of Lind *et al.* (1998), who measured Cd accumulation in the kidneys and livers of mice fed a semi-synthetic control feed supplemented with wheat bran, sugar beet fibre, and carrots (all Cd-containing) as well as $CdCl_2$. After mixing, all diets had a nominal total Cd concentration of approximately 0.05 $mg \cdot kg^{-1}$. Cadmium concentrations in the kidney and liver tissues were expressed relative to the cadmium intake; this fractional accumulation for the wheat bran supplemented diet was lower than that for the sugar beet, carrot, or $CdCl_2$ supplemented diets, which had similar fractional accumulations. This suggests that the Cd in bran was less available to both liver and kidney than Cd from the other foods, or from $CdCl_2$. The bran was not removed from any of the wheat diets used in our study (i.e. the $Cd(NO_3)_2 \cdot 4H_2O$ was supplemented to a bran-containing diet), so it is difficult to compare our data with those of Lind *et al.*

(1998); however, bran seems to be an important dietary component for reducing the bioavailability of Cd, independent of whether or not the Cd is incorporated into the bran. Our bioaccumulation indices for Cd in durum wheat were consistent with the fractional accumulations of Cd determined by Lind *et al.* (1998); for all diets, in both studies, less than 25% of the ingested Cd was accumulated in kidney and liver tissue.

The data of Moberg Wing (1993) who looked at accumulation of plant-incorporated Cd in liver and kidney tissues of rats fed diets containing wheat bran, wheat endosperm, whole wheat, and wheat endosperm supplemented with $CdCl_2$, are consistent with our study. The accumulation of Cd from the whole wheat diet was lower than that from the $CdCl_2$ supplemented diet; the accumulations of Cd from the two endosperm diets (unsupplemented and supplemented with $CdCl_2$) were similar when the values reported in the paper are corrected for the amount of Cd in 1g of the diet. Their data suggest that the dietary bioavailability of plant-incorporated Cd may be dependent on the plant tissue into which it is incorporated. Phytates in the endosperm tissue were 10x lower than in whole wheat; their purported role in decreasing dietary bioavailability of Cd may be the reason for the similar bioavailabilities between the two endosperm diets. Phytates are known to complex Cd in plant tissues, and since they are insoluble, are thought to reduce the dietary absorption of Cd. As with the Lind *et al.* (1998) study, however, the $CdCl_2$ was not supplemented to a bran-containing diet, as was done in our study with the $Cd(NO_3)_2 \cdot 4H_2O$, so it is difficult to closely compare our work to theirs. The studies are certainly dissimilar in that the fractional accumulations for kidney and liver in the Moberg Wing (1993) study (calculated by us to be 0.3% - 1.7%) were much lower than those observed in our study (3% - 25%) although those for kidney were higher than those for liver, in both studies.

Our study demonstrates that in a short-term exposure, the bioavailability of Cd to kidney and liver tissues of mice was not influenced by plant incorporation of this metal into whole wheat, relative to the addition of Cd to whole wheat as a soluble inorganic salt. Our study was of short term, limited by the amount of wheat with plant-incorporated Cd available. It appears from our work that the presence of whole wheat (including dietary bran) reduces the bioavailability of Cd, whether or not the Cd is incorporated into the wheat or wheat bran at the time of ingestion. The larger conclusion that can be drawn from the three studies is that the transfer of Cd to mammalian organs appears reasonably well estimated using diets that are supplemented with soluble inorganic Cd salts, and that the modification of Cd bioavailability likely occurs in the gut during the process of digestion. The inconsistencies among these various studies, on the other hand, may point to the potential for other plant tissue constituents in the diet to influence the dietary bioavailability of Cd.

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