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Cadmium toxicokinetics and bioaccumulation in turtles: trophic exposure of *Trachemys scripta elegans*

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Abstract Ecotoxicological data in reptiles are mainly represented by field studies reporting the tissue burden of wild-captured individuals but much less is known regarding the processes of uptake, depuration, accumulation and the effects of inorganic contaminants in these species. In the present study, the accumulation, the path and the effects of exposure to cadmium (Cd) through diet intake were investigated in female red eared slider turtles, *Trachemys scripta elegans*. In the first phase of the experiment, turtles underwent an acclimatization period during which they were fed a control diet. In the second phase, the turtles were exposed to cadmium through a $CdCl_2$ supplemented-diet with increased environmentally relevant concentrations for a period of 13 weeks. Following this, the turtles went through a third phase, a recovery phase of 3 weeks, during which they were fed uncontaminated food. Blood and feces were collected during the three phases of the experiment. The turtles were euthanized at the end of the experiment and organ samples collected. The Cd-concentrations in blood remained stable over the course of the experiment while Cd-concentrations in feces increased with time and with the amount of Cd ingested. The proportional accumulation in liver and kidney together was comprised between 0.7 and 6.1% and they represented the main organs of accumulation. Cd accumulated in the organs in the following order of concentration: kidney > liver > pancreas > muscle. In terms of burden in organs, the Cd-burden was the highest in liver followed by kidney and pancreas. The proportional accumulation decreased as Cd ingestion increased, suggesting that at a higher dose of Cd, assimilation decreased. Mineral content of the liver and pancreas became modified according to Cd level; increasing dietary Cd exposure increased concentrations of zinc and iron in liver and copper in pancreas in a dose-dependent manner. Accumulation of Cd had no effect on survival, food consumption, growth, weight or length suggesting no effect of the treatment on female turtle body condition.

Keywords Cadmium · Trophic transfer · Assimilation · Distribution · Elimination · Reptiles

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Introduction

Environmental pollution has been cited as one of the main threats affecting reptile populations (Gibbons et al. 2000). However, most ecotoxicological research on reptiles relies on the assessment of tissue concentrations from field-captured individuals (Hopkins 2006) and few studies have focused on the fate and the effects of contaminants (Ganser et al. 2003; Hopkins et al. 2002; Mann et al. 2006, 2007). Nevertheless, assessing the fundamental processes of distribution and accumulation and kinetics such as the uptake and depuration rates of contaminants is necessary in order to understand the mechanisms of bioavailability, trophic transfer, the path taken by contaminants in the body and the biological effects on reptiles.

Among environmental contaminants, cadmium (Cd) is a potential environmental hazard known to be deleterious to biological function by disrupting cellular homeostasis of essential metal ions such as copper (Cu), zinc (Zn) and calcium (Ca) (Noel et al. 2004). Cd is a relatively rare element in the earth's crust and occurs naturally in the environment with Zn, Cu and lead (Pb) in sulfide ores

(Nordberg et al. 2007). With human activities, natural concentrations are increased as Cd is continuously introduced into the atmosphere as a result of mining, smelting, refining sulfide ores, combustion of fossil products and waste incineration. Global consumption of Cd can be seen in the use of Cd in nickel–cadmium batteries, paints, pigments, corrosive coatings, plastic stabilizers and phosphate fertilizers used for the improvement of agricultural soils (Jarup 2003; NCM 2003; Bertin and Averbeck 2006). As Cd accumulates in plants (Simon et al. 1996) and in the food chain (Croteau et al. 2005), Cd can readily be transferred from one organism to another by trophic transfer, despite the fact that Cd is not known to be essential to any organism. The increasing worldwide pollution of terrestrial and aquatic systems therefore raises toxicological concerns for organisms exposed to pollutants such as Cd via trophic transfer (de Vries et al. 2007; Schwarzenbach et al. 2006).

Dietary transfer, accumulation, kinetics and the effects of Cd have been widely studied in mammals (Chan et al. 2004; Lind et al. 1997; Matsuno et al. 1991; Schilderman et al. 1997), whereas little is still known about reptiles (snakes: Hopkins et al. 2002; lizards: Mann et al. 2007). In those studies, individual animals were contaminated with Cd via food, and distribution in the organs was investigated. However, there is no literature available regarding Cd toxicity levels in reptiles. Metal toxicity occurs when toxic elements displace nutrient elements from their metabolic site (Sunda and Huntsman 1998). Chronic exposure to environmental Cd may disturb essential mineral metabolism at low doses (Noel et al. 2004). Cd has a high affinity for Zn binding sites and it can displace this essential element from pre-existing complexes, leading to disruption of target protein (Bertin and Averbeck 2006). In mammalian cells, Cd can affect aspects of the cell cycle: proliferation, differentiation and DNA replication and repair. Cd can also affect the antioxidant defense system, induce oxidative stress (the denaturation of proteins and lipid peroxidation) and affect enzyme activities (Bertin and Averbeck 2006; Beyersmann and Hechtenberg 1997; Lopez et al. 2006). At the organism level, Cd is associated with renal and skeletal damage and cancer, and is highly embryotoxic and teratogenic (Beyersmann and Hartwig 2008; Burger 2008; Jarup et al. 1998; Noel et al. 2004).

Numerous authors have therefore argued for an increased research effort to investigate the trophic transfer of Cd, its distribution and accumulation, and the biological effects induced by a known dose of contaminant ingested in reptiles (Burger 2008; Hopkins 2000; Mann et al. 2007). Risk assessment may be biased if the toxic threshold established for another class of vertebrates is used for reptiles, where those vertebrates differ in terms of their biological and physiological characteristics (e.g., long life span, high trophic position, and ectothermic physiology; Hopkins et al. 2002). While chelonians have received far more attention than crocodiles or squamates in ecotoxicological field studies (Bishop et al. 1998; de Solla et al. 2007), there is a great lack of laboratory-controlled studies of turtles and, to our knowledge, there is no study on kinetics or on the accumulation of contaminant in chelonians. Indeed, turtles are less logistically practical than squamates for use in the experimental assessment of contamination. As such experimental studies require the euthanizing of individuals at the end of the experiment, the use of the red eared slider turtle, *Trachemys scripta elegans*, is an interesting model that bypasses many difficulties related to the study of wild chelonians: this species, considered to be an ecological pest in Europe, can be raised in controlled conditions for experimental approaches.

The current experiment aimed therefore to study the trophic transfer of Cd in the red-eared slider turtle, and to assess the bioavailability, kinetics, and distribution of Cd from a contaminated diet. We investigated the uptake of Cd in blood, its elimination in feces and its distribution in organs in turtles fed a Cd-contaminated diet at environmentally relevant concentrations.

Materials and methods

Turtles

Thirty-two red-eared sliders turtles (*Trachemys scripta elegans*) were obtained from Alligator Bay Reptilarium (Manche, France) where they were reared in an outdoor pool. All animals sampled were mature female adults in order to have the biggest individuals, but their age was not determined. The turtles were transported in March 2009, just after the end of hibernation, to the Aquarium-Museum (University of Liège), weighed and measured (weight and plastron length, mean $\pm$ SD $=$ 1331 $\pm$ 343 g and 18.4 $\pm$ 2.1 cm respectively). Individuals were then randomly allocated to one of 8 pools ($n = 4$ females per pool). Pools (130 $\times$ 85 $\times$ 46 cm) were provided with an aquatic section heated to 26°C and a terrestrial section where turtles were able to lie under a warm point at 30°C. Pools were also equipped with a UV lamp and a filter, and the water was changed every week to ensure water quality. The photoperiod was progressively increased depending on the season from 8:16 h (light:dark) in March to 12:12 h in July and August.

Preparation of diet treatments

Turtles were allocated into either a control group (C) or into one of the three groups receiving a Cd-supplemented diet treatment (T1, T2, T3). The Cd-supplemented diet

treatments were designed to have increased Cd-concentrations in food in order to expose turtles to a range of relevant environmental doses of Cd through trophic transfer. Known concentrations of Cd in the prey of reptiles and in Cd-contaminated aquatic habitat (Ganser et al. 2003; Hopkins et al. 2001; Nagle et al. 2001; Rowe et al. 2001; Ward and Mendonca 2006) were used as the reference for the Cd concentration levels used in the diets treatments of the experiment. The three groups of turtles exposed to the diet treatments received a $CdCl_2$-suplementation via their food as follows: 0.400 μg Cd g^{-1} diet (T1: low dosage group), 0.575 μg Cd g^{-1} diet (T2: medium dosage group) and 0.950 μg Cd g^{-1} diet (T3: high dosage group). The commercial turtle diet (standard pellet diet meeting the requirements of adult aquatic turtles, Natural Aquatic Turtle Food Maintenance, Zoomed) for each treatment was contaminated with Cd aqueous solutions prepared from $CdCl_2$ of sufficient concentration to reach the final desired concentration for the diet treatment. This solution was sprayed onto the food (350 ml of solution/3.5 kg of food) while the food was being homogenized in a rotating tank. Food was then kept refrigerated (4°C) prior to analysis and distribution to the turtles. Uncontaminated food was sprayed in the same way with deionized water and stored in the same conditions. Samples of both contaminated and uncontaminated food were analyzed for Cd-concentrations prior to feeding the animals.

Phases of the experiment

During the total duration of the experiment (19 weeks, 57 meals), each turtle was fed 3 times a week. Food was distributed until females were no longer interested in the pellets, and quantities given were recorded for each turtle in each pool. All turtles initially went through an adaptation phase in order to become acclimatized to the food and pools. This phase was also needed to assess the quantity of pellets eaten by each turtle for each meal as a percentage of its body mass. During this phase, all turtles were fed a control diet. After this 3-week adaptation period, control turtles continued to be fed the control diet. Meanwhile, in order to reproduce dietary exposure (the main source of environmental exposure to Cd for reptiles), the three treatment groups (T1, T2, T3, each group containing 8 females allocated into 2 replicates) received the corresponding $CdCl_2$-supplemented diet for 13 weeks. Finally, a phase of recovery occurred during which all turtles were fed control food for 3 weeks.

Sampling

Blood sampling was performed during the three phases of the experiment (at day 19, 33, 54, 68, 84, 98, 112, 133 after the arrival of the turtles in the aquarium). Blood was drawn from the femoral vein using single use needles, plastic syringes and blood collection tubes containing heparin to prevent clotting. Samples were collected 24 h after a meal. At each blood sampling, turtles were also measured and weighed to adjust the quantity of pellets given for meals. Fecal material was collected from each pool as often as possible using a net and was stored in plastic containers. At the end of the experiment, all individuals were anesthetized and euthanized. At necropsy, kidneys, liver, muscle and pancreas were sampled and weighed. All samples were frozen (−20°C) until trace element analysis.

Analytical procedures

After being weighed and lyophilized, samples of food, feces, blood, kidney, liver, muscle and pancreas were homogenized before being digested and analyzed for trace element concentrations. Approximately 100 mg for blood samples and 300 mg for other samples were digested in Teflon tubes with concentrated nitric acid (2–5 ml), deionized water and H_2O_2 in a microwave oven (20 min at between 0 and 600 Watts). After cooling, samples were diluted to 50 ml with deionized water in a volumetric flask. Samples were analyzed by an Inductively Coupled Plasma-Mass Spectrometer (ICPMS, Elan DRC II) to determine Cd, Cu, Zn, Ca and iron (Fe) concentrations in all tissues. Concentrations were expressed in μg g^{-1} dry weight. The quality assurance program included analysis of reagent blanks, sample duplicates, digestion duplicates, and certified reference materials (Seronorm™, trace elements whole blood, for whole blood analysis and DOLT3-liver, National Research Council Canada, for food, feces and organ analysis). Analyses were repeated throughout each set of analyses to ensure the accuracy and precision of the method. Mean percentage recoveries for trace elements in certified reference materials ranged from 89 to 107% for Seronorm™ material and from 94 to 108% for DOLT3-liver material.

Statistical analysis

We first tested whether the turtles, randomly distributed between the different diet treatments, were similar in terms of weight, length and trace element concentrations in blood and feces at the beginning of the experiment by performing a one-way ANOVA. Moreover, in each diet treatment, we had eight female turtles distributed between two pools (four turtles per pool), which provided an extra source of variability. The Student's *t*-test was performed for each diet treatment in order to test the effect of the same diet treatment pools on the weight, length and concentrations of the trace elements at the end of the contamination period in blood and feces. We then performed ANOVAs to

investigate the effect of diet treatment and the effect of time on weight, length, and concentrations of trace elements in blood and feces. When the interaction between variables was significant, a post-hoc Tukey test was performed to compare the mean concentrations between the four different diets. For the bioaccumulation of Cd in organs, we investigated the Cd-burden and Cd-concentration of organs according to the dose of Cd ingested by turtles (continuous variable) rather than as a function of the diet treatment given (categorical variable). Indeed, since each experimental unit (pool) consisted of three or four turtles for which the combination of food consumption and Cd-concentration was unique, there was in fact no true replication of any dose of Cd ingested. Linear regressions were therefore performed to test the relationship between Cd-burden and Cd-concentrations in organs when regressed against the amount of Cd ingested. The normality of all dependent variables was tested before statistical analysis. Significance was assumed at $P \leq 0.05$. Computations were performed with Statistica 6·0 (StatSoft Inc. 2001). When necessary, data were log10 transformed in order to adhere more closely to assumptions of the statistical model.

Results

Food consumption and body condition of the turtles

Mineral composition was verified in all diets by ICP-MS and found to be within the target for Cd-concentrations (Table 1). Three females died during the acclimatization phase ($n = 1$ in control group, $n = 1$ in T1 and $n = 1$ in T2), the hibernation being a critical phase for the species and mortality is known to be high at he end of the hibernation, but none died during the exposure phase in any treatment. Moreover, food refusals occurred for several meals in 4 females in the low and medium treatment groups and these females were therefore removed from further analysis. These females apart, total food intake was stable over the course of the experiment and among turtles and pools (mean ± SD = 1.3 ± 0.2% of turtle body mass) indicating no avoidance of food in any treatment, and there was no significant difference in food intake between treatments ($F_{3,316} = 2.63$, $P = 0.051$). On the basis of Cd-concentrations in the diet treatments and on the amount of food ingested (sum of each meal for each turtle), the estimated total Cd intake over the experiment was (mean ± SE) 189.1 ± 21.5 µg Cd ($n = 3 + 4 = 7$, range 82.4–244.8 µg Cd) for the control group, 324.7 ± 45.8 µg Cd ($n = 2 + 3 = 5$, range 201.8–455.1 µg Cd) for group T1, 447.4 ± 63.2 µg Cd ($n = 2 + 3 = 5$, range 292.0–661.5 µg Cd) for group T2 and 723.3 ± 68.6 µg Cd ($n = 4 + 4 = 8$, range 509.1–1032.7 µg Cd) for group T3.

At the beginning of the experiment, the turtles assigned to one of the four diet treatments did not differ in their weight, length of plastron or trace element concentrations analyzed in blood and feces ($P > 0.05$ in all cases). At the end of the experiment, turtles in the two pools receiving the same diet treatment did not show significant difference in their weight, length of plastron or concentrations of trace elements in blood and feces or organs ($P > 0.05$ in all cases). For further statistical analyses, all samples from the same diet treatment were therefore pooled. All turtles exhibited positive growth over the 5-month study without a marked difference between diets (data not shown). This suggests that there were no effects of dietary Cd on final body mass ($F_{3,21} = 0.05$, $P = 0.984$) or length ($F_{3,21} = 0.45$, $P = 0.718$) at the end of the exposure. Moreover, liver and kidney fresh weights at the end of the study and kidney and liver somatic indexes (organ mass/body mass) were not related to diet, suggesting no effect of treatment on organ mass ($F_{3,21} = 0.26$, $P = 0.853$ for kidney mass, $F_{3,21} = 0.17$, $P = 0.911$ for liver mass, $F_{3,21} = 0.43$, $P = 0.732$ for kidney SI and $F_{3,21} = 1.14$, $P = 0.353$ for liver SI).

Blood and feces Cd kinetics

Cd-concentrations in blood remained very low in the turtles of each treatment group and during the whole course of the experiment (Fig. 1). Cd treatment had no effect on blood concentration at the end of the exposure phase ($F_{3,18} = 0.548$, $P = 0.656$) and Cd blood concentrations did not vary between the beginning and the end of the exposure phase ($F_{3,34} = 1.61$, $P = 0.205$, Fig. 1). Nor did the Cd-concentrations in blood at the end of the recovery phase vary for any treatment ($F_{3,35} = 0.55$, $P = 0.652$).

Cd-concentrations in feces were much higher than in blood. The level of fecal elimination was obtained for each pool by grouping feces collected at 2 weeks intervals. Cd-concentrations in feces were not statistically different between treatments in the first 2 weeks of exposure ($F_{3,4} = 4.26$, $P = 0.099$). However, during the exposure phase, Cd-concentrations in feces varied significantly with time ($F_{1,12} = 29.22$, $P < 0.001$) and between diet treatments ($F_{3,12} = 29.49$, $P < 0.001$), and the interaction between treatment and time was significant ($F_{3,12} = 7.10$, $P = 0.005$) (Fig. 1). During the recovery phase, Cd-concentrations in feces also varied significantly with time ($F_{1,14} = 18.58$, $P < 0.001$) and between diet treatments ($F_{3,14} = 16.07$, $P < 0.001$). Their interaction was also significant ($F_{3,14} = 6.22$, $P = 0.007$). After 3 weeks of recovery, Cd-concentrations in feces were similar to those of the beginning of the experiment ($F = 0.67$, $P = 0.423$). Post-hoc Tukey tests showed significant differences

Table 1 Trace element analysis of diets: basal diet (commercial pellets for aquatic turtles), control diet (pellets treated with deionized water) and $CdCl_2$ supplemented-diet (T1: low dosage, T2: medium dosage, T3: high dosage)

Elements (μg g-1)	Basal, control and cadmium supplemented diets			T2	T3
	Basal diet	Control	T1		
Cd	0.238 ± 0.005	0.249 ± 0.007	0.415 ± 0.010	0.571 ± 0.044	0.956 ± 0.003
Ca	15440 ± 698	13200 ± 247	13260 ± 442	13837 ± 496	13888 ± 527
Fe	276 ± 15	246 ± 5	240 ± 9	257 ± 9	249 ± 4
Cu	11.3 ± 0.2	11.2 ± 0.2	10.9 ± 0.4	13.7 ± 4.6	11.5 ± 0.9
Zn	144 ± 2	139 ± 1	136 ± 6	143 ± 5	141 ± 2.1
Se	0.665 ± 0.022	0.619 ± 0.041	0.663 ± 0.094	0.732 ± 0.060	0.692 ± 0.015
Pb	0.175 ± 0.026	0.142 ± 0.012	0.132 ± 0.006	0.231 ± 0.161	0.177 ± 0.028

Values are mean ± SD of three determinations

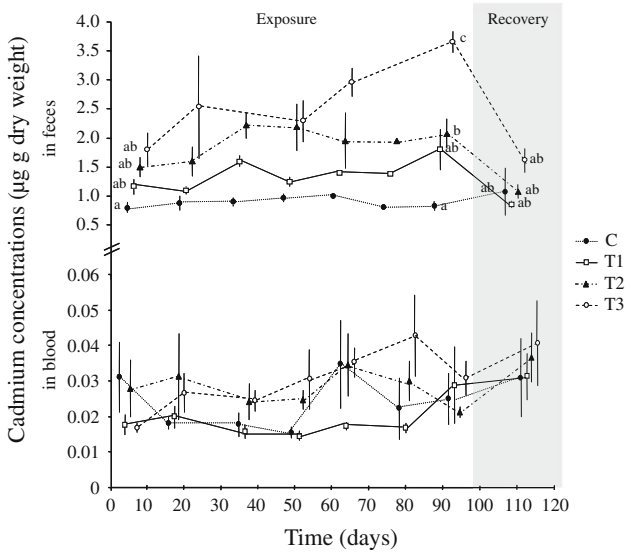

Fig. 1 Dynamics of Cd-concentrations in blood and feces during the exposure phase and recovery phase. Feces in the high dosage treatment could not be collected for certain periods of the experiment (absent samples) resulting in missing values for two periods of the kinetics of depuration

between treatments and time in feces concentration of Cd during the exposure and recovery phases (Fig. 1).

Cd distribution and bioaccumulation

Turtles fed Cd-contaminated food accumulated Cd in their organs and the accumulation was different between target organs ($F_{3,79} = 83.80$, $P < 0.001$), with the highest concentrations in kidney > liver > pancreas > muscle, but the highest Cd-burden in liver > kidney > pancreas (the amount in muscle was not quantified because muscle mass was not assessed). Moreover, there was a positive and significant relationship between the dose of Cd ingested through the diet and Cd-burden and Cd-concentration in liver and kidney (Fig. 2). By contrast, concentrations of Cd in pancreas and muscle and the Cd-burden in pancreas did not vary with the amount of Cd ingested (Fig. 2).

The results also provide indications on the proportional accumulation in liver and kidney (% of Cd absorbed by gastrointestinal tracts from the dose of Cd ingested via contaminated food) and Cd accumulation in organs. In this study, the proportional accumulation of Cd in kidney and liver together over the whole experiment ranged between 0.7 and 6.1% of the total Cd ingested (with proportional accumulation ranging from 0.1 to 1.3% for kidney and 0.7 to 5.8% for liver). A significant negative correlation was observed between proportional accumulation in liver and kidney together and the dose of Cd ingested ($F_{1,23} = 6.49$; $P = 0.017$, Fig. 3).

Homeostasis of essential elements

In blood and feces, Cd diet treatment had no effect on concentrations of essential elements such as Ca, Fe, Cu, Zn during the exposure phase ($P > 0.05$ in all cases). In contrast, element concentrations in liver and pancreas were weakly but positively modified in relation to Cd-concentration. In liver, Fe and Zn increased when Cd increased ($R^2 = 0.21$, $P = 0.022$ and $R^2 = 0.27$, $P = 0.008$ respectively) and in pancreas, Cu increased when Cd increased ($R^2 = 0.21$, $P = 0.032$).

Discussion

In reptiles and other vertebrates, Cd is mainly accumulated in liver, kidney, and pancreas and in the intestinal mucosa (Chan et al. 2004; Mann et al. 2007; Rie et al. 2001; Vogiatzis and Loumbourdis 1998; Weigel et al. 1984; Xu and Wang 2002). Intestinal mucosa is rather an organ of transport (Chan et al. 2001), and once Cd is absorbed through gastrointestinal tracts into the bloodstream, Cd associates reversibly with plasma proteins (i.e. albumin)

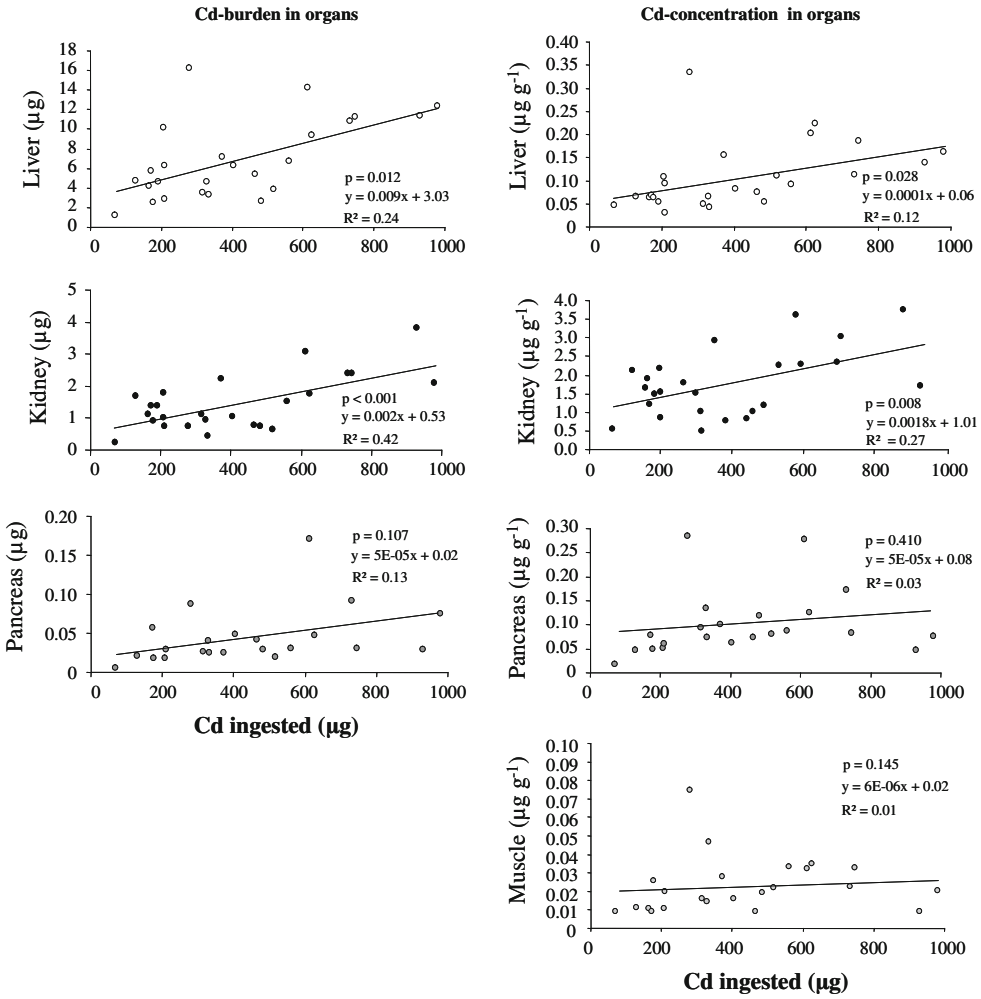

Fig. 2 Relationship between ingested Cd (µg) and Cd-burden or Cd-concentration in liver, kidney, pancreas, and muscle of female red eared slider turtles fed Cd-contaminated food at an environmental dose

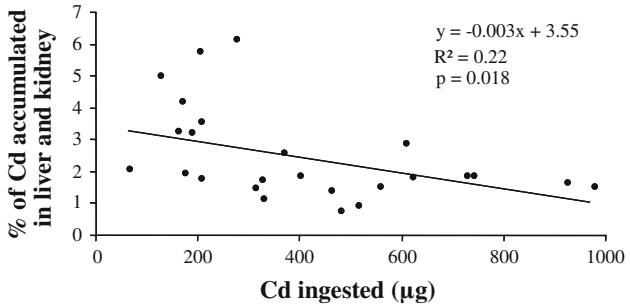

Fig. 3 Relationship between the proportional accumulation in liver and kidney and total Cd ingested by female red eared slider turtles during the exposure phase

and is delivered first to the liver where it binds to metallothionein (MT), a family of cysteine-rich, low molecular weight proteins (Klaassen et al. 1999). MTs have the capacity to bind both essential and non essential elements (such as Zn, Cu, selenium for essential elements and Cd, mercury, silver, arsenic for non essential) through the thiol group of its cysteine residues. The MT complex is then redistributed to the kidney where it becomes concentrated (Zalups and Ahmad 2003). Liver and kidney are known to accumulate together about half of the Cd body burden (Nordberg et al. 2007).

This general pattern of organ distribution is verified in our study on red-eared slider turtles. We showed that Cd accumulated in tissues in a dose-dependent manner in the following decreasing order of Cd-burden liver > kidney > pancreas and of Cd-concentration kidney > liver > pancreas > muscle. The tissue burden and concentration in both liver and kidney confirmed the expected relationship between dose and accumulation, in that as the dose of ingested Cd increased, so did accumulation in these storage organs. The ability of kidneys to accumulate higher concentrations of Cd than liver is consistent with a transfer of Cd from blood to liver, where Cd binds to MT, followed by a remobilization and subsequent transport from liver to kidney where Cd bioaccumulates to the greatest concentration (Nordberg et al. 2007). However, in terms of burden, the liver accumulates a greater burden of Cd than the kidney. In our study, liver, by virtue of its mass (154.5 ± 43.7 g), accounted for up to 5.8% of the ingested dose of

Cd, but concentrations remained low. The distribution of Cd in mainly kidney or mainly liver is the result of complex interactions between parameters such as the treatment dose size (low doses vs. high doses; Ando et al. 1998), dosing regime (single dose vs. chronic exposure; Lind et al. 1997), type of contamination (administration of Cd-MT vs. CdCl2; Groten et al. 1991).

Our results on levels of gastrointestinal absorption are consistent with levels reported in various vertebrate experiments ranging from 0.5 to 16% (Neathery and Miller 1975; Xu and Wang 2002, Hispard et al. 2008; Mann et al. 2006; Zalups and Ahmad 2003) according to the Cd-concentration in diet that individuals were fed. In our study, the proportional accumulation in liver and kidney depended on the amount of Cd ingested with decreasing assimilation efficiency when ingestion increased, suggesting that as the dose of Cd increases, assimilation decreases. In the turtles that ingested the highest quantities of Cd, proportional accumulation was as low as 1% in kidney and liver together. Other studies have already reported such low rates of assimilation (Chan et al. 2001) and the same tendency for a decreasing absorption rate with an increasing Cd intake (Matsuno et al. 1991; Weigel et al. 1984). Dietary Cd-concentration is therefore an important parameter that impacts the toxicokinetic of uptake and the fractional gastro-intestinal uptake (saturable uptake kinetic of the membranes) that depends on dose size (Andersen et al. 2004).

Following oral exposure, most of the Cd ingested is not absorbed enterically and are excreted in the feces (Schilderman et al. 1997). The values found for proportional accumulation in liver and kidney in our study ranged between 0.7 and 6.1% in liver and kidney together suggesting that the assimilation efficiency in the whole body is about 1.4–12.2% if 50% of the Cd body burden is stored in the kidney and liver (Nordberg et al. 2007). This suggests that 90% of Cd ingested or more is eliminated through feces, which is therefore the main route of elimination (Gregus and Klaassen 1986; Schilderman et al. 1997). Cd-concentration in feces may therefore be used as an indicator of recent Cd contamination through trophic exposure, if fecal elimination reflects the dose ingested. However, in this study, Cd concentrations in feces varied over time and therefore can not be used as a reliable estimate for Cd oral exposure. Cd concentration in feces suggest that gastro-intestinal uptake of Cd was highest during the first weeks of exposure, after which assimilation decreased significantly, reflecting reduced binding of Cd in the membranes of gastro-intestinal tracts. Higher levels of Cd assimilation in the first weeks have already been reported in lizards and have been related to accumulation and therefore saturation of the binding sites for Cd transport (Mann et al. 2006). Intestinal absorption of Cd is characterized by a high accumulation of Cd within the intestinal mucosa and a low rate of subsequent transport of Cd into the blood. In order to be absorbed into the digestive system, the divalent ion (Cd^{2+}) interacts with and competes for binding sites on membrane proteins involved in the transport of essential elements (such as Ca, Fe, and Zn) into target epithelial cells, possibly through some form of ionic mimicry (Beckett et al. 2007; Zalups and Ahmad 2003).

At the end of the exposure phase in the present study, the ratio of the fecal Cd levels of the different diets reflected the ratio of the diet Cd-concentration of the respective treatments, suggesting a tendency for a dose-dependent Cd concentrations in feces and reflecting a reduced binding of Cd on the membranes in the gut and intestines. Such concentration ratios between feces and food have already been observed in rats after extended oral administration of Cd (Weigel et al. 1984). Moreover, we found that after cessation of Cd trophic exposure, 3 weeks were needed to recover initial Cd-concentrations in feces (levels before exposure).

Blood concentrations are generally used as an indicator of exposure and are expected to vary with recent diet exposure (Nordberg et al. 2007). But in our study, Cd-concentrations in blood were very low and stable over time despite the Cd intake from contaminated diet. The low concentrations used in this study (non lethal environmental dose) combined with a rapid clearance of Cd from the blood after ingestion (Liu et al. 1996; Rie et al. 2001; Schilderman et al. 1997) could explain the lack of variation of Cd-concentrations in blood. Cd-concentrations in the blood are therefore not a useful indicator of the degree of recent exposure when doses of exposure are low. Increased blood Cd-concentrations may be observed for high diet dosage only (exposure to supplemented $CdCl_2$ diet at high dosage of 50–200 ppm, Noel et al. 2004). Obviously, only elevated amounts of ingested Cd would lead to noticeable increases in Cd blood concentrations. In our experiment, although blood concentrations are very low, transfer of Cd across the gut into the bloodstream did occur because accumulation was observed in storage organs as expected.

The investigation of the biological effects of Cd at environmental concentrations is also an important step in reptile conservation. In our study, all turtles survived to the exposure phase and exhibited normal growth and body conditions. Cd trophic exposure did not therefore have any effect on the body or organ mass of the turtles whatever the diet treatment received. However, the present results suggest that Cd can alter the mineral metabolism of reptiles exposed to low doses of Cd by oral intake. We showed that increasing dietary Cd exposure increased both hepatic Fe and Zn concentrations and pancreatic Cu concentrations in a dose-dependent manner. The liver is known to be a critical organ in the homeostasis of essential metals

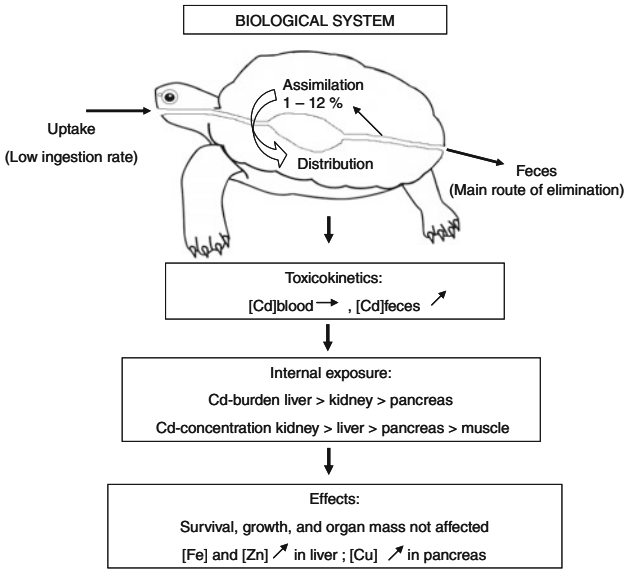

Fig. 4 Illustration of the processes of the fate and effects of a pollutant in organisms. Uptake, distribution, accumulation and elimination are shown and the main results of the kinetics, distribution and effects of this study on Cd in turtles are summarized

including Ca, Cu, Fe and Zn. Similar changes have already been reported in previous studies on small mammals showing increased Zn concentrations in rat livers after long term oral exposure to a low dose Cd-supplemented diet and the increased concentration of Zn in liver was likely due to the de novo synthesis of MT induced by Cd administration (Nakagawa et al. 2004). The displacement of Cu and Fe from intracellular proteins by Cd has also been reported by Waisberg et al. (2003), which suggests that the increased concentrations of ionic Cu and Fe could cause oxidative stress through Fenton reactions. Further studies are therefore needed to investigate the deleterious effects of environmental contamination on reptiles at the individual and population level so that prediction of the impact of contaminants on reptiles and conservation efforts can be effective.

Bioaccumulation of Cd in the turtles of our study was the result of a dynamic process between the rate of uptake from diet and the rate of loss through feces. The mechanisms of uptake, distribution, accumulation and depuration of Cd through trophic exposure illustrated in our study are summarized in Fig. 4. Understanding such processes is an important step in reptile conservation. Because reptile populations are currently threatened by environmental pollution, the use of non lethal samples to assess of the level of contamination and the potential risk of pollution on reptile populations is required. In this study, blood and feces have not been shown as relevant indicator of recent Cd trophic exposure, so as shed skin, blood and tail clips that have been tested in Hopkins et al. (2001). The challenge now is to find suitable tissues that can be easily collected and that represent a reliable indicator of exposure by trophic transfer.

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