Potential arsenic exposures in 25 species of zoo animals living in CCA-wood enclosures

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HIGHLIGHTS

- Daily inorganic As dose from zoo animal foods was 0.22–7.8 μg/kg bw/day.
- Total As concentrations in soils of zoo animal enclosures were 1.0–110 mg/kg.
- Endangered zoo animals live in soils with As above USEPA Eco-SSLs for avian and mammal species.
- Dislodgeable As on CCA-wood beams where primates sit was 4.6–111 μg/100 cm².
- Marmoset hair had 6.37 mg/kg As compared to a reference value of 0.21 mg/kg.

GRAPHICAL ABSTRACT

Abstract

Animal enclosures are often constructed from wood treated with the pesticide chromated copper arsenate (CCA), which leaches arsenic (As) into adjacent soil during normal weathering. This study evaluated potential pathways of As exposure in 25 species of zoo animals living in CCA-wood enclosures. We analyzed As speciation in complete animal foods, dislodgeable As from CCA-wood, and As levels in enclosure soils, as well as As levels in biomarkers of 9 species of crocodilians (eggs), 4 species of birds (feathers), 1 primate species (hair), and 1 porcupine species (quills). Elevated soil As in samples from 17 enclosures was observed at 1.0–110 mg/kg, and enclosures housing threatened and endangered species had As levels higher than USEPA’s risk-based Eco-SSL for birds and mammals of 43 and 46 mg/kg. Wipe samples of CCA-wood on which primates sit had dislodgeable As residues of 4.6–111 μg/100 cm², typical of unsealed CCA-wood. Inorganic As doses from animal foods were estimated at 0.22–7.8 μg/kg bw/d. Some As levels in bird feathers and crocodilian eggs were higher than prior studies on wild species. However, hair from marmosets had 6.37 mg/kg As, 30-fold greater than the reference value, possibly due to their inability to methylate inorganic As. Our data suggested that elevated As in soils and dislodgeable As from CCA-wood could be important sources of As exposure for zoo animals.

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1. Introduction

Chromated copper arsenate (CCA) is a water-soluble pesticide that extends the service life of outdoor wood structures by 20–40 years. CCA was withdrawn from use in most residential applications in 2004 because of health concerns about arsenic (As) exposure from CCA-wood (Hemond and Solo-Gabriele, 2004). However, CCA-treated poles, fencing, and plywood are still commonly used in areas where animals are housed, including barns, feedlots, and zoos. Depending on the intended use of the wood, As concentrations are typically 1500–5000 mg/kg (AWPA, 1998).

Human exposure to As is a public health issue because of its systemic effects and carcinogenicity from chronic low dose exposures (WHO, 2010). Typical background As concentrations in US soils are 3–4 mg/kg, although concentrations can be significantly higher in areas adjacent to CCA-wood (Townsend et al., 2003; Chirenje et al., 2003; Gress et al., 2014). Like humans, animals are commonly exposed to As through ingestion of As-contaminated water and food (Eisler, 2003; Gress et al., 2014; ATSDR, 2007). Additionally, CCA-wood can contaminate soil within enclosures, which can be ingested by animals during grooming and/or from eating food on the ground (Goessler and Braeuer, 2014). Systemic health effects from intermediate and chronic exposure to As in laboratory animals are similar to those observed in humans and include gastrointestinal, neurological, reproductive, liver, kidney and blood-related disorders (ATSDR, 2007).

To identify potential health hazards to wildlife from chronic exposure to soil contaminants at hazardous waste sites, USEPA established ecological soil screening levels (Eco-SSLs), which are the maximum soil concentrations to protect animal health (USEPA, 2003). The As Eco-SSL for avian species is 43 mg/kg and for mammals is 46 mg/kg. However, there are no Eco-SSLs for reptiles, amphibians or soil-dwelling invertebrates because of a lack of relevant toxicological data (USEPA, 2005a,b). Zoo animals are vulnerable to chronic exposures to environmental contaminants because they are typically confined in enclosures for years (Gupta and Bakre, 2013). Few studies have evaluated As exposures in zoo animals. Comparison of As in the blood of free-ranging and captive Matschie's tree kangaroos (Dendrolagus matschiei) in Papua New Guinea found 42.6 µg/L As in the blood of captive animals (n = 5) and <1 µg/L in free-ranging ones (Travis et al., 2012).

Hair, nails and feathers can be useful non-invasive biomarkers for animals to monitor their chronic As exposure because As is known to accumulate in these tissues (Burger, 1994; Smith et al., 2008a,b). Feathers, quills and hair are composed of sulfur-rich keratin proteins to which As binds during growth. The amount of As in these tissues reflects exposure during a specific time period, but their use in health risk assessment can be limited because tissue concentrations have not been well-correlated with exposure doses (Yanez et al., 2005; Marchiset-Ferlay et al., 2012). Metal concentrations in reptile and avian eggs have been used as indicators of embryonic exposure, although metal distribution between shells, membranes and egg contents varies between species and also have not been correlated with environmental concentrations or health effects (Burger and Gochfeld, 2001; Xu et al., 2006). Marco et al. (2004) reported As translocation from contaminated nesting substrate into reptile eggs, indicating the potential for embryonic exposure to soil contaminants. Arsenic can also accumulate in external reptilian structures including scutes and shells, which are comprised of similar keratin found in mammals and bird feathers. Analyses of metals known to cause skin disease found significantly higher levels of As (1.58 vs. 0.63 mg/kg) in ill or dying Desert tortoises (Gopherus agassizii) with cutaneous dyskeratosis compared to healthy tortoises, with As being concentrated in keratin in the shells (Berry et al., 2001).

For most species, there are little reference data on As levels in tissues from healthy animals. There is also a lack of information about species-specific exposure factors, which could result in inaccurate dose estimations when observational and experimental data on surrogate species is used in ecological risk assessments (Smith et al., 2008a,b; Weir et al., 2010). Because of a lack of uniformity in study design and the low number of individuals and species represented in most ecotoxicology studies, there is a need for research that provides practical data in species-specific toxicity assessments (Gardner and Oberdorster, 2006; Grillitsch and Schiesari, 2010). For example, most animal species detoxify inorganic As via methylation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), which are excreted in urine (Vahter, 2002). The inability of marmoset and tamarin monkeys (Callithrix jacchus) to methylate As due to their deficiency in methyltransferase was identified by Zakhrayan et al. (1996). Their inability to methylate As leads to As bioaccumulation in tissues, with the animals receiving a 0.4 mg As/kg bw IV-administered dose accumulating 0.91 mg/kg in the liver (Vahter and Marafante, 1985).

Identifying potential As exposure pathways in zoo animals living in enclosures constructed of CCA-wood is essential to reduce avoidable health risks from chronic As exposure, particularly in endangered species. The objective of this study was to assess the impact of CCA-wood structures on As exposures in zoo animals by: (1) measuring As concentrations in soils inside enclosures and on wipe samples of CCA-wood, (2) determining As speciation in animal foods, and (3) analyzing As concentrations in the feathers, eggs, hair, and quills from 15 species of zoo animals. These were compared to scarce data in the literature when possible. This is the first study reporting As concentrations in the biomarkers of 15 species of zoo animals living in CCA-wood enclosures, including threatened and endangered species.

2. Materials and methods

2.1. Zoo characteristics and animal species

The commercial zoo in this study is an accredited member of the Association of Zoos and Aquariums (AZA) and has numerous animals enrolled in the AZA Species Survival Plan® (SSP) Program, which include threatened or endangered animal species. Zoos that participate in SSP Programs engage in conservation activities, including active breeding programs to maintain genetic diversity. Like many zoos, animal enclosures at this zoo are constructed of CCA-treated poles, posts, plywood and planks (Fig. 1). Sawdust was collected from 3 new CCA-wood poles being used in the construction of animal enclosures. Sawdust was digested using EPA Method 3050B and metal concentrations were determined with inductively coupled plasma mass spectrometry (ICP-MS; PerkinElmer, NexION 300).

This study evaluated potential pathways of As exposure in a total 25 species of zoo animals living in CCA-wood enclosures. We analyzed As speciation in complete animal foods (5 types), dislodgeable As from CCA-wood (4 primate species) and As levels in enclosure soils (17 species), as well as As levels in biomarkers of 15 species including 9 species of crocodilians (eggs), 4 species of birds (feathers), 1 primate species (hair), and 1 species of porcupine (quills).

2.2. Sampling of soils, water, foods and dislodgeable As residues

Soil samples were collected from 17 enclosures housing 8 crocodilian, 4 primate, 3 avian, 1 tortoise and 1 porcupine species. Twelve of the species were enrolled in the AZA SSP® Program. Because of the lack of uniformity in enclosure design and size, sampling plans varied between enclosures. A minimum of 3 soil samples were collected from bare soil at a depth of 2.5 cm in each enclosure and at least 45 cm away from CCA-wood. Where possible, samples were collected from areas where animals were observed to sit or lie. Soil core samples were also collected at a depth of 15 cm. All soil samples were digested following EPA Method 3050B and analyzed for As by graphite furnace atomic absorption.
spectrophotometry (GFAAS, Varian AA240Z). Water samples from ponds located in enclosures and from streams in open areas of the zoo were collected, filtered with a 0.2 μm polyester filter and also analyzed by GFAAS. Samples of 5 commercial complete animal foods were digested using the protocol for biological samples and As speciation was conducted using HPLC-ICP-MS (Zhao et al., 2015). QA/QC protocols included the use of blanks, spikes, duplicates and NIST SRM 2710a (Montana soil) and 2976 (mussel tissue).

Dry wipe samples were collected from the surface of dry CCA-wood within enclosures of 4 primate species (Table 1). We followed NIOSH Method 1900, using a 50 cm² polyester wipe (Texwipe, TX 1009) under ~1.1 kg pressure. After sampling, wipes were placed in digestion tubes and 50 mL 10% HNO3 was added. Tubes were placed in a 65 °C water-bath for 16 h prior to filtration using 0.45 μm polyester filters (Environmental Express, South Carolina) (Stilwell et al., 2003; CPSC, 2005). The goal of wipe sampling was to remove dislodgeable As residues that are easily accessible through casual contact (CPSC, 2005; USEPA, 2005a,b).

2.3. Tissue sample collection, digestion and analysis

Biomarkers from 15 species of zoo animals were collected, including newly-laid eggs from 9 crocodilian species, feathers from 4 avian species, quills from 1 porcupine, and hair from the backs of 2 adult and 1 juvenile Geoffroy’s marmosets. The samples were placed in clean bags and stored in a refrigerator at 4 °C. All biological tissues were cleaned 3 times using DI water and acetone, and dried at 60 °C for 2 d. The calamus was removed from feathers prior to digestion.

Feather and hair samples were weighed and placed in 50 mL Teflon digestion tubes, to which 10 mL trace metal grade concentrated HNO3 was added. Samples underwent hot block digestion at 110 °C until the volume was reduced to 5 mL. Once cooled, 5 mL of 30% H2O2 was added and again heated at 110 °C for 2 h. This was followed by addition of DI water to 50 mL, filtration with 0.2 μm filters and analysis by GFAAS. QA/QC included the use of blanks, spikes, triplicates and the use of NIST SRM 2976 (mussel).

### Table 1

<table>
<thead>
<tr>
<th>Food sample</th>
<th>Dimethyl arsenic acid (μg/kg)</th>
<th>Arsenite (μg/kg)</th>
<th>Arsenate (μg/kg)</th>
<th>Total inorganic As (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Softbill bird</td>
<td>ND</td>
<td>17.0 ± 1.0 (39%)</td>
<td>27.0 ± 1.4 (61%)</td>
<td>44.0</td>
</tr>
<tr>
<td>Game bird</td>
<td>6.0 ± 2.0</td>
<td>122 ± 51 (73%)</td>
<td>45.0 ± 0.1 (27%)</td>
<td>167</td>
</tr>
<tr>
<td>Crocodilian</td>
<td>55 ± 0.4</td>
<td>229 ± 25 (83%)</td>
<td>39.0 ± 0.1 (15%)</td>
<td>268</td>
</tr>
<tr>
<td>Primate solid</td>
<td>0.52 ± 0.30</td>
<td>12.9 ± 0.93 (30%)</td>
<td>30.0 ± 2.1 (70%)</td>
<td>42.9</td>
</tr>
<tr>
<td>Primate gel</td>
<td>2.6 ± 0.25</td>
<td>3.20 ± 0.09 (17%)</td>
<td>15.7 ± 2.0 (83%)</td>
<td>18.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Mean bodyweight (kg)</th>
<th>Complete food type</th>
<th>Food ingestion rate (g/d)</th>
<th>As concentration in food (μg/kg)</th>
<th>As ingested daily (μg/kg bw/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crocodilian</td>
<td>68</td>
<td>Pellets</td>
<td>56</td>
<td>268</td>
<td>0.22</td>
</tr>
<tr>
<td>Gamebird</td>
<td>1.1</td>
<td>Pellets</td>
<td>47</td>
<td>167</td>
<td>17.1</td>
</tr>
<tr>
<td>Primate</td>
<td>0.1</td>
<td>Pellets</td>
<td>3.5</td>
<td>42.9</td>
<td>0.30</td>
</tr>
<tr>
<td>Primate</td>
<td>0.5</td>
<td>Gel</td>
<td>40</td>
<td>18.9</td>
<td>0.76</td>
</tr>
</tbody>
</table>

*Method detection limit = 1 μg/kg
Whole eggs were cleaned with 3 alternating rinses of DI water and acetone while being lightly brushed with a soft-bristle brush to remove soil. Egg content and shell were then separated and dried at 60 °C for 3 d prior to homogenization. One gram of sample was placed in 50 mL digestion tube and 9 mL HNO3 + 1 mL HCl of trace metal grade was added. Tubes were placed on a 95 °C hot block and samples digested for 2 h. When cool, 5 mL of 30% H2O2 were added and again heated at 95 °C until a volume of 2 mL was reached. Double DI water was added to a final volume of 12 mL and samples placed in the refrigerator over-night. Prior to analysis with GFAAS, samples of egg content were transferred to 15 mL tubes and centrifuged at 4200 rpm for 10 min then 9 mL of sample was placed in tubes for analysis. Egg shell digestate was prepared for analysis by filtration with 0.45 μm filters.

2.4. Quality control and statistical analysis

The As recovery for NIST SRM 2710a (Montana soil) and 2976 (mussel tissue) was 89.3–96.5%, averaging 92.7%. Due to the limited sample size provided by the zoo, data were presented as the mean of all replicates with standard error where possible. Using one-way analysis of variance (ANOVA), significant differences were determined by comparing means using Tukey’s multiple range tests, at p < 0.05 using JMP®10 PRO (SAS Institute Inc., Cary, NC, 1989–2010).

3. Results and discussion

3.1. As in soils and on CCA-wood may be sources of animal exposures

To identify potential sources of animal exposure to As, we analyzed As concentrations in 5 animal diets, 17 enclosure soils, and dislodgeable As from CCA-wood. Since inorganic As is more toxic to animals than organic As (ATSDR, 2007), we analyzed As speciation in commercial foods for crocodilian, avian and primate species using HPLC-ICP-MS (Zhao et al., 2015). Inorganic As was found in all foods, with crocodilian and avian products having the highest As concentrations at 268 μg/kg and 167 μg/kg, respectively (Table 1). Daily As doses via food ingestion would vary between species due to differences in food intake and body weights. Estimation of As doses for marmosets and tamarins was 1.8 μg/kg bw/d, while avian species being fed with gamebird product, such as the ground-dwelling Cabot’s tragopan (Tragopan caboti), could ingest up to 7.8 μg/kg bw/d (Table 1). Even though the As content was higher in crocodilian food, a 68 kg crocodilian was estimated to ingest only 0.22 μg As/kg bw/d. The USEPA Eco-SSL estimates the no observable adverse effect levels (NOAEL) at 2.24 mg/kg bw/d for avian species and 1.04 mg/kg bw/d for mammals. Even though there was insufficient toxicological data to establish a NOAEL for reptiles, food ingestion of As was unlikely a significant source of inorganic As for crocodilians at this zoo. All samples of pond water collected within enclosures and from natural sources on-site had As concentrations below 2 μg/L, indicating drinking water was not a significant source of As for animals at this zoo either.

The CCA-wood posts used in construction of enclosures had mean concentrations of 5227 mg/kg Cr, 4710 mg/kg As, and 3266 mg/kg Cu (data not shown). The average total As concentrations in soil samples from 8 crocodilian enclosures (80–200 m2 in size) were 12–71 mg/kg (Fig. 2A; Table 2), with all samples being elevated above background soil As concentrations of 2.50–3.90 mg/kg (data not shown). The highest arsenic concentrations in soil samples from 6 enclosures were higher than the risk-based Eco-SSL for birds (43 mg/kg) or mammal species (46 mg/kg) (Table 2), leading to speculation about the potential for adverse health impacts from chronic exposure to As via incidental soil ingestion in these crocodilians.

In comparison, the average soil As concentrations in 4 primate enclosures were lower at 1.8–22 mg/kg, with those housing Geoffroy’s marmoset and Goeldi’s monkey being the highest at 19 and 22 mg/kg (Fig. 2B; Table 2). Core samples taken within the marmoset enclosure showed elevated soil As at 15 cm depth (7.2 mg/kg) (data not shown). Primates are not typically ground-dwelling animals and were observed on the tree branches and CCA-wood beams within enclosures (Fig. 1C). However, ingestion of contaminated soil could occur while eating foods on the ground and during normal grooming activities.

In addition to As from soils, ingestion of dislodgeable As on CCA-wood during grooming and nursing is another exposure pathway. Primate enclosures were constructed with a frame of CCA-wood posts and beams surrounded by metal wire mesh, and there were numerous areas within each enclosure where primates directly contact unsealed wood (Fig. 1A, C). Wipe sampling of CCA-wood beams in primate enclosures found dislodgeable As residues of 4.60–111 μg/100 cm2 (data not shown). This amount of dislodgeable-As on the wood is comparable to levels reported in a review of CCA-wood wipe studies (Hemond and Solo-Gabriele, 2004). USEPA evaluated potential health risks to children after contact with CCA-wood during play activity, concluding that young children having regular contact with CCA-wood could be exposed to As in amounts associated with an elevated risk of cancer, primarily from ingestion of residues during hand-mouth behaviors (Zartarian et al., 2006). It is reasonable to assume that primates were also exposed to dislodgeable-As after prolonged hours of hand contact with CCA-treated wood. Because primates have different behaviors than children, including frequent grooming behaviors, the same assumptions cannot be used to estimate an As dose in primates from ingestion of dislodgeable As. However, the concentrations of dislodgeable-As from CCA-wood suggested that the residues were at levels that could be associated with adverse health impacts, particularly in primate species vulnerable to As bioaccumulation such as marmosets and tamarins.

3.2. As concentrations in the eggs of 9 crocodilian species

Eggs have long been used as a biological indicator of metal exposures but concentrations in different egg compartments have not been
correlated with species-specific exposure levels. The use of eggs for biomonitoring is useful for spatial comparisons between populations or for assessing impacts on a particular population over time (Burger, 1994; Burger and Gochfeld, 2001). Arsenic concentrations in the egg shells and contents of nine crocodilian species found inter and intra-species variability in both As concentrations and partitioning between egg compartments, which was noted in the few studies that assessed As levels (Fig. 3).

Both As concentrations in the egg contents and egg shell varied greatly among the 9 crocodilian species, with a mean concentration of 272 ± 46 μg/kg in the content and 94 ± 75 μg/kg in the shell. In the egg content, as ranged from 192 μg/kg in Saltwater crocodiles (Crocodylus porosus) to 342 μg/kg in Smooth-fronted caiman (Paleosuchus trigonatus) (Fig. 3). In comparison, As concentrations in egg shells varied by 6-fold, from 0.5 μg/kg in Johnston’s crocodile (Crocodylus johnsonii) to 166 μg/kg in American alligators (Alligator mississippiensis). However, there was no correlation between As concentrations in egg contents and egg shells (r < 0.02), i.e., higher As contents in egg contents were not necessarily associated with higher As contents in egg shells, which was observed in prior studies on crocodilians and birds. Generally speaking, As content was greater in egg contents than egg shells, with the lowest As in egg content being greater than the highest As in egg shells (192 vs. 166 μg/kg; Fig. 3). However, it was unclear why As concentrations in egg contents and shells varied so widely and the mechanisms controlling As partitioning between egg contents and shells in different species are unknown. As concentrations in egg content indicate direct embryonic As exposure during incubation (Marco et al., 2004).

Most studies evaluated crocodilian exposures to Hg, Cd and Pb by measuring metal concentrations in muscle tissue or organs, with only few studies assessing As concentrations in eggs. Ogden et al. (1974) found As concentrations of 60–80 μg/kg (averaging 70 μg/kg) in 5 eggs from American crocodiles (Crocodylus acutus) and 50–200 μg/kg (averaging 120 μg/kg) in 4 eggs from American alligators. Xu et al. (2006) measured As concentrations in 10 eggs from 3 critically endangered Chinese alligators (Alligator sinensis), averaging 262 μg/kg As in egg-shells and 474 μg/kg in egg contents. It is unknown whether the high As concentrations in eggs from those captive alligators imply they were exposed to As, although the area where they reside (Changxing Nature Reserve and Breeding Center, Huzhou, China) is near battery

**Table 2**

<table>
<thead>
<tr>
<th>Order</th>
<th>Common name</th>
<th>Scientific name</th>
<th>IUCN status(^a)</th>
<th>Mean and range of soil As content (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crocodilia</td>
<td>American alligator</td>
<td>Alligator mississippiensis</td>
<td>Low risk</td>
<td>24 ± 2 (19-25)</td>
</tr>
<tr>
<td>Crocodilia</td>
<td>Broad-snout caiman</td>
<td>Caiman latirostris</td>
<td>Low risk</td>
<td>71 ± 33 (48-109)</td>
</tr>
<tr>
<td>Crocodilia</td>
<td>Chinese alligator</td>
<td>Alligator sinensis(^b)</td>
<td>Crit Endangered</td>
<td>39 ± 31 (8.9-71)</td>
</tr>
<tr>
<td>Crocodilia</td>
<td>Cuban crocodile</td>
<td>Crocodylus rhombifer(^b)</td>
<td>Crit Endangered</td>
<td>28 ± 32 (5.3-51)</td>
</tr>
<tr>
<td>Crocodilia</td>
<td>Dwarf caiman</td>
<td>Paleosuchus palpebrosus</td>
<td>Low risk</td>
<td>41 ± 18 (29-54)</td>
</tr>
<tr>
<td>Crocodilia</td>
<td>Malayan gharial</td>
<td>Tomistoma schlegelii(^b)</td>
<td>Vulnerable</td>
<td>12 ± 4.6 (6.6-17)</td>
</tr>
<tr>
<td>Crocodilia</td>
<td>Orinoco crocodile</td>
<td>Crocodylus intermedius</td>
<td>Crit Endangered</td>
<td>22 ± 8.0 (13-27)</td>
</tr>
<tr>
<td>Crocodilia</td>
<td>Slender-snout croc</td>
<td>Meccistops cataphractus(^b)</td>
<td>Crit Endangered</td>
<td>44 ± 33 (26-67)</td>
</tr>
<tr>
<td>Primates</td>
<td>Geoffroy's marmoset</td>
<td>Callithrix geoffroyi(^b)</td>
<td>Low risk</td>
<td>19 ± 4.7 (14-24)</td>
</tr>
<tr>
<td>Primates</td>
<td>Goeldi's monkey</td>
<td>Callimico goeldii(^b)</td>
<td>Vulnerable</td>
<td>22 ± 7.5 (13-27)</td>
</tr>
<tr>
<td>Primates</td>
<td>Cotton-top tamarin</td>
<td>Leontopithecus rosalia(^b)</td>
<td>Endangered</td>
<td>4.6 ± 0.8 (3.7-5.0)</td>
</tr>
<tr>
<td>Primates</td>
<td>Pygmy marmoset</td>
<td>Cebeuila pygmaea(^b)</td>
<td>Low risk</td>
<td>1.8 ± 0.8 (2.0-2.3)</td>
</tr>
<tr>
<td>Bucerotiformes</td>
<td>Red-knobbed hornbill</td>
<td>Aceros cassidix</td>
<td>Vulnerable</td>
<td>53 ± 52 (6.3-110)</td>
</tr>
<tr>
<td>Accipitriformes</td>
<td>Cape Griffon vulture</td>
<td>Gyps coprotheres(^b)</td>
<td>Vulnerable</td>
<td>7.5 ± 7.0 (2.9-16)</td>
</tr>
<tr>
<td>Piciformes</td>
<td>Toco toucan</td>
<td>Ramphastos toco(^a)</td>
<td>Low risk</td>
<td>4.5 ± 1.9 (2.6-6.5)</td>
</tr>
<tr>
<td>Testudines</td>
<td>Galapagos tortoise</td>
<td>Chelonoidis nigra(^b)</td>
<td>Vulnerable</td>
<td>20 ± 19 (4.8-41)</td>
</tr>
<tr>
<td>Rodentia</td>
<td>Porcupine</td>
<td>Coendou prehensilis(^b)</td>
<td>Low risk</td>
<td>26 ± 6.4 (19-31)</td>
</tr>
</tbody>
</table>

\(^a\) IUCN is International Union for Conservation of Nature
\(^b\) Enrolled in AZA Species Survival Plan® (SSP) Program

**Fig. 3.** The As concentrations in egg shells and contents from 9 zoo crocodilians compared to reference values (Ogden et al., 1974; Xu et al., 2006; Booyens, 2011). The values were expressed as mean plus one standard deviation with n = 3–10 and mean with n = 2. Mugger crocodiles, Chinese alligators, and Saltwater crocodiles are endangered species.
manufacturing plants and coal-powered electricity generating plants (Liu, 2013). Booyens (2011) reported averaged As concentration of 100 μg/kg in eggshells and 31 μg/kg As in egg contents from 28 eggs of Nile crocodiles (Crocodylus niloticus). Mean As concentrations in egg shells and contents from crocodilians at this zoo (94 and 272 μg/kg) were below those reported by Xu et al. (2006) for captive Chinese alligators (474 μg/kg) but those in egg contents were more than 9-fold higher than those reported in Nile crocodiles (31 μg/kg). Arsenic in both egg shells and contents can be mobilized and taken up by the developing embryo during incubation, which is most sensitive to As exposure, leading to developmental defects (Marco et al., 2004).

3.3. As concentrations in porcupine quills, bird feathers, and marmoset hair

Quills, feathers and hair can also serve as potential non-invasive biomarkers of contaminant exposure in animals (Yanez et al., 2005; Smith et al., 2008a,b). The soil As concentrations in the 20 m² porcupine enclosure ranged from 19 to 31 mg/kg, averaging 26 mg/kg (Table 2). The porcupines at this zoo spend considerable time inside their nesting box made from CCA-wood. Similar to mammals, it is assumed that As would accumulate in the keratin-rich quills over time. Porcupines have an estimated 30,000 quills of varying length, which are regularly shed, with regrowth occurring at a rate of 0.5 mm/d, so we compared As concentrations in those of <6 cm and ≥6 cm. Porcupine quill As concentrations were 0.38–0.91 mg/kg, with quills ≥6 cm in length having 3 times more As than longer quills, indicating possible dilution with growth, which has been noted in bird feathers (Fig. 4; Bortolotti, 2010).

There is no previous study on As exposures in porcupines or data on expected values in healthy animals. D’Have et al. (2006) analyzed As concentrations in keratin-rich hair and feathers from wild European hedgehogs (Erinaceus europaeus) to evaluate correlations between As concentrations in the biomarkers and concentrations in various tissues. They found that As was preferentially accumulated in the spines, averaging 1.24 mg/kg (Fig. 4). Although the porcupines in this study were potentially exposed to As-contaminated soil and dislodgeable As residues in their CCA-wood enclosure, As concentrations in the quills were lower than those found in hedgehog spines. Of course, they are different species so the data may not be comparable. It may also be that wild hedgehogs are exposed to higher levels of As than the porcupines from ingestion of soil-dwelling invertebrates. In addition, it is possible that the frequent loss of quills may have served as a means of As detoxification for the porcupines, leading to lower As concentrations in quills, as hedgehogs do not normally shed spines (Fig. 4).

Besides porcupines, As concentrations were also determined in the feathers from 4 diverse species of birds. The Crested fireback (Lophura ignita) and Cabot’s tragopan (T. caboti) are ground-dwelling omnivores, the Cape Grifon vulture (Gyps coprotheres) is a carnivore and nests upon the ground (Fig. 1D) and the Red-knobbed hornbill (Aceros cassidix) is an omnivore that perches in tree branches. The traditional use of feathers for biomonitoring contaminant exposures has led to an extensive database on As concentrations in some species. However, due to differences in experimental design and species studied, results are not easily compared (Bortolotti, 2010). In this study, As concentrations in the feathers varied significantly among species, averaging 3.1, 2.8, 1.5, and 1.2 mg/kg in Red-knobbed hornbill, Cape Grifon vulture, Cabot’s tragopan and Crested fireback, respectively (Fig. 5). These numbers were within the range of As concentrations in the feathers of aquatic Greylag goose, Red knot and Grey plover species (Lucia et al., 2010), as well as terrestrial Barn owls (Fig. 5) (Dauwe et al., 2003). Lebedeva (1997) reported that As concentrations in the tissues from aquatic and predatory bird species could accumulate higher As concentrations because of their high-As diet. Besides incidental ingestion of contaminated soils while preening or eating, birds consuming gamebird food may experience elevated chronic exposure to As (Table 1). The ingestion of soil invertebrates is another As exposure pathway, which could be particularly significant in small, ground-dwelling birds such as the Crested fireback and Cabot’s tragopan.

Analysis of 9 hair samples from 2 adult and a 2-year-old juvenile Geoffroy’s marmoset living in one enclosure found 6.54 ± 0.31 mg/kg As, with no significant difference between animals (data not shown). Ambeskovic et al. (2013) reported As concentrations of 0.16–0.26 mg/kg in hair from 24 captive marmosets, which was age-dependent. Lee et al. (2012) reported As concentrations of 0.65 mg/kg in the hair of rhesus monkeys (Macaca mulatta) from China. The As concentrations in marmosets’ hair in this study were 30-fold higher than those reported by Ambeskovic et al. (2013) and 10-fold higher than in the rhesus monkeys. In addition to As exposure from the soil and wood surfaces, the juvenile marmoset could have experienced transplacental fetal exposure from his mother. Vahter (1994) found that marmosets acutely exposed to 0.4 mg As/kg body weight accumulated the highest concentration in the liver (0.91 mg/kg), representing >10% of the total administered dose, with increased amounts also being found in keratinized tissues. One marmoset (7 year old) in this study was euthanized in 2014 due to complications from chronic liver disease, which has been associated with As exposure in other animals (ATSDR, 2007). The high As concentrations observed in the hair of marmosets, as a vulnerable sub-group within the community of zoo animals, indicate that they may have suffered from As exposure, however, further study is needed.

4. Environmental implication

Recognition that soil around CCA-wood structures becomes contaminated with As from normal weathering was well-documented over a decade ago (Townsend et al., 2003; Stilwell et al., 2003). Concern
about potential health effects from ingestion of dislodgeable As residue led the CPSC, USEPA and the US Department of Agriculture (USDA) to issue guidance that people wash hands after contact with CCA-wood and keep pets from playing in soil near CCA-wood structures (CPSC, 2011). Although risks were identified years ago, CCA-wood is still commonly used in agricultural and commercial settings where animals are in regular contact with dislodgeable As residues and As-contaminated soil resulting from CCA-wood enclosures. Captive animals also commonly chew on wood, leading to concern about using CCA-wood in enclosure construction (Schweinfurth and Chou, 2011).

USEPA’s Eco-SSLS are estimates of maximum soil concentrations below which no adverse health effect is anticipated in most chronically exposed species. Due to a lack of relevant toxicological data, As Eco-SSLS for amphibians, reptiles and soil invertebrates are unavailable. The soils in zoo enclosures showed elevated As levels, with some enclosures having concentrations higher than Eco-SSLS for mammals and avian species (USEPA, 2005a,b). In this study, soils in 4 avian enclosures were sampled, with the Hornbill enclosure having soil As at 6.9–110 mg/kg, averaging 53 mg/kg (Table 2), which was higher than the Eco-SSL of 43 mg/kg. Even though the Hornbills at this zoo reproduce successfully, of the 4 species sampled, the As concentrations in Hornbill’s feathers were the highest at 3.2 mg/kg. In the absence of an As Eco-SSL for reptiles, crocodilians living in enclosures with elevated soil As concentrations, including critically endangered Chinese alligators (A. sinensis), Orinoco crocodiles (Crocodylus intermedius), Cuban crocodiles (Crocodylus rhombifer) and Slender-snout crocodiles (Mecistops cataphractus), may benefit from further investigation into potential impacts on their reproductive capability.

Our ability to accurately estimate As exposure doses in these animals and predict whether adverse health effects were likely hindered by a lack of species-specific exposure factors, but As in some biomarkers, including feathers, eggs, and hair, were higher than those in previous studies. In particular, As concentrations in hair from marmosets at this zoo were 30-fold higher than levels in marmosets living in a laboratory setting, suggesting potential As toxicity. Even though the soil As concentrations in all primate enclosures were below the Eco-SSL for mammals, marmosets’ inability to metabolize arsenic makes them especially vulnerable to chronic exposures to As-contaminated soil and dislodgeable As residues.

The variety of 25 animal species in this study exhibited a broad range of physical and behavioral characteristics that directly influence their exposure to environmental contaminants. The use of Eco-SSLS as a general screening tool does not adequately account for species-specific and site-specific variables that can influence As exposures. In the zoo setting, animals can have long-term, daily exposure to contaminants, which is of a particular concern for threatened and endangered species whose reproductive success is especially important. To minimize potential As exposure to animals, zoos could replace CCA-wood with non-toxic alternatives in enclosure construction.

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References


