ORGANOCHLORINE PESTICIDES AND MERCURY IN COTTONMOUTHS (AGKISTRODON PISCIVORUS) FROM NORTHEASTERN TEXAS, USA

THOMAS R. RAINWATER,* KEVIN D. REYNOLDS, JACLYN E. CAÑAS, GEORGE P. COBB, TODD A. ANDERSON, SCOTT T. MCMURRY, and PHILIP N. SMITH

The Institute of Environmental and Human Health, Department of Environmental Toxicology, Texas Tech University, Box 41163, Lubbock, Texas 79409-1163, USA

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Abstract—Despite their ecological importance and global decline, snakes remain poorly studied in ecotoxicology. In this study, we examined organochlorine (OC) pesticide and mercury accumulation in cottonmouths (Agkistrodon piscivorus) living on a contaminated site in northeastern Texas (USA). Mercury and p,p'-dichlorodiphenyl dichloroethylene (p,p'-DDE) were detected in all snakes examined. Other OCs, including p,p'-dichlorodiphenyltrichloroethane (p,p'-DDT), methoxychlor, aldrin, and heptachlor, also were detected, but less frequently. Concentrations of p,p'-DDE were higher in fat than in liver, while mercury concentrations were highest in liver, followed by kidney and tail clips. One animal contained the highest mercury concentration yet reported for a snake (8,610 ng/g). Mercury concentrations in liver and kidney were higher in males than females, while no intersex differences in p,p'-DDE concentrations were observed. Concentrations of p,p'-DDE in fat were correlated positively with body size in male cottonmouths but not females, suggesting a slower rate of accumulation in females. Body size strongly predicted mercury concentrations in liver, kidney, and tail clips of both sexes. Tail clips were strong predictors of mercury in liver and kidney in males but not females, suggesting possible sex-dependent differences in mercury toxicokinetics. Both long-term field studies and controlled laboratory investigations are needed to adequately assess the response of cottonmouths to chronic contaminant exposure.

Keywords—Mercury  Organochlorine pesticides  Cottonmouths  Snakes  Texas

INTRODUCTION

Environmental contamination has been identified as one of six major threats to reptile populations worldwide and may be a significant contributing factor to their current global decline [1]. However, despite their decline and integral role in natural ecosystems [1], reptiles largely have been ignored in ecotoxicological research and are the least studied of all vertebrates with regard to environmental contaminants [2–6]. To understand adequately how pollution affects an ecosystem, all components of the ecosystem must be examined [5,6]. Multiple reports recently have encouraged an increased emphasis on reptilian ecotoxicology by underscoring the paucity of basic toxicological information on reptiles and highlighting several characteristics that make these animals excellent study organisms for ecotoxicological investigations [5–10]. However, ecological risk assessments and other contaminant-related ecosystem monitoring programs still rarely include reptiles [5,6,10].

Of the relatively few studies that have examined exposure and response of reptiles to environmental pollutants, most have focused on crocodilians and turtles. Snakes and lizards have received comparatively little attention and remain grossly understudied [5–9]. Snakes occupy multiple ecological niches and are vital to the structure and function of various aquatic and terrestrial ecosystems [11]. Over the last three decades, multiple studies have demonstrated the potential utility of snakes as indicators of environmental contamination [2,3,6–9], but only recently have studies examined the effects of certain xenobiotics on snakes in controlled experiments [12,13]. These recent studies are encouraging, and highlight the importance of both laboratory investigations and continued field studies in assessing contaminant accumulation in snakes and potential impacts on wild populations.

Cottonmouths (Agkistrodon piscivorus) are heavy-bodied, semiaquatic pit vipers found throughout the southeastern United States [14] and have been one of the most-frequently examined species regarding contaminant accumulation in snakes [15–20]. Cottonmouths are useful indicators of environmental contamination due to their high trophic status, long life span, and small home ranges [14,21,22] that make them highly susceptible to persistent pollutant exposure and accumulation. The objective of this study was to examine accumulation of organochlorine (OC) pesticides and mercury in cottonmouths living on a contaminated site in northeastern Texas (USA).

MATERIALS AND METHODS

Study area and sample collection

This study was conducted on the Longhorn Army Ammunition Plant (LHAAP), a U.S. Environmental Protection Agency National Priorities List Superfund site located in Harrison County, northeastern Texas (Fig. 1) [23]. The LHAAP is located within the Caddo Lake watershed, which extends into Harrison and Marion Counties in Texas and Caddo Parish in northwest Louisiana (USA). The site encompasses approximately 3,200 ha of mixed hardwood bottomlands and upland pine forests and is drained by four principal lotic systems: Goose Prairie Creek, Central Creek, Harrison Bayou, and Saunders Branch (Fig. 1). Site contamination, involving over 200 chemicals, occurred as a result of various military activities from 1942 to 1997 [24]. Organochlorine pesticides, par-
particularly DDT, likely were used on the site, whereas mercury was not [24]. Rather, the presence of mercury on the LHAAP is likely the result of atmospheric deposition from nonpoint-source emissions [24].

Few data are available concerning OC and mercury contamination of the LHAAP. The OCs $p,p'$-dichlorodiphenyldichloroethylene ($p,p'$-DDE), $p,p'$-dichlorodiphenyldichloroethane ($p,p'$-DDD), aldrin, and dieldrin have been detected in soils from the site, but a contaminant concentration was reported only for aldrin (2.540 ng/g ppb) [24]. Mercury has been detected in groundwater on the LHAAP (3.3 μg/L), but concentrations in soils and sediments have not been examined [24]. However, sediment samples collected from two sites on Caddo Lake, Texas (USA), indicating the three drainages from which cottonmouths were collected for organochlorine pesticide and mercury analyses: Goose Prairie Creek, Central Creek, and Harrison Bayou. Black dots indicate approximate capture locations of the 19 snakes collected.

In April 2002, 19 cottonmouths (Goose Prairie Creek, $n = 8$; Central Creek, $n = 5$; Harrison Bayou, $n = 6$) were collected from the site using Pillstrom tongs (Fort Smith, AR, USA) and placed in holding bags for no longer than 2 h until transportation to a field laboratory. Snakes were then sacrificed, and snout-vent length (SVL), body mass, and sex were determined postmortem (Table 2). Animals were then frozen at $-20^\circ$C and later shipped on ice to Texas Tech University (Lubbock, TX, USA) where they were stored at $-80^\circ$C. Snakes were later necropsied, and tissues were collected for OC and mercury analyses. Livers were divided into four approximately equal aliquots; one was analyzed for OCs, one was analyzed for mercury, and the remaining two were saved for separate studies. Left kidneys were collected for mercury analysis. All visible fat bodies were removed, and the entire sample from each snake was analyzed for OCs. Two snakes contained insufficient fat for analysis. Last, a tail clip consisting of the distal 2 cm of the tail was collected from each snake using surgical scissors for mercury analysis. All samples were labeled and stored at $-80^\circ$C.

**Sample analyses**

**Organochlorines.** Cottonmouth fat and liver samples were analyzed for OC pesticides using standard methods with modifications as necessary to improve quantitation [25]. Fat samples (mean ± standard error) = 2.97 ± 0.44 g; range = 0.80–6.20 g; $n = 17$) and liver aliquots (mean = 1.74 ± 0.16 g; range = 0.82–3.54 g; $n = 19$) were weighed and mixed with 10 g anhydrous sodium sulfate, transferred to 33-ml extraction cells, and fortified with two internal standards, tetrachlorom-xylene and decachlorobiphenyl. Samples were then extracted with methylene chloride using a Dionex 200 accelerated solvent extractor (Sunnyvale, CA, USA) under the following parameters: Pressure = 1,500 psi, temperature = 100°C, extract-

<table>
<thead>
<tr>
<th>Drainage</th>
<th>$p,p'$-DDE Mean</th>
<th>95% CI</th>
<th>Mercury Mean</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goose Prairie Creek</td>
<td>2.96</td>
<td>1.79–5.88</td>
<td>559.59</td>
<td>421.88–741.55</td>
</tr>
<tr>
<td>Central Creek, Harrison Bayou</td>
<td>4.03</td>
<td>2.42–6.73</td>
<td>143.21</td>
<td>132.34–154.9</td>
</tr>
</tbody>
</table>

Table 1. Geometric means and 95% confidence intervals (CI) of $p,p'$-dichlorodiphenyldichloroethylene ($p,p'$-DDE) and mercury concentrations (ng/g) detected in two sediment cores collected from Caddo Lake, Texas (USA), one near the mouth of Goose Prairie Creek and one near the mouths of Central Creek and Harrison Bayou. Means were calculated using contaminant concentrations for individual core subsamples (Goose Prairie Creek: $n = 9$; Central Creek, Harrison Bayou: $n = 8$) reported by Wilson [24].
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Table 2. Sex, number, and body size of cottonmouths collected from three drainages on the Longhorn Army Ammunition Plant, Texas (USA), for examination of organochlorine pesticide and mercury accumulation.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Drainage</th>
<th>n</th>
<th>Mean a (± SE)</th>
<th>Range</th>
<th>Mean a (± SE)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Central Creek</td>
<td>4</td>
<td>77.0 ± 9.0</td>
<td>53.8–96.3</td>
<td>579.61 ± 170.65</td>
<td>168.91–922.66</td>
</tr>
<tr>
<td></td>
<td>Goose Prairie Creek</td>
<td>4</td>
<td>72.5 ± 9.8</td>
<td>47.5–92.5</td>
<td>515.11 ± 183.63</td>
<td>123.95–936.04</td>
</tr>
<tr>
<td></td>
<td>Harrison Bayou</td>
<td>3</td>
<td>63.1 ± 6.2</td>
<td>52.4–73.8</td>
<td>292.47 ± 74.96</td>
<td>171.54–429.67</td>
</tr>
<tr>
<td>Females</td>
<td>Central Creek</td>
<td>1</td>
<td>55.9</td>
<td>NA</td>
<td>153.65</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Goose Prairie Creek</td>
<td>4</td>
<td>60.5 ± 3.0</td>
<td>54.1–67.2</td>
<td>236.26 ± 23.22</td>
<td>192.51–290.15</td>
</tr>
<tr>
<td></td>
<td>Harrison Bayou</td>
<td>3</td>
<td>53.8 ± 5.7</td>
<td>42.6–61.1</td>
<td>201.12 ± 49.50</td>
<td>110.35–280.73</td>
</tr>
</tbody>
</table>

a Arithmetic mean.

RESULTS

Five OC pesticides were detected in cottonmouth fat and liver samples collected at the study site: p, p'-DDE, p, p'-DDT, methoxychlor, aldrin, and heptachlor. The predominant OC found was p, p'-DDE, occurring in 100% of fat samples (n = 17) and 80% of liver samples (n = 19) examined. Fat samples also were found to contain p, p'-DDT (mean = 6 ng/g, 95% confidence interval = 2–22 ng/g; occurrence = 29%) and methoxychlor (mean = 2 ng/g; 95% confidence interval = 1–4 ng/g; occurrence = 12%), while liver samples also contained

OC pesticides

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p,p'\text{-DDT} \text{ (mean = 2 ng/g; 95\% confidence interval = 1–4 ng/g; occurrence = 16\%)}$, methoxychlor (2 ng/g; 95\% confidence interval = 1–2 ng/g; occurrence = 0.1\%), aldrin (1 ng/g; 95\% confidence interval = 1–2 ng/g; occurrence = 0.1\%), and heptachlor (1 ng/g; 95\% confidence interval = 1–2 ng/g; occurrence = 0.1\%). The high frequency of occurrence of p,p'\text{-DDE residues in fat and liver permitted intersex and inter-drainage (males only) comparisons of p,p'\text{-DDE concentrations and provided adequate sample sizes to examine relationships between cottonmouth body size and contaminant loads in these tissues. Overall, the mean p,p'\text{-DDE concentration in cottonmouth fat was significantly higher than in liver (}F = 63.29; degrees of freedom [df] = 1; p < 0.0001; Table 3). Comparison of mean p,p'\text{-DDE concentrations in fat and liver using SVL as a covariate indicated no differences between sexes and among the three drainages.}

Snout-vent length and body mass both were significantly correlated to p,p'\text{-DDE concentrations in cottonmouth fat, but no significant relationships existed between these two measures of body size and p,p'\text{-DDE in snake liver (Table 4). Specifically, 39\% of the variation in p,p'\text{-DDE in fat was explained by SVL, while 33\% was explained by body mass. When p,p'\text{-DDE concentrations in fat were examined by sex, SVL and body mass were both significantly correlated to p,p'\text{-DDE in males but not in females (Table 4).}}

**Mercury**

Mercury was detected in 100\% of tissue samples collected from cottonmouths in this study. Overall, the mean mercury concentration in snake livers was significantly higher than in kidneys and tail clips (}F = 10.68; df = 2; p < 0.0001), though concentrations in the latter two tissues were not significantly different (Table 5). In addition, mercury concentrations in liver (}F = 10.93; df = 1; p = 0.0048) and kidney (}F = 11.29; df = 1; p = 0.0043) were significantly higher in males than females, though concentrations in tail clips did not differ between sexes. Males were significantly larger than females (}F = 4.73; df = 1; p = 0.0441), but intersex differences in mercury concentrations were size-dependent for kidney mercury only (}F = 6.01; df = 1; p = 0.0269). No differences in tissue mercury concentrations in males were observed among the three drainages.

Overall, both measures of snake body size were significantly correlated to mercury concentrations in all three tissues examined (Table 6). Specifically, SVL explained 69, 64, and 67\% of the variation in mercury in liver, kidney, and tail clips, respectively. Likewise, body mass explained 61, 59, and 69\% of the variation in mercury in liver, kidney, and tail clips, respectively. When mercury concentrations in these tissues were examined separately by sex, SVL, and body mass were correlated significantly to mercury in each tissue for both males and females (Table 6).

Cottonmouth tail clips were effective in predicting mercury concentrations in both liver and kidney when tissues for males and females were examined together (Figs. 2 and 3). However, when sexes were analyzed separately, mercury in tail clips strongly predicted mercury in livers and kidneys of males but were poor predictors of mercury in female tissues (Figs. 2 and 3).

**DISCUSSION**

Results of this study indicate that cottonmouths living on LHAAP are exposed to and accumulate multiple OC pesticides and mercury. Based on the snake tissues examined, OC and mercury contamination generally appears to be homogeneous among the three drainages sampled. However, further investigations are needed to examine the relationships between contaminant concentrations in cottonmouths and those in soil and sediment from their respective collection sites.

Organochlorine pesticide concentrations did not differ between male and female snakes, and overall, cottonmouths appeared to accumulate OCs as they increased in size. However,
when sexes were examined separately, males appeared to accumulate $p,p'\text{-DDE}$ in fat with increasing body size, while females did not. Interestingly, in the only other study to have examined sex- and size-related differences in contaminant concentrations in snakes, the same pattern was observed. Santos et al. [27] determined that concentrations of DDTs (primarily $p,p'\text{-DDE}$) in carcass lipids of wild vipersnakes (*Natrix maura*) increased significantly with carcass mass in adult males but not adult females. The authors speculated this to be an indication of a slower rate of OC accumulation in female snakes, likely as a result of periodic reductions in OC body burdens through maternal transfer [27]. This is likely the case in the present study as well. Although reproductive condition specifically was not examined, six of the seven (86%) female cottonmouths for which OCs in fat were examined were of reproductive size ($\geq 45$ cm SVL [21]). Fleet et al. [15] found high OC (including DDE) concentrations (396 $\mu$g/g [ppm]) in fat bodies of cottonmouth embryos from southeast Texas, and these concentrations were similar to those detected in the fat of the maternal female (432 $\mu$g/g). This finding confirms that maternal transfer of OCs occurs in cottonmouths and suggests that in snakes, as in other reptiles [28], mobilization of OC-laden fat during vitellogenesis and subsequent incorporation into developing follicles serves as a mechanism by which adult females deurate lipophilic contaminants [16,27]. Although our analyses may be hampered by a small sample size, periodic reductions in $p,p'\text{-DDE}$ concentrations through maternal transfer at least partially may explain a slower rate of $p,p'\text{-DDE}$ accumulation in adult females.

It should be noted that OC concentrations detected in cottonmouths in this study are a reflection of both contaminant accumulation and body condition of individuals at the time of sampling [29], and sample collection in a different year or time of year might yield different results. Natural variability and fluctuations in cottonmouth growth rates [22] influence age of sexual maturity and ultimately fat body lipid dynamics [30,31], which in turn can influence OC concentrations recovered in fat samples. For example, recovery of OC pesticides in fat of a breeding adult female living in a homogeneously contaminated habitat would be expected to be greater at times when the snake is storing fat (e.g., in the fall just before brumation [31]) rather than during or shortly following periods in which fat is mobilized to meet energy demands (e.g., brumation, pregnancy [31]). The snakes in this study were collected in mid-April, just following spring emergence [22], and age (mature or immature) and reproductive status (breeding or nonbreeding) of females likely influenced their rate of lipid depletion during brumation. It is possible that despite deputation of OCs in some individuals as a result of maternal transfer, accumulation of $p,p'\text{-DDE}$ in female fat may in fact increase with body size as it appears to in males, but this relationship may be masked by temporal variability in lipid reserves and subsequent variability in $p,p'\text{-DDE}$ concentrations following lipid mobilization. Long-term studies in which OC concentrations are determined in both fat and blood samples collected from the same individuals multiple times in a year are needed to examine adequately fluctuations in OC concentrations with respect to annual and seasonal variation in fat body lipid stores [30,31].

Few studies specifically have examined the effects of OC exposure on snakes. However, direct applications of OCs to snakes, both intentionally and accidentally, have demonstrated that these reptiles are highly sensitive to acute exposure [32–34]. Interestingly, of the few reports available on acute OC exposure in snakes, three involve cottonmouths. In a 1946 field study to test the effects of DDT on ticks and nontarget wildlife, cottonmouths commonly were observed on research plots experimentally treated with DDT [35]. Following chemical application, convulsions and paralysis were observed in other snake species on treated plots, but cottonmouths exhibited no overt signs of intoxication [35]. Exposure to DDT was not confirmed analytically in the snakes, so it is unknown whether

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### Table 5. Geometric means of mercury concentrations (ng/g wet mass) detected in cottonmouth tissues from the Longhorn Army Ammunition Plant, Texas (USA). Numbers in parentheses indicate the number of samples analyzed in a group. 95% confidence intervals appear below means.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Combined</th>
<th>Central Creek</th>
<th>Goose Prairie Creek</th>
<th>Harrison Bayou</th>
<th>Sample group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>739.4 (19)</td>
<td>1,187.4 (5)</td>
<td>859.4 (8)</td>
<td>407.6 (6)</td>
<td>Males Females All</td>
</tr>
<tr>
<td>Kidney</td>
<td>211.4 (19)</td>
<td>351.9 (5)</td>
<td>240.3 (8)</td>
<td>116.6 (6)</td>
<td>Sex</td>
</tr>
<tr>
<td>Tail clip</td>
<td>163.1 (19)</td>
<td>205.1 (5)</td>
<td>167.2 (8)</td>
<td>130.3 (6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>123.6-215.1</td>
<td>85.3-493.2</td>
<td>105.1-265.9</td>
<td>77.6-218.9</td>
<td></td>
</tr>
</tbody>
</table>

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### Table 6. Results of linear regression analysis of tissue mercury concentrations as a function of snout-vent length (SVL, cm) and body mass (g) in cottonmouths from the Longhorn Army Ammunition Plant, Texas (USA). Sample sizes: Males, $n = 11$; females, $n = 8$.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>SVL $R^2$</th>
<th>SVL $p$</th>
<th>Mass $R^2$</th>
<th>Mass $p$</th>
<th>Liver</th>
<th>Kidney</th>
<th>Tail clip</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>All</td>
<td></td>
<td>Males</td>
<td>Females</td>
<td>All</td>
</tr>
<tr>
<td>Liver</td>
<td>0.86</td>
<td>0.70</td>
<td>0.69</td>
<td></td>
<td>0.75</td>
<td>0.79</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>$&lt;0.0001$</td>
<td>0.0093</td>
<td>$&lt;0.0001$</td>
<td></td>
<td>0.0006</td>
<td>0.0031</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Mass</td>
<td>0.812</td>
<td>0.57</td>
<td>0.61</td>
<td></td>
<td>0.77</td>
<td>0.68</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>0.0002</td>
<td>0.0303</td>
<td>0.0001</td>
<td></td>
<td>0.0004</td>
<td>0.0122</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
the presumably unaffected cottonmouths exhibited a greater tolerance to DDT or if they received a lower dose or were not exposed. Similarly, 18 of 21 snakes in a pen sprayed with a 5% DDT-kerosene solution exhibited tremors and paralysis, and died less than a day after application [33]. Although one of the dead snakes included a cottonmouth, the three surviving snakes were also cottonmouths. The author attributed the survival of these individuals to their sheltered position in the pen during chemical application rather than a greater tolerance to DDT [33]. Conversely, two captive juvenile cottonmouths dusted with 10% DDT powder to control mites exhibited convulsions, side-to-side head whipping, tail writhing, and mouth gaping for 3 d following chemical application and eventually died within approximately 1 to 3 weeks [32].

A series of field studies in the 1970s found site-specific differences in OC accumulation and species abundance in snakes living on a contaminated agricultural site (Old River Slough) and reference site (Navasota River) in southeastern Texas [15–17]. In 1971, \( p,p' \)-DDE concentrations in snakes at Old River Slough were over 100-fold higher than in snakes from Navasota River [15]. In addition, egg-laying species were common at Navasota River but essentially absent from Old River Slough.

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**Fig. 2.** Relationship between mercury concentrations in cottonmouth tail clips and liver samples collected from the Longhorn Army Ammunition Plant, Texas (USA).

**Fig. 3.** Relationship between mercury concentrations in cottonmouth tail clips and kidney samples collected from the Longhorn Army Ammunition Plant, Texas (USA).
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River Slough. However, by 1974 and 1975 (following the ban on DDT in 1972), \( p,p' \)-DDE concentrations in snakes from Old River Slough had declined in the three most frequently sampled species, and the abundance of egg-laying species had increased greatly [16]. During these studies, diamondback water snakes (\( Nerodia rhombifer \) (live bearers)) also were common at Navasota River but consistently absent from Old River Slough [15–17]. However, when Old River Slough was surveyed again in the mid-1990s, this species was the most common species encountered [20]. Clark et al. [20] speculated that the absence of diamondback water snakes at Old River Slough in the 1970s was the result of higher OC pesticide concentrations at the site at that time (based on snake fat samples), and the abundance of the species in the mid-1990s reflects the dissipation of OC residues at the site over the ensuing 20 years. Although the observed changes in species abundance at Old River Slough also may have been influenced by other natural or anthropogenic perturbations (e.g., changes in plant composition, flooding, additional chemical inputs), these observations suggest that the effects of \( p,p' \)-DDE and other OCs on snakes may be species-specific, with some species exhibiting high sensitivity.

Concentrations of \( p,p' \)-DDE in cottonmouths sampled in the present study are notably lower than those reported for cottonmouths and other snakes in southeastern Texas [15,16]. Cottonmouths collected from Old River Slough in the 1970s consistently contained the highest mean \( p,p' \)-DDE concentrations of all snakes examined [15,16]. Mean \( p,p' \)-DDE concentrations in cottonmouths collected from the site in 1971 and 1974 through 1975 are 662- and 252-fold higher, respectively, than the mean concentration detected in cottonmouths at the LHAAP [15,16]. Additionally, the mean \( p,p' \)-DDE concentration in nine other snake species collected from Navasota River in 1971 was twofold higher than that found in the LHAAP cottonmouths [15]. These data suggest exposure of cottonmouths to OC pesticides on the LHAAP is relatively low and concentrations detected in cottonmouths sampled in this study may be considered background concentrations.

Though little is known concerning the effects of OC pesticides on snakes, even less is known concerning exposure and response of snakes to mercury [7,10,36]. To our knowledge, only nine studies have reported mercury exposure in wild snakes [7,10,20], and only two reports are available concerning the toxicity of mercury to snakes [36,37]. Wolfe et al. [36] fed methylmercury to garter snakes (\( Thamnophis sirtalis \)) at concentrations up to 280 \( \mu g/g \) and observed no overt signs of toxicity in treated snakes or their young. Conversely, Bazar et al. [37] fed methylmercury to juvenile corn snakes (\( Elaphe guttata \)) at 1.0, 2.5, 6.0, and 12.0 \( \mu g/g \) and observed 100% mortality in the 12.0 \( \mu g/g \) dose group. Although more research clearly is needed, these two studies suggest mercury toxicity in snakes may be both species- and age-dependent.

In the present study, mercury was detected in all tissue samples analyzed, and consistent with reports for other reptiles [7], concentrations were highest in liver. The liver of one cottonmouth (male, SVL = 96.3 cm, mass = 787.8 g; largest snake sampled) contained 8,610 ng/g mercury, the highest mercury concentration yet reported for a snake [7,10,20]. Although data relating tissue mercury concentrations to effects in snakes are unavailable, similar concentrations (1,000–13,700 ng/g) in the liver of water birds (primarily ducks and herons) have been associated with decreased reproductive fitness, alterations in growth and behavior, and mortality [36,38]. Male cottonmouths exhibited higher mercury concentrations than females in both liver and kidney, but the intersex difference in liver mercury was not size-dependent. Whether this is an artifact of small sample size or reflects a female-specific mode of mercury depuration (e.g., maternal transfer) is unknown. However, unlike \( p,p' \)-DDE, mercury in all tissues examined increased with body size in females as well as males. This supports previous studies suggesting that, although maternal transfer of metals (including mercury) occurs in reptiles [7,39], it does not appear to be a significant route of elimination for adult females [7]. Burger [40] found mercury concentrations in the skin of hatching pine snakes (\( Pituophis melanoleucus \)) to be almost twofold higher than concentrations in the body and speculated that ecdysis (shedding of skin) may be a major route of mercury elimination in both sexes. Recent laboratory investigations have examined elimination of other trace metals in shed skins [41], but the extent of mercury depuration in snakes through ecdysis remains unknown.

Recent evidence of declining reptile populations [1] has underscored the need for nondestructive sampling techniques for assessing contaminant accumulation in these animals. Tail clips recently have shown promise as nondestructive tissue samples for determining trace metal contamination in snakes [41,42]. In the present study, mercury concentrations in tail clips were correlated highly with those in both liver (\( R^2 = 0.91 \)) and kidney (\( R^2 = 0.88 \)) in male cottonmouths, but were poorly correlated with mercury in female tissues (liver: \( R^2 = 0.21 \); kidney: \( R^2 = 0.24 \); Figs. 2 and 3). This suggests a possible sex-dependent difference in mercury toxicokinetics such that in males, mercury accumulation in tail clips is proportional to that in liver and kidney, while in females it is not. The reason for such a difference is unclear and again may be an artifact of small sample size. Based on this limited data set, however, it appears tail clips may be useful as nondestructive samples for evaluating mercury accumulation in male cottonmouths.

This study demonstrates that cottonmouths living on the LHAAP accumulate OC pesticides and mercury. To examine more closely the utility of these snakes as indicators of site contamination, future studies should attempt to correlate contaminant concentrations in cottonmouth tissues with concentrations in soils and sediments within the home range of sampled individuals. Although OCs and mercury have been detected in cottonmouths from multiple areas in the southeastern United States, these snakes appear to remain abundant locally, possibly suggesting a tolerance to low-level contaminant exposures. Stafford et al. [17] found that of five snake species sampled from southeast Texas, cottonmouths had the highest microsomal oxidase activity and the lowest DDE concentrations in fat, and attributed the snake’s abundance in the area to its ability to degrade enzymatically OC pesticides. In addition, in one of the few controlled studies to examine the effects of xenobiotics on snakes, Hopkins et al. [13] recently reported that exposure to high concentrations of coal combustion wastes had no adverse effects on multiple biological endpoints tested in banded water snakes (\( Nerodia fasciata \)). However, the authors emphasized that reproductive endpoints have not yet been examined and may serve as more sensitive indicators of contaminant effects on snakes and other reptiles [13]. At the time of snake collection in the present study, cottonmouths appeared to be abundant on the site, suggesting a stable population with successful and sustained reproduction (several juveniles and yearlings were observed but not col-
lected). However, following a 14-year population study of cottonmouths in northeastern Texas, Ford [22] cautioned that short-term observations of this species may give a false perception of abundance. Thus, long-term studies are clearly needed to assess adequately the response of cottonmouth and other snake populations to chronic contaminant exposure. Controlled laboratory studies also are needed to more clearly define the toxicodynamics and toxicokinetics of OC pesticides and metals in snakes and subsequent effects on snake reproduction.

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