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Renal pathologies in giant toads (*Bufo marinus*) vary with land use

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ABSTRACT

A variety of human land uses involve the release of toxins into the environment. Wildlife live alongside humans across this array of land uses and thus, are exposed to varying chemical milieus. Kidneys are the principle excretory organs for vertebrates and excessive or chronic exposure to exogenous toxins can lead to renal pathology and renal failure. Although studies have linked chemical exposure to specific renal diseases across diverse taxa, none compare renal lesions occurring in wildlife living in different types of human-modified landscapes. We identify lesions characteristic of renal stress, including toxin exposure, in 82 giant toad (*Bufo marinus*) males living in habitats ranging from suburban to agricultural. In a previous study [McCoy K.A., Bortnick L.J., Campbell C.M., Hamlin H.J., Guillette L.J., Jr., St. Mary C.M. Agriculture Alters Gonadal Form and Function in Bufo marinus. Environ Health Persp; in press.], these individuals were examined for gonadal abnormalities, which were significantly and positively associated with percentage of agriculture at the collection site. Thus, we hypothesized the same association for renal abnormalities. We scored gross anatomical abnormalities and used light microscopy to identify tubular and interstitial lesions that have been associated with toxicant exposure in other organisms, including humans. Renal lesions indicative of tubular disease were observed at one suburban and two agricultural sites, whereas interstitial lesions were most severe at one agricultural site. Although there was no relationship between frequency of renal disease and proportion of agriculture in the collection vicinity, the renal lesions we identify are consistent with toxin exposure and are similar to those found in human drug abusers and patients suffering medication-induced nephropathy. This is the first study to describe renal lesions in a wild amphibian species and investigate the distribution of renal lesions across human altered landscapes. Indentifying the chemicals inducing renal lesions across these landscapes, their toxicological mechanisms, and their implications on wildlife health will help us devise strategies to mitigate the impacts of toxins on humans and animals living in human-modified environments.

1. Introduction

Many pollutants are broadly distributed due to their wide-scale use, run off, and fallout in precipitation (Thurman and Cromwell, 2000; Sparling et al., 2001; Lie et al., 2003; Davidson and Knapp, 2007). Even areas once believed to be pristine are now known to be affected by environmental pollutants (e.g. (Thurman and Cromwell, 2000; Lie et al., 2003). As a result,

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environmental contaminants are a global health concern for humans and wildlife (Colborn and Clement, 1992; Colborn et al., 1993; Guillette et al., 1995; Vos et al., 2000; Guillette and Gunderson, 2001; Edwards et al., 2006; Norris and Carr, 2006).

Urban and suburban areas are typically polluted with polyaromatic hydrocarbons (PAHs) which are associated with road runoff (Maltby et al., 1995; Crosbie and Chow-Fraser, 1999), whereas agricultural areas are more often polluted with mixtures of pesticides used to control weed, insect, and rodent populations (Kreuger, 1998; Crosbie and Chow-Fraser, 1999). Importantly, these associations are so well documented that PAH and pesticide (i.e. metolachlor) concentrations have been used to estimate the proportion of urbanized and agricultural land, respectively, among different watersheds (Standley et al., 2000). Clearly, both urban and agricultural habitats are polluted with chemicals that can potentially induce renal pathologies. Indeed, studies have linked exposure to environmental pollutants with renal disease; however, none have compared renal pathologies occurring in animals living in different types of human-dominated landscapes.

Kidneys are the principle excretory organs for many vertebrates, first filtering toxins from the blood, then concentrating and excreting them, so excessive or chronic exposure to exogenous toxins can lead to renal lesions. Many endogenous metabolic toxins and exogenous contaminants are removed from the body via active uptake across the tubules which make them a target for toxin-induced pathologies (Sweet, 2005). For example, tubular necrosis occurs in humans that have ingested large quantities of medications or pesticides, or (chronically) in wildlife that live in polluted habitats (Chan et al., 1998; Ortiz et al., 2003; Rankin, 2004). However, it can also be caused by ischemia (restricted blood and oxygen supply). This necrosis leads to loss of tubular brush boarders, epithelial cell thinning, vacuolization, and tubular cell degeneration (Kern, 1999; Ortiz et al., 2003).

The tissue around tubules (interstitium) is also an important target for toxin-induced disease such as interstitial nephritis. Chronic interstitial nephritis can occur after long term exposure to pharmaceuticals or environmental toxins, and is further associated with interstitial fibrosis, an irreversible renal injury characterized by the accumulation of matrix proteins in the interstitium (Eddy, 1996; Kern, 1999; Ortiz et al., 2003).

Renal lesions have been associated with pollutant exposure in a diverse array of wildlife. For example, herring gulls (*Larus argentatus*), from regions of the Great Lakes (USA) that are contaminated with planar halogenated aromatic hydrocarbons (e.g., used in solvents and some pesticides), have been diagnosed with interstitial nephritis (Fox et al., 2007). Several species of fish were diagnosed with tubular necrosis, and desquamation and vacuolization of tubular epithelial cells after an accidental discharge of lindane (an organochlorine insecticide) into the Barbate River (Spain) (Ortiz et al., 2003). Polar bears sampled from eastern Greenland had several renal lesions that were correlated with body burdens of specific contaminants. The occurrence of interstitial fibrosis was correlated with polybrominated diphenyl ether (a flame retardant) concentrations in polar bear blubber, whereas diffuse glomerular capillary wall thickening was found to be associated with chlordane (a pesticide) concentrations (Sonne et al., 2006a). This study is particularly striking, as these compounds must be transported to this arctic region from more populated areas highlighting that toxins are globally distributed and a global health concern.

In humans, toxin-induced renal disease typically does not involve the glomeruli. However, Baltic grey seals (*Halichoerus grypus*), polar bears (*Ursus maritimus*), and sledge dogs (*Canis familiaris*) exposed to dichloro-diphenyl-trichloroethane (DDT), or polychlorinated biphenyls (PCBs) had glomerular lesions including large hyaline bodies, diffuse thickening of the glomerular capillary walls, and sclerosis (Bergman et al., 2001; Sonne et al., 2006a, 2007a). Although studies of the effects of pollutants on renal physiology have been conducted across many taxa, no studies have characterized toxin-induced renal damage in wild amphibians.

The goal of this study was to characterize renal lesions consistent with toxin exposure in the giant toad (*Bufo marinus*), and to investigate the association between those pathologies and the nature of their environment (i.e., regional land use). These same individuals have been examined for gonadal abnormalities (McCoy et al., in press). In that study, male toads (but not females) from more agricultural collection sites (higher percentage of agriculture in the surrounding 5.6 km^2 area) were significantly more likely to show feminized and/or demasculinized reproductive traits (including secondary sexual characteristics, gonadal morphology, and circulating testosterone). Thus, we hypothesized that abnormalities of the kidneys (as a component of the urogenital system) might be similarly affected by degree of agricultural exposure.

2. Methods

2.1. Characterizing land use type

We collected *B. marinus* from five sites in South Florida with different suburban and agricultural land use patterns. Land use type for each site was determined by importing Google Earth digital satellite images (downloaded August 20, 2007) and a distance key of each site into Image Pro-Plus (Media Cybernetics Inc) image analysis program. We centered a 5.6 km^2 grid (with 9 ~ 622 m^2 cells) over each collection site. The home range of *B. marinus* is ~2 km^2 (Zug et al., 1975), so this grid includes all of the area likely to be experienced by a toad from each local site. Then we estimated the percentage of agriculture within each cell of the grid and calculated the mean percentage of agriculture for each site. Lake Worth and Wellington, had zero agriculture and were the most suburban sites. Homestead (34% agriculture) included a mixture of agricultural fields, buildings, and roadways, and thus, consisted of both suburban and agricultural land. Canal Point (51% agriculture) was largely agricultural but was bordered on one side by Lake Okeechobee, whereas Belle Glade was almost entirely agricultural land (93% agriculture).

Toads collected from suburban areas were often found in shopping center parking lots where we can assume they are exposed to oil residues (polyaromatic hydrocarbons PAHs) (Maltby et al., 1995; Crosbie and Chow-Fraser, 1999), antifreeze (e.g., ethylene glycol), and car exhaust. Toads collected from agricultural areas were collected from within agricultural