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Insights into reptile dermal contaminant exposure: Reptile skin permeability to pesticides

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HIGHLIGHTS

- We determined the permeability of reptile ventral skin samples to four pesticides.
- Reptile skin had relatively low permeability with log Kp values <-3.5.
- The greatest permeability was seen in moderately lipophilic pesticides.
- Mammalian skin is likely an adequate surrogate for reptiles in risk assessment.

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ABSTRACT

There is growing interest in improving ecological risk assessment exposure estimation, specifically by incorporating dermal exposure. At the same time, there is a growing interest in amphibians and reptiles as receptors in ecological risk assessment, despite generally receiving less research than more traditional receptors. Previous research has suggested that dermal exposure may be more important than previously considered for reptiles. We measured reptile skin permeability to four pesticides (thiamethoxam, malathion, tebuthiuron, trifluralin) using ventral skin samples. All four pesticides penetrated the skin but generally had low permeability. There was no apparent relationship between physicochemical properties and permeability coefficients. Malathion had a significantly greater permeability rate at all time points compared to the other pesticides. Tebuthiuron had a greater permeability than thiamethoxam. Reptiles and mammals appear to have similar skin permeability suggesting that dermal exposure estimates for mammals may be representative of reptiles.

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1. Introduction

There has been a growing interest to improve ecological risk estimates by developing contaminant exposure models that more realistically simulate exposures in the wild (Butcher and Nielsen, 1996). Specifically, researchers have indicated that dermal contaminant exposure should be addressed in avian risk assessments (Mineau, 2011) and could also be important for amphibians (Van Meter et al., 2014) and reptiles (Weir et al., 2010, 2014). Experimental evidence has shown that dermal exposure can be a significant exposure route immediately following a pesticide spray (Driver et al., 1991). Exposure modeling simulations have also indicated that for some taxa such as reptiles, dermal exposure could be relatively more important than for other taxa (Weir et al., 2010). Reptiles are generally the least studied vertebrate taxon in ecotoxicology (Hopkins, 2000; Sparling et al., 2010) and performing accurate reptile ecological risk assessments are hindered by the lack of both exposure and effects data (Weir et al., 2010). Studies to better characterize and understand dermal exposure in reptiles will help to improve risk estimates for this taxon (Weir et al., 2014, 2015).

Understanding and predicting dermal exposure to contaminants is a common element of human health risk assessments (e.g., at superfund sites: USEPA, 2004), and there are standardized methods for estimating the permeability of human skin to contaminants (Moody, 2000; Bronaugh et al., 1986; see Holmgaard

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et al., 2014 for a review). These methods generally make use of an excised section of skin placed between a "donor" solution spiked with the chemical and a "receptor" solution on the interior of the skin to receive the dose. Previous research using a similar design found that amphibian skin is far more permeable than mammal skin (Quaranta et al., 2009). The relative permeability of reptile skin compared to other vertebrate taxa is currently unknown.

While reptile skin has been suggested to be fairly impermeable (Snodgrass et al., 2008), there is no particular reason to assume the scaled skin of reptiles is any less permeable than other amniotes. Reptiles, birds, and mammals contain alpha keratin in the "soft" skin but reptiles and birds (in some areas, e.g., feet and legs) also contain the "hard" layer of beta keratin (Alibardi, 2003) which comprises the scales. It is unlikely that an additional keratin layer would have a significant role in preventing chemical absorption. Experiments to understand how desert reptiles withstand desiccation have found that keratin provides toughness with little effect on water permeability, while lipids provide the opposite (Roberts and Lillywhite, 1980). Further, avian skin can contain lipid bodies that help prevent water loss and function similarly to mammalian lamellar bodies (Menon and Menon, 2000). The combined evidence suggests that reptiles and mammals may have similar skin permeability, but no experiments using contaminants on reptile skin have yet been performed.

The goal of this experiment was to provide a first step in understanding the permeability of reptile skin and compare reptile skin permeability to previous data with amphibians and mammals. We exposed excised reptile belly skin to four pesticides and quantified recovery of doses and estimated permeability rates (Kp) for comparison with previous studies. Our methods and results have important implications for refining reptile dermal exposure models to improve ecological risk assessment.

2. Materials and methods

2.1. Study organisms

Male, adult, western fence lizards, *Sceloporus occidentalis*, were acquired from a colony maintained at Oklahoma State University (Talent et al., 2002). The husbandry of this species has been described elsewhere (Weir et al., 2014). Briefly, lizards were held individually in plastic containers (11 cm deep × 15.5 cm wide × 28.5 cm long) with 1 kg washed sand as substrate. Lizards were provided a small water dish (10 mL volume) for *ad libitum* drinking and were fed 2 large mealworms (*Tenebrio sp.* approximate weight = 0.15 g) every other day. The lizards were provided a 14:10 light dark cycle and a heat lamp was provided for 6 h each day for thermoregulation. To maximize use of animals in research, lizards used for obtaining skin samples in this experiment were associated with other experiments (IACUC Protocol #s: 13012 and 11066), and skin was collected only from lizards that were not exposed to contaminants in other experiments (i.e., control lizards).

2.2. Pesticides

We chose four pesticides, which span a range of log Kow values and a variety of functional groups including thiamethoxam, malathion, tebuthiuron, and trifluralin. Selection of these particular chemicals (and associated physicochemical properties) was designed to create generalizable results that could be applied to other pesticides. The physicochemical characteristics of these four pesticides are summarized in Table 1. All pesticides used were technical grade (>98% purity); we weighed out 40 mg of each pesticide to which 1 mL of acetone was added to achieve a nominal concentration of 40 mg/mL for spiking donor solutions. We added 50 μL of the 40 mg/mL stock solution of each pesticide to 20 mL of donor solution to achieve a nominal donor concentration of 100 μg/mL with a total of 2000 μg of each pesticide. We chose 100 μg/mL in order to increase the probability of detection if a pesticide had low permeability, not to represent a realistic exposure scenario.

2.3. Exposure setup

We created a system similar to the previously published methods for an "infinite" dosing solution (Fig. 1, Moody, 2000). Both the donor and receptor sides of the exposure cell received a salt solution containing 112 mM of NaCl, 5 mM of KCl, 1 mM of $CaCl_2$, and 2.5 mM of $NaHCO_3$ (Quaranta et al., 2009). A "donor" solution was created by pipetting 50 μL of the standard solutions previously described for each pesticide. A stir bar was placed in each donor solution to homogenize the solution and to allow constant contact between the skin surface and the pesticides in solution. Following spiking the donor solution, we allowed the stir bar to homogenize the sample for 30 s, which also allowed the acetone to volatilize prior to addition of the skin and receptor segment of the exposure cell. We excised a section of ventral (belly) skin from Western fence lizards (n = 8) during necropsies from other experiments. Skin samples were not stored for any extended period. Instead, skin samples were immediately placed into the exposure system. Skin samples were placed over the mouth of a 20 mL scintillation vial and secured using a rubber O-ring and then wrapped in Parafilm around the sides of the mouth of vial. In this way the area of skin available to the donor solution was the area of the opening of the scintillation vial (2.01 cm^2). Scintillation vials had a small hole burned into the bottom to allow access with a syringe during experiments. The hole was covered with Parafilm to prevent any evaporation of the receptor solution during experiments. Overall, our experimental setup worked well with no leaking and minimal difficulty in administering or extracting solution.

2.4. Sample collection and extraction

We collected a 5 mL water sample from the receptor solution at 4, 8, and 24 h. At 24 h we also collected a 5 mL sample of the donor solution and kept the skin for analysis. Samples were frozen at −20 °C until extraction. We had two difficulties in chemical extraction. First, some pesticides were lipophilic while others were highly hydrophilic (Table 1). Second, we could not find a single chemical analysis method that was optimal for all 4 pesticides in a single solvent (e.g., trifluralin in water, see *Chemical Analysis* below). Therefore, to each aqueous sample we added 5 mL of optima grade hexane to extract more lipophilic compounds while water samples contained more hydrophilic compounds. Skin samples had 5 mL of hexane and 5 mL of the salt solution added. Samples were placed on a shaker for 24 h. Following the 24 h extraction period, a 4 mL sample was taken of both the hexane and aqueous layers. These 4 mL samples were concentrated to 1 mL. For hexane, samples were concentrated using a rotary evaporator, for water samples evaporation occurred in a fume hood for 24 h.

2.5. Chemical analysis

All samples were sent to the Mississippi State Chemical Laboratory for analysis. Samples were analyzed using liquid chromatography coupled to mass spectrometry detection (LC-MS). The LC-MS system was an Agilent 1290UPLC coupled to a 6430 MS triple quad. The column used was an Agilent Poroshell 120 EC-C18 (2.1 × 50.0 mm). Reverse-phase LC was accomplished with mobile phases of (A) water with 5 mM ammonium formate and (B) methanol with 5 mM ammonium formate. We used a gradient

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