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SOIL ORGANIC MATTER CONTENT EFFECTS ON DERMAL PESTICIDE BIOCONCENTRATION IN AMERICAN TOADS (*BUFO AMERICANUS*)

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Abstract: Pesticides have been implicated as a major factor in global amphibian declines and may pose great risk to terrestrial phase amphibians moving to and from breeding ponds on agricultural landscapes. Dermal uptake from soil is known to occur in amphibians, but predicting pesticide availability and bioconcentration across soil types is not well understood. The present study was designed to compare uptake of 5 current-use pesticides (imidacloprid, atrazine, triadimefon, fipronil, and pendimethalin) in American toads (*Bufo americanus*) from exposure on soils with significant organic matter content differences (14.1% = high organic matter and 3.1% = low organic matter). We placed toads on high- or low-organic matter soil after applying individual current-use pesticides on the soil surface for an 8-h exposure duration. Whole body tissue homogenates and soils were extracted and analyzed using liquid chromatography–mass spectrometry to determine pesticide tissue and soil concentration, as well as bioconcentration factor in toads. Tissue concentrations were greater on the low-organic matter soil than the high-organic matter soil across all pesticides (average ± standard error; 1.23 ± 0.35 ppm and 0.78 ± 0.23 ppm, respectively), and bioconcentration was significantly higher for toads on the low-organic matter soil (analysis of covariance $p = 0.002$). Soil organic matter is known to play a significant role in the mobility of pesticides and bioavailability to living organisms. Agricultural soils typically have relatively lower organic matter content and serve as a functional habitat for amphibians. The potential for pesticide accumulation in amphibians moving throughout agricultural landscapes may be greater and should be considered in conservation and policy efforts. *Environ Toxicol Chem* 2016;35:2734–2741. © 2016 SETAC.

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INTRODUCTION

Disease, pollution, habitat loss, climate change, and agriculture have all been identified as leading factors globally in amphibian loss [1–6]. Agricultural practices that lead to altering wetland and forested habitats, as well as perpetually using chemicals on plant matter and soils, may be particularly harmful to amphibian populations [7,8]. Among larval amphibians, acute and chronic exposure to specific agro-chemicals is known to cause deformities, delayed development, altered production of hormones, and mortality [9–11]. Juvenile and adult amphibian response to these chemicals has not been studied as thoroughly as the embryonic and larval stages, although both field and laboratory data indicate pesticide exposure in postmetamorphic life stages alters liver metabolism [12], survival [13,14], and reproduction [15]. Lethal and sublethal effects of pesticides have been documented across all amphibian life stages, which increases their susceptibility to field exposures of residual and recently applied compounds.

Agricultural landscapes serve as active amphibian breeding grounds despite their seemingly poor habitat value [16–18]. Tadpoles are exposed to pesticides in breeding ponds in agricultural areas, as well as ponds located downwind of pesticide application sites [19,20]. Activity of adults and dispersal of metamorphs to and from agricultural ponds occurs in most species from spring through late summer or early fall [16], a time that coincides with pesticide applications on farm fields and crops. In addition, as many as 80% of amphibian migrations to and from breeding ponds in Germany were found to coincide with insecticide, fungicide, or herbicide applications on crops [18]. During this time, migrating amphibians are likely to come in contact with the active ingredients of multiple pesticides, although the number and type will vary between crops and years [18]. Smalling et al. [17] reported significant concentrations of current-use pesticides in both water and sediment samples from a field study of agricultural and urban amphibian breeding habitats across the US where pesticides are actively applied. A field study of pond-breeding amphibians (*Pseudacris regilla*) in habitats downwind of major agricultural areas in California revealed significant body burdens of current-use pesticides in frog tissues, water, and sediment samples [21]. Similarly, water samples from 6 restored and reference wetlands in an Iowa landscape dominated by corn and soybean agriculture had measurable concentrations of more than 30 current-use pesticides, and associated soil samples contained 14 different pesticides [22]. Postmetamorphic chorus (*Pseudacris triseriata*) and leopard frogs (*Lithobates pipiens*) collected from these 6 wetlands also had quantifiable tissue concentrations of 17 current-use pesticides [22]. Although the effects of pesticide exposure in adult amphibians have been understudied, agrochemical exposure may be contributing to global decline.

In terrestrial landscapes, dermal contact with contaminated soil and plant matter may lead to bioconcentration, as well as lethal and sublethal effects in amphibians. Although several studies have quantified pesticide tissue concentrations in wild amphibian populations [22,23], isolating the exact route of exposure in field surveys is challenging. For amphibians traversing or residing in agricultural areas during pesticide application, direct dermal contact through aerial deposition or spray drift may lead to significantly greater uptake [24] and higher

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risk of mortality [13,14]. Whereas direct exposure to pesticides contributes to higher body burdens and lethality, indirect contact with contaminated soil also leads to bioconcentration [25] and decreased brain cholinesterase activity [26]. Diet may provide an additional mechanism for pesticide accumulation, although preliminary data indicates dermal routes of exposure are more significant for both amphibians and reptiles [26,27]. Distinct amphibian skin properties such as the pelvic patch and specialized water protein channels may contribute to increased dermal uptake of pesticides during postmetamorphic life stages that experience soil exposure [6,8,28,29].

Although the physiological structure of the amphibian dermis may facilitate pesticide uptake, soil properties may ultimately dictate bioavailability of pesticides in terrestrial habitats. The organic matter fraction of soil readily binds to pesticides, potentially decreasing the availability of pesticides adhering to biological matter. Soil partition coefficient (K_{oc}) is a comprehensive measure used to indicate how mobile a given pesticide is after interacting with the solid, carbon fraction of soils. Hydrophobic pesticides generally have a higher K_{oc} value than hydrophilic compounds that preferentially bind to the aqueous phase of soils [30,31]. Soils with lower organic matter content are less likely to bind to pesticides, rendering these chemicals more bioavailable to animal tissues [30]. A basic understanding of soil organic carbon content and soil-specific K_{oc} values may be important to indicate pesticide bioavailability and potential bioconcentration in amphibians.

The unique chemical structure of a pesticide combined with soil properties will alter its subsequent bioavailability to living organisms. Studies of pesticide accumulation or effects in amphibians through terrestrial exposures vary with the type of soil used, ranging from potting or gardening soil [26] to natural wetland soils [32]. Variation in soil type presents a challenge for conservation practitioners and policy makers, because inference across data sets may be impractical without a basic measure of bioavailability differences. Laboratory determined K_{oc} values for 5 pesticide active ingredients were a top predictor of amphibian tissue concentration and skin permeability in statistical models, indicating the significance of organic carbon content as a contributor to bioavailability and accumulation [25]. Beyond this modeling effort, limited data existed on the relationship between soil organic carbon and pesticide bioavailability in amphibians. For metals, James et al. [33] reported increased cadmium uptake in toads as a function of soil sand content, indicating that lower organic matter content is associated with magnified bioavailability. Accumulation of pesticides in amphibians and associated lethal and sublethal effects may depend, at least partially, on soil carbon content. Given the lack of data on the relationship between soil carbon content and pesticide accumulation in amphibians, the present study was designed to evaluate dermal uptake of 5 current-use pesticide active ingredients that span a gradient of hydrophobicity or water solubility (imidacloprid, atrazine, triadimefon, fipronil, and pendimethalin; Table 1) by soil type. We predicted that amphibian body burdens (and resulting bioconcentration factors) would be a function of soil carbon content, with greater bioconcentration in individuals exposed to pesticides on sandy soil with low organic matter content, compared to soil rich in organic matter.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents were obtained from Fisher Scientific. Experiments were conducted with pesticide active ingredients (purity $\geq$ 98%). Imidacloprid, atrazine, triadimefon, fipronil, and pendimethalin were obtained from the US Environmental Protection Agency (USEPA) National Pesticide Standard Repository.

Table 1. Pesticide type, maximum labeled allowable application rate ($\mu g\,cm^{-2}$) and water solubility at 20 °C ($mg\,L^{-1}$).

Pesticide	Type	Application rate	Water solubility
Imidacloprid	Insecticide	5.7	510
Atrazine	Herbicide	22.9	30
Triadimefon	Fungicide	2.7	260
Fipronil	Insecticide	1.1	3.78
Pendimethalin	Herbicide	69.8	0.3

Soil collection

The 2 different soil types in the present study were characterized by the University of Georgia's Soil, Plant, and Water Lab in Athens, Georgia, USA. In December 2012, a Plott series soil (PLE) was collected from the Coweeta Long-term Ecological Research site in Otto, North Carolina, USA. The PLE soil was classified as a fine-loam with up to 14.1% organic matter (Table 2). In January 2013, an Orangeburg loamy-sand soil (OLS) with up to 3.1% organic matter was collected from the Joseph Jones Ecological Research Center in Newton, Georgia (Table 2). Both sites are considered relatively pristine within their respective study areas, have not received direct application of pesticides in recent history (J. Knoepp, USDA Forest Service, Otto, NC and L. Smith, Joseph Jones Ecological Research Center, Newton, GA, respectively, personal communication) and tested negative for detectable pesticide concentrations in control samples. After collection, the soils were stored in a walk-in cooler at 4 °C in a USEPA laboratory in Athens, Georgia or at Washington College, Chestertown, Maryland.

K_{oc} *determination*

To calculate the organic carbon normalized adsorption coefficient (K_{oc}) on various soil types for each pesticide's active ingredient, an adsorption-desorption using a batch-equilibrium method was used [34]. Briefly, 2 soil types were air-dried and passed through a 2 mm sieve to remove large debris. Tier I (preliminary study) K_{oc} determination consisted of liquid chromatography–mass spectrometry (LC–MS) analysis of control (0.01 M calcium chloride [$CaCl_2$] with pesticide active ingredient), blank (soil with 0.01 M $CaCl_2$), and pesticide test soil to solution ratios 1:1, 1:5, and 1:10 (pesticide active ingredient, soil, and 0.01 M $CaCl_2$) kept on a shaker table and sampled at 4 h, 8 h, 24 h, 48 h and, occasionally, 72 h or 96 h for the more polar pesticides. All samples were done in duplicate. At each time interval, a blank, control, and 2 soil to solution ratios were removed from the shaker, and a 1 mL aliquot of the sample was transferred to a 2 mL centrifuge tube containing a 0.2 μm filter. The samples were centrifuged for 9 min at 3250 rpm; afterward, a 200 μL aliquot of the filtrate was transferred to a GC vial with an insert and analyzed on a LC–MS. Tier I studies with soil to solution ratios of 1:1, 1:5, and 1:10 indicated the 1:1 ratio generated the best data with the shortest equilibration time; the 1:1 ratio was therefore used exclusively for the Tier 2 (adsorption kinetics) study. After completing the Tier 2 study, the distribution coefficient (K_d) was first determined and then used to estimate the organic carbon normalized adsorption coefficient (K_{oc}) as outlined in the Adsorption-Desorption using a Batch-Equilibrium Method [34].

Table 2. Soil characteristics for Plott series (PLE) and Orangeburg loamy-sand (OLS) soils[a]

Soil type	pH	BS	CEC	% OM	% Sand	% Silt	% Clay
PLE	4.4 (0.04)	14.3 (1.48)	16.0 (0.26)	14.2 (0.32)	54.6 (0.67)	22.0 (0)	23.3 (0.67)
OLS	4.5 (0.02)	34.3 (0.23)	7.2 (0.04)	3.1 (0.04)	80.5 (0.70)	12.8 (0.70)	6.1 (0)

[a]Data reported as mean (+/– standard error). Soils tested by the University of Georgia's Soil, Plant and Water Laboratory, Athens, GA, USA.
BS = base saturation (%); CEC = cation exchange capacity (meq/100g); % OM = percentage of organic matter.

Amphibian care

Adult American toads (*Bufo americanus*) were purchased from Reptile City in Texas and shipped to Washington College in Maryland in May 2014. On arrival, they were placed in 415 L outdoor polyethylene tanks lined with moist sphagnum moss, leaf litter, and woody debris to simulate a terrestrial habitat. All toads were fed cultured fruit flies and purchased crickets for 3 wk until experimentation began in June 2014.

Pesticide exposure study

The pesticide exposure study is considered a conservative scenario exposure designed to maximize water uptake across the dermis on contaminated soils. All toads were dehydrated overnight for 12 h in clean, unlined polyethylene tanks held in the laboratory at Washington College prior to pesticide exposure. Exposures were initiated between 7:00 AM and 9:00 AM in the laboratory following toad dehydration. Experimental units were 0.94 L Pyrex® glass bowls. Bowls were cleaned with methanol (MeOH) prior to experimentation, then filled with 150 g of either PLE soil or OLS soil. Pesticide active ingredients were dissolved in 75 mL of 100% MeOH and sprayed over the entire bowl surface at the maximum labeled allowable application rates (Table 1). The control treatment was 75 mL of 100% MeOH. For pesticide application, bowls were grouped by active ingredient and surfaces of 6 bowls were sprayed simultaneously to minimize spraying time between pesticide treatments. Pesticides were applied using compressed air propellant Preval Spray Gun® canisters attached to gradated, clean glass jars. A single pesticide active ingredient was applied to each bowl to avoid synergistic or ameliorative effects; after application, bowls were placed in a fume hood in the laboratory overnight to allow for MeOH evaporation from the soil surfaces prior to exposure. The following morning, soils were rehydrated with spring water by spraying the surface of the 6 bowls with a total of 300 mL of water (50 mL per bowl).

Immediately after soil rehydration, 1 American toad was placed into each bowl. The top of each bowl was secured with a mesh screen to keep the toad inside the experimental unit and in contact with the contaminated soil. Experimental units were arranged in a completely randomized design and held at 18.8 °C to 20 °C and 65% to 70% relative humidity in the laboratory for an 8 h exposure. The experiment was a randomized design completed over 2 consecutive days. Due to the logistics and timing of applying different pesticides to experimental chambers, all frogs exposed to the same pesticide treatment were tested on the same day; however, the sequence of pesticides tested was randomized over the 2-d period. Atrazine and imidacloprid exposures were completed on day 1, followed by fipronil, triadimefon, and pendimethalin on day 2. In total, 72 toads were used in the present study, with 6 toads exposed to each soil type and pesticide active ingredient or control treatment combination. At the end of the 8 h exposure study, all toads were placed in prelabeled clean amber jars and euthanized in a freezer at –80 °C. On termination of the experiment, a soil sample was collected from each experimental unit (72 samples) for pesticide analysis.

Soil and amphibian extraction

Extraction methods for both amphibians and soils are detailed in Van Meter et al. [24,25]. Briefly, 5 mL of methanol (MeOH) was added to each sample, followed by sonication, vortexing, and centrifugation. The supernatant was collected and each sample was extracted again, for a total of 2 extractions per sample. The resulting supernatant from the second extraction was combined with the first extract supernatant and evaporated to 1 mL under nitrogen gas. For final pesticide extraction, 10 mL of Milli-Q water, 3 mL methyl-*tert*-butyl ether (MTBE), and sodium sulfate were added to each sample. The MTBE layer was transferred off the top of the final sample, centrifuged, and 1 mL of the final extract was analyzed using LC-MS after being blown down with nitrogen and reconstituted with 30% methanol.

In addition to the 5 active pesticide ingredients, soil and frog tissue samples were scanned for primary metabolites. When metabolites were detected, their concentrations were summed with that of the associated parent compound as follows: desethyl-atrazine and deisopropyl atrazine with atrazine; triadimenol with triadimefon; and fipronil sulfone with fipronil. We did not detect quantifiable metabolites of either pendimethalin or imidacloprid. After analysis, bioconcentration factors (BCFs) were determined for each species and pesticide as:

$$\mathrm{BCF} = \mathrm{C_f}/\mathrm{C_s}$$

where C_f is the frog whole-body tissue concentration and C_s is the average composite soil concentration within each treatment, both at the end of the 8 h exposure. Whereas BCFs typically refer to accumulation of contaminants from an aquatic medium at steady state, they also describe dietary and dermal accumulation in terrestrial environments, as presented in prior studies [26,35]. In addition, amphibian surface area (cm^2) was determined as a function of body weight (BW_f) following [36]:

$$\text{Surface area} = 1.131 \times (\mathrm{BW_f}^{0.579})$$

LC–MS instrumentation

All samples were analyzed on an Agilent 1100 Series HPLC linked to a 6120 mass spectrometer. For K_{oc} determination, using the batch slurry method, an Eclipse XDB-C18 (3.5 μm particle size, 3.0 mm × 150 mm; Agilent Technologies) quantified pesticide active ingredients following the procedure in Van Meter et al. [24]. Briefly, the initial mobile phase was 70% water with 0.1% formic acid (A) and 30% acetonitrile with 0.1% formic acid (B). Starting conditions were held for 2 min, ramped to 90% B over 16 min, and held for 4 min, before returning to initial conditions of 30% B and re-equilibrated for 5 min (total run time of ~30 min). Samples were analyzed in positive electrospray ionization (ESI) from 0 min through 19 min, then switched to negative ESI from 19 min through 23 min, and back to positive ESI from 23 min through 30 min, using selected ion monitoring mode. Switching between positive and negative ESI was due to the respective elution times of the pesticide active ingredients. The m/z ions were 216 and 174 for atrazine, 294 and 225 for triadimefon, 256 and 175 for imidacloprid, 330 and 435 for fipronil, and 212 and 282 for pendimethalin. The ion for the internal standard, tetraconzole, was monitored at 372 m/z. For metabolites, m/z ions in positive ESI were 174 and 104 for deisopropyl atrazine, 188 and 146 for desethyl-atrazine, 296 and 227 for triadimenol. Fipronil sulfone was detected in negative ESI with m/z ions 451 and 415.

Statistical analysis

We report descriptive statistics for each pesticide for the laboratory-determined K_{oc} values, soil concentrations, and homogenized amphibian tissue residue concentrations. In addition, we report descriptive statistics for amphibian pesticide uptake by calculating a continuous dependent variable, BCF, which is derived by dividing the homogenized tissue concentration of an exposed amphibian within its experimental unit by the mean composite soil concentration within its treatment. We also used one-way analysis of covariance (ANCOVA) to test for the significance of covariates on the calculated BCFs within the context of a paired comparison design. Selected covariates included soil type (a proxy for organic carbon content), amphibian surface area, laboratory-determined K_{oc} values and pesticide active ingredient as a factor. The experimental procedure implemented a randomized block design to allow for the blocking variable (the 5 pesticide active ingredients) to be treated as a confounding variable [37] to statistically control for the large variation in treatment application rates and amphibian uptake across the different pesticides tested. Soil types with their associated organic carbon contents were therefore tested as a fixed effect factor. This approach allows for more general inference across all the pesticides rather than having test results for each individual pesticide active ingredient. Distributions of pesticides in tissue residues are assumed to be normal via the Central Limit Theorem as an averaged concentration of millions of cells within each amphibian in its experimental unit. Sample sizes were insufficient to robustly test for heteroscedasticity. Reported statistics include F-value, degrees of freedom (*df*), and a *p* value. All statistical analyses were performed in R Ver 3.2.2 [38].

RESULTS

Soils and K_{oc}

When assessing the mobility of organic pesticides in soil, K_{oc} values are helpful. Low sorption coefficients indicate low ratios of the adsorbed pesticide mass in soil relative to the amount of total carbon and indicate more mobile pesticides. Higher K_{oc} values indicate higher soil sorption, impeding mobility. Our previous modeling efforts indicated that laboratory determined K_{oc} values were better predictors of amphibian pesticide body burdens than literature-derived K_{oc} values [24]; therefore, we only report laboratory K_{oc} values in the present study. Contradicting trends in laboratory-determined K_{oc} were observed across pesticides in the 2 soil types (Table 3). For the high organic matter PLE soil, the more hydrophilic pesticides (imidacloprid and atrazine) had lower K_{oc} values and were not as strongly adsorbed, whereas the most hydrophobic pesticide (pendimethalin) had the highest observed sorption rate. Although the K_{oc} values were less variable on the OLS soil, adsorption by the 5 active pesticide ingredients showed the opposite trend, that is, hydrophilic pesticides were more strongly adsorbed than the more hydrophobic (Table 3). Generally, pesticides were more mobile on the OLS soil compared to the PLE soil, indicating that uptake by amphibians may be greater on lower organic matter soil. The K_{oc} value was similar on both PLE and OLS soils for atrazine and triadimefon (Table 3). There was a larger divergence in K_{oc} values between soil types for the more hydrophilic (imidacloprid) and hydrophobic (fipronil and pendimethalin) pesticides tested (Table 3); in particular, our laboratory-determined K_{oc} value for pendimethalin was much greater on the PLE than the OLS soil (6.43 and 1.73, respectively), indicating that pendimethalin is more bioavailable for uptake on the low organic carbon soil.

Pesticide concentrations on soils were consistent within active ingredients across soil types. Mean active ingredient concentrations on the PLE and OLS soils, respectively, were: imidacloprid 1.4 ppm (± 0.4 standard error [SE]) and 0.9 ppm (± 0.1 SE); atrazine 26.8 ppm (± 3.6 SE) and 21.2 ppm (± 2.9 SE); triadimefon 5.1 ppm (± 0.9 SE) and 4.5 ppm (± 0.7 SE); fipronil 1.8 ppm (± 0.4 SE) and 1.1 ppm (± 0.2 SE); and pendimethalin 14.5 ppm (± 2.4 SE) and 11.3 ppm (± 1.7 SE).

Amphibians

Toad mass averaged 12.0 g (± 0.4 g SE) and calculated surface area averaged 5.5 cm^2 (± 0.1 cm^2 SE). All pesticide-treated frogs had quantifiable concentrations in their tissues, whereas pesticides were not detected in any of the control frogs or soils. Pesticide tissue concentrations were consistently higher among toads exposed on OLS soil than those on PLE soil

Table 3. Laboratory-determined K_{oc} values for both Plott series (PLE) and Orangeburg loamy sand (OLS) soils, as well as reported literature values as determined by the Log K_{ow} and MCI methods for the 5 active pesticide ingredients tested

	Imidacloprid	Atrazine	Triadimefon	Fipronil	Pendimethalin
Laboratory determined values					
Soil type					
PLE	2.56	2.30	3.03	4.24	6.43
OLS	3.65	2.63	3.01	2.86	1.73
Literature reported values					
Method	Imidacloprid	Atrazine	Triadimefon	Fipronil	Pendimethalin
Log K_{ow}	1.53	2.12	2.78	4.00	4.19
MCI	2.99	2.35	2.48	3.77	3.75

Table 4. Pesticide tissue concentration in ppm (mean [standard error]) among American toads (*Bufo americanus*) after 8 h exposure on Plott series (PLE) or Orangeburg loamy sand (OLS) soil

Soil type	Active ingredient				
	Imidacloprid	Atrazine	Triadimefon	Fipronil	Pendimethalin
PLE	0.04 (0.01)	0.60 (0.14)	0.18 (0.05)	0.10 (0.02)	2.95 (0.58)
OLS	0.04 (0.01)	1.95 (0.94)	0.33 (0.04)	0.16 (0.03)	3.69 (0.82)

(Table 4). The average percentage difference in pesticide body burdens between toads on OLS relative to PLE soil was 106% for atrazine, 57% for triadimefon, 46% for fipronil, and 22% for pendimethalin. The K_{oc} values for these 4 active ingredients indicate bioavailability or mobility should generally increase on lower organic matter OLS soil, which is consistent with our tissue concentration results (Tables 3 and 4). Tissue concentrations of imidacloprid were slightly higher (13% on average) for toads exposed on PLE than OLS soil. This is consistent with our experimental K_{oc} results, because bioavailability or mobility of imidacloprid decreased slightly on the OLS soil (Table 3).

Average BCF for the 5 active pesticide ingredients in the 2 soil types tested ranged from 0.01 in toads exposed to atrazine on PLE soils to 0.61 in toads exposed to pendimethalin on OLS soil (Figure 1). Bioconcentration factors were, on average, 23%, 288%, 104%, 183%, and 61% greater on OLS soil relative to PLE for imidacloprid, atrazine, triadimefon, fipronil, and pendimethalin, respectively. The paired comparison design tested across all active ingredients found that toads in the OLS soil treatment had significantly higher BCF values relative to toads in the PLE soil treatment (ANCOVA soil effect $F = 11.0$, $df1 = 1$, $df2 = 53$, $p = 0.002$). In the OLS soil treatment, BCF values ranged from 0.02 to 0.07 for imidacloprid, 0.02 to 0.30 for atrazine, 0.04 to 0.11 for triadimefon, 0.02 to 0.20 for fipronil, and 0.13 to 0.61 for pendimethalin. As the water solubility of the pesticide active ingredient decreased in the OLS soil, the average BCF increased. However, this trend was not as apparent in PLE soils, where the average BCF values for 4 of the 5 pesticides tested were very similar (Figure 1). In the PLE soil treatment, BCF values ranged from 0.01 to 0.06 for imidacloprid, 0.01 to 0.05 for atrazine, 0.02 to 0.08 for triadimefon, 0.02 to 0.09 for fipronil, and 0.10 to 0.36 for pendimethalin. Bioconcentration factors were highest for pendimethalin on both soil types (Figure 1). Amphibian surface area did not have a significant effect on BCF (ANCOVA surface area effect $F = 0.9$, $df = 1$, $df2 = 53$, $p = 0.764$).

DISCUSSION

Pesticide uptake through dermal exposure in terrestrial amphibians is important for a better understanding of toxicokinetics and has implications for the amphibians' conservation status. Agricultural landscapes may present particularly serious challenges to amphibians during all life stages, including postmetamorphosis and adulthood when moving throughout cultivated fields [16,18,39]. Given current

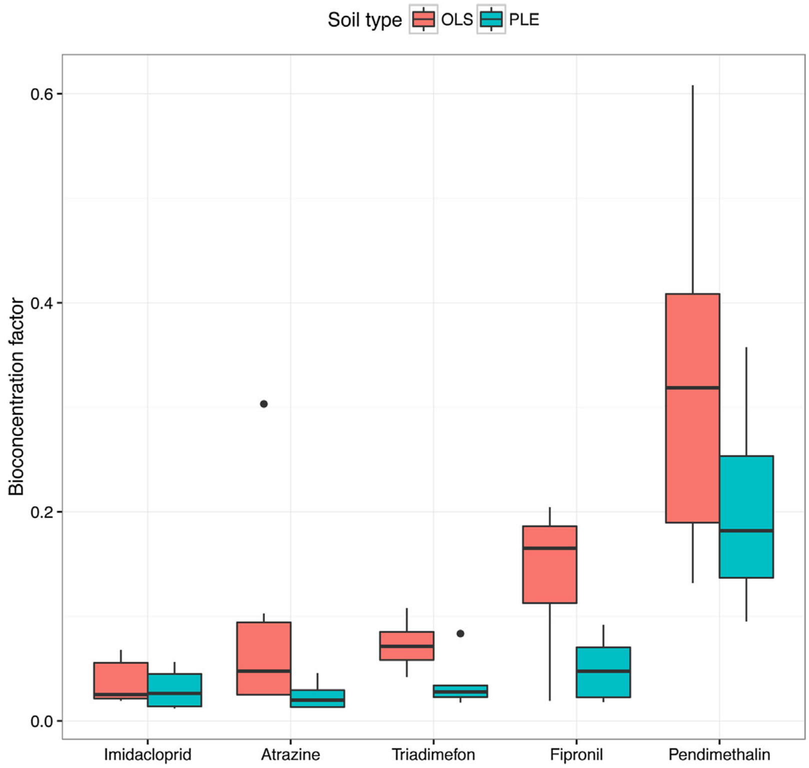

Figure 1. Bioconcentration factors for American toads (*Bufo americanus*) exposed to 1 of 5 active pesticide ingredients on low organic matter (OLS) or high organic matter (PLE) soil. Box values corresponding to the 25th percentile, 50th percentile, and 75th percentile are shown. Whiskers extend to the maximum and minimum data values that are within 1.5 times the interquartile range above and below the 75th percentile and 25th percentile; asterisks indicate outliers beyond this range.

and historical use of pesticides throughout agricultural areas, amphibian exposure to soils and associated dermal uptake during juvenile and adult phases is a viable, but understudied, exposure pathway. Pesticides have been found in tissues of terrestrial amphibians in both urban and rural habitats [22,23,40], and laboratory studies indicate that dermal absorption is a viable route of exposure [25,26,41]. As indicated by the present study, soils display a range of characteristics that may alter the mobility or bioavailability of contaminants to amphibians for uptake and bioconcentration, with amphibians in agricultural habitats with relatively lower organic matter soils likely receiving higher pesticide exposures.

Organic matter content of agricultural soils plays a significant role in how a pesticide behaves on the soil surface. After application, pesticides interact with water in the pore spaces of the soil matrix and with soil particles themselves. The extent to which a pesticide adsorbs to soil is largely dependent on the organic matter content of that particular soil, because the organic matter fraction functions as a nonpolar surface and will aggregate with nonpolar pesticides [30]. Many nonpolar pesticides will therefore readily adsorb to high organic matter soils (such as the PLE soil in the present study) and be less bioavailable for accumulation by terrestrial organisms. More polar pesticides may be adsorbed by clay particles in low organic matter soils, becoming less bioavailable, or they may dissolve in the water fraction of the soil matrix, making them available for accumulation across amphibian dermis [30]. Current and previous data [24,25] demonstrate that amphibians readily accumulate pesticides from soil during rehydration of contaminated water in the soil matrix.

Other factors that affect the sorptive properties of pesticides in soils include, but are not limited to, pH, moisture content of the soil, temperature, and water solubility [30]. Ping et al. [42] found that increased temperature decreased adsorption of imidacloprid on soils due to higher water solubility at higher temperatures. Furthermore, imidacloprid adsorption decreased with increasing pH likely because of increased polarity of organic matter in the soils [42]. Given the complexity of soil-sorption properties across pesticides, Wauchope et al. [30] suggested that to understand behavior of a given pesticide on a particular soil type, K_{oc} determinations are necessary. This may not be practical for all research studies, however, given the wide variation in soil types used and the time-intensive nature of K_{oc} determinations. In instances where K_{oc} cannot be, or has not been, measured for a particular pesticide and soil combination, basic soil analyses to understand organic matter content will be more practical and offer a proxy for estimating pesticide sorption characteristics.

We used 2 soils with significantly different organic matter content to evaluate differences in K_{oc} and determine how bioavailability and subsequent bioconcentration by amphibians would be affected. Our laboratory-determined K_{oc} values follow similar trends of increasing adsorption with increasing hydrophobicity as literature-reported values for the molecular connectivity index and log K_{ow} (octanol–water partition coefficient) methods ([43–46]; Table 3). Although general trends are similar, our laboratory-based K_{oc} values allow a more accurate evaluation of variations in pesticide soil sorption between specific soils in our experiment. Through these analyses, we found that pesticides adsorbed more strongly to the high organic matter soil and were more mobile on the lower organic matter soil. When combined with analyses of bioconcentration in amphibians, we were better able to estimate pesticide mobility and amphibian uptake of pesticides on 2 different soil types. Toads exposed to pesticides on low organic matter soil had higher body burdens and BCF values for the 5 active pesticide ingredients we tested, compared to those on high organic matter soil. Given that conventional agricultural practices reduce the organic matter fraction in soils over time [47,48], amphibians in these areas may be more susceptible to pesticide accumulation through dermal exposure. Although they bioconcentrate more pesticide from lower organic matter soils, we do not know how variations in soil might alter associated pesticide effects on amphibians. Laboratory and field-based experiments looking at amphibian uptake or effects of pesticides from soils should report soil characteristics, particularly organic matter content, to improve foundational understanding of pesticide mobility and bioavailability across soil types.

Bioavailability of contaminants on soils is an important consideration for terrestrial, benthic, and fossorial organisms. James et al. [33] studied bioavailability of cadmium to American toads (*Bufo americanus*) across a range of sandy soils with variable water-holding capacities. Juvenile toads were placed on cadmium-contaminated soil in hibernation chambers for 5 mo to 6 mo for a long-term exposure. Their uptake of cadmium increased with increasing cadmium concentration and on soils containing more sand. They also experienced lowered mass loss over the hibernation period on sandier soils, which is important for maintaining water balance. This, however, left the toads more susceptible to cadmium uptake through water bioavailability [33]. In a city close to Shanghai, China, Tang et al. [40] sampled soils and terrestrial food web organisms, including the Chinese toad (*Bufo gargarizans*), from agricultural areas. They quantified bioaccumulation of hexachlorobutadiene, a persistent organic pollutant, and organochlorine phosphate pollutants (OCPs). Concentrations of hexachlorobutadiene were lower than all OCPs measured in the soil samples and likely reflect historical use of pesticides in the area. Toads bioaccumulated many OCPs, particularly those that fed on earthworms. Although hexachlorobutadiene was found in toad tissues, it was not believed to biomagnify through the food chain indicating uptake from the terrestrial environment [40]. Exposure to pesticides through dermal contact with soils and plant matter is an important route of accumulation that should be considered in conservation and regulatory efforts.

Simultaneous exposure to multiple pesticides in agricultural habitats is very likely. Lenhardt et al. [18] studied the breeding movements of 4 amphibian species throughout a 700 ha cultivated landscape in Germany, which coincided with agricultural pesticide applications from February through May. Forty-three different pesticides (herbicides, fungicides, and insecticides) were applied at 3 farms during this time. An average of 12% to 42% of captured amphibians were moving across these landscapes at times coinciding with pesticide application or persistence on soils and vegetation after application. Most pesticide applications consisted of numerous active ingredients applied in tandem; therefore, amphibians were exposed to multiple pesticides during movement across the terrestrial landscape [18]. Smalling et al. [22] found 14 pesticide active ingredients and 3 pesticide metabolites in sediment samples from wetlands actively draining agricultural fields in Iowa. In amphibians collected from these wetlands, they found complex mixtures of pesticides and 17 pesticide products in tissue samples [22]. Glyphosate is known to cause mortality in amphibians [49,50] and its mobility in soils can be affected by the presence of phosphate fertilizers [51]. Berger et al. [39] reported that more than 60% of amphibians caught at an agricultural field site in Germany were moving over the landscape during the same time fertilizers were being applied. Interception by vegetation during the growing season may reduce pesticide exposure and related mortality among amphibians [52], indicating there is likely variation in pesticide accumulation and effects across seasons. Research efforts focusing on collecting data on co-exposure to multiple chemical stressors in terrestrial amphibians needs to be a priority because it is clear amphibians come in contact with a cocktail of chemicals in agricultural environments.

The present study focused on a suite of 5 different active pesticide ingredients spanning a range of water solubility and pesticide types (Table 1). Pesticides are applied or used as a formulation that contains both active and inactive ingredients. Inactive ingredients serve many functions, including extending the shelf-life of the product, improving solubility of the active ingredient, increasing penetration or stickiness of the active ingredient to plants and soil, and controlling spray drift [53]. Several studies have looked at variations in pesticide formulations on terrestrial phase amphibians, highlighting potentially adverse effects of inactive ingredients [13,14,50]. Great Plains and New Mexico spadefoot toads (*Spea multiplicata* and *cognatus*, respectively) exposed to 1 of 4 glyphosate-based herbicide formulations on low organic matter soil for 48 h showed considerable variability in survival based on formulation [50]. Brühl et al. [14] found significant differences in the mortality of the European common frog (*Rana temporaria*) exposed to 2 fungicide formulations on soil that contained pyraclostribin as the active ingredient, but differed in the percentage of naptha, an inactive ingredient. They suggested that variations in the amount of inactive ingredients may also play a role in amphibian mortality and impairments [14]. The present study was not designed to evaluate formulations, however, we encourage future research on bioconcentration and associated effects among amphibians exposed to formulated products on soils of varying organic matter content because this may provide important data typifying field conditions.

Amphibian species may differ in their susceptibility to pesticides. We used adult American toads, because they make long migrations between upland habitats and breeding ponds [54]. Dermal properties vary by species and can be associated with life history. In particular, terrestrial amphibian species such as toads have thicker skin than frogs and are more resistant to desiccation [55]; toad skin is also more permeable than frog skin [56]. Our previous study comparing 7 amphibian species [24] found that degree of terrestriality may be important in understanding pesticide accumulation, but not as important as pesticide properties such as water solubility and K_{oc}. In the present study, we used adults that have a larger surface area, but a smaller surface area-to-volume ratio, compared to juvenile amphibians. Surface area did not have an effect on bioconcentration of pesticides in the present study; therefore, a study of juvenile amphibians that compares uptake on different soils is needed. Although toads may spend more time in direct contact with pesticide-contaminated soils than other species, the data presented in the present study are intended to be broadly applied across amphibians having dermal contact with pesticides on a soil medium.

The present study demonstrates higher amphibian body burdens across a set of pesticides for exposures occurring on soils with relatively lower organic matter content soil. This highlights the importance of organic matter content as an exposure determinant and adds to existing evidence of the significance of the dermal pathway in amphibian risk assessment. Further investigation should include a more robust experimental design (more than 2 soil organic matter content treatments) to estimate a sufficient regression relationship between organic matter content and amphibian body burdens. We also urge researchers estimating soil-based exposure and effects for terrestrial-phase amphibians to measure and report organic matter content in their results to add to the scant data on this relationship. For vertebrate chemical risk assessments, data from mammals and bird studies are often used as a proxy for amphibian exposure and effects; however, amphibians are unique within vertebrates due to the dermal pathway. This, combined with the lack of amphibian exposure data, compromises our ability to extrapolate exposure and overall risk from other vertebrate studies. Insufficient data also means that defensible general-form models that determine reasonable exposure levels are in short supply. This also contributes to current uncertainty regarding terrestrial-phase amphibian exposure estimation and protection.

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Data availability—Data and statistical analyses available at: https://github.com/puruckertom/VanMeteretal2016_ple_v_ols

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