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BIOACCUMULATION OF 2,4,6-TRINITROTOLUENE AND POLYCHLORINATED BIPHENYLS THROUGH TWO ROUTES OF EXPOSURE IN A TERRESTRIAL AMPHIBIAN: IS THE DERMAL ROUTE SIGNIFICANT?

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Abstract—Tiger salamanders (*Ambystoma tigrinum*) were exposed via soil and/or food (earthworms) to 2,4,6-trinitrotoluene (TNT) and a PCB mixture (Aroclor 1260) at environmentally relevant concentrations. Four exposures were considered: (1) uncontaminated food + uncontaminated soil (control group); (2) contaminated soil + uncontaminated food (dermal group); (3) contaminated food + uncontaminated soil (oral group); and, (4) contaminated soil + contaminated food (dual-exposure group). The chemical exposure was estimated for each group by analysis of both soil and earthworms. Body burdens of TNT and its primary metabolites were highest in the dermal groups while PCB burdens were highest in the oral groups. Concentrations of the primary TNT metabolites evaluated, 2-amino-dinitrotoluene (DNT) and 4-amino-DNT, exceeded that of unmetabolized TNT and accumulated to 116 and 670 ng/g, respectively. These results provide evidence that dermal exposures to nitroaromatics in terrestrial salamanders may make an important contribution to total body burden and thus may be important when considering the health consequences of such exposures. Further, the demonstration of the accumulation of TNT and TNT metabolites in a primitive vertebrate may have food web modeling implications.

Keywords—Salamander Dermal 2,4,6-Trinitrotoluene Bioaccumulation *Ambystoma tigrinum*

INTRODUCTION

Present legislation requires an assessment of risk to the environment (e.g., wildlife) from exposures to anthropogenic contamination. Currently, several guidance documents exist that are useful [1,2]. Although these documents agree that all potential exposure pathways should be evaluated, few suggestions concerning dermal exposures to wildlife have been made.

Given the variety of organisms comprising any particular terrestrial community and the lack of species-specific data available and applicable to dermal exposure pathways, it is clear why there are few ecological risk assessments that quantify and/or characterize risk from dermal exposures. However, the potential for dermal exposures to some compounds may in fact be considerable in organisms with less impermeable integument. This is logical in fauna that actively respire or osmoregulate through their skin (e.g., amphibians [3]).

In addition, the relatively recent declines in amphibian populations and increases in the rate of developmental abnormalities have provided incentives for investigating these issues [4–6]. The use of amphibians as sentinel species in toxicity evaluations has been suggested by various investigators [7–9]. Furthermore, the contribution of amphibians to the biomass of communities has been shown to be considerable, in some cases exceeding that of nesting birds and equaling that for small mammals [10]. These attributes, coupled with the higher trophic level of urodeles and the terrestrial life history of mole salamanders (family: Ambystomidae) provided the basis for using tiger salamanders (*Ambystoma tigrinum*) as a model to investigate questions concerning exposures to xenobiotics in a soil matrix. *Ambystoma tigrinum* are fairly long-lived (>10 years) [11]. Furthermore, they (and members of their genus) exist predominantly below the soil surface [12,13] and presumably have relatively small home ranges [14].

We chose to investigate the relative contribution of the dermal in relation to the ingestion pathway of exposure to a nitroaromatic, 2,4,6-trinitrotoluene (TNT) in a realistic scenario (in situ) for *A. tigrinum*. The relatively unique chemical/physical properties of TNT compared to those of other organic compounds and the number of military sites where TNT is a soil contaminant of concern provided the initiative for this project [15]. In addition, Aroclor 1260 (PCB mixture) was also used in combination with TNT because the fate and transport of PCBs in the food chain have been well characterized [16,17].

MATERIALS AND METHODS

Treatment preparation

Soil was collected (from a location where species of Ambystomidae were known to be present), dried, pulverized, and sifted through two screens (Nalgene, 1 mm² and 0.5 mm² mesh, Nalgene, Rochester, NY, USA). A PCB mixture (Aroclor 1260; Alltech, Deerfield, IL, USA) was transferred to an acetone diluent, added and mixed into the dry, processed soil, and allowed to evaporate. A portion of this stock mixture was then mechanically mixed for 20 min using a Hobart feed mixer

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(model A120T, Hobart, Troy, OH, USA) in a 12-qt stainless steel bucket to achieve an Aroclor 1260/soil concentration of 67 μg/g dry weight. A redistilled stock of crystalline TNT was obtained from the U.S. Army Research Laboratory (Aberdeen Proving Ground, MD) and determined to be at least 99.4% pure through flame ionization detection, gas chromatography. The TNT crystals were weighed and added to the previously mixed soil containing PCBs to achieve a concentration of 1,000 μg/g dry weight. These test concentrations were determined through a 10-d preliminary range-finding study exposing *A. tigrinum* individuals to 1/10 LC50 concentrations in two, 2/10 log intervals above and below (i.e., five concentrations) [18,19]. The concentrations selected for the 28-d study were found not to produce overt toxicity in the preliminary assay (unpublished data). Manual mixing (vs using a total acetone diluent for PCBs) of the final soil mixture was necessary to preserve the natural microflora of the soil to best emulate natural conditions. Soils used for the control treatments were treated identically to the methods above except without the addition of PCBs or TNT. Approximately 125 g of control soil or chemically treated soil were added to each 2-gallon terrarium (Carolina Biological, Burlington, NC, USA) and subsequently hydrated with approximately 20 ml deionized water.

Salamanders were divided into one of four exposure groups: (1) untreated soil and untreated food (control), (2) treated soil and untreated food (dermal), (3) untreated soil and treated food (oral), and (4) treated soil and treated food (dual-exposure group). Earthworms (*Lumbricus terrestris*) and redworms (*Lumbricus rubellus*; Carolina Biological) were exposed to either of two soil treatments (i.e., untreated, for control and dermal treatments; or treated, PCBs + TNT for oral and dual-exposure treatments) from the identical soil stocks previously discussed. Treated earthworms were fed ad libitum to salamanders in the appropriate treatments. Worms were maintained in the treated soil for a minimum of 10 d prior to feeding. Earthworms served as the only food for the salamanders during the course of the treatments.

Animal husbandry

Adult tiger salamanders (n = 48; field collected and obtained from NASCO, Ft. Atkinson, WI, USA) were evenly distributed by weight into four treatments to minimize any age-specific differences (ANOVA, $df = 3$, $p > 0.96$, overall; $\bar{x} = 34.6 \pm 1.52$ g). Sex could not be determined reliably in this group of animals and thus was unknown at the initiation of treatment. Given the sporadic availability of these animals and the established experimental chronology, animals were placed into treatment upon arrival. All animals were housed individually. Husbandry techniques generally followed Jaeger [20] and included proper temperature monitoring, maintaining proper humidity, and ensuring that at least one worm was added, per animal, every 2 d. More food was added as necessary. Presence or absence of food and/or observation of feeding determined consumption. Animals exhibiting profound overt stress (e.g., excess mucus production, cutaneous/subcutaneous septicemia, i.e., red-leg or moribund) were treated and removed from the study. Animals were exposed for 28 d, euthanized by decapitation, and necropsied, with the bulk of the tissues retained for the chemical analysis of body burdens.

Toxicity

To investigate potential mechanisms and other potential responses to treatment, basic indices of toxicity were measured. These included weekly body weight measures (bw) and organ/bw measures of spleen, liver, and kidney at necropsy. Slices of these excised organs were taken for subsequent histological examination.

Chemical analyses

Soil and food samples were pooled for each treatment and gathered at the beginning, midpoint, and end of the study. Entire carcasses of salamanders with the exception of small slices taken for histological examination were immediately frozen at −30°C until analyzed. The TNT concentrations were determined by the U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM), Directorate of Laboratory Sciences. Samples for PCB analysis were prepared and analyzed by the Pesticide and Organic Chemistry Program in accordance with a modified USACHPPM-POCP SOP 37.2, solvent extraction with gel-permeation clean-up, electron-capture, gas chromatography. Samples for explosive analysis were prepared and analyzed by the Military Unique and Special Chemistry Program in accordance with USACHPPM-MUSCP SOP 51.4. Estimated concentrations were used (i.e., J values) when available if estimable below the method detection limit (MDL). Duplicates were averaged. Concentrations not detected were assumed to be zero. This research was conducted in accordance with Good Laboratory Practices where appropriate.

Data analysis

This study was a factorial design, with the two factors of exposure consisting of the dermal and ingestion pathways. Salamanders were exposed to TNT/PCBs either in their food, soil, both food and soil, or neither food nor soil. The data were tested for normality using a Shapiro–Wilk W test at the $p < 0.05$ level. If the data failed to fit either a normal or log-transformed distribution, they were ranked and analyzed using the ranks. To test the hypotheses of dermal and ingestion effects and their interaction, a two-way ANOVA was used. To test the equal effects of the four treatments, a one-way ANOVA was used. Statistical significance was defined at the $p < 0.05$ level; adequate power was defined as 80% or greater. The mean and standard error of the mean were reported for the data having a normal distribution. The geometric mean and the geometric standard error of the mean were reported for the data having a lognormal distribution. The median and the 5th and 95th percentiles were reported for data that had neither distribution.

A replicate study using *Ambystoma maculatum* (Glade Herp, Fort Meyers, FL, USA) was conducted to investigate the possibility of TNT/PCB facilitated uptake. Sufficient numbers of *A. tigrinum* were not available for this investigation, thus, the syngeneric *A. maculatum* were used. These methods were identical to those described above with the following exceptions: the four treatment groups were composed of a control, TNT-exposed, PCB-exposed, and TNT/PCB-exposed animals. All contaminated treatments consisted of exposure to contaminated soil and food.

RESULTS

Soil concentrations

Initial soil concentrations of TNT at the time of mixing, prior to hydration and 10 d prior to treatment was 1,101 μg/g (Table 1). Polychlorinated biphenyls were considered relatively stable and not analyzed until the beginning of treatment.

Table 1. Soil and worm concentrations of 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2-amino-DNT), 4-amino-2,6-dinitrotoluene (4-amino-DNT), and Aroclor 1260 in μg/g dry weight

	Soil (μg/g)				Worms (μg/g)			
	TNT	2-amino-DNT	4-amino-DNT	PCB[a]	TNT	2-amino-DNT	4-amino-DNT	PCB[a]
Sample dry at mixing	1,101	<1.3	<1.3	ND[b]				
Ambystoma tigrinum assay								
Control—initial sample	<0.23	<0.23	<0.23	0.12	<0.02	<0.01	<0.01	<0.040
Control—midpoint	<0.23	<0.23	<0.23	0.40	<0.02	<0.01	<0.01	<0.040
Control—final	<0.23	<0.23	<0.23	1.27	<0.02	<0.01	<0.01	<0.040
PCB + TNT—initial sample	79.0	38.0[c]	38.0[c]	56.6	0.13	17.3	12.1	6.34
PCB + TNT—midpoint	76.0	15.5[c]	15.5[c]	34.0	0.12	1.82	1.62	0.33
PCB + TNT—final	60.0	12.0[c]	12.0[c]	45.0	0.02	0.14	0.15	2.33
Ambystoma maculatum assay								
Control—initial sample	<1.8	<1.8	<1.8	<1.0	<0.05	<0.05	<0.05	<0.05
Control—midpoint	<2.0	<2.0	<2.0	<1.0	<0.05	<0.05	<0.05	<0.05
Control—final	<1.7	<1.7	<1.7	<1.0	<0.05	<0.05	<0.05	<0.05
TNT—initial sample	738	<1.7	<1.7	<1.0	1.8	5.6	6.4	<0.05
TNT—midpoint	164	60	90	<1.0	0.45	3.4	3.5	<0.05
TNT—final	184	56	82	<1.0	0.33	2.10	2.0	<0.05
PCB—initial sample	<1.7	<1.7	<1.7	55.6	<0.05	<0.05	<0.05	2.13
PCB—midpoint	<1.9	<1.9	<1.9	56.5	<0.05	<0.05	<0.05	5.07
PCB—final	<1.8	<1.8	<1.8	60.8	<0.05	<0.05	<0.05	1.09
TNT + PCB—initial sample	692	<1.6	<1.6	62.3	0.86	3.9	4.1	2.58
TNT + PCB—midpoint	123	60	91	71.3	0.38	2.75	2.85	3.65
TNT + PCB–final	214	61	91	55.5	0.40	2.25	1.95	3.30

[a] The method detection level for PCBs in soil was <0.10 μg/g.
[b] ND = no data.
[c] Method used failed to separate analytes; totals evenly divided between analytes for 2-and 4-amino-DNT.

However, natural attenuation of TNT in soil reduced the soil concentration dramatically (about one order of magnitude) in 10 d and reached an approximate steady state at about 15 d after initial hydration (i.e., 5 d after the start of treatment). This was duplicated in the *A. maculatum* study. The PCB concentrations were variable, which may be due to an uneven distribution of compound and/or may be an artifact of pooled sampling. However, average concentrations across each sample interval approximated the intended mixed concentration. All control soils had undetectable explosive residues. Control soils had background concentrations of Aroclor 1260 <1.3 μg/g.

Food concentrations

Concentrations of TNT in the worms were highest at the beginning of treatment (i.e., after 10 d of initial exposure; Table 1). Concentrations of primary metabolites (2- and 4-amino-dinitrotoluene [DNT]) were also detected at this time. The latter were found to be higher than parent compound concentrations in both experiments. Mean concentrations of PCBs in worms were stable for both studies (though variable), in the range of one order of magnitude less than the soil concentrations.

Body burdens

Because logistical problems prevented a sustained quarantine, infrequent incidences of septicemia (i.e., red-leg) occurred, irrespective of treatment. Moreover, appetite was varied within and among treatments. Therefore, only animals exposed (20 d) and those that had eaten (two times during the experiment) were included in the analyses. Of these, the average salamander ate 6.11 ± 0.55 times.

Power was adequate, greater than 98% in the dermal comparisons that were made for TNT, 2-amino-DNT, and for 4-amino-DNT (Table 2). The data for the PCBs did not confirm a

Table 2. Distribution, power, and statistical test results of compounds detected in *Ambystoma tigrinum* body burdens

Compound	Distribution	Power: two-way ANOVA	Two-way ANOVA	Power one-way ANOVA	One-way ANOVA
2,4,6-Trinitrotoluene	Lognormal	Dermal—99% Ingestion—22% Interaction—5%	Dermal $p < 0.001$	99%	Overall group effect $p < 0.001$
amino-2,6-dinitrotoluene	Lognormal	Dermal—99% Ingestion—75% Interaction—36%	Dermal $p < 0.001$ Ingestion $p = 0.01$	99%	Overall group effect $p < 0.001$
2-amino-2,6-dinitrotoluene	Lognormal	Dermal—99% Ingestion—97% Interaction—12%	Dermal $p < 0.001$ Ingestion $p = 0.001$	99%	Overall group effect $p < 0.001$
PCBs	Neither	NA	Interaction $p = 0.023$	NA	Overall group effect $p = 0.004$

Table 3. Concentrations of 2,4,6-trinitrotoluene (TNT); 2-amino-4,6-dinitrotoluene (2-amino-DNT); 4-amino-2,6-dinitrotoluene (4-amino-DNT); and Aroclor 1260 in *Ambystoma tigrinum* and *Ambystoma maculatum* in μg/kg dry weight[a]

Treatments	TNT	2-Amino-DNT	4-Amino-DNT	PCBs
A. tigrinum				
Control	0.00 ± 0.00 A (6)	0.00 ± 0.00 A (6)	0.00 ± 0.00 A (6)	9.0 (5.0–110) A (5)
Oral	2.39 ± 0.19 A (6)	16.46 ± 0.58 B (6)	22.10 ± 0.82 B (6)	1,960.0 (1,010–2,870) B (6)
Dermal	16.78 ± 0.47 B (6)	67.03 ± 0.35 C (6)	532.79 ± 0.49 C (6)	550.0 (500–570) A (4)
Dual-exposure	29.97 ± 1.34 B (6)	291.95 ± 2.19 C (6)	971.63 ± 2.46 C (6)	1,965 (1,210–3,170) B (6)
A. maculatum				
Control	0.00 ± 0.00 (2)	0.00 ± 0.00 (2)	0.00 ± 0.00 (2)	150.0 ± 5.00 (2)
TNT	170.0 ± 0.00 (2)	940.0 ± 46.0 (2)	755.0 ± 85.0 (2)	ND (2)
PCB	0.00 ± 0.00 (3)	0.00 ± 0.00 (2)	0.00 ± 0.00 (2)	2,136.7 ± 960 (3)
PCB/TNT	<49.0 (1)	1,450.0 ± 0.00 (1)	840.0 ± 0.00 (1)	4,770.0 ± 0.00 (1)

[a] All values are presented with geometric means, SEM; except medians and 5% to 95% for PCB data. Treatment values followed by different letters are different at $p < 0.05$. Sample sizes are noted in parentheses.

normal or a lognormal distribution; therefore the power was not determined. Analyses of these data were performed on the ranks. No other biotransformation product of TNT approached the concentrations of these primary reduction products.

The results of the two-way ANOVA revealed a dermal contribution for TNT uptake but no ingestion contribution or interaction. This implies that dermal exposures to TNT/PCBs made the greatest contribution to the bioaccumulation of TNT. The results of the one-way ANOVA showed an overall group effect. The control and the oral groups had lower TNT concentrations than the dermal and dual-exposure groups (Table 3).

The two-way ANOVA showed both a dermal and an ingestion effect, contributing to 2-amino-DNT concentrations, but no significant interaction. This implies that both dermal and ingestion exposures to PCBs/TNT contributed to the bioaccumulation of 2-amino-DNT. The results of the one-way ANOVA revealed an overall group effect. The control and oral groups had lower 2-amino-DNT concentrations than the dual-exposure group.

The results of the two-way ANOVA demonstrated a dermal effect and an ingestion effect compared to controls, but no interaction for 4-amino-DNT. This implies that both dermal and ingestion exposure to PCBs/TNT were related to the bioaccumulation of 4-amino-DNT. The one-way ANOVA resulted in an overall group effect. The control and the oral groups had lower concentrations of 4-amino-DNT than the dermal and dual-exposure groups.

The results of the two-way ANOVA revealed an ingestion/dermal interaction contributing to PCB burdens. The one-way ANOVA showed an overall group effect. The control group had lower PCB concentrations than the oral and dual-exposure groups only.

Because availability of animals was limited for the *A. maculatum* study, each treatment included six individuals. At the completion of the study, four of six controls, two of six TNT-, five of six PCB-, and two of six PCB/TNT-treated animals survived until necropsy, too few to do meaningful statistical comparisons. However, mean concentrations of the compounds of interest were calculated for comparison purposes. These were comparable to those of the previous study in *A. tigrinum*.

Treatment-related effects

No treatment-related changes in relative organ weight were found (Fig. 1). There were no significant changes in body mass over the course of the exposures (ANOVA; $p > 0.67$ for all treatments).

Tissues were processed using routine histological techniques, stained with hematoxylin and eosin, and examined by light microscopy. Because these animals were field collected and thus previous histories were not known, these results must be considered preliminary. Histopathological examinations of the spleen, liver, and kidney revealed that an increased incidence of alterations was associated with contaminant exposure (i.e., oral, dermal, and dual-exposure) relative to control animals (data not shown). Briefly, kidneys of all control animals were essentially normal, with no obvious patterns of kidney changes among treatments. However, some salamanders ($n = 3$) in the dual-exposure group exhibited generalized renal nephrosis and/or necrosis. Livers and spleens were adversely affected in all but one animal in the dual-exposure group. In spleens, the predominant effect included focal to multifocal necrosis with varying degrees of severity (i.e., size of the necrotic area). Three animals in the dermal group displayed essentially normal tissue in all three organs, while others had incidences of liver and spleen necrosis and focal to diffuse splenic lymphoid loss. All animals in the oral group displayed varying degrees of liver necrosis; one spleen was characterized with mild, diffuse lymphoid loss. A few cases of parasitic infestations were noted.

Some individuals in both studies, predominantly the *A. maculatum* study were affected by septicemia and skin ulcerations. Cultured samples from infections in animals in the *A. maculatum* study indicated the presence of *Staphylococcus epidermis* and *Staphylococcus hominis*, two ubiquitous bacteria. An investigation of septicemia in one *A. tigrinum* individual detected *Vibrio* sp. Histological examinations of these ulcerated skin lesions (predominanlty associated with the caudal region) were equivocal. Because the number of cases were roughly equivalent across treatments, the exact causes are unknown. However, the development of these incidences is consistent with shipping-related stresses. Fewer cases were observed as the studies progressed.

DISCUSSION

Risks from exposure to xenobiotics are rarely assessed in amphibians, although many indications have been reported that these animals may represent sensitive sentinel organisms with potential efficacy in toxicological studies [7–9]. Permeable integument, complex life histories, and physiological meta-

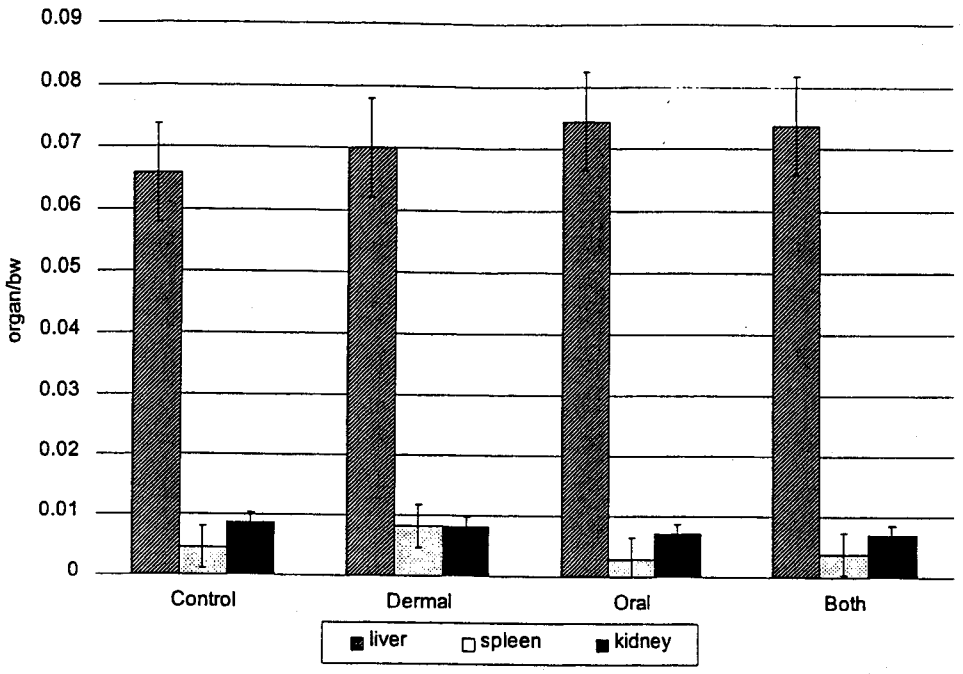

Fig. 1. Relative organ/body weight ratios of *Ambystoma tigrinum* according to treatment.

morphosis add to the difficulties in assessing risks of chemical exposure to amphibians, particularly in a modeled effort. However, given these constraints, we have attempted to evaluate the relative importance of two routes of exposure (dermal and oral) in a terrestrial amphibian genus and have identified a significant p in the estimation of risk to these organisms when the dermal route is not considered. A similar result was reported for American toads (*Bufo americanus*), where dermal exposures to methoxychlor in water contributed an order of magnitude greater to the body burdens than the oral exposures [21].

The amphibian integument is a complex, vital organ. The majority of respiration occurs through the gas exchange capabilities of the skin, as much as 70% in mole salamanders (family Ambystomidae) to 95% in species of lungless salamanders (family Plethodontidae) [3]. Many species of these families are predominantly terrestrial [12–14], including the *Ambystoma* spp. considered in the present studies. Given these physiological differences between salamander skin and the skin of other terrestrial nonamphibian vertebrate species, it is reasonable to assume that amphibian integument may differ in its permeability to certain pollutants within the soil matrix, as compared to the skin of many other terrestrial vertebrate species. Therefore, as the results of this study indicate, any comprehensive ecological risk evaluation neglecting the dermal route of exposure in amphibians would likely underestimate the total systemic exposure and result in inaccurate characterization of potential effects.

In mammals, TNT has been shown to absorb well across the skin. Using ^{14}C-labeled TNT in a soil matrix, 3 to 5% of the label was found to traverse excised pig skin in an ex vivo situation [22]. These data were consistent with in vivo assays conducted by the same author, leading to the conclusion that rmal absorption was a predominant route of exposure for NT uptake from a contaminated soil matrix applied to the skin. Considering the relatively thin, moist, physiologically active integument of many amphibians, it seemed reasonable to hypothesize that dermal absorption may similarly constitute a significant route of TNT uptake in species such as *A. tigrinum* and *A. maculatum*. Although differences were observed (i.e., in species, media, and compound), our results were consistent with the findings of Hall and Swineford [21].

The relative contribution of contaminated food consumption to overall body burden of chemical pollutants has been the focus of several studies in aquatic organisms [23,24], although less so for terrestrial vertebrate systems [25,26]. Measures of fat solubility (often evaluated through the *n*-octanol/water partition coefficient, K_{ow}) have been used to determine the probability and magnitude of uptake for organic compounds through ingestion [27,28] and are heavily relied upon in risk assessments. However, field verification and replication of food-chain exposures are often problematic [29–31].

Salamanders are opportunistic foragers. A study investigating the metabolic demands and food availability of *Plethodon jordani* revealed that many individuals operated on a periodic daily negative energy budget [32]. Rainfall events and ambient temperature were criteria cited as most important in determining capture rate and, as such, suggest that some species are pulse feeders, eating when opportunities are maximized. If this is true of *Ambystoma,* then oral exposures may vary and be most important during (or after) pulse-feeding events. We found appetite to be varied, often most voracious once weekly, though food was offered every other day. Moreover, capture rate of earthworms was less than accurate, capturing prey in about one in three attempts when hand fed (personal observation). In these species, estimation of food intake would require a greater understanding of food capture rates, as well as an understanding of differential fat accumulation and metabolism that may liberate lipophilic compounds and thus periodically enhance systemic contaminant concentrations.

Ignoring these criteria and making daily estimates on exposure are likely to be inaccurate and suggest the need for species-specific, holistic evaluations in the determination of chemical bioaccumulation in potential target/sentinel species such as terrestrial salamanders. These studies were therefore designed to evaluate the relative contributions of oral and/or

dermal exposures to chemical uptake in salamanders and the potential differences between the exposures of two dissimilar compounds in a realistic setting.

These studies proved difficult for several reasons, including obtaining a sufficient number of similar-sized research animals and maintaining sufficient numbers healthy for the duration of the studies (particularly the *A. maculatum* study). This is common in studies that depend on field-collected individuals. The loss of study animals to septicemia seemed to be related to shipping-related stresses, because incidences of such were not associated with treatment and decreased with time. This is consistent with other studies we have conducted where extended periods of quarantine were necessary until all incidences of septicemia had ceased. Further, other confounding variables associated with field-collected amphibians must also be considered (e.g., age differences, parasite load, previous environmental stresses, bacterial exposures, etc.). Logistical constraints did not allow for an extended quarantine in these assays, but these and other confounding criteria must be considered in the proper evaluation of these data. However, we consider it unlikely that these influences would have any significant effect on the alteration of contaminant load in the total burden of these salamanders. A subsequent study conducted with only healthy *A. tigrinum* has corroborated these exposure data (unpublished data).

The most interesting observation in these studies is the importance of the dermal route of exposure to the accumulation of TNT and particularly the primary metabolic reduction products of TNT, which may be of toxicological importance to higher trophic level species. Specifically, though both a dermal and ingestion route were shown to be important to the total body accumulations, the dermal route was found to be more important than the oral route for the bioaccumulation of both the parent compound and its metabolites in salamanders. In contrast, PCB burdens were found to be highest in salamanders exposed by the oral route. The latter observation is reasonable, considering the lipophilic nature of PCBs and the moist skin of the study salamanders. An understanding of the specific physiochemical properties of chemicals that are most important in amphibian dermal uptake is greatly needed for any future model development that accurately characterizes exposure.

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