Science of the Total Environment 630 (2018) 517–525

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Derivation of avian dermal LD50 values for dermal exposure models using in vitro percutaneous absorption of [^{14}C]-atrazine through rat, mallard, and northern bobwhite full thickness skin

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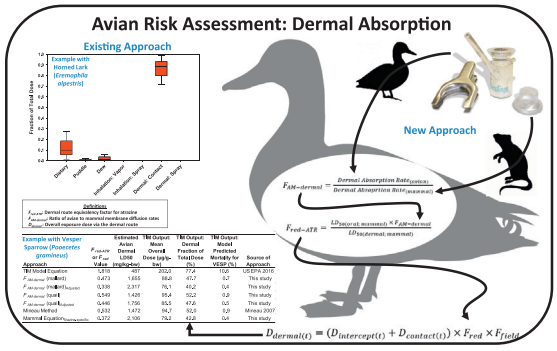
HIGHLIGHTS

- Estimation of avian dermal LD50s is critical for assessing chemical risk to birds.
- Current models generate high dermal exposure estimates.
- These models also generate low dermal LD50 estimates.
- Empirical dermal absorption data were used for an alternative approach.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 December 2017
Received in revised form 15 February 2018
Accepted 17 February 2018
Available online 24 February 2018

Editor: Henner Hollert

Keywords:
Atrazine
Avian dermal absorption
Avian risk assessment
Dermal exposure modeling
Terrestrial investigation model

ABSTRACT

Understanding dermal exposure is important for higher-tier avian ecological risk assessments. However, dermal exposure and toxicity are often unknown for avifauna. The US EPA's Terrestrial Investigation Model (TIM) uses a method to estimate avian dermal LD50 values (and ultimately dermal exposure) that frequently results in unusually high dermal exposure and low dermal LD50 estimates. This is primarily a result of using organophosphate and carbamate toxicity data to develop the oral-dermal relationship. An estimated dermal LD50 is necessary to generate a dermal route equivalency factor that normalizes potency relative to oral toxicity within the dermal pathway dose equation. In this study, atrazine dermal absorption experiments were conducted with mallard, northern bobwhite, and rat skin. These data were used to derive an avian-mammal dermal route equivalency factor for atrazine and introduce a new approach for estimating dermal LD50 values and ultimately predicting exposure via the TIM dermal pathway. Compared to the default TIM method, this new approach yielded TIM output with lower mean total dose, lower dermal fraction of total dose, greater oral fraction of total dose, and reduced model predicted mortality for atrazine. In addition, the new approach was compared with other methods for estimating avian dermal LD50 values such as those proposed for use with mammalian data and physico-chemical properties and a triazine-specific oral-dermal equation using mammalian LD50 data. The three alternative approaches resulted in output similar to one another and different from the default TIM methods. These results indicate that a dermal route equivalency factor derived from empirical data provides a higher avian dermal LD50 estimate that is consistent with other methods. In addition, the use of this dermal route equivalency factor results in greatly reduced modeled atrazine risk to birds than previously reported in US EPA risk assessments using TIM.

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https://doi.org/10.1016/j.scitotenv.2018.02.206
0048-9697/

1. Introduction

Understanding dermal exposure is important for higher-tier ecological risk assessments of crop protection chemicals to avifauna (Hudson et al., 1979; Mineau, 2011, 2012). Historically, generation of dermal absorption and toxicity data have focused on mammals (e.g., rat and mouse) as a surrogate for human exposure, while dermal-based toxicity data for birds, reptiles, and amphibians have been comparatively sparse (Mineau, 2012; Weir et al., 2015). For birds, most of our knowledge regarding the role of dermal exposure and the relationship between toxicity endpoints (i.e., median lethal doses [LD50 values]) from dermal exposure and toxicity endpoints from dietary exposure is almost exclusively based on organophosphate, carbamate, and organochlorine class pesticides (Schafer et al., 1973; Hudson et al., 1979; Driver et al., 1991; Mineau, 2012).

Despite the lack of avifauna-specific data across other chemical classes and mechanisms of action, understanding the contribution of dermal exposure is an important component for higher-tier, probabilistic avian risk assessment models such as the U.S. Environmental Protection Agency's (US EPA) Terrestrial Investigation Model (TIM) (US EPA, 2015). The TIM predicts fractions of exposure attributed to multiple exposure routes including dietary, drinking water from puddles, drinking water from dew, inhalation of volatilized pesticide, inhalation of spray, dermal contact with contaminated foliage, dermal exposure through direct spray, and spray drift transport to edge habitats. From each of these routes, the model predicts an overall exposure estimate for assessing risk. In recent avian risk assessments that have utilized TIM, dermal exposure dose estimates for some species have been approximately nine times higher than oral exposure dose estimates. This has also led to an elevated overall predicted exposure dose (e.g., US EPA, 2016). The goal of this study was to examine the mechanics of the dermal exposure model component of TIM to assess the validity of these estimates and outline proposed alternative approaches as they relate to atrazine.

2. Model background and rationale

The US EPA's TIM estimates exposure to pesticides via multiple routes including dietary, drinking water, inhalation, dermal, and spray (US EPA, 2015). The dermal exposure component of TIM utilizes a ratio of oral to dermal LD50 values (mg/kg-bw) called the dermal route equivalency factor (F_{red} [unitless]) that functions to normalize potency relative to oral toxicity (i.e., creates an oral dose equivalency):

$$F_{red} = \frac{LD50_{(avian\ oral)}}{LD50_{(avian\ dermal)}} \quad (1)$$

The F_{red} has an important role in the TIM calculation for overall exposure dose via the dermal route (i.e., D_{dermal} [μg/g-bw]) which includes both contact and aerial interception terms. It acts as a multiplicative factor in the dermal pathway equation:

$$D_{dermal(t)} = \left(D_{intercept(t)} + D_{contact(t)}\right) \times F_{red} \times F_{field} \quad (2)$$

However, avian dermal toxicity data are not part of many data packages and a modeled LD50 value must be estimated. Within TIM, dermal LD50s are estimated using a regression equation of avian dermal and oral LD50 (mg/kg-bw) data (Fig. 1) for organophosphate (OP) and carbamate (CA) insecticides:

$$\log LD50_{(dermal)} = 0.84 + 0.62 \times \log LD50_{(oral)} \quad (3)$$

For compounds lacking dermal LD50 data, estimates are derived by solving for the dermal LD50 using oral LD50 data and the slope and intercept of this regression equation. Historically, there has been criticism regarding the strength of the relationships between oral and dermal LD50 values within this dataset (i.e., $R^2 = 0.30$) (US EPA, 2004). In addition, the equation above has been applied to pesticides with different mechanisms of action despite the underlying data consisting almost exclusively of cholinesterase (ChE) inhibitors. In 2004, the US EPA convened a Federal Insecticide Fungicide and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) that included a review of the dermal LD50 estimation approach in TIM (US EPA, 2004). The SAP concluded that this approach should not be used to interpret dermal exposure beyond the OPs (US EPA, 2004). They cited that the relationship between toxicity and exposure between oral and dermal exposure routes is not strong and that the approach should be verified with other species and compounds prior to being used universally in risk assessments.

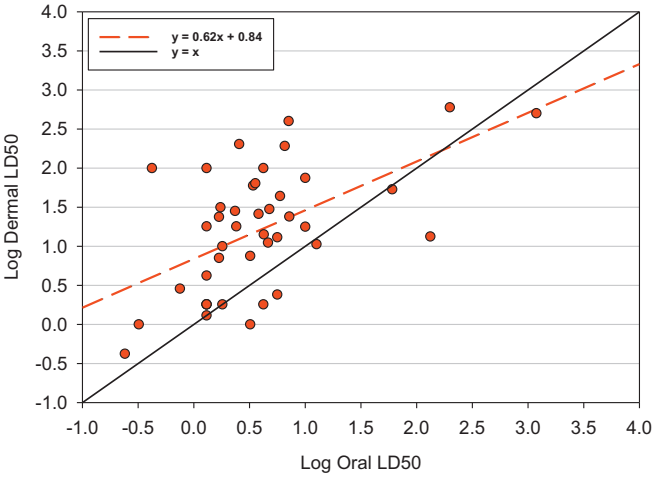

Fig. 1. Data and equation used for estimating avian dermal LD50s in US EPA's Terrestrial Investigation Model (TIM). The data are reproduced from Fig. H-1 in Appendix H from US EPA (2015). The solid line is for reference to a 1:1 relationship and to identify the inflection point at log oral LD50 = 2.20997.

Compounds that are relatively non-toxic to birds, such as atrazine, with comparatively higher oral LD50 values are outside the primary data cloud for OPs and CAs. Furthermore, compounds with high oral LD50 values suffer from an issue with the inflection point when using the OP and CA equation (Eq. (3)) to estimate dermal LD50 values. The inflection point where the TIM oral-dermal equation crosses the 1:1 line is approximately log oral LD50 = 2.20997 (Fig. 1). Thus, for oral LD50 values greater than the inflection point, dermal LD50 values are always lower. The inflection point issue is likely an artifact of the weak relationship between oral and dermal OP and CA LD50 values and translates into a significant flaw in the current TIM approach for estimating avian dermal LD50 values for less toxic compounds.

For relatively non-toxic compounds with high oral LD50 values, this approach results in calculated dermal LD50 values that may be significantly biased low (i.e., much greater toxicity than the oral LD50). In addition, this bias results in a F_{red} value of >1 and a multiplicative effect on the overall dermal exposure estimate in TIM (Eq. (2)). For example, using the current approach, an oral LD50 of 2000 mg/kg-bw would correspond to an avian dermal LD50 of 770 mg/kg-bw and an F_{red} of 2.6. Estimates of dermal exposure from interception and contact would then be multiplied by the F_{red} value of 2.6. In some examples, this has led to the fractional dose attributed to the dermal route to be several times greater (e.g., atrazine, US EPA, 2016), and in some cases up to nine times greater (e.g., horned lark, Fig. 2), than the fractional dose attributed to food ingestion for relatively non-toxic compounds. This is not representative of use patterns, toxicity profiles, and physicochemical properties of the compound examined. Generally it is expected for dermal LD50s to be greater than oral LD50s. This was observed for avian data on OP's and carbamates (84.1% dermal LD50 > oral LD50; $n = 44$; Appendix H, US EPA, 2015), avian data across many types of chemicals (84.5% dermal LD50 > oral LD50; $n = 97$; Mineau, 2012), mammal data across many types of chemicals (91.4% dermal LD50 > oral LD50;

$n = 35$; Mineau, 2012), mammal data for triazines (Table 1), and across other taxa for compounds varying in chemical class (Schafer et al., 1973; Hudson et al., 1979; Henderson et al., 1994; Brooks et al., 1998; Weir et al., 2015).

The magnitude of the F_{red} anomaly on overall exposure estimates in risk assessments is not inconsequential and has led to elevated model outputs including estimates of avian mortality (TIM) and reproductive output from the Markov Chain Nest Productivity Model (i.e., MCnest) that are used to inform risk assessments and regulatory decision making (US EPA, 2016).

One potential approach to solve this problem is the use of an avian to rat dermal absorption correction factor. A precedence for such a correction factor exists in TIM, as an analogous approach is used for avian inhalation toxicity. Specifically, estimates of an avian inhalation LD50 from oral LD50 data is derived from rat LD50 data using an avian-mammal pulmonary tissue correction factor. When avian inhalation toxicity data are available TIM uses the following equation to derive the oral dose equivalency factor (F_{re} [unitless]):

$$F_{re} = \frac{LD_{50(oral;avian)}}{LD_{50(inhalation;avian)}} \quad (4)$$

The F_{re} is used to convert inhalation dose to oral dose equivalents (US EPA, 2015). However, when avian inhalation toxicity data are not available, TIM uses the relationship between the rat acute oral and inhalation LD50 values to derive a route equivalency factor (F_{re}). This F_{re}, however, must account for differences in respiratory physiology between birds and mammals. The US EPA successfully did this by identifying pulmonary membrane (PM) diffusion rate estimates for birds and mammals to calculate a ratio of the avian to mammal pulmonary membrane diffusion rates (F_{AM} [unitless]) (US EPA, 2004):

$$F_{AM} = \frac{PM\ Diffusion\ Rate_{(avian)}}{PM\ Diffusion\ Rate_{(mammal)}} \quad (5)$$

The relative diffusion rate across the pulmonary membrane is typically between 2.4 and 3.4 times greater in birds than mammals. F_{AM} is then used to produce a modified inhalation route equivalency factor

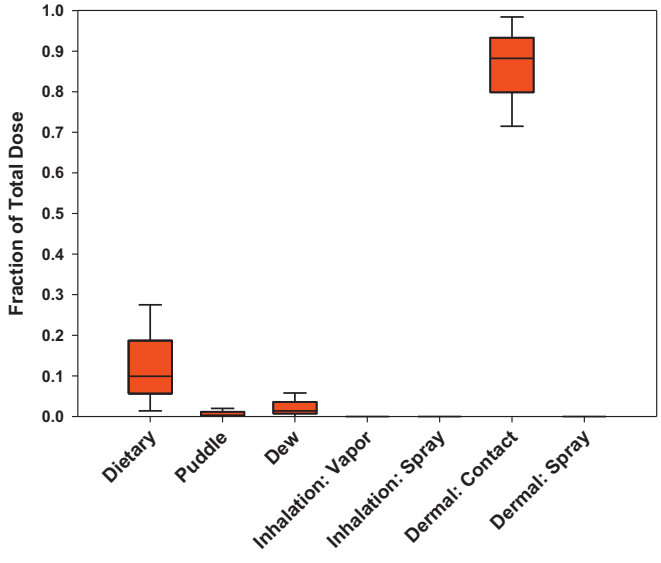

Fig. 2. Fractional atrazine doses for seven exposure pathways for horned lark (*Eremophila alpestris*) predicted by the Terrestrial Investigation Model (TIM) using the default model approach to estimate the avian dermal LD50. Using this approach, the model suggests that approximately 88.2% of the total atrazine dose is attributed to the dermal contact pathway. All TIM model inputs are provided in the Supplemental Data.

Table 1
Mammal oral and dermal LD50s for selected triazines. All LD50s are mg/kg-bw.

Compound	Rat oral LD50	Log rat oral LD50	Mammal dermal LD50[a]	Log mammal dermal LD50	Source
Atrazine	1869	3.27	>3100	3.49	US EPA, 2016, WHO, 2007
Terbuthylazine	1000	3.00	>2000	3.30	US EPA, 1995
Propazine	>5050	3.70	>5050	3.70	US EPA, 1998
Simazine	>5000	3.70	>2000	3.30	US EPA, 2006
Ametryn	1009	3.00	>2020[b]	3.31	US EPA, 2005
Prometryn	1802	3.26	>3170	3.50	US EPA, 1996

[a] All rat data except where noted.
[b] Rabbit.

for birds using mammal toxicity data:

$$F_{re} = \frac{LD_{50(oral;mammal)} \times F_{AM}}{LD_{50(inhalation;mammal)}} \quad (6)$$

Utilizing the same rationale and approach, an avian-mammal dermal route equivalency factor for atrazine is proposed using data generated from a standardized dermal absorption study with mallard, northern bobwhite, and rat skin. To estimate a ratio of avian to mammal epidermal membrane diffusion rates ($F_{AM\text{-}dermal}$) the following approach is proposed:

$$F_{AM-dermal} = \frac{Dermal\ Absorption\ Rate_{(avian)}}{Dermal\ Absoprtion\ Rate_{(mammal)}} \quad (7)$$

$F_{AM\text{-}dermal}$ will be used to calculate a new F_{red} called $F_{red\text{-}ATR}$ that is specific for atrazine using the following equation which will replace Eq. (1):

$$F_{red-ATR} = \frac{LD_{50(oral;mammal)} \times F_{AM-dermal}}{LD_{50(dermal;mammal)}} \quad (8)$$

Ultimately we propose to use $F_{red\text{-}ATR}$ as a replacement for F_{red} in the calculation of the overall dermal exposure estimate for atrazine (Eq. (2)). Here data generated to derive an avian-mammal dermal route equivalency factor ($F_{AM\text{-}dermal}$) for atrazine using mallard, northern bobwhite, and rat dermal absorption data are reported. Atrazine is specifically addressed as a case-study; however, the broader goal is to develop a framework and approach that can be used for other compounds and applied universally such as the approach used for F_{AM} within TIM to more accurately depict potential risks to avifauna. In addition to introducing this new approach, the results are compared with other methods for estimating avian dermal LD50 values such as those proposed for use with physico-chemical properties (Mineau, 2007) and a triazine-specific oral-dermal equation using mammalian LD50 data.

3. Materials and methods

3.1. Dermal absorption study: general

A dermal absorption study was conducted to: 1) determine the suitability of mallard (*Anas platyrhynchos*) and northern bobwhite (*Colinus virginianus*) skin as in vitro models for predicting dermal absorption in these species and 2) evaluate and compare the dermal absorption of atrazine relative to skin obtained from the standard rat model species (*Rattus norvegicus*). The study was conducted following OECD regulatory guidelines and Buist et al. (2017). Eight replicate samples of skin were obtained from four individuals of each species (mallard, northern bobwhite, and rat) for the study. Complete details of the study can be found in the Supplemental information.

Test materials included radiolabelled [^{14}C]-atrazine (radiochemical purity: 97.2%; specific activity: 61.8 μCi/mg), technical grade atrazine (purity: 97.5%), formulated atrazine (AAtrex® 4 L; density: 1.1 g/cm^3), and blank formulation (AAtrex® 4L Blank: contains all formulation components except atrazine).

3.2. Dermal absorption study: preparation of avian and rat dermal membranes

Each sample of mallard and northern bobwhite skin was removed from frozen storage and allowed to reach ambient temperature. The area of interest was a downy feathered region below the wing junction for mallard and a small featherless region below the wing junction for quail and was removed using a scalpel. Fine downy mallard feathers were removed using clippers and the thickness of the skin (approximately 700 μm for mallard and 100 μm for northern bobwhite) was measured using a micrometer. For mallards, it was estimated that the excised skin would be sufficient for at least 2 static cells (0.64 cm^2 exposed area). An attempt was made to dermatome the skin sample to a standard depth of 400 μm. However, the high fat content resulted in the blade being unable to grip/cut the skin. It was therefore concluded that it would be necessary to use full-thickness mallard skin for the dermal absorption test. For quail, it was estimated that the excised skin would be sufficient for at least 1 static cell (0.64 cm^2 exposed area). No attempt was made to prepare split-thickness skin as the membrane was already very thin. It was therefore concluded that it would be necessary to use full-thickness quail skin for the dermal absorption test.

Eight samples of full-thickness rat skin were obtained from four adult male Crl:CD (SD) rats aged between 6 and 8 weeks old with bodyweights between 200 and 250 g from Charles River Margate, UK. On arrival on dry ice, the samples were stored in a freezer set to maintain a temperature of −20 °C until they were used in the study. The age, sex, weight range, and strain of the animal from which the skin was taken were recorded.

3.3. Dermal absorption study: confirmation of radiochemical purity of [^{14}C] atrazine

An aliquot (10 μL) of [^{14}C]-atrazine was dissolved in acetonitrile (500 μL) to make a radiolabeled stock solution. A UV standard was prepared by dissolving atrazine (G30027) primary standard (3.10 mg) in acetonitrile (3 mL). The UV standard (10 μL) was added to the radiochemical solution (100 μL) and analyzed by high performance liquid chromatography (HPLC). The radiochemical purity of [^{14}C]-atrazine was determined by HPLC using the following equipment and conditions: HPLC Model: Agilent 1260; Radio-detector Model: Beta-Ram 4; Scintillant: ProFlow G+; Scintillant Flow Rate: 2.0 mL/min; Flow Cell: Liquid (200 μL); Column: Inertsil ODS 3 (150 mm × 4.6 mm, 5 μm); Solvent A: Acetonitrile:Water (1:1, v/v); Mobile Phase Conditions: Isocratic; Run Time: 30 min; Flow Rate: 1.0 mL/min; Column Temperature: 25 °C; Auto-sampler Temperature: 4 °C; and U.V. Detector Wavelength: 210 nm.

Data were captured by Laura software (LabLogic). The chemical authenticity of the radiolabeled test item was confirmed by co-chromatography with non-radiolabeled test item. The radiochemical purity of [^{14}C]-atrazine was determined to be 98.1%.

3.4. Dermal absorption study: preparation of [^{14}C]-atrazine labeled formulation rate

Zirconium silicate beads (TypZ 0.8 1.0 mm) were weighed into a 4.5 mL Fast Prep® tube. [^{14}C]-atrazine radiochemical was transferred into the tube and the solvent was removed under a gentle stream of nitrogen gas. AAtrex 4L Blank was also added and contents were mixed by FastPrep 24™. CIPAC D Water was added in small aliquots and the contents were mixed by FastPrep 24™. A magnetic stir bar was added and the formulation was stirred continuously. Six aliquots (6.4 μL) were taken into vials, mixed with methanol:scintillation fluid and analyzed by liquid scintillation counting.

3.5. Dermal absorption study: static diffusion cell apparatus and procedures

A static diffusion cell system (PermeGear Inc.) was used for these assays. The static diffusion cells were placed in a manifold on a magnetic stir plate heated via a circulating water bath to maintain the skin surface temperature at 32° ± 1 °C. The diffusion cell temperatures (ranging from 32.1° to 32.6 °C) were calibrated prior to mounting of the skin sample. The surface area of exposed skin within the cells was 0.64 cm^2. The receptor chamber volume was 5 mL, with each receptor chamber individually marked with the actual volume.

The receptor fluid chosen for use in this study was phosphate buffered saline containing polyoxyethylene 20 oleyl ether (PEG, 6%, w/v), sodium azide (0.01%, w/v), streptomycin (0.1 mg/mL), and penicillin (100 units/mL). The pH of the receptor fluid was checked and adjusted to pH 7.35.

The receptor chambers were placed in a manifold and connected to a circulating waterbath. Magnetic stir bars were placed in the receptor fluid chambers which were filled with receptor fluid. Once ambient temperature was reached, sections of skin (1.5 × 1.5 cm) were cut and mounted in the diffusion cells between the donor and receptor chamber. The donor chamber was tightened into place with a clamp. Cells were visually checked to ensure no cells were leaking and no air bubbles were present in the receptor fluid chamber.

For an application of 10 μL/cm^2 over a 0.64 cm^2 application area, 6.4 μL of formulation was applied to each skin sample to result in the highest labeled formulation concentration (25.2265 g/L). Theoretically, if 100% of atrazine was absorbed the resulting concentration in the receptor fluid would be 32.3 mg/L.

The [^{14}C]-atrazine labeled rate dilution was applied evenly over the *stratum corneum* surface of 8 samples of rat, mallard, and northern bobwhite skin using a Rainin MR25 positive displacement pipette set to deliver 6.4 μL (10 μL/cm^2). The donor chambers of the cells were not occluded. Seven representative aliquots (mock doses) of the test preparation were dispensed into vials at the time of dosing, mixed with methanol:scintillation fluid, and analyzed by liquid scintillation counting.

Receptor fluid aliquots were collected at 2, 4, 6, 8 and 12-h post dose. All receptor fluid samples were analyzed by liquid scintillation counting. The exposure was terminated by washing and drying the skin at 6 h post dose. After an 18-h post exposure monitoring period (i.e., at 24 h post dose) the skin was again washed, dried, and then analyzed. The donor chamber was transferred to a pre-weighed container with ultrapure water (40 mL). The skin was removed from each cell and placed on a piece of tissue to remove any remaining receptor fluid from the underside of the skin. This tissue was placed into the pre-weighed receptor chamber wash container for that particular cell.

The *stratum corneum* was removed with 20 successive tape strips using Scotch® tape. The skin sample was rotated 90° after each tape strip. If any epidermis was removed, the rotation of the skin between each tape strip was stopped. Each tape strip was placed into an individual vial containing methanol:scintillation fluid and then analyzed by liquid scintillation counting. The skin under the cell flange (unexposed skin) was cut away from the exposed skin. The exposed and unexposed skin samples were placed into separate vials containing Solvable® (2 mL). The skin samples were placed into a waterbath set to 60 °C to aid solubilisation. Stannous chloride solution (0.2 g/mL in ethanol; 150 μL) and scintillation fluid were added to each skin sample. Samples were analyzed by liquid scintillation counting.

Donor chambers were extracted in solvent for 30 min before sonication (10 min). Duplicate weighed aliquots (1 mL) of the sample were removed, mixed with scintillation fluid, and analyzed by liquid scintillation counting.

The bulk receptor fluid was removed from each receptor chamber and retained in a vial. This was split into two parts, with 2.5 mL of each sample being transferred into a new vial. Scintillation fluid was added and all receptor fluid samples were analyzed by liquid scintillation counting.

The receptor chambers were rinsed with solvent (20 mL). The solvent was pooled as a single sample into a pre-weighed receptor wash pot. Duplicate weighed aliquots (1 mL) of the sample were removed, mixed with scintillation fluid, and analyzed by liquid scintillation counting.

All samples were counted together with representative blanks using a liquid scintillation analyzer (Packard 2100-TR) with automatic quench correction by external standard. Where scintillation fluid was added to the samples, the volume was 10 mL. Where methanol:scintillation fluid was added, the volume was 12 mL. Representative blank sample values were subtracted from sample count rates to give net d.p.m. per sample. Prior to analysis, samples were allowed to stabilize with regard to light and temperature. Full details on dermal absorption study methods can be found in the Supplemental data.

3.6. Approach 1 for estimating avian dermal LD50: avian-mammal dermal route equivalency factor using dermal absorption data

An avian-mammal dermal route equivalency factor was derived using data generated from the standardized atrazine dermal absorption study on mallard, quail, and rat skin. The ratio of avian to rat epidermal membrane diffusion rates was used to determine $F_{AM\text{-}dermal}$ (Eq. (7)). The $F_{AM\text{-}dermal}$ for both mallard and northern bobwhite, the rat oral LD50 for atrazine (1869 mg a.i./kg-bw), and the rat dermal LD50 for atrazine (3100 mg a.i./kg-bw) were used to calculate $F_{red\text{-}ATR}$ (Eq. (8)). Finally, $F_{red\text{-}ATR}$ was used in lieu of F_{red} for estimating the avian dermal LD50 for atrazine (Eq. (1)).

3.7. Approach 2 for estimating avian dermal LD50: Mineau method

A second approach to estimate avian dermal LD50 values was initially described by Hudson et al. (1979) and further advanced by Mineau (2007, 2012). The underpinnings of this method is to utilize the vast quantity of mammalian dermal toxicity data to generate a dermal toxicity index (DTI [unitless]) for each compound and use this information to predict avian dermal toxicity. Mineau (2007, 2012) used 51 oral-dermal comparisons for birds and found that model fit was vastly improved if direct-acting toxicants (i.e., compounds in which the parent molecule causes toxicity) were separated from indirect-acting toxicants (e.g., organophosphates in which the oxon metabolite is the cholinesterase-inhibitor). There is a significant physiological difference in the mechanism of action (MOA) between these two groups of chemicals as one involves direct metabolism to become the active molecule. The data from which the TIM equation (Eq. (3)) is generated does not distinguish between direct- and indirect-acting chemicals. Model fit was improved further by considering additional physico-chemical parameters within the equation. For direct-acting toxicants, Mineau (2007) proposed the following equation for deriving avian DTIs and it included vapor pressure (*VP* [mPA]), molecular weight (*MW*), and oral-dermal toxicity ratios for rats (*Rat DTI*) as input parameters and was associated with an $R^2 = 0.84$:

$$Avian\ DTI = -0.711436 + 0.792714 \times Rat\ DTI + 0.093169 \times MW^{0.5} + 0.096683 \times log\ VP \quad (9)$$

The atrazine *Avian DTI* can be calculated using the above equation with atrazine-specific input parameters. The atrazine *Rat DTI* (i.e., ≥ 2.78) was estimated using the equation from Mineau (2012):

$$Rat\ DTI = \log\left(\frac{oral\ LD50}{dermal\ LD50} \times 1000\right) \quad (10)$$

using parameter values for the rat oral LD50 = 1869 mg a.i./kg-bw and rat dermal LD50 > 3100 mg a.i./kg-bw (US EPA, 2016). For atrazine, vapor pressure is 0.040 mPA (3.0×10^{-7} mm Hg) at 20 °C, thus, *log VP* = −1.40 and MW = 215.7 (US EPA, 2016).

3.8. Approach 3 for estimating avian dermal LD50: triazine-specific mammal oral-dermal equation

A third approach for estimating avian dermal LD50s again was based on available mammal toxicity data and examined the relationships between acute oral and dermal toxicity data within a chemical class. This approach invokes an assumption that dermal absorption dynamics are similar between birds and mammals within a class of compounds, resulting in similar oral-dermal LD50 ratios for both taxa. Using the regression equation of mammalian oral and dermal LD50s, avian dermal LD50s were solved using the oral LD50.

Mammal oral and dermal LD50 data were collected for 6 triazines including: atrazine, terbuthylazine, simazine, propazine, prometryn, and ametryn (Table 1). A triazine specific oral-dermal relationship was expressed by the equation:

$$\log LD50_{(dermal)} = 2.576 + 0.2583 \times \log LD50_{(oral)} \quad (11)$$

All of the mammal dermal LD50 values were unbounded (i.e., the actual LD50 value was greater than the highest test concentration), while only 2 of 6 oral LD50 values were unbounded. The model fit for this equation was $R^2 = 0.25$. The true relationship between the oral and dermal LD50 is likely a steeper slope and a broader gap between the oral and dermal LD50 values. This would result in a higher F_{red}. Thus, estimated avian dermal LD50 values using this approach are likely conservative and lower than true values.

3.9. TIM modeling

The US EPA's TIM Version 3.0 was used to conduct simulations of atrazine use on avian responses. All atrazine related TIM input parameters were consistent with those used in US EPA (2016) and listed in the Supplemental data with the exception of the slope of the avian oral LD50 in which the correct slope (i.e., 3.836) was used corresponding to the northern bobwhite acute oral LD50. Avian life history and species specific TIM input parameters were obtained from Etterson et al. (2017) and are also listed in the Supplemental data. The model was run for a focal species (i.e., vesper sparrow, [*Pooecetes gramineus*]) using each approach for estimating the avian dermal LD50. During each run, all parameters remained the same except for the estimated avian dermal LD50 from each approach.

In addition, the model was used to compare predicted mortality between using a dermal LD50 estimated from the US EPA approach (i.e., method currently used in TIM) and the avian dermal LD50 estimated using the $F_{AM\text{-}dermal}$ approach newly described here from empirical dermal absorption data (i.e., Approach 1). Specifically, we selected the $F_{AM\text{-}dermal}$ corresponding to the quail adjusted potentially absorbable dose to be consistent with the quail LD50 used as the endpoint in the risk assessment. Thus, rat toxicity and corresponding dermal absorption data were used with quail toxicity and corresponding dermal absorption data. Comparisons between TIM output including mean total dose, dermal fraction of total dose, oral ingestion fraction of total dose, and model predicted mortality for the two approaches were made for eight individual species, many of which were focal species in US EPA (2016). In addition, visual comparisons were made of the probability distribution functions from the two approaches for multiple feeding guilds (i.e., insectivore, frugivore, granivore, and omnivore) and habitat use categories (i.e., field and edge).

4. Results

4.1. Dermal absorption study: rat

A total of 8 samples of rat split thickness skin membranes, obtained from 4 different animals, were dosed topically with [^{14}C]-atrazine at the labeled rate (25.2265 g/L). Overall, the absorption profiles were similar for all samples, with the absorption of [^{14}C]-atrazine increasing up to 24 h and a wash-in effect increasing the rate of absorption following the termination of exposure at 6 h.

The mean absorption rate of [^{14}C]-atrazine was 0.26 μg equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 6.13 μg equiv./cm^2 (2.28% of the applied dose). Following the skin wash at 6 h, 57.57% of the applied dose of [^{14}C]-atrazine was washed off. At 24 h post dose, a further 13.77% was removed during the skin wash. A proportion of the dose applied was recovered from the donor chamber (0.43%), exposed skin (8.48%), and receptor chamber wash (0.15%). The mean total recovery was 98.94% of the applied dose. The distribution by mass of [^{14}C]-atrazine radioactivity and absorption profiles can be found in the Supplemental data.

The total absorbed dose, consisting of the amount measured in the receptor fluid and the receptor chamber wash, was 2.43% of the applied dose (6.54 μg equiv./cm^2). The dermal delivery, consisting of the total absorbed dose and the amount measured in the exposed skin, was 10.90% of the applied dose (29.35 μg equiv./cm^2). The potentially absorbable dose, consisting of the dermal delivery and the amount measured in tape strips 3 to 20, was 24.66% of the applied dose (66.36 μg equiv./cm^2). In accordance with EFSA guidance, a multiple of the standard deviation value based on the number of accepted replicates (in this case 0.84 × s.d. based on 8 replicates) was added to the mean potentially absorbable dose to generate a final value (i.e., adjusted potentially absorbable dose) of 31.25% of the applied dose (Buist et al., 2017). A summary of the absorbed dose, dermal delivery, potentially absorbable dose, and adjusted potentially absorbable dose results is provided in Table 2.

4.2. Dermal absorption study: mallard

A total of 8 samples of mallard full-thickness skin membranes, obtained from 4 different animals, were dosed topically with [^{14}C]-atrazine at the labeled rate (25.2265 g/L). Absorption profiles appeared to fall into 2 groups, with 5 of the 8 samples having similar profiles and the remaining 3 samples having similar profiles. Despite the grouping, all the data were considered to capture the full variation among the samples.

The mean absorption rate of [^{14}C]-atrazine was 0.66 μg equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 15.72 μg equiv./cm^2 (5.84% of the applied dose). Following the skin wash at 6 h, 69.26% of the applied dose of [^{14}C]-atrazine was washed off. At 24 h post dose, a further 7.76% was removed during the skin wash. A proportion of the dose applied was recovered from the donor chamber (0.53%), exposed skin (9.39%), and receptor chamber wash (0.50%). The mean total recovery was 100.41% of the applied dose. The distribution by mass of [^{14}C]-atrazine radioactivity and absorption profiles can be found in the Supplemental data.

The total absorbed dose, consisting of the amount measured in the receptor fluid and the receptor chamber wash, was 6.34% of the applied dose (17.06 μg equiv./cm^2). The dermal delivery, consisting of the total absorbed dose and the amount measured in the exposed skin, was 15.72% (42.33 μg equiv./cm^2) of the applied dose. The potentially absorbable dose, consisting of the dermal delivery and the amount measured in tape strips 3 to 20, was 19.33% of the applied dose (52.02 μg equiv./cm^2). The adjusted potentially absorbable dose (i.e., 0.84 × s.d. based on 8 replicates added to the mean potentially absorbable dose) was 28.44% of the applied dose (Buist et al., 2017). A summary of the absorbed dose, dermal delivery, potentially absorbable dose, and adjusted potentially absorbable dose results is provided in Table 2.

4.3. Dermal absorption study: northern bobwhite

A total of 8 samples of northern bobwhite full thickness skin membranes, obtained from 6 different animals, were dosed topically with [^{14}C]-atrazine at the labeled rate (25.2265 g/L). Absorption profiles were similar in shape but variable in magnitude.

The mean absorption rate of [^{14}C]-atrazine was 1.12 μg equiv./cm^2/h during the 24 h experimental period. The amount penetrated at 24 h, as measured in the receptor fluid, was 26.84 μg equiv./cm^2 (9.97% of the applied dose). Following the skin wash at 6 h, 74.98% of the applied dose of [^{14}C]-atrazine was washed off. At 24 h post dose, a further 6.42% was removed during the skin wash. A proportion of the dose applied was recovered from the donor chamber (1.43%), exposed skin (2.59%), and receptor chamber wash (0.48%). The mean total recovery was 97.09% of the applied dose. The distribution by mass of [^{14}C]-atrazine radioactivity and absorption profiles can be found in the Supplemental data.

The total absorbed dose, consisting of the amount measured in the receptor fluid and the receptor chamber wash, was 10.45% of the applied dose (28.12 μg equiv./cm^2). The dermal delivery, consisting of the total absorbed dose and the amount measured in the exposed skin, was 13.04% (35.09 μg equiv./cm^2) of the applied dose. The potentially absorbable dose, consisting of the dermal delivery and the amount measured in tape strips 3–20, was 13.82% of the applied dose (37.19 μg equiv./cm^2). The adjusted potentially absorbable dose (i.e., 0.84 × s.d. based on 8 replicates added to the mean potentially absorbable dose) was 23.14% of the applied dose (Buist et al., 2017). A summary of the absorbed dose, dermal delivery, potentially absorbable dose, and adjusted potentially absorbable dose results is provided in Table 2.

4.4. Approach 1 for estimating avian dermal LD50: avian-mammal dermal route equivalency factor using dermal absorption data

Four scenarios were used to calculate the potentially absorbable dose and the adjusted potentially absorbable dose for both mallard and northern bobwhite. From the dermal absorption data, $F_{AM\text{-}dermal}$ values ranged from 0.560 to 0.910 and $F_{red\text{-}ATR}$ ranged from 0.338 to 0.549 for the four scenarios (Table 3). Avian dermal LD50 values were estimated using the empirical dermal absorption data and ranged from 1426 to 2317 mg/kg-bw (Table 4).

Table 2
Results of the dermal absorption study with three species: rat (*Rattus norvegicus*), mallard (*Anas platyrhynchos*), and northern bobwhite (*Colinus virginianus*). Values are mean ± standard deviation (s.d.). The adjusted potentially absorbable dose followed EFSA guidance (Buist et al., 2017) and was calculated by subjecting the s.d. to a multiplication factor based on sample size and adding this value to the mean of the potentially absorbable dose. For a sample size of eight, the multiplication factor was 0.84.

Absorption parameter	Rat (%)	Mallard (%)	Bobwhite quail (%)
Absorbed dose	2.43 ± 0.61	6.34 ± 6.02	10.45 ± 10.92
Dermal delivery	10.90 ± 5.94	15.72 ± 10.66	13.04 ± 11.07
Potentially absorbable dose	24.66 ± 7.84	19.33 ± 10.85	13.82 ± 11.09
Adjusted potentially absorbable dose	31.25	28.44	23.14

4.5. Approach 2 for estimating avian dermal LD50: Mineau method

The calculated *Avian DTI* for atrazine was ≤2.73 which is equivalent to an $F_{red} \leq 0.532$ using the equation:

$$F_{red} = 0.001 \times 10^{Avian\ DTI} \quad (12)$$

Solving for the avian dermal LD50 using the atrazine oral LD50 and Eq. (2) yields a dermal LD50 of ≥1472 mg a.i./kg-bw (Table 4). The Mineau (2007) method generated a lower bound dermal LD50 that is nearly double the oral LD50 value of 783 mg a.i./kg-bw.

4.6. Approach 3 for estimating avian dermal LD50: triazine-specific mammal oral-dermal equation

Using the triazine-specific mammal oral-dermal equation and the avian oral LD50 of 783 mg/kg-bw, the estimated avian dermal LD50 was 2106 mg a.i./kg-bw with a corresponding avian F_{red} for atrazine of 0.372 (Table 4). The estimated dermal LD50 of 2106 mg a.i./kg-bw is consistent with dermal LD50 values estimated using our dermal absorption approach (i.e., $F_{AM\text{-}dermal}$ approach) as well as the Mineau (2007) method.

4.7. TIM modeling

TIM was used to obtain exposure and acute effects output using estimated avian dermal LD50 values from each of the approaches. Model outputs that were compared among approaches included the mean overall total dose, the dermal fraction of the total dose, and model predicted mortality (Table 4). Using the current approach within TIM, the overall total estimated dose (i.e., the summed dose from all exposure pathways) was 202.0 μg/g-bw. Alternatively, total dose estimated using avian LD50 values generated from the three alternative approaches described here ranged from 76.1 to 95.4 μg/g-bw, which is 2.7 to 2.1 times lower than the default approach in TIM. This total dose pattern is largely driven by the fraction attributed to the dermal exposure route. Following the TIM calculations for estimating the avian LD50, the modeled dermal fraction was 77.4% of the total dose; whereas, the alternative approaches generated a modeled dermal fraction ranging from 40.2 to 52.2%. This ultimately affected the model predicted mortality for vesper sparrow with the TIM approach yielding 10.6% mortality while the alternative approaches yielded 0.4 to 0.9% (i.e., there was 26.5 to 11.8 times greater predicted percent mortality following the default TIM approach).

The model was also used to assess response differences between dermal LD50 estimates using the TIM approach and the dermal absorption ratio approach (i.e., using $F_{AM\text{-}dermal}$, approach 1). For $F_{AM\text{-}dermal}$ calculated for the quail adjusted potentially absorbable dose, the corresponding estimated avian dermal LD50 was 1756 mg/kg-bw (Table 4). The TIM approach for estimating avian dermal LD50 values yielded 487 mg/kg-bw (Table 4). Within each species examined, model output following the default TIM approach always yielded greater mean total dose, greater dermal fraction of total dose, lower oral fraction of total dose, and greater model predicted mortality (Table 5). Model predicted mortality using the $F_{AM\text{-}dermal}$ approach was 64.4 to 97.4% lower than the TIM approach for the eight focal species tested (Table 5), demonstrating a significant reduction in model output. Variation among these reductions in model predicted mortality is likely driven by variability in life history parameter inputs among species.

In terms of probability distribution functions from the two approaches, for every feeding guild (i.e., insectivore, granivore, frugivore, and omnivore) and for field or edge species status, probability distribution functions were shifted toward zero on the x-axis indicating reduced risk when using Approach 1 employing the empirically derived dermal absorption data (Supplemental data, Fig. S1).

5. Discussion

For each of the three alternative approaches for estimating avian dermal LD50 values described here the atrazine dermal LD50 was higher than the oral LD50. The US EPA's approach for estimating avian dermal LD50 values in TIM returns a substantially lower dermal LD50 than oral LD50 for any oral LD50 used in the equation with a value >log 2.20997 (i.e., 162.2 mg a.i./kg-bw). Thus, for compounds that are relatively non-toxic to birds, use of the current OP/CA equation in TIM likely does not accurately estimate dermal LD50 values. This idea is supported by the triazine mammal data in which all dermal LD50 values are higher than oral LD50 values. In addition, considering the data used to generate the oral-dermal equation in TIM, 85.7% of the oral-dermal data pairs had a higher dermal LD50 than the oral LD50 (US EPA, 2015). Similar patterns of higher dermal LD50 values (or toxicity endpoints) than oral LD50 values (or toxicity endpoints) were also observed for OPs and CAs in both birds and mammals (Hudson et al., 1979; Henderson et al., 1994; Mineau, 2007, 2012); for pyrethroids, anticoagulants, and chemicals with other mechanisms of action to quelea (*Quelea quelea*) and house sparrow (*Passer domesticus*) (Schafer et al., 1973) and brown tree snakes (*Boiga irregularis*) (Brooks et al., 1998); and for a pyrethroid and organochlorine to western fence lizards (*Sceloporus occidentalis*) (Weir et al., 2015). From a mechanistic viewpoint, it is expected that avian oral LD50 values would be typically lower than dermal LD50 values given that acute oral avian studies introduce a dose via gavage of bioavailable pesticide directly into a bird's highly permeable digestive tract. Conversely, dermal exposure is slower (Henderson et al., 1994) and occurs over time across various barriers such as feathers and less permeable epidermal layers, thus allowing for some metabolism and elimination before body burdens reach lethal levels. It should be noted, however, that patterns between oral and dermal toxicity of a chemical can be highly dependent on physicochemical properties, mode of action, detoxification processes, bioactivation processes (if relevant), and location of dermal exposure.

The exposure scenario described in this study represents a worst case scenario as featherless avian skin was used in the dermal absorption experiments. Not only does this build in conservatism on exposure but also correlates to avian dermal LD50 studies, many of which exposure occurred via bare underwing skin (Mineau, 2012). Furthermore, featherless underwing skin would be the most comparable to hairless rat skin used in standard dermal absorption studies as our objective was to compare dermal absorption patterns between birds and mammals.

The three alternative approaches discussed here also provide dermal LD50 estimates that are relatively similar to one another. Two of these (i.e., Approach 2 [Mineau method] and Approach 3) utilize only mammal data. Approach 1 utilizes both mammal and avian data and facilitates

Table 3
Mallard (*Anas platyrhynchos*) and northern bobwhite (*Colinus virginianus*) potentially absorbable dose (PAD) and adjusted potentially absorbable dose following EFSA guidance (PAD_{EFSA}) (Buist et al., 2017). $F_{AM\text{-}dermal}$ and $F_{red\text{-}ATR}$ were calculated from rat, mallard, and northern bobwhite dermal absorption data. $F_{AM\text{-}dermal}$ was calculated using Eq. (7). $F_{red\text{-}ATR}$ was calculated with Eq. (8) using the rat oral LD50 (1869 mg a.i./kg-bw), rat dermal LD50 (3100 mg a.i./kg-bw), and the $F_{AM\text{-}dermal}$ values.

Species	Absorption parameter	Avian dermal absorption (%)	Mammal dermal absorption (%)	$F_{AM\text{-}dermal}$	$F_{red\text{-}ATR}$
Mallard	PAD	19.33	24.66	0.784	0.473
Northern Bobwhite	PAD	13.82	24.66	0.560	0.338
Mallard	PAD_{EFSA}	28.44	31.25	0.910	0.549
Northern Bobwhite	PAD_{EFSA}	23.14	31.25	0.740	0.446

Table 4
Estimated avian dermal LD50s and calculated $F_{red\text{-}ATR}$ or F_{red} using four different approaches (i.e., TIM model equation, the $F_{AM\text{-}dermal}$ approach from avian dermal absorption data, the Mineau (2007) approach, and a triazine specific mammal equation). For the $F_{AM\text{-}dermal}$ approach, values were calculated absorption data from two different species (i.e., mallard [*Anas platyrhynchos*] and northern bobwhite [*Colinus virginianus*]) and two different absorption values (i.e., mean values and EFSA-adjusted values). Also shown are the modeled total dose, fraction of overall total dose attributed to the dermal exposure route, and modeled predicted mortality for vesper sparrow (VESP) (*Pooecetes gramineus*) from EPAs TIM exposure model. For all calculations, the northern bobwhite oral LD50 for atrazine (783 mg/kg-bw) was used (US EPA, 2016).

Approach	$F_{red\text{-}ATR}$ or F_{red} value	Estimated avian dermal LD50 (mg/kg-bw)	TIM output: mean overall dose (μg/g-bw)	TIM output: dermal fraction of total dose (%)	TIM output: model predicted mortality for VESP (%)	Source
TIM model equation	1.818[a]	487[b]	202.0	77.4	10.6	US EPA, 2016
$F_{AM\text{-}dermal}$ (mallard)	0.473[c]	1,655[d]	88.8	47.7	0.7	This study
$F_{AM\text{-}dermal}$ (mallard)-Adjusted	0.338[c]	2,317[d]	76.1	40.2	0.4	This study
$F_{AM\text{-}dermal}$ (quail)	0.549[c]	1,426[d]	95.4	52.2	0.9	This study
$F_{AM\text{-}dermal}$ (quail)-adjusted	0.446[c]	1,756[d]	85.5	47.6	0.5	This study
Mineau method	0.532[e]	1,472[f]	94.7	52.0	0.9	Mineau, 2007
Triazine-specific mammal equation	0.372[g]	2,106[h]	79.2	42.8	0.4	This study

Solved using.
[a] Eq. (1) after estimating avian dermal LD50 using the TIM model approach (Eq. (3)).
[b] Eq. (3).
[c] Eq. (8) using calculated $F_{AM\text{-}dermal}$ values from Table 3.
[d] Eq. (1) after determining $F_{red\text{-}ATR}$ and using in lieu of F_{red}.
[e] Eq. (9) and then conversion of *Avian DTI* to F_{red} with Eq. (12).
[f] Eq. (1).
[g] Eq. (1) after estimating avian dermal LD50 using Eq. (11).
[h] Eq. (11).

confirmation of the mammal only methods. Thus, consistency among these three approaches further indicates that using mammal data as a surrogate may be feasible for estimating avian dermal LD50 values in the TIM framework. Use of mammal data for avian dermal risk assessments has historically been proposed (Hudson et al., 1979; Mineau, 2007, 2012). Furthermore, it is also consistent with a pattern observed for reptiles (Weir et al., 2016), suggesting similarity across taxa to mammals. Weir et al. (2016) reported that skin permeability (i.e., Kp) for four agricultural chemicals was in close agreement between published values for hairless mice and western fence lizards (*Sceloporus occidentalis*) based on empirically-derived values generated using a skin permeability apparatus with donor and receptor cells. Use of mammal data as surrogates for avian dermal absorption is further supported by the dermal absorption data from the present study. Here it is demonstrated that absorption of atrazine by underwing skin in both mallard and northern bobwhite was similar to rat dermal absorption dynamics. These data can be used for potentially two TIM inputs: (1) estimates of avian dermal LD50 values as demonstrated with Approach 1 that can be input directly into the appropriate cell on the pesticide parameters window, and (2) to parameterize the dermal absorption fraction for calculation of dermal exposure through direct interception ($D_{intercept(t)}$) (US EPA, 2015).

This study demonstrates that the default approach in TIM for estimating avian dermal LD50 values is inaccurate and varies greatly from the three proposed alternative approaches. Fundamentally, a mathematical relationship between oral and dermal toxicity for compounds primarily with the same MOA should not be applied universally across all compounds of varying MOAs. Indeed, a previous SAP concluded that this approach should not be used to interpret dermal exposure beyond the OPs (US EPA, 2004). The SAP suggested that the relationship of toxicity between oral and dermal exposure routes in the current TIM

Table 5
TIM model output for eight passerine species with avian dermal LD50s estimated from two different approaches: the EPA default equation within TIM and an approach deriving an avian-mammal dermal route equivalency factor from dermal absorption data (i.e., $F_{AM\text{-}dermal}$ based on quail and EFSA adjusted). Thus, the avian dermal LD50 of 1756 m/kg-bw was used as a TIM input value for the $F_{AM\text{-}dermal}$ approach. For each species TIM output can be compared side by side for the following responses: mean total dose, dermal fraction of total dose, oral fraction of total dose, and model predicted mortality.

Avian dermal LD50 estimate approach	Species[a]	Mean total dose (±s.d.) (μg/g-bw)	Dermal fraction of total dose (±s.d.) (%)	Oral fraction of total dose (±s.d.) (%)	Model predicted mortality (%)
TIM equation	AMRO	115.5 ± 72.2	55.3 ± 0.1	44.2 ± 0.1	2.07
$F_{AM\text{-}dermal}$	AMRO	71.0 ± 44.2	25.9 ± 0.1	73.5 ± 0.1	0.37
TIM equation	COYE	173.7 ± 136.9	53.5 ± 12.7	46.3 ± 12.7	15.58
$F_{AM\text{-}dermal}$	COYE	100.3 ± 83.9	20.2 ± 7.0	79.5 ± 7.1	5.55
TIM equation	DICK	171.2 ± 97.2	70.7 ± 7.7	28.5 ± 7.7	8.36
$F_{AM\text{-}dermal}$	DICK	81.2 ± 42.6	37.0 ± 8.2	61.5 ± 8.5	0.87
TIM equation	EAKI	176.2 ± 107.3	53.2 ± 9.7	46.4 ± 9.8	10.46
$F_{AM\text{-}dermal}$	EAKI	102.7 ± 60.7	22.9 ± 7.0	76.6 ± 7.2	2.72
TIM equation	FISP	199.7 ± 104.6	80.6 ± 5.7	18.0 ± 5.7	14.28
$F_{AM\text{-}dermal}$	FISP	77.0 ± 34.8	49.5 ± 7.9	46.9 ± 8.4	0.71
TIM equation	GRSP	184.7 ± 105.7	75.8 ± 6.4	23.4 ± 6.3	12.14
$F_{AM\text{-}dermal}$	GRSP	78.2 ± 40.4	44.0 ± 6.7	54.1 ± 7.0	0.78
TIM equation	HOLA	262.8 ± 67.9	88.4 ± 3.0	9.9 ± 2.7	14.49
$F_{AM\text{-}dermal}$	HOLA	94.2 ± 17.7	67.8 ± 7.5	27.4 ± 7.0	0.38
TIM equation	VESP	202.1 ± 89.2	77.4 ± 6.2	21.4 ± 6.2	10.61
$F_{AM\text{-}dermal}$	VESP	85.5 ± 33.1	47.6 ± 7.6	49.5 ± 7.6	0.52

[a] Species are denoted by their four letter American Ornithological Union alpha code and included American robin (AMRO) (*Turdus migratorius*), common yellowthroat (COYE) (*Geothlypis trichas*), dickcissel (DICK) (*Spiza americana*), eastern kingbird (EAKI) (*Tyrannus tyrannus*), field sparrow (FISP) (*Spizella pusilla*), grasshopper sparrow (GRSP) (*Ammodramus savannarum*), horned lark (HOLA) (*Eremophila alpestris*), and vesper sparrow (VESP) (*Pooecetes gramineus*).

approach should be verified with other species and compounds prior to being used universally in risk assessments.

This study also demonstrates that selection of the method for estimating avian dermal LD50 values can have significant influence on certain TIM outputs such as overall total dose (Table 4), the dermal fraction of the total dose (Table 4), model predicted mortality (Table 4), and probability distribution functions (Supplemental data, Fig. S1). These outputs indicated significantly elevated levels of model predicted mortality when using the default TIM approach that generates lower dermal LD50 estimates than the data-derived oral LD50 values. These elevated model outputs are not inconsequential as they are used to inform risk assessments and assist in regulatory decision making (US EPA, 2016).

6. Conclusion

This study demonstrated that a new approach for estimating avian dermal LD50 values using empirical dermal absorption data results in lower estimated total dose, lower dermal fraction of total dose, greater oral fraction of total dose, and reduced predicted mortality from the currently used regulatory risk assessment model for birds (i.e., TIM) in the US. Data from this new approach and the dermal absorption study can be used to advance current wildlife dermal risk models, including transfer rates in EFSA higher-tier dermal exposure models (EFSA, 2009) and dermal absorption data that can inform model input parameters such as dermal absorption fraction (DAF) in both TIM and EFSA models.

In addition, the new approach was compared with other previously described methods for estimating avian dermal LD50 values based on mammalian data and physico-chemical properties. All three alternative approaches resulted in outputs similar to one another and different from the default TIM method. These results indicate that a dermal route equivalency factor derived from empirical data provides better avian dermal LD50 estimates. These data also validate previously described approaches that utilize mammalian data and physicochemical parameters (Hudson et al., 1979; Mineau, 2007, 2012; Fryday et al., 2014) that can be used for estimating avian dermal toxicity across chemical classes. In addition, the use of this dermal route equivalency factor results in greatly reduced modeled atrazine risk to birds than previously reported in US EPA risk assessments using TIM (US EPA, 2016).

Supplemental data and information are available at the journal website. The raw data from the dermal absorption study are provided in the Supplemental Data and TIM output for each model run can be requested from the authors. Supplementary data to this article can be found online at doi:https://doi.org/10.1016/j.scitotenv.2018.02.206.

Acknowledgements

The dermal absorption study was funded by Syngenta Crop Protection, LLC and we thank F. Toner for his assistance with the study. We thank M. Hammond, P. Hubbard, R. Moore, R. O'Boyle, and D. Temple at EAG Laboratories for assistance on obtaining and processing mallard and northern bobwhite skin samples and four anonymous manuscript reviewers.

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