

Estimating terrestrial dermal amphibian pesticide exposures for regulatory use

Terrestrial Vertebrates Risk Assessment to Pesticides SETAC Latin America Symposium

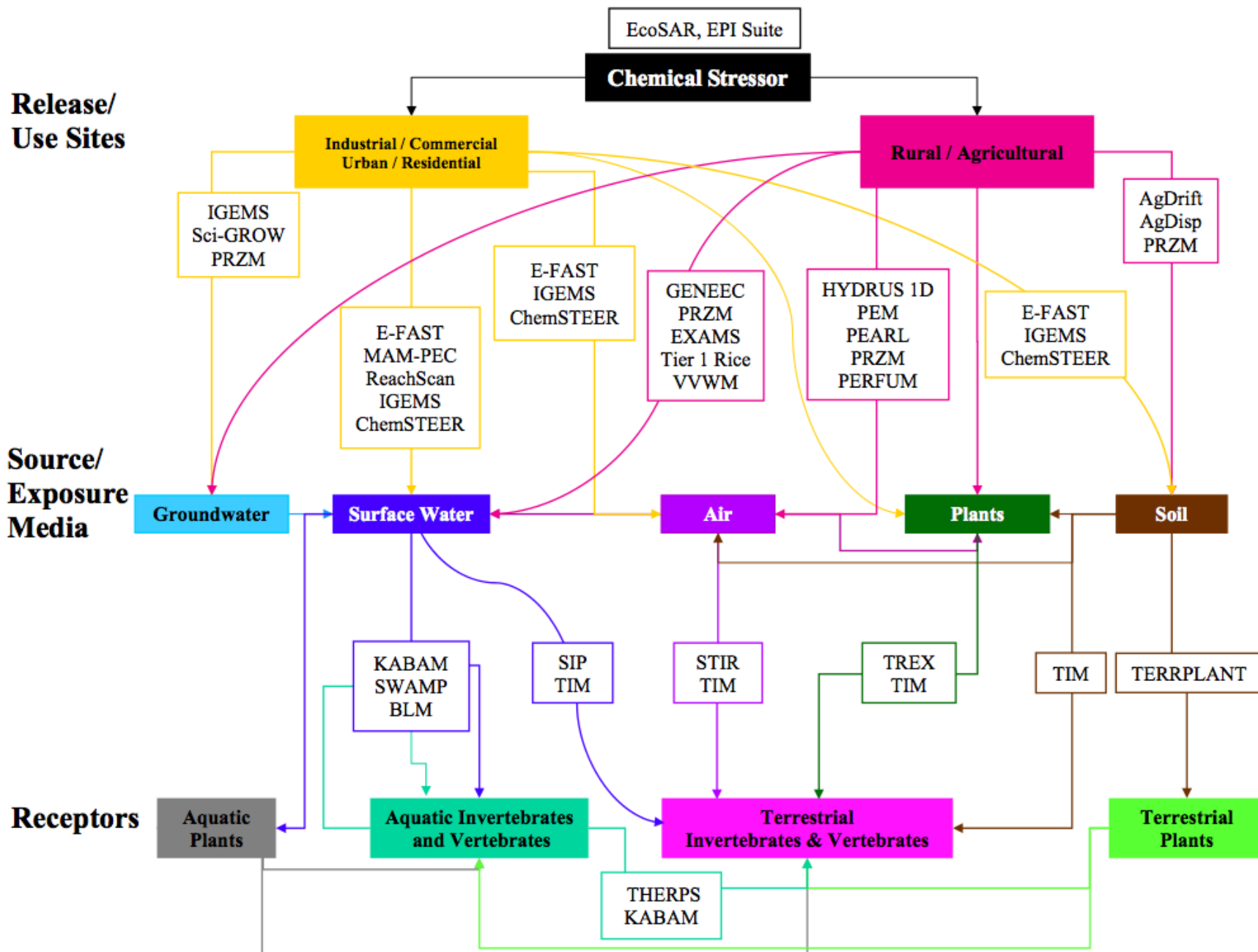
Tom Purucker (purucker.tom@epa.gov)



Ecological Risk Estimation for Registration and Effects Assessment

- Tiered ecological risk for pesticides (effects determination, registration)
- Must clear external review
- Complex problem- many combinations of:
 - species,
 - chemicals,
 - physical settings,
 - data sources,
 - application rates/methods;
 - often a spatial component.
- Many lines of evidence and models are evaluated for a pesticide registration.





Technical Overview of Ecological Risk Assessment – Analysis Phase: Exposure Characterization

About Exposure Characterization

Table of Contents for Technical Overview of

Dietary Exposure for Reptiles and Amphibians through Food Items

For terrestrial reptiles and amphibians, EPA uses a modified version of T-REX called [T-HERPS](#) (Terrestrial Herpetofaunal Exposure Residue Program Simulation) to estimate dietary exposure. The allometric equations in T-REX have been adjusted to account for the lower metabolic rate and food intake of herptiles compared to birds. Information concerning T-HERPS can be found on the [Models for Pesticide Risk Assessment](#) website.

- source(s) of the pesticide and what is exposed (e.g., plants, animals, media),

[Templates](#)

- [Ecotoxicity Categories](#)

- [Analysis - Exposure Characterization](#)

- fate and t

- how often
concern t

Dermal Exposure to Birds, Mammals, Reptiles, and Amphibians

Currently, EPA is developing a model (Dermal Uptake Screening Tool (DUST)) to estimate exposure to birds, mammals, reptiles, and amphibians through the dermal route. DUST compares a ratio of exposure to toxicity and then compares this ratio to a limit of concern to determine if dermal exposure warrants further exploration. After the model is finalized, it will be used as a qualitative tool to screen out pesticides that are not of concern when considering exposure through the dermal route.

Public consultation on the Draft Scientific Opinion on the state of the science of pesticide risk assessment for amphibians and reptiles

Deadline: 24 May 2017

[Document](#)  (7.18 MB)

[Privacy statement](#)  (78.67 KB)

EFSA has launched an open consultation on the draft Scientific Opinion on the state of the science on pesticide risk assessment for amphibians and reptiles. This document proposes the scientific basis for developing a future risk assessment scheme. The coverage of the risk to amphibians and reptiles by current risk assessments for other

Subject area



Pesticides



The PPR Panel was tasked to provide a scientific opinion on the state of the science on pesticide risk assessment for amphibians and reptiles. Concerns had been raised that the current risk assessment of pesticides may not sufficiently cover the risk to amphibians and reptiles. The opinion should provide the scientific basis for potentially developing a guidance document for pesticide risk assessment for amphibians and reptiles.

The Panel concludes that exposure of amphibians and reptiles to pesticides does occur, and that this exposure may lead to decline of populations and harm individuals, which would be of high concern. Therefore, a specific environmental risk assessment (ERA) scheme is needed for for these groups.

Amphibian Dermis and Exposure

Gas & water
exchange

Water transport
via
aquaporins

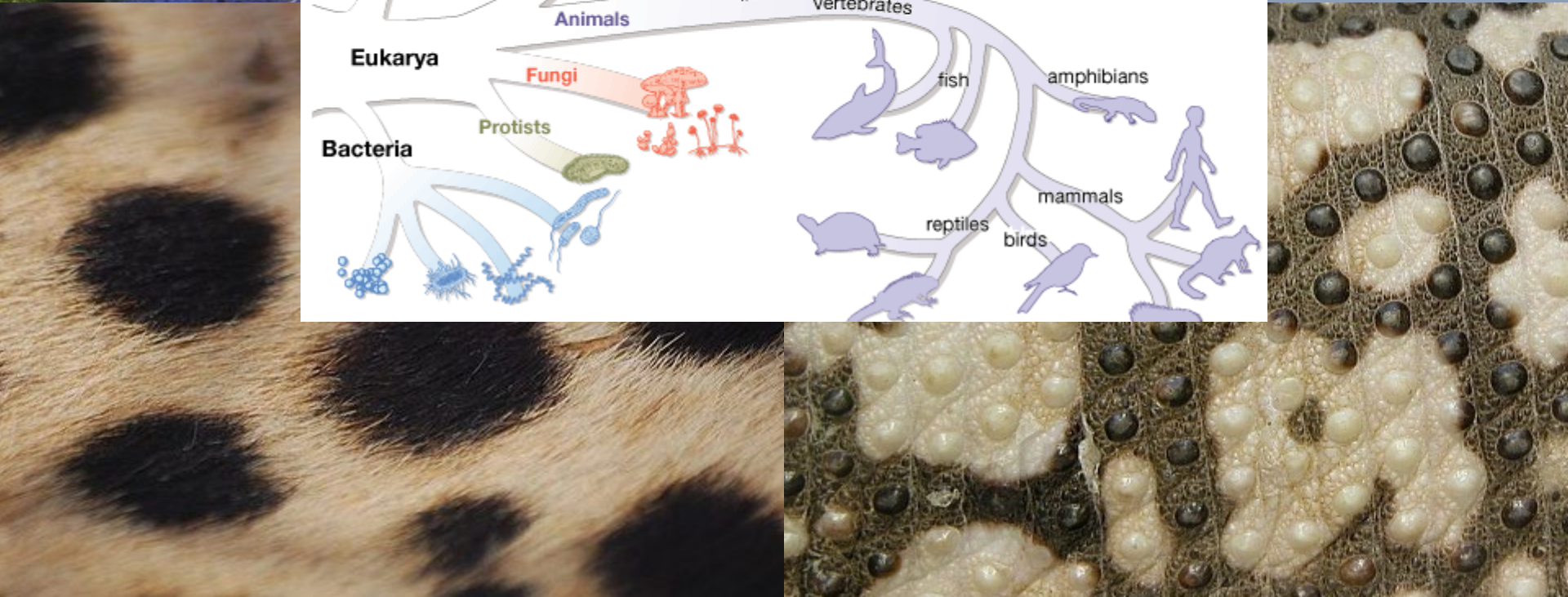
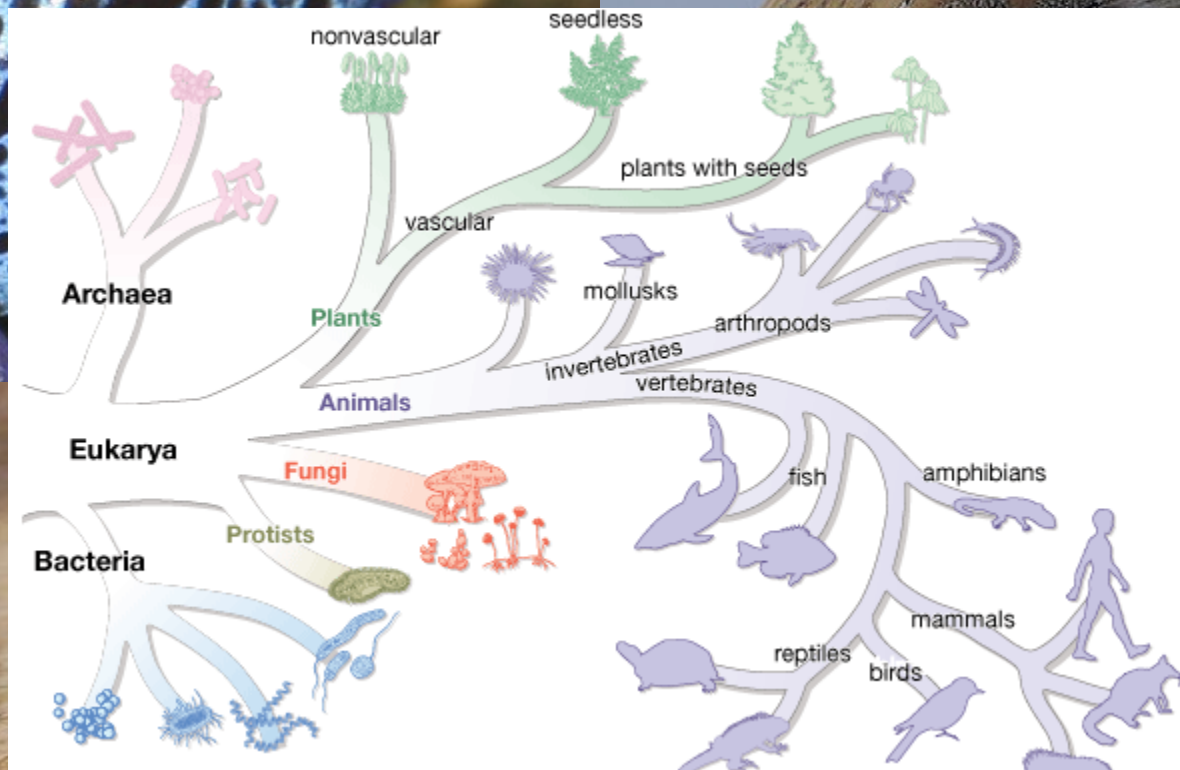
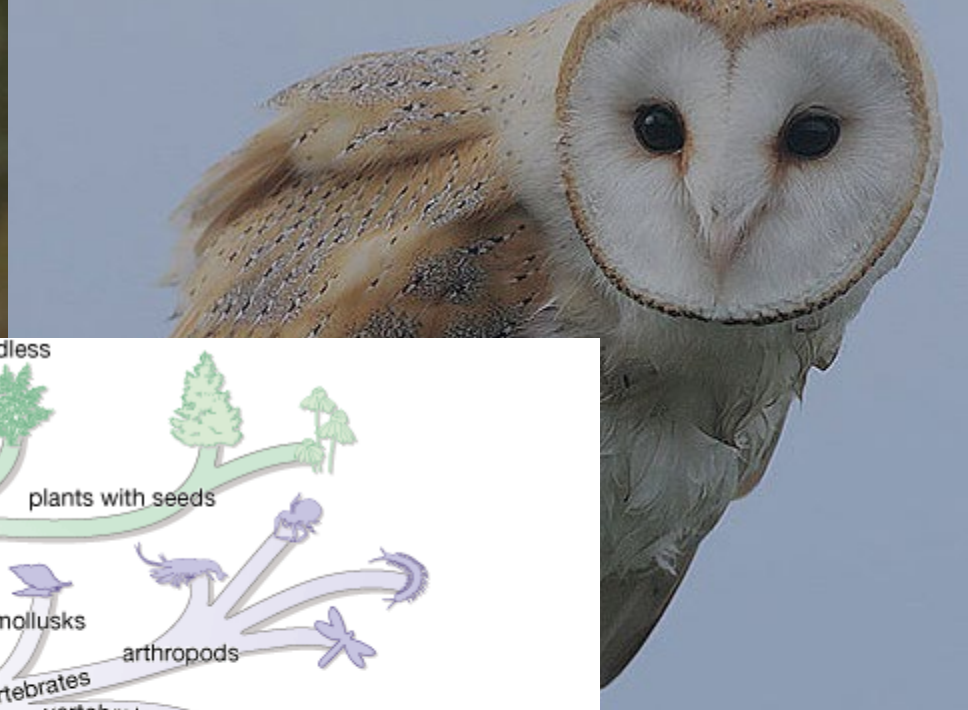
Highly vascularized seat
patch

Comparatively thinner
stratum corneum

Lack outer
hydrophobic
barrier

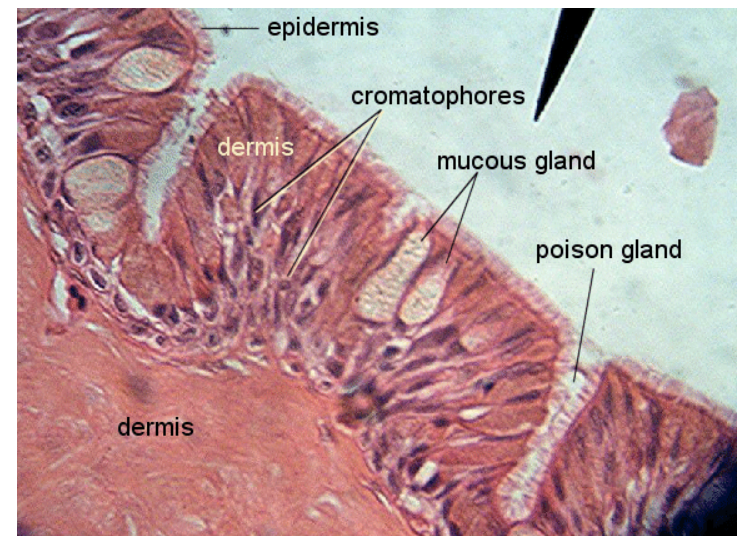
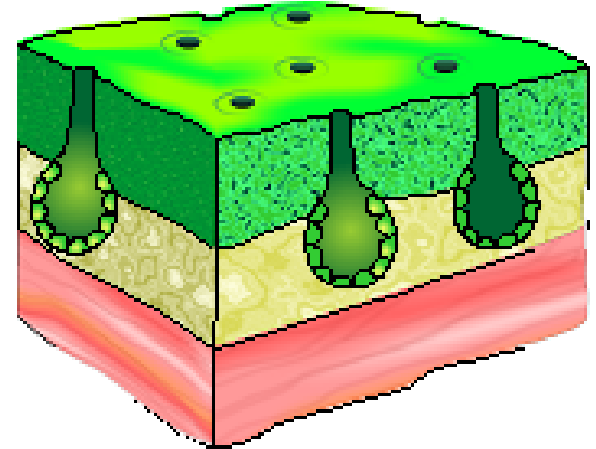
Less keratinized

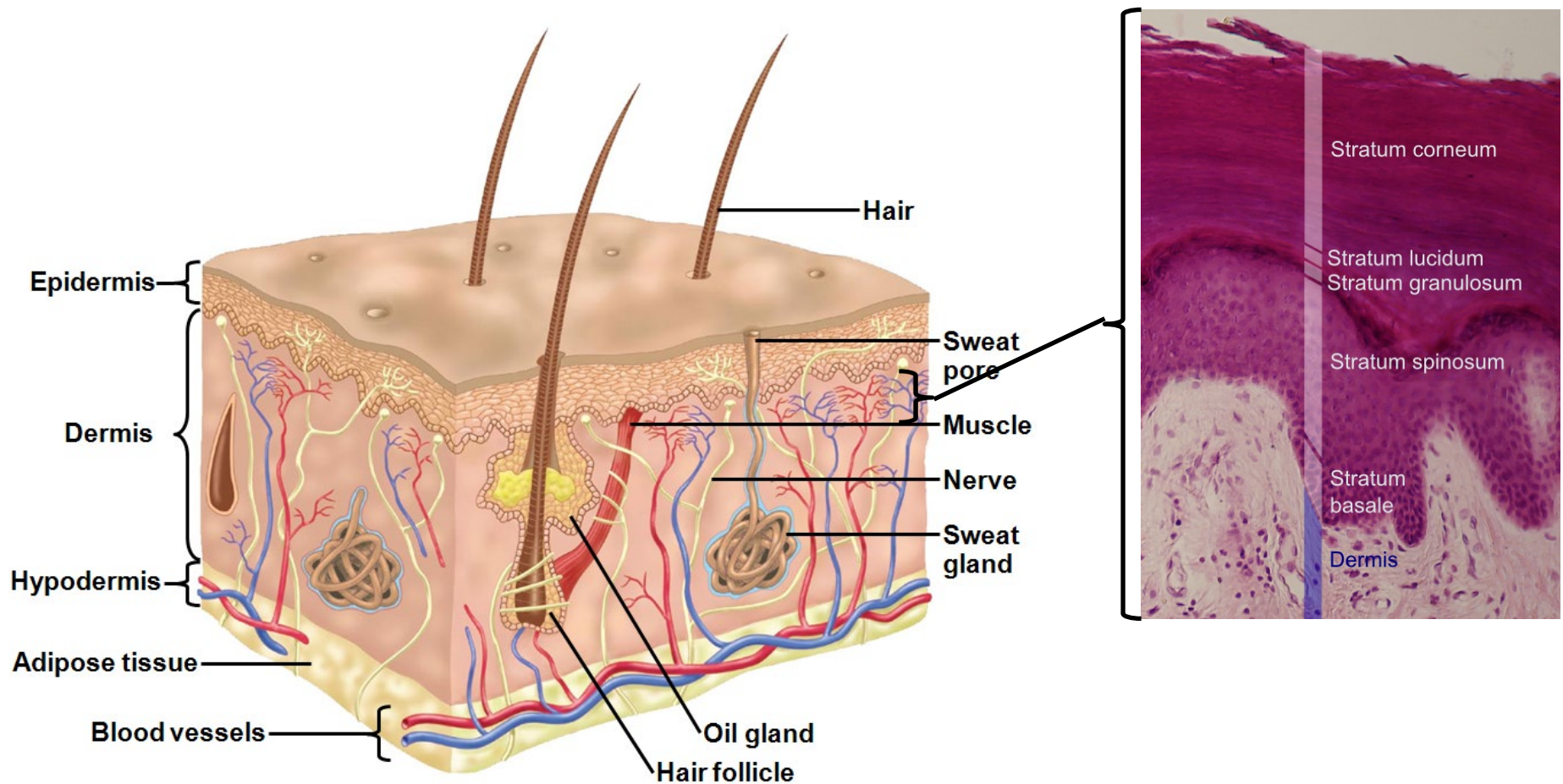




Amphibian dermis structural differences

- Relatively thinner
- Thin stratum corneum
- No external hydrophobic barrier
- Less keratinized
- High rates of gas and water exchange
- Seal patch as preferential pathway
- Physiological properties change over life history





Hydrophilic (low K_{ow}) and lipophilic (high K_{ow}) molecules have separate pathways for dermal exposure in humans

For humans, lipophilic molecules get the most attention with a focus on non-ionic (neutral, lipophilic) chemicals for dermal.

The seat patch

Amphibian seat patch is a preferential path for osmotic water uptake. Water potential dependent.

Amphibian seat patch is crenulated - effective surface area for uptake is much larger than the actual size of the patch.

Movement exposes seat patch to pesticides sprayed on bare soil and leaf surfaces.



Behavioral Assumptions are Important



Bundesarchiv, Bild 183-21377-0001
Foto: Gerbeth | 21. September 1953



Burrowing behavior

Irrigated farm systems are preferential habitats, terrestrial amphibians prefer soils with high moisture content.

Amphibians (often) burrow, overnight or overwinter, to rehydrate - 100% contact with soil can be a significant portion of exposure.

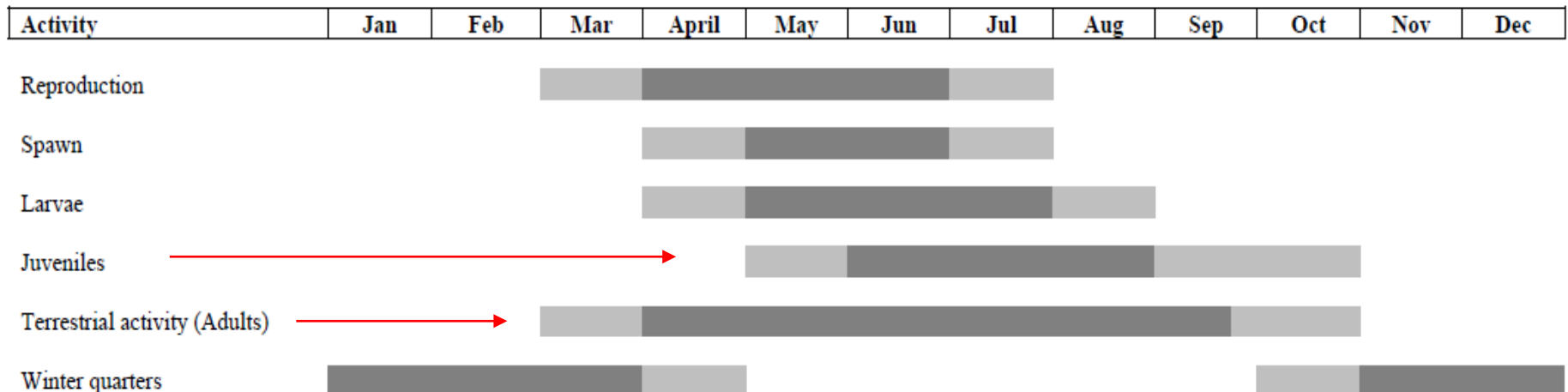
Also while burrowing for long periods of time they ingest shedded skin, adding a dermal component to the ingestion scenario.

Agriculture Habitat

Adult dispersal to and metamorph dispersal from breeding ponds can coincide with pesticide applications



Seasonal activity of *Hyla arborea* in Germany based on information in Pfeffer et al. (2011). Darker area represents the main period of activity.



Amphibian Movement Behavior

- Seasonal, Night/Day
- Additive exposures over time
- Specific behaviors can increase or decrease exposures



What does this mean for dermal pesticide exposure?

The more terrestrial an amphibian's life cycle is, the more likely it is to use the skin to regulate its water content in order to maintain hydration.

Also, terrestrial amphibians are more likely to use soil water or puddles as rehydration sources – with higher pesticide concentrations.

Behavior can amplify exposures beyond 'conservative' screening assumptions, or decrease exposures.

Amphibian dermal contact can be a key exposure pathway compared to non-amphibian receptors.

Dermal contact approaches for eco risk

Higher trophic level ecological risk assessment endpoints are usually mammals and/or birds (surrogates)

Dermal exposure is generally assumed to be negligible for birds and mammals (Suter 2006)

Hope (1995) recommended 2 dermal models:

1) organism is exposed to all contamination in soil it is in contact with while at rest

2)

$$D_{der} = \frac{C A F_d F_b B_d}{W}$$

These one-size-fits-all models can seem conservative, but they may underestimate

Estimating dermal dose with Kp

$$D_{der} = \frac{C K_p A T B_d F_b}{W}$$

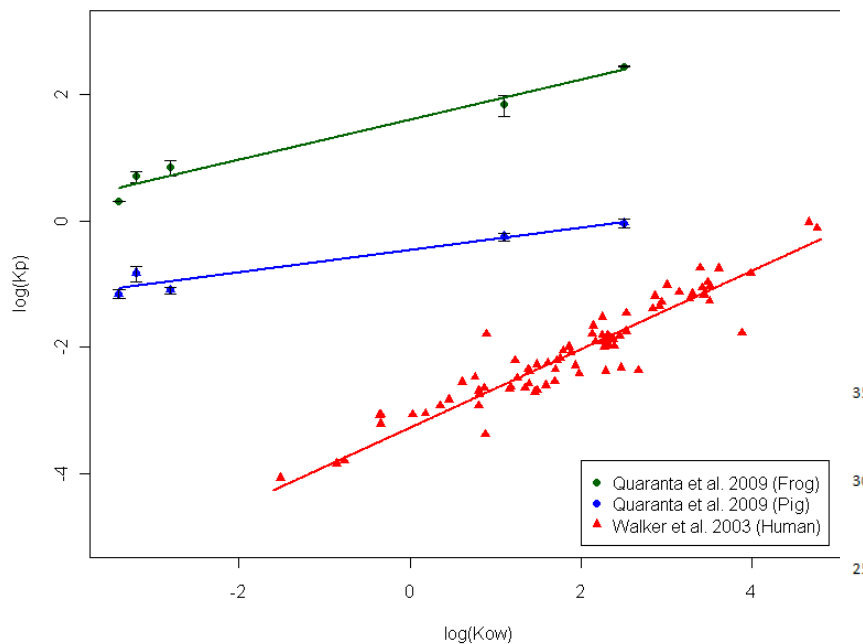
Physical differences in skin causes differences in empirical permeability rates – cm/hour

Number of studies available for mammals, few for non-mammals.

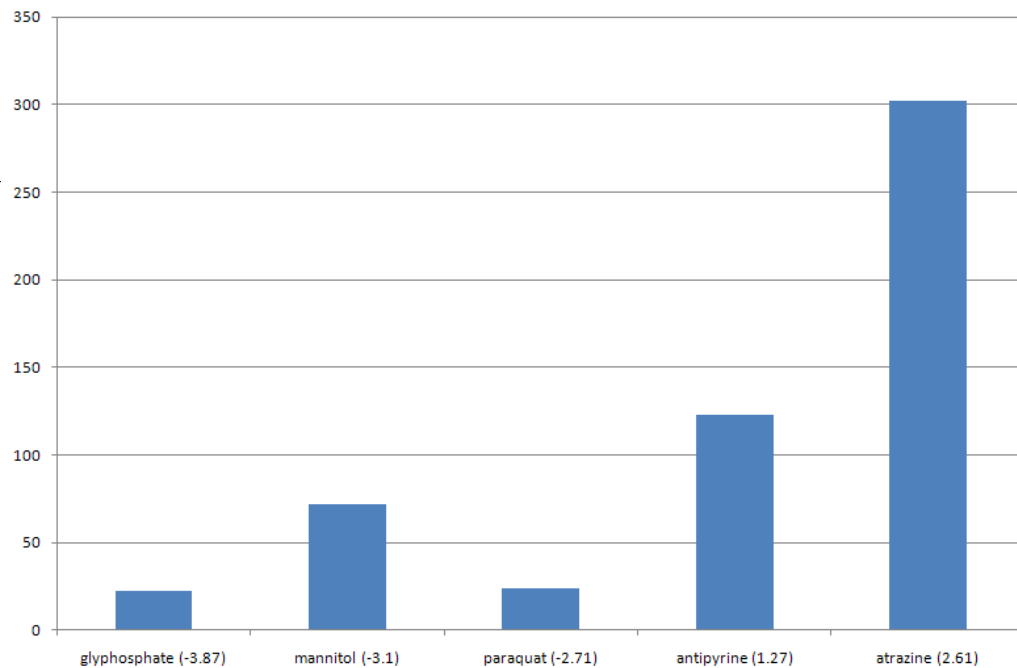
Permeability coefficient is primarily a function of hydrophobicity - $\log(K_{ow})$ - and molecular volume or perhaps could be empirical

Measured dermal permeability coefficients

Permeability Coefficient Data



Frog/Pig Permeability Ratio (Quaranta et al. 2009)



Initial modeling study--Relative pathway exposures

$$D_{\text{Diet}}(\text{mg/kg BW/d}) = \text{FMR}(\text{kcal/d}) / (\text{BW}(\text{g})(C_{\text{insects}}(\text{mg/kg})) / (1.7 \text{ kcal/g}))$$

$$D_{\text{der}} = \frac{C K_p A T B_d F_b}{W}$$

Calculate exposure for diet (Weir et al. 2010) and dermal for amphibians, birds, mammals, reptiles.

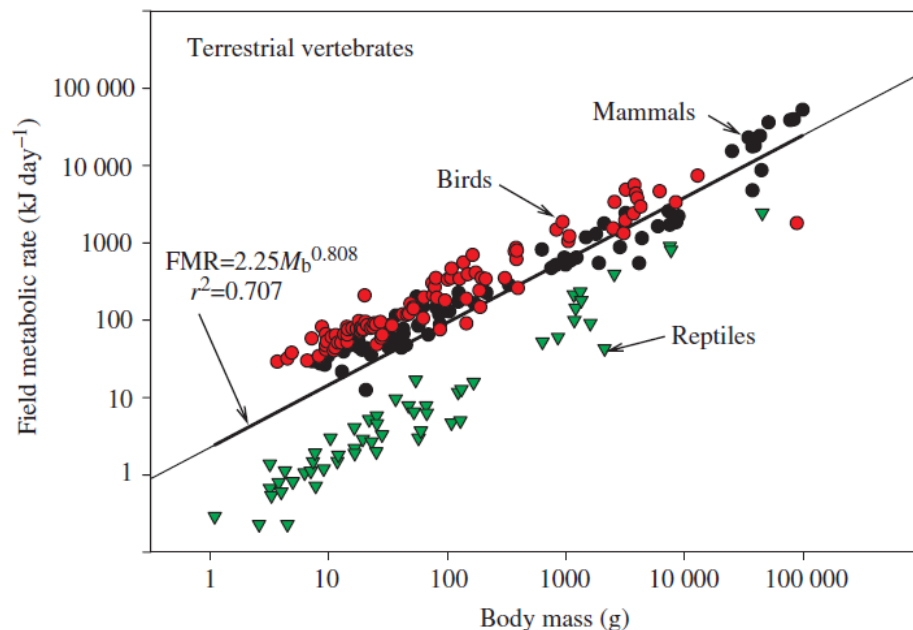
Solve for range of appropriate body weights for insectivores in each class (instead of representative receptor).

Range of K_{ow} .

Compare total dose between classes and relative contribution of dermal.

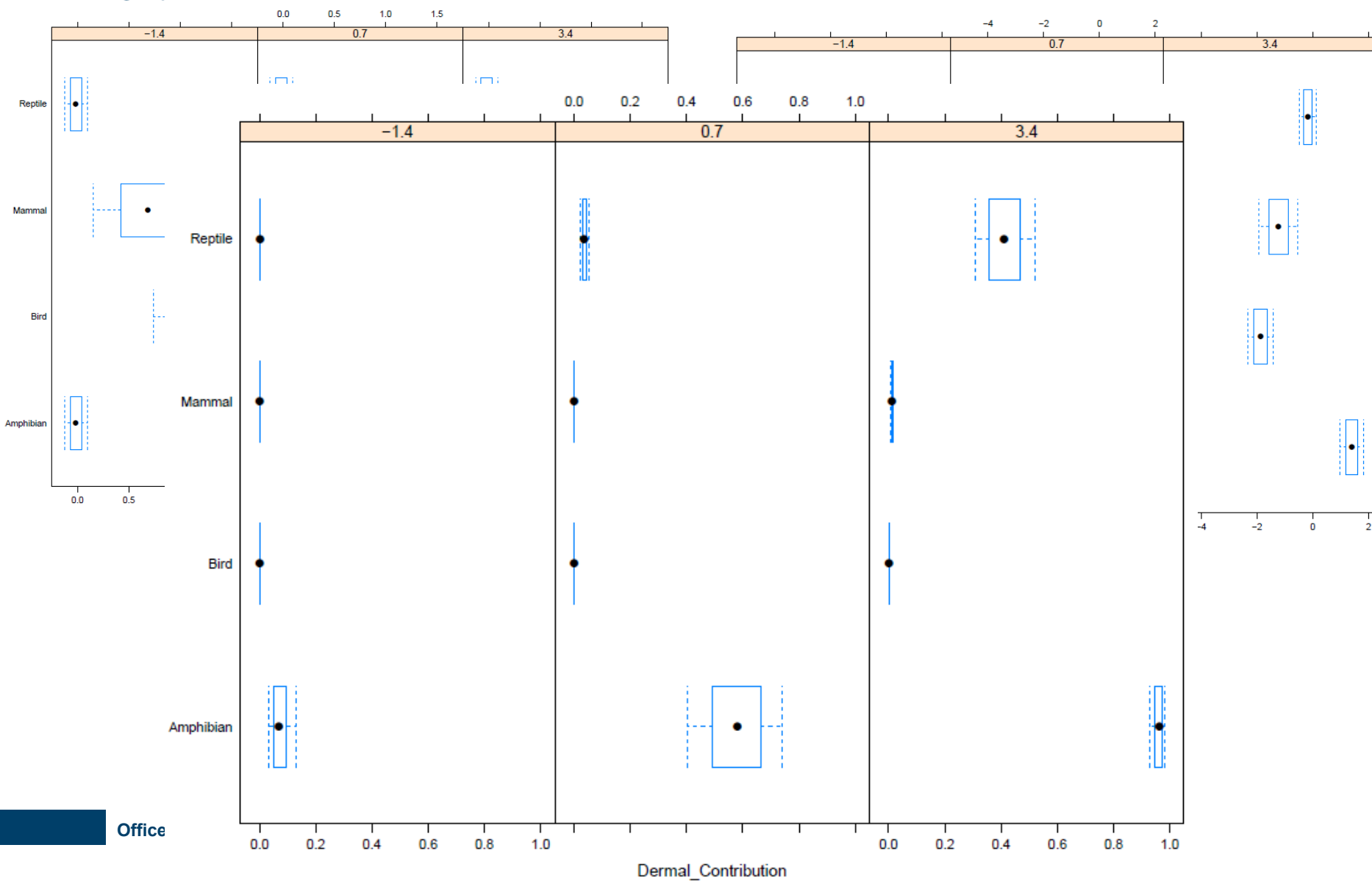
No tox comparisons, just looking at exposure.

Field Metabolic Rates (Nagy 2005)



Weir et al. 2010. Ecological risk of anthropogenic pollutants to reptiles: Evaluating assumptions of sensitivity and exposure. *Environmental Pollution*, 158:3596-3606.

MC of Dietary, Dermal and Relative Dose Estimates



Initial Modeling Exercise Conclusions

Calculated dietary and dermal doses,
parameterized for Amphibians, Birds, Mammals,
Reptiles

Amphibians/Reptiles may get significant percentage
of dose from dermal, Birds/Mammals not so much.

Significant uncertainties and data limitations for
assessing dermal exposure: needs – data!, dermal
properties, aquaporin impacts, seal patches, soil
water, chemical property effects, etc.

We decided to conduct some amphibian exposure
studies.

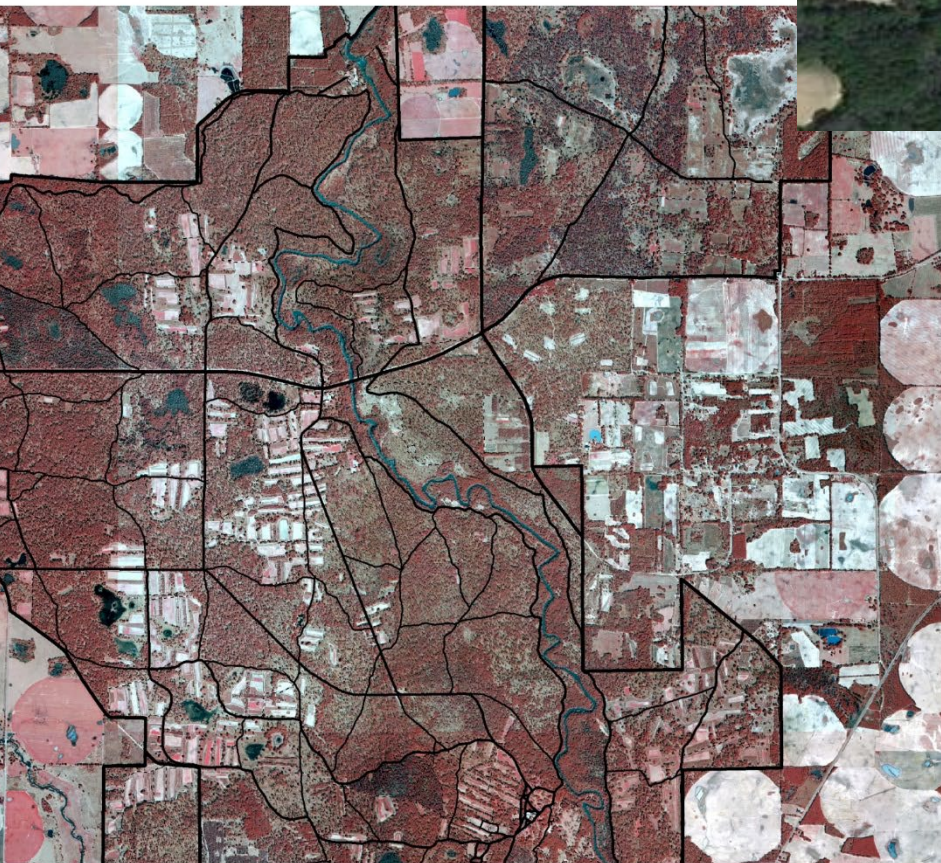
Rearing Amphibians



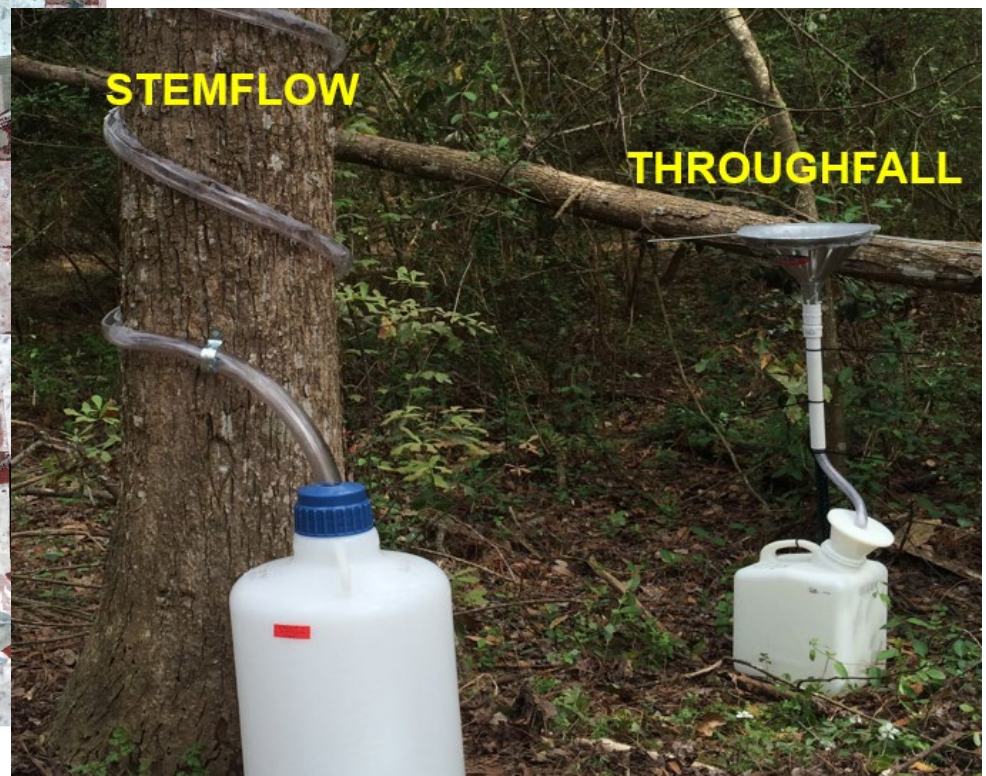
Exposure Methods



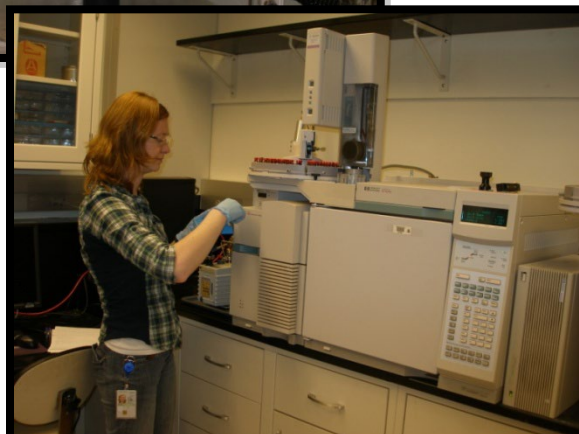
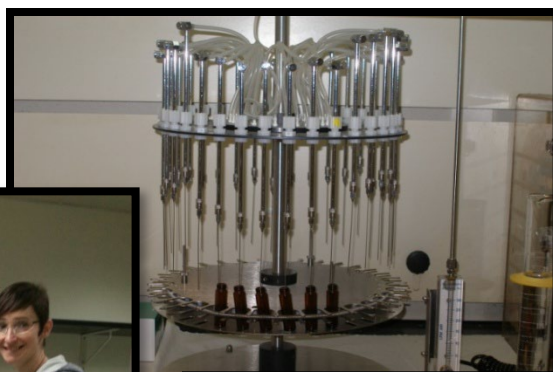
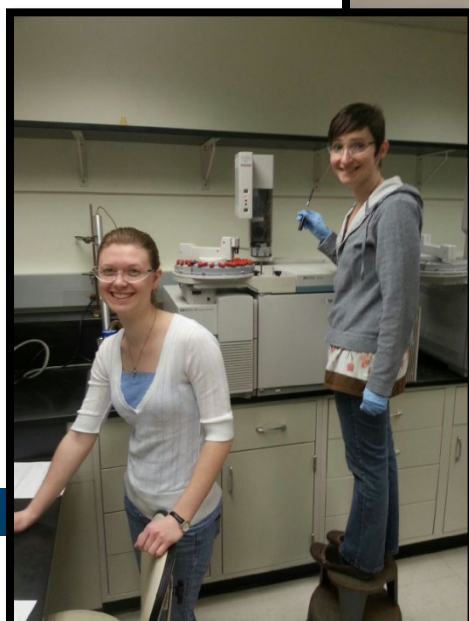
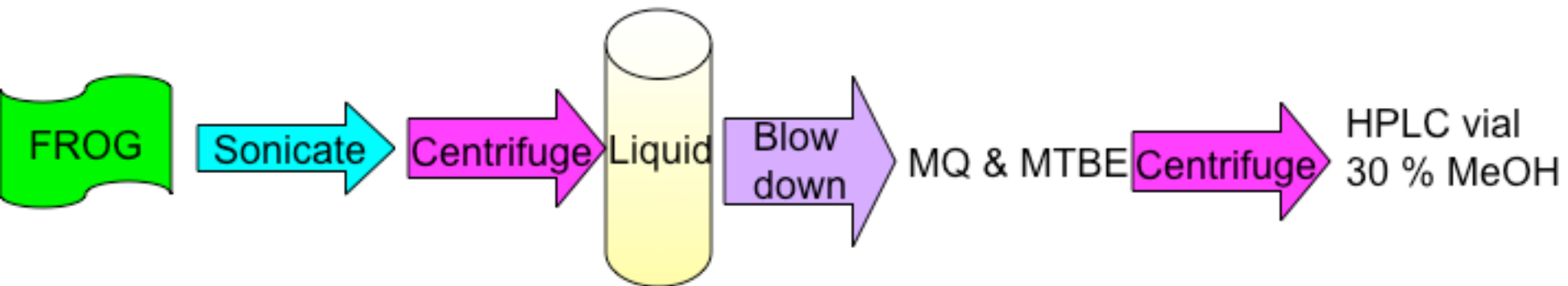
Field sites



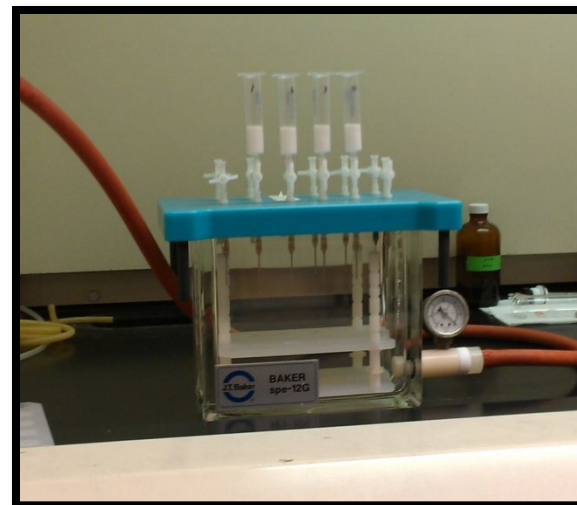
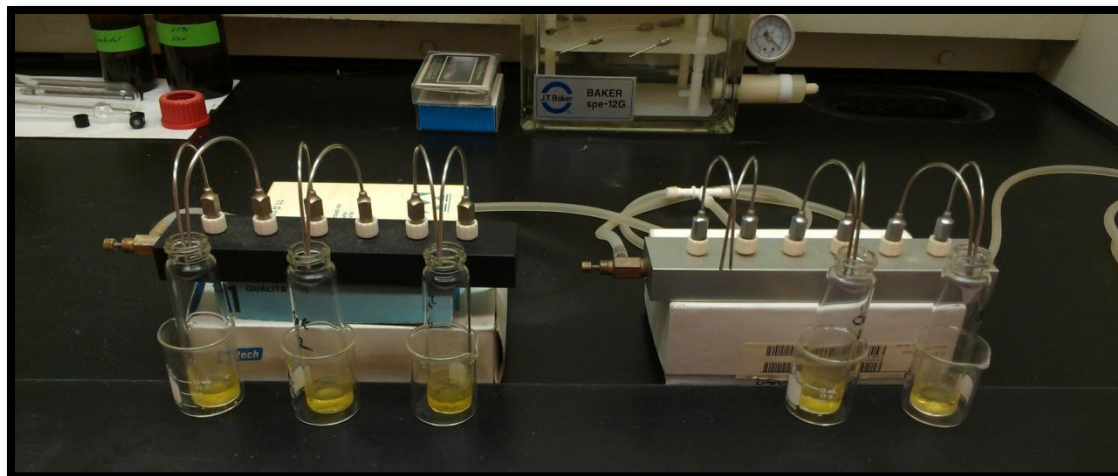
2,000 1,000 0



Sample Extraction



Protocol Development



Whole body frog tissue, livers, soil, water extraction methods

Methanol and MTBE solvents

GC/MS & LC/MS analysis of pesticide body burdens, metabolomics

Study	N	BCF	Species	Pesticides	Treatments
Henson-Ramsey et al. 2008	9	0.015 – 0.133	<i>Ambystoma tigrinum</i>	Malathion	Soil application
Van Meter et al. 2014	106	0.005-0.614	<i>Lithobates sphenoccephala</i> , <i>Anaxyrus fowleri</i> , <i>Hyla versicolor</i> , <i>Acris crepitans</i> , <i>Gastrophysa carolinensis</i> , <i>Hyla gratiosa</i> , <i>Hyla cinerea</i>	Imidacloprid, triadimefon, fipronil, pendimethalin	Soil application
Van Meter et al. 2015	90	0.013 – 1.16	<i>Hyla gratiosa</i> , <i>Hyla cinerea</i>	Imidacloprid, atrazine, triadimefon, fipronil, pendimethalin	Direct spray versus soil application
Van Meter et al. 2016	96	0.01-0.61	<i>Anaxyrus americanus</i>	Imidacloprid, atrazine, triadimefon, fipronil, pendimethalin	Exposures on soil with contrasting organic matter content
Glinski et al. 2018a	300	0.00014-2.76	<i>Lithobates sphenoccephala</i> , <i>Anaxyrus fowleri</i>	Atrazine, triadimefon, metolachlor, chlorothalonil, imidacloprid	Soil exposures after varying dehydration times
Glinski et al. 2018b	60	NA	<i>Anaxyrus fowleri</i>	Atrazine, triadimefon, fipronil	Soil application
Van Meter et al. 2018	137	NA	<i>Lithobates clamitans</i>	Atrazine, metolachlor, 2,4-D, malathion, propiconazole	Soil applications including pesticide mixtures
Glinski et al. 2018c	192	0.0004-4.84	<i>Lithobates sphenoccephala</i>	Bifenthrin, metolachlor, triadimefon	Soil applications including pesticide mixtures
Van Meter et al. 2019	96	NA	<i>Lithobates sphenoccephala</i>	Atrazine, alachlor	Exposures on soil, including mixtures
Glinski et al. 2021	24	0.18-4.63	<i>Lithobates sphenoccephala</i>	Bifenthrin, chlorpyrifos, trifloxystrobin	Exposures on soil versus surface water
Van Meter et al. 2022	48	0.19-2.47	<i>Lithobates sphenoccephala</i>	Atrazine, alachlor	Exposures on soil, including mixtures

Selected Research Questions

- Is hydrophobicity (using K_{ow} as a proxy) a useful predictor like it is on other terrestrial vertebrates?
 - Answer: not so much. Van Meter et al. 2014, Environmental Pollution.
- Is there a significant difference between direct (aerial) and indirect (soil) exposures for dermal uptake?
 - Answer: Yes, significant difference. Van Meter et al. 2015. Environmental Contamination and Toxicology.
- Does soil organic carbon content impact amphibian uptake?
 - Answer: Yes, high OC content significantly negatively impacts uptake. Van Meter et al. 2016
- Can we quantify hepatic microsomal metabolic rates of pesticides with known degradates?
 - Yes, for commonly applied pesticides in south Georgia. Glinski et al. 2018a.
- Can we identify changes in metabolic profiles for exposed amphibians versus controls?
 - Yes, Snyder et al 2017, Aquatic Toxicology. Van Meter et al 2018, Glinski et al 2018b
- Can we quantify forested exposure concentrations experienced by treefrogs?
 - Yes, pesticide concentrations characterized in stemflow and canopy throughfall in south Georgia. Glinski et al. 2018c.
- Does the hydration status of an exposed amphibian affect dermal uptake?
 - Answer: Yes, but in the opposite way that we expected. Glinski et al 2018c.

Amphibian Exposure Database

Each published study tests a hypothesis but also added another terrestrial amphibian exposure database to the literature.

Species used: Barking treefrog (*Hyla gratiosa*), Green treefrog (*Hyla cinerea*), American toad (*Bufo americanus*), Southern leopard frog (*Lithobates sphenoccephala*), Fowler's toad (*Anaxyrus fowleri*), Eastern narrowmouth toad (*Gastrophryne carolinensis*)

Amphibians reared in Athens (field collected adults -> fertilized eggs -> metamorph stage)

Pesticides used in the lab: Imidacloprid, Atrazine, Triadimefon, Fipronil, Pendimethalin, Metolachlor, Bifenthrin, Tebiconazole, Chlorothalonil.

Pesticide applications at maximum labeled rates.

Untargeted analyses of field caught amphibians and associated exposure concentrations in south Georgia

We have combined these data sets into a single data set for testing amphibian dermal exposure models


Collated dataset is now published (in press IEAM) and publicly available

https://github.com/amphibian-exeff/purucker_dermalcollation

amphibian-exeff / purucker_dermalcollation Public Notifications

<> Code Issues Pull requests Actions Projects Wiki Security Insights

master 1 branch 0 tags Go to file Code

 puruckertom Update README.md 0dbc871 on Aug 8, 2021 144 commits

data_in	fix 2019/21 input	9 months ago
data_out	updated all final rmd stuff, and added in script	9 months ago
graphics	fix 2019/21 input	9 months ago
notebooks	add jupyter notebook for bayesian inference/mcmc	4 years ago
pdf	adding Glinski 2019	3 years ago
src	updated all final rmd stuff, and added in script	9 months ago
.gitignore	add jupyter notebook for bayesian inference/mcmc	4 years ago
README.md	Update README.md	8 months ago
runtime.txt	create runtime.txt to load snapshot of R	4 years ago

README.md

About
Code and data repository for Purucker ST, Snyder MN, Glinski DA, Van Meter RJ, Garber K, Cyterski MJ, Sinnathamby S, Henderson WM, 2021. Estimating dermal contact soil exposure for amphibians.
Readme
1 star
4 watching
0 forks

Releases
No releases published

Packages
No packages published

Metadata

```
1 # -----
2 Metadata for amphib_dermal_collated.csv
3
4 "Estimating dermal contact soil exposure for amphibians"
5 Purucker ST, Snyder MN, Glinski DA, Van Meter RJ, Garber K, Cyterski MJ, Sinnathamby S, Henderson WM.
6 # -----
7
8 amphib_dermal_collated.csv is the final collated data set containing all amphibian dermal exposure studies.
9
10
11 Column labels and description of the column for the csv file.
12
13 app_rate_g_cm2 - application rate of pesticide in g/cm^2
14 application - application method
15                 (overspray, soil, indirect)
16 body_weight_g - body weight of exposed amphibian in grams
17 chemical - pesticide used in the study
18 exp_duration - duration of exposure experiment in hours
19 formulation - commercial formulation or active ingredient dissolved in Methanol
20                 (commercial formulation = 1, active ingredient dissolve in Methanol = 0)
21 sample_id - sample identifier used in the original study
22 soil_conc_ugg - soil concentration of pesticide in ug/g
23 soil_type - type of soil used in the study if available
24                 (PLE = Plott series soil, OLS = Orangeburg loamy-sand soil, NA)
25 source - manuscript where data originated from
26 species - amphibian species name
27           (Barking treefrog, Cricket frog, Fowlers toad, Gray treefrog, Green treefrog, Leopard frog, Mole salamander, Narrowmouth toad)
28 tissue conc_ugg - amphibian tissue concentration in ug/g
```

1158 observations of
tissue residues in 11
amphibian species
across 14 different
pesticides.

Dataset

		app_rate_g_cm2	application	body_weight_g	chemical	exp_duration	formulation	sample_id	soil_conc_ugg	soil_type	source	species	tissue_conc_ug
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5	4	5.58e-06	overspray	2.91551	imidacloprid	8	0	HGO4	0.47984644	PLE	rvm2015	Barking treefrog	0.718277624
6	5	5.58e-06	overspray	2.49115	imidacloprid	8	0	HGO5	0.47984644	PLE	rvm2015	Barking treefrog	1.08903614
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15	14	2.3e-05	overspray	2.36942	atrazine	8	0	HGOA4	15.10490982	PLE	rvm2015	Barking treefrog	17.83079959
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17	16	1.11e-06	overspray	2.73317	fipronil	8	0	HGOF1	3.492483429	PLE	rvm2015	Barking treefrog	2.400285765
18	17	1.11e-06	overspray	2.02542	fipronil	8	0	HGOF2	3.492483429	PLE	rvm2015	Barking treefrog	1.658107241
19	18	1.11e-06	overspray	2.12849	fipronil	8	0	HGOF3	3.492483429	PLE	rvm2015	Barking treefrog	1.662784049
20	19	1.11e-06	overspray	1.73265	fipronil	8	0	HGOF4	3.492483429	PLE	rvm2015	Barking treefrog	2.07396123
21	20	1.11e-06	overspray	1.52771	fipronil	8	0	HGOF5	3.492483429	PLE	rvm2015	Barking treefrog	1.915890199
22	21	2.85e-06	overspray	1.72511	triadimefon	8	0	HGOT1	8.451291713	PLE	rvm2015	Barking treefrog	0.636157453
23	22	2.85e-06	overspray	1.94887	triadimefon	8	0	HGOT2	8.451291713	PLE	rvm2015	Barking treefrog	0.972799964
24	23	2.85e-06	overspray	1.55295	triadimefon	8	0	HGOT3	8.451291713	PLE	rvm2015	Barking treefrog	0.397699131
25	24	2.85e-06	overspray	2.37346	triadimefon	8	0	HGOT4	8.451291713	PLE	rvm2015	Barking treefrog	0.642604155
26	25	2.85e-06	overspray	1.57685	triadimefon	8	0	HGOT5	8.451291713	PLE	rvm2015	Barking treefrog	0.48091159
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28	27	5.69e-06	soil	2.26328	imidacloprid	8	0	HGI2	2.565991685	PLE	rvm2015	Barking treefrog	0.210770534
29	28	5.69e-06	soil	1.27439	imidacloprid	8	0	HGI3	2.565991685	PLE	rvm2015	Barking treefrog	0.50673308
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32	31	1.98e-05	soil	2.44812	pendimethalin	8	0	HGP1	13.61565316	PLE	rvm2015	Barking treefrog	0.409017886
33	32	1.98e-05	soil	2.46997	pendimethalin	8	0	HGP2	13.61565316	PLE	rvm2015	Barking treefrog	0.257508419

Exposure data set utility

This data set can be used to test the protectiveness and accuracy of amphibian dermal exposure models

- We have tested screening models to ensure that they do not underpredict body burdens
- Can be used by us (and others) to evaluate screening and higher tiered models of dermal exposure

This is only one aspect of amphibian exposure modeling, but an important area where not much info was available

Testing existing/proposed models

We have used this dataset to test the dermal component of TIM and a proposed EFSA modification

Can also be used to test other surrogate models in use, proposed models at different tiers

Comparing to available field data to estimate differences between post-exposure field and laboratory body burdens

Lower-tier models can be evaluated to ensure that they do not underpredict body burdens, higher-tier models can be evaluated/calibrated for accuracy while ensuring protectiveness

TIM Dermal Exposure Algorithm (Direct Interception)

Dermal exposure from applied pesticide droplets is considered for each time step representing a pesticide application for aerial, airblast and ground applied sprays (See **Section 1.4.2**). The dermal exposure dose from direct interception ($D_{\text{intercept}(t)}$) is calculated by considering the pesticide application rate relationship to the surface area and BW of the bird (**Equation 6.2**; **Table 6.1**). The dermal interception model assumes that pesticide deposition occurs in a manner consistent with a horizontal surface in the treatment area. Surface area calculation of a bird for the interception model assumes that the upper half of the bird in the field is exposed as a result of either ground or aerial spray applications. Therefore, the total surface area of the bird is multiplied by 0.5. The total surface area of a bird is calculated using the allometric equation for relating BW to surface area (USEPA, 1993; **Equation 6.3**). The dermal adsorption fraction (DAF) is used to account for pesticide specific data that define a fraction of the pesticide mass present on the bird that is actually absorbed by the bird. These data may be submitted by the registrant (non-guideline study) or obtained from the literature. When no data are available to parameterize DAF, the default value is 1. In this equation, a factor of 11.2 is used to convert the units of the application rate, which are lb a.i./A, to the metric units needed to generate a concentration value expressed in $\mu\text{g a.i./g-bw}$.

Equation 6.2.
$$D_{\text{intercept}(t)} = \frac{(A_{\text{rate}} * 11.2) * (SA_{\text{total}} * 0.5) * \text{DAF}}{BW}$$

Equation 6.3.
$$SA_{\text{total}} = 10 * BW^{0.667}$$

EFSA Modification

Dermal exposure

For the calculation of the dermal dose (Tables 60, 61 and 62), it was assumed that the animal is oversprayed in field at the full rate (worst-case assumption of no crop interception), only upper side exposed (half of its surface) and that 100% is absorbed. The application rate was assumed to be 1 kg/ha (as for oral uptake).

Amphibians

In Wildlife exposure factors handbook (USEPA), equations are provided to calculate the skin area surface (S_{skin}) with a power function of the animals' weight (p 3–14 or 514/572):

$S_{\text{skin}} (\text{cm}^2) = 1.131 W^{0.579} (\text{g})$ (all frogs) (less protective when compared with two other models)

$S_{\text{skin}} (\text{cm}^2) = 0.953 W^{0.725} (\text{g})$ bull frog

$S_{\text{skin}} (\text{cm}^2) = 0.997 W^{0.712} (\text{g})$ green frog

$S_{\text{skin}} (\text{cm}^2) = 8.42 W^{0.694} (\text{g})$ salamanders

The allometric equations for body surface area from the US EPA exposure handbook are identical with the ones from Hutchinson et al. (1968).

The formula for *Hyla arborea* from Hutchinson et al. (1968) was added to the species from the Wildlife exposure handbook.

$$SA = 0.905 \times W^{0.823}$$

SA = surface area in cm²

W = body weight in g

Table 60: Dermal exposure calculation from overspray for different groups of amphibians

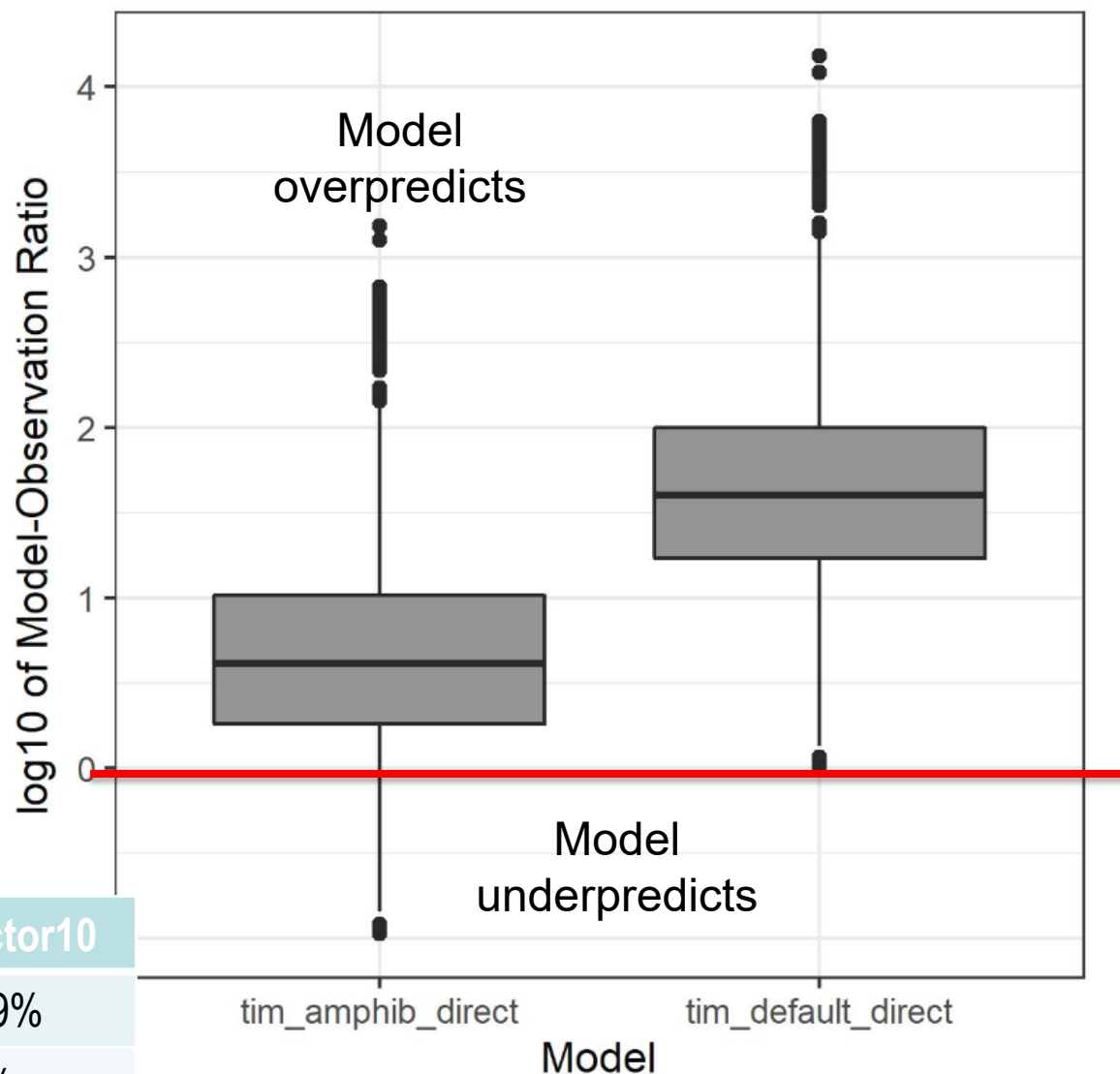
Amphibians	Body weight (g)	Total surface (cm ²) ^(a)	Dermal absorption%	Applied rate (kg/ha)	Applied rate (mg/cm ²)	Dermal dose (mg/kg bw)
Green frog	85	23.5744	100	1	0.01	1.387
Bull frog	500	86.2673	100	1	0.01	0.863
All frogs	100	16.2728	100	1	0.01	0.814
<i>Hyla arborea</i>	1.4	1.1937	100	1	0.01	4.263
<i>Hyla arborea</i>	11	6.5120	100	1	0.01	2.96
Salamander	50	127.1737	100	1	0.01	12.717

bw: body weight.

(a): For the calculation of the dermal dose, the total surface was divided by 2 assuming that only the upper side of the animal is exposed.

Model ratios: (Model_Predictions/Observations)

Comparison of
model predictions
to observations



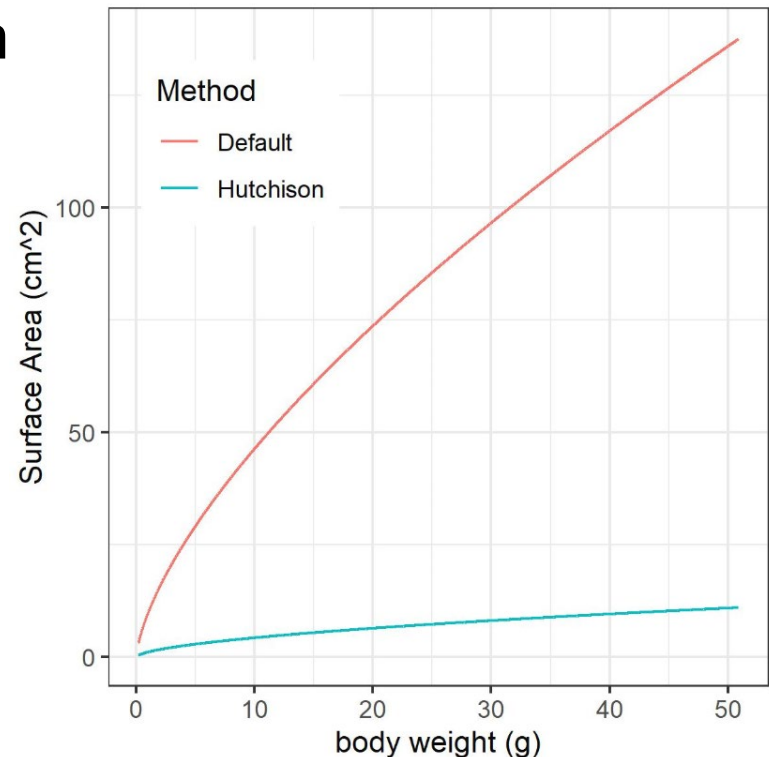
model	under	Factor10
tim_default_direct	0%	11.9%
tim_amphib_direct	11.3%	63%

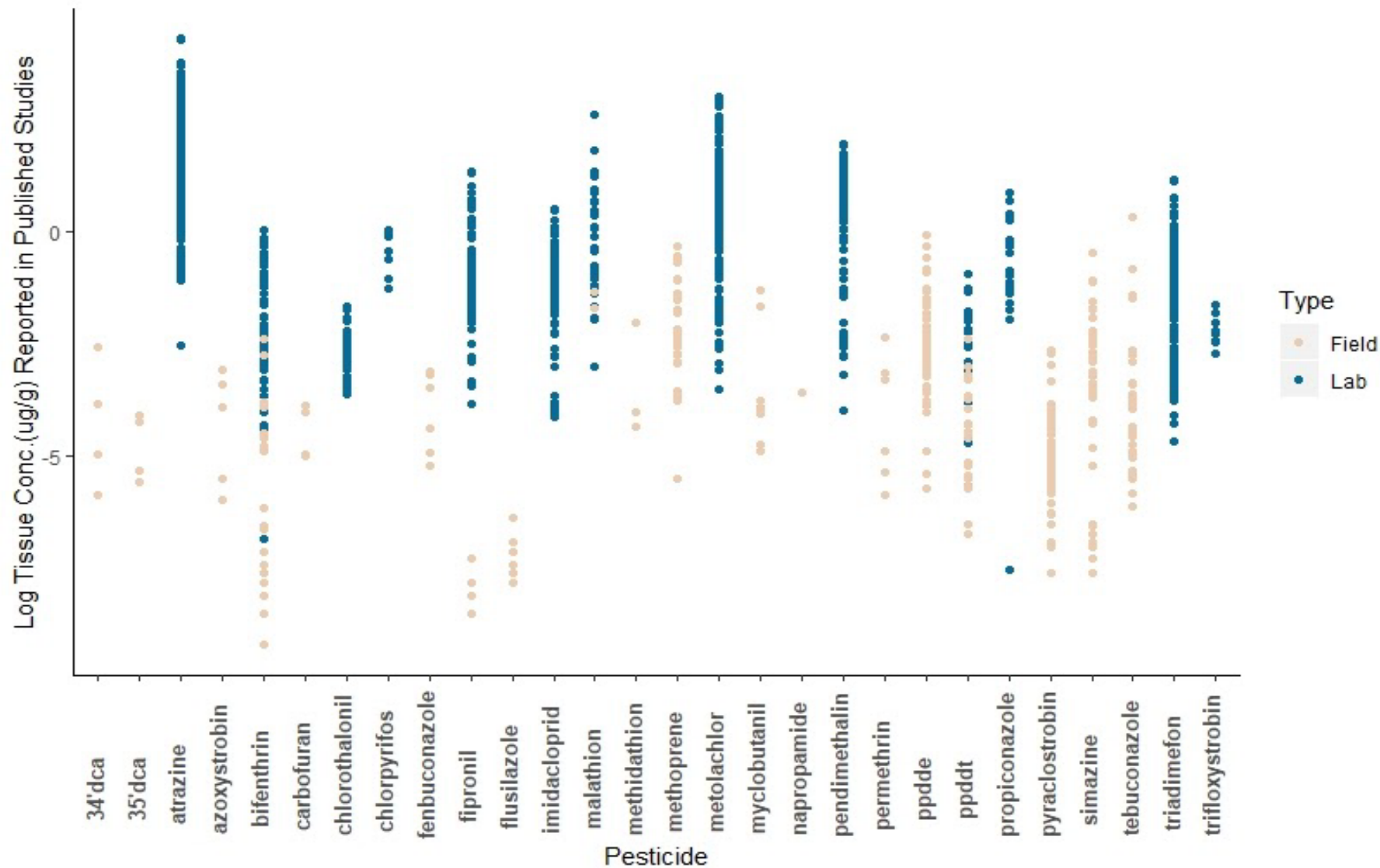
Why the difference?

We have used this dataset to test the dermal component of TIM (based on birds) and a proposed EFSA modification (using amphibian surface area)

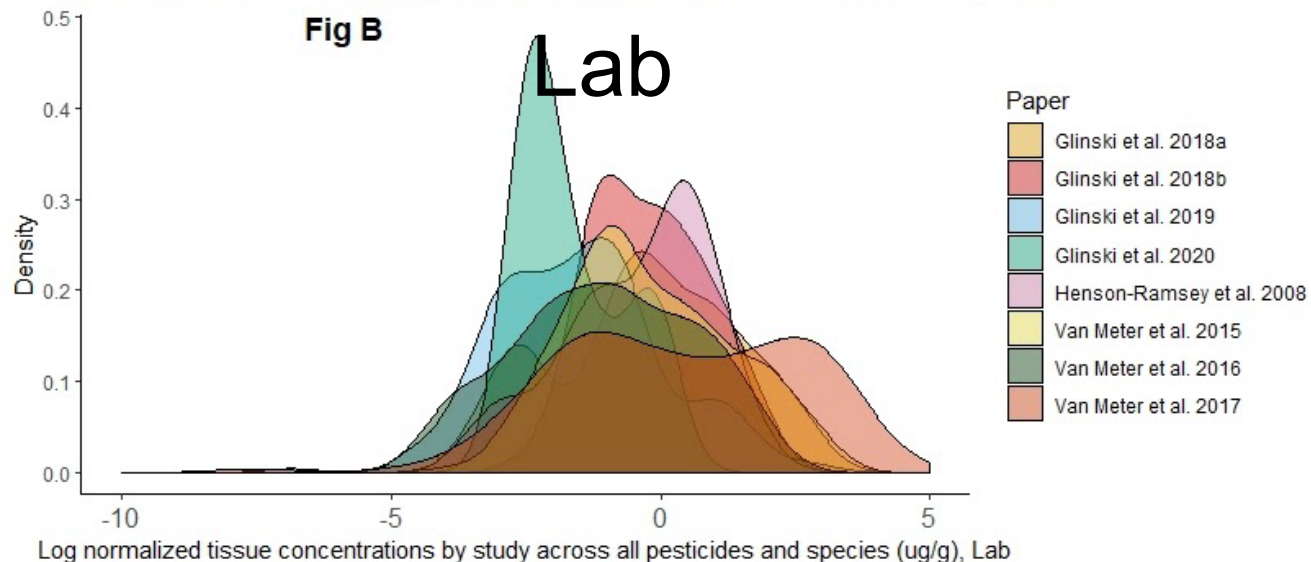
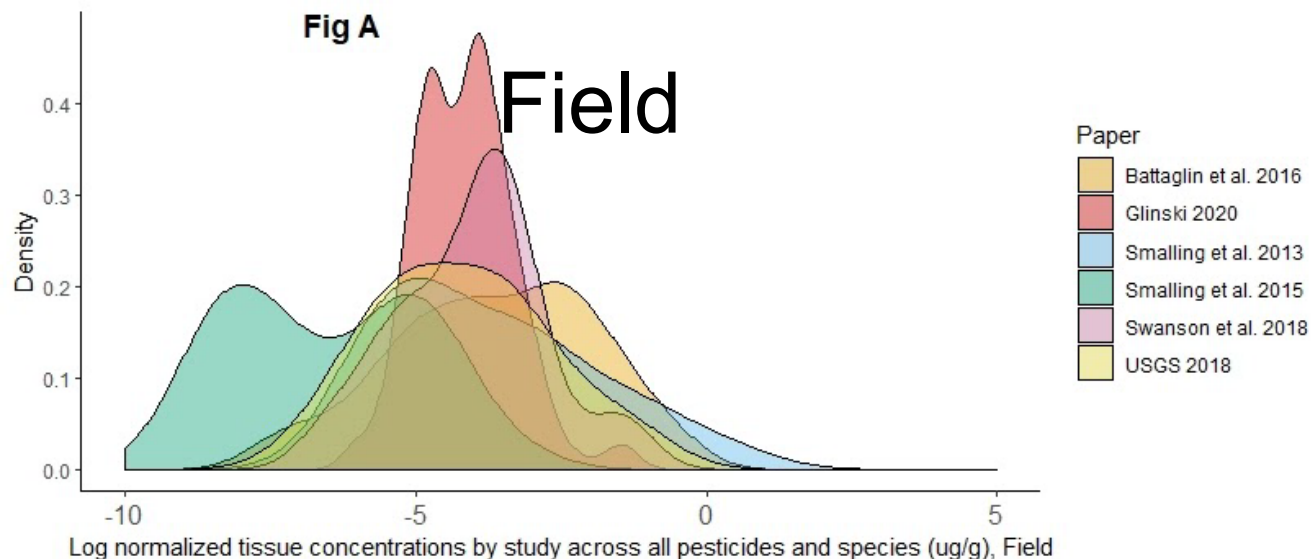
Can also be used to test other surrogate models in use, proposed models at different tiers

EFSA uses Hutchison modification





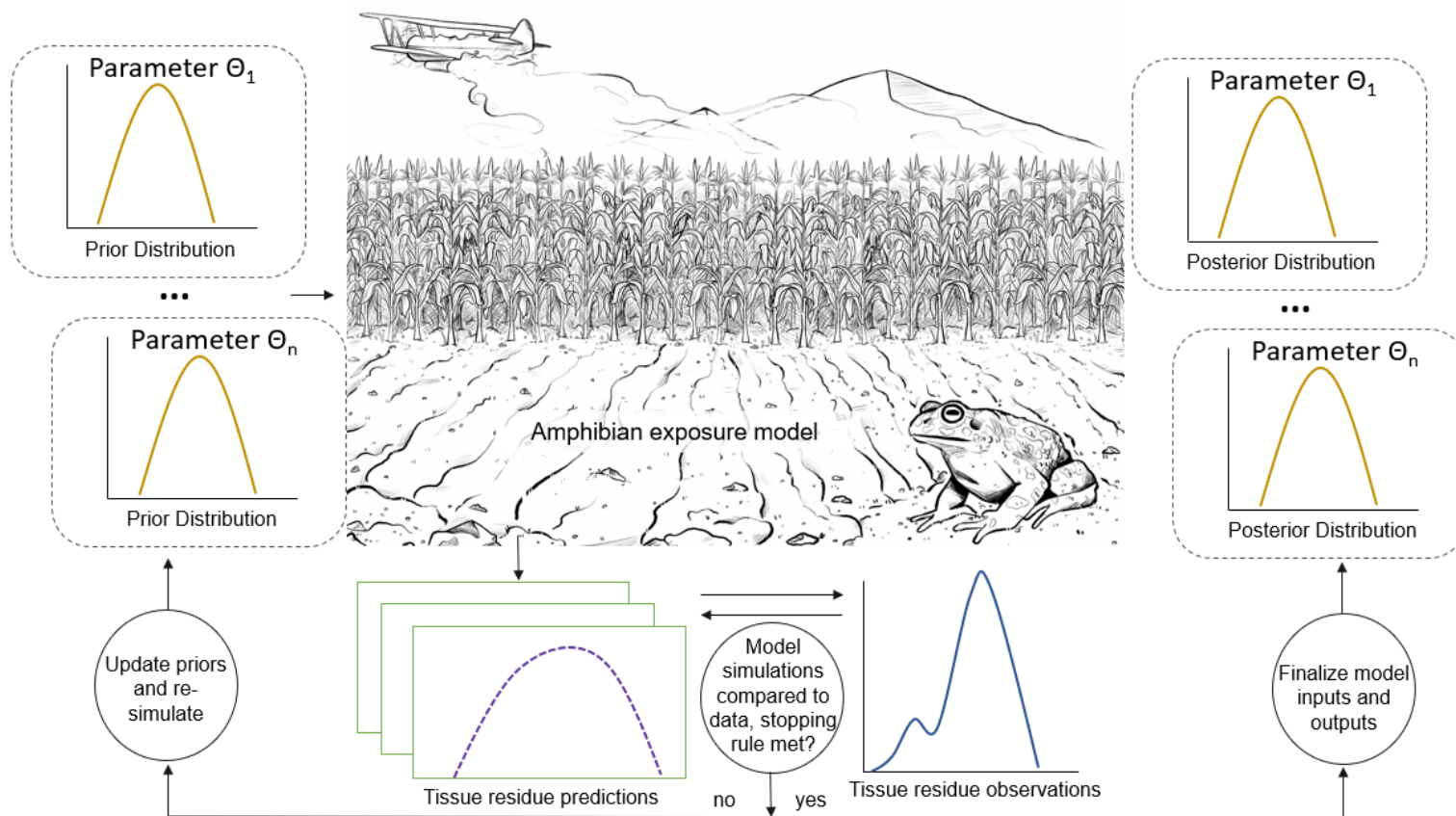
Field v Lab Data (Pesticides combined)



Ongoing work

We are also leveraging this dataset to parameterize and test alternative candidate models

Trying different model constructs, physical properties, exposure assumptions, etc.



Status and Next Steps

Dermal exposure for amphibians is an important route for many species and habitat combinations (T-Rex covers ingestion, TIM adds dermal)

We have published a collated data set that can be used to evaluate existing and proposed dermal exposure algorithms

The USEPA current TIM dermal approach is protective (though perhaps not for the right reasons)

The proposed EFSA modification with amphibian surface area modifications is logical but problematic (for screening)

This data set can be used to evaluate new fit-for-purpose amphibian-specific exposure algorithms at different tiers

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