User's Guide

T-HERPS Version 1.0

(**Terrestrial Herpetofaunal Exposure Residue Program Simulation**)

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Acknowledgements

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EPA uses the model T-HERPS to estimate dietary exposure and risk to terrestrial-phase amphibians and reptiles from pesticide use. This guidance document serves as a User's Guide to T-HERPS version 1.0 and discusses the following items:

- Summary of changes made to T-REX (v. 1.3.1) in the development of T-HERPS (v. 1.0);
- Guidance on using RQs generated by T-HERPS (v. 1.0); and
- Limitations and uncertainties in T-HERPS

Additional information on T-REX inputs and equations not discussed in this document may be found in the T-REX (version 1.3.1) User's Guide (U.S. EPA, 2006). T-HERPS is currently approved for assessing exposure and risk to the California red-legged frog (CRLF) (*Rana aurora draytonii*) and terrestrial-phase herptiles with similar dietary behavior as the CRLF. Future versions of T-HERPS will allow for an evaluation of dietary exposures of additional terrestrial herptiles, including herbivores.

1. Introduction

In conducting ecological risk assessments, EPA currently uses birds as surrogates for terrestrial-phase amphibians and reptiles. However, reptiles and amphibians are poikilotherms (body temperature varies with environmental temperature), while birds are homeotherms (temperature is regulated, constant, and largely independent of environmental temperatures). Therefore, reptiles and amphibians (collectively referred to as herptiles in this guidance) tend to have much lower metabolic rates and lower caloric intake requirements than birds or mammals. As a consequence, birds are likely to consume more food than amphibians or reptiles on a daily dietary intake basis, assuming similar caloric content of the food items. This difference is evident by comparing the estimated caloric requirements for free-living iguanid lizards (Iguanidae) to those of passerines (U.S. EPA, 1993):

$$\text{Iguanid: } FMR = 0.0535(Wt)^{0.799} \qquad \text{(EQ 1)}$$

$$\text{Passerine: } FMR = 2.123(Wt)^{0.749} \qquad \text{(EQ 2)}$$

where FMR = free-living metabolic rate [kcal/day]
Wt = body mass [g]

Given the similar exponents on the allometric functions, the free-living metabolic rate of birds can be 40 times higher than reptiles of similar weight, with differences narrowing as body weight increases. Consequently, use of an avian food intake allometric equation as a surrogate to herptiles is likely to result in an over-estimation of exposure for reptiles and terrestrial-phase amphibians.

Because there was a need to evaluate dietary exposure to terrestrial-phase amphibian species (*e.g.*, California red-legged frog, CRLF) and other amphibians and reptiles, EPA developed the terrestrial model T-HERPS. In developing T-HERPS, T-REX (version 1.3.1) was modified to allow for an estimation of food intake for herptiles using the same basic procedure that T-REX uses to estimate avian food intake.

Although this version of T-HERPS may be used to evaluate both reptiles and terrestrial-phase amphibians, some of the guidance presented in this document focuses on assessment of the CRLF, given the current need to assess exposures and associated risks to this species.

2. Summary of Changes in T-REX to Allow for Food Intake Estimation for Herptiles

2.1. Food Intake Allometric Equation

Equation 3 is an insectivore iguanid food ingestion rate that was implemented in T-HERPS to allow for estimation of daily food ingestion for herptiles (Nagy, 1987 as cited in U.S. EPA, 1993):

$$FI = 0.013(Wt)^{0.773} \quad \text{(EQ 3)}$$

Where FI = food ingestion [g/day].

The insectivore food intake equation was chosen to be consistent with the California red-legged frog diet. Therefore, this version of T-HERPS should not be used to estimate potential exposures to herbivores.

The T-HERPS-implemented Equation 3 replaces the following equivalent allometric equation that is used in T-REX (v. 1.3.1.) to estimate food ingestion rates of birds, reported by Nagy (1987) and cited in U.S. EPA (1993):

$$FI = 0.648(Wt)^{0.651} \quad \text{(EQ 4)}$$

The iguanid allometric equation (EQ 3) is used to estimate the food ingestion rate of herpetofauna. It is assumed that since both reptiles and amphibians are poikilothermic, they have similar caloric requirements.

The assumption that use of the iguanid lizard allometric equation results in a reasonable approximation of terrestrial-phase amphibian food intake was tested. For this analysis, measured food intake values reported for juvenile bullfrogs (*Rana catesbeiana*) by Modzelewski and Culley (1974, as cited in U.S. EPA, 1993) were compared to estimates derived using Equation 3 for the same body weight range. This analysis showed that food intake values for juvenile bullfrogs in the Modzelewski and Culley (1974) study are reasonably approximated using the allometric equation for iguanid lizards. The data in juvenile bullfrogs reported daily food intake values that ranged from approximately 3% to 7% of their body weight. Estimates of daily food intake using T-HERPS for the same range of body weights ranged from approximately 3% to 5% body weight.

2.2. Addition of small mammals and amphibians as potential dietary items

The current version of T-REX (v. 1.3.1) evaluates exposure from consumption of grasses, plants, insects, seeds, and fruits. However, some herpetofauna consume small mammals and other amphibians. Because larger CRLFs may consume up to approximately half of their diet from consumption of larger prey (vertebrates), the potential exposure from these food sources needs to be evaluated. There is uncertainty in estimated exposure concentrations (EEC) resulting from consumption of contaminated prey species; therefore, simplifying assumptions were made in T-HERPS that likely result in a conservative estimate of exposure in most cases (See Section 4.3 for discussion of uncertainties). EECs resulting from consumption by herpetofauna (*e.g.*, CRLF) of small mammals and herpetofauna (*e.g.*, prey) that have consumed contaminated food items are estimated using procedures outlined in Sections 2.2.1 and 2.2.2.

2.2.1. EECs from Consumption of Prey Herpetofauna

In order to assess potential exposures to CRLF via consumption of a pesticide contained in herpetofauna, concentrations of the pesticide in the prey item must first be estimated. The basis for the herpetofaunal prey item EEC is the oral daily dose for the prey item. Daily dose is calculated using methodology in T-REX (v. 1.3.1) with incorporation of Equation 3 as described in Section 2.1. The prey herptile is assumed to eat small insects. Then, assuming the entire prey species is consumed, the daily dose calculated for the prey herptile species (mg/kg-bw) is equal to the dietary exposure concentration (mg/kg-food item = ppm). Therefore, the resulting estimated dietary concentration in small prey amphibians (ppm) can be used in the same manner as other standard food items represented in T-REX (plants, insects, fruits, etc., with estimates of residue levels from the Kenaga nomogram) to estimate potential dose-based exposures. In other words, exposure is a function of residue level in the prey item and food intake of the assessed species.

For the CRLF assessment, the weight of the prey item was based on data for the Pacific tree frog (*Pseudacris regilla*), which has been reported to be a dietary item of the CRLF (CA OEHHA, 1999). The user can alter the weight of the prey amphibian as needed for species specific assessments.

2.2.2. EECs from Consumption of Prey Mammals

For mammals that serve as prey to the CRLF, an alternative method for estimating exposure (EECs) is used. This alternative method is necessary because the weight of a small mammal that may be consumed is larger than the estimated daily food intake, resulting in an underestimation of acute exposures (see Section 4.3). Two mammalian EECs are calculated by T-HERPS by assuming the prey mammal consumes either (1) short grass or (2) large insects.

Potential exposures from consumption of contaminated mammals is calculated in T-HERPS using the following steps: (1) estimated daily dose for a mammal (mg/kg-bw) of user defined size is calculated using methodology identical to that incorporated into T-REX (version 1.3.1.); (2) the mass of pesticide consumed (mg) by the assessed species is calculated by multiplying the weight of the prey item (kg-bw) by the dose in the prey item (mg/kg-bw); (3) the resulting EEC (mg/kg-bw) for the assessed herpetofaunal species is then calculated as the pesticide mass consumed (mg/bw of assessed species (kg-bw)). Uncertainties associated with this calculation are discussed in Section 4.

The assessor may choose the body weight of the prey item consumed by the assessed species. For the CRLF, prey mammals are assumed to be 35 grams, which is the high-end weight of a deer mouse (U.S. EPA, 1993). However, alternative body weights can be entered to evaluate the potential effect of body weight on EECs and RQs (discussed further in Section 4).

2.3. Water content of food items

Water content of potential food items is used to convert the dry weight food intake estimate to wet weight for use in Equation 3. Water contents of various potential food items of wildlife are reported in Tables 4-1 and 4-2 of U.S. EPA (1993). Given that availability of particular food items will vary across locations and time, the use of the highest mean water content of the taxonomic group (*e.g.*, terrestrial invertebrates) consumed by the assessed species is recommended at this time. For the CRLF, water content of terrestrial-phase insects (69% water; U.S. EPA, 1993) is used in the dose calculation for small terrestrial-phase CRLFs, and a water content of terrestrial herptiles (85% water; U.S. EPA, 1993) is used for the dose-calculation for larger terrestrial-phase CRLFs.

2.4. Body Weight of Assessed Animals

In the T-HERPS spreadsheet, up to three body weights of herpetofauna can be entered. They are referred to as small, medium, and large animals in T-HERPS; however, any three values may be entered. Fewer than three values may also be entered.

For the CRLF, data from Fellars (2007) summarized in Table 1 below were used to define the range of terrestrial-phase red-legged frog body weights that are used as default body weights in Cells D29 to D31. Frogs collected by Fellars (2007) were collected from Point Reyes National Seashore and may not be reflective of the range of weights for the species over its entire range. However, these data are considered the best available information for the species.

Table 1. Summary Statistics for California Red-Legged Frog Size Data (Fellars (2007).

Statistic	Length (cm)	Weight (g)
Number of Observations (N)	545	545
Mean	6.1	37
SD	3.7	43
Minimum	2.5	1.4
Median	4.7	9.9
Maximum	13	238

These body weights are also used by the risk assessor to provide insight into the relevance of some food items. For example, terrestrial phase CRLFs reported by Fellers (2007) were as small as approximately 1 gram. It is unlikely that a small mammal will be consumed by a 1 gram frog. Therefore, RQs are not calculated for small terrestrial phase herpetofauna that consume mammals. Therefore, RQs in the summary tables of the “print results” worksheet are not calculated for all body weight/food item combinations. The user should consider the body weight of the assessed species and the body weight assumptions of the prey items when evaluating the RQs from T-HERPS.

3. Guidance on Using RQs Generated by T-HERPS in Effects Determinations of the CRLF

The following guidance should be considered by risk assessors in developing RQs and effects determinations for direct effects to terrestrial-phase CRLFs. The risk assessor should note that all available lines of evidence, in addition to RQs generated by T-HERPS, should be considered when making an effects determination.

Currently, RQs from T-HERPS are calculated only if the "standard" avian RQs calculated by T-REX (v.1.3.1) exceed the endangered species LOC for acute or chronic exposures. If avian RQs do not exceed the endangered species avian LOC of 0.10, then RQs that incorporate the food intake allometric equation for herptiles would presumably not exceed LOCs because of the lower food intake of herptiles relative to birds. In situations where the avian RQ is less than the LOC, the effects determination for dietary exposures to terrestrial-phase amphibians is "no effect" and no further evaluation is required. However, the uncertainty section of the risk characterization should include a discussion of the food intake assumptions as they relate to the conservative nature of the "no effect" determination (see Section 4).

If avian RQs calculated by T-REX (v.1.3.1) exceed any avian LOC, a preliminary "may effect" determination is made by the risk assessor, and RQs that incorporate estimates of dietary exposure for terrestrial-phase herpetofauna may be used to further characterize LOC exceedances. If both avian and herpetofauna RQs exceed the endangered species avian LOC, then the effects determination is "likely to adversely affect" (LAA). However, the uncertainties discussed in Section 4 of this document should be discussed in the effects determination. If avian RQs exceed any LOC, but none of the RQs that incorporate Equation 3 exceed LOCs, then the effects determination may be "may affect, but not likely to adversely affect" (NLAA). However, all available lines of evidence should be considered when making an effects determination.

4. Limitations and Uncertainties in T-HERPS

4.1. Exposure Pathways Not Quantified in T-HERPS

T-HERPS evaluates potential exposures to terrestrial-phase herpetofaunal species resulting from consumption of **terrestrial** organisms. T-HERPS does not estimate EECs from consumption of aquatic organisms. If the assessed chemical does not bioaccumulate, then the absence of quantifying potential exposures from consumption of aquatic animals is unlikely to impact the conclusions of the assessment. However, if the assessed chemical does bioaccumulate in aquatic organisms, the consumption of aquatic organisms could be an important exposure source, and this should be captured in the risk characterization.

Consistent with the standard assessment process for terrestrial organisms, T-HERPS does not evaluate a number of potential exposure routes, including dermal exposures, water intake/submersion, or inhalation. For some pesticides, each of these exposure routes could be significant for terrestrial-phase frogs. If any lines of evidence are available to allow for characterization of the potential importance of these potential exposure routes, then these should be discussed in the risk characterization.

4.2. Use of Avian Toxicity Data as a Surrogate for Herpetofauna

In the absence of data on terrestrial herpetofauna, T-HERPS uses avian toxicity data as a surrogate for risk estimation. Although differences in sensitivity may be expected, the lack of available toxicity data on reptiles and amphibians precludes a robust comparison to birds. This represents a source of uncertainty in the estimated risks to amphibians and reptiles. In order to address this uncertainty, the following text could be included in the uncertainty section of a CRLF risk assessment:

> Toxicity data for terrestrial-phase amphibians were not available for use in this assessment. Therefore, avian toxicity data were used as a surrogate for risk estimation. There is uncertainty regarding the relative sensitivity of herptiles and birds to pesticide X. If birds are substantially more or less sensitive than the California red-legged frog, then risk would be over or under estimated, respectively.

4.3. Uncertainties in the Mammal and Herptile Prey Item EEC

T-HERPS calculates EECs for terrestrial-phase herptiles that consume mammals and other terrestrial-phase herptiles. The amount of chemical estimated to be in the prey animal, in most cases, is thought to be a conservative estimate of potential dietary exposure because T-HERPS assumes that a small prey animal is consuming its daily intake of contaminated food before being consumed by the assessed species. Depuration of the pesticide from the prey item due to excretion or metabolism has not been included in the estimation. Therefore, the EECs for chemicals that are short-lived in an animal are expected to represent an over-estimate of exposure. However, for chemicals that are bioaccumulative and are not readily degraded or excreted in an animal, the resulting exposure estimates could be low-end estimates because body burdens within the prey species would be expected to increase over time for bioaccumulative chemicals, resulting in potential body burdens that exceed the estimated daily dose calculated by T-HERPS. In addition, potential residues on the surface of potential prey items (*e.g.*, in the fur) were not estimated by T-HERPS. Additional residues would be expected to be on the prey item surface as well as within the prey item. Residues could be on prey items by several pathways, including direct deposition of spray drift or by contact of the prey animal with contaminated soil or foliage.

In addition, the mammal prey item assessment assumes consumption of a 35-gram mammal by the assessed species. A body weight of 35 grams was chosen because it represents a higher end body weight of deer mice (U.S. EPA, 1993). Use of larger sized prey mammals would result in higher dose-based RQs, but lower dietary-based RQs. It is uncertain if dose-based or dietary-based RQs are more appropriate for this exposure pathway. Therefore, in cases where neither dietary-based nor dose-based RQs exceed LOCs, effects of using a smaller mammal prey item (*i.e.*, 15 grams) on the dietary-based RQs should be considered by the assessor.

4.4. Uncertainties Associated with the Food Intake Allometric Equation

The daily food intake is estimated in T-HERPS, using an iguanid lizard allometric equation as presented in U.S. EPA (1993). This equation is used in T-HERPS to estimate potential exposures to all herptiles, including the CRLF. Allometric equations specific for terrestrial-phase amphibians have not been identified. To test the assumption that use of the iguanid lizard allometric equation results in a reasonable approximation of terrestrial-phase amphibian food intake, measured food intake values reported for juvenile bullfrogs (*Rana catesbeiana*) of various weights reported by Modzelewski and Culley (1974, as cited in U.S. EPA, 1993) were compared to estimates derived using the iguanid food intake allometric equation incorporated into T-HERPS for the same body weight range.

The analysis suggests that food intake values for juvenile bullfrogs in the Modzelewski and Culley (1974) study are reasonably approximated using the allometric equation for iguanid lizards. The data in juvenile bullfrogs reported daily food intake values that ranged from approximately 3% to 7% of their body weight. Estimates of daily food intake using T-HERPS for the same range of body weights (13 grams to 100 grams) ranged from approximately 3% to 5% body weight daily. This analysis suggests that use of the iguanid lizard allometric equation results in a reasonable approximation of food intake reported for terrestrial phase frogs.

An additional uncertainty of T-HERPS is associated with temperature influence on the food intake allometric equation. Given that terrestrial-phase frogs are poikilothermic, temperature may impact feeding rate. Temperature has not specifically been incorporated into the food ingestion allometric equation, and is not directly considered in T-HERPS.

4.5. Uncertainties Associated with the Feeding Behavior of the Assessed Species

The allometric equation used to estimate daily food intake assumes a typical or constant food intake rate daily. In reality, the amount of food consumed (and, therefore, potential exposures to pesticides) may vary significantly from day to day, depending on a number of factors including availability of particular food items and energy needs.

T-HERPS estimates potential exposures for a number of food items. EECs for a particular food item are calculated with the assumption that one food item is consumed daily. Terrestrial-phase herptiles may receive 100% of their daily diet from one food item for a particular day, especially if larger prey, such as a small mammal, is available. However, many terrestrial-phase herptiles (including the California red-legged frog) may consume a variety of food items in a given day. T-HERPS estimates potential exposures resulting from consumption of a range of food items for the purpose of giving a high-end and low-end bounding estimate. All exposure values may be used in characterizing potential exposures.

References

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