Appendix G. T-HERPS Methodology and example output from T-HERPS model

G.1. Summary of Changes in TREX to Allow for Food Intake Estimation for Herptiles

G.1.1 Food Intake Allometric Equation

Equation 1 is an 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, equation 3-13, page 3-7).

$$\text{FI (g/day)} = 0.013\ \text{Wt}^{0.773}\ \text{(g)} \qquad \text{(EQ 1)}$$

Equation 1 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):

$$\text{FI (g/day)} = 0.648\ \text{Wt}^{0.651} \qquad \text{(EQ 2)}$$

The iguanid allometric equation presented in U.S. EPA (1993) (EQ 1) is used to estimate the food ingestion rate of herpatofauna. 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 1 for the same body weight range. Conclusions from this analysis are 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 range 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.

G.1.2. 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 herpatofauna consume small mammals and other amphibians. Larger CRLFs may consume up to approximately half of their diet from consumption of larger prey (vertebrates). Therefore, there is a need to evaluate potential exposure from these food sources. There is uncertainty in EECs 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 herpatofauna (e.g. CRLF) of small mammals and herpatofauna (e.g. prey) that have consumed contaminated food items are estimated using procedures outlined in Sections 2.2.1 and 2.2.2.

G.1.2.1. EECs from Consumption of Prey Herpatofauna

In order to assess potential exposures to CRLF via consumption of a pesticide contained in herpatofauna, concentrations of the pesticide in the prey item must first be estimated. The basis for the herpatofaunal 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 1 as previously described. 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 (i.e., 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 treefrog (*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,

G.1.2.2. EECs from Consumption of Prey Mammals

For mammals that serve as prey to the CRLF, an alternative method for derivation of EECs is used. This is 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 herpatofaunal 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 were 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).

G.1.2.3. Water content of food items

Water content of potential food items is used to convert the dry weight food intake estimate calculated using Equation 1 to wet weight. Water contents of various potential food items of wildlife are presented in Tables 4-1 and 4-2 of U.S. EPA (1993) and are included in Figures 1 and 2 below. A summary of mean water content values for various broad taxonomic groups is reported below:

Terrestrial Invertebrates:	84%, earthworms
Terrestrial Insects	69%, grasshoppers and crickets
Terrestrial Vertebrates:	67% to 68%, birds and mammals
Terrestrial Plants:	88%, young grasses
Terrestrial Reptiles and Amphibians:	85%, frogs and toads

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%) is used in the dose-calculation for small terrestrial-phase CRLFs, and a water content of terrestrial herptiles (85%) is used for the dose-calculation for larger terrestrial-phase CRLFs.

Table 4-1. Gross Energy and Water Composition of Wildlife Foods: Animal Prey (values expressed as mean [standard deviation]^n where n = number of studies)

Type of food	kcal/g wet wt	% H_2O	kcal/g dry wt	References
Aquatic				
invertebrates				
bivalves (without shell)	0.80	82 (4.5)^3	4.6 (0.35)^4	1,2,3,4,5,6
crabs (with shell)	1.0 (0.21)^5	74 (6.1)^5	2.7 (0.45)^4	1,2,3,7
shrimp	1.1 (0.24)^4	78 (3.3)^7	4.8 (0.31)^6	1,3,4,6,7
isopods, amphipods	1.1	71-80	3.6 (0.78)^3	4,6,7
cladocerans	0.74	79-87	4.8 (0.62)^{14}	2,4
insect larvae			5.3 (0.37)^8	1,4
vertebrates				
bony fishes	1.2 (0.24)^{18}	75 (5.1)^{18}	4.9 (0.38)^{18}	7
Pacific herring	2.0 (0.43)^3	68 (3.9)^3	6.1 (0.50)^4	8,9
small fish (e.g., bluegill)			4.1 (0.47)^3	1,7
Terrestrial				
invertebrates				
earthworms[a]	0.78-0.83	84 (1.7)^3	4.6 (0.36)^4	1,7
grasshoppers, crickets	1.7 (0.26)^3	69 (5.6)^{11}	5.4 (0.16)^4	1,10,11
beetles (adult)	1.5	61 (9.8)^5	5.7-5.9	1,10,11
mammals				
mice, voles, rabbits	1.7 (0.28)^{14}	68 (1.6)^4	5.0 (1.3)^{17}	12,13,14
birds				
passerines				
with peak fat reserves[b]			7.8 (0.18)^{10}	15
with typical fat reserves	1.9 (0.07)^3	68	5.6 (0.34)^{13}	10,14,15,16
mallard (flesh only)	2.0	67	5.9	10
gulls, terns	1.9		4.4	1
reptiles and amphibians				
snake, lizards	1.4	66	4.5 (0.28)^5	14,17
frogs, toads	1.2	85 (4.7)^3	4.6 (0.45)^3	12,14

Note: For Tables 4-1 and 4-2, a single value represents the results of a single study on one species, and should not be interpreted as a mean value or a value indicating no variation in the category. Two values separated by a hyphen indicate that values were obtained from only two studies.

[a]Not including soil in gut, which can constitute one-third of the wet weight of an earthworm.
[b]Peak fat reserves occur just prior to migration. Typical fat reserves are for resident passerines or migratory species during nonmigratory seasons.

References: (1) Cummins and Wuycheck, 1971; (2) Golley, 1961; (3) Tyler, 1973; (4) Jorgensen et al., 1991; (5) Pierotti and Annett, 1987; (6) Minnich, 1982; (7) Thayer et al., 1973; (8) Ashwell-Erickson and Elsner, 1981; (9) Miller, 1978; (10) Collopy, 1975; (11) Bell, 1990; (12) Górecki, 1975; (13) Golley, 1960; (14) Koplin et al., 1980; (15) Odum et al., 1965; (16) Duke et al., 1987; (17) Congdon et al., 1982.

Figure 1. Water and caloric content of various potential animal food items.

Table 4-2. Energy and Water Composition of Wildlife Foods: Plants (values expressed as mean [standard deviation]^n where n = number of studies)

Type of food	kcal/g wet wt[a]	% H_2O	kcal/g dry wt	References
Aquatic				
algae	0.41-0.61	84 (4.7)^3	2.36 (0.64)^4	1,2,3
aquatic macrophytes		87 (3.1)^3	4.0 (0.31)^{12}	1,2,4
emergent vegetation		[45-80][b]	4.3 (0.13)^3	1,2,4
Terrestrial				
monocots				
young grasses	1.3	70-88	4.2	5,6
mature dry grasses		7-10	4.3 (0.33)^5	1,5,7,8
dicots				
leaves		85 (3.5)^3	4.2 (0.49)^{57}	9
roots			4.7 (0.43)^{52}	9
bulbs, rhizomes			3.6 (0.68)^3	2,7,10
stems, branches			4.3 (0.34)^{51}	9
seeds		9.3 (3.1)^{12}	5.1 (1.1)^{57}	6,9,11,12
fruit				
pulp, skin	1.1 (0.30)^3	77 (3.6)^3	2.0 (3.4)^{28}	10,13
pulp, skin, seeds			2.2 (1.6)^{10}	10

Note: For Tables 4-1 and 4-2, a single value represents the results of a single study on one species, and should not be interpreted as a mean value or a value indicating no variation in the category. Two values separated by a hyphen indicate that values were obtained from only two studies.

[a] Few determinations of the energy content of plants have been made on a wet-weight basis because plants fluctuate widely in water content depending on environmental conditions.

[b] Values in brackets represent total range of field measurements, instead of values from only two studies, as for the remainder of the table. Buchsbaum and Valiela (1987) found the water content of the emergent marsh vegetation *Spartina alterniflora*, *S. patens*, and *Juncus gerardi* to decrease over a summer from 80 to 60 percent, 70 to 45 percent, and 78 to 61 percent, respectively, as the marsh dried. In contrast, they found a submerged macrophyte to maintain water content within a few percent throughout the season.

References: (1) Cummins and Wuycheck, 1971; (2) Jorgensen et al., 1991; (3) Minnich, 1982; (4) Boyd and Goodyear, 1971; (5) Davis and Golley, 1963; (6) Drozdz, 1968; (7) Golley, 1960; (8) Kendeigh and West, 1965; (9) Golley, 1961; (10) Karasov, 1990; (11) Dice, 1922; (12) Robel et al., 1979; (13) Levey and Karasov, 1989.

Figure 2. Water and caloric content of various potential plant food items.

G.2. Body Weight of Assessed Amphibian

Up to three body weights of herpatofauna can be entered. A small, medium, and large value considered representative of the range of body weights of the assessed species may be entered.

For the CRLF, data from Fellars (2007) were used to define the range of terrestrial-phase red-legged frog body weights (Table 2). Frogs 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 2. 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

For the small sized terrestrial phase CRLF, RQs are not calculated for the terrestrial phase herpatofauna or the mammal food item given that a 1.4 gram frog would not likely eat animals that are larger than its body weight. 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.

G.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 to be 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 herpatofauna may be used to further characterize LOC exceedances. If both avian and herpatofauna 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 1 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.

G.4. Limitations and Uncertainties in T-HERPS

G.4.1. Exposure Pathways Not Quantified in T-HERPS

T-HERPS evaluates potential exposures to terrestrial-phase herpatofaunal 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.

G.4.2. Use of Avian Toxicity Data as a Surrogate for Herpatofauna

In the absence of data on terrestrial herpatofauna, 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.

G.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 was not 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 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.

G.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 were not 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 range 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.

G.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.

G.5. References

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Example output from T-HERPS model (Peanuts, 8 applications of 1.125 lbs a.i./Acre, 14-Day re-application interval)

Summary of Risk Quotient Calculations Based on Upper Bound Kenaga EECs

Upper Bound Kenaga, Acute Terrestrial Herpatofauna Dose-Based Risk Quotients											
Size Class (grams)	**Adjusted LD50**	**EECs and RQs**									
		Broadleaf Plants/ Small Insects		**Fruits/Pods/ Seeds/ Large Insects**		**Small Herbivore Mammals**		**Small Insectivore Mammal**		**Small Amphibians**	
		EEC	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**	**EEC**	**RQ**
1.4	158.00	4.32	0.03	0.48	0.00	N/A	N/A	N/A	N/A	N/A	N/A
37	158.00	4.25	0.03	0.47	0.00	N/A	N/A	N/A	N/A	0.15	0.00
238	158.00	2.78	0.02	0.31	0.00	5.23	0.03	0.33	0.00	0.10	0.00