APPENDIX J. Back Calculation of Acute LD50 and LC50 Values for No Mortality Test Results

The acute RQ values used to compare to the acute endangered species LOC are calculated using the LD_{50} (or LC_{50}) (*e.g.*, acute RQ = acute EEC/LD_{50}). However, in some cases a definitive acute LD_{50} (or LC_{50}) is not always provided in a study, such as in a limit guideline test (*e.g.*, LD_{50} >limit dose). For such cases where the highest dose level (*e.g.*, limit dose) does not result in mortality, the use of this limit dose as the LD_{50} (*i.e.*, concentration that results in 50% mortality of the exposed population) in the acute RQ calculations potentially overestimates risk. This is expressed by reporting a less than value for the calculated acute RQ. For example, with a LD_{50} >5000 mg/kg-diet and an \EEC of 2500 mg/kg, the acute RQ would be reported as <0.5 [1]. This value is then compared to the acute LOC as though it is not a "less than" value to identify if there is potential for acute risks to listed species. Rather than reporting an acute RQ value, an alternative approach is to simply state if the EEC value is above or below 1/20 or 1/10 of the limit dose for aquatic exposures and terrestrial exposures, respectively. The 1/20 and 1/10 values are comparable to the $LD_{0.05}$ and $LD_{0.1}$ values, respectively, for a dose-response relationship with a probit slope of 4.5, which is the basis for the derivation of the acute endangered LOC values (EPA, 1986).

Both of the approaches described above for dealing with limit dose results have been used in EFED, and they both suffer from the same weakness in that the proportion of mortality, $\hat{p}$, for the limit test result is assumed to be 0.50 in the evaluation of risk to listed species even though no mortality was observed; the soundness of this assumption, however, is not evaluated. For these two approaches, this assumption is made because of not knowing where on the dose-response curve the tested dose response actually occurs. Although there is no mortality observed, given the number of test organisms (typically between 7 and 100 test organisms, depending on the taxa) it can not be concluded with confidence that the true *p* is at or below 0.001 (*i.e.*, $LD_{0.1}$) or 0.0005 ($LC_{0.05}$) for terrestrial and aquatic organisms, respectively. For example, the Binomial Theorom dictates that if 10 organisms are tested and no mortality is observed, the upper 95% confidence limit on the proportion of mortality is 0.31 (from Table 4 in Conover, 1980). This means that the estimate of the true proportion of mortality may actually be as high as 0.31 when no organisms die out of the 10 exposed. Therefore it can not be concluded with confidence that the proportion of mortality at the limit dose is at or below 0.001 (or 0.0005).

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[1] $\text{Acute RQ} = EEC / LD_{50} = 2500 \text{ mg/kg} / > 5000 \text{ mg/kg} = < 0.5$

However, an improvement on these approaches is to actually take into consideration a reasonable estimate of the true proportion of mortality for limit test results, such as the 95% UCL on $\hat{p}$, provided by application of the Binomial Theorem. The 95% UCL on $\hat{p}$ can then be used in a rearrangement of the Hill *et al*. (1975) dose-response equation to solve for the LD_{50} (or LC_{50}), which can then be used in estimating risk.

The 95% UCL value on $\hat{p}$ for binomial data can be easily obtained from sources such as Table 4 in Conover (1980), or using the Wilson interval (or score interval) from Table A.1 in Brown *et al*. (2001) or calculating the Jeffreys prior interval (Brown *et al*., 2001). The estimated proportion of mortality from the study for the limit test dose is calculated ($\hat{p}$ = number dead divided by the number exposed) and this value along with the number exposed are used either to look up the 95% UCL on $\hat{p}$ from a table or to calculate it (Conover, 1980; Brown *et al*. 2001).

The Hill *et al*. (1975) dose response model is written to solve for any point on the dose-response curve when the LD_{50} and probit slope is known:

$$\log LD_p = \log LD_{50} + (\text{probit } p - 5)/b$$ **Equation 1**

where:

p = any percent mortality of interest on the dose-response curve;

LD_p = the dose which corresponds to being lethal to p% of the exposed test population;

LD_{50} = the dose which is lethal to 50% of the exposed test population;

b = the probit dose-response slope; and

5 = the probit for 50% mortality.

Rearrangement of the Hill *et al*. (1975) equation by subtracting the term (probit p -5) /b from both sides provides for a solution of the LD_{50} when any point on the dose-response (p, LD_p) and the slope is known:

$$\log LD_{50} = \log LD_p - (probit\ \ p - 5)/b$$ **Equation 2**

In this rearranged equation p can be set to be equal to the 95% UCL on $\hat{p}$ (x 100 for percent) and the limit dose is the corresponding LD_p value. The probit of p is obtained from a table such as Table I in Finney (1977). The probit slope is the default of 4.5 [or the 95% lower or upper bound slope of 2 and 9, respectively] used in setting the acute endangered LOC values (EPA, 1986).

$$\log\ LD_{50} = \log\ LD_{95\%\ UCL\ \hat{p}} - {(probit\ (95\%\ UCL\ \ \hat{p}) - 5)}\big/{4.5}$$ **Equation 3**

Using Equation 3, LD_{50} and LC_{50} values were back-calculated for mallard duck and bobwhite quail dose-based and dietary-based acute studies, and the honey bee acute contact study with clomazone where the highest dose or concentration tested resulted in no mortality. The resulting values and inputs are summarized in the following table.

Test Species / Source	**Test dose**	**Number of organisms tested**	**95% UCL on $\hat{p}$ x 100**[1]	**Probit for 95% UCL on $\hat{p}$ x 100** [2]	**Back-calculated LD_{50} (or LC_{50}) at Slope 9**	**Back-calculated LD_{50} (or LC_{50}) at Slope 4.5**	**Back-calculated LD_{50} (or LC_{50}) at Slope 2**
Bobwhite quail	2250 mg/kg bw	10	31	4.504	2554 mg/kg bw	2900 mg/kg	3983 mg/kg bw
Bobwhite quail dietary	5000 ppm	10	31	4.504	5677 ppm	6445 ppm	8851 ppm
Mallard duck dietary	5620 ppm	10	31	4.504	6380 ppm	7244 ppm	9948 ppm
Rat acute	5000mg/kg-bw	10	31	4.504	5677 mg/kg bw	6445 mg/kg-bw	8851 mg/kg bw
Rainbow Trout-acute	500 ppb	30	12	3.8250	675 ppb	912 ppb	1934 ppb
Bluegill-acute (assume conc as reported)	6700ppb	30	12	3.8250	9050 ppb	12,223 ppb	25916 ppb

[1] Obtained 95% confidence limits on binomial from Table 4 in Conover (1980) for the number of organisms tested and p = 0 (*i.e.*, no mortality).
[2] Obtained from Table I of Finney (1977) for transformation of percentages to probits.

$$\boxed{\log\ LD_{50} = \log\ LD_{95\%\ UCL\ \hat{p}} - {(probit\ (95\%\ UCL\ \ \hat{p}) - 5)}\big/{4.5}}$$

	Log (slope 4.5)	LD50 (slope 4.5)	Log (slope 2)	LD50 (slope 2)	Log (slope 9)	LD50 (slope 9)
clomozone	3.988151636	9731	4.360596	22940	3.839174	6905
bobwhite acute	3.46240474	2900	3.60018	3983	3.40729	2554

			3		4	
bobwhite diet	3.809192227	6445	3.94697	8851	3.75408	5677
					1	
mallard diet	3.859958538	7244	3.99773	9948	3.80484	6380
			6		7	
rat acute	3.809192227	6445	3.94697	8851	3.75408	5677
					1	

Upper Bound Kenaga Residues For RQ Calculation

Chemical Name:	0
Use	0
Formulation	Slope 9
Application Rate	0 lbs a.i./acre
Half-life	0 days
Application Interval	0 days
Maximum # Apps./Year	0
Length of Simulation	1 year

Acute and Chronic RQs are based on the Uppe
Kenaga Residues.

The maximum single day residue estimation is u
both the acute and reproduction RQs.

**RQs reported as "0.00" in the RQ tables belc
<0.01 in your assessment. This is due to rou
figure issues in Excel.**

Endpoints			
Avian	Bobwhite quail	LD50 (mg/kg-bw)	2554.00
	Bobwhite quail	LC50 (mg/kg-diet)	5677.00
	Mallard duck	NOAEL(mg/kg-bw)	0.00
	Bobwhite quail	NOAEC (mg/kg-diet)	0.00
Mammals		LD50 (mg/kg-bw)	5677.00
		LC50 (mg/kg-diet)	0.00
		NOAEL (mg/kg-bw)	0.00
		NOAEC (mg/kg-diet)	0.00

Dietary-based EECs (ppm)	Kenaga Values
Short Grass	#DIV/0!
Tall Grass	#DIV/0!
Broadleaf plants/sm Insects	#DIV/0!
Fruits/pods/seeds/lg insects	#DIV/0!

Avian Results

Avian Class	Body Weight (g)	Ingestion (Fdry) (g bw/day)	Ingestion (Fwet) (g/day)	% body wgt consumed	FI (kg-diet/day)
Small	20	5	23	114	2.28E-02
Mid	100	13	65	65	6.49E-02
Large	1000	58	291	29	2.91E-01
Granivores	20	5	5	25	5.06E-03
	100	13	14	14	1.44E-02
	1000	58	65	6	6.46E-02

Avian Body Weight (g)	Adjusted LD50 (mg/kg-bw)
20	1839.98
100	2342.38
1000	3308.70

Mammalian Class	Body Weight	Adjusted LD50	Adjusted NOAEL
Herbivores/ insectivores	15	12477.08	0.00
	35	10095.29	0.00
	1000	4366.52	0.00
Grainvores	15	12477.08	0.00
	35	10095.29	0.00
	1000	4366.52	0.00

Upper Bound Kenaga Residues For RQ Calculation

Chemical Name:	0
Use	0
Formulation	Slope 4.5
Application Rate	0 lbs a.i./acre
Half-life	0 days
Application Interval	0 days
Maximum # Apps./Year	0
Length of Simulation	1 year

Acute and Chronic RQs are based on the Uppe
Kenaga Residues.

The maximum single day residue estimation is u
both the acute and reproduction RQs.

**RQs reported as "0.00" in the RQ tables belo
<0.01 in your assessment. This is due to rou
figure issues in Excel.**

Endpoints			
Avian	Bobwhite quail	LD50 (mg/kg-bw)	2900.00
	Bobwhite quail	LC50 (mg/kg-diet)	6445.00
	Mallard duck	NOAEL(mg/kg-bw)	0.00
	Bobwhite quail	NOAEC (mg/kg-diet)	0.00
Mammals		LD50 (mg/kg-bw)	6445.00
		LC50 (mg/kg-diet)	0.00
		NOAEL (mg/kg-bw)	0.00
		NOAEC (mg/kg-diet)	0.00

Dietary-based EECs (ppm)	Kenaga Values
Short Grass	#DIV/0!
Tall Grass	#DIV/0!
Broadleaf plants/sm Insects	#DIV/0!
Fruits/pods/seeds/lg insects	#DIV/0!

Avian Results

Avian Class	Body Weight (g)	Ingestion (Fdry) (g bw/day)	Ingestion (Fwet) (g/day)	% body wgt consumed	FI (kg-diet/day)
Small	20	5	23	114	2.28E-02
Mid	100	13	65	65	6.49E-02
Large	1000	58	291	29	2.91E-01
Granivores	20	5	5	25	5.06E-03
	100	13	14	14	1.44E-02
	1000	58	65	6	6.46E-02

Avian Body Weight (g)	Adjusted LD50 (mg/kg-bw)
20	2089.25
100	2659.71
1000	3756.95

Mammalian Class	Body Weight	Adjusted LD50	Adjusted NOAEL
Herbivores/ insectivores	15	14165.02	0.00
	35	11461.01	0.00
	1000	4957.24	0.00
Grainvores	15	14165.02	0.00
	35	11461.01	0.00
	1000	4957.24	0.00

Upper Bound Kenaga Residues For RQ Calculation

Chemical Name:	0
Use	0
Formulation	Slope 2
Application Rate	0 lbs a.i./acre
Half-life	0 days
Application Interval	0 days
Maximum # Apps./Year	0
Length of Simulation	1 year

Acute and Chronic RQs are based on the Uppe
Kenaga Residues.

The maximum single day residue estimation is u
both the acute and reproduction RQs.

**RQs reported as "0.00" in the RQ tables belo
<0.01 in your assessment. This is due to rou
figure issues in Excel.**

Endpoints			
Avian	Bobwhite quail	LD50 (mg/kg-bw)	3983.00
	Bobwhite quail	LC50 (mg/kg-diet)	8851.00
	Mallard duck	NOAEL(mg/kg-bw)	0.00
	Bobwhite quail	NOAEC (mg/kg-diet)	0.00
Mammals		LD50 (mg/kg-bw)	8851.00
		LC50 (mg/kg-diet)	0.00
		NOAEL (mg/kg-bw)	0.00
		NOAEC (mg/kg-diet)	0.00

Dietary-based EECs (ppm)	Kenaga Values
Short Grass	#DIV/0!
Tall Grass	#DIV/0!
Broadleaf plants/sm Insects	#DIV/0!
Fruits/pods/seeds/lg insects	#DIV/0!

Avian Results

Avian Class	Body Weight (g)	Ingestion (Fdry) (g bw/day)	Ingestion (Fwet) (g/day)	% body wgt consumed	FI (kg-diet/day)
Small	20	5	23	114	2.28E-02
Mid	100	13	65	65	6.49E-02
Large	1000	58	291	29	2.91E-01
Granivores	20	5	5	25	5.06E-03
	100	13	14	14	1.44E-02
	1000	58	65	6	6.46E-02

Avian Body Weight (g)	Adjusted LD50 (mg/kg-bw)
20	2869.47
100	3652.98
1000	5159.97

Mammalian Class	Body Weight	Adjusted LD50	Adjusted NOAEL
Herbivores/ insectivores	15	19453.00	0.00
	35	15739.55	0.00
	1000	6807.84	0.00
Grainvores	15	19453.00	0.00
	35	15739.55	0.00
	1000	6807.84	0.00