Data Evaluation Report on the acute toxicity of Glyphosate-Cosmo Flux® Coca Mix End Use Product to African clawed frogs (*Xenopus laevis*) MRID 468736-01

Data Requirement:

EPA DP Barcode	D331321
OECD Data Point	203 (adapted)
EPA MRID	468736-01
EPA Guideline	OPPTS 850.1075 (adapted)

Test material: **Glyphosate-Cosmo Flux® Coca Mix**
Purity: 18% Glyphosate

Common name: Glyphosate
Chemical name: CAS name: Glyphosate Isopropyl amine salt
CAS No. 38641-94-0

Primary Reviewer: Thomas Steeger, PhD. **Date:** 5/26/06
Environmental Fate and Effects Division,
Office of Pesticide Programs,
Environmental Protection Agency

Secondary Reviewer: Kristina Garber **Date:** 8/23/06
Environmental Fate and Effects Division,
Office of Pesticide Programs,
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Tertiary Reviewer: Silvia Termes, Ph.D. **Date:** 10/31/06
Environmental Fate and Effects Division
Office of Pesticide Programs,
Environmental Protection Agency

EPA PC Code: 417300 (glyphosate, N-(phosphonomethyl) glycine)

Date Evaluation Completed: May 26, 2006

CITATION: Sutherland, C. A., T. Z. Kendall, H. O. Krueger. 2006. Glyphosate-Cosmo-Flux®--Coca Mix: A 96-hr static-renewal acute toxicity test with the African clawed-frog tadpole (*Xenopus laevis*). Report No. 628A-101. Wildlife International, Ltd., 8598 Commerce Dr, Easton, Md 21601. Inter-American Drug Abuse Control Commission (CICAD), Organization of American States. Study dates: March 20 – May 24, 2006

I. EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, African clawed frog tadpoles (*Xenopus laevis*) were exposed to glyphosate-Cosmo-Flux® Coca mix at nominal concentrations of 0, 1.3, 2.5, 5.2, 10 and 21 mg/L (measured concentrations of 0.14, 0.27, 0.56, 1.1 and 2.3 mg acid equivalents glyphosate/L) under static-renewal conditions. The 96-h LC_{50} was 10 mg/L formula (1.1 mg a.e. glyphosate/L). The NOEC value, based on mortality and sub-lethal effects, was 1.3 mg/L formula (0.14 mg a.e. glyphosate /L). Tadpoles treated at concentrations ≥ 2.5 mg/L formula (≥0.27 mg a.e. glyphosate /L) exhibited mortality and/or overt signs of toxicity. From 48 - 96 h, most of the tadpoles in the 10 mg/L (1.1 mg a.e. glyphosate/L) treatment group were observed to be smaller in size than controls. Based on the results of this study, glyphosate-Cosmo Flux® Coca mix would be classified as moderately toxic (LC_{50} >1-10 mg/L) to *X. laevis* in accordance with the classification system of the U.S. EPA.

There is some uncertainty regarding the concentration of glyphosate in the test concentrations in terms of milligrams acid equivalents. However, this toxicity study is classified scientifically sound.

II. MATERIALS AND METHODS

A. GUIDELINE FOLLOWED:

Protocol based on procedures outline in OECD Guideline for testing of chemicals, 203: Fish, Acute Toxicity Test; U. S. Environmental Protection Agency Series 850—Ecological Effects Test Guidelines (draft), OPPTS Number 850.1075: Fish Acute Toxicity Test, Freshwater and Marine, and ASTM Standard E729-96, Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians

B. COMPLIANCE: Conducted in compliance with Good Laboratory Practice Standards as published by the U. S. Environmental Protection Agency in 40 CFR Pars 160 and 792, 17 August 1989, OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17), and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999.

C. MATERIALS:

1. Test Material Coca Spray Max—Glyphosate Herbicide containing Glyphosate Isopropyl Ammonium

Description: Liquid

Lot No./Batch No.: Not reported; Wildlife International ID No. 7567

Purity: 18% Glyphosate
Other chemical species not identified

Stability of Compound Under Test Conditions: Not identified

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: Ambient conditions

2. Test organism:

Species: African clawed frog *(Xenopus laevis)*

Age at test initiation: Tadpoles
Gosner Stage 25 or Nieuwkoop-Faber Stage 47

Weight at study initiation: Not reported

Length at study initiation: Not reported

Source: Xenopus One, Dexter, Michigan 48130

D. STUDY DESIGN:

1. Experimental Conditions

Range-finding Study: Not reported.

Definitive Study: Details of experimental parameters in **Table 1.**

Table 1. Experimental Parameters

Parameter	**Details**	**Remarks (*Guideline Criteria)***
Acclimation	Duration: 1 d Conditions: 30 L acclimation tank, Temp. 21.2 – 21.3 °C, pH 8.6, D.O. ≥8.4 mg/L Feeding: 2.6 mL Sera Micron® (77 mg/mL) Health: Mortality approximately 2% during holding period	*(EPA: minimum of 12 d required, 14 d recommended for acclimation)* *(EPA: Pretest mortality must be <5%)*
Test Duration	96 h	*(EPA/OECD requires 96 h)*
Test condition	Static renewal Complete water change every 24 h	*(EPA: Must provide reproducible supply of toxicant)*
Aeration	Not reported	(*EPA: no aeration; OECD permits aeration*)
Test vessel	Material: Glass (beaker) Size: 2 L Fill Volume: 1 L	
Source of dilution water	Filtered well water	*(EPA 1975: Soft reconstituted water or water from a natural source, **not** dechlorinated tap water; OECD permits dechlorinated tap water.)*

Parameter	Details	Remarks (*Guideline Criteria*)
Water parameters	Hardness: 140 mg/L as $CaCO_3$ pH: 7.5 – 8.1 DO: 4.5 – 8.5 mg/L TOC: not reported Particulate Matter: TDS not reported Metals: <levels of detection Pesticides: <levels of detection Chlorine: Not reported Temperature: 21.4 – 22.2°C Intervals of water quality measurement: 24 h	*(Hardness: EPA: 40 - 48 mg/L as $CaCO_3$; OECD allows 10 -250 mg/L as $CaCO_3$)* *(pH EPA: 7.2 - 7.6; OECD: 6.0 - 8.5)* (Dissolved Oxygen: EPA: *Static: > 60% during 1st 48 hrs and >40% during 2nd 48 hrs; OECD ≥ 80% saturation value)* *(EPA water quality: measured at beginning of test and every 48 h)*
Number replicates/group	Control: 2 Treatment: 2	*(EPA: 2 replicates per test concentration are preferred)*
Number organisms per replicate/group	10	*(EPA: minimum 7 organisms/replicate, 10 are recommended; OECD requires at least 7 fish/replicate)*
Biomass loading rate	10 tadpoles/L	These are relatively crowded conditions for an amphibian study. *(Static: # 0.8 g/L at # 17EC, # 0.5 g/L at > 17EC; flow-through: # 1 g/L/d; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through)*
Test concentrations	Nominal (formula): 1.3, 2,5, 5.0, 10, 20 mg/L Measured (formula): 1.3, 2,5, 5.2, 10, 21 mg/L Measured: 0.14, 0.27, 0.56, 1.1, and 2.3 mg a.e. glyphosate/L)	Measured values were 100-108% of nominal at 0, 24 and 96 hour measurements. Formula concentrations were confirmed by measuring the amount of glyphosate by HPLC and then back calculating the concentration of the overall formulated product based

Parameter	Details	Remarks (*Guideline Criteria)*
		on 10.9% glyphosate. *(EPA/OECD: Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geo-metric series)*
Solvent	Water	*(EPA: Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD: solvent not to exceed 100 mg/L.)*
Lighting	16 h light (280 lux), 8 h dark 30-minute transition Similar to natural sunlight	*(EPA: 16 h light/8 h dark; OECD: 12 -16 h photoperiod)*
Feeding	0.18 mL Sera Micron® (77 mg/mL)/d	*(EPA/OECD: No feeding during the study)*
Recovery of glyphosate	LOQ = 0.367 mg/L LOD = 0.040 mg/L	
Positive control	NA	

2. Observations: See **Table 2.**

Table 2. Observations

Criteria	Details
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and behavioral effects
Observation intervals	4, 24, 48, 72 and 96 h after test initiation *(EPA/OECD requires: minimally every 24 h)*
Raw data	Measured test concentrations, water characteristics, cumulative mortality and clinical observations.

III. RESULTS and DISCUSSION:

A. MORTALITY:

According to report, at test termination, mortality in the 2.5, 5.2 and 10 mg/L treatment groups was 5, 0 and 55% respectively. All tadpoles in the 21 mg/L treatment group were dead within 24 h. Observations of mortality and reported LC_{50} values at 24, 48, 72 and 96 hours are located in **Table 3.**

Table 3. Effect of Glyphosate-Cosmo Flux Coca Mix on mortality of *Xenopus laevis*.

Measured value for formula in mg/L (mg a.e. glyphosate/L)	Observation period							
	24 h		48 h		72 h		96 h	
	# Dead	Cumulative mortality (%)	# Dead	Cumulative mortality (%)	# Dead	Cumulative mortality (%)	# Dead	Cumulative mortality (%)
Control (<LOQ)	0	0	0	0	0	0	0	0
1.3 (0.14)	0	0	0	0	0	0	0	0
2.5 (0.27)	0	0	0	0	0	0	1	5
5.2 (0.56)	0	0	0	0	0	0	0	0
10 (1.1)	0	0	1	5	1	5	11	55
21 (2.3)	20	100	20	100	20	100	20	100
LC_{50} 95% CI	14 (1.6) 10-21 (1.1-2.3)		14 (1.6) 10-21 (1.1-2.3)		14 (1.6) 10-21 (1.1-2.3)		9.6 (1.1) 5.2-21 (0.56-2.3)	

B. NON-LETHAL TOXICITY ENDPOINTS:

From 48 h to 96 h, the surviving tadpoles in the 10 mg/L treatment were observed to be smaller in size compared to controls. One tadpole each in the 2.5 and 5.2 mg/L treatment groups was observed to be smaller in size between 72 and 96 h. Observations of sublethal effects and resulting NOEC and LOEC values at 24, 48, 72 and 96 hours are located in **Table 4.**

Table 4: Sub-lethal effects observed in surviving tadpoles exposed to Glyphosate-Cosmo-Flux® Coca Mix.

Measured value for formula in mg/L (mg a.e. glyphosate/L)	number affected per Observation period			
	24 h	48 h	72 h	96 h
Control	AN	AN	AN	AN
1.3 (0.14)	AN	AN	AN	AN
2.5 (0.27)	AN	AN	1 small/weak	AN
5.2 (0.56)	AN	AN	1 small	1 small
10 (1.1)	AN	17 Small	17 Small/weak 2 lying on bottom	9 Small/weak (all surviving fish)
21 (2.3)	NA[1]	NA[1]	NA[1]	NA[1]
NOEC	10 (1.1)	5.2 (0.56)	1.3 (0.14)	1.3 (0.14)
LOEC	21 (2.3)	10 (1.1)	2.5 (0.27)	2.5 (0.27)

AN = appear normal

[1]No tadpoles survived in this treatment group at this observation time.

C. REPORTED STATISTICS:

LC_{50} values were calculated by nonlinear interpolation method. The reported 96 h LC_{50} value based on a binomial distribution was 9.6 mg/L (1.1 mg a.e.glyphosate/L). The NOEC was determined by the study author using "visual interpretation" of mortality and sublethal effects data. The 96 h NOEC for mortality and sublethal effects was 1.3 mg/L (0.14 mg a.e. glyphosate /L).

D. VERIFICATION OF STATISTICAL RESULTS:

The reviewer utilized binomial distribution to verify the reported statistical results. Outputs from the statistical analysis are located in Appendix 1 and 2. The results are summarized as:

LC_{50}:	9.27 mg/L	(1.1 mg a.e./L)
95% C.I for LC_{50}:	5.2 – 20.7 mg/L	(0.56 – 2.3 mg a.e./L)
NOAEL:	5.2 mg/L	(0.56 mg a.e./L)
Probit Slope:	4.92	
95% C.I. for probit:	0 - 13 (Poor fit on probit dose response curve)	

E. STUDY DEFICIENCIES:

Loading rate (10 tadpoles/L) is greater than preferred (1 tadpole/L); however, based on controls, this did not appear to have an adverse effect on the study.

Acute toxicity studies are typically conducted without feeding.

The acclimation period of 1 day was not sufficient to meet the EPA guideline requirement of 12 days.

F. REVIEWER'S COMMENTS:

Dissolved oxygen levels by 96 h had dropped to 4.5 mg/L (53% saturation in the second highest treatment group and the general decline in dissolved oxygen in spite of 24-hr renewals suggests that the loading rate was too high.

Mean measured concentrations, based on a reanalysis of the raw data using Statistical Analysis Software (SAS Institute, Release 8.02), are 1.3, 2.5, 5.2, 10.1 and 20.7 mg/L (0.14, 0.27, 0.56, 1.1 and 2.3 mg a.e./L). Measured concentrations ranged between 96.6 to 103% of nominal. Measured concentrations for the matrix fortification (spiked) samples ranged from 96 to 106%. Although log-10 probit analysis using SAS was attempted, the data do not lend themselves to this analysis since the goodness of fit was poor (Appendix 1). LC_{50} values were verified using a binomial distribution analysis (ToxWin) (Appendix 2).

Because the material used in this test was a formulated product, the toxicity observed in test organisms is attributed to the formulated product as a whole. The actual ingredient or combination of ingredients in the formula which caused the toxicity is uncertain. One active ingredient of this formulated product was identified as glyphosate. Although toxicity values were calculated by the study author in terms of the glyphosate component of the formulated product, the toxicity of that product cannot necessarily be attributed to glyphosate. To identify the actual ingredients causing the toxicity observed in this test, further tests would be required involving other ingredients in the formulation, as well as combinations of those ingredients.

There is no information about how the "Glyphosate-Cosmo Flux Mix" ("Coca-Mix" and "Poppy Mix" (MRID 468736_02)) were prepared and stored prior to its arrival at the laboratory. Even though glyphosate is stable to abiotic hydrolysis, it is unlikely that the water used was sterile. Since the major route of transformation is metabolism under aerobic conditions, the concentration of glyphosate in the mix received by the laboratory may have been lower than in the freshly prepared mixes. The metabolite aminomethyl phosphonic acid (AMPA) is the major metabolite of glyphosate. Formation of AMPA during mix preparation and receiving times is feasible.

The concentrations of glyphosate in the test media were determined by high-performance liquid chromatography (HPLC) after derivatization of glyphosate. A retention time of 7.4 minutes was used in the "Coca Mix" to quantify the glyphosate derivative, but 5.6 minutes for the "Poppy Mix". This

apparent difference is not discussed. Theoretically, the derivatized product of a same analyte should show approximately the same retention times under the same analytical conditions. In a representative chromatogram of a matrix fortification sample, "Poppy Mix" (Appendix 3.10), two different chemical species are apparent. One elutes at approximately 5.5 (claimed to be "derivatized glyphosate" in the "Poppy Mix study) and the other at about 7.3 (claimed to be "derivatized glyphosate" in the "Coca Mix", suggesting that another chemical species could be derivatized (AMPA?). In the "Coca Mix", there is only one elution at approximately 7.4 minutes.

Regardless of other issues, the "derivatized glyphosate" can only form from the "glyphosate acid" and, therefore, it is legitimate to express concentrations in terms of "mg ae/L", as presented in the report's conclusion section.

According to the sponsor, the mix was prepared using glyphosate as the isopropylamine salt. The sponsor claimed that the mix purity was"18% glyphosate" without specifying if this is expressed in terms of "glyphosate acid equivalents" or in terms of "glyphosate isopropylamine salt". That is, the "Coca Mix" contains 18% "Glyphosate"

For the sake of argument, let us assume that it was expressed in percent of the isopropylamine salt. The glyphosate acid equivalents in a mole of glyphosate isopropylamine salt is,

Glyphosate acid equivalents (a.e.) = Molecular weight of glyphosate acid/Molecular weight of the isopropylamine salt

a.e. in "Coca Mix"= 169/228 = 0.74

If the sponsor expressed the composition if the mix in terms of "18%" isopropylamine salt, then in terms of a.e. it corresponds to,

% a.e in "Coca Mix" = (18% x 0.74)= 13.3%

On page 14 it reads that "Glyphosate content in the mix was 10.9%". Assuming that that the supplied mix was expressed in terms of a.e., 10.9% is still well below its theoretical percent (13.3 %) mix supplied by the sponsor.

On page 16, it is not clear why glyphosate "is one of the active ingredients" in the mix. This is misleading and incorrect because glyphosate (glyphosate acid" is the pesticide (herbicide) active ingredient. It is the chemical that specifically binds and inhibits the enzyme 5-enolpyruvylshikimate 3-phosphate (ESPS) synthase. This enzyme is the sixth enzyme on the shikimate pathway and it is essential for the biosynthesis of aromatic amino acids On the other hand, Cosmo Flux is a surfactant used as an adjuvant in the mix. The role of the surfactant is to lower the surface tension of the mix solution to improve wetting of the hydrophobic plant surfaces.

IV. CONCLUSIONS:

Although there is uncertainty regarding the concentration of glyphosate in terms of milligrams of acid equivalents, this study is scientifically sound and provides useful information on the acute toxicity of formulated endproduct to tadpoles of African clawed frogs. The results are summarized below. Since the 96-hr LC_{50} value falls between 1 and 10 mg a.e./L, the formulated endproduct (glyphosate-Cosmo-Flux® coca mix) is classified as moderately toxic to African clawed frog tadpoles under the conditions tested.

Results Synopsis

Test Organism Age:	Nieuwkoop-Faber Stage 47 (7 d post-fertilization)
Test Type:	Static Renewal
LC_{50}:	9.6 mg/L formula (1.1 mg a.e. glyphosate/L)
95% C.I. for LC_{50}:	5.2-21 mg/L formula (0.56 -2.3 mg a.e. glyphosate/L)
NOEL:	1.3 mg/L formula (0.14 mg a.e. glyphosate/L)
Probit Slope:	NA

Appendix 1. Results of reviewer's statistical analysis using SAS.

SAS Output

MEASURED CONCENTRATIONS IN MG/L AND MG A.E./L 43
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Obs	TREAT	_TYPE_	_FREQ_	NOMINAL	MEASURE	PRCT
1	1.3	0	5	1.3320	0.14380	102.462
2	2.5	0	5	2.5340	0.27380	101.360
3	5.0	0	5	5.1960	0.56180	103.920
4	10.0	0	5	10.1380	1.09800	101.380
5	20.0	0	3	20.7333	2.26333	103.667

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS EXPOSURE TO COCA MIX 44
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Probit Procedure

Iteration History for Parameter Estimates

Iter	Ridge	Loglikelihood	Intercept	Log10(DOSE)
0	0	-69.314718	0	0
1	0	-32.180279	-1.928397374	2.0816375444
2	0	-25.068656	-3.194950354	3.3681689256
3	0	-23.174552	-4.238652485	4.4031250436
4	0	-22.978331	-4.71100312	4.8713226027
5	0	-22.976234	-4.766577781	4.9271508238
6	0	-22.976234	-4.767193131	4.9277793172

Model Information

Data Set	WORK.D
Events Variable	RESPONSE
Trials Variable	N
Number of Observations	10
Number of Events	32
Number of Trials	100
Name of Distribution	NORMAL
Log Likelihood	-22.97623352

Last Evaluation of the Negative of the Gradient

Intercept	Log10(DOSE)
4.2852169E-8	-5.52261E-8

Last Evaluation of the Negative of the Hessian

	Intercept	Log10(DOSE)
Intercept	23.421434409	22.052316598
Log10(DOSE)	22.052316598	22.00234359

Algorithm converged.

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Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	21.3971	3	<.0001
L.R. Chi-Square	10.4863	3	0.0149

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	5

All variances and covariances have been multiplied by the heterogeneity factor H= 7.1324.

Please check to be sure that the large chi-square (p < 0.0001) is not caused by systematic departure from the model. A t value of 3.18 will be used in computing fiducial limits.

Analysis of Parameter Estimates

Variable	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq	Label
Intercept	1	-4.76719	2.32535	4.2029	0.0404	Intercept
Log10(DOSE)	1	4.92778	2.39917	4.2187	0.0400	

Estimated Covariance Matrix

	Intercept	Log10(DOSE)
Intercept	5.407270	-5.419551
Log10(DOSE)	-5.419551	5.756024

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
0.96741206	0.20293117

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Estimated Covariance Matrix
for Tolerance Parameters

MU	SIGMA

MU	0.012699	0.001244
SIGMA	0.001244	0.009762

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PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS EXPOSURE TO COCA MIX 48
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Probit Procedure

Probit Analysis on DOSE

Probability	DOSE	95% Fiducial Limits	
0.01	3.12841	.	.
0.02	3.55339	.	.
0.03	3.85248	.	.
0.04	4.09396	.	.
0.05	4.30150	.	.
0.06	4.48642	.	.
0.07	4.65509	.	.
0.08	4.81149	.	.
0.09	4.95828	.	.
0.10	5.09736	.	.
0.15	5.71594	.	.
0.20	6.26067	.	.
0.25	6.76920	.	.
0.30	7.26097	.	.
0.35	7.74852	.	.
0.40	8.24138	.	.
0.45	8.74805	.	.
0.50	9.27710	.	.
0.55	9.83813	.	.
0.60	10.44297	.	.
0.65	11.10723	.	.
0.70	11.85303	.	.
0.75	12.71414	.	.
0.80	13.74685	.	.
0.85	15.05694	.	.
0.90	16.88413	.	.
0.91	17.35773	.	.
0.92	17.88730	.	.
0.93	18.48826	.	.
0.94	19.18333	.	.
0.95	20.00802	.	.
0.96	21.02233	.	.
0.97	22.34003	.	.
0.98	24.22042	.	.
0.99	27.51063	.	.

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS EXPOSURE TO COCA MIX 49
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Probit Procedure

Iteration History for Parameter Estimates

Iter	Ridge	Loglikelihood	Intercept	Log10(DOSE)

0	0	-69.314718	0	0
1	0	-28.172423	-0.327716274	1.6687866768
2	0	-17.194493	-0.534128779	3.0839314751
3	0	-9.4306308	-0.888366349	5.260571905
4	0	-3.6019279	-1.574847799	8.6650510934
5	0	-0.9023342	-2.425289726	12.63209476
6	0	-0.2530684	-3.057734815	15.645822582
7	0	-0.076348	-3.571429939	18.122958775
8	0	-0.0240451	-4.013857009	20.271008111
9	0	-0.007787	-4.408167052	22.193677542
10	0	-0.0025712	-4.767376838	23.950293127
11	0	-0.0008611	-5.099565665	25.578151669
12	0	-0.0002916	-5.410125707	27.102389815
13	0	-0.0000996	-5.702859497	28.540869543
14	0	-0.0000342	-5.980565738	29.906817346
15	0	-0.0000118	-6.245375266	31.210352798
16	0	-4.1152E-6	-6.498955338	32.459425726
17	0	-1.436E-6	-6.742640035	33.660418596
18	0	-5.029E-7	-6.977516943	34.81854931
19	0	-1.7666E-7	-7.204486789	35.938149712
20	0	-6.2228E-8	-7.424305673	37.022863699
21	0	-2.1972E-8	-7.637615743	38.075791648
22	0	-7.7752E-9	-7.844967964	39.099597951
23	0	-2.7567E-9	-8.04683933	40.09659259
24	0	-9.791E-10	-8.243646109	41.068794026
25	0	-3.483E-10	-8.435754177	42.017978401
26	0	-1.241E-10	-8.623487188	42.945718528
27	0	-4.427E-11	-8.807133118	43.853415145
28	0	-1.581E-11	-8.986949549	44.742322246
29	0	-5.653E-12	-9.163167998	45.613567798
30	0	-2.024E-12	-9.335997469	46.468170832
31	0	-7.25E-13	-9.505627413	47.307055663
32	0	-2.598E-13	-9.672230204	48.131063807
33	0	-9.326E-14	-9.835963224	48.940964026
34	0	-3.331E-14	-9.99697063	49.737460861
35	0	-1.221E-14	-10.15538486	50.521201921
36	0	-4.441E-15	-10.31132794	51.292784131
37	0	-2.22E-15	-10.46491258	52.05275913
38	0	0	-10.61624315	52.801637951
39	0	0	-10.61624315	52.801637951

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS EXPOSURE TO COCA MIX 50

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Probit Procedure

Model Information

Data Set	WORK.E
Events Variable	RESPONSE
Trials Variable	N
Number of Observations	10
Number of Events	20
Number of Trials	100
Name of Distribution	NORMAL
Log Likelihood	0

Last Evaluation of the Negative of the Gradient

Intercept	Log10(DOSE)

1.060313E-15 -5.57583E-16

Last Evaluation of the Negative of the Hessian

	Intercept	Log10(DOSE)
Intercept	4.069785E-14	6.787234E-15
Log10(DOSE)	6.787234E-15	2.126709E-15

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	0.0000	3	1.0000
L.R. Chi-Square	0.0000	3	1.0000

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	5

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of 1.96.

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS EXPOSURE TO COCA MIX 51
07:20 Friday, May 26, 2006

Probit Procedure

Analysis of Parameter Estimates

Variable	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq	Label
Intercept	1	-10.61624	7247727.7	0.0000	1.0000	Intercept
Log10(DOSE)	1	52.80164	31705428	0.0000	1.0000	

Estimated Covariance Matrix

	Intercept	Log10(DOSE)
Intercept	5.2529557E13	-1.676442E14
Log10(DOSE)	-1.676442E14	1.0052341E15

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
0.20105897	0.01893881

Estimated Covariance Matrix
for Tolerance Parameters

	MU	SIGMA
MU	9237087159.5	234133332.14
SIGMA	234133332.14	129323560.78

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS EXPOSURE TO COCA MIX 52
07:20 Friday, May 26, 2006

Probit Procedure

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS EXPOSURE TO COCA MIX 53
07:20 Friday, May 26, 2006

Probit Procedure

Probit Analysis on DOSE

Probability	DOSE	95% Fiducial Limits	
0.01	1.435492	.	.
0.02	1.452658	.	.
0.03	1.463656	.	.
0.04	1.471984	.	.
0.05	1.478793	.	.
0.06	1.484614	.	.
0.07	1.489736	.	.
0.08	1.494337	.	.
0.09	1.498534	.	.
0.10	1.502408	.	.
0.15	1.518554	.	.
0.20	1.531509	.	.
0.25	1.542712	.	.
0.30	1.552843	.	.
0.35	1.562289	.	.
0.40	1.571306	.	.
0.45	1.580080	.	.
0.50	1.588762	.	.
0.55	1.597493	.	.
0.60	1.606412	.	.
0.65	1.615684	.	.
0.70	1.625513	.	.
0.75	1.636187	.	.
0.80	1.648156	.	.
0.85	1.662217	.	.
0.90	1.680080	.	.
0.91	1.684423	.	.
0.92	1.689154	.	.
0.93	1.694372	.	.
0.94	1.700218	.	.
0.95	1.706910	.	.
0.96	1.714806	.	.
0.97	1.724563	.	.
0.98	1.737619	.	.
0.99	1.758398	.	.

Appendix 2. TOXWIN Output from reviewer's statistical analysis.

Steeger Glyphosate Xenopus LC50 in mg/L

CONC.	NUMBER	NUMBER EXPOSED	PERCENT DEAD	BINOMIAL DEAD
	PROB. (PERCENT)			
20.7	20	20	100	9.536742E-05
10.1	20	11	55	41.19014
5.2	20	0	0	9.536742E-05
2.5	20	1	5	2.002716E-03
1.3	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 5.2 AND 20.7 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.666855 mg/L

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	2.661529	7.1327320	

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.927781
95 PERCENT CONFIDENCE LIMITS =-3.111496 AND 12.96706

LC50 = 9.277118
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 5.124961
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

Steeger Glyphosate in mg a.e./L

CONC	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
2.3	20	20	100	9.536742E-05
1.1	20	11	55	41.19014
.56	20	0	0	9.536742E-05
.27	20	1	5	2.002716E-03
.14	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .56 AND 2.3 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.052044 mg a.e./L

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND 100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS G
H
GOODNESS OF FIT PROBABILITY
6 2.524779
6.788605

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.867916
95 PERCENT CONFIDENCE LIMITS =-2.866985 AND 12.60282

LC50 = 1.010688
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .5542747
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY