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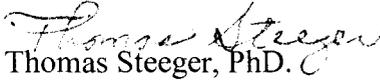
**Data Evaluation Report on the acute toxicity of Glyphosate-Cosmo Flux<sup>®</sup> Poppy Mix End Use Product to African clawed frogs (*Xenopus laevis*)** MRID 468736-02

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**Data Requirement:**

EPA DP Barcode D331321  
OECD Data Point 203 (adapted)  
EPA MRID 468736-02  
EPA Guideline OPPTS 850.1075 (adapted)

**Test material:** **Glyphosate-Cosmo Flux<sup>®</sup> Poppy Mix**  
**Purity:** 0.0205% Glyphosate (reported)  
**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,  
Environmental Protection Agency

**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<sup>®</sup> -- Poppy Mix: A 96-hr static-renewal acute toxicity test with the African clawed frog tadpole (*Xenopus laevis*). Report No. 628A- 102. 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

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## **I. EXECUTIVE SUMMARY:**

In a 96-h acute toxicity study, African clawed frog tadpoles (*Xenopus laevis*) were exposed to glyphosate-Cosmo-Flux<sup>®</sup> poppy mix at nominal concentrations of 0, 1.3, 2.5, 5.0, 10, 20 mg/L (measured concentrations of 0.12, 0.22, 0.44, 0.92 and 1.8 mg acid equivalents glyphosate/L) under static-renewal conditions. The 96-h LC<sub>50</sub> was 16 mg/L formula (1.3 mg a.e. glyphosate/L). The no mortality concentration and the NOEC were 11 mg/L (0.92 mg a.e. glyphosate/L) and 5.3 mg (0.43 mg a.e. glyphosate/L), respectively. Overt signs of toxicity were observed in the group exposed to 11 mg/L (0.92 mg a.e. glyphosate/L). Based on the results of this study, Cosmo Flux poppy mix would be classified as moderately (LC<sub>50</sub>>1-10 mg a.i./L) toxic 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 and provides useful information on the toxicity of glyphosate-Cosmo-Flux<sup>®</sup> poppy mix to *X. laevis* based on nominal concentrations.

## **II. MATERIALS AND METHODS**

### **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

### **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.

### **A. MATERIALS:**

#### **1. Test Material**

Opium Poppy Spray Max—Glyphosate Herbicide containing Glyphosate Isopropyl Ammonium

#### **Description:**

Liquid

#### **Lot No./Batch No.:**

Not reported; Wildlife International ID No. 7568

**Purity:** 0.0205% 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  
and Nieuwkoop-Faber Stage 46-47 (mean 47)

**Weight at study initiation:** Not reported

**Length at study initiation:** Not reported

**Source:** Xenopus One, Dexter, Michigan 48130

## B. STUDY DESIGN:

### 1. Experimental Conditions

**Range-finding Study:** Not reported

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

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**Table1. Experimental Parameters**

<b>Parameter</b>	<b>Details</b>	<b>Remarks (<i>Guideline Criteria</i>)</b>
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 <sup>®</sup> (77 mg/mL) Health: Mortality approximately 2% during holding period.	<i>(EPA: minimum of 12 d required, 14 d recommended for acclimation)</i>  <i>(EPA: Pretest mortality must be &lt;5%)</i>
Test duration	96 h	<i>(EPA/OECD requires: 96 h)</i>
Test condition	Static renewal Complete water change every 24 h	<i>(EPA: Must provide reproducible supply of toxicant)</i>
Aeration	Not reported	<i>(EPA: no aeration; OECD permits aeration)</i>
Test vessel	Material: Glass (beaker) Size: 2 L Fill Volume: 1 L	
Source of dilution water	Filtered well water	<i>(EPA 1975; Soft reconstituted water or water from a natural source, not dechlorinated tap water; OECD permits dechlorinated tap water.)</i>

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Parameter	Details	Remarks ( <i>Guideline Criteria</i> )
Water parameters	Hardness: 132 pH: 7.4 – 8.1 DO: 5.1 – 8.5 mg/L TOC: not reported TDS: not reported Metals: <levels of detection Pesticides: <levels of detection Chlorine: Not reported Temperature: 21.2 – 22.3°C Intervals of water quality measurement: 24 h	( <i>Hardness: EPA: 40 - 48 mg/L as CaCO<sub>3</sub>; OECD allows 10 -250 mg/L as CaCO<sub>3</sub>)</i> ( <i>pH EPA: 7.2 - 7.6; OECD: 6.0 - 8.5)</i> ( <i>Dissolved Oxygen: EPA: Static: &gt; 60% during 1<sup>st</sup> 48 hrs and &gt;40% during 2<sup>nd</sup> 48 hrs; OECD ≥ 80% saturation value)</i>  ( <i>EPA water quality: measured at beginning of test and every 48 h</i> )
Number of replicates/group	Control: 2 Treatment: 2	( <i>EPA: 2 replicates per test concentration are preferred</i> )
Number of organisms per replicate	10	( <i>EPA: minimum 7 organisms/replicate, 10 are recommended; OECD requires at least 7 fish/replicate</i> )
Biomass loading rate	10 tadpoles/L	These are relatively crowded conditions for an amphibian study.  ( <i>Static: # 0.8 g/L at # 17EC, # 0.5 g/L at &gt; 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</i> )
Test concentrations:	Nominal (formula): 1.3, 2.5, 5.0, 10, 20 mg/L Measured (formula): 1.4, 2.7, 5.3, 11, 22 mg/L Measured: 0.12, 0.22, 0.44, 0.92, and 1.8 mg a.e. glyphosate/L	Measured values were 101-116% 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 on 7.15% glyphosate.

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Parameter	Details	Remarks ( <i>Guideline Criteria</i> )
		<i>(EPA/OECD: Control &amp; 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series)</i>
Solvent	Water	<i>(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.)</i>
Lighting	16 h light (280 lux) 8 h dark 30-minute transition Similar to natural sunlight	<i>(EPA: 16 h light/8 h dark; OECD: 12-16 hours photoperiod)</i>
Feeding	0.18 mL Sera Micron <sup>®</sup> (77 mg/mL)/d	<i>(EPA/OECD: No feeding during the study)</i>
Recovery of glyphosate	LOQ = 0.559 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 <i>(EPA/OECD requires: minimally every 24 h)</i>
Raw data	Measured test concentrations, water characteristics, cumulative mortality and clinical observations.

**III. RESULTS and DISCUSSION:**

**A. MORTALITY:**

All of the tadpoles in the 22 mg/L were dead within 48 hours. Tadpoles treated with 11 mg/L exhibited no mortality but expressed overt signs of toxicity. The no mortality concentration was 11 mg/L formula (0.92 mg a.i. glyphosate/L). Observations of mortality and reported LC<sub>50</sub> values at 24, 48, 72 and 96 hours are located in **Table 3**.

**Table 3. Effect of Glyphosate-Cosmo Flux Poppy 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
1.4 (0.12)	0	0	0	0	0	0	0	0
2.7 (0.22)	0	0	0	0	0	0	0	0
5.3 (0.44)	0	0	0	0	0	0	0	0
11 (0.92)	0	0	0	0	0	0	0	0
22 (1.8)	9	45	20	100	20	100	20	100
LC <sub>50</sub>	>22 (>1.8)		16 (1.3)		16 (1.3)		16 (1.3)	
95% CI	NA		11-22 (0.92-1.8)		11-22 (0.92-1.8)		11-22 (0.92-1.8)	

**B. NON-LETHAL TOXICITY ENDPOINTS:**

According to report, all tadpoles in the negative control group and the 1.4, 2.7, 5.3 mg/L treatment groups appeared healthy and normal throughout the test. At test termination, tadpoles in the 11 mg/L treatment group appeared smaller in size compared to controls. 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 effect of Glyphosate-Cosmo-Flux<sup>®</sup> Poppy Mix on *Xenopus laevis*.

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 (<LOQ)	AN	AN	AN	AN
1.4 (0.12)	AN	AN	AN	AN
2.7 (0.22)	AN	AN	AN	AN
5.3 (0.44)	AN	AN	AN	AN
11 (0.92)	AN	7 small (13 AN)	7 small (13 AN)	7 small (13 AN)
22 (1.8)	2 lethargic, 1 surfacing	NA <sup>1</sup>	NA <sup>1</sup>	NA <sup>1</sup>
NOEC	11 (0.92)	5.3 (0.44)	5.3 (0.44)	5.3 (0.44)
LOEC	22 (1.8)	11 (0.92)	11 (0.92)	11 (0.92)
AN = appear normal				
<sup>1</sup> No tadpoles survived in this treatment group at this observation time.				

**C. REPORTED STATISTICS:**

LC<sub>50</sub> values were calculated by nonlinear interpolation method. The reported 96-hr LC<sub>50</sub> value based on a binomial distribution was 16 mg/L (1.3 mg a.e. glyphosate /L). The no mortality concentration and NOEC were determined by the study author using “visual interpretation” of mortality and sublethal effects data. The no mortality concentration and the NOEC were 11 mg/L (0.92 mg a.e. glyphosate /L) and 5.3 mg (0.43 mg a.e. glyphosate /L), respectively.

**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<sub>50</sub>: 15.8 mg/L (1.28 mg a.e./L)  
 95% C.I. for LC<sub>50</sub>: 11.1 – 21.6 mg/L (0.92 – 1.79 mg a.e./L)  
 NOAEL: 5.3 (0.44 mg a.e./L)  
 Probit Slope: Not applicable  
 95% C.I. for Probit: Not applicable

**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; however, a longer acclimation period was not possible if the test animals were to be exposed during sensitive developmental windows.

There is uncertainty associated with the measured formula concentrations. It is unclear how the percent active equivalents can be as low as 0.0205% when measured concentrations show that it is roughly 8%. The methods section of the report indicated that formula concentrations were confirmed by measuring the amount of glyphosate by HPLC and then back-calculating the concentration of the overall formulated product based on 7.15% glyphosate. This is inconsistent with the reported purity of 0.0205% and the 8% difference between glyphosate and measured formula concentrations. This uncertainty should be cleared up by the submitter by providing information confirming 1) the percent of glyphosate in the formula; 2) the method for measuring the formula concentrations in the test solutions; and 3) the measured concentrations of formulated product in the test concentrations.

#### **F. REVIEWER'S COMMENTS:**

Dissolved oxygen levels by 96 hours had dropped to 5.1 mg/L (60% saturation in the second highest treatment group; the general decline in dissolved oxygen concentrations over the study period suggests that the loading rate (10 tadpoles/L) is too high.

Mean measured concentrations, based on a reanalysis of the raw data using Statistical Analysis Software (SAS Institute, Release 8.02), are 1.4, 2.8, 5.3, 11 and 22 mg/L (0.12, 0.22, 0.44, 0.92 and 1.8 mg a.e./L. Measured concentrations ranged between 105 to 109% of nominal. Measured concentrations for the matrix fortification (spiked) samples ranged from 96 to 106%. Although log<sub>10</sub> probit analysis using SAS was attempted, the data do not lend themselves to this analysis since there are less than two concentrations at which the percent dead is between 0 and 100 (Appendix 1). LC<sub>50</sub> values were verified using a binomial distribution analysis (ToxWin) (Appendix 2).

Table 4 indicates the mean measured formula concentration as 5.1 mg/L while p. 9 and 16 indicate that this concentration is 5.3 mg/L. The correct value should be indicated. In this DER, the concentration is referred to as 5.3 mg/L.

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” (MRID 468736\_01) and “Poppy Mix”) 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 “0.0205 % glyphosate” without specifying if this is expressed in terms of “glyphosate acid equivalents” or in terms of “glyphosate isopropylamine salt”. This reported purity appears to be very low when compared to the “Coca Mix” formulation (MRID 468736-01).

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

$$\text{a.e. in "Poppy Mix"} = 169/228 = 0.74$$

If the sponsor expressed the composition of the mix in terms of “0.0205%” isopropylamine salt, then in terms of a.e. it corresponds to,

$$\% \text{ a.e. "Poppy Mix"} = (0.0205\% \times 0.74) = 0.0152 \%$$

On page 14, it states that the glyphosate content of 7.15% glyphosate in the “Glyphosate-Cosmo

Flux<sup>®</sup>- Poppy Mix. This is way above the percent glyphosate provided by the sponsor.

#### **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 for African clawed frogs. The results are summarized below. Since the 96-hr LC<sub>50</sub> value (1.3 mg a.e./L) falls between 1 and 10 mg a.i./L, the formulated endproduct (glyphosate-Cosmo-Flux<sup>®</sup> Poppy mix) is classified as moderately toxic to African clawed frog tadpoles under the conditions tested.

##### **Results Synopsis**

Test Organism Size/Age:	Nieuwkoop-Faber Stage 47 (7 days post-fertilization)
Test Type:	Static Renewal
LC <sub>50</sub> :	16 mg/L formula (1.3 mg a.e. glyphosate/L)
95% C.I for LC <sub>50</sub> :	11-22 mg/L formula (0.92 to 1.8 mg a.e. glyphosate /L)
NOEL:	5.3 mg/L formula (0.44 mg a.e. glyphosate/L)
Probit Slope:	NA

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

SAS Output

Measured versus Nominal Concentrations and Percent of Nominal						
Obs	TREAT	_TYPE_	_FREQ_	NOMINAL	MEASURE	PRCT
1	1.3	0	5	1.418	0.1174	109.077
2	2.5	0	5	2.706	0.2240	108.240
3	5.0	0	5	5.262	0.4352	105.240
4	10.0	0	5	11.080	0.9180	110.800
5	20.0	0	5	21.620	1.7920	108.100

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS 11

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS 12

Probit Procedure

Model Information

Data Set	WORK.D
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)
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1.065813E-15	4.910254E-16
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Last Evaluation of the Negative of the Hessian

Intercept	Log10(DOSE)
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Intercept	4.371589E-14	5.076931E-14
Log10(DOSE)	5.076931E-14	5.991032E-14

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

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of 1.96.

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS

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Probit Procedure

Analysis of Parameter Estimates

Variable	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq	Label
Intercept	1	-66.87510	37990377	0.0000	1.0000	Intercept
Log10(DOSE)	1	56.12835	32452074	0.0000	1.0000	

Estimated Covariance Matrix

	Intercept	Log10(DOSE)
Intercept	1.4432688E15	-1.223057E15
Log10(DOSE)	-1.223057E15	1.0531371E15

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
1.19146728	0.01781631

Estimated Covariance Matrix for Tolerance Parameters

	MU	SIGMA
MU	7564274323.6	179390751.51
SIGMA	179390751.51	106109856.95

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS (in mg/L)

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Probit Procedure

Probit Analysis on DOSE

Probability	DOSE	95% Fiducial Limits	
0.01	14.12604	.	.
0.02	14.28490	.	.
0.03	14.38661	.	.
0.04	14.46360	.	.
0.05	14.52654	.	.
0.06	14.58032	.	.
0.07	14.62764	.	.
0.08	14.67013	.	.
0.09	14.70889	.	.
0.10	14.74466	.	.
0.15	14.89367	.	.
0.20	15.01318	.	.
0.25	15.11647	.	.
0.30	15.20983	.	.
0.35	15.29686	.	.
0.40	15.37990	.	.
0.45	15.46068	.	.

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0.50	15.54058	.	.
0.55	15.62090	.	.
0.60	15.70294	.	.
0.65	15.78819	.	.
0.70	15.87853	.	.
0.75	15.97659	.	.
0.80	16.08651	.	.
0.85	16.21559	.	.
0.90	16.37947	.	.
0.91	16.41930	.	.
0.92	16.46268	.	.
0.93	16.51051	.	.
0.94	16.56409	.	.
0.95	16.62542	.	.
0.96	16.69775	.	.
0.97	16.78712	.	.
0.98	16.90665	.	.
0.99	17.09678	.	.

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS

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PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS

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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)
1.074336E-15	-6.62345E-16

Last Evaluation of the Negative of the Hessian

	Intercept	Log10(DOSE)
Intercept	4.516726E-14	3.643828E-15
Log10(DOSE)	3.643828E-15	1.215743E-15

Algorithm converged.

Goodness-of-Fit Tests

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

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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 18

Probit Procedure

Analysis of Parameter Estimates

Variable	DF	Estimate	Standard	Chi-Square	Pr >	ChiSq Label
			Error			
Intercept	1	-6.32193	5403750.1	0.0000	1.0000	Intercept
Log10(DOSE)	1	57.93863	32937165	0.0000	1.0000	

Estimated Covariance Matrix

	Intercept	Log10(DOSE)
Intercept	2.9200515E13	-8.751986E13
Log10(DOSE)	-8.751986E13	1.0848569E15

Probit Model in Terms of Tolerance Distribution

	MU	SIGMA
	0.10911422	0.01725964

Estimated Covariance Matrix for Tolerance Parameters

	MU	SIGMA
MU	6856774143.1	158635009.09
SIGMA	158635009.09	96271870.254

PROBIT ANALYSIS (LOG10) OF XENOPUS LAEVIS MORTALITY AFTER 96 HOURS (in mg a.e./L) 20

Probit Procedure

Probit Analysis on DOSE

Probability	DOSE	95% Fiducial Limits	
0.01	1.172094	.	.
0.02	1.184861	.	.
0.03	1.193033	.	.
0.04	1.199218	.	.
0.05	1.204272	.	.
0.06	1.208591	.	.

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**Data Evaluation Report on the acute toxicity of Glyphosate-Cosmo Flux<sup>®</sup> Poppy Mix End Use Product to African clawed frogs (*Xenopus laevis*)** MRID 468736-02

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0.07	1.212391	.	.
0.08	1.215803	.	.
0.09	1.218915	.	.
0.10	1.221786	.	.
0.15	1.233746	.	.
0.20	1.243335	.	.
0.25	1.251621	.	.
0.30	1.259109	.	.
0.35	1.266088	.	.
0.40	1.272745	.	.
0.45	1.279220	.	.
0.50	1.285625	.	.
0.55	1.292061	.	.
0.60	1.298634	.	.
0.65	1.305463	.	.
0.70	1.312699	.	.
0.75	1.320552	.	.
0.80	1.329353	.	.
0.85	1.339685	.	.
0.90	1.352799	.	.
0.91	1.355986	.	.
0.92	1.359456	.	.
0.93	1.363282	.	.
0.94	1.367568	.	.
0.95	1.372473	.	.
0.96	1.378258	.	.
0.97	1.385403	.	.
0.98	1.394958	.	.
0.99	1.410153	.	.

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Use Product to African clawed frogs (*Xenopus laevis*)** MRID 468736-02

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

Glyphosate *Xenopus* 96-hr LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
21.6	20	20	100	9.536742E-05
11.1	20	0	0	9.536742E-05
5.26	20	0	0	9.536742E-05
2.71	20	0	0	9.536742E-05
1.42	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 11.1 AND 21.6 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 15.48418 (mg/L)

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

Glyphosate *Xenopus* LC50

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.792	20	20	100	9.536742E-05
.918	20	0	0	9.536742E-05
.4352	20	0	0	9.536742E-05
.224	20	0	0	9.536742E-05
.1174	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT .918 AND 1.792 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 1.282597 (mg a.e./L)

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS

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