ProtectGuard

48-Hour Acute Toxicity Test Report

Ceriodaphnia dubia Pimephales promelas

March 2008

Prepared for:

Guard Industry (UK) Ltd. 7200 The Quorum Oxford Business Park Oxford OX4 2JZ United Kingdom

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48-HOUR LC50 PRODUCT REPORT

Client Guard Industry Sample ProtectGuard	
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INTRODUCTION

A product identified as ProtectGuard was delivered to Huther and Associates Inc. on February 25, 2008. Two acute toxicity tests were requested: a static acute 48-hour definitive toxicity test using Ceriodaphnia dubia (water flea) and a static acute 48-hour definitive toxicity test using Pimephales promelas (fathead minnow). Test procedures followed recommended methods contained in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition", EPA-821-R-02-012, October 2004 (Attachment A).

C. dubia and P. promelas are two freshwater aquatic indicator organisms frequently used to evaluate the potential toxicity of a compound or an effluent. The acute toxicity of a compound or effluent is generally measured using a multi-concentration, or definitive test, consisting of a control water and a minimum of five increasing concentrations of product added to control water. The tests are designed to provide dose-response information, expressed as the concentration that is lethal to 50% of the test organisms (LC50).

SAMPLE PREPARATION

The product was initially prepared for testing by adding a wide range of product concentrations to laboratory water (rangefinding tests). There was 100% survival to both species in the initial rangefinding tests. A second set of rangefinding tests were conducted using higher concentrations of product. Based on the results of the second set of rangefinding tests, definitive test solutions were conducted using a narrower range of concentrations.

The initial rangefinding test concentrations for both species were 50, 100, 200, 400, 800, 1600, 3200 and 6400 ml/L (ppm). The second set of rangefinding test concentrations were 5,000, 10,000, 20,000, 40,000, 60,000, 80,000 and 100,000 ml/L (ppm). The definitive test concentrations for both species were 4,000, 6,000, 8,000, 10,000, 12,000, 14,000, 16,000 and 18,000 ml/L (ppm).

Laboratory water/control water was distilled, deionized water reconstituted with reagent grade chemicals to a hardness of 160 mg/L as CaCO₃ an alkalinity of 110 mg/L and a pH of 8.20.

TEST DESIGN Ceriodaphnia dubia

The 48-hour static, non-renewal, definitive *Ceriodaphnia dubia* test was conducted in 20 mL beakers containing 10 mL of test solution. The test was initiated March 5, 2008 following completion of the rangefinding tests. Five *C. dubia* neonates, less than 24-hours old, were added to each of the four replicate beakers per concentration. Neonates were fed a concentration of *Selenastrum capricornutum* plus cerophyll extract while in holding prior to test initiation. The test was conducted for 48-hours during which survival was recorded daily.

A control of four replicate beakers containing five *C. dubia* neonates each in laboratory water was conducted concurrently with the test. The test was completed on March 7, 2008. Survival data were statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the lethal concentration to fifty percent (50%) of the test population (LC50).

RESULTS Ceriodaphnia dubia

The following estimated LC50 value was determined for ProtectGuard:

48-	Hour	Defin	itive	Test

Conc. (ml/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
4,000	20	20	0	100.0
6,000	20	20	0	100.0
8,000	20	16	4	80.0
10,000	20	18	2	90.0
12,000	20	9	11	45.0
14,000	20	3	17	15.0
16,000	20	0	20	0.0
18,000	20	0	20	0.0

Percent Spearman-Karber	0.0%
Estimated LC50 (ml/L):	11,271.83
95% Upper C.L. (mg/L):	12,066.41
95% Lower C.L. (mg/L):	10,529.57

TEST DESIGN Pimephales promelas

The 48-hour static, non-renewal, definitive *Pimephales promelas* test was conducted in 250 mL beakers containing 200 mL of test solution. The test was initiated March 5, 2008 following completion of the rangefinding tests. Ten *P. promelas* larvae were added to each of two replicate beakers per concentration. Larvae originated from laboratory cultures and were six days old at test initiation. Larvae were fed laboratory cultured *Artemia* nauplii during test exposure. The test was conducted for 48-hours during which survival was recorded daily.

A control of two replicate beakers containing ten *P. promelas* larvae each in laboratory water was conducted concurrently with the test. The test was terminated on March 7, 2008. Survival data were statistically analyzed using the Trimmed Spearman-Karber point estimate test to determine the lethal concentration to fifty percent (50%) of the test population (LC50).

RESULTS
Pimephales promelas

The following estimated LC50 value was determined for ProtectGuard:

	48-Hou	· Definitive	Test	
Conc. (ml/L)	# exposed	# alive	#dead	% survival
Control	20	20	0	100.0
4,000	20	20	0	100.0
6,000	20	20	0	100.0
8,000	20	20	0	100.0
10,000	20	20	0	100.0
12,000	20	10	10	50.0
14,000	20	1	19	5.0
16,000	20	0	20	0.0
18,000	20	0	20	0.0

Percent Spearman-Karber Trim:

0.00%

Estimated LC50 (ml/L):

12,001.79

95% Upper C.L. (ml/L):

12,493.42

95% Lower C.L. (ml/L):

11,529.51

DISCUSSION AND CONCLUSIONS

Ceriodaphnia dubia were determined to be the more sensitive species to ProtectGuard with an estimated 48-hour LC50 concentration of 11,271.93 ml/L. The estimated 48-hour LC50 concentration to *Pimephales promelas* was 12,001.79 ml/L.

Currently, there is no universal aquatic toxicity classification for chemicals. The US uses an aquatic toxicity classification system for pesticides while a proposed aquatic toxicity chemical classification system between the US and Europe is known as the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

The following Table compares both classification systems (Attachment B):

GHS	US Pesticide Aquatic	Toxicology Classification
Classification		LC50 (ml/L; ppm)
Category 1	super	<0.01
Category 1	extreme	0.01-0.10
Category 1	high	0.11 - 1.0
Category 2	moderate	1.1 - 10.0
Category 3	slight	11.0 - 100.0
	minimal	>100.0

Neither of the classification systems address chemicals with LC50 values exceeding 1,000 ml/L. It is therefore concluded that even if the product was applied shortly before a rainfall run-off event or drift occurred during application, this product would pose minimal, if any, acute toxicity in the receiving body of water.

RAW DATA

48-HOUR CERIODAPHNIA DUBIA SURVIVAL

CLIENT:

PROTECTGUARD PT4572

PROJECT#:

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48-HOUR PIMEPHALES PROMELAS SURVIVAL

CLIENT:

Protect Guard

PROJECT#:

PT4577

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48-HOUR PIMEPHALES PROMELAS SURVIVAL

CLIENT:

PROTECT GUARD

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Ph: (940) 387-1026

Fax: (940) 387-1036

48-HOUR CERIODAPHNIA DUBIA SURVIVAL

CLIENT:

PROTECT GUARD

RANGEFINDAG 712

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48-HOUR CERIODAPHNIA DUBIA SURVIVAL

CLIENT:

PROJECT#:

Protect Guard

Pryson
Definitive Test

	NUMBER ORGANISMS, 0 HRS		SMS,	NUMBER ORGANISMS, 24 HRS			NUMBER ORGANISMS, 48 HRS							
CONC. (%)	A	В	E	Ð	A	В	C	D	Δ	В	<u>c.</u>	Ð	MEAN	CV%
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48-HOUR PIMEPHALES PROMELAS SURVIVAL

CLENT:

Protect Guard

PROJECT#:

PT4572

Definitive

Test

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Ph: (940) 387-1026

Fax: (940) 387-1036

n	48- Hour Acute To	oxicity Summary Fo	orm Ext	1072
Permittee: Protect Guard		Organism		-
Outfall: Definitive TEAT		_		
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egin Time/ Date 3/5/06	/		- , ,	
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3-1 48°H- 24.0 3	756 1095	الإي الناوي	1080 1 02	到[62] [2] [2] [2] [2] [2] [2] [2] [2] [2] [
			: Cerio	1615
Begin Time/ Date _3/5/08	1615	End Date	Time 3/7/08	
Company Have Selection				
Samp		pH (mg/L) of So	olutions 1.37	Amiysta
Dare Time Temp No.	Aciu G CO	8,000 16,000	12 000 14 000	76,000
	' <u>-</u>	\$15 \$12	814 814	813 CC
3-5 Start -25.0	820 816			
3-6 24HF 240 F/				
3-6 Renew 25.0 2			823 823	822 mm
3-7 48 HF. 240 2	900 P33	- 1824 - 1821 -	国のなどは国国のかり	
	7 (A.) (A.) (A.)			
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		0 5,000 10,00	0 (2.000 14.00	5 76 000
	EDDE SEC	ac 750		00
3-5 Start 25.0	756 756	245 30 725	7 734 738	
3-6 24世 24.0				
3-6 Renew 25.0 Q				ENTRE CONTRACTOR
3-7-48 AL 240		58 168	160 E 100 E 100	1609 III MW
The second secon				
	Hardness	TAlcalming Cond	uct and ResideCize a	Decidor (mb)
Samp PH No.	DO Jung/I CaC03	mg/I CaCO3		mpress and the second
1			/ /_	
The street of th	/ /	/ /		
2		<u> </u>		
Con. 8:20 7	56 160	110 76	8	

			71	<u> 48- Hour</u>	Acute Tox	cicity Summ	nary rui	LILL	Fat		
Permittee:	: Prod	ect Gu	3C4			O:	rganism:				
Outfall: _	DEE	JUITIVE	TOT								-
Lab ID N	o.:	P74	<u> エクク</u>			- 듄	nd Date/	Time	3/7/08	1545	
rin Tir	ne/ Da	te _ 3/5	-108	1547		_					
			المتعدد المتعدد	- new transfer							Analysc
		Temp	Samp			pH(m	dE) of Soluti				
Date	Time		No	16 000	20,000		1,000				66
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3-5	Start	250						u feine 19inu = 15i Pil (North Holl)			Land Park Control of the Control of
3-6	24-H .	24.0			gajiwa garati <u>se</u> li	Commence of the control of the same				_	
3-6	Renew	25.0	2		- n		89				Mm-
3-7	48 Hiz	24. D	2	8	811			The tree to	Loin Liverille . P. P. G. Stram	- P(L) and (1) the Page	
							ediores de la comp				
			Sámo:			DO (ng/E)Yof Solu				Analyst
Date.	Time	Temp	y No≝				9,000				
				T /	20,000	The state of the s	754			. <u> </u>	1 ac
3-5	Start	25,0		1729	726						
3-6	24 日口	94.0					FORT HEARING		Total Best Taxas		-
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3-13	48'Hi_	130 5	i a	1699	689		69 7				
	177	tri lati di mana				0	rganism:		<u>rio</u>	11 -	
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Desm.	. 1111-1	,								建设型型	
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Dare	Time	Temps	Sampe No.								
				19,000	<u> </u>		7000	Marie Company	Service Control of the Control of th		AC.
3-5	Start	25.0	1	812	509		816				
	24 Hi		Total Control of the Control								
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3-G	48 Hi	and the Land of Prince Street, and the Land			811		819=				
3-7		- 194,0		E-1-1200							
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			Samo			HOLD DO) (mg/E) of S	olinon:			
Date		Tem	Samp	18 CQ	0 00	8 6	4.000				70
					7,20		756	ļ			1/(
3-5	Star	0.000		729							
36	-24 E		O PETE	据图 學便報				**************************************	1		
3-6	Rene	w 25.0	<u> </u>								AAAAE
	#E481		The second second	温 [099]			WA)				
	ALLEG LIST STREET	<u> </u>					11-11-11-11-11-11-11-11-11-11-11-11-11-	East Season		sependar (m)	
-5575					Jardiness 15 JE CaCO3151	PAlkalmiry.	====iCondi		Resid CIZ	Dechlor (ml Na2SZ03 mg/tell	
Date	Sin No				IE CaCO3	HIPTE GICOS	umnos/		理型运输	mg/ls	
		ļļ						/			
		<u> </u>		/-			1				
		2					#		<u></u>	 	
	C C	on. 8.	20 7	256	160	110_					
		□ ∪∴	<u> </u>		<u></u>				 -		<u></u>

LC50 DETERMINATIONS

DATE: MARCH 20 DURATION: 48 H TEST NUMBER: 1

TOXICANT : PROTECTGUARD

SPECIES: CERIODAPHNIA DUBIA

RAW DATA:	Concentration	Number	Mortalities
	(PPM)	Exposed	
	.00	20	0
	6000.00	20	0
	8000.00	20	4
	******10,000	20	2
	****** 12,000	20	11
	****** 14,000	20	17
	****** 16,000 RN	20	20

SPEARMAN-KARBER TRIM: .00%

 SPEARMAN-KARBER
 ESTIMATES:
 LC50:
 11271.83

 95%
 LOWER
 CONFIDENCE:
 10529.57

 95%
 UPPER
 CONFIDENCE:
 12066.41

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

DATE: MARCH 20 TEST NUMBER: 1 DURATION: 48 H

TOXICANT : PROTECTGUARD

SPECIES: PIMEPHALES PROMELAS

RAW DATA:	Concentration	Number	Mortalities
	(PPM)	Exposed	
	.00	20	0
	8000.00	20	0
	****** 10,000	20	0
	****** 12,000	20	10
	****** 14,000	20	19
	****** 16,000 BH	20	20

.00% SPEARMAN-KARBER TRIM:

SPEARMAN-KARBER ESTIMATES: LC50: 12001.79

11529.51 12493.42 95% LOWER CONFIDENCE: 95% UPPER CONFIDENCE: