BRL

BIO RESEARCH LABORATORIES, INC.

2897 152nd Avenue N.E. / Redmond, WA 98052-5514 (425) 869-4224 / FAX: (425) 869-4231

Web Site: www.bioresearch.com / E-mail: info@bioresearch.com

September 8, 2006

RECEIVED

Pam Covey WA State Deptartment of Ecology Manchester Laboratory 7411 Beach Drive East Port Orchard, WA 98366

SEZ 18 ZIIIIS DEPT. OF ECOLOGY

Fax#: 1-360-871-8850

Enclosed is one original and one copy of the completed study per your request:

BRL#

Client Code

60598

Drost, Maralco: Station ID B-9-D, 06/13/06 Time 1620 Sample # 244011

5,000mg/kg

The bioassay on the above referenced sample were completed in accordance with WAC 173-303, and DOE 80-12, revised May, 1999.

Bio-Research Laboratories performs sample studies by using the EPA Good Laboratory Practices set forth in the 40 CFR (163.80-1). Our laboratory is recognized as an *Accredited Laboratory* by the State of Washington Department of Ecology. We are also licensed by the United States Department of Agriculture (registration 91 R 043).

Bio-Research is committed to providing you with excellent laboratory services. Please call if you have any questions regarding your test results. We thank you for the opportunity to have been of service to you.

Sincerely,

John J. Majnarich, Ph.D.

President & Scientific Director

JJM:src Enclosure

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RECEIVED

LABORATORY REPORT

DEPT. OF ECOLOGY

ACUTE ORAL RAT TOXICITY TEST

FOR

Washington Dept of Ecology

Performed by:

BIO RESEARCH LABORATORIES, INC.

Laboratory Ref. Number: 60598

Client Sample Code: Drost, Maralco: Station D B-9-D, 06/13/06 Time 1620 Sample # 244011

Study Director

warren C. Ladiges, DVM

Date

President & Scientific Director

John J. Majnarich, Ph.D.

Data

September 8, 2006

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C. Dosing:

"Dose" is defined as the amount of test substances administered. This is expressed as weight of the test substance in grams or milligrams per unit weight of the test animal. Ten rats were dosed by gavage with 5000 mg of the sample material per 1000 grams of body weight. A group of ten rats were dosed with DI water by gavage as the control group. All of the animals received the appropriate concentration and approximate volume of the dosing solution. The volume did not exceed 2 mL per rat.

D. Test Duration:

The test began on August 24, 2006 and ended on September 7, 2000. The rats were observed for 14 days for mortality and clinical pharmacological or toxicological signs.

E. Sample Test Substance:

An evaluation of the integrity of the test samples was made upon receipt as to packaging deficiency, proper weight of sample for testing, possible mishandling during shipping, or any other visible defects. Any deficiencies were noted and used in the final interpretation of the data.

Date of Delivery: August 8, 2006

Physical & Chemical Characteristics: Dark grey, rocky soil like material

Client Reference Code: Drost, Maralco: Station ID B-9-D, 06/13/06 Time 1620

Sample # 244011

Vehicle: Water was used as a suitable vehicle. The test substance was

then administered in a single dose by gavage.

F. Observation of Animals:

Observations were made for any toxicity effect immediately after dosing at one and four hours and daily thereafter for a period of 14 days. From cageside the rats were carefully observed daily for the following:

- 1. The skin and fur.
- 2. Eyes and mucous membranes.
- 3. Respiratory system.
- 4. Circulatory system.
- 5. Autonomic and central nervous system.
- 6. Somatomotor activity and behavior pattern.
- 7. Tumor, convulsions, salivation, diarrhea, lethargy, and coma.

The individual weight of the animals was determined immediately before the test substance was administered, weekly, and at death. At termination of the test all of the surviving rats were weighed and sacrificed.

G. **Gross Necropsy:**

Gross necropsy was performed on the on the rats that were sacrificed at termination. The gross necropsy included examinations of:

- 1.
- The external surface of the body.
 The thoracic and abdominal cavities and their contents. 2.

III. RESULTS

A. Body Weight:

The mean weekly body weight for the rats dosed 0 mg/kg (control), and 5000 mg/kg, are shown in the following table:

Table I: Mean Weekly Body Weight

Sample/Dosage	Me	an Body Wt. (gra	The second secon				
	day 0 (8/24/06)	day 7 (8/31/06)	day 14 (9/07/06)	14 Day Wt. Gain (g/rat)			
Control, male (5) 0 mg/kg	214	274.8	306.4	78.2			
Control, female (5) 0 mg/kg	182.2	216.6	221.4	39.2			
Sample #60598, male (5) 5000 mg/kg	213.8	275	308	94.2			
Sample #60598, female (5) 5000 mg/kg	185.4	216.6	224.4	39			

Number in parentheses () is the number of animals results are based on.

III. RESULTS (Cont.)

B. <u>Cageside Observation:</u>

All of the rats appeared healthy and ate normally. No abnormal behavior was observed.

C. Mortality:

There were no mortalities.

D. Gross Necropsy:

All the animals appeared normal and healthy. There were no signs of typical toxicity effects. All the organs examined on necropsy appeared normal. Specifically the organs examined macroscopically were the liver, spleen, kidneys, adrenals, bladder, ovaries (females), testes (males), heart, lungs, and thymus gland.

Table 2. Raw Data Sheet - Sample #60598 - Controls (0 g/kg)

DATA SHEET FOR ACUTE ORAL RAT TOXICITY TEST

Toxicant:

DI Water

Bio Research Laboratories Sample# 60598

Industry:

WA Dept. of Ecology.

Analyst: JM, WL, LP

Collector:

BRL

Beginning Time/Date: 10:30am, 08/24/06

Date Sample Collected: 08/24/06

Ending Time/Date: 10:30am 09/07/06

Test Organism: Sprague Dawley rats

Dosage Level: 0g/kg

RAT#	W	eight (gn	OBSERVATIONS AND DATES															COMMENTS		
	0	7	14		4hr	8/	8/	8/	8/	8/	8/	8/	9/	9/	9/	9/	9/	9/	9/	Gross Necropsy
'						25	26	27	28	29	30	31	01	02	03	04	05	06	07	
11M	210	267	293	0g/kg	1	1	1	$\sqrt{}$	1	$\sqrt{}$	1	1	$\sqrt{}$	1	\checkmark	$\sqrt{}$	√	1	√]	Normal Organs
12M	223	283	317	0g/kg		1	1	1		$\sqrt{}$	1	\checkmark	$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	Normal Organs
13M	215	279	314	0g/kg		$\sqrt{}$	1	$\sqrt{}$	$\sqrt{}$		1	1	$\sqrt{}$	1		1	1	\checkmark	$\sqrt{}$	Normal Organs
14M	210	277	313	0g/kg			1	1					$\sqrt{}$	$\sqrt{}$			1	$\sqrt{}$		Normal Organs
15M	212	268	295	0g/kg	1		√	1	1				$\sqrt{}$	$\sqrt{}$				√	$\sqrt{}$	Normal Organs
16Fe	179	206	222	0g/kg	1		1		1		1	$\sqrt{}$		1		1	$\sqrt{}$	7		Normal Organs
17Fe	181	225	220	0g/kg	1	1	1	√			$\sqrt{}$	1	$\sqrt{}$	\checkmark		\checkmark		1	$\sqrt{}$	Normal Organs
18Fe	191	223	224	0g/kg	1	1		7	1			$\sqrt{}$	1					7		Normal Organs
19Fe	178	214	215	0g/kg	V	1	$\sqrt{}$	1		$\sqrt{}$	1	$\sqrt{}$	$\sqrt{}$	V			7	7	$ \sqrt{ }$	Normal Organs
20Fe	182	215	226	0g/kg		V		1	1	1	1			1	1		1	7	$ \sqrt{ }$	Normal Organs
Initials	JM,LP	LP	JM,LP	JM,LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	JM,WL

^{*} $\sqrt{\ }$ = no abnormal behavior

Table 3. Raw Data Sheet - Sample #60598 - Drost, Maralco: Station ID B-9-, 06/13/06 Time 1620 Sample # 244011 (5000 mg/kg)

DATA SHEET FOR ACUTE ORAL RAT TOXICITY TEST

Toxicant: Drost, Maralco: Station ID B-9-, 06/13/06 Time 1620 Sample # 244011

Bio Research Laboratories Sample# 60598

Industry:

WA Dept of Ecology

Analyst: JM, WL, LP

Collector:

WA Dept of Ecology

Beginning Time/Date: 10:30am, 08/24/06

Date Sample Collected: 06/13/06

Ending Time/Date: 10:30am 09/07/06

Test Organism: Sprague Dawley rats

Dosage Level: 5000mg/kg

RAT#	W	eight (gn	Dose OBSERVATIONS AND DATES															COMMENTS		
	0	7	14		4hr	8/	8/	8/	8/	8/	8/	8/	9/	9/	9/	9/	9/	9/	9/	Gross Necropsy
						25	26	27	28	29	30	31	01	02	03	04	05	06	07	
1M	224	279	315	5000mg/kg	$\sqrt{}$	1	4	1			$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	7	1	√	$\sqrt{}$	$\sqrt{}$	1	Normal Organs
2M	214	276	312	5000mg/kg	1	√	$\sqrt{}$	\checkmark	1	1	$\sqrt{}$	$\sqrt{}$	\checkmark	\checkmark	$\sqrt{}$	$\sqrt{}$	\forall	$\sqrt{}$	1	Normal Organs
3M	220	287	323	5000mg/kg	1	$\sqrt{}$	1	1	$\sqrt{}$	$\sqrt{}$	\checkmark	$\sqrt{}$	\checkmark	$\sqrt{}$	√	√ .	\checkmark	\checkmark	$\sqrt{}$	Normal Organs
4M	211	268	296	5000mg/kg	$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	1	$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	1	$\sqrt{}$	$\sqrt{}$	1	1		$\sqrt{}$	$\sqrt{}$	Normal Organs
5M	200	265	294	5000mg/kg	1	1	$\sqrt{}$	$\sqrt{}$	1		$\sqrt{}$	$\sqrt{}$		1	$\sqrt{}$		√	1	1	Normal Organs
6Fe	185	212	228	5000mg/kg	1	$\sqrt{}$	$\sqrt{}$	1	1	1	$\sqrt{}$	\checkmark	√		$\sqrt{}$		$\sqrt{}$	$\sqrt{}$	$\sqrt{}$	Normal Organs
7Fe	190	215	226	5000mg/kg	$\sqrt{}$	$\sqrt{}$			1	1	√	\checkmark	√	$\sqrt{}$	1	1	$\sqrt{}$	\forall	$\sqrt{}$	Normal Organs
8Fe	179	214	225	5000mg/kg			1	$\sqrt{}$	1	1	$\sqrt{}$		7	1	1	7	$\sqrt{}$	1	1	Normal Organs
9Fe	189	220	218	5000mg/kg	1	1	√		1	1	1		V	√,		1	1	1	1	Normal Organs
10Fe	184	222	225	5000mg/kg		1				1	√		1	$\sqrt{}$	1		√	1	$\sqrt{}$	Normal Organs
Initials	JM,LP	LP	JM,LP	JM,LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	LP	JM,WL

IV. <u>DISCUSSION AND CONCLUSIONS</u>

The purpose of this acute oral toxicity test was to provide information on rat health hazards likely to arise from a single oral exposure. Data from an acute study serves as a basis for classification, labeling, and packaging. Also, data is evaluated to determine whether the median lethal dose (LD_{50}) was below or above the administered dose.

"LD₅₀ oral (median lethal dose)" is a statistically derived single dose of a substance that can be expected to cause death in 50 percent of the animals when administered by the oral route. The LD₅₀ value is expressed in terms of weight of the test substance (g, mg) per unit weight of the test animal (e.g., mg/kg).

According to the Washington State Hazardous Waste Regulation (WAC 173-303) and Washington State Department of Ecology (DOE) "Acute Oral Rat Toxicity Test" (DOE 80-12, revised August 1996), a waste substance can be classified as an extremely hazardous waste (i.e., $LD_{50} \le 100$ mg/kg) or a dangerous waste (i.e., $LD_{50} \le 5000$ mg/kg). The death of two or less test animals per dose will be statistical evidence that the LD_{50} is greater than the standard at the 95 percent confidence level.

BRL Sample #60598: (5000 mg/kg)

In conclusion, with no mortalities associated with toxicity at the 5000 mg/kg dose, this sample is <u>not</u> considered to be a dangerous waste. The average weight gain over the two week period was 78.2g for the control males versus 94.2g for the test males per rat. The weight gains of the control and test males were comparable. The control females gained a total of 39.2g versus the test females which gained a total of 39g per rat over the same two week period. The total weight gains were comparable in both the test and the control groups.

V. PROFESSIONAL STAFF

John J. Majnarich, Ph.D., President & Scientific Director

Warren C. Ladiges, DVM; Director of Veterinary Services

Lisa A. Pedigo, B.S., Laboratory Manager