Santafé de Bogotá, D.C., March 4, 1 999 AFAR-015-99

Messrs SALDER LTDA Santafé de Bogotá City

Re: Analysis of Foam Repellent NOPIKEX®

Dear Mr. Ardila:

This is in response to your request of March 2 in which you ask that we clarify two items of the Analysis Report AFARI-097-98, which corresponds to the biological assays conducted in our Department on the product NOPIKEX®:

- 1. In Item 2, the treated animals used correspond to **Mouse spp, sex Female**.
- 2. In the NOTE of the last part of the report it says "The following results correspond only to the samples received and assayed", it should read "The above mentioned results correspond only to the samples received and assayed".

Also we wish to send to you the Summary Tables of the Lethal Dose 50 results, as well as the respective graph.

REPORT OF THE ACUTE TOXICITY STUDY [LD50] OF THE PRODUCT NOPIKEX [Insect Repellent]

Specie:MOUSESex:FemaleStrain:I.C.R

Administration: Oral [by intubation]

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Origin: Bioterium of the Pharmacology Department

Universidad Nacional [National University]

Diet: Dog food Kenn-L by Purina and water, ad libitum

Environmental Conditions: 12 h light - 12 h darkness. Temperature

20°C±2°C. Relative humidity 70%. Steel cages with rice

husks for bedding.

Numbers of Doses: 5

Group Control: 1

Number of animals per dose: Ten

Report of corporal weights of the animals at each dose [logarithmical]

Dose A: 3.73 g/Kg of corporal weight

Corporal Weight [Days]

Days Mouse	0	3	5	7	9	11	13	15
1	35	33	32	32	32	33	32	31
2	33	33	32	34	35	35	34	33
3	37	36	36.5	35	37	36	34	34
4	37	37	36.5	36	37	38	36	36
5	34	34.5	35	34	33	33	32.5	32
6	36	36	35.5	35	35	35	34	34
7	35	34	33	33	32	32.5	32	32
8	34	34	33	34	35	34	34	33
9	33	34	33	33	34	33	33	33
10	35	34	34	33	33	34	34	34.5

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Dose B: 5.32 g/Kg Corporal Weight

Corporal Weight [Days]

Corporal Weight [Days]												
Days	0	3	5	7	9	11	13	15				
Mouse												
1	28	29	29	30	29.5	28	27	27				
2	25	24	25	26	25	26	26	26				
3	26	26	27	25	24	25	24.5	25				
4	30	31	31	32	31	31	31	31				
5	32	32	31	31	30	30	29	29				
6	30	30	31	31	30	29.5	29	30				
7	26	26	27	27	26	26	25.5	25				
8	25	25.5	26	26	26	25.5	25	25				
9	31	31	31	32	31	30	30	30.5				
10	28	28	28	27.5	27	27	27	27				

Dose C: 7.48 g/Kg Corporal Weight

Corporal Weight [days]

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	0	3	5	7	9	11	13	15			
1	33	34	34.5	34	33.5	33	33	32	Wh: 1.8 WR: 0.6		
2	37	36	35	35.5	33	+					
3	35	35	34	34	34	33	32.5	32	Wh: 1.7 WR: 0.7		
4	34	34	34	33	31.5	30	30	29.5	Wh: 1.6 WR: 0.6		
5	31	31	30	29	28	+					
6	35	34	32	31.5	30	29	28	28	Wh: 1.5 WR: 0.5		
7	37	37	36	35	35	34.5	33	32	Wh: 1.6 WR: 0.7		
8	34	33	33	+							
9	35	35	34	32.5	31	30	30	28	Wh: 1.5 WR: 0.5		
10	34	33	32	30	30	29.5	28	28	Wh: 1.6 WR: 0.5		

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Dose D: 10.52 g/Kg Corporal Weight

Corporal Weight [Days]

days Mouse	0	1	3	5	7	9	11	13	15		
1	35	34	32	32	31.5	30	30	29	28	Wh: 1.6 WR: 0.6	
2	34	33	32	+							
3	31	30	29	28.5	28	26	+				
4	34	33	32	31.5	+						
5	34	33	33	32.5	32	32	31	30	28	Wh: 1.7 WR: 0.7	
6	32	32	31.5	31	30	+					
7	36	36	35	34	34	33	31.5	30	29	Wh: 1.75 WR: 0.7	
8	31	30	29.5	28	27	+					
9	30	30	28	27	+						
10	32	32	31	30.5	29	28	28	27	26	Wh: 1.6 WR: 0.5	

Dose E: 14.92 g/Kg Corporal Weight

Corporal Weight [Days]

Days Mouse	0	1	3	5	7	9	11	13	15
1	27	+							
2	30	28	27	27	26	26.5	25	25	24
3	29	+							
4	30	+							
5	30	+							
6	28	+							
7	28	+							
8	30	+							
9	29	+							
10	30	+							

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Control Group: Isotonic Saline Solution

_Days Mouse	0	1	3	5	7	9	11	13	15	
1	34	34	35	34	34	34	35	34.5	35	Wh: 2.0 WR: 0.6
2	32	32	32	33	32	31.5	32	32	32	Wh: 2.05 WR: 0.8
3	33	33	34	33	34	33.5	33	34	34	Wh: 2.3 WR: 0.7
4	30	31	31	30	30	31	31	32	31	Wh: 1.7 WR: 0.6
5	31	31	32	32	31	32	32	33	33	Wh: 1.9 WR: 0.7
6	32	32	32	31	32	31.5	32	33	32	Wh: 1.9 WR: 0.8
7	34	34	33	34	33	32	33	33	33	Wh: 1.85 WR: 0.7
8	32	33	34	34	33	34	33.5	34	34	Wh: 1.9 WR: 0.6
9	30	31	31	32	32	31.5	32	32	32	Wh: 2.0 WR: 0.7
10	31	31	30	31	32	31.5	32	32	32	Wh: 1.9 WR: 0.6

Dose g/ kg	# Deaths	# Alive	Dead Accumulated	Alive Accumulated	% Mortality
Control Group Group E 14.92 Group D 10.52 Group C 7.48 Group B 5.32 Group A 3.73	0 9 6 3 0	10 0 4 7 10 10	- 18 9 3 0	- 1 50 12 22 32	0 90 60 30 0

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LETHAL DOSE FIFTY MIRROR CURVE 50 30 D Α L Ε 25 Α V D 40 Е Α 20 \mathbf{C} A 30 - \mathbf{C} \mathbf{C} C U 20 -15 U M M U U L L 10 -10 A T A T E - 5 Е D D 0 10 Do g/Kg

CUTTING POINT: 8.70 G/Kg

Calculation of the DL₅₀:

$$Log DL_{50} = Log 7.48 * + (50-30) Log 10.52 60-30 7.48 DL_{50} 8.70 g/Kg$$

COMMENTARY:

From the third day after treatment, the general aspect of the females deteriorated, the hair lost its shine and presented piloerection. The fecal feces turned darker and lacked lubrication.

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At the time of necropsy the animals which survived the fifteen days of observation, hypothermia was apparent, the lungs were hypothermic and in the stomach and intestines the presence of gas was quite noticeably.

The weight relationship of the liver and kidneys with respect to corporal weight was not significant for none of the animals.

We hope to have responded to your request.

Sincerely,

Signed:

ROBERTO PINZÓN SERRANO

Director
Department of Pharmacology

JAIME H. ROJAS

Head Consulting Services and Extension Unit