REPORT ON ACUTE DERMAL TOXICITY TEST: FIXED DOSE PROCEDURE – Biovectrol 20EM
Batch no: BN2005062701
Manufacture Date: 27/06/2005
Sponsor: OKADA ECOTECH PTE LTD, Blk 1 Pasir Panjang Road #07-15/17, Alexandra Distripark, Singapore 118478.
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Materials and Methods

Test substance: Biovectrol 20EM, Batch no: BN2005062701;
Manufacture Date: 27/06/2005
Test was conducted from 6 Feb to 20 Feb 2006
Sample received was a concentrate brownish in colour viscous solution, with a characteristic aroma. Test was conducted on the undiluted liquid.

The procedure was a modification of the OECD GUIDELINE FOR TESTING OF CHEMICALS – Proposal for a New DRAFT GUIDELINE 434: Acute Dermal Toxicity – Fixed Dose Procedure. DRAFT GUIDELINE 14 May 2004 (1st Version).

A dose of 2ml/kg (approx 2000 mg/kg) body weight was used for dermal application.

1. Ten female Sprague Dawley (SD) rats, 8-12 weeks old, were obtained from the Laboratory Animals Centre, NUS and acclimatized at Animal Holding Unit, Kent Ridge, for 5 days. Animals were caged individually. Room was kept at 22-26°C, humidity at 40-70% with 12 hour light and 12 hour dark cycle. They were fed a conventional laboratory diet with unlimited supply of drinking water.
2. After the initial Sighting test using 1 female rat at 2ml/kg rat on 25 Jan 2006, 5 female rats were randomly chosen and allocated as treatment group and the other 5 female rats were to act as control group.
3. Each rat was anesthetized with an approximate dose of 0.2ml per 100 g rat of a cocktail (20 ml working solution) containing 4.8 ml ketamine (2mg/ml), 3.2 ml medetomidine (1mg/ml) and 12ml sterile water.
4. When the rat was sedated, its weight was recorded, hair on its back was shaved to expose an area of 4 x 5 cm skin for dermal application of the test substance.
5. Five female rats were treated with **Biovectrol 20EM** at a dose of **2ml/kg rat** being applied on to exposed skin. The other 5 female rats were used as Control and dosed with water, at 2ml/kg rat. A specially constructed Elizabethan collar was used to prevent rats from ingesting the test substance.
6. All animals recovered from anesthesia in about an hour and were monitored. At the end of 24 h exposure period, the collar was detached and the test substance was washed off with water. The rats were observed at least once daily during working days until day 15. Body weights, feed and water consumption were also monitored every 2-4 days.

7. At day 15, body weights were recorded and the rats euthanised. Post mortem was performed and gross pathology observations made.

Results - for details, please refer to tables below.

Mortality: None of the rats died over the testing period

Observations: Dermal application of **Biovectrol 20EM** did not seem to affect the rats much after they recovered from anesthesia. No other untoward clinical signs were observed during the rest of the test period.

Raw Data: Weight gain and food and water consumption after dosing of **Biovectrol 20EM** were comparable between the test group and the control.

Necropsy: All rats survived to Day 15. Euthanasia was carried out by overdose of CO_2 inhalation. No abnormality was observed during gross necropsy of all rats.

Conclusion

The Acute Dermal LD_{50} for **Biovectrol 20EM** in rats cannot be determined accurately from this method but is considered to be much greater than 2 ml/kg body weight (approximately equivalent to 2000 mg/kg body weight).

Biovectrol 20EM *is considered to have acute dermal toxicity of the least hazard category,* ***category 5/Unclassified*** *in the Globally Harmonised System (GHS) classification of chemicals to cause acute toxicity.*

"TESTING AT DOSES ABOVE 2000 MG/KG
2. Exceptionally, and only when justified by specific regulatory needs, the use of an additional upper fixed dose level of 5000 mg/kg may be considered. Recognising the need to protect animal welfare, testing in animals in Category 5 ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test would have a direct relevance for protecting human health." (OECD New Draft Guideline 434, 14 May 2004 (1st version) Annex 4, page 13)

Acute dermal toxicity tests on animal species do not provide information on chronic toxicity or other adverse effects of the chemical in other species and human.

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Date April 3rd 2006

Acute Dermal Toxicity Test of Biovetrol 20EM

Summary

Dosage: 2 ml/kg
Date: Start 6/2/2006; End 20/2/2006
Route of administration: Dermal
Animals used: Female SD rats (8-12 weeks)
Operators: JL, LEY, TYQ

Rats	Body Weight (g)	Dose (ml)	Weight on 15 day (g)	Observations	
				1st Day	2-15th day
Control (water)					
C1	169	0.34	192	Sedated but	All normal
C2	185	0.37	211	awake after	
C3	202	0.40	242	about an hour	
C4	171	0.34	205	Normal	
C5	178	0.36	217		
Test (Biovetrol 20EM)					
20EM1	194	0.39	228		
20EM2	177	0.35	210	Sedated but	All normal
20EM3	202	0.40	229	awake after	
20EM4	183	0.37	219	about an hour	
20EM5	181	0.36	210	Normal	
Mean for Control	181	0.36	213		
Mean for 20EM	187	0.37	219		

Records of Body Weights, food intake and water intake in Rats for Day 1 – Day 3 after dermal application of water (C1-C5) and 20EM (20EM1-20EM5)

Rats	day1 (6.02.06)			day 2 (7.02.06)					day 3 (08.02.06)				
	body wt (g)	food (g)	water (g)	body wt (g)	food (g)	food intake(g)	water (g)	water intake(g)	body wt (g)	food (g)	food intake(g)	water (g)	water intake(g)
C1	169	401	670	168	364	37	650	20	169	305	59	632	18
C2	185	403	671	182	389	14	650	21	181	368	21	634	16
C3	202	403	658	203	379	24	625	33	206	359	20	604	21
C4	171	400	676	164	389	11	635	41	172	360	29	603	32
C5	178	402	674	184	383	19	592	82	185	361	22	567	25
20EM1	194	401	685	191	383	18	658	27	200	367	16	630	28
20EM2	177	400	656	177	383	17	627	29	179	373	10	596	31
20EM3	202	402	666	197	389	13	646	20	201	370	19	620	26
20EM4	183	404	644	180	393	11	617	27	185	377	16	590	27
20EM5	181	400	667	190	387	13	638	29	193	371	16	610	28
mean for control	181			180		21		39	183		30		22
mean for 20EM	187			187		14		26	192		15		28

	mean food intake Day 5-15	mean water intake Day 5-15
mean for control	31	29
mean for 20EM	29	32

Records of Body Weights, food intake and water intake in Rats for Day 5 – Day 8 after dermal application of water (C1-C5) and 20EM (20EM1-20EM5)

Rats	day 5 (10.02.2006)							day 8 (13.02.2006)				
	body wt (g)	old food (g)	new food (g)	food intake(g)	old water (g)	new water (g)	water intake(g)	body wt (g)	food (g)	food intake(g)	water (g)	water intake(g)
C1	172	260	653	45	590	676	42	178	636	17	612	64
C2	187	262	611	106	512	679	122	197	541	70	617	62
C3	207	316	612	43	555	680	49	221	488	124	592	88
C4	178	310	610	50	546	692	57	189	533	77	609	83
C5	186	300	660	61	520	642	47	199	585	75	561	81
20EM1	201	316	645	51	576	685	54	208	559	86	610	75
20EM2	179	339	620	34	553	678	43	189	540	80	599	79
20EM3	206	323	630	47	570	697	50	213	539	91	618	79
20EM4	190	337	659	40	536	640	54	206	583	76	545	95
20EM5	189	327	656	44	555	680	55	198	562	94	581	99
mean for control	186			61			63	197		73		76
mean for 20EM	193			43			51	203		85		85

Records of Body Weights, food intake and water intake in Rats for Day 12 – Day 15 after dermal application of water (C1-C5) and 20EM (20EM1-20EM5)

Rats	day 12 (17.02.2006)						day 15 (20.02.2006)				
	body wt (g)	food (g)	food intake(g)	old water (g)	new water (g)	water intake(g)	body wt (g)	food (g)	food intake(g)	water (g)	water intake(g)
C1	190	480	156	528	528	84	192	296	184	463	65
C2	206	448	93	454	454	163	211	383	65	385	69
C3	234	397	91	496	496	96	242	325	72	420	76
C4	198	445	88	573	573	36	205	377	68	433	140
C5	211	476	109	461	461	100	217	382	94	379	82
20EM1	217	464	95	528	528	82	228	387	77	454	74
20EM2	202	454	86	361	692	238	210	378	76	611	81
20EM3	228	431	108	531	531	87	229	356	75	457	74
20EM4	214	484	99	440	440	105	219	405	79	355	85
20EM5	203	421	141	444	444	137	210	322	99	325	119
mean for control	208		107			96	213		97		86
mean for 20EM	213		106			130	219		81		87