

**REPORT ON ACUTE ORAL 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,
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Materials and Methods

Test substance: Biovectrol 20EM, Batch no: BN2005062701.

Manufacture Date: 27/06/2005

Test was conducted from 27 Dec to 11 Jan 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 420, GUIDELINE FOR TESTING OF CHEMICALS - Acute Oral Toxicity – Fixed Dose Procedure. Adopted 17th December 2001.

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

1. Nine 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. Before test substance was orally administered, food was withheld overnight.
2. Initial Sighting test with 1 female rat was carried out on 20 Dec 2006 with a gavage of 2ml/kg rat of Biovectrol 20EM. For this Main study, 6 female rats were randomly chosen and allocated as treatment group. Body weights were recorded just before dosing. They were dosed by gavage with **Biovectrol 20EM** at a dose of **2ml/kg rat**.
3. The other 3 female rats were used as Control and dosed with water, at 2ml/kg rat.
4. All animals were observed over 2 hours in the first instance after dosing, and then every hour for the next 2 hours. Thereafter, observations were made at least once daily during working days until day 15/16. Body weights, feed and water consumption were also monitored.
5. At day 15/16, 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 during the test period

Observations: After oral administration of **Biovectrol 20EM**, rats were slightly subdued and some were gasping for 1-2 hours before they recovered. No other untoward clinical signs were observed during the rest of the test period.

Raw Data: Weight gain and food and water consumption were reduced in the first 3 days after dosing of **Biovectrol 20EM** when compared to control. Final weight gain at Day 16 was comparable between the test group and the control.

Necropsy: All rats survived to Day 16. Euthanasia was carried out by overdose of CO₂ inhalation. No abnormality was observed during gross necropsy of all rats.

Conclusion

The LD₅₀ for **Biovectrol 20EM** in rats cannot be determined accurately from this method but is considered to be much greater than 2 ml/kg (approximately equivalent to 2000 mg/kg) body weight.

Biovectrol 20EM is considered to have acute oral 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 of 5000 mg/kg is discouraged and should only be considered when there is a strong likelihood that the results of such a test would have a direct relevance for protecting animal or human health." (OECD 420 Annex 4, page 13/14)

Acute oral 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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Acute Oral Toxicity Test of Biovetrol 20EM

Summary

Dosage: 2 ml/kg

Date: Start 27/12/2005; End 11/01/2006

Route of administration: Oral gavage

Animals used: Female SD rats (8-12 weeks)

Operators: JL, LEY, TYQ

Rats	Body Weight (g)	Dose (ml)	Weight on 16 day (g)	Observations	
				1st Day	2-16th day
Control (water)					
C1	219	0.44	269	All slightly subdued	All normal
C2	206	0.41	242		
C3	211	0.42	267		
Test (Biovetrol 20EM)					
20EM1	232	0.46	257		
20EM2	222	0.44	253	All subdued	Did not feed well
20EM3	221	0.44	265	and some gasping	for 2 days
20EM4	210	0.42	254	for 1-2 hour, but	but became normal
20EM5	214	0.43	252	recovered later	after that
20EM6	211	0.42	249		
Mean for Control	212	0.42	259		
Mean for 20EM	218	0.44	255		

Acute oral toxicity test: Okada Biovetrol 20EM
Original Report

Records of Body Weights, food intake and water intake in Rats for Day 1 – Day 3 after gavage of water (C1-C3) and 20EM (20EM1-20EM5)

Rats	day1			day 2			day 3				
	body wt (g)	food (g)	water (g)	body wt (g)	food (g)	water (g)	body wt (g)	food Intake (g)	water Intake (g)		
C1	219	501	673	232	470	31	231	448	22	586	46
C2	206	532	599	211	509	23	218	490	19	551	19
C3	211	455	673	232	424	31	230	402	22	605	24
20EM1	232	560	671	236	546	14	224	536	10	644	9
20EM2	222	553	690	221	540	13	203	532	8	671	4
20EM3	221	592	670	232	562	30	235	536	26	602	29
20EM4	210	580	697	218	556	24	219	540	16	635	30
20EM5	214	554	683	218	532	22	220	515	17	608	50
20EM6	211	559	693	205	545	14	206	527	18	651	24
mean for control	212			225			226		21		30
mean for 20EM	218			222			218		16		24

mean for control	mean food intake 5-16day (g)	mean water intake 5-16 days (g)
	22	34
mean for 20EM	23	29

D: Lhs – acute oral toxicity test- Okada Biovetrol 20EM

Records of Body Weights, food intake and water intake in Rats for Day 4 – Day 8 after gavage of water (C1-C3) and 20EM (20EM1-20EM5)

Rats	day 4						day 8					
	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 Intake (g)	water Intake (g)	food Intake (g)	water Intake (g)
C 1	230	426	736	22	547	675	39	241	648	88	648	527
C2	216	471	709	19	529	686	22	260	629	80	629	597
C3	231	378	720	24	571	648	34	248	618	102	618	510
20EM1	228	510	786	26	610	644	34	239	723	63	723	531
20EM2	216	513	744	19	639	683	32	236	671	73	671	543
20EM3	239	519	743	17	570	664	32	253	644	99	644	552
20EM4	225	513	720	27	598	676	37	232	644	76	644	563
20EM5	217	500	733	15	556	680	52	238	626	107	626	518
20EM6	224	508	787	19	616	674	35	232	682	105	682	554
mean for control	226			22			32	250		90		125
mean for 20EM	225			21			37	238		87		127

Records of Body Weights, food intake and water intake in Rats for Day 11 – Day 16 after gavage of water (C1-C3) and 20EM (20EM1-20EM5)

Rats	day 11				day 16						
	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	263	589	59	376	689	151	269	465	124	426	263
C2	236	589	40	534	668	63	242	469	120	570	98
C3	262	563	55	418	695	92	267	445	118	529	166
20EM1	244	681	42	457	676	74	257	549	132	539	137
20EM2	237	624	47	453	681	90	253	500	124	530	151
20EM3	252	599	45	478	642	74	265	450	149	515	127
20EM4	240	593	51	489	689	74	254	437	156	527	162
20EM5	257	580	46	427	672	91	252	433	147	532	140
20EM6	236	631	51	479	692	75	249	480	151	545	147
mean for control	254		51			102	259		121		176
mean for 20EM	244		47			80	255		143		144