

**CERTIFICATE OF ANALYSIS**

TIMCARE BIOTECH SDN BHD  
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Certificate No : CN/nOTS0070/0116  
Sample Log Code : nOTS0037/0116  
Sample Received Date : 13-Jan-2016  
Complete Analysis Date : 25-Feb-2016  
Date Issue : 25-Feb-2016

Singapore Branch: E-ORGANIC SOLUTIONS PTE. LTD

**Sample description** : TIMCARE GSO DIGESTER - EPPEWOS-04 (120212016)

<u>Probiotic Bacteria</u>	
Pediococcus pentosaceus	3.0 x10 <sup>5</sup> cfu / g
Lactobacillus plantarum	4.0 x10 <sup>5</sup> cfu / g
Acetobacteraceti	7.0 x10 <sup>5</sup> cfu / g
Bacillus amyloliquefaciens	8.0 x10 <sup>7</sup> cfu / g
Pichia farinosa	4.0 x10 <sup>5</sup> cfu / g
<u>Main Enzymes</u>	g (µmole/g x min)
Amylase	217.11
Protease	2.50
Lipase	3.50
Pectinase	2.50
Cellulase	14.10
Xylanase	8.10
β-glucanase	70.20

- Dosing was 10 mL/kg for oral administered

**Method used:** OECD Guideline for Testing Chemicals, Guideline 420 (2001)  
Acute oral toxicity – Fixed Dose Procedure

**Test procedures:**

Female mice (nulliparous and non-pregnant) were used for oral toxicity study. In the sighting study, a female mouse was fasted overnight and administered with a single oral dose of Eppwos 300 mg/kg. The animal was observed closely for any symptoms of toxic effects for the first 4 hours. The onset and duration of the toxic symptoms observed are recorded. The animal was monitored for 24 hours. Since the first animal survived and did not show any sign of toxic symptom till 48 hours, a second female mouse was administered with a higher dose at 2000 mg/kg and observed as before. Since the second animal also survived for more than 48 hours, the main study is carried out. The third, fourth, fifth and sixth female mice were administered with 2000 mg/kg dose of Eppewos and observed as before. The animal that survived the dosing is continued to be observed at least once a day for 14 days before being sacrificed for necropsies.



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**Results:**

Table 1 shows the animal's body weight changes over the two weeks observation period. The starting dose chosen for this test was 300 mg/kg based on recommendation by OECD Guideline 420 (whereby there is no prior toxicity information available on the test chemical). The first female mouse given Eppewos at 300 mg/kg survived after 48 hours observation period with no signs of toxicity. Thus, the next dose tested according to the guideline was 2000 mg/kg. The mouse given this dose also survived with no sign of toxicity after 48 hours observation period. Based on these results, subsequently 4 mice were dosed at 2000 mg/kg in the main test and observed for 14 days. None of the animals showed any signs of toxicity as shown in Table 2. All the animals showed weight gain of between 0.4 – 1.3 g weekly over the two weeks observation period. No death was recorded in this study. Necropsy done at the end of the study found no changes or abnormalities in gross organs observations. All the major organs (liver, heart, lung and kidneys) appeared normal.

Table 1: The effect of oral administration of different doses of Eppewos on female mice body weight, toxic symptoms and internal organs.

Rat No.	Dose (mg/kg)	Weight of rat (g)				Onset of Death (h)	Toxic Symptoms	Internal organs
		week 0	week 1	week 2	at death			
1	300	20.5	21.7	23.0	-	-	Normal	Normal
2	2000	24.0	24.7	25.9	-	-	Normal	Normal
3	2000	22.4	22.9	23.9	-	-	Normal	Normal
4	2000	23.5	23.9	24.9	-	-	Normal	Normal
5	2000	22.3	23.2	23.9	-	-	Normal	Normal
6	2000	18.2	19.0	19.4	-	-	Normal	Normal

Table 2: The cageside observation of oral administration of Eppewos on female mice.

Test seq.	Animal ID	Dose (mg/kg)	Days																		
			0	1	2	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
			30 mins	1 hr	2 hr	4 hr															
1	1	300	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	2	2000	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	3	2000	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	4	2000	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	5	2000	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	6	2000	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N = Normal

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**Discussion:**

The oral LD50 of Eppewos was found to be more than 2000 mg/kg in the female mice. No abnormal cageside observation was found in orally-treated animals. The body weight of the treated animals which survived the 2 weeks study period increased progressively suggesting that Eppewos does not influence the food intake of the animals. Necropsy findings showed no changes or abnormalities in all the major organs.

**Conclusion:**

Acute oral LD50 of Eppewos in the female mice is greater than 2000 mg/kg.



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