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TEST REPORT

STUDY REPORT NUMBER: MB-**AOT**-33-08

Study Completion Date: 14 JAN 2009

Title: ACUTE ORAL TOXICITY STUDY

Natural Sago Hydrogel

Study Sponsor	**Testing Facility**
Rumbia Bio-Tech Sdn Bhd Blok 43 Alurtron MOSTI Agensi Nuklear Malaysia Jalan Dengkil, Bangi 43000 Kajang SELANGOR.	**Makmal Bioserasi** Universiti Kebangsaan Malaysia, 43600 UKM Bangi, SELANGOR.

Study Director : Assoc. Prof Dr. Md Anuar Osman

Quality Assurance Personnel : Nurdiana Ishak

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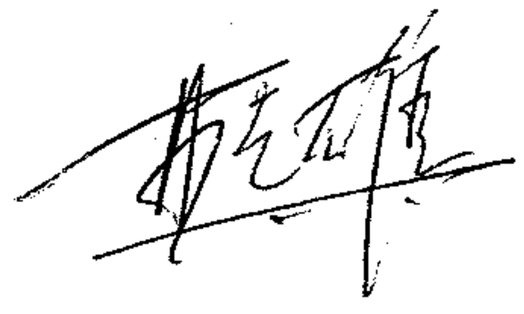
ACUTE ORAL TOXICITY STUDY (LIMIT TEST)

Study Director	**Signature**
Assoc. Prof. Dr. Md. Anuar Osman *DVM (Pak), M. Sc (W. Aust), Ph.D (Murdoch)*	
	Date: 14/1/09
Sponsor	**Signature**
Rumbia Bio-Tech Sdn Bhd	
	Date: 22/3/09

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TEST REPORT - Acute Oral Toxicity Study (Limit Test)

SUMMARY

Acute Oral Toxicity Study of Natural Sago Hydrogel (LIMIT TEST)

Protocol reference : Protocol AL - AOT

Test method used : OECD Guideline 401

Study completion date : 14/1/09

Study reference number : AOT-20-36-08

Study report number : MB-AOT-33-08

Job number : 33-08-RBT

Test material : Natural Sago Hydrogel

Conditions of use : Neat

1. OBJECTIVE

To evaluate the toxic potential of a test material by determining the short term adverse effects following an oral administration in rats.

2. EXPERIMENTAL PROCEDURE

Animals: Fifteen Sprague Dawley albino rats; ten for test group (5 males, 5 females) and five for negative control (3 males, 2 females).

Weight: Test group : 92.40 – 104.00 g
Control group : 85.60 – 105.50 g

Acclimatization period	8 August – 15 August 2008
Date of treatment	15 August 2008
Observation period	15 August – 29 August 2008
End of test	29 August 2008

Treatment

Following a 7-day acclimatization period in the laboratory, two groups of animals were treated with a single oral dose of 2000 mg/kg of the test material fed through gastric intubation using a plastic gavage tube attached to a 3ml syringe.

3. OBSERVATIONS

The rats were observed for mortality, signs of gross toxicity, respiratory effects, tremors, convulsion, diarrhea and other abnormal behavioral effects such as walking backwards.

Clinical Observation

Animals were observed for morbidity and mortality at 1 hour and 3 hours after dosing on the first day and once daily thereafter for 14 days.

Body weight

Body weights were obtained and recorded prior to initiation, once weekly and at termination (day 14).

Upon termination, all rats were euthanized with diethyl ether. The major organ systems of the cranial, thoracic and abdominal cavities were grossly examined.

Gross Necropsy

All animals in the study were subjected to a full, detailed gross necropsy which included careful examination of the external surfaces of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The brain, spleen, liver, heart, pancreas, lungs, kidneys and stomach were appropriately trimmed of any adherent tissue and individually weighed.

4. RESULT

Based on the observations and raw data generated there was no death or remarkable loss of body weight and no adverse reaction in the group of animals treated with 2000 mg/kg body weight of test material. Gross necropsy of the brain, spleen, liver, heart, pancreas, lungs, kidneys and stomach showed no abnormal. The single acute median lethal dose of Natural Sago Hydrogel was greater than 2000 mg/kg of body weight when administrated once orally via gastric intubation to male and female albino rats.

5. CONCLUSION

The single acute median lethal dose of **Natural Sago Hydrogel** was greater than 2000 mg/kg of body weight when administrated once orally. The test material is therefore considered 'non-toxic' under the condition of this study.

TEST REPORT

1.0 SPONSOR OF TEST MATERIAL

1.1	**Name**	: Rumbia Bio-Tech Sdn Bhd
1.2	**Address**	: Blok 43 Alurtron MOSTI Agensi Nuklear Malaysia Jalan Dengkil, Bangi 43000 Kajang SELANGOR.
1.3	**Study report number**	: MB-AOT-33-08
1.4	**Job number**	: 33-08-RBT

2.0 DETAILS OF TEST MATERIAL

2.1	**Name**	: Natural Sago Hydrogel
2.2	**Intended use of test material**	: N/A
2.3	**Test material reference**	: HS-33-RBT0708
2.4	**Study reference number**	: AOT-20-36-08
2.5	**Lot number**	: N/A
2.6	**Date received**	: 15 July 2008
2.7	**Expiry date**	: 2009
2.8	**Appearance**	: Solid (sheet form)
2.9	**Colour**	: Translucent
2.10	**Quantity**	: 13 packets
2.11	**Storage**	: Room temperature
2.12	**Solubility**	: N/A
2.13	**Condition of use**	: Neat
2.14	**Dates of study**	: 15 August – 29 August 2008
2.15	**End of test**	: 29 August 2008
2.16	**pH**	: N/A

3.0 LABORATORY FACILITY

3.1	**Name**	: Makmal Bioserasi
3.2	**Address**	: Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, SELANGOR.

3.3 Project staff

3.3.1 Study Director
Assoc. Prof. Dr. Md. Anuar Osman
DVM (Pak), M. Sc (W. Aust), PhD (Murdoch)

3.3.2 Study Personnel
Mohd Hisyam Syabril Yaacob
Mimi Norhilda Mohd Hatta
Nurhardyawati Ismail

3.3.3 Quality Assurance Personnel
Nurdiana Ishak

3.4 Address of correspondence

MEDIAMAL TESTING & CONSULTANCY SERVICES (MTES)
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Tel/Fax: 03-91737022
Tel: 03-91726629

c/o
Fadhilah Manap

3.5 Study Timetable

3.5.1	Acclimatization period	8 August – 15 August 2008
	Date of treatment	15 August 2008
	Observation period	15 August – 29 August 2008
3.5.2	End of test	29 August 2008

3.6 Environment and Husbandry

3.6.1 Species and strain
Sprague Dawley albino rats.

3.6.2 Supplier
Animal Breeding Facility of the Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor.

3.6.3 Number of animal and sex
Fifteen Sprague Dawley albino rats; ten for test group (5 males, 5 females) and five for negative control (3 males, 2 females).

3.6.4 Body weight at initiation of treatment

Weight: Test group	: 92.40 – 104.00 g
Control group	: 85.60 – 105.50 g

3.6.5 Housing
Animals were housed in a plastic caging with saw dust bedding.
Size of cage: Plastic cage internal dimension: 22cm by 15cm by 37cm. Two animals were placed in one cage.

3.6.5.1 Animal Room Temperature
23°C to 25°C

3.6.5.2 Photoperiod
12 hour light/dark cycle.

3.6.5.3 Acclimatization period
Seven days.

3.6.6 Diet
Mouse pellet: Barastoc Animal Feed

3.6.7 Water
Pre-filtered tap water *ad libitum* continuously supplied in water dispensing bottles.

3.7 Pre-Treatment Procedures

3.7.1 Check for ill health
On arrival and just before the beginning of treatment to ensure only healthy animals were used in the study.

3.7.2 Body weight
All animals were weighed on the day just before treatment, once weekly and at termination of study (day 14).

3.7.3 Acclimatization period
At least 5 days between arrival of animals and start of treatment.

3.7.4 Selection and allocation of animal
Rats were selected randomly at the start of the acclimatization period.

3.7.5 Identification

Animals	: Individually numbered (permanent marker pen) on the tail.
Cages	: Labeled with animal number, sex of animal and study reference number.
Animal	: Each rat was marked with a color code (permanent marker pen) on the tail and given a sequential animal number assigned to study reference number, which constitutes a unique identification system.

4.0 TEST METHOD

4.1 Name of test: Acute Oral Toxicity Study of Natural Sago Hydrogel (Limit Test)

4.2 Objective
To evaluate the toxic potential of a test material by determining the short term adverse effects following an oral administration in rats.

4.3 Significance and Use
In the assessment and evaluation of the toxic characteristics of a chemical, the determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from a short-term exposure by the oral route. Data from an acute study serve as a basis for classification and labeling. It is an initial step in establishing a dosage regimen in subchronic and other studies and could provide initial information on the mode of toxic action of a substance.

4.4 Material

4.4.1 Test system
Fifteen Sprague Dawley albino rats; Ten for test group (5 males, 5 females) and five for negative control (3 males, 2 females).

4.4.2 Miscellaneous
A 3ml syringe and a plastic tube attached to the syringe.

4.5 Preparation of Test Material

4.5.1 Test material for Oral Administration
An appropriate amount of test material was suspended in normal saline and used as an oral suspension.

4.5.2 Route and Rationale of Test material Administration
The route of test material administration was by oral gastric intubation. This is the route of exposure in human and is the accepted technique for assessment of acute oral toxicity.

4.5.3 Method of Test material Administration
Individual doses of test material were calculated based on body weights obtained just prior to dosing.
The test material was administered orally via gastric intubation by using a plastic gavage tube attached to an appropriate syringe.

4.5.4 Dose Level/Group/Treatment Regimen
Two groups of 5 rats were administered with a single dose of 2000 mg/kg test material per animal.

4.6 Description of Test Procedure

4.6.1 Preparation and Selection of Animals

4.6.1.1 On the day before initiation, animals were weighed and the skin, fur and eyes were examined for any abnormalities. The animals were fasted for at least 4 hours by removing feed from their cages while continuing with non-stop water supply.

4.6.1.2 Treatment and Observation Period
Each animal was administered with 2000 mg/kg of test material using a plastic gavage. After administration, the animals were returned to their cage and feeding was resumed after 3 hours of dosing.

Rats were observed for mortality, signs of gross toxicity and behavioral changes. Changes on the fur, and eyes were observed. Respiratory effects, tremors, convulsion, diarrhea and other effects such as walking backwards were also observed for.

Clinical Observation
Animals were observed for morbidity and mortality at 1 hour and 3 hours post dosing on the first day, and once daily thereafter for 14 days.

Body weight
Body weights were obtained and recorded once prior to initiation of test, once during observation and on the fourteenth day prior to necropsy.

Gross Necropsy
All animals in the study were subjected to a full, detailed gross necropsy which includes careful examination of the external surfaces of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The brain, spleen, liver, heart, pancreas, lungs, kidneys and stomach were trimmed of any adherent tissue, as appropriate and individually weighed.

4.7 Scoring Method

Acute Oral Toxicity Study of Natural Sago Hydrogel in rats.

Table 1a: The observed features are presented as below:

Fur Changes	A
Eyes Changes	B
Respiratory Effect - Increased respiratory depth-slow, labored respiration - Decreased respiratory depth-slow, rapid respiration	C a b
Motor activity - Increased motor activity- speed of movement increased - Decreased motor activity- lethargy, does not respond to external stimuli	D a b
Convulsion - Tonic convulsion-sustained spasm with head arched backward - Clonic convulsion- short choppy spasm with head arched toward stomach - Mixed convulsions-combination of clonic and tonic	E a b c
Walking backwards and/or Ataxia-inability to coordinate bodily movement (gross wobbling)	F
Diarrhea	G
Death-self-explanatory	H

Table 1b: Severity Score of Observed Changes

Changes	**Score**
Normal/No changes	0
Mild	1
Moderate	2
Severe	3

4.8 Interpretation of Results

The rats were visually assessed for signs of toxicity within 1 hour, 3 hours after feeding and once daily thereafter for 14 days. Any sign of abnormalities were recorded and tabulated. The single acute median lethal dose would be greater than 2000 mg/kg if less than 50% of death occured in the test group of animals.

5.0 NUMBER OF TEST MATERIAL REPLICATE

Ten rats.

6.0 CONTROL

Five rats as negative control.

7.0 QUALITY ASSURANCE

The final report was audited in agreement with the raw data records and for compliance with the protocol and Standard Operating Procedures of Makmal Bioserasi. Dates of inspections and audits performed during the study and the dates of reporting of the inspection and audit findings to the Study Directors are presented in the Quality Assurance Statement.

8.0 DEVIATIONS FROM APPROVED PROTOCOL

None.

9.0 RECORDS TO BE MAINTAINED

A copy of this signed report, together with the protocol and all raw data generated during the study are retained in Makmal Bioserasi archive.

10.0 DATES OF TEST PROCEDURES

Acclimatization period	8 August – 15 August 2008
Date of treatment	15 August 2008
Observation period	15 August – 29 August 2008
End of test	29 August 2008

11.0 SUMMARY OF RESULTS

11.1 Treatment

Test material : Natural Sago Hydrogel
Study reference number : AOT-20-36-08

11.2 Morbidity / Mortality

Animals were examined daily. These observations were recorded in the study raw data documentation and reported.

11.3 Clinical Observation (Table 2)

All animals appeared normal throughout the study.

Table 2: Observed Changes during Treatment and Observation Period.

Group	Animal		Day 1		Observation (Day)												
	Number	Sex	1 hr	3 hrs	2	3	4	5	6	7	8	9	10	11	12	13	14
			A-H	A-H	A-H	A-H	A-H	A-H	A-H	A-H	A-H	A-H	A-H	A-H	A-H	A-H	A-H
1	rt341	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rt342	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rt343	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rt344	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rt345	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	rt346	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rt347	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rt348	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rt349	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rt350	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	rc86	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rc87	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rc88	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rc89	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	rc90	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

M : Male F : Female

Refer Table 1a and Table 1b for observation features.

11.4 Body Weight

Individual body weight of animals were recorded prior to initiation, once weekly and again on the day after completion. There was no loss in body weight of animals in treatment group at the end of the test period.

Table 3: Individual Body Weight and Doses - Group Treated with Natural Sago Hydrogel (Group 1 and Group 2) and Group Treated with normal saline (Control).

Group	Animal number	Test animal sequences	Body weight (g) Initial Day 0	Body weight (g) Initial Day 7	Body weight (g) End Day 14	Doses (ml)
1 (Male)	rt341	35-1	104.0	154.1	199.0	2.08
	rt342	35-2	103.8	171.3	235.7	2.08
	rt343	35-3	101.4	156.1	165.0	2.03
	rt344	35-4	98.3	147.8	152.2	1.97
	rt345	35-5	100.4	156.9	196.3	2.01
2 (Female)	rt346	35-6	101.3	144.2	163.2	2.03
	rt347	35-7	94.7	127.5	148.1	1.89
	rt348	35-8	95.6	129.3	111.6	1.91
	rt349	35-9	92.4	133.6	115.6	1.85
	rt350	35-10	99.5	137.2	165.5	1.99
Mean			**99.14**	**145.80**	**165.22**	
Control	rc86	1	105.5	168.0	212.0	2.11
	rc87	2	96.8	141.5	163.0	1.94
	rc88	3	85.6	132.9	183.0	1.71
	rc89	4	101.0	141.8	161.6	2.02
	rc90	5	95.6	136.4	161.4	1.91
Mean			**96.90**	**144.12**	**176.20**	

11.5 Gross Necropsy

11.5.1 The brain, spleen, liver, heart, pancreas, lungs, kidneys and stomach appeared normal in gross necropsy.

11.5.2 Organ Weight Data

Table 4a: Weight of organ (gram) and percentage weight of organ upon necropsy - Test animal

Group	Animal number	Sex	Weight (g) and Percentage Weight of Organ (%)															
			Brain		Spleen		Liver		Heart		Pancreas		Lungs		Kidneys		Stomach	
1	rt341	M	1.5	0.75	0.4	0.20	9.0	4.52	0.7	0.35	0.5	0.25	1.1	0.55	1.9	0.95	1.4	0.70
	rt342	M	1.5	0.64	0.7	0.30	11.7	4.96	0.8	0.34	0.6	0.25	1.3	0.55	2.1	0.89	1.5	0.64
	rt343	M	1.7	1.03	0.6	0.36	8.8	5.33	0.7	0.42	0.6	0.36	1.0	0.61	1.9	1.15	1.4	0.85
	rt344	M	1.5	0.99	0.6	0.39	9.5	6.24	0.7	0.46	0.5	0.33	1.0	0.66	1.6	1.05	1.3	0.85
	rt345	M	1.5	0.76	0.5	0.25	8.8	4.48	0.7	0.36	0.7	0.36	1.4	0.71	1.8	0.92	1.6	0.82
2	rt346	F	1.6	0.98	0.4	0.25	8.8	5.39	0.7	0.43	0.6	0.37	1.1	0.67	1.5	0.92	1.3	0.80
	rt347	F	1.3	0.88	0.4	0.27	7.0	4.73	0.6	0.41	0.5	0.34	1.1	0.74	1.8	1.22	1.3	0.88
	rt348	F	1.5	1.34	0.3	0.27	5.2	4.66	0.4	0.36	0.5	0.45	0.9	0.81	1.4	1.25	0.9	0.81
	rt349	F	1.7	1.47	0.3	0.26	6.4	5.54	0.6	0.52	0.4	0.35	0.9	0.78	1.5	1.30	1.0	0.87
	rt360	F	1.5	0.91	0.4	0.24	8.6	5.20	0.5	0.30	0.6	0.36	1.0	0.60	1.8	1.09	1.4	0.85
Mean			**1.53**	**0.98**	**0.46**	**0.28**	**8.38**	**5.11**	**0.64**	**0.40**	**0.55**	**0.34**	**1.08**	**0.67**	**1.73**	**1.07**	**1.31**	**0.81**

Table 4b: Weight of organ (gram) and percentage weight of organ upon necropsy - Control animal

Group	Animal number	Sex	Weight (g) and Percentage Weight of Organ (%)															
			Brain		Spleen		Liver		Heart		Pancreas		Lungs		Kidneys		Stomach	
Control	rc86	M	1.7	0.80	0.6	0.28	11.3	5.33	0.8	0.38	0.6	0.28	1.2	0.57	2.4	1.13	1.5	0.71
	rc87	M	1.7	1.04	0.5	0.31	9.1	5.58	0.6	0.37	0.6	0.37	1.1	0.67	1.6	0.98	1.1	0.67
	rc88	M	1.5	0.82	0.7	0.38	9.3	5.08	0.7	0.38	0.6	0.33	1.3	0.71	2.0	1.09	1.5	0.82
	rc89	F	1.6	0.99	0.3	0.19	9.5	5.88	0.7	0.43	0.7	0.43	1.0	0.62	1.6	0.99	1.5	0.93
	rc90	F	1.6	0.99	0.3	0.19	8.6	5.33	0.7	0.43	0.5	0.31	1.0	0.62	1.4	0.87	1.2	0.74
Mean			**1.62**	**0.93**	**0.48**	**0.27**	**9.56**	**5.44**	**0.70**	**0.40**	**0.60**	**0.34**	**1.12**	**0.64**	**1.80**	**1.01**	**1.36**	**0.77**

M : Male F : Female

12.0 INTERPRETATION OF RESULTS

Based on the observations and raw data generated there was no death or remarkable loss of body weight and no adverse reaction in the group of animals treated with 2000 mg/kg body weight of test material. Gross necropsy of the brain, spleen, liver, heart, pancreas, lungs, kidneys and stomach showed no abnormal. The single acute median lethal dose of Natural Sago Hydrogel was greater than 2000 mg/kg of body weight when administrated once orally via gastric intubation to male and female albino rats.

13.0 CONCLUSION

The single acute median lethal dose of **Natural Sago Hydrogel** was greater than 2000 mg/kg of body weight when administrated once orally. The test material is therefore considered 'non-toxic' under the condition of this study.

14.0 REFERENCE

OECD Guideline for Testing of Chemicals, Acute Oral Toxicity. 401.1987.

15.0 VERIFICATION

I the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

.....

Assoc. Prof. Dr. Md. Anuar Osman
Study Director

14/1/09
.....
Date

16.0 QUALITY ASSURANCE INSPECTIONS STATEMENT

The Quality Assurance Unit randomly selects intervals for QA inspections prior to study initiation. Records of the findings of these inspections are kept in file. The summary below provides verification of statements made in the final report section that addresses QA audits.

Inspections were made of:

DATE	PROCEDURE INSPECTED
13/1/09	Raw data
-	Draft report
13-14/1/09	Final report

Findings reported to:

Study Director 14/1/09

.....

Nurdiana Ishak
Quality Assurance Personnel