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TEST REPORT

EVALUATION OF NANO COLLOIDAL ARGENTUM IN THE ACUTE ORAL TOXICITY STUDY ON RATS (UP-AND-DOWN-PROCEDURE [UDP] – LIMIT TEST)

Job No. J209/13

Report No. R209/13/B19/27

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Study Initiation Date:
12 August 2013

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19 August 2013

Experimental End Date:
07 September 2013

Study Completion Date:
12 September 2013

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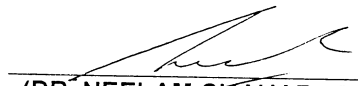
APPROVAL SIGNATURES

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



12 SEP 2013

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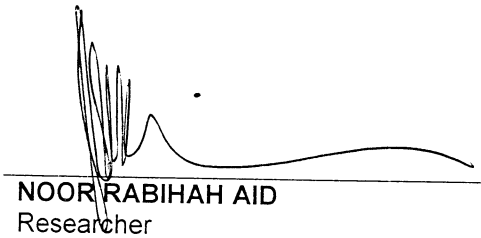

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KEY PERSONNEL PARTICIPATING IN THIS STUDY

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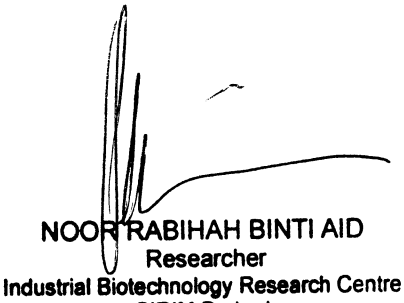

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SUMMARY

EVALUATION OF NANO COLLOIDAL ARGENTUM IN THE ACUTE ORAL TOXICITY STUDY ON RATS (UP-AND-DOWN-PROCEDURE [UDP] – LIMIT TEST)

An acute oral toxicity study was conducted on rats to determine the toxicity potential of Nano Colloidal Argentum from a single dose via the oral route. The acute oral toxicity Up-and-Down Procedure (UDP) – Limit Test as described in the OECD Guidelines for Testing of Chemicals No 425 – is a sequential test which uses a maximum of five animals. Animals are dosed in sequential manner with the next animal receiving the same dose only if the first animal survives the limit dose. A starting dose of 2000 mg/kg was selected based on recommendation from the BALB/c 3T3 NRU cytotoxicity test (Report No R205/13/B19/03).

The Nano Colloidal Argentum was freshly prepared prior to dosing. The test item was mixed in distilled water and subsequently vortexed for 5 minutes.

An acute oral toxicity was initially conducted on one female rat. The first animal received a single dose administration of Nano Colloidal Argentum at 2000 mg/kg body weight. This animal survived a 48-hour observation. Therefore four additional animals were sequentially dosed at approximately 24-hour intervals. A total of five animals were tested.

All animals were observed individually for mortality, signs of gross toxicity and behavioural changes once during the first 30 minutes after dosing. Special attention was given during the first 4 hours and periodically during 48 hours post-dosing. Daily observation was carried out for 14 days. Body weights were recorded prior to dosing and again on Day 7 and Day 14 (termination). Necropsies were performed on all animals at terminal sacrifice.

No mortality was observed within the 14 days procedure. All animals gained body weight, appeared normal and did not demonstrate any abnormal behaviour during the observation period.

Under the conditions of this study, the Nano Colloidal Argentum showed a median lethal oral dose (LD50) of greater than 2000 mg/kg body weight. Therefore the Nano Colloidal Argentum is classified as Category 5 according to the Globally Harmonised System for the classification of chemicals.

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1.0 OBJECTIVE

The objective of this study was to provide information on health hazards likely to arise from a short term exposure to Nano Colloidal Argentum by the oral route. A median lethal oral dose or LD50 will be determined as described in the OECD Guidelines for Testing of Chemicals No 425. The LD50 value is a statistically derived single dose of a test that can be expected to cause death in 50 per cent of animals when administered by the oral route, expressed in terms of weight of test item per unit weight of test animal (mg/kg). The results allow ranking and classification according to the Globally Harmonized System for the classification of chemicals which cause acute toxicity.

2.0 STUDY TIMETABLE

Acclimatization: 12 August 2013 – 18 August 2013

Dosing dates: 19 August 2013 - 24 August 2013

Observation: 19 August 2013 – 07 September 2013

Necropsy: 02 September 2013 – 07 September 2013

3.0 MATERIALS

3.1 Test Item

3.1.1 Test item: Nano Colloidal Argentum

3.1.2 Lot/Batch No.: Not Provided

3.1.3 Date received: 21 June 2013

3.1.4 Physical appearance: Liquid

3.1.5 Colour: Clear

3.1.6 Physical Chemical Properties Data: Not provided

3.1.7 Quantity: Approximately 100 mL

3.1.8 pH: Not provided

3.1.9 Storage condition: Refrigerated

3.1.10 Solubility: Water soluble

3.1.11 Stability: Not provided

3.1.12 Expiration date: Not provided

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3.2 **Test System**

3.2.1 Species/Strain: Rat/Sprague Dawley (SD)

3.2.2 Number of animals: 5

3.2.3 Sex: Female (all animals were nulliparous and non-pregnant)

3.2.4 Age at Study Start: Young adult (8 weeks)

3.2.5 Body weight: 209 -226 g at experiment start

3.2.6 Source: A Sapphire Enterprise, 45 Jalan Indah 1/22, Taman Universiti Indah, 43300 Seri Kembangan, Selangor Darul Ehsan.

4.0 METHOD

4.1 **Husbandry**

4.1.1 Housing: The animals were individually housed in suspended polycarbonate cages on metal racks. Saw dust was placed beneath each cage and was changed twice a week.

4.1.2 Animal room temperature: 19-23 °C

4.1.3 Photoperiod: 12 hour light/dark cycle

4.1.4 Food: Gold Chain mouse pellet

4.1.5 Water: Filtered tap water was supplied *ad libitum* through a 250 mL water dispenser bottle. The water was changed twice a week.

4.2 **Identification**

4.2.1 Cage: Each cage was identified with a card cage displaying study title, strain, age, group, cage number and treatment period.

4.2.2 Animal: Each animal was identified prior to dosing by a label on its tail using a permanent marker. The label stayed with the animal throughout the study.

4.3 **Initial Consideration**

The test item has been classified as non-corrosive (Report No R208/13/B19/11) according to dermal irritation test (Report No R208/13/B19/05) which was classified as non-irritant/irritant. Therefore, oral administration is unlikely to cause marked pain and distress to the animals.

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5.0 PROCEDURE

5.1 Preparation of Animals

5.1.1 Acclimatization: Each animal was individually caged for a minimum of 5 days to allow for acclimatization. On the day of and prior to dosing, each animal was examined to be in good health condition.

5.1.2 Fasting of animals: Each animal was fasted overnight by withholding food but not water, prior to dosing. Food was returned to the animals approximately 3 hours after dosing.

5.1.3 Body weights: Each animal was individually weighed on Day 0, just before administration of the test item (initial).

5.2 Preparation of Test Item

5.2.1 Preparation of test item: The test item was freshly prepared prior to dosing. Approximately 1.0 g of the test item was weighed and transferred into a universal bottle. Distilled water measuring approximately 5 mL was added into the universal bottle to give a final concentration of approximately 0.2 g/mL. The bottle was then vortexed for 5 minutes.

5.3 Oral Administration

5.3.1 Dose level: Each animal was individually dosed with the test item at 2000 mg/kg body weight. The starting dose was selected based on recommendation from the BALB/c 3T3 NRU cytotoxicity test (Report No R205/13/B19/03).

5.3.2 Dose volume: 1 mL per 100 g body weight. Dosage was calculated based on the weight of the animal on the day of dosing.

5.3.3 Oral administration: Oral administration on each rat was conducted using a round-headed stainless steel gavage tube measuring 2 inches long and 18G in diameter, fitted with a syringe.

5.3.4 Limit Test: The first animal was dosed at 2000 mg/kg body weight. This animal survived a 48-hour observation. Therefore four additional animals were sequentially dosed, one at a time, at approximately 24-hour intervals. A total of five animals were tested. This dosing sequence procedure is presented in Table 1.

5.4 Observation

5.4.1 Cage-side observation: The animals were individually observed for mortality and signs of illness, injury or abnormal behaviour, once during the first 30 minutes after dosing, periodically during the first 48 hours (with special attention given during the first 4 hours), and daily thereafter for a total of 14 days.

5.4.2 Body weight: Each animal was individually weighed on Day 7 and on Day 14 (termination) after dosing.

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5.4.3 Pathology: All animals were sacrificed on Day 14 and gross necropsies were performed. Microscopic examination was performed on selected vital organs. The histopathological information may be considered for further toxicity studies.

6.0 RESULT AND DISCUSSION

6.1 Cage-side Observation

Individual cage-side observations are presented in Table 2. All animals survived. Throughout the 14-day observation period, all animals appeared active and healthy.

6.2 Body Weight

Individual body weights are presented in Table 3. All animals gained body weight over the 14-day observation period.

6.3 Pathology

Individual gross necropsy observations are presented in Table 4. At sacrifice times, gross necropsies showed no abnormalities for any of the animals.

7.0 CONCLUSION

Under the conditions of this study, the Nano Colloidal Argentum showed LD50 of greater than 2000 mg/kg body weight. Therefore the Nano Colloidal Argentum is classified as Category 5 according to the Globally Harmonised System for the classification of chemicals

8.0 RETENTION OF RECORDS AND TEST ITEM

Two original signed final reports were prepared. One report will be forwarded to the Sponsor. The other report, together with all generated raw data is maintained at the Industrial Biotechnology Research Centre Archives. IBRC will maintain these records for a period of ten years. After this time, the Sponsor will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by IBRC.

9.0 REFERENCES

9.1 OECD (2008), Acute Oral Toxicity – Up-and-Down Procedure (UDP), OECD Guidelines for Testing of Chemicals No. 425, OECD, Paris.

9.2 Principles and Methods of Toxicology, 5th Ed (2008). Edited by A Wallace Hayes, CRC Press.

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Table 1 Dosing Sequence Procedure

Dosing Sequence	Animal No.	Dosing Date	Short-Term Outcome (48 hour)	Long-Term Outcome (14 Days)
1	R021	19 August 2013	O	O
2	R022	21 August 2013	O	O
3	R023	22 August 2013	O	O
4	R024	23 August 2013	O	O
5	R025	24 August 2013	O	O

O – Survival, X – Death

Table 2 Individual cage-side observation

Dosing Sequence	Animal No.	Findings	Day of Occurrence
1	R021	Active and healthy	Maintained over the 14-day observation period
2	R022		
3	R023		
4	R024		
5	R025		

Table 3 Individual body weights

Dosing Sequence	Animal No.	Body Weight (g)		
		Day 0	Day 7	Day 14
1	R021	209	216	223
2	R022	226	230	239
3	R023	215	222	235
4	R024	213	229	235
5	R025	219	227	233

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Table 4 Individual gross necropsy observations

Dosing Sequence	Animal No.	Necropsy Date	Tissue/Organ	Findings
1	R021	02 September 2013	All tissues/organs	No gross abnormalities
2	R022	04 September 2013	All tissues/organs	No gross abnormalities
3	R023	05 September 2013	All tissues/organs	No gross abnormalities
4	R024	06 September2013	All tissues/organs	No gross abnormalities
5	R025	07 September 2013	All tissues/organs	No gross abnormalities

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