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TEST REPORT

EVALUATION OF QUANTUM ION IN THE ACUTE ORAL TOXICITY STUDY ON RATS (UP-AND-DOWN-PROCEDURE [UDP] – MAIN TEST)

Job No. J734/20

Report No. R734/20/B19/27

Sponsor:
Eva Energy Sdn Bhd
12, Jalan Bandar 20,
Pusat Bandar Puchong,
47160, Puchong,
Selangor Darul Ehsan.

Test Facility:
Industrial Biotechnology Research Centre (IBRC),
Building 19, SIRIM Berhad

Study Initiation Date:
29 June 2020

Experimental Start Date:
30 June 2020

Experimental End Date:
22 July 2020

Study Completion Date:
24 July 2020

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APPROVED SIGNATORIES

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.

(SYAMIMI KHALID)
Reviewer
Industrial Biotechnology Research Centre

26/8/2020
Date

(JUANI MAZMIN HUSIN)
Analyst
Industrial Biotechnology Research Centre

26/8/2020
Date

SUMMARY

EVALUATION OF QUANTUM ION IN THE ACUTE ORAL TOXICITY STUDY ON RATS (UP-AND-DOWN-PROCEDURE [UDP] – MAIN TEST)

An acute oral toxicity study was conducted on rats to determine the toxicity potential of **QUANTUM ION** from a single dose via the oral route. In acute oral toxicity Up-and-Down Procedure (UDP) – Main Test, single animals were dosed in sequence at 48 h intervals. A starting dose of 175 mg/kg was selected based on for this test.

QUANTUM ION was extracted in reverse osmosis water at 37±1°C for 72±2 hours. The test extract was allowed to cool to room temperature before being administered to the rats.

The first animal received a single dose administration of **QUANTUM ION** at 175 mg/kg body weight. This animal survived a 48-hour observation and therefore the second animal received higher dose at 550 mg/kg. This animal survived a 48-hour observation and therefore the next animal received the higher dose at 2000 mg/kg. This animal survived a 48-hour observation and therefore the last two animal received the highest dose at 2000 mg/kg. Based on Acute Oral Toxicity (Guideline 425) Statistical Program" (AOT425StatPgm) with the default sigma of 0.5, the dose level increased from 175mg/kg to 550 mg/kg and to 2000 mg/kg as each level was dosed. 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, **QUANTUM ION** showed a median lethal oral dose (LD50) of greater than 2000 mg/kg body weight. Therefore, **QUANTUM ION** is classified as Category 5 according to the Globally Harmonised System for the classification of chemicals which is for substances which are of relatively low acute toxicity but which under certain circumstances, may pose a hazard to vulnerable populations. The **QUANTUM ION** is under the category of "Not Classified" according to CLASS Regulation 2013 where it does not meet the classification criteria for any hazard class. (Annex 1 and Annex II)

TEST REPORT

CONTENTS

1.0 OBJECTIVE

The objective of this study was to provide information on health hazards likely to arise from a short term exposure of **QUANTUM ION** by the oral route. A median lethal oral dose or LD50 will be determined. 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: 04 June 2020 – 11 June 2020

Dosing dates: 30 June 2020 – 08 July 2020

Observation: 30 June 2020 – 22 July 2020

Necropsy: 14 July 2020 – 22 July 2020

Data Analysis: 22 July 2020 - 24 July 2020

3.0 MATERIALS

3.1 Test Item

3.1.1 Test item: **QUANTUM ION**

3.1.2 Sample Marking.: Copper Ionic

3.1.3 Date received: 29 June 2020

3.1.4 Physical appearance: Liquid

3.1.5 Colour: Clear Blue

3.1.6 Physical Chemical Properties Data: Not provided

3.1.7 Quantity: 1 bottle

3.1.8 pH: Not provided

3.1.9 Storage condition: Room Temperature

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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 (12 weeks)

3.2.5 Body weight: 201 g – 213 g at experiment start

3.2.6 Source: Laboratory Animal and Facility Management, Faculty of Pharmacy, UiTM Puncak Alam Campus, Shah Alam, Selangor.

3.2.7 Animal Ethic Approval No.: SIRIM-IACUC/IBRC/B19-27/0002

4.0 METHOD

4.1 **Husbandry**

4.1.1 Housing: The animals were individually housed in Individual Ventilated Cages (IVC) System. Corn cob bedding was placed beneath each cage and was changed once a week.

4.1.2 Animal room temperature: 19-25 °C

4.1.3 Photoperiod: 12 hour light/dark cycle

4.1.4 Food: Altromin rodent maintenance diet, 10 mm pellets feed for rat.

4.1.5 Water: Reverse osmosis 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.

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 Approximately 175 mg, 550 mg and 2000 mg of the test item was weighed individually and transferred into a universal bottle respectively. Reverse Osmosis water measuring approximately 10 mL was added into the each universal bottle to give a final concentration of approximately 175 mg/mL, 550 mg/mL and 2000 mg/mL respectively.

5.2.2 The test item was extracted in reverse osmosis water at 37±1°C for 72±2 hours. The test extract was allowed to cool to room temperature before being administered to the rats.

5.3 Oral Administration

5.3.1 Dose level: Dosing was initiated using the default dose level 175 mg/kg, sigma of 0.5 and approximately 3.2 multiplier dose progression.

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 Main Test: A single animals were dosed in sequence at 48 h intervals (Table 1). The first animal received a single dose administration of **QUANTUM ION** at 175 mg/kg body weight. This animal survived a 48-hour observation and therefore the second animal received higher dose at 550 mg/kg. This animal also survived a 48-hour observation and therefore the third animal received higher dose at 2000 mg/kg. This animal survived a 48-hour observation and therefore the last two animals received highest dose at 2000 mg/kg. Based on Acute Oral Toxicity (Guideline 425) Statistical Program" (AOT425StatPgm) with the default sigma of 0.5, the dose level increased from 175mg/kg to 550 mg/kg and to 2000 mg/kg as each level was dosed. A total of five animals were tested.

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.

5.4.3 Pathology: All animals were sacrificed on Day 14 and gross necropsies were performed. Macroscopic examination was performed on selected vital organs.

5.5 **Statistical Analysis**

5.5.1 After each level was conducted, the short-term and long-term outcomes were input into the Oral Toxicity (Guideline 425) Statistical Program" (AOT425StatPgm) . When the stopping criteria were engaged, the LD50 and 95% confidence intervals were calculated.

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, **QUANTUM ION** showed a median lethal oral dose (LD50) of greater than 2000 mg/kg body weight. Therefore, **QUANTUM ION** is classified as Category 5 according to the Globally Harmonised System for the classification of chemicals which is for substances which are of relatively low acute toxicity but which under certain circumstances, may pose a hazard to vulnerable populations. The **QUANTUM ION** is under the category of "Not Classified" according to CLASS Regulation 2013 where it does not meet the classification criteria for any hazard class.
(Annex 1 and Annex II)

8.0 RETENTION OF RECORDS AND TEST ITEM

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.

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.

9.3 ISO 10993 Biological evaluation of medical devices Part 12: Sample preparation and reference materials. Fourth edition 2012-07-01

9.4 Guidelines for the Application of Special Management of Scheduled Waste. Appendix II: Properties of wastes which render them hazardous.

9.5 LWI-238-27: Acute Oral Toxicity – Up-and-Down Procedure (UDP)

9.6 Globally Harmonised System of Classification and Labelling of Chemicals. Eighth revised edition. United Nations New York and Ganeva Copyright, 2019. ST/SG/AC.10/30/Rev.8

9.7 Industry Code of Practice on Chemicals Classification and Hazard Communication 2014. Copyright of the Department of Occupational Safety and Health, Malaysia.

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Figure 1 Acute Oral Toxicity (Guideline 425) Statistical Program

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Date/Time: Wednesday, 22 July, 2020, 10:42:23 AM
Data file name: J734.dat
Last modified: 22/7/2020 10:42:23 AM

Test/Substance: Enter test description.
Test type: Main Test
Limit dose (mg/kg): 2000
Assumed LD50 (mg/kg): Default
Assumed sigma (mg/kg): 0.5

Recommended dose progression: 2000, 550, 175, 55, 17.5, 5.5, 1.75

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	R086	175	0	0
2	R087	550	0	0
3	R088	2000	0	0
4	R089	2000	0	0
5	R090	2000	0	0

(X = Died, 0 = Survived)

Dose Recommendation: The main test is complete.

Stopping criteria met: 3 at Limit Dose.

SUMMARY OF LONG-TERM RESULTS:

Dose	0	X	Total
175	1	0	1
550	1	0	1
2000	3	0	3
All Doses	5	0	5

Statistical Estimate based on long term outcomes:

The LD50 is greater than 2000 mg/kg.

Table 1 Dosing Sequence Procedure

Dosing Sequence	Animal No.	Dosing Date	Short-Term Outcome (48 hour)	Long-Term Outcome (14 Days)
1	R086	30 June 2020	O	O
2	R087	02 July 2020	O	O
3	R088	04 July 2020	O	O
4	R089	06 July 2020	O	O
5	R090	08 July 2020	O	O

O – Survival, X – Death

Table 2 Individual cage-side observation

Dosing Sequence	Animal No.	Findings	Day of Occurrence
1	R086	Active and healthy	Maintained over the 14-day observation period
2	R087		
3	R088		
4	R089		
5	R090		

Table 3 Individual body weights

Dosing Sequence	Animal No.	Body Weight (g)		
		Day 0	Day 7	Day 14
1	R086	201	215	227
2	R087	211	228	239
3	R088	205	218	229
4	R089	208	218	229
5	R090	213	229	239

Table 4 Individual gross necropsy observations

Dosing Sequence	Animal No.	Necropsy Date	Tissue/Organ	Findings
1	R086	14 July 2020	All tissues/organs	No gross abnormalities
2	R087	16 July 2020	All tissues/organs	No gross abnormalities
3	R088	18 July 2020	All tissues/organs	No gross abnormalities
4	R089	20 July 2020	All tissues/organs	No gross abnormalities
5	R090	22 July 2020	All tissues/organs	No gross abnormalities

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Annex I Label Elements for Acute Toxicity

	Category 1	Category 2	Category 3	Category 4	Category 5
Symbol	Skull and crossbones	Skull and crossbones	Skull and crossbones	Exclamation mark	*No symbol*
Signal word	Danger	Danger	Danger	Warning	Warning
Hazard statement:					
–Oral	Fatal if swallowed	Fatal if swallowed	Toxic if swallowed	Harmful if swallowed	May be harmful if swallowed

Annex II Classification Results

Phrases used in classification results	Explanation
"Classification not possible"	In case insufficient or no data are available for classifying a substance or mixture after searching information sources described in this ICOP, the substance shall be designated as "Classification not possible".
"Not applicable"	Substances or mixture with physical properties, chemical structure or groups not relevant to the hazard class being considered. For example: • Considering a hazard class of "flammable solids", a substance or mixture whose normal state is a liquid or a gas is designated as "Not applicable". • Considering a hazard class of "organic peroxides", a substance which does not contain –O–O– structure is designated as "Not applicable". • Considering a hazard class of "oxidizing liquids", a substance or mixture which does not contain oxygen, fluorine or chlorine is designated as "Not applicable".
"Not classified"	If a substance or mixture does not meet the classification criteria for any hazard class, then it is "Not classified" for that respective hazard class.