QUALITY TOXICOLOGICAL SERVICES

**OECD GLP CERTIFIED**

# REPORT

## STUDY TITLE

Acute Oral Toxicity Study

of **Edible Oil containing *Vijaya Extract* (10%)**

in Rat

(OECD Guideline No. 423)

Date : 09 February 2022

## SPONSOR

**Savikalpa Sciences Pvt. Ltd.**
A 1/6 Panchsheel Enclave, 2nd Floor
New Delhi- 110017, India

## TEST FACILITY

**INTOX PVT. LTD.**
375, Urawade, Tal. Mulshi, Dist. Pune, Maharashtra, 412 115, INDIA.
Tel. : +91-20-66548700 Fax : +91-20-66548799 Email : info@intoxlab.com
www.intoxlab.com

Report No.: R/20939/AOR/22

Number of Pages : 31

# CONTENTS

# STATEMENT OF GLP COMPLIANCE

The Study No. 20939, entitled 'Acute Oral Toxicity Study of **Edible Oil containing *Vijaya Extract* (10%)** in Rat' (OECD Guideline No. 423) was performed in compliance with the OECD Principles of Good Laboratory Practice (OECD, 1998).

No unforeseen circumstances were observed which might have affected the quality or integrity of the study. This report represents a true and accurate record of the results obtained.

I accept the responsibility for validity of the data, as well as the interpretation, analysis, documentation and reporting of the results.

The report contains 31 pages including contents, tables and certificates.

Mrs. C. C. Magar
**Study Director**

09-02-2022
Date

# STATEMENT BY THE TEST FACILITY MANAGEMENT

The Study No. 20939, entitled 'Acute Oral Toxicity Study of **Edible Oil containing *Vijaya Extract* (10%)** in Rat' (OECD Guideline No. 423) was performed at INTOX PVT. LTD., Pune.

Management of the Test Facility had made available all the resources necessary for conduct of the study in compliance with the OECD Principles of GLP.

The Test Facility Management also hereby approves this report for issue :

Dr. M. P. Pore

09-02-2022

Date

# QUALITY ASSURANCE STATEMENT

The Study No. 20939, entitled 'Acute Oral Toxicity Study of **Edible Oil containing *Vijaya Extrac* (10%)** in Rat' (OECD Guideline No. 423) was subjected to periodic inspections by the Quality Assurance Unit in compliance with the OECD Principles of Good Laboratory Practice (OECD 1998).

Dates of conduct of these audits, the critical phases inspected and the dates on which findings o these audits were reported to the Study Director and the Test Facility Management have beer presented below. No inspection led to findings, which would have impaired this study in any way.

This report has been audited by the Quality Assurance Unit, INTOX PVT. LTD. It is an accurate account of the raw data generated and of the procedures followed.

| Dates of Inspection | Type of Inspection | Phases of the Study Inspected | Reporting Dates to the Study Director | Reporting Dates to the Test Facility Management |
|---|---|---|---|---|
| 10-12-2021 | Study Plan Audit | Draft Study Plan | 10-12-2021 | 10-12-2021 |
| 10-12-2021 | Study Plan Audit | Final Study Plan | 10-12-2021 | 10-12-2021 |
| 17-12-2021 | Study Audit | Animal Weighing, Treatment, (Test item weighing, dose formulation, and exposure of test item), Clinical Observations | 17-12-2021 | 17-12-2021 |
| 21-01-2022 | Report Audit | Draft Report | 21-01-2022 | 21-01-2022 |
| 09-02-2022 | Report Audit | Final Report | 09-02-2022 | 09-02-2022 |

**Mrs. P. A. Bhambure** M.Sc.
Quality Assurance Unit : Pallavi

Date : 09-02-2022

## STUDY INFORMATION

| | | |
|---|---|---|
| **Study No.** | : | **20939** |
| **Report No.** | : | **R/20939/AOR/22** |
| **Study Title** | : | Acute Oral Toxicity Study of **Edible Oil containing *Vijaya Extract* (10%)** in Rat (OECD Guideline No. 423) |
| **Sponsor** | : | **Savikalpa Sciences Pvt. Ltd.** A 1/6 Panchsheel Enclave, 2nd Floor New Delhi- 110017, India |
| **Test Facility** | : | **INTOX PVT. LTD.** 375, Urawade, Tal. Mulshi, Dist. Pune – 412115, Maharashtra, INDIA |

### STUDY SCHEDULE

| | | |
|---|---|---|
| **Study Initiation Date** | : | 10 December 2021 |
| **Experimental Starting Date** | : | 10 December 2021 |
| **Dates of Treatment** | | |
| **Step-1** | : | 17 December 2021 |
| **Step-2** | : | 22 December 2021 |
| **Experimental Completion Date** | : | 05 January 2022 |

| | | |
|---|---|---|
| **Study Director** | : | **Mrs. C. C. Magar** |

## PRINCIPAL PERSONNEL PARTICIPATED IN THE STUDY

| Name and Educational Qualification | Responsibility and Function |
|---|---|
| **Chhaya C. Magar** M.Sc. | **Study Director**<br>Overall responsible for conduct of the study |
| **Pooja K. Ghadage** M.Sc. | **Study Personnel**<br>Assistance in conduct of the study |
| **Akash B. Vaidya** M.V.Sc. | **Study Veterinarian**<br>Veterinary services |
| **Dr. A. R. Chaure** M.V.Sc. | **Study Pathologist**<br>Gross pathology evaluations |

**Address of all those listed above;**
**INTOX PVT. LTD.**
375, Urawade,
Tal. Mulshi,
Dist. Pune – 412 115,
Maharashtra, INDIA

# 1. SUMMARY

Acute oral toxicity study of **Edible Oil containing *Vijaya Extract* (10%)** in Wistar rats was performed as per Organization for Economic Co-operation and Development (OECD) Guidelines for Testing of Chemicals, Section 4, No. 423 - Acute Oral Toxicity - Acute Toxic Class Method, adopted by the council on 17 December, 2001 and Center for drug evaluation and Research (CDER)- Single dose acute toxicity for pharmaceutical, adopted August 1996. The method uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonised System (GHS) for classification of chemicals which cause acute toxicity.

In this study, single oral administration of the test item was made to groups of three male and three female rats in step-wise manner to assess its acute toxicity.

In **step-1** of the study, the undiluted test item was administered to three male and three female rats, at the dose of **2000 mg/kg** body weight. Test item did not induce any clinical signs and mortality in treated rats. The body weight gain of male and female rats was not affected during the observation period. No gross pathological changes were observed in any of the rats, as evident at terminal necropsy.

In **step-2**, three male and three female rats when further tested at the dose of **2000 mg/kg** body weight; test item did not induce any abnormal clinical signs and mortality in treated rats. The body weight gain of male and female rats was not affected during the observation period. No gross pathological changes were observed in any of the rats, as evident at terminal necropsy.

Based on these results and according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) – Ninth Revised Edition (GHS - ST/SG/AC.10/30/Rev.9); United Nations, New York and Geneva, 2021; eISBN 978-92-1-005213-9 and Commission Directive 1272/2008 of 16 December 2008 adapting to technical progress for the 28$^{th}$ time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the Classification, Packaging and Labelling of Dangerous Substances. Official Journal of the European Communities OJL 225 pp. 1-333, the test item, **Edible Oil containing *Vijaya Extract* (10%)** has to be classified in **GHS Category 5 or unclassified** for the obligatory labelling requirement for oral toxicity, the corresponding **$LD_{50}$ value** lying between **2000 < ATE ≤ 5000** or **> 5000 mg/kg** body weight respectively.

# 2. INTRODUCTION

## 2.1 OBJECTIVE

The objective of this acute oral toxicity study in rat was to assess the toxic characteristics of **Edible Oil containing *Vijaya Extract* (10%)** when administered orally by gavage in a single dose.

In the assessment and evaluation of toxic characteristics of a substance determination of acute toxicity by oral route is one of the initial steps. This study would provide information on health hazards likely to arise from acute overdosage in man. Data from the acute study would serve a basis for establishing a dosage regimen for subchronic studies and may provide initial information on the mode of toxic action of the substance.

The results of this study allows ranking of the test item according to the 'Globally Harmonised System' (GHS) for classification of chemicals which cause acute toxicity.

## 2.2 REGULATORY REFERENCES

### 2.2.1 TEST GUIDELINES

The study was conducted in compliance with Study Plan No. **P/20939/AOR/22**, approved by the Sponsor. The Study Plan incorporated the recommendations made in following regulatory guidelines:

1) OECD Guideline No. 423, 'Acute Oral Toxicity – Acute Toxic Class Method'. The Organization for Economic Co-operation and Development (OECD) guidelines for the Testing of Chemicals, adopted by the council on 17 December 2001.

2) Center for drug evaluation and Research (CDER): Single dose acute toxicity for pharmaceutical, adopted August 1996.

### 2.2.2 GOOD LABORATORY PRACTICE

The study was conducted in compliance with the principles of Good Laboratory Practice as set forth in: OECD, 1998; OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, 'OECD Principles on Good Laboratory Practice' ENV/MC/CHEM(98)17 (as revised in 1997).

## 2.3. STANDARD OPERATING PROCEDURES

All the procedures described in this study, were performed in compliance with the 'Standard Operating Procedures' currently in force at INTOX PVT. LTD.

## 2.4. SAFETY PRECAUTIONS

Gloves, goggle and face mask were used in addition to protective body garments and footwear to ensure adequate personal health and safety and to avoid inhalation and skin contact with the test item.

## 2.5. ANIMAL WELFARE

The study was performed as per the Study Plan, approved by Institutional Animal Ethics Committee (Form B) and the relevant certificate of approval has been maintained at the Test Facility. The study was performed under the conditions recommended by the 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Guidelines for Laboratory Animal Facility', published in The Gazette of India, December 15, 1998, and the US publication by National Research Council, Institute of Laboratory Animal Resources, 2011, 'Guide for the Care and Use of Laboratory Animals', National Academy Press, Washington, DC.

# 3. MATERIALS AND METHODS

## 3.1 TEST ITEM

### 3.1.1 TEST ITEM INFORMATION

| | | |
|---|---|---|
| Test Item Name | : | **Edible Oil containing *Vijaya Extract* (10%)** |
| Characteristics (Physical State) | : | Greenish viscous oil |
| Batch Number | : | SS01 |
| Purity | : | 10% strength of active |
| Storage Condition | : | Room Temperature (27 ± 9 °C), kept away from light |
| Date of Manufacture | : | 16-08-2021 (DD-MM-YYYY) |
| Date of Expiry | : | 15-08-2022 (DD-MM-YYYY) |
| Sponsor | : | **Savikalpa Sciences Pvt. Ltd.**<br>A 1/6 Panchsheel Enclave, 2nd Floor<br>New Delhi- 110017, India |
| Manufactured & Supplied by | : | **Savikalpa Sciences Pvt. Ltd.**<br>Ward No. 2, Complex Industrial Area<br>Chhindwara- 480001, M.P., India |

### 3.1.1 CHARACTERISATION OF TEST ITEM

Characterisation of test item was performed by the Sponsor. The Sponsor has provided an authorized 'Certificate of Analysis' which identified the test item with the batch used for the study, manufacturing date, expiry date and other physical parameters. The 'Certificate of Analysis' is included in this report.

### 3.1.3 PREPARATION OF THE TEST ITEM

The test item, **Edible Oil containing *Vijaya Extract* (10%)**, being a liquid, was administered in undiluted form based on density (0.8 g/mL).

## 3.2 TEST SYSTEM AND MANAGEMENT

### 3.2.1 SELECTION OF ANIMAL SPECIES

| | | |
|---|---|---|
| Species and justification for the selection of the species | : | Rat (*Rattus norvegicus*); The regulatory guidelines for this test has preferred rat among the species of rodents. |
| Strain and justification of selection of strain | : | Wistar; The strain was selected due to its availability in requisite numbers. |
| Sex | : | Both male and female were used for this study as per the Sponsor's requirement. The selected females were nulliparous and non-pregnant. |
| Source | : | INTOX PVT. LTD. |
| Age at start of study | : | 8 weeks |
| Body weight range at start of the treatment (Day -1) | : | Male : 170 g to 183 g<br>Female: 140 g to 145 g<br>Body weights of the animals fell in an interval within ±20% of the mean weight of any previously dosed animals in the study. |
| Route of administration and justification of its choice | : | Oral, through gavage. The oral route is an anticipated route of exposure in humans. |
| No. of dose groups | : | Two: Step-1 : 2000 mg/kg ; Step-2 : 2000 mg/kg |
| No. of animals per dose group | : | Three per sex |
| Veterinary examination | : | Prior to assignment to the study, the animals were subjected to a veterinary examination to ensure that the selected rats were in a good state of health. |

### 3.2.2 HOUSING AND FEEDING CONDITIONS

| | | |
|---|---|---|
| Environmental conditions | : | The experimental animal room was supplied with fresh and filtered air, with 10 to 15 air changes per hour. The room was air conditioned with temperature between 19 to 25 $^{o}$C, relative humidity 30 to 70% and illumination cycle set to 12 hours light and 12 hours dark. |

| | | |
|---|---|---|
| Accommodation | : | Animals were housed in room number AR-05 in the experimental animal facility of INTOX PVT. LTD., maintained under appropriate barriers.<br><br>Animals were housed in sterilised solid bottom polypropylene cages [size: 42 cm (L) x 29 cm (W) x 19 cm (H)] with stainless steel grill tops, facilities for food and water bottle, and with bedding of clean and sterilised paddy husk. Cages were suspended on movable stainless steel racks.<br><br>Animals were group housed, with three animals of similar sex of same dose group being housed in one cage. |
| Diet | : | 'Altromin' brand pelleted rat feed manufactured by M/s Altromin Spezialfutter GmbH & Co. KG, Germany, and supplied by ATNT Laboratories, Mumbai was provided *ad libitum*. The diet has been tested and certified to be free from undesired levels of contaminants. |
| Water | : | Potable water passed through 'Aquaguard' water filter was provided *ad libitum* in sterilized bottles with stainless steel sipper tubes.<br><br>The drinking water has been tested and certified for potability and the water source has been verified to be free from undesired levels of contaminants. |

### 3.2.3 PREPARATION OF ANIMALS

| | | |
|---|---|---|
| Acclimatization | : | The animals were acclimatized for a period of 7 to 12 days in the experimental room before start of the treatment. |
| Selection | : | The animals were randomly assigned to three animals per group. |
| Identification | : | Each animal was assigned a unique identification (ID) number, which was specified on individual cage tag. The animals were also identified by individual tail numbering. |
| Nutritional conditions | : | The rats were fasted overnight prior to dosing. Food was offered at period of 3 to 4 hours after dosing. |

## 3.3 STUDY DESIGN

### 3.3.1 EXPERIMENTAL DESIGN AND ALLOTMENT OF ANIMALS

The toxicity of the test item was assessed by stepwise treatment of animals. Three male and female rats were used per step. Absence or presence of compound-related mortality of the animals dosed at one step determined the next step, i.e. either no further testing is needed or dosing of three additional animals with the same dose or dosing of three additional animals at the next higher / lower dose level.

Following their treatment, the rats were observed for incidence of mortality and signs of toxicity for 14 days.

**STUDY DESIGN**

| Step | Group | Dose (mg/kg) | Female rats | | |
|---|---|---|---|---|---|
| | | | Nos. per group | Animal IDs. | |
| | | | | Male | Female |
| 1 | G1 | 2000 | 6 | RI9409 to RI9411 | RI9412 to RI9414 |
| 2 | G2 | 2000 | 6 | RI9415 to Rk9417 | RI9418 to RI9420 |

### 3.3.2 RATIONALE FOR SELECTION OF DOSE LEVELS

Published information on the active ingredient revealed that the intravenous $LD_{50}$ value of **Edible Oil Extract** in rats is 16500 mg/kg body weight (Source: www.thegoodscentscompany.com/data/vg1666551.html).

Based on the available information, study was started at the dose of 2000 mg/kg body weight.

### 3.3.3 ADMINISTRATION OF THE TEST ITEM

The test item was administered by oral gavage to each rat as a single dose using a suitably graduated syringe and a stainless steel intubation needle (16 G). The dose administered to individual rat was adjusted according to its body weight that was recorded just before dosing and the density of test item i.e. 0.8 g/mL. Treatment record of individual animal is presented in following table.

## TABLE 1

### INDIVIDUAL ANIMAL TREATMENT RECORD

| Step | Group | Animal ID. | Dose (mg/kg) | Body Weight (g) (After Fasting) | Individual Animal Dose Volume (mg) | Individual Animal Dose Volume (ml) | Date of Dosing (DD-MM-YYYY) | Time of Dosing |
|---|---|---|---|---|---|---|---|---|
| 1 | G1 | R9409 | 2000 | 169 | 338 | 0.42 | 17-12-2021 | 09:58 AM to 10:00 AM |
| | | R9410 | | 162 | 324 | 0.41 | | |
| | | R9411 | | 172 | 344 | 0.43 | | |
| | | RI9412 | | 129 | 258 | 0.32 | | 10:01 AM to 10:03 AM |
| | | RI9413 | | 130 | 260 | 0.33 | | |
| | | RI9414 | | 134 | 268 | 0.34 | | |
| 2 | G2 | RI9415 | 2000 | 160 | 320 | 0.40 | 22-12-2021 | 10:23 AM to 10:25 AM |
| | | RI9416 | | 167 | 334 | 0.42 | | |
| | | RI9417 | | 165 | 330 | 0.41 | | |
| | | RI9418 | | 131 | 262 | 0.33 | | 10:26 AM to 10:28 AM |
| | | RI9419 | | 133 | 266 | 0.33 | | |
| | | RI9420 | | 132 | 264 | 0.33 | | |

**EXAMPLE OF CALCULATION OF INDIVIDUAL ANIMAL DOSE VOLUME:**

Body weight of animal = 169 g

$$\text{Intended Dose for the animal (mg)} = \frac{\text{Body weight of the aimal (g) X Dose (mg/kg)}}{1000}$$

$$= \frac{169 \times 2000}{1000} = 338 \text{ mg}$$

$$\text{Intended Dose for the animal (mL)} = \frac{\text{Intended Dose for the animal (mg)}}{\text{Density (g/mL)}}$$

$$= \frac{338}{0.8} = 422.5 \text{ µL} = 0.42 \text{ mL}$$

## 3.4 OBSERVATIONS

### 3.4.1 MORTALITY

All animals were observed for mortality twice a day throughout the observation period.

### 3.4.2 CLINICAL SIGNS

On the day of dosing, all animals were observed for signs of toxicity and death, periodically during the first 24 hours with special attention given during the first 4 hours (i.e. at 10 minutes, 30 minutes, 1 hour, 2 and 4 hours following dosing) and thereafter they were observed once a day for 14 days after treatment.

Cageside observations included changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. The appearance, progress and disappearance of the signs were recorded.

### 3.4.3 BODY WEIGHTS

The body weights of rats were individually recorded at one day prior to dosing (day -1), on the day of dosing (day 0, fasting body weight), on day 7 and at termination on day 14. Weight gain and group mean values were computed (over day -1 body weight).

### 3.4.4 NECROPSY AND HISTOPATHOLOGY

At end of the study, all animals were weighed and humanely sacrificed by carbon dioxide asphyxiation. All animals in the study were subjected to a complete necropsy and the gross pathological changes were recorded.

Histopathological examination was not carried out in the absence of treatment related gross pathological changes.

## 3.5 INTERPRETATION OF RESULTS

In principle, the acute toxic class method is not intended to allow the calculation of a precise $LD_{50}$ value, but it does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.

The test item is classified according to the Globally Harmonized System (GHS) for classification of chemicals which cause acute toxicity; Ninth Revised Edition (GHS - ST/SG/AC.10/30/Rev.9); United Nations, New York and Geneva, 2021; eISBN 978-92-1-005213-9, United Nations, 2021 and Commission Directive 1272/2008 of 16 December 2008 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the Classification, Packaging and Labelling of Dangerous Substances. Official Journal of the European Communities OJL 225 pp. 1-333.

. The test item is classified to GHS hazard categories according to the following convention:

| **Experimentally Obtained Range of $LD_{50}$ (mg/kg)** | **GHS (Hazard) Category** |
|---|---|
| ATE ≤ 5 | Category 1 |
| 5 < ATE ≤ 50 | Category 2 |
| 50 < ATE ≤ 300 | Category 3 |
| 300 < ATE ≤ 2000 | Category 4 |
| 2000 < ATE ≤ 5000 | Category 5 |
| > 5000 | Unclassified |

ATE = Acute Toxicity Estimate

# 4. RESULTS

## 4.1 STEP-1: STARTING DOSE - GROUP G1: 2000 mg/kg Body Weight

### 4.1.1 MORTALITY AND CLINICAL SIGNS (Table 2)

When dosed to three male and three female rats at the dose of 2000 mg/kg body weight, **Edible Oi containing *Vijaya Extract* (10%)** did not cause any mortality and abnormal clinical signs on the da of dosing and also throughout the observation period of 14 days following dosing.

### 4.1.2 BODY WEIGHTS (Table 3)

The body weight gain of male and female rats was not adversely affected during the 14 days observation period, following dosing.

### 4.1.3 NECROPSY (Table 4)

No gross pathological alterations were encountered in any of the rats when sacrificed at termination of the study.

## 4.2 STEP-2: GROUP G2: 2000 mg/kg Body Weight

### 4.2.1 MORTALITY AND CLINICAL SIGNS (Table 2)

When further tested on three male and three female rats at the dose of 2000 mg/kg body weight, **Edible Oil containing *Vijaya Extract* (10%)** did not cause any mortality and abnormal clinical signs on the day of dosing and also throughout the observation period of 14 days following dosing.

### 4.2.2 BODY WEIGHTS (Table 3)

The body weight gain of male and female rats was not adversely affected during the 14 days observation period, following dosing.

### 4.2.3 NECROPSY (Table 4)

No gross pathological alterations were encountered in any of the rats when sacrificed at termination of the study.

## 5. CONCLUSION

Acute oral toxicity study of **Edible Oil containing *Vijaya Extract* (10%)** in Wistar rats was performed as per Organization for Economic Co-operation and Development (OECD) Guidelines for Testing of Chemicals, Section 4, No. 423 - Acute Oral Toxicity - Acute Toxic Class Method, adopted by the council on 17 December, 2001 and Center for drug evaluation and Research (CDER) -Single dose acute toxicity for pharmaceutical, adopted August 1996.

Based on these results and according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) – Ninth Revised Edition (GHS - ST/SG/AC.10/30/Rev.9); United Nations, New York and Geneva, 2021; eISBN 978-92-1-005213-9 and Commission Directive 1272/2008 of 16 December 2008 adapting to technical progress for the 28$^{th}$ time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the Classification, Packaging and Labelling of Dangerous Substances. Official Journal of the European Communities OJL 225 pp. 1-333, the test item, **Edible Oil containing *Vijaya Extract* (10%)** has to be classified in **GHS Category 5 or unclassified** for the obligatory labelling requirement for oral toxicity, the corresponding **$LD_{50}$ value** lying between **2000 < ATE ≤ 5000** or **> 5000 mg/kg** body weight respectively.

## 6. ARCHIVES

The following shall be retained in the Archives of INTOX Pvt. Ltd. for periods starting from the date of submission of final report, as specified below.

| Material | Study Plan | Raw Data | Draft and Final Report | Sample of Test Item |
|---|---|---|---|---|
| Period of Archiving | A period, whichever is less, among the period covering three GLP inspection / certification cycles undergone by the Test Facility, or Nine years after completion of the study | | | Till Expiry Date of the Test Item |

## 7. QUALITY ASSURANCE UNIT REVIEW

The Quality Assurance Unit has conducted inspections at various phases of the study as per the Principles of Good Laboratory Practice (OECD, 1998). The dates on which the findings of these inspections are reported to the Study Director and to Test Facility Management have been specified in this report.

This report has been reviewed by Quality Assurance Unit comparing individual findings against raw data and comparing the statement and results presented in the report with individual data presented in the respective tables of the report.

## 8. REFERENCES


1. Commission Directive 1272/2008 of 16 December 2008 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the Classification, Packaging and Labelling of Dangerous Substances. Official Journal of the European Communities OJL 225 pp. 1-333.

2. OECD, 1998: OECD Series on principles of Good Laboratory Practice and Compliance Monitoring, Number 1, 'OECD Principles on Good Laboratory Practice' ENV/MC/CHEM(98)17 (as revised in 1997).

3. OECD, 2001: OECD Guideline No. 423, 'Acute Oral Toxicity – Acute Toxic Class Method'. The Organization for Economic Co-operation and Development (OECD) guidelines for the Testing of Chemicals, adopted by the council on 17 December 2001.

4. OECD, 2001: OECD series on testing and assessment, Number 33; Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures; ENV/JM/MONO (2001) 6.

5. Globally Harmonized System of Classification and Labelling of Chemicals (GHS) – Ninth Revised Edition (GHS - ST/SG/AC.10/30/Rev.9); United Nations, New York and Geneva, 2021; eISBN 978-92-1-005213-9.

6. OECD, 2000: Guidance Document on the Recognition, Assessment and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation. OECD Environmental Health and Safety Publications. Series on Testing and Assessment No. 19.

7. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Guidelines for Laboratory Animal Facility', published in The Gazette of India, December 15, 1998.

8. The US publication by National Research Council Institute of Laboratory Animal Resources, 2011, 'Guide for the Care and Use of Laboratory Animals', National Academy Press, Washington, DC.

9. Center for drug evaluation and Research (CDER): Single dose acute toxicity for pharmaceutical, adopted August 1996.

# 9. TABLES

# TABLE 2

## INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY

### MALE RATS

| Animal ID. | Incidence of Clinical Signs / Mortality Observed after Dosing on | | | | | | | | | | | | | | | | | | Mortalit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | | | | | Day | | | | | | | | | | | | | |
| | Min | | Hours | | | | | | | | | | | | | | | | |
| | 10 | 30 | 1 | 2 | 4 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
| **Step: 1** | | | | | | | | | **Group: G1** | | | | | | | | | | **Dose: 2000 mg/kg** | |
| R9409 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| R9410 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | 0 / 3 |
| R9411 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| **Step: 2** | | | | | | | | | **Group: G2** | | | | | | | | | | **Dose: 2000 mg/kg** | |
| RI9415 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| RI9416 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | 0 / 3 |
| RI9417 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |

N – No abnormal clinical signs

Day 0 is the day of dosing  Min – Minutes

* Number of animals died / number of animals treated

# TABLE 2 (Cont.)

## INDIVIDUAL ANIMAL CLINICAL SIGNS AND MORTALITY

### FEMALE RATS

| Animal ID. | Incidence of Clinical Signs / Mortality Observed after Dosing on | | | | | | | | | | | | | | | | | | Mortality |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | | | | | Day | | | | | | | | | | | | | |
| | Min | | Hours | | | | | | | | | | | | | | | | |
| | 10 | 30 | 1 | 2 | 4 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
| **Step: 1** | | | | | | | | | **Group: G1** | | | | | | | | | **Dose: 2000 mg/kg** | | |
| RI9412 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| RI9413 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | 0 / 3 |
| RI9414 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| **Step: 2** | | | | | | | | | **Group: G2** | | | | | | | | | **Dose: 2000 mg/kg** | | |
| RI9418 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| RI9419 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | 0 / 3 |
| RI9420 | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |

N – No abnormal clinical signs

Day 0 is the day of dosing  Min – Minutes

* Number of animals died / number of animals treated

# TABLE 3

## INDIVIDUAL ANIMAL BODY WEIGHTS (g)

### MALE RATS

| Step | Group and Dose (mg/kg) | Animal ID. | Day -1 (Before fasting) | Day 0 (After Fasting) | Day 7 | % Gain (Day -1 to 7) | Day 14 | % Gain (Day -1 to 14) |
|---|---|---|---|---|---|---|---|---|
| 1 | G1 2000 | R9409 | 182 | 169 | 195 | 7.14 | 208 | 14.29 |
| | | R9410 | 177 | 162 | 191 | 7.91 | 204 | 15.25 |
| | | R9411 | 183 | 172 | 197 | 7.65 | 211 | 15.30 |
| | | **Mean** | **180.67** | **167.67** | **194.33** | **7.57** | **207.67** | **14.95** |
| | | **± S. D.** | **3.21** | **5.13** | **3.06** | - | **3.51** | - |
| | | **n** | **3** | **3** | **3** | - | **3** | - |
| 2 | G2 2000 | RI9415 | 170 | 160 | 182 | 7.06 | 195 | 14.71 |
| | | RI9416 | 176 | 167 | 190 | 7.95 | 203 | 15.34 |
| | | RI9417 | 177 | 165 | 191 | 7.91 | 205 | 15.82 |
| | | **Mean** | **174.33** | **164.00** | **187.67** | **7.64** | **201.00** | **15.29** |
| | | **± S. D.** | **3.79** | **3.61** | **4.93** | - | **5.29** | - |
| | | **n** | **3** | **3** | **3** | - | **3** | - |

S. D. – Standard Deviation; n – Number of Animals

# TABLE 3 (Cont.)

## INDIVIDUAL ANIMAL BODY WEIGHTS (g)

### FEMALE RATS

| Step | Group and Dose (mg/kg) | Animal ID. | Day -1 (Before fasting) | Day 0 (After Fasting) | Day 7 | % Gain (Day -1 to 7) | Day 14 | % Gain (Day -1 to 14) |
|---|---|---|---|---|---|---|---|---|
| 1 | G1 2000 | RI9412 | 140 | 129 | 152 | 8.57 | 165 | 17.86 |
| | | RI9413 | 140 | 130 | 153 | 9.29 | 167 | 19.29 |
| | | RI9414 | 145 | 134 | 158 | 8.97 | 171 | 17.93 |
| | | **Mean** | **141.67** | **131.00** | **154.33** | **8.94** | **167.67** | **18.36** |
| | | **± S. D.** | **2.89** | **2.65** | **3.21** | - | **3.06** | - |
| | | **n** | **3** | **3** | **3** | - | **3** | - |
| 2 | G2 2000 | RI9418 | 140 | 131 | 153 | 9.29 | 166 | 18.57 |
| | | RI9419 | 142 | 133 | 156 | 9.86 | 170 | 19.72 |
| | | RI9420 | 142 | 132 | 157 | 10.56 | 170 | 19.72 |
| | | **Mean** | **141.33** | **132.00** | **155.33** | **9.90** | **168.67** | **19.34** |
| | | **± S. D.** | **1.15** | **1.00** | **2.08** | - | **2.31** | - |
| | | **n** | **3** | **3** | **3** | - | **3** | - |

S. D. – Standard Deviation; n – Number of Animals

# TABLE 4

## INDIVIDUAL ANIMAL FATE AND NECROPSY FINDINGS

### MALE RATS

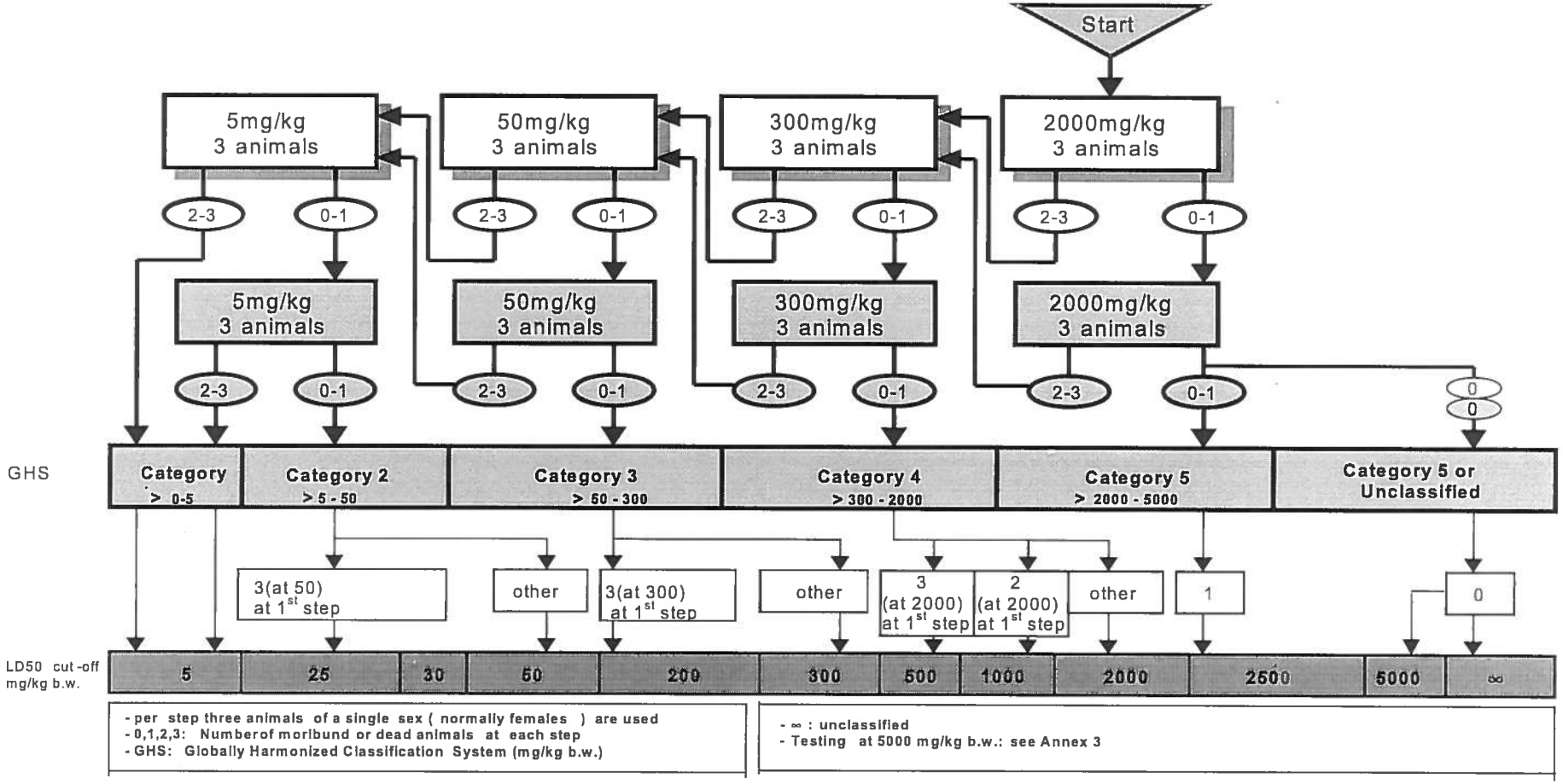
| Animal ID. | Fate | Day of Sacrifice | Necropsy Findings |
|---|---|---|---|
| **Step: 1** | | **Group: G1** | **Dose: 2000 mg/kg** |
| R9409 | TS | 14 | NAD |
| R9410 | TS | 14 | NAD |
| R9411 | TS | 14 | NAD |
| **Step: 2** | | **Group: G2** | **Dose: 2000 mg/kg** |
| RI9415 | TS | 14 | NAD |
| RI9416 | TS | 14 | NAD |
| RI9417 | TS | 14 | NAD |

### FEMALE RATS

| Animal ID. | Fate | Day of Sacrifice | Necropsy Findings |
|---|---|---|---|
| **Step: 1** | | **Group: G1** | **Dose: 2000 mg/kg** |
| RI9412 | TS | 14 | NAD |
| RI9413 | TS | 14 | NAD |
| RI9414 | TS | 14 | NAD |
| **Step: 2** | | **Group: G2** | **Dose: 2000 mg/kg** |
| RI9418 | TS | 14 | NAD |
| RI9419 | TS | 14 | NAD |
| RI9420 | TS | 14 | NAD |

NAD - No abnormalities detected TS - Terminal Sacrifice

## ANNEX 2d: TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT



# SAVIKALPA SCIENCES PRIVATE LIMITED

| CERTIFICATE OF ANALYSIS | |
|---|---|
| Product Name : Edible Oil containing *Vijaya* Extract (10%) | |
| Batch No : SS01 | |
| Manufactured By: Savikalpa Sciences Pvt. Ltd. | Manufacturing Date: Aug - 2021<br>Expiry Date: Aug - 2022 |
| Storage condition: Store at room temperature in a cool and dry place | |

| S. No | TESTS | RESULTS | |
|---|---|---|---|
| 1. | Description | Greenish viscous oil | |
| 2. | pH | Complies as per specification | |
| 3. | Phase separation | No phase separation | |
| 4. | Spreadability | Easily spreadable | |
| 5. | Viscosity | Complies as per specification | |
| | **Parameter** | **Result** | **Limit** |
| 6. | Microbiological analysis<br>Total Aerobic Microbial Count (TAMC)<br>Total Yeast and Mold Count (TYMC) | <br>Complies<br>Complies | <br>NMT-1000 cfu/gm<br>NMT-$10^2$ cfu/gm |
| 7. | Heavy Metals<br>Arsenic<br>Lead | <br>Not detected<br>Not detected | <br>Less than 2 ppm<br>Less than 20 ppm |
| 8. | Test for Aflatoxins<br>B1<br>B1+B2 +G1+G2 | <br>Not detected<br>Not detected | <br>NMT 2 ppb<br>NMT 5 ppb |
| | REMARKS: Complies as per specifications | | |

| | Prepared By | Approved by |
|---|---|---|
| Name | Dr. Kriti Soni | Raghav Priyadarshi |
| Signature | | |
| Date | 30.06.2021 | 15.11.2021 |

SAVIKALPA SCIENCES PVT. LTD.



A1/6 PANCHSHEEL ENCLAVE, 2ND FLOOR, NEW DELHI – 110017, INDIA

सत्यमेव जयते

**GOVERNMENT OF INDIA**
**Department of Science and Technology**
**National Good Laboratory Practice (GLP) Compliance Monitoring Authority (NGCMA)**

# Certificate of GLP Compliance

Based on the Inspection and the subsequent follow-up actions

## Intox Pvt. Ltd.,
## 375, Urawade, Tal. Mulshi, Pune -412115

is certified capable of conducting the below-mentioned tests/studies in compliance with Organization for Economic Co-operation & Development (OECD) Principles of GLP:

- **Physical-chemical Testing (Including 5 Batch Analysis)**
- **Toxicity studies**
- **Mutagenicity studies**
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Studies on Behavior in Water, Soil and Air; Bioaccumulation**
- **Residue Studies**
- **Analytical and Clinical Chemistry Testing**
- **Others**

The specific areas of expertise, types of chemicals and test systems are listed in annexure overleaf.

## Validity: March 15, 2019 – March 14, 2022

This certificate is subject to the condition that the test facility complies with the NGCMA's Document No. GLP-101 "Terms & Conditions of NGCMA for obtaining and maintaining GLP certification by a test facility" and OECD Principles of GLP.

Certificate No. : GLP/C-131/2019
Issue Date : 06-05-2019

भारत सरकार ★ Govt. of India
राष्ट्रीय जी एल पी अनुपालन निगरानी प्राधिकरण
National GLP Compliance Monitoring Authority
सत्यमेव जयते
विज्ञान और प्रौद्योगिकी विभाग ★ Dept. of Science and Technology

**(Dr. Neeraj Sharma)**
Head, NGCMA

National GLP Compliance Monitoring Authority (NGCMA)

# Annexure to Certificate of GLP Compliance No. GLP/C-131/2019

## Areas of Expertise:

- **Physical-chemical Testing (Including 5 Batch Analysis)**
- **Toxicity studies**
  - Acute Toxicity
  - Acute Eye Irritation
  - Acute Skin Irritation
  - Acute Skin Sensitization
  - Repeated Dose Toxicity
  - Reproductive Toxicity
- **Mutagenicity Studies**
  - Bacterial Reverse Mutation Test (AMES Test)
  - Chromosomal Aberration Test (*In-vivo* and *In-vitro*)
  - Micronucleus Assay (*In-vivo* and *In-vitro*)
  - Cell Gene Mutation Test
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Studies on Behavior in Water, Soil and Air; Bioaccumulation**
- **Residue Studies**
- **Analytical and Clinical Chemistry Testing**
- **Others**
  - Efficacy Studies/ Bioassays
  - Method Development

**Types of Chemicals:** Industrial Chemicals, Pesticides, Pharmaceuticals, Veterinary Drugs, Cosmetics, Food Additives and Feed Additives.

**Test Systems:**
Rat, Mouse, Rabbit, Guinea Pig, Fresh Water Fish, Algae, Daphnia, Honeybee, Earthworm, Chicken, Pigeon, Quail, Cell Lines (CHO, Mouse Lymphoma, Reconstructed Human Epidermis and Statens Serum Institute Rabbit Cornea) and *Salmonella typhimurium* (TA 97a, TA 98, TA 100, TA 102 and TA 1535).

भारत सरकार ★ Govt. of India
राष्ट्रीय जी एल पी अनुपालन निगरानी प्राधिकरण
National GLP Compliance Monitoring Authority
विज्ञान और प्रौद्योगिकी विभाग ★ Dept. of Science and Technology

**(Dr. Neeraj Sharma)**
Head, NGCMA