citoxlab
Safety and Health Research

FINAL REPORT

6-methoxy-harmalan:
Acute Oral Toxicity Study in Rats

Study code: 18/068-001P

Study Director:

10 December 2018

GENERAL INFORMATION

STUDY TITLE:	6-methoxy-harmalan: Acute Oral Toxicity Study in Rats
TEST ITEM:	6-methoxy-harmalan
SPONSOR:	SODEVAL S.A.S.
	Address: 3 boulevard de Verdun, 86000 Poitiers, France
STUDY MONITOR:	Jean-Bernard Fourtillan
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STUDY DIRECTOR:	
TEST FACILITY MANAGEMENT:	
QUALITY ASSURANCE*:	
RESPONSIBLE PERSONS*:	

* Other trained, competent personnel worked on the study as required.

STATEMENT OF THE STUDY DIRECTOR

This study has been performed in accordance with the Study Plan, OECD 423 (17th December 2001), Commission Regulation (EC) No 440/2008 of 30 May 2008, B.1.tris, and the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 42/2014. (VIII. 19.) EMMI decree of the Ministry of Human Capacities which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.).

No chemical analysis of the dose formulation was performed as part of this study. Traceability (equipment used, quantities of test item weighed) of dosing form preparations was checked and revealed no abnormalities of consequences. Furthermore, for this study, the formulations were prepared just before the treatment. Consequently, the absence of dose formulation analysis data was considered not to prejudice the overall GLP status of the study and the scientific reliability of the study conclusions.

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study.

Signature: Date: 10 December 2018

STATEMENT OF THE MANAGEMENT

According to the conditions of the research and development agreement between SODEVAL S.A.S. (as Sponsor) and Citoxlab Hungary Ltd. (as Test Facility) the study titled "6-methoxy-harmalan: Acute Oral Toxicity Study in Rats" was performed in compliance with the Principles of Good Laboratory Practice.

Signature: Date: 10 Dec 2018

QUALITY ASSURANCE STATEMENT

Study Code: 18/068-001P

Study Title: 6-methoxy-harmalan: Acute Oral Toxicity Study in Rats

Test Item: 6-methoxy-harmalan

This study has been inspected and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the Study Director and to Management. The dates of such inspections and of the report audit are given below:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
07 August 2018	Study Plan	07 August 2018	07 August 2018
09 August 2018	Treatment	09 August 2018	09 August 2018
19 October 2018	Draft Report	19 October 2018	19 October 2018
10 December 2018	Final Report	10 December 2018	10 December 2018

Signature: Date: 10 December 2018

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1. SUMMARY

The single-dose oral toxicity study with 6-methoxy-harmalan was performed according to the acute toxic class method (OECD 423 and Commission Regulation (EC) No 440/2008 of 30 May 2008, B.1.tris) in Crl:WI Wistar female rats.

Four groups of three female Crl:WI rats were treated with the test item at the dose levels of 2000, 300 and 50 mg/kg body weight (bw) (Group 1, Group 2, Group 3 and Group 4).

A single oral treatment was carried out by gavage for each animal after an overnight food withdrawal. Food was made available again 3 hours after the treatment. The test item was administered at the dose levels of 2000, 300 and 50 mg/kg bw.

Initially, three females (Group 1) were treated at a dose level of 2000 mg/kg bw. All animals died rapidly, therefore the next three females (Group 2) were treated at the dose level of 300 mg/kg bw. All animals died rapidly again, therefore the next three females (Group 3) were treated at the dose level of 50 mg/kg bw. No mortality was observed in this group; therefore, a confirmatory group (Group 4) was treated at the same dose level. No mortality was observed in the confirmatory group; therefore, no further testing was required according to OECD 423 and Commission Regulation (EC) No 440/2008 of 30 May 2008, B.1.tris.

Clinical observations were performed at 30 minutes, 1, 2, 3, 4 and 6 hours after dosing and daily for 14 days thereafter or until death. Additional observations were performed. Body weight was measured on Days -1, 0 and in case of surviving animals on Day 7 and before necropsy (Day 14). All animals were subjected to a necropsy and a macroscopic examination.

RESULTS

Mortality

3 out of 3 animals died on Day 0 (within 10 minutes after treatment) at the dose level of 2000 mg/kg bw.

3 out of 3 animals died on Day 0 (within 1 hour after treatment) at the dose level of 300 mg/kg bw.

No mortality occurred at the dose level of 50 mg/kg bw, all 6 animals survived the 14-day observation period.

Clinical Observations

At the dose level of 2000 mg/kg bw, the following test item related symptoms were observed until death: continuous tremors on the whole body (3 out of 3 animals), tonic convulsion on the whole body (3 out of 3 animals) and vocalisation (3 out of 3 animals).

At the dose level of 300 mg/kg bw, the following test item related symptoms were observed until death: vocalisation (3 out of 3 animals), hunched back (3 out of 3 animals), prone position (3 out of 3 animals), continuous tremors on the whole body (3 out of 3 animals), tonic convulsion on the whole body (3 out of 3 animals), piloerection (3 out of 3 animals), reddish frothy material in the oral cavity (1 out of 3 animals) and reddish coloured discharge in the oral cavity (1 out of 3 animals).

At the dose level of 50 mg/kg bw, the following test item related symptoms were observed on the day of treatment: hunched back (6 out of 6 animals), slight or moderate incoordination (6 out of 6 animals), continuous tremors on the whole body (6 out of 6 animals), slightly or moderately decreased activity (5 out of 6 animals) and vocalisation (4 out of 6 animals). All animals were symptom-free from Day 1 until the end of the observation period.

Body Weight and Body Weight Gain

There were no treatment related body weight changes in the surviving animals. Body weights were within the range commonly recorded for this strain and age.

Macroscopic Findings at Necropsy

All of the animals dosed at 2000 or 300 mg/kg bw died on Day 0 of the study. The lungs were not collapsed and dark red or red, the digestive content in the stomach, duodenum and jejunum was mucoid and yellow.

There was no evidence of the macroscopic changes at necropsy in the animals dosed at 50 mg/kg bw.

CONCLUSION

Under the conditions of this study, the acute oral LD_{50} value of the test item 6-methoxy-harmalan was found to be between 50 and 300 mg/kg bw in female Crl:WI Wistar rats.

According the GHS criteria, classification of 6-methoxy-harmalan can be ranked as "Category 3" for acute oral exposure.