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HMR. Acute oral toxicity in the rat.

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STUDY REPORT

SC 4304 - 99017

Sponsor study number 1904 - 001

**HMR ACUTE ORAL TOXICITY
IN THE RAT**

**HMR AKUUTTI TOKSISUUS ROTASSA
ORAALISEN ANNOSTELUN JÄLKEEN**

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SafetyCity

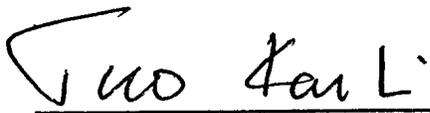
Preclinical Contract Research in University of Turku

Key Words toxicity, HMR, rat, oral

1 GENERAL

1.1 SIGNATURES

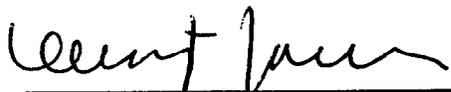
Study director



Tero Karhi

22.12.2000
date

Management



Ulla-Marjut Jaakkola
Responsible Director

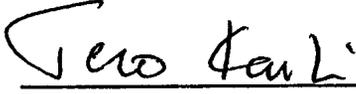
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date

1.2 STATEMENT OF COMPLIANCE

SafetyCity study number SC 4304 – 99017
Sponsor study number 1904 - 001
Test Item Hydroxymatairesinol (HMR)
Study Director Tero Karhi
Study name **HMR ACUTE ORAL TOXICITY IN THE RAT**
Tutkimuksen nimi **HMR AKUUTTI TOKSISUUS ROTASSA ORAALISEN
ANNOSTELUN JÄLKEEN**

The report is a complete and accurate account of the methods employed and the data obtained.

Study Director



Tero Karhi

22.12.2017
date

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1.4 ABBREVIATIONS

OECD	Organisation for Economic Co-operation and Development
NOAEL	No Observable Adverse Effect
SC	SafetyCity
SPF	specific pathogen free
TI	test Item
p	Page
pp	Pages
G	group
g	gram

1.5 SUMMARY

The purpose of this study was to investigate the acute toxicity of the test item , hydroxymatairesinol (HMR) after single oral dose (by gavage) in the rat with the Fixed Dose method. The aim was to clarify the lowest toxic dose and to gain information of the time course of onset of toxicity, possible recovery and target organs of the toxicity.

A sighting study (preliminary dose-finding) was performed. Single rats were dosed with oral doses of 2 000 and 1 000 mg/kg.

On the basis of the sighting study the highest nonlethal single dose of 2 000 mg/kg was selected and administered to five (5) females and five (5) males. These animals were observed for fourteen (14) days for clinical signs of test item effects.

Gross necropsy was performed to all animals and all macroscopic signs were recorded. Clinical signs were observed twice a day. Body weights were recorded at dosing and at the end of the study.

Results

The test item gave no clinical symptoms or test item related macroscopic changes in necropsy with the fixed dose of 2 000 mg/kg.

No toxicity was detected. The NOAEL is above the tested dose of 2 000 mg/kg

1.6 GUIDELINES

The study confirm the following guidelines

- OECD Guidelines for the Testing of Chemicals: 420, Acute Oral Toxicity – Fixed Dose Method, Adopted by the Council on 17th Jul 1992, Paris.
- Council directive 75/318/EEC of 20 May 1975 on the approximation of the laws of Member States relating to analytical, pharmacotoxicological and clinical standards and protocols in respect of the testing of medicinal products (OJ No L 147 of 9.6.1975, p1)
- Asetus Kokeellisiin ja muihin tieteellisiin tarkoituksiin käytettävien selkärankaisten eläinten suojelemiseksi tehdyn eurooppalaisen yleissopimuksen voimaansaattamisesta. Suomen säädöskokoelma n:o 1360/90. Helsinki, 21 joulukuuta 1990
- European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, European Treaty Series No. 123, (EU n:o 609/86)(Official Journal of the European Communities No L 358)Strasbourg 24th November 1986

1.7 ANIMAL CARE AND USE COMMITTEE APPROVAL

The study has been approved by Animal Care and Use Committee in University of Turku, approval number. 923/99 and by Länsi-Suomen Läninhallitus LSLH-1999-1352/Ym-23

1.8 SPONSOR

Hormos Medical Oy
BioCity
Tykistökatu 6 A
FIN-20520 Turku
FINLAND

Contact person; Research Director Lauri Kangas
Study Monitor; Quality Assurance person Seija Hannula 02 - 333 7664

1.9 RESEARCH LABORATORIES

University of Turku
SafetyCity / Tero Karhi
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FIN-20520 TURKU
FINLAND

Central animal laboratory / Merja Tieaho
BioCity
Tykistökatu 6
FIN-20520 TURKU
FINLAND

1.10 STUDY DIRECTOR

Tero Karhi M.Sc, Research assistant
Duties of the study director, necropsy and sampling, macroscopic pathology

Deputy study director
Merja Tieaho M.Sc.

1.11 PERSONNEL INVOLVED IN THE STUDY

Central animal laboratory
Katja Sikka, animal technician
Anitta Niittymäki, animal technician
Matti Salminen, equipment service

SafetyCity
Maija Liisa Hoffrén, medical laboratory technologist
Jere Lindén D.V.M. pathologist

1.12 TIME TABLE

Start of animal acclimatisation	22. 09.1999
Study initiation date	20.10.1999
Experimental starting date	20.10.1999
Dosing	20.10.-26.10.1999
Experimental completion date	09.11.1999

The day of dosing was designed as study day 0 for each animal. Accordingly on day 1 each animal had been in the study for 1 day.

2 MATERIALS AND METHODS.

2.1 OBJECTIVE / PURPOSE OF THE STUDY

The purpose of this study was to investigate the acute toxicity of the test item , hydroxymatairesinol (HMR) after single oral dose (by gavage) in the rat with the Fixed Dose method. The aim was to clarify the lowest toxic dose and to gain information of the time course of onset of toxicity, possible recovery and target organs of the toxicity.

2.2 STUDY DESIGN

2.2.1 Sighting study

First a sighting study was performed. Based on the personal information of the substance low toxicity was expected and a maximal dose of 1 000 mg/kg was selected. Further the dose was raised to 2 000 mg/kg.

2.2.2 Main study

A dose of 2 000 mg/kg was chosen on basis of the sighting study.

Dose mg/kg	Number of animals	
	♀	♂
2 000	6	5
1 000	1	-

2.3 TEST SYSTEM

2.3.1 Species and strain

Rat, Sprague Dawley: Hsd:SD

2.3.2 Origin of the animals

Harlan Winkelmann GmbH, Germany

2.3.3 Quality

SPF

2.3.4 Quarantine / acclimatisation

No quarantine. Acclimatisation period was 12 days.
Only clinically healthy animals were accepted into the study.

2.3.5 Rationale for species selection

There is plenty of background information of the rat in acute toxicity testing and the rat is recommended in the guidelines

2.3.6 Animal identification

The animals were identified with colour coded numbering to the tail. The markings were renewed when needed during the observation period. Cages were respectively marked with colour coded cage cards indicating study number, animal numbers, group number, sex, strain, in life study period and the name of the study director.

2.3.7 Number of animals in the study

Totally 12 animals , 7 females and 5 males
Age and weight at the start of the study
11 - 13 wk Females 152-319 g, males 236-402 g

2.3.8 Randomisation and grouping

No actual randomisation was performed. The animals were weighed and assigned to the dose group used.

2.4 HOUSING CONDITIONS

The room temperature was +20 - +23°C, relative humidity 31-57%. Lighting was artificial, 12 h light and 12 h dark (1900-0700). The room number was 131 (Biocity animal unit, Turku).

2.4.1 Animal care

The animals were taken care according to the routine procedures in the animal center. Clean cages with clean bedding were changed twice a week. Fresh water was changed once a week with bottle change once a week. Food and water were given *ad libitum*.

2.4.2 Number of animals per cage

2-3 animals/cage

2.4.3 Cage type

Polycarbonate Macrolon III

2.4.4 Bedding

Aspen chips (Tapvei Oy, Kaavi, Suomi, batch no: 3855 and 3851).

2.4.5 Fodder

RM1 E SQC (SDS England), nonsterilised, *ad libitum*.

2.4.6 Water

Normal tap water, *ad libitum*

2.5 TEST ITEM AND DOSING SOLUTIONS

2.5.1 Definitions

	Test item	Vehicle
Name	HMR	PEG 300 with 20 % ethanol
Manufacturer	Hormos Medicals Ltd.	
Batch number	00799	
Expiry date	15.8.2001	-
Manufacturing date	15.8.1999	-

The test item was dry, white granular substance. The test item was stored in the original plastic container in ambient room temperature and protected from light.

Vehicle control

No vehicle control was used

2.5.2. Formulation

The test item was first dissolved in alcohol (99.5 % ethanol) . The ethanol solution was further dissolved into polyethyleneglycol PEG 300 to give 20 % alcohol concentration. The following test item concentrations were used in dosing solutions for each dose group.

Dose mg/kg	Test item concentration mg/ml
2 000	200
1 000	100

2.5.3 Dosing

The test substances were administered orally by gavage in a volume of 10 ml/kg. The individual dosing volume was according to the weighing result on the day of dosing. Before dosing the animals were fasted overnight.

2.6 ANIMAL HANDLING

2.6.1 Daily handling

The dosing was performed during morning hours. At the same time the animals were observed for signs of toxicity. In the afternoon the clinical general condition of the animals was checked.

2.6.2 Weighing

The animals were weighed on the day of dosing (study day 0) and at necropsy.

2.6.3 Anaesthesia

The animals were euthanased with carbon dioxide (CO₂).

2.7 CLINICAL OBSERVATIONS

2.7.1 Clinical signs

In connection with dosing the clinical signs of toxicity or altered behaviour were recorded.

2.7.2 Mortality

Records were kept of the time of death.

2.8 STUDY TERMINATION AND NECROPSY

The animals were weighed and sacrificed with carbon dioxide (CO₂). A gross necropsy was performed and macroscopic signs were recorded.

2.9 STATISTIC

No statistics was used.

2.10 DEVIATIONS FROM STUDY PROTOCOL

The animals were not weighed 7 day after dosing due to a human error. This does not affect the overall purpose of the study.

2.11 ARCHIVING

All material listed below and relating to this study will be stored in the archives of SafetyCity, Tykistökatu 4, 20520 Turku, Finland, for 10 years.

Study report including

- Test substance information and control substance information
- Test system information
- Test conditions

Results

- Animal weights and weight gain
- Clinical signs of toxic symptoms
- The nature, quality, quantity and frequency of clinical signs
- Animal weights and organ weights at necropsy
- Necropsy findings
- Histological evaluation of the heart tissue muscles
- Results of the statistical calculations

At the end of 10 years, the Sponsor will be contacted for the disposal of the archived material. However, at his request, part of all the archived material may be sent to the sponsor before the end of the 10-year period. Even after the 10-year period SafetyCity will retain an electronic copy and a hardcopy of the study protocol and study report.

No data or specimen will be discarded without the sponsor's content.
Transportation and further archiving will be the responsibility of the Sponsor.

3 RESULTS

3.1 SIGHTING STUDY

A dose of 1 000 mg/kg and 2 000 mg/kg was administered to two female rats each receiving one dose.

3.2 MAIN STUDY

On basis of the sighting study a single dose of 2 000 mg/kg was administered to five male and five female rats. The animals were observed for fourteen days after which a gross necropsy was performed.

3.3 CLINICAL SIGNS

No abnormal clinical signs was recorded.

3.4 VIABILITY/MORTALITY

All animals survived the test in good condition

3.5 BODY WEIGHTS AND WEIGHT GAIN

The individual body weights are presented in Appendix 1 on p 15.
All the animals surviving the planned observation period had a normal weight gain.

3.7 NECROPSY

No test item related macroscopical changes was recorded. The incidental finding in lung was probably due to accidental rupture of a rib before the test period or a developmental disorder (Appendix 2. P. 16).

4 DISCUSSION

The test item HMR gave no clinical signs nor macroscopic changes in necropsy.

5 CONCLUSION

No toxicity was observed. NOAEL was above 2000 mg/kg and could not be determined .

No target organs could be identified.