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**Study code: 3000-4207**  
(Old code 1903007)

● **Study title:**

Effects on motor co-ordination in mice.  
Hydroxymatairesinol.

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## Study Report

### EFFECTS ON MOTOR CO-ORDINATION IN MICE

#### HYDROXYMATAIRESINOL

Study number: P11.8-1999

Date: 20.8.2002 (version 2)

Sponsor:

Hormos Medical Ltd.  
Tykistökatu 6A  
FIN-20520 Turku  
FINLAND

Sponsor Study number: 1903007

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## PreFa

Preclinical Pharmacology Research Unit  
University of Turku

Key Words

Hydroxymatairesinol (HMR), safety pharmacology, motor co-ordination, rotarod -test

## 1. GENERAL

### 1.1. SIGNATURES

### 1.2. APPROVAL OF THE STUDY PLAN

Title Effects on motor co-ordination in mice; Hydroxymatairesinol

PreFa study number: P11.8-1999

Sponsor study number: 1903007

Testi item: Hydroxymatairesinol (HMR)

This Report version 2 replaces the 1<sup>st</sup> version dated 15.8.2000. Following change have been made:

1. **Section 2.3.3. Rationale for dose selection:** Reference to a study demonstrating the antitumor activity of HMR has been added.
2. **Summary, line 1:** The age of the animals used in the Study has been corrected (previously 6 weeks)

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

  
Aapo Honkanen  
Study Director

20.8.2002  
date

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#### 1.4. PURPOSE OF THE STUDY

The purpose of this study was to assess safety pharmacological properties of the compound Hydroxymatairesinol (HMR) by assessing its effect on motor co-ordination in mice.

In addition to HMR, the effects of another compound, HTS-101 were tested in the same experiment. Same control group (vehicle treatment) and reference compound-treated groups were used in the evaluation of these compounds. The results from HMR and HTS are reported separately.

#### 1.5. SUMMARY

The motor co-ordination of the 7-8 -weeks-old NMRI mice was tested with a rotarod apparatus. The animals were first trained with the apparatus using a rotation speed of 15 rpm while in the final test trials a rotation speed of 20 rpm was used. The time period the animals were able stay on the rotating rod (300 s in maximum in the test trial) was recorded,

Mice were selected to the experiment according to their performance in the training sessions. A mouse was accepted to the experiment when it was able to stay for at least one minute on the rotating rod in at least 5 training trials. A final training session was conducted before the drug administrations on the test day.

Diazepam significantly impaired the motor performance of the mice, while all tested doses of HMR were without effect. Almost all animals in the vehicle- and HMR-treated groups stayed on the rod for maximal 300 sec (median 300 sec), while in the dizepam-treated group, the median time on the rod was 25 sec. The results of the study indicate that HMR (10-100 mg/kg, p.o.) does not impair the motor co-ordination of the mice in the rotarod test.

#### 1.6. GUIDELINES

The study procedures described were based on the guidelines listed below:

- 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. APPROVAL FROM THE ANIMAL CARE AND USE COMMITTEE

The study has a permission from the animal care and use committee of University of Turku n:o 922/99.

## 1.8. SPONSOR

Hormos Medical Ltd.  
Tykistökatu 6A  
FIN-20520 Turku  
FINLAND

## 1.9. RESEARCH LABORATORIES

University of Turku  
PreFa/Preclinical Pharmacology Research Unit  
Tykistökatu 6 B  
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Central Animal Laboratory  
BioCity  
Tykistökatu 6B  
FIN-20520 Turku  
Finland

CRST/Biometrics  
Kiinamylynkatu 10  
FIN-20520 Turku

## 1.10. STUDY DIRECTOR

Aapo Honkanen M.Sc. (Pharm.), Project Manager

## 1.11. PERSONNEL INVOLVED IN THE STUDY

PreFa/Department of Pharmacology and Clinical Pharmacology  
Esa Korpi, MD, Ph.D. Professor of Pharmacology  
Aapo Honkanen, Project Manger  
Elisa Riuttala, Laboratory Technician

CRST(Clinical Research Services Turku)/Biostatistics  
Esa Wallius

## 1.12. TIME TABLE

Start of animal acclimatisation:	26.1.2000
Experimental starting date:	15.2.2000
Experimental completion date:	25.2.2000

## 2. MATERIALS AND METHODS

### 2.1. TEST SYSTEM/SUBJECTS

Experimental animals:	NMRI mice, HsdWin:NMRI
Age/weight:	7-8 weeks/35 g $\pm$ 3 g (mean $\pm$ S.D.)
Source:	Harlan, Netherlands
Number of animals in the study:	40
Number of animals/group:	8
Acclimatisation period:	3-4 weeks before start of the experiment
Principles for selection into test groups:	Animals were randomly allotted into various test groups. Mean body weights of each group at randomization were not significantly different from each other (analysis of variance).
Identification of animals:	The animals were marked on their tails with codes in different colors
Grounds for selection of species:	Mice are commonly used in studies of this type

### 2.2. ENVIRONMENTAL CONDITIONS

Animal care:	The animals were cared and checked daily by the experimenters and/or personnel of the Central Animal Laboratory. The bedding of the animals was changed twice and water bottles once a week.
Number of animals/cage:	2-4 mice/cage.
Cage Type:	Polycarbonate Macrolon III (Scanbur AS, Denmark).
Bedding:	Aspen chips (Tapvei Oy Kaavi, Finland). The results of the analysis for specified contaminants are attached (Appendix 3.)
Water:	Community tap water, <i>ad libitum</i> , except during the experiments. The results of the analysis for specified contaminants are attached (Appendix 4.)
Fodder:	RM1 (E) SQC, Special Diet Service, Witham Essex, England. Certificate detailing nutritional composition and levels of specified contaminants is attached (Appendix 5.)

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Ambient temperature: 21 ± 2.5 °C  
Humidity: 50 % ± 15 %  
Illumination: 12-h dark/light cycle; lights on from 7.00 to 19.00 and lights off from 19.00 to 7.00.  
Room numbers: Experimental Room 313, BioCity, C-department  
Colony Room 309, BioCity, C-department

## 2.3. REAGENTS

### 2.3.1. Test compounds

#### Hydroxymatairesinol (HMR, mw. 374)

Vehicle: PEG 300 Sigma (Chemicals Co, St Louis, MO, USA)  
Batch: 00799  
Storage: at 4 °C, desiccated, protected from direct light

### 2.3.2. Reference compounds

#### Diazepam (mw. 284.74)

Manufacturer: Sigma Chemicals Co, St Louis, MO, USA  
Vehicle: PEG 300  
Lot: 105F0451  
Batch: 07/98  
Storage: at room temperature protected from direct light

### 2.3.3. Rationale for dose selection

In the experiments assessing the pharmacodynamic efficacy of HMR ,e.g. antitumor activity (Saarinen et al. Nutrition and cancer 2000 (36):207-216) a dose 15 mg/kg, (p.o.) have been found to be effective.

Thus the doses selected for the present study (10, 30 and 100 mg/kg, p.o.) were within this therapeutic range or exceeded that.

### 2.3.4. Preparation and handling of test compound solutions

Fresh test compound solutions were prepared on each experimental day. HTS-101 and reference compound diazepam were dissolved in Polyethylene glycol 300 (PEG 300). Solutions were sonicated at 40 °C for 8-15 min

## 2.4. EXPERIMENTS

### 2.4.1. Procedure

The motor co-ordination of the mice was tested with a rotarod apparatus (Ugo Basile model 7650, Comerio, Italy). The animals were first trained with the apparatus using a rotation speed of 15 rpm. In the final test trials a rotation speed of 20 rpm was used. The time period the animal were able stay on the rotating rod (300 s in maximum in test trial) was recorded,

#### Training of the mice for rotarod experiment

Mice were selected to the experiment according to their performance in the training sessions. A mouse was accepted to the experiment when it was able to stay for at least one minute on the rotating rod in at least 5 training trials. Each mouse was allowed to practice in 5 -10 1-min trial twice a day on a day preceding the drug testing day. If a mouse did not fulfil the criteria, it was discarded. A final training session was conducted before the drug administrations on the test day, and the animal had to fulfil the criteria in this trial in order to be accepted to the experiment.

### 2.4.2. Administration of compounds

Vehicle or different doses of HMR or reference compound diazepam will be given p.o. 1 h before the animals will be placed on the rotating rod.

**Table 2.1. Treatments**

Groups	Treatment	Dose
I	Vehicle (PEG 300)	-
II	Diazepam	20 mg/kg
III	HMR	10 mg/kg
IV	HMR	30 mg/kg
V	HMR	100 mg/kg

$n_i = 8, n = 40$

### 2.4.3. Data collection

The time period the animals were able stay on the rotating rod was recorded, 300 sec in maximum. The rotarod apparatus record the time when animal falls down from the rotating rod automatically and this value was then entered into the worksheet manually.

### 2.4.4. Statistics

The data were not normally distributed, so non-parametric tests were employed. The data were first tested with Kruskal-Wallis non-parametric ANOVA, and when possible pairwise comparisons were done with Mann-Whitney test with Bonferroni adjustment. Medians, and quartiles were calculated for each group.

#### 2.4.5. Termination of the experiments

At the end of the experiment, all surviving animals will be sacrificed with CO<sub>2</sub>.

### 3. ARCHIVING

Study plan, final report and original data from different experiments are retained in the archive of PreFa (Tykistökatu 6B) at least for 10 years following approval of final report. After that, the further treatment of the documentation is decided together with the Sponsor. The documentation or parts of it may be delivered to the Sponsor on request before 10-year term. No data or documentation will be destroyed without permission from the Sponsor.

### 4. DEVIATIONS FROM STUDY PLAN

Instead of 15 rpm, rotation speed of 20 rpm was used in the final test trials. This modification was made to increase the sensitivity of the test. The Sponsor was informed about the modification.

### 5. RESULTS

#### 5.1. BODY WEIGHTS

Average ( $\pm$  S.D.) body weights of the animals in different treatment groups are shown in table 5.1. The effects of different treatments on motor co-ordination are shown in table 5.2. There was no differences in the body weights of the animals between the groups ( $F = 0.51$ ,  $p = 0.73$ , ANOVA).

**Table 5.1.** Average weight of the animals in each treatment group.

Group	Treatment	Mean	S.D.	MIN	MAX	n <sub>i</sub>
I	Vehicle	34	2	32	38	8
II	Diazepam 20	35	2	31	38	8
III	HMR 10	35	3	32	39	8
IV	HMR 30	34	2	30	36	8
V	HMR 100	35	2	32	38	8

#### 5.2. EFFECTS OF HMR ON MOTOR CO-ORDINATION

Diazepam significantly impaired the motor performance of the mice, while all tested doses of HMR were without effect. Kruskal-Wallis test revealed a significant effect of treatment ( $p < 0.001$ ), and pairwise comparison confirmed that only diazepam-treated group differed significantly from the vehicle-treated control group (vehicle vs. diazepam  $p < 0.05$ ).

**Table 5.2.** Effects of HTS-101 and diazepam, a reference compound, on ability of the mice to walk on rotating rod (20 rpm).

Group	Treatment	Median	25th Percentile	75th Percentile	MIN	MAX	n <sub>i</sub>
I	Vehicle	300	300	300	37	300	8
II	Diazepam 20	25	20	74	15	300	8
III	HTS 10	300	284	300	124	300	8
IV	HTS 30	300	262	300	38	300	8
V	HTS 100	300	226	300	39	300	8

## 6. CONCLUSION

These results indicated that hydroxymatairesinol (HMR) (10-100 mg/kg, p.o.) does not impair the motor co-ordination of the mice in the rotarod test.

## 7. DISTRIBUTION OF THE REPORT

The Report is written in duplicate, one original copy being retained in the Archives of PreFa and one delivered to the Sponsor.

### Appendices

1. Values from the individual animals
2. Statistics
3. Report from analysis of bedding for contaminants
4. Report from analysis of water for contaminants
5. Report from analysis of fodder for nutritional composition and levels of specified contaminants.