FREY-TOX GERMANY

FINAL REPORT

SPONSOR: Chrysamed Vertrieb GmbH
Karl-Emminger-Str. 14-16
A-5020 Salzburg
Austria

CHRYSAMED INSEKTIZID

ACUTE ORAL TOXICITY STUDY IN THE RAT

AUTHOR: A. Vaeth

Telephone +49 (0) 35353119
Telefax +49 (0) 353521380
Osteroda 38, D-04916 Herzberg (Elster)

CONTENTS

1. GLP-COMPLIANCE 3
2. QUALITY ASSURANCE CLAIM 4
3. PERSONNEL RESPONSIBLE FOR THE STUDY 5
4. SUMMARY 6
5. INTRODUCTION 7
6. MATERIALS AND METHODS 7
6.1. Test item 7
6.2. Vehicle 8
6.3. Animals 8
6.4. Housing 8
6.5. Bedding 8
6.6. Diet 9
6.7. Drinking water 9
7. STUDY PROCEDURE 9
7.1. Sighting study 9
7.2. Main study 9
8. OBSERVATIONS 10
9. BODY WEIGHT 10
10. NECROPSY 10
11. ARCHIVES 10
12. RESULTS 10
12.1. Sighting study 10
12.2. Main study 11
13. CONCLUSION 11

ANNEX OF TABLES

Table 1
Body weight 12

Table 1, cont.
Body weight 13

Table 2
Key to daily observations 14

Table 3
Daily observations 15

Table 3, cont.
Daily observations 16

1. GLP-COMPLIANCE

The investigation described in this report "CHRYSAMED INSEKTIZID - Acute Oral Toxicity Study in the Rat" was carried out under my supervision and responsibility and in accordance with the principles of Good Laboratory Practice (GLP) according to ChemG from July 2nd, 2008.

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

FREY-TOX GmbH
November 24th, 2008

A. Vaeth, biologist-university degree
Study Director

2. QUALITY ASSURANCE CLAIM

The Quality system by FREY-TOX GmbH complies with the OECD principles of Good Laboratory Practice.

Short-term studies of the type described in this final report "CHRYSAMED INSEKTIZID - Acute Oral Toxicity Study in the Rat" are inspected by the Quality Assurance Unit in compliance with the principles of Good Laboratory Practice. Study-based, Facility-based and Process-based inspections are carried out regularly. Documented inspection reports are communicated to the study director and to the management.

Date of most recent study-based inspection: September 10th, 2008

Date of report to study director and management: September 10th, 2008

This final report has been audited by the Quality Assurance Unit and was found to be an accurate description of the methods and procedures used during the conduct of the study and an accurate reflection of the raw data.

Dr. D. Daske
Quality Assurance

Date

3. PERSONNEL RESPONSIBLE FOR THE STUDY

Study Director	A. Vaeth, biologist-university degree
Quality Assurance	Dr. D. Daske
Sponsor Monitor	Mr. Y. Aktas, Dipl.-Ing.

4. SUMMARY

The acute oral toxicity in rats was determined according to the method recommended in the OECD Guideline No. 420, "Acute Oral Toxicity - Fixed Dose Procedure", December 2001, and the council regulation (EC) No 440/2008 “B.1 bis. Acute Oral Toxicity - Fixed Dose Procedure”, May 2008.

The study was initiated with a sighting study, in which one female rat was given CHRYSAMED INSEKTIZID in the maximum dose step of 2000 mg/kg b.w. Within the first hours piloerection and a hunched posture were observed in this rat.

Based on the results from the sighting study the main study was carried out with four more female animals each given the maximum dose step of 2000 mg/kg b.w.

All animals in the main study survived the treatment and showed piloerection and a hunched posture within the first hours.

Under the experimental conditions described in this final report, a highest non-lethal dose of 2000 mg/kg b.w. was determined for CHRYSAMED INSEKTIZID, Batch: T08030402A. The test item shall not be classified or shall be classified into GHS category 5, respectively. Harmful effects if swallowed were not shown.

5. INTRODUCTION

The objective of this study was to assess the acute toxicity of CHRYSAMED INSEKTIZID administered as a single oral dose followed by an observation period of 14 days.

The present study has been conducted in accordance with the method recommended in the OECD Guideline No. 420, "Acute Oral Toxicity - Fixed Dose Procedure", December 2001, and the council regulation (EC) No 440/2008 “B.1 bis. Acute Oral Toxicity - Fixed Dose Procedure”, May 2008.

The rat was selected as the test system because of its proven suitability in toxicological studies.

The animals for the study arrived on September 30th, 2008 and October 14th, 2008. The experimental phase was carried out between October 6th, 2008 and November 4th, 2008.

This final report describes the procedures used and the results obtained.

6. MATERIALS AND METHODS

6.1. Test item

Test item:	CHRYSAMED INSEKTIZID
Chemical name:	Solution
Labelling of the original container:	Chrysamed Universal Haushaltsinsektizid T08030402A Inhalt: 500 ml
Test item name for report:	CHRYSAMED INSEKTIZID
Batch:	T08030402A
Activity/ Purity:	0.12 % Permethrin and 0.06 % Esbiothrin
Description of the test item:	milky-liquid
pH:	7.2
Volatility:	like water
Arrival of test item:	September 22nd, 2008
Stability/ Expiry date:	stable for at least 2 years
Storage of the test item:	to protect against sunbeams (room temperature)

Test item characterization (purity, solubility and stability etc.) was the responsibility of the sponsor according to available statements from August 28th, 2008, September 4th, 2008, September 18th, 2008, September 19th, 2008 and September 25th, 2008. The test item was labelled with the Lab. No. 03188 for this study. The test result relates to the above mentioned test item supplied by the sponsor.

6.2. Vehicle

Water.

6.3. Animals

The main study was performed in 4 female SPF Wistar rats of the stock Crl:WI from Charles River Deutschland GmbH, D-97633 Sulzfeld each. On the day of dosing the rats weighed from 146 g – 168 g. An acclimatization period of at least 5 days was allowed. Another female animal was used in the sighting study.

6.4. Housing

The study took place in animal room No. 4 provided with filtered air at a temperature of 22°C ± 3°C, relative humidity being at least 30 % and preferably not exceed 70 % and air changes 10 times/ hour. The room was illuminated to give a cycle of 12 hours light and 12 hours darkness. Light was on from 6 am to 6 pm.

The rats were kept in transparent macrolone cages (type 3-180, floor area 810 cm^2) with two or three in each cage. The cages were cleaned and the bedding changed at least twice a week.

6.5. Bedding

Bedding was "Lignocel-Fasern" from Altromin, D-32791 Lage, Lippe. Regular analyses for relevant possible contaminants are performed. Certificates of analysis are retained.

6.6. Diet

A pelleted complete rodent diet "Altromin 1314" from Altromin GmbH, D-32791 Lage, Lippe, was available *ad libitum*. Analyses for major nutritive components and relevant possible contaminants are performed regularly on the diet and certificates are retained.

6.7. Drinking water

The animals had free access to bottles with domestic quality drinking water acidified with hydrochloric acid to pH 2.5 in order to prevent microbial growth. Analyses for possible contaminants are performed regularly. Certificates of analysis are retained.

7. STUDY PROCEDURE

The test item was administered orally by gavage to rats fasted overnight prior to dosing. After dosing diet was withheld for four more hours. Dosing in the sighting study took place on October 7th, 2008 at 8:30 am and the main study on October 21st, 2008 between 8:00 and 8:10 am.

7.1. Sighting study

The study was initiated with a sighting study:

One female rat was given CHRYSAMED INSEKTIZID in the maximum dose step of 2000 mg/kg b.w. Piloerektion and a hunched posture were observed in this rat.

7.2. Main study

Based on the results from the sighting study it was decided to carry out the main study with four more female animals given the maximum dose step of 2000 mg/kg b.w.

The dose volume administered was 10 ml/kg b.w. in both sighting and main study.

8. OBSERVATIONS

Each rat was observed 30 min., 2, 4 and 6 hours after the administration and thereafter daily for a period of 14 consecutive days.

9. BODY WEIGHT

Body weight was recorded on days 0, 7 and 14.

10. NECROPSY

All rats were killed by inhalation of CO_2 on day 14 and subjected to a gross necropsy examination.

11. ARCHIVES

For a period of max. 15 years the following material relating to the study will be retained in the archives of FREY-TOX GmbH:

Study plan, study plan amendments and correspondence
Test items
Animal records
Raw data
Final report

After the end of the storage period FREY-TOX GmbH will contact the sponsor for instructions whether the material should be transferred, retained or destroyed.

12. RESULTS

12.1. Sighting study

The animal included in the sighting study survived the treatment.

The body weight is shown in Table 1. The rat had a normal body weight gain during the study period.

The clinical signs of the rat observed daily throughout the study are presented in Table 3. A key to daily observations is listed in Table 2.

Animal No. 1 showed a hunched posture and piloerection 30 min and 2 hrs after the application of the test item. Piloerection was still observed after 4 hrs, too. After 6 hrs and from day 1 to the end of the observation period on day 14 no abnormalities were revealed.

The post mortem inspection revealed no pathological abnormalities.

12.2. Main study

None of the female rats died on account of the treatment nor did they show severe signs of toxicosis.

The group mean body weight and individual values are shown in Table 1. The rats had a normal body weight gain during the study period.

The clinical signs of the rats observed daily throughout the study are presented in Table 3. A key to daily observations is listed in Table 2.

Animal No. 2, No. 3, No. 4 and No. 5 showed a hunched posture and piloerection 30 min, 2 hrs and 4 hrs after the application of the test item. Piloerection was still observed after 6 hrs, too. From day 1 to the end of the observation period on day 14 no abnormalities were revealed.

Necropsy

The gross necropsy of the animals revealed no pathological abnormalities.

13. CONCLUSION

Under the experimental conditions described in this final report, a highest non-lethal dose of 2000 mg/kg b.w. was determined for CHRYSAMED INSEKTIZID, Batch: T08030402A. The test item shall not be classified or shall be classified into GHS category 5, respectively. Harmful effects if swallowed were not shown.

CHRYSAMED INSEKTIZID

ACUTE ORAL TOXICITY STUDY IN THE RAT

BODY WEIGHT

SIGHTING STUDY

Individual values [g]

Animal No.	dose mg/kg b.w.	Sex	Day 0	Day 7	Day 14
1	2000	Female	150	202	222

MAIN STUDY

Group mean values [g]

dose mg/kg b.w.	sex	Day 0			Day 7			Day 14		
		$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
2000	female	156	9	4	200	9	4	230	9	4

$\bar{x}$ Mean value
SD Standard deviation
n Number of animals

CHRYSAMED INSEKTIZID

ACUTE ORAL TOXICITY STUDY IN THE RAT

BODY WEIGHT

MAIN STUDY

Individual values [g]

Animal No.	Dose mg/kg b.w.	Sex	Day 0	Day 7	Day 14
2	2000	female	156	206	237
3	2000	female	146	196	224
4	2000	female	154	190	220
5	2000	female	168	209	238

CHRYSAMED INSEKTIZID

ACUTE ORAL TOXICITY IN RATS

KEY TO DAILY OBSERVATIONS

A Normal behaviour
B Piloerection
C Salivation
D Apathy
E Hunched posture/ abdominal rigidity
F Body weight loss or emaciation
G Vomitting
H Diarrhoea
I Constipation
J Compulsive behaviour/ Pruritus
K Tremor
L Paresis
M Discharge, abnormal
N Anaemia
O Blood around nose and eyes
P Dehydration
Q Dyspnea
R Cyanosis
S Ataxia
T Paralysis
U Comatose
V Moribund

CHRYSAMED INSEKTIZID

ACUTE ORAL TOXICITY STUDY IN THE RAT

DAILY OBSERVATIONS

SIGHTING STUDY

Animal No.	Dose mg/kg b.w.	Sex	After dosing				Day after dosing						
			30 min	2 hrs	4 hrs	6 hrs	1	2	3	4	5	6	7
1	2000	female	BE	BE	B	A	A	A	A	A	A	A	A

Animal No.	Dose mg/kg b.w.	Sex	Day after dosing						
			8	9	10	11	12	13	14
1	2000	female	A	A	A	A	A	A	A

CHRYSAMED INSEKTIZID

ACUTE ORAL TOXICITY STUDY IN THE RAT

DAILY OBSERVATIONS

MAIN STUDY

Dose: 2000 mg/kg b.w.

Animal No.	Dose mg/kg b.w.	Sex	After dosing				Day after dosing						
			30 min	2 hrs	4 hrs	6 hrs	1	2	3	4	5	6	7
2	2000	female	BE	BE	BE	B	A	A	A	A	A	A	A
3	2000	female	BE	BE	BE	B	A	A	A	A	A	A	A
4	2000	female	BE	BE	BE	B	A	A	A	A	A	A	A
5	2000	female	BE	BE	BE	B	A	A	A	A	A	A	A

Animal No.	Dose mg/kg b.w.	Sex	Day after dosing						
			8	9	10	11	12	13	14
2	2000	female	A	A	A	A	A	A	A
3	2000	female	A	A	A	A	A	A	A
4	2000	female	A	A	A	A	A	A	A
5	2000	female	A	A	A	A	A	A	A