LABORATORY of PHARMACOLOGY and TOXICOLOGY

LPT

LPT Report No. 23118

ACUTE ORAL TOXICITY STUDY OF DRYFLEX I/II IN RATS

- according to EC method B.1 tris (2004/73/EC) and OECD guideline 423 (ATC method) -
- Limit Test -

Sponsor:

SGS INSTITUT FRESENIUS GmbH
Im Maisel 14
65232 Taunusstein
Germany

Study conducted by:

LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG
Redderweg 8
21147 Hamburg
Germany

Contact person:

Dr. H. Lebertz

Contact person:

Dr. phil. J. Leuschner

July 30, 2008

This report consists of 25 pages.

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STATEMENT OF COMPLIANCE

ACUTE ORAL TOXICITY STUDY OF
DRYFLEX I/II
IN RATS

- according to EC method B.1 tris (2004/73/EC) and
OECD guideline 423 (ATC method) -
- Limit Test -

The study was performed in compliance with:

- 'Good Laboratory Practice' Regulations of the EC enacted in Germany in the 'Chemikaliengesetz' [Chemicals Act], current edition;

- 'OECD Principles of Good Laboratory Practice', Document Nos. 1 and 13, ENV/MC/CHEM (98) 17 and ENV/JM/MONO (2002) 9, respectively.

The following regulations were considered:

- United States Food and Drug Administration Good Laboratory Practice Regulations - 21 Code of Federal Regulations, Part 58, current edition;

- Japanese Guidelines for Non-clinical Studies of Drugs Manual 1995; Guidelines for Toxicity Studies of Drugs. Japanese Ministry of Health and Welfare.

There were no deviations from the 'Good Laboratory Practice' Regulations. Raw data obtained during the performance of the study are accurately reflected.

Jö. Hafe — 30.7.08

Dr. rer. nat. J. Haferkorn
Study Director

Date

QUALITY ASSURANCE STATEMENT

Based on a quality assurance review, it was concluded that this report accurately reflects the raw data for the study. Methods, procedures and observations are correctly and completely described in the report.

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Study Plan dated May 28, 2008.

Date of control	Criteria	Date of report to the Study Director and the Management
28 May 2008	Study Plan	28 May 2008
16 Jun 2008	General inspection of acute toxicity studies: administration, evaluation, animal housing, raw data	16 Jun 2008
30 Jul 2008	Final report	30 Jul 2008

Approved and submitted by:

Dipl. Biol. S. Steuer
Director of Quality Assurance Unit (QAU)
pp Dipl. Biol. O. Hannemann

30. July 2008
Date

1. SUMMARY

Test system	**Acute toxicity, oral, in rats according to OECD guideline 423 and EC method B.1 tris (2004/73/EC) - ATC method**
Test item	**Dryflex I/II**
Vehicle	The test item was used as supplied
Dose level	2000 mg/kg b.w. (limit test)
No-effect dose level	2000 mg/kg b.w., by oral administration
Dose level with first intolerance reactions	> 2000 mg/kg b.w., by oral administration
Lowest lethal dose level	> 2000 mg/kg b.w., by oral administration
LD_{50}	Exceeding 2000 mg/kg b.w., by oral administration

Under the present test conditions, a single oral administration of 2000 mg Dryflex I/II/kg b.w. to rats did not reveal any signs of toxicity. No mortality occurred. All animals gained the expected body weight.

No pathological changes were observed at necropsy.

Dr. rer. nat. J. Haferkorn
Study Director

30.7.08
Date

2. GENERAL INFORMATION

2.1 Aim of experiment

The test item was given to rats by oral administration to obtain information on the toxicity, in particular lethality, of the test item.

The Acute Toxic Class Method was employed to establish the required information for hazard assessment and hazard classification.

2.2 Sponsor / Test Facility / Responsible personnel

Sponsor	SGS INSTITUT FRESENIUS GmbH Im Maisel 14 65232 Taunusstein Germany Phone: +49 - 6128 – 744 7722 E-mail: herbert.lebertz@sgs.com
Test Facility	**LPT** Laboratory of Pharmacology and Toxicology GmbH & Co. KG Redderweg 8 21147 Hamburg Germany Phone: +49 - 40 - 70 20 20 E-mail: LPT-Hamburg@t-online.de
Study director	Dr. rer. nat. J. Haferkorn **LPT**, Redderweg 8, 21147 Hamburg, Germany
Deputy study director	Dr. phil. J. Leuschner
Management	Dr. rer. nat. A. Winkler
Conduct of study	Dr. rer. nat. J. Haferkorn
Animal husbandry	G. Stehr
Veterinarian	Dr. med. vet. G. Rohde
Quality Assurance Unit (QAU)	Dipl. Biol. S. Steuer
Code number of the study in the raw data	23118

2.3 Rules and regulations

This study was carried out in compliance with:

- EC method B.1 tris: Acute toxicity (oral) - Acute Toxic Class Method (2004/73/EC);
- OECD Guidelines for the Testing of Chemicals No. 423 - Acute oral toxicity - Acute Toxic Class Method, adopted December 17, 2001.

In addition, the 'Good Laboratory Practice' Regulations were considered (see the Statement of Compliance and the enclosed GLP Certificate of the Test Facility **LPT**).

Standard Operating Procedures (SOPs)	All work was carried out according to Standard Operating Procedures which were followed for all stages of the study; they may be inspected in those divisions which were engaged in the study and in the Quality Assurance Unit (QAU).
Staff safety	The standard safety precautions operating within the department were applied to this study.

2.4 Archive

Archives of data and specimens	All specimens, raw data and other documents generated at **LPT** during the course of this study, together with a second print of the final report are stored in the **LPT** archives as required by the 'Chemikaliengesetz' [Chemicals Act]. **During the study:** in the depot **LPT**, Redderweg 8 21147 Hamburg, Germany **After reporting:** written raw data, specimens and the second print of the final report in Archive 11 **LPT**, Redderweg 8 21147 Hamburg, Germany The final report will be archived by the Sponsor.
Duration of storage	According to the periods laid down in the German 'Chemikaliengesetz' [Chemicals Act]; afterwards the Sponsor will decide on further use.

2.5 Study dates

Start of study

Date of Study Plan	May 28, 2008
Start of the experimental phase	June 2, 2008
1st dosing	June 16, 2008

Study termination

Termination of the in-life phase	July 2, 2008
Date of the final report	July 30, 2008

2.6 Study Plan Deviations

The study was conducted in accordance with the Study Plan agreed upon. There were no deviations from this Study Plan.

3. TEST ITEM

3.1 Identification of the test item

After receipt at **LPT** the test item was inspected; batch number, amount and characteristics (colour, consistency and density) were determined and compared with information given by the Sponsor; an identification sheet was filed with the raw data.

Test item	Parameter	LPT Identification	Sponsor Identification
Dryflex I/II	colour consistency density	slight brownish liquid 1.16 g/cm^3	none none none

No further identification was carried out by **LPT**.

3.2 Description

Designation	Dryflex I/II
Batch no.	LQ12A1214
Receipt no.	39844
Date of receipt	May 21, 2008
Characteristics	Liquid
Storage conditions	At room temperature, protected from light
Stability (expiry date)	August 2008
Content	No 'Certificate of Analysis' was available to **LPT**
Retention sample of the test item	Stored at **LPT** Laboratory of Pharmacology and Toxicology GmbH & Co. KG Archive 11 Redderweg 8 21147 Hamburg Germany

3.3 Preparation of the test item

The test item was used as supplied. The administration volume amounted to 1.72 mL/kg b.w. as the density of the test item was 1.16 g/cm^3.

4. METHOD

4.1 Principle of the ATC-test method

This procedure permits the identification of the 'acute-toxic-class' (ATC), a measurement of the acute toxicity by the oral route.

The test item is administered orally by gavage at a single dose level to a group of experimental animals. The dose used is selected from a series of defined dose levels. Due to the small number of animals used with this method, there is no need to perform a range finding test.

The test item is tested using a stepwise procedure, each step uses three female animals. The results of each step determine if:

- no further testing is needed,
- the next step will be performed with the same dose,
- the next step will be performed at the next higher or next lower dose level.

Starting at 2000 mg/kg b.w.

- Testing at 2000 mg/kg b.w.:

Three animals of one sex (preferably females) are treated at 2000 mg/kg b.w. (first step). If two to three animals die, testing at 300 mg/kg b.w. should be performed.
If no to one animal dies, the test item should be retested (second step) with 2000 mg/kg b.w., using three animals of the same sex.
If, in this second step, two to three animals die, testing at 300 mg/kg b.w. should be performed. If, in this second step, no to one animal dies, no further testing is necessary.

- Testing at 300 mg/kg b.w.:

If the results of the test at 2000 mg/kg b.w. indicate the need for further testing at a lower dose level.
Three female animals are treated at 300 mg/kg b.w. (first step).
If two or three animals die, testing at 50 mg/kg b.w. should be performed.
If fewer than two animals die, the test item should be retested (second step) with 300 mg/kg b.w., using three animals of the same sex.
If, in this second step, two or three animals die, testing at 50 mg/kg b.w. should be performed. If, in this second step, no to one animal dies, no further testing is necessary.

- Testing at 50 mg/kg b.w.:

If the results of the test at 300 mg/kg b.w. indicate the need for further testing at a lower dose level.
Three female animals treated at 50 mg/kg b.w. (first step).
If two or three animals die, testing at 5 mg/kg b.w. should be performed.
If fewer than two animals die, the test item should be retested (second step) with 50 mg/kg b.w., using three animals of the same sex.
If, in this second step, two or three animals die, testing at 5 mg/kg b.w. should be performed. If, in this second step, no to one animal dies, no further testing is necessary.

4.2 Animals / Animal maintenance

Species / Strain / Stock	Rat / CD / Crl: CD(SD)
Supplier	Charles River Laboratories, Research Models and Services, Germany GmbH Sandhofer Weg 7 97633 Sulzfeld Germany
Selection of species	International recommendations; EC and OECD guidelines
Sex	Female
Number of animals	6 female animals (Limit Test)
Group	1 dose level group of 6 female animals
Body weight (at start of administration)	165 - 181 g
Age (at start of administration)	49 - 51 days
Identification of animals	By coloured marks and cage label
Duration of experiment	At least 5 adaptation days 1 test day 2 recovery weeks

Diet

Commercial diet, ssniff® R/M-H V1534 (ssniff Spezialdiäten GmbH, 59494 Soest, Germany; see Appendix 1: Composition of the diet) served as food. Feeding was discontinued approx. 16 hours before administration; only tap water was then available *ad libitum*.

Periodic analysis of the food for contaminants based on EPA/USA[1] is conducted at least twice a year by LUFA-ITL[2] (see Appendix 1: Limitation for contaminants in the diet). Certificates of analysis of the composition and for contaminants were provided by the manufacturer and are QAU archived.

Housing

Granulated textured wood (Granulat A2, J. Brandenburg, 49424 Goldenstedt, Germany) was used as bedding material for the cages. The cages were changed and cleaned twice a week.

Periodic analysis of the bedding material for contaminants based on EPA/USA is conducted at least once a year by LUFA-ITL (see Appendix 1: Limitation for contaminants in the bedding material).

During the 14-day observation period the animals were kept in groups of 3 animals in MAKROLON cages (type III) at a room temperature of 22°C ± 3°C (maximum range) and a relative humidity of 55% ± 15% (maximum range). Deviations from the maximum range caused for example during cleaning procedures are dealt with in SOPs.

The rooms were lit (about 150 lux at approx. 1.50 m room height) and darkened for periods of 12 hours each.

Drinking water

Drinking water in bottles was offered *ad libitum*.

Drinking water is examined according to the 'Deutsche Trinkwasserverordnung 2001' [German Regulations on drinking water 2001] by the Hamburger Wasserwerke, 20539 Hamburg, Germany, at least four times a year (see Appendix 1: Limitation for contaminants in the drinking water).

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1 EPA/USA, Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules, Federal Register 44, 27334 - 27375, May 1979

2 Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Institut für Tiergesundheit und Lebensmittelqualität GmbH, 24107 Kiel, Germany

In addition, drinking water samples taken at LPT are analysed by LUFA-ITL once a year for means of bacteriological investigations according to the 'Deutsche Trinkwasserverordung 2001, Anlage 1 [German Regulations on drinking water, 2001, Addendum 1].

Certificates of analysis of diet, drinking water and bedding material are QAU archived.

4.3 Administration / Dose level

Route of administration	Oral, by gavage
Selection of route of administration	According to OECD/EC guidelines
Vehicle	The test item was used as supplied
Dose level	2000 mg/kg b.w. (limit test)
Administration volume	1.72 mL/kg b.w.

Protective clothing:
All personnel handling the animals met the requirements for strict cleanliness. All experimental manipulations were performed by the designated personnel wearing a sterile cap, mask, gown and gloves.

4.4 Evaluation

Following administration, observations were made and recorded systematically with individual records being maintained for each animal. Observations were performed before and immediately, 5, 15, 30 and 60 min, as well as 3, 6 and 24 hours after administration. All animals were observed for a period of 14 days.

During the follow-up period of two weeks, changes of skin and fur, eyes and mucous membranes, respiratory and the circulatory, autonomic and central nervous system and somatomotor activity, as well as behaviour pattern were observed at least once a day until all symptoms subsided, thereafter each working day. Attention was also paid to possible tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

Observations on mortality were made at least once daily to minimize loss of animals during the study. Individual body weights were recorded before administration of the test item and thereafter in weekly intervals up to the end of the study. Changes in weight were calculated and recorded.

At the end of the experiments, all animals were sacrificed, dissected and inspected macroscopically. All gross pathological changes were recorded. No histopathology was carried out as no macroscopical findings were noted at autopsy.

The LD_{50} value was ranked exceeding 2000 mg/kg b.w..

5. RESULTS

In this experiment Dryflex I/II was examined for acute toxicity after a single oral administration to rats.

Under the present test conditions, a single oral administration of 2000 mg Dryflex I/II/kg b.w. to rats did not reveal any signs of toxicity. No mortality occurred. All animals gained the expected body weight.

No pathological changes were observed at necropsy.

See table 1 for a summary, table 2 for the individual clinical signs, table 3 for individual body weights and table 4 for necropsy findings.

Acute oral toxicity study of
Dryflex I/II
in rats

TABLE 1 S u m m a r i z e d R e s u l t s

Symptoms/ Criteria	Dryflex I/II 2000 mg/kg b.w. (n = 3) females (first step)	females (second step)
Clinical signs	none	none
mortality		
within 6 h	0	0
within 24 h	0	0
within 7 d	0	0
within 14 d	0	0
mean body weight (in g)		
start	173.3	172.3
after 7 days	208.0 (+20.0)	204.3 (+18.6)
after 14 days	232.0 (+33.9)	226.7 (+31.6)
inhibition of body weight gain	none	none
necropsy findings	none	none

in brackets: body weight gain in %, compared with the start value
h = hours
d = days

Acute oral toxicity study of
Dryflex I/II
in rats

TABLE 2 Clinical signs

Test day	1	1	1	1	1	1	1	2	3	4	5	6	7	8	9-14d
Time after administration	0'	5'	15'	30'	60'	3h	6h	24h							
Animal no./sex Clinical signs															
2000 mg Dryflex I/II/kg b.w.															
1 f none															
2 f none															
3 f none															
4 f none															
5 f none															
6 f none															

f = female
0' = immediately after dosing

h = hour
' = minute

Acute oral toxicity study of
Dryflex I/II
in rats

TABLE 3 Body weight

Animal no./ sex	Individual body weight (g) / Body weight gain (%)		
	TD 0	TD 8	TD 15
		2000 mg Dryflex I/II/kg b.w.	
1 f	170	210 (+23.5)	228 (+34.1)
2 f	169	198 (+17.2)	231 (+36.7)
3 f	181	216 (+19.3)	237 (+30.9)
Mean	173.3	208.0 (+20.0)	232.0 (+33.9)
SD	6.7	9.2	4.6
4 f	174	214 (+23.0)	236 (+35.6)
5 f	178	206 (+15.7)	227 (+27.5)
6 f	165	193 (+17.0)	217 (+31.5)
Mean	172.3	204.3 (+18.6)	226.7 (+31.6)
SD	6.7	10.6	9.5

TD = test day
TD 0 = test day 1 immediately before dosing
f = female
SD = standard deviation
in brackets: body weight gain in %, compared with the start value

Acute oral toxicity study of
Dryflex I/II
in rats

TABLE 4 Macroscopic post mortem findings

Animal no./ sex	Affected Organ / Finding
2000 mg Dryflex I/II/kg b.w.	
1 f	no pathological findings
2 f	no pathological findings
3 f	no pathological findings
4 f	no pathological findings
5 f	no pathological findings
6 f	no pathological findings

f = female

APPENDIX 1

Composition of the Diet; Limitation for Contaminants in the Diet, Drinking Water and Bedding Material

Composition of the diet

Standard Diet for Rats and Mice
ssniff® R/M-H V1534
(ssniff Spezialdiäten GmbH, 59494 Soest, Germany)

Ingredients
(average % content in the diet)

crude protein	19.0
crude fat	3.3
crude fibres	4.9
ash	6.4

Metabolizable Energy

(MJ/kg)	12.8

Amino Acids
(average % content in the diet)

lysine	1.00
methionine	0.30
Met + Cys	0.65
glycine	0.80
leucine	1.30
isoleucine	0.76
arginine	1.14
phenylalanine	0.85
Phe + Tyr	1.43
tryptophan	0.25
histidine	0.44
aspartic acid	1.61
glutamic acid	3.90
valine	0.88
threonine	0.68
proline	1.25
alanine	0.79
serine	0.89

Minerals
(average % content in the diet)

calcium	1.00
phosphorus	0.70
sodium	0.24
magnesium	0.22
potassium	0.91

Trace Elements
(average content in mg per 1 000 g of diet)

manganese	69
copper	16
zinc	94
iodine	2.2
iron	179
selenium	0.3
cobalt	2.1

Vitamins
(additive per 1 000 g of diet)

vitamin A	15 000 IU
vitamin D_3	1 000 IU
vitamin E	110 mg
vitamin B_1	18 mg
vitamin B_2	23 mg
vitamin B_6	21 mg
vitamin B_{12}	100 µg
biotin	525 µg
pantothenic acid	43 mg
choline chloride	2 990 mg
folic acid	7 mg
nicotinic acid	135 mg
vitamin K (as menadione)	5 mg
inositol	100 mg

Fatty Acids
(%)

C 14:0	0.01
C 16:0	0.47
C 16:1	0.01
C 18:0	0.08
C 18:1	0.62
C 18:2	1.80
C 18:3	0.23
C 20:0	0.01
C 20:1	0.02
C 20:5	-
C 22:6	-

Limitation for contaminants in the diet [ppb]

	min.	max.
Aflatoxin (B_1, B_2, G_1, G_2), total		5
Lindane		20
Heptachlor		20
Malathion		2 500
DDT (Total)		100
Dieldrin		20
Cadmium		160
Arsenic		1 000
Lead		1 500
Mercury		100
Selenium	100	600
PCB		50

Limitation for contaminants in the drinking water (mg/L)

	max.
Iron	0.2
Manganese	0.05
Ammonium	0.5
Chloride	250
Arsenic	0.01
Lead	0.01
Cadmium	0.005
Chromium	0.05
Cyanide	0.05
Fluoride	1.5
Nickel	0.02
Nitrite	0.5
Nitrate	50
Mercury	0.001
Vinylchloride	0.0005
Acrylamide	0.0001
Benzene	0.001
Boron	1
Bromate	0.01
Selenium	0.01
Antimony	0.005
Copper	2
Aluminium	0.2
Sodium	200
Sulphate	240

Polycyclic aromatic hydrocarbons

- Benzo-(b)-fluoroanthene		
- Benzo-(k)-fluoroanthene		
- Benzo-(ghi)-perylene		
- Indeno-(1,2,3-cd)-pyrene	total	0.0001
- Benzo-(a)-pyrene		0.00001

		max.
Chlorinated organic compounds		
Trihalogenemethane including Trichloromethane, Bromodichloromethane, Dibromochloromethane and Tribromomethane	total	0.05
- 1,2-Dichloroethane		0.003
- Tetrachloroethene and Trichloroethene		0.01
- Epichlorohydrine		0.0001
Organic chemical compounds used as pesticides and biocides including their toxic metabolites except for	maximum of 0.0001/substance	
- Aldrin		0.00003
- Dieldrin		0.00003
- Heptachlor		0.00003
- Heptachloroepoxide		0.00003
	maximum total of	0.0005
Tritium [Bq/L]		100
pH	between	6.5 and 9.5

Limitation for contaminants in the bedding material (in mg/kg)

	max.
Aflatoxin (B_1)	0.01
Chlordane	0.05
Endrin	0.02
Fluorine	150.00
Lindane	0.10
Heptachlor and epoxide	0.03
DDT, DDE, DDD	0.05
Dieldrin and aldrin	0.02
Arsenic	2.00
Lead	5.00
Mercury	0.10
Nitrite (Na-Nitrite)	15.00
HCB	0.03

APPENDIX 2

GLP Certificate of the Test Facility LPT

FREIE UND HANSESTADT HAMBURG
Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz

GLP – Bescheinigung / Statement of GLP Compliance

(gemäß/according to § 19b Abs.1 und Anhang 2 des Chemikaliengesetzes
in der Neufassung vom 20. Juni 2002
(BGBl. I S. 2090) in der geltenden Fassung)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP - Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive2004/9/EC at:

[X] Prüfeinrichtung/Test facility [ ] Prüfstandort/ Test site

Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address:

LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG
Redderweg 8
21147 Hamburg

Prüfungen nach Kategorien/ Areas of Expertise (gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

Kategorie 2, 3, 4 und 9 (Sicherheitspharmakologie und Auftragsarchiv)

Datum der Inspektion/ Date of Inspection:
(Tag.Monat.Jahr/day.month.year)

23., 24. und 25.11.2004

Die/Der genannte Prüfeinrichtung /Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung / diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility/ test site is able to conduct the aforementioned studies in compliance with the Principles of GLP

Hamburg, den 20.4.2007

Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz 33 Hamburg

Lettau
Amtsleiter