

**REPORT FOR
Experimental study**

of food supplement "MAOLO"

**on male and female white mice after the oral
administration and in vitro cytotoxicity study,**

**according to the contract dated 03.07.2009 between Agency for
Quality and product safety – SMILO EOOD and the Faculty of
Pharmacy at Medical University – Sofia.**

MANUFACTURER: SMILO EOOD, Sofia, Bulgaria,

DATE : 20.07.2009

SUBJECT

Experimental study of food supplement "MAOLO" on male and female white mice after the oral administration and in vitro cytotoxicity study

SPONSOR

“Agency for Quality and product safety – SMILO” EOOD,
Sofia, r.a. “Yavorov”, building 23, entr. 1 app. 3., represented by
Dr. Atanas Smilov Smilov

RESEARCHERS

Prof. Dr. N. Danchev, DM

ch.assistant dr. Georgi Momekov DF

ch.assistant dr. I. Nikolova, DM,
M. Yordanova - laboratory technician
M. Tomova – tech. Assistant

RESEARCH CENTRE

Department of "Pharmacology, Pharmacotherapy and
Toxicology"
Pharmaceutical Faculty,
Sofia, Bulgaria,
2 “Dunav” str.
1000 Sofia, Bulgaria,
tel: + 3592-9236582
e-mail: nikolai.danchev@gmail.com

1. Introduction

The company "SMILO" EOOD has provided the following food supplement in hard gelatin capsules for acute toxicity study after oral administration of male and female white mice and in vitro cytotoxicity study:

2. Materials and Methods

2.1. Materials and Methods

2.1.1. Food supplement MAOLO®:

Composition – plant extracts of:

<i>Hibisci sabdariffae flos</i>	40 mg
<i>Pinus Sibirica Du Tour</i>	25 mg
<i>Curcuma longa/xanthorrhizae rhizoma</i>	18 mg
<i>Origanum majorana</i>	15 mg
<i>Elettaria cardamomum</i>	12 mg
<i>Crocus sativus</i>	10 mg
<i>Artemisia dracunculus</i>	10 mg
<i>Coriandrum sativum</i>	
<i>Levisticum officinale</i>	
<i>Petroselinum crispum/neapolitanum</i>	
<i>Hyssopus officinalis</i>	
<i>Lichen islandicus</i>	< 4 mg

Excipients: sorbitol, lactose, silicon dioxide.

2.2. An acute toxicity study

2.2.1. Laboratory animals

To determine the acute toxicity of the products were used 36 white mice line H-(18 male and 18 female) weighing 18-20 gr., from Bulgarian Academy of Sciences' kennel, located in the town of Slivnitsa, Bulgaria. The mice were divided into 4 groups of 9 animals in each group. By the beginning of the experiment, animals were kept for a week in Pharmacy Faculty's vivarium an certified as a livestock breeding to acclimatize, being applied a rearing and feeding mode like the one in the kennel. All animals used in the experiment were healthy and without any physiological abnormalities.

Type	mice
Breed	Line H
Age to experiment	8-10 weeks
Bodyweight	18-20 gr.
Sex	Male female
Quantity of animals	18
Food	standard, certified
Drinking	water, autodinking
Air Temperature	20 - 24°C
Humidity	30-70%
Lighting	to 325 lux at a distance of one meter from the floor; 12 hours a light/dark
Noise	up to 85 dB
Route of administration of the test substances	oral
Duration of administration	once
Monitoring duration of any animal	14 days
Solution types	aqueous solutions
Statistical method of data processing	ISO 10993 - 11 and Eur. Ph. - 4 ed.

To determine the median lethal dose (LD50), the test product was dissolved in isotonic (0.9%) sodium chloride solution and administered orally, in one dose in a volume of 0.2 ml/10 grams body weight. In the first days after the administration of each animal was

4. Conclusions

The food supplement MAOLO was tested for acute toxicity in mice and *in vitro* for cytotoxicity in a model of intestinal epithelium.

According to ISO 10993-11, LD50, the value represents material's quantity administrated once, which causes death to 50% of the treated with the test substance animals. This value is used to determine the short-term toxicological potential (acute toxicity) of the test substances in a particular route of administration, in this case orally, which is the future route of administration of the supplements in humans. In determining the acute oral toxicity, it was found that MAOLO's LD50 is > 10,000 mg/kg bodyweight. According to Hodge and Sterner's classification this value refers to Class V, i.e. to the group of non-toxic substances after white male and female mice's oral administration.

The cytotoxicity results obtained give reason to conclude that the food supplement MAOLO® is a non-toxic product in terms of human cell line - intestinal epithelium *in vitro* model even when applying in supraphysiological concentrations.

In conclusion, the food supplement MAOLO, provided by the Agency for Quality and product safety – SMILO EOOD, is practically non-toxic in oral administration on male and female white mice and shows no cytotoxicity at intestinal epithelium *in vitro* model (cell line HT-29).

20.07.2009

Signature: (illegible)

Prof. dr. N. Danchev, D.M. Head of Department

"Pharmacology, Pharmacotherapy and Toxicology", Faculty of Pharmacy, Sofia

Ch. assistant dr. Georgi Momekov, Signature (illegible)

Ch. assistant dr. I. Nikolova, D.M. Signature (illegible)

Laboratory Technician M. Yordanova Signature (illegible)