



**SUBCHRONIC ORAL TOXICITY-  
RODENT: 90 DAY STUDY IN RATS  
ACCORDING TO OECD-  
GUIDELINE 408 and EFSA Guidance  
on conducting repeated-dose 90-day oral  
toxicity study in rodents on whole food/feed**



Issue No. ....  
Page No.: 1/4

## **90-day feeding study of rats with Monsanto MON 810 maize**

### **Amendment to the Study plan Study No: 311957 - A / 13/ GLP**

#### **Amendment No1**

**3.12.2013**

#### **Reason for the amendment:**

**Deviations from the Study plan No: 311957 - A / 13/ GLP. Any deviation from the study plan is marked in red and the respective page number is given**

#### **1. Page 1 (affiliation changed):**

**Test Site 1:** TOPALAB, s.r.o., Kameničná 7, 04015 Košice  
**Histopathology** Laboratory of histopathology, Komenského 73,  
041 81 Košice

#### **2. Page 14 (time schedule specified in more detail)**

Test feeds arrival		Planned date: March 18-20, 2013
Arrival of animals		March 27, 2013
Starting of the treatment	Males 1-8 of each group	April 2, 2013
		April 3, 2013
	Males 9-16 of each group	
	Females 1-8 of each group	April 4, 2013
	Females 9-16 of each group	April 5, 2013





**SUBCHRONIC ORAL TOXICITY -  
RODENT: 90 DAY STUDY IN RATS  
ACCORDING TO OECD -  
GUIDELINE 408 and EFSA Guidance  
on conducting repeated-dose 90-day oral  
toxicity study in rodents on whole food/feed**



Issue No. ....  
Page No.: 3/4

1. personal disposition for forced progress (40 animals per day will be necropsied) :

- In the special laboratory animals will be **anaesthetized** by person No. 1
- **Blood taking** from the abdominal vessel will be done by person No. 2
- **Blood processing** and transport to the competent laboratory – person No. 3
- **Animal transport** to the necropsy room on the same floor by person No. 4
- Removal and weighing of **selected organs** for "omics" study and their preparation - person No. 5
- Necropsy of **thorax part** body - person No. 6
- Necropsy of **abdominal part** body - person No. 7
- Necropsy of **genital organs** - person No. 8
- **Weighing of organs** in line with OECD guideline - person No. 9
- Decapitation and necropsy of the **head** including brain by person No. 10
- All organs will be stored into Formaline except omics samples which are immediately frozen in liquid Nitrogen and stored at -80°C - controlled by person No. 11
- All steps are inspected by QA

## **8. Page 23 (Haematology)**

At the end of the study, **few days** before sacrifice, blood samples from the tail vein will be taken from all animals for haematological examination **after 12 hours fasting**. EDTA will be used as anticoagulant. Blood samples will be stored under room temperature (17-25°C) maximum up to 4 hours until measurement. Haematological analysis will be performed in accordance with SOP: ŠPP/IMU/M002 using Haematological analyzer Sysmex K-4500, SYSMEX TOA Medical Electronics Co. LTD, Japan.

Parameters scheduled for examination are

- Erythrocyte Count (RBC)
- Haematocrit (HT)
- Haemoglobin (Hb)
- Mean Corpuscular Haemoglobin (MCH)
- Mean Corpuscular Haemoglobin Concentration (MCHC)
- Mean Cell Volume (MCV)
- Leukocyte Count (WBC)
- Differential Leukocyte Count
- Platelet Count (PLT)

- The assessment of “Activated Partial Thromboplastin Time and Prothrombin Time (PT) from citrate-treated plasma” was not included, as they cannot be measured at SZU

## **9. Page 24 (Clinical Chemistry)**

- At the end of the study, **in anaesthesia** before sacrifice, blood samples from the **abdominal vessel** ~~tail vein~~ will be taken from all animals for blood chemistry examination after **18** ~~12~~ hours fasting.

- Parameters will include total protein (TP), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP), creatinine (CREA), urea **nitrogen**, fasting blood glucose, total bilirubin (TBIL), total cholesterol, triglycerides, Na, K, Ca, Cl, P.



**SUBCHRONIC ORAL TOXICITY-  
RODENT: 90 DAY STUDY IN RATS  
ACCORDING TO OECD-  
GUIDELINE 408 and EFSA Guidance  
on conducting repeated-dose 90-day oral  
toxicity study in rodents on whole food/feed**



Issue No. ....  
Page No.: 4/4

### **10. Page 27 (Histopathology)**

Organs and tissues preserved in neutral buffered 10% formalin will be shipped to TOPALAB for histopathological evaluation in accordance with SOPs: TPLB 08 – Tissue processing; ŠPP TPLB 09 - Procedure for histopathological slides evaluation. Complete microscopic examination of the tissues listed above will be performed in accordance with the OECD TG 408 on 16 animals ~~from each group~~ the high dose (33% inclusion of DKC6667-YG) and control group (33% inclusion of DKC6666). The different treatment groups were identified by Gijs Kleter without informing SZU and the histological laboratory about the identity of each group.

### **11. Page 29 (Archiving)**

Under the Code Number 311957 - A / 13/ GLP

### **12. Page 30 (Distribution)**

- ~~1 Copy~~ Issue No 1: Sponsor
- ~~1 Copy~~ Issue No 2: Study Director
- ~~1 Copy~~ Issue No 3: Study Monitor
- ~~1 Copy~~ Issue No 4: QA Manager