



**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.: 1/3

90-day feeding study of rats with PioneerMON 810 maize

Amendment to the Study Plan No: 311957 - B/ 13/ GLP

Amendment No1

3.12.2013

Reason for the amendment:

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

1. Page 14 (time schedule specified in more detail)

Test feeds arrival		Planned date: April 8-10, 2013
Arrival of animals		April 10, 2013
Starting of the treatment	Males 1-8 of each group	April 16, 2013
	Males 9-16 of each group	April 17, 2013
	Females 1-8 of each group	April 18, 2013
	Females 9-16 of each group	April 19, 2013
Necropsy of the animals in successive steps		July 15 - 18, 2013
Histology	Slides preparation	August 30 – September 30, 2013
	Histology evaluation	September 30 – December 15, 2013
Final report – draft to Sponsor:		January 2014



**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.: 2/3

2. Page 22 (Ophthalmologic examination)

Using an ophthalmoscope, we will examine the eyes of all animals prior to the administration of the test feeds and ~~at the termination of the study in the week 12 (for technical and organisational reasons)~~

3. Page 23 (PROCEDURES FOR SAMPLE COLLECTION)

Sample collection and tissue processing will be more detailed:

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 Autopsy 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

4. Page 23 (Haematology)

At the end of the study, ~~3 - 5 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~~



**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.: 3/3

5. Page 24 (Clinical Chemistry)

- At the end of the study, in anaesthesia before sacrifice, blood samples from the ~~tail-vein~~ abdominal vessel will be taken from all animals for blood chemistry examination after 12-18 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 bilirubine (TBIL), total cholesterol, Triglycerides, Na, K, Ca, Cl, P.

6. 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 animals from ~~each group~~ the high dose (33% inclusion of PR33D48) and control group (33% inclusion of PR32T16). The different treatment groups were identified by Gijs Kleter without informing SZU and the histological laboratory about the identity of each group.