Assessment and Further Discussion on Relevance of Perceived Elevation in Testicular Atrophy for [redacted] Project Number 2060/0011 (Glyphosate Technical: Mouse Oncogenicity Study)

Introduction

A question has been raised concerning the perceived increased incidence of testicular atrophy in mice exposed to technical Glyphosate via dietary inclusion at a dose level of 5000 ppm for a continuous period of eighteen months when compared with control animals. This report will demonstrate that the statistically significant effect reported in the original report for "testis 2" is an artefact created by inappropriate data management and that there is in fact no effect on testes atrophy at all.

Details

A statistical significance was seen for the incidence of testicular atrophy (irrespective of severity grade) for "Testis 2" at the highest exposure level compared with controls. A similar finding was not observed for a similar assessment for "Testis 1". The following will clarify the observation and provide rationale for a further analysis of the data:

1. Clarification of the term Testis 1 and Testis 2. At macroscopic *post mortem* examination of animals at both interim (decedent) and study termination (terminal kill) necropsy, the dissected tissues were weighed and/or preserved in fixative for subsequent histology and histopathological examination: The method of tissue preservation and histological preparation of the testes was as follows:

    I. Both testes were preserved in the same tissue sample pot. No attempt was made to differentiate the origin of each testis from the left or right side of the body.

    II. A histological preparation both, testes were embedded in the same wax block. At this point it must be emphasised that the positioning of each testis (from either left or right side of the animals body) within the block was entirely random.

    III. Subsequent slide production from wax blocks produced samples of sections of both testes but with no indication from what side of the body they were derived.

Histopathological examination of slides of testes therefore, was conducted in a standard fashion. Lack of knowledge of origin of location of each testis (either right or left side of the body) has meant that the pathologist randomly assigned the terminology.

Testis 1 First testis on slide examined.

Testis 2 Second testis examined.

As a consequence of this randomised approach in the preparation and examination of this paired organ (testis) there occurred a random and entirely fortuitous difference in incidence of testicular atrophy in Testis 2 for treated animals compared with controls but not a similar finding for Testis 1.

2. Re-Analysis of the data

Since there is no distinguishable difference between the tissue samples attributed the label "testis 1" from those labelled "testis 2" then clearly the only way to analyse the incidence of lesions found within these tissues is to treat them as a single source and combine the incidence.

The following table shows the incidence of unilateral or bilateral testicular atrophy of mice in the control and high dose group. Where a mouse had different grades of lesion in each testis, it is included in the more severe of the categories. The data has been checked to ensure that an individual mouse only appears once in the table below. (When treated unilaterally, a mouse with bilateral atrophy would appear in both testis 1 and testis2)

Table 2 Distribution of severity grades for testicular atrophy (unilateral/bilateral).

Severity Grade	Incidence of unilateral or bilateral testicular atrophy	
	Control 0 ppm	High Dose 5000 ppm
Total examined	39	35
Absent	15	18
Minimal	13	8
Slight	4	3
Moderate	1	1
Marked	4	4
Severe	2	1
Total affected (regardless of grade)	24	17

The distribution of severity grades for combined testis results shows no significant trend towards increased severity of finding. In addition the majority of affected animals were for minimal severity only. Due to the clear lack of any effect, no statistical analysis of this data has been performed.

Conclusion

The method of data presentation in the study report ([redacted] project number 2060/0011) where the individual testis identification at Histopathology has resulted in an apparent increased incidence of testicular atrophy at the highest dose level. A re-evaluation of the data has shown that the original differentiation of the data into arbitrarily assigned "Testis 1" and "testis 2" was not appropriate. The data has been re-presented as a combined testes finding whereby the incidence and distribution of severity grades for the paired organ have been presented. This demonstrates that there is no increase in incidence of testicular atrophy for animals receiving 5000 ppm, the highest administered dose when compared to the controls..

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