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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Phosmet Mouse Oncogenicity Study - Response to Concerns Raised by Esther Rinde

Tox. Chem. No. 543

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On August 13, 1986, Jack Quest requested, on your behalf, that I respond in writing to several concerns* raised by Esther Rinde regarding the results of the phosmet mouse oncogenicity study. These concerns focused on the apparent increase in the incidence of two tumor types in treatment groups of male $B6C_3F_1$ mice: adenoma of the Harderian gland and lymphoma of the lymph nodes. The two concerns will be addressed in sequence.

Concern #1: Increased Incidence of Harderian Gland Adenomas in Male $B6C_3F_1$ Mice.

* COPY ATTACHED.

The following data were abstracted from Appendix C, table 3 (Terminal Sacrifice) of the report.

Incidence of Harderian Gland Adenomas in Male $B6C_3F_1$ Mice at Terminal Sacrifice (Days 366 to 736)

	Dose (ppm)			
Tumor Type	0	5	25	100
Adenoma	3/49 (6%)	7/50 (14%)	4/49 (8%)	9/50 (18%)*
Adenocarcinoma	0	2/50 (4%)	0	0
Adenoma or adenocarcinoma	3/49 (6%)	9/50 (18%)	4/49 (8%)	9/50 (18%)*

(No Harderian gland tumors were observed prior to Day 366.)

An informal opinion was obtained from Bert Litt concerning the statistical significance of the occurrence of these tumors. He expressed the opinion that there was a significant positive trend for adenomas, and for adenomas and adenocarcinomas combined. In addition, there was a statistically significant difference between the 0 and 100 ppm groups with respect to adenomas. There was also a statistically significant difference between the 0 and 5 ppm groups with respect to adenomas and adenocarcinomas combined. His overall opinion was that "all the damage was done beginning at the 5 ppm level."

The following issues were considered in the analysis of these data:

1. Was there a statistically significant increase in Harderian gland tumors?

   There was a statistically significant increase in the incidence of adenomas in the 100 ppm group and in the incidence of adenomas and adenocarcinomas in the 5 ppm group when compared to controls. However, it is probably inappropriate to combine adenomas and adenocarcinomas in the analysis. Adenocarcinomas occur only in the 5 ppm group, not in the 25 and 100 ppm groups, thus this tumor type is not likely to be related to treatment. No significance was achieved when the incidence of adenomas in the 25 ppm and control groups were compared. There is a potential that the increased incidence of adenomas observed in the 100 ppm group may be a high-dose effect.

* It should be noted that the incidence is 9/60 (15%) if one includes the mice sacrificed at 12 months.

2. Was there a dose-response relationship demonstrated?

   There was a positive trend for the occurrence of adenomas. However, there was considerable variability in the incidence of adenomas among the control and treatment groups. The incidence was moderate in the control (6%) and 25 ppm (8%) groups. But the incidence was high in the 5 ppm (14%) and 100 ppm (18%) groups. Normally, it would be expected that the tumor incidence would increase in direct proportionality with increasing dose. This did not occur with respect to the 5 and 25 ppm groups even though there was a fivefold difference between the dose levels.

3. Was there a decrease in latency period as dose increased?

   One mouse in each of the 5 and 25 ppm groups was observed to have Harderian gland tumors that died prior to terminal sacrifice. The mouse in the 5 ppm group with an adenocarcinoma died on day 678, whereas the mouse in the 25 ppm group with an adenoma died on day 611. No mouse in the 100 ppm group with a Harderian gland tumor died prior to sacrifice. Hence, there was no apparent decrease in the latency period. This estimate of the latency period is necessarily poor due to the excellent survival of mice in all groups which would equate to poor sampling across time.

4. Was there an increase in the degree of malignancy as dose increased?

   Adenocarcinoma of the Harderian gland occurred in two mice in the 5 ppm group. There were no occurrences of adenocarcinomas in mice in the 25 and 100 ppm groups indicating no increase in malignancy as dose increased.

5. Was there an increase in the incidence of Harderian gland tumors above that reported for historical controls?

   Historical control data reported by Goodman et al. (1985) using 2343 male mice from studies conducted by NTP indicate a mean of 2.1% for Harderian gland adenomas with a range of 0 to 12%. For adenocarcinomas, the mean was 0.1% with a range of 0 to 2%. The incidence of adenomas in the 5 and 100 ppm groups

exceeded the historical control range. The incidence of adenocarcinoma in the 5 ppm group exceeded by twofold the historical control range. Although the incidence of adenomas in the 5 and 100 ppm groups exceed the range of historical control data, the use of historical control data for this tumor type may be of limited value since the incidence of a related tumor type, adenocarcinoma, was double that of historical control data and its occurrence was clearly unrelated to treatment.

In conclusion, the occurrence of adenoma of the Harderian gland varies considerably from group to group and probably reflects normal biological variation.

Concern #2: Increased Incidence of Lymphoma in Lymph Nodes in Male $B6C_3F_1$ Mice.

The following data were extracted from Appendix C, table 3 (Terminal Sacrifice) of the report. The incidence of lymphoma of the lymph nodes in male mice in the 0, 5, 25, and 100 ppm groups was 2/48 (4%), 2/18 (11%), 8/18 (44%), and 0/48 (0%), respectively.

Lymphoma occurs at a wide variety of sites, such as the mesenteric lymph nodes, Peyer's patches, spleen, and liver (Goodman et al. 1985). To limit the analysis of the occurrence of lymphoma to one site (lymph nodes) is an oversimplification which can, as in this case, lead to an erroneous conclusion. Although, not directly indicated in the review of the phosmet mouse oncogenicity study, an analysis of the incidence of lymphoma was conducted by examining its occurrence in each of the 480 mice in the study. This was accomplished by examining each individual animal's histopathology sheet for all tissue examined. (This information could not be obtained by a cursory examination of the summary data presented in Appendix C, table 3 [Terminal Sacrifice].) Thus an extensive evaluation has been reconducted. The incidence of lymphoma in the 0, 5, 25, and 100 ppm groups at "terminal sacrifice (Days 366 to 736)" is 3/49 (6%), 2/50 (4%), 8/49 (16%), and 1/50 (2%), respectively. Lymphoma was not observed prior to Day 366, as expected, since lymphoma rarely occurs in $B6C_3F_1$ mice prior to 18 months (Goodman et al. 1985).

An informal opinion was obtained from Bert Litt concerning the significance of the occurrence of this tumor. He concluded that there were no significant differences when the phosmet treated groups were compared (pairwise) to the control group.

Historical control data obtained from 2343 male $B6C_3F_1$ mice used in NTP studies indicate a mean incidence of 12.7 percent and range of 2 to 32 percent (Goodman et al. 1985). Two mice in the 25 ppm group with lymphoma died prior to terminal sacrifice. Mice with lymphoma in all other groups survived to sacrifice. There was no decrease in the latency period as dose increased.

In conclusion, the incidence of lymphoma was not increased in male $B6C_3F_1$ mice by the administration of phosmet in the diet.

This reviewer acknowledges that the apparent increase in the incidence of adenoma of the Harderian gland may be subject to several interpretations. However, in the absence of any arguments to the contrary we believe our interpretation to be correct. Additionally, it is our opinion that the occurrence of lymphoma was unrelated to treatment is self-evident.

References

Goodman, D.G.; Boorman, G.A.; Strandberg, J.D. (1985) Selection and use of the $B6C_3F_1$ mouse and F344 rat in long-term bioassays for carcinogenicity. In Handbook of Carcinogen Testing.

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