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

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NOV - 5 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Phosmet - Potential Oncogenicity

Tox. Chem. No. 543

FROM: William B. Greear, M.P.H. William B. Greear 10/28/86
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: George LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C) ABK 11/3/86

and

Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

This memorandum is being written to clarify the issue concerning the potential oncogenicity of phosnet. On June 30, 1986, the Peer Review Committee met in order to deliberate on the potential oncogenicity of phosmet. The committee determined that phosmet is a "tentative Class C oncogen." The conclusions reached by the Peer Review Committee always supercede the conclusions reached by individual reviewers and are thus, definitive (refer to SOP #4000 - Toxicology Branch Peer Review). The committee recommended that the rat oncogenicity study be repeated and that several mutagenicity studies, including mammalian cells in culture, be conducted. A copy of the Peer Review Committee memorandum on phosmet is attached.

Attachment

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

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OCT 21 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Phosmet (Imidan)

FROM: John A. Quest, Ph.D., Team Leader JAQ
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: George LaRocca, Product Manager #15
Insecticide Rodenticide Branch
Registration Division (TS-769)

The Toxicology Branch Peer Review Committee met on June 30, 1986 to evaluate the data base on Phosmet, with particular reference to the oncogenic potential of the chemical.

A. Individuals in Attendance:

1. Peer Review Committee (Signatures indicate concurrence with peer review unless otherwise stated.

Theodore M. Farber

William Burnam

Richard Hill

Reto Engler

Bernice Fisher

Diane Beal

for Robert Beliles

Judith Hauswirth

John A. Quest

Esther Rinde

2. Scientific Reviewers: (Non-Committee members responsible for presentation of data; signature indicate technical accuracy of Committee report.)

Albin B. Kocialski

William B. Greear

3. Peer Review Committee in absentia: (Committee members who were not able to attend the discussion; Signatures indicate concurrence with the overall conclusions of the Committee).

Anne Barton

Reto Engler

Stephen Johnson

Bertram Litt

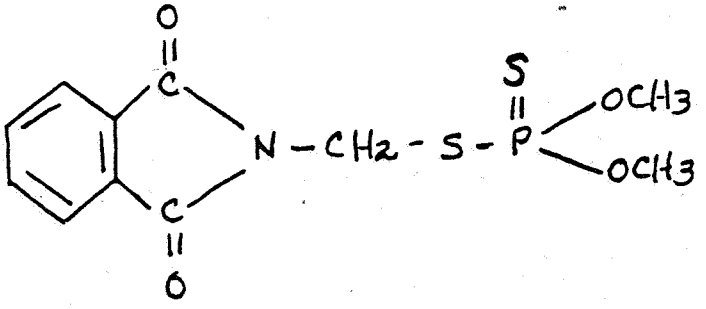
B. Material Reviewed:

The material available for review consisted of an overview summary of the data (Greear/Kocialski memorandum of June 13, 1986), and appendices to the above data summary relating to the metabolism of Phosmet, structure-activity relationships of similar chemicals, summary tables on the incidence of tumors in mice, Toxicology Branch statistical analyses of the mouse tumors, historical control data, DER's of toxicology studies, "one-liner" information on the Phosmet data base, and a copy of the WHO report (1976 meeting) on Phosmet.

C. Overview of Toxicology Issues:

Phosmet is a systemic broad-spectrum organophosphate insecticide and acaricide that is registered for use on a variety of crops and on domestic animals. It is currently available under the trade names Imidam, Appa, Prolate, and R-1504. The registrant for the chemical is Stauffer Chemical Company, Richmond, California. The primary oncogenicity concern of the Peer Review Committee on the chemical was the finding of an increased incidence of liver adenomas and carcinomas in male and female $B6C3F_1$ mice at the highest dose level tested in a two-year study performed by Stauffer Chemical Company from April 1981 to May 1983.

Structure:



[N-(mercaptomethyl)phthalamide S-(o,o dimethyl phosphosodithioate]

D. Evaluation of the Evidence:

1. Mouse Oncogenicity Study of Phosmet:

Phosmet was administered in the diet to 60 $B6C3F_1$ mice/sex/dose level at doses of 0, 5, 25 and 100 ppm for 2 years. Ten mice/sex/dose group underwent interim sacrifice at 12 months after the start of the study. The study was conducted by Stauffer Chemical Company between April, 1981 and May, 1983. The following incidence patterns of liver tumors in male and female mice suggestive of a compound related effect were observed.

Liver Tumor Type & Observation Time	Sex	Dose (ppm) 0	5	25	100
Interim Sacrifice[a]					
Adenoma	M	0/11(0%)	1/10(10%)	1/10(10%)	2/10(20%)
Carcinoma	M	0/11(0%)	0/10(0%)	1/10(10%)	1/10(10%)
Combined	M	0/11(0%)	1/10(10%)	2/10(20%)	3/10(30%)T
Final Kill[b]					
Adenoma	M	9/49(18%)	9/50(18%)	11/50(20%)	19/50(38%)T*
Carcinoma	M	14/49(28%)	11/50(22%)	10/50(20%)	13/50(26%)
Combined	M	23/49(47%)	20/50(40%)	21/50(42%)	32/50(64%)T
Total (Interim Sacrifice & Final Kill)[c]					
Adenoma	M	9/60(15%)	10/60(17%)	12/60(20%)	21/60(35%)T*
Carcinoma	M	14/60(23%)	11/60(18%)	11/60(18%)	14/60(23%)
Combined	M	23/60(38%)	21/60(35%)	23/60(38%)	35/60(58%)T*
Final Kill[b]					
Adenoma	F	5/49(10%)	4/50(8%)	5/48(10%)	9/50(18%)T
Carcinoma	F	5/49(10%)	4/50(8%)	3/48(6%)	9/50(18%)T
Combined	F	10/49(20%)	8/50(16%)	8/48(16%)	18/50(36%)T
Total (Interim[c]	F	5/60(8%)	4/60(7%)	5/58(9%)	9/60(15%)
Sacrific & Final	F	5/60(8%)	4/60(7%)	3/58(5%)	9/60(15%)
Kill)	F	10/60(17%)	8/60(14%)	8/58(14%)	18/60(30%)

a = Interim sacrifice values were obtained at study week 52 for 10 mice/sex/dose level except for the control group where 11 mice/sex were sacrificed.

b = Final kill values include mice sacrificed at study were 104 plus all intercurrent deaths occurring after study week 52.

c = Total values include interim sacrifice value plus final kill and intercurrent deaths occurring after study week 52.

T Signficant ($p < 0.05$) positive dose-related trend using Cochran-Armitage Trend Test.

* $p < 0.05$ compared to controls using Fisher-Exact Test.

An elevated incidence of liver tumors was observed in both male and female $B6C3F_1$ mice. In the male mice, tumors were observed both at the interim kill period (52 weeks) and at the time of final sacrifice (104 weeks). At the interim kill period, there was a statistically significant ($p < 0.05$) positive trend for adenomas/carcinomas combined. In addition, at the final sacrifice period, there were statistically significant ($p < 0.05$) positive trends for adenomas per se, and for adenomas/carcinomas combined. The incidence of adenomas was also significantly ($p < 0.05$) elevated in high dose male mice, whereas the incidence of carcinomas was not increased. When the total number of animals for which tissues were histologically examined at interim sacrifice plus the final kill period were evaluated, there were also statistically significant ($p < 0.05$) positive trends for adenomas per se, and for adenomas/carcinomas combined. The incidences of adenomas per se, and adenomas/carcinomas combined, were also significantly ($p < 0.05$) elevated in high dose male mice. In addition, there was evidence for liver hyperplasia in the high dose males as indicated by the appearance of clear cell foci both at interim sacrifice (0% controls, 0% low dose, 0% mid dose, and 10% high dose) and at final sacrifice (8% controls, 12% low dose, 10% mid dose, and 26% high dose). In the female mice positive trends for tumors were observed only at the time of final sacrifice (104 weeks) but not at the interim kill-period (52 weeks). At the final sacrifice period, there were statistically significant ($p < 0.05$) positive trends for adenomas per se, carcinomas per se, and for adenomas/carcinomas combined. However, none of these individual tumor types were significantly elevated in high dose female mice. Finally, as shown in the table, results similar to these were obtained in female mice when the total number of animals for which tissues were histologically examined in the study at interim sacrifice plus the final kill period were evaluated. Although, there was some indication of liver cell proliferation in females at interim sacrifice when no tumors occurred (i.e., clear cell foci occurred in 0% of control, 0% low dose, 9% mid dose, and 20% high dose females), there were no clear cell foci observed in female livers at the final sacrifice period when positive trends for tumors were noted. (According to Dr. Kasza, the occurrence of hepatic clear cell foci represents a proliferative change which may or may not progress to real hyperplasia.)

In summary, Phosmet produced an increase in hepatocellular adenomas (also reflected as an increase in the incidence of adenomas/carcinomas combined) at the highest dose level tested (100 ppm) in male mice. No significant increase in carcinomas occurred, indicating that there was

no progression of benign tumors to malignancy. However, there was evidence for liver cell proliferation in male mice. There was also an indication from the interim sacrifice results that the liver tumors occurred in male rats with a reduced latency. Phosmet also produced positive trends for adenomas, carcinomas, and both tumor types combined, in female mice, but none of these tumors were significantly elevated at the highest dose level tested (100 ppm), there was no hyperplasia, and no indication that the tumors occurred with a reduced latency period.

The Phosmet liver tumor data was compared with historical control tumor data from the NTP data base (Goodman et. al., Handbook of Carcinogen Testing, pg. 291, 1985). For male mice the comparison indicated that at the time of the final kill, the increased incidences of adenomas (38%) and adenomas/carcinomas combined (64%) seen in high dose male mice exceeded the historical range of these tumor types seen in the NTP studies (i.e., range of 0-24% for adenomas, and range of 16-58% for adenomas/carcinomas combined). For female mice the comparison indicated that at the time of the final kill, the increased incidences of carcinomas (18%) and adenomas/carcinomas combined (36%) seen in high dose female mice exceeded the historical range of tumor types seen in the NTP studies (i.e., range of 0-15% for carcinomas, and range of 0-20% for adenomas/carcinomas). The Committee also had historical data available from a single study in $B6C3F_1$ mice conducted at Stauffer Chemical Company but found it to be of limited use since only average tumor values and therefore no range data were available.

In the chronic mouse bioassay, the highest dose (i.e., 100 ppm) tested appeared to approximate a MTD level in both males and females. The effects seen at this dose included: a) reductions in plasma and brain cholinesterase activity; b) increases in liver weight in males ; c) microscopic pathological changes in the livers of male rats (degeneration and vacuolation of individual cells and foci of clear cells) and in female rats (midzonal degenerative vacuolation, necrotizing inflammation, and cell necrosis of individual liver cells); d) convulsions, stomach mucosal hyperplasia, testicular atrophy, and perivasculitis of muscle in males; and e) inflammation of the stomach and duodenum, and myometrial atrophy of the uterus in females.

2. Rat Oncogenicity Study of Phosmet:

Phosmet was administered in the diet to 25 Charles River strain albino rats/sex/dose level at doses of 0, 20, 40, and 400 ppm for 2 years. The animals were originally fed doses of 0, 10, 20, and 200 ppm for the first 3 weeks after which the doses were increased to compensate for differences in food intake. The study was conducted by Stauffer Chemical Company in the 1960's. No tumors were observed that were considered to be related to treatment with Phosmet. However, the Committee noted that there appeared to be a larger proportion of rats sacrificed at the end of the study in the mid and/or high dose groups that showed be presence of pituitary adenomas and thyroid adenomas. The incidences of pituitary adenomas were: a) Males: 3/10 or 30%, control; 1/10 or 10%, low dose; 6/10 or 60%, mid dose; and 4/7 or 57%, high dose; and b) Females: 6/7 or 35%, controls; 3/13 or 23%, low dose; 5/10 or 50%, mid dose; and 6/12 or 50%, high dose. The incidences of thyroid adenomas were: a) Males: 0/13 or 0%, controls; 0/13 or 0%, low dose; 2/13 or 15%, mid dose; and 2/14 or 14% high dose; and b) Females: 1/19 or 5%, controls; 2/14 or 14%, low dose; 0/19 or 0%; mid dose; and 2/16 or 13%, high dose. The Committee recognized that the numbers of animals sacrificed at the end of the study were too small to fully evaluate these tumor responses. In addition, although the study might be considered acceptable according to Toxicology Branch criteria for studies conducted many years ago, it is inadequate according to modern standards in terms of the number of surviving animals available for gross and histopathological examination. As a consequence, and in order to provide for a more complete data base on the oncogenicity of Phosmet, the Committee recommended that a new rat oncogenicity be initiated for the chemical. This recommendation was reached with the particular concern that new uses for Phosmet may be requested by the registrant in the future.

The limited data available for the rat study made it difficult to determine whether or not an MTD level was tested. The toxicological effects that were reported at the highest dose level tested, i.e., 400 ppm, included decreases in plasma, brain and erythrocyte cholinesterase levels of greater than 20% (the Committee recognized that changes of + 20% in these parameters are common based on experimental technique), weight loss ranging from -13% to -17% in males and 0% to -10% in females at various intervals during the study, and "moderate" liver cell vacuolation. Based on the data available the Committee determined that the mid dose level, i.e., 40 ppm was a

NOEL, and that the highest dose level tested, i.e., 400 ppm, may have been close to a MTD level. This observation, however, did not dissuade the Committee from recommending that a repeat oncogenicity study be performed in the rat using larger numbers of animals per dose group (i.e., according to Subpart F Guidelines).

E. Additional Toxicity Data:

1. Two-Year Dog Toxicity Study:

A 2-year study of Phosmet was conducted in purebred Beagles. The chemical was administered in the diet to 3 dogs/sex/dose level at doses of 0, 20, 40 and 400 ppm. This NOEL was 40 ppm. The LEL was 400 ppm based upon the finding of brain and erythrocyte cholinesterase inhibition. No unusual target organ effects were observed.

2. Metabolism Studies:

Three similar metabolism studies were performed in which single oral doses of ^{14}-C Phosmet (doses ranging from 19 to 31 mg/kg) were administered to Long-Evans rats. The animals were sacrificed 3 to 5 days after dosing. Absorption from the GI tract appeared to be rapid and fairly complete. After 72 to 96 hours, approximately 78-79% of the administered radioactivity (RA) was elminated in the urine and approximately 18-19% of the RA was eliminated in the feces. The major water soluble urinary metabolites have been tentatively identified as phthalamic acid (51-54%), phthalic acid (16-21%), and a derivative of phthalic acid (7-9%). The major water soluble metabolite tentatively identified in the feces was phthalamic acid. No unchanged Phosmet was found in the urine. Less than 0.04% of the RA was eliminated as CO_2. Tissue residues accounted for 2.6 to 3.5% of the administered RA. There did not appear to be any selective localization of RA in any tissue.

3. Mutagenicity Assays:

Phosmet was evaluated in several mutagenicity tests. The chemical was found to be positive only when tested in S. typhimurium strain TA-100 without metabolic activation. In contrast, the chemical was found to be negative in all other tests. There included tests in S. typhimurium strains TA-98, TA-1535, TA-1537 and TA-1538 with and without metabolic activation, a test in S. typhimurium strain TA-100

with metabolic activation, and a reversion assay with E. coli strains B/r WP2 hcr^+ and WP2 hcr^- and a rec^- assay with B. subtilis H17 Rec^+ and M45 Rec^- without metabolic activation. The Committee noted that no mutagenicity study of Phosmet was performed in mammalian cells in culture.

4. Reproduction and Teratology Studies:

The Committee briefly considered data from several reproduction/teratology studies that were in the "one-liner" information on Phosmet. In a 3-generation reproduction study in rats, no adverse reproductive effects occurred at the HDT (80 ppm). In a one-generation reproductive/teratology study in rabbits, no adverse reproductive or teratological effects occurred at the HDT (60 mg/kg). In four other teratology studies performed in monkeys, rats (2 studies) and rabbits, no teratologic effects were noted at HDT levels ranging from 8 to 35 mg/kg.

5. Structure-Activity Considerations:

Phosmet contains the mercaptomethylphthalimide moiety as its primary structural configuration. A computer based search on the Chemical Information System (CIS) generated a list of 16 additional chemicals that also contained this moiety (see appendix B of Greear/Kocialski memorandum of June 13, 1986 attached to this report). A literature search conducted over several National Library of Medicine databases failed to uncover any studies to indicate that any of the 16 chemicals are carcinogenic or mutagenic. Phosmet is also structurally similar to Folpet, which contains the phthalimide moiety, and is known to produce intestinal tumors in mice and to be mutagenic in in vitro systems. However, Folpet, unlike Phosmet, contains a side chain which is thought to convert to thiophosgene, a highly reactive chemical believed to be responsible for producing the intestinal tumors in mice.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding toxicology data on Phosmet to be important in a weight of the evidence determination of oncogenic potential.

1. Phosmet produced a significantly elevated incidence of hepatocellular adenomas (also reflected as an increase in adenomas/carcinomas combined) in male $B6C3F_1$ mice at the highest dose level tested (i.e., 100 ppm). The adenomas occurred with a reduced latency and exceeded NTP historical control incidences, but did not progress to carcinomas.

2. Phosmet was associated with significant positive dose-related trends for liver adenomas and for liver carcinomas in female $B6C3F_1$ mice. The tumors did not occur with a reduced latency, and were not significantly elevated at the highest dose level tested. However, the carcinomas (but not the adenomas) did exceed the NTP historical incidence for carcinomas.

3. The highest dose tested (100 ppm in the diet) in the chronic mouse bioassay appeared to approximate a maximum tolerated dose (MTD) in both males and females.

4. Phosmet was not reported to be oncogenic when administered in the diet to Charles River albino rats at doses ranging from 20 to 400 ppm. This test, however, was not considered to be an acceptable negative study by present day standards due to the fact that only very small numbers of animals were histopathologically evaluated after 2 years. The Committee recommended that a repeat rat study be performed with Phosmet.

5. Phosmet was tested for mutagenicity in a series of in vitro microbial assays. A positive mutagenic response was obtained only in one bacterial strain (S. typhimurium TA-100) whereas negative results occurred in tests in several other procaryote strains (S. typhimurium TA-1535, TA-1537, TA-1538, and TA-98; E. coli B/W WP2 hcr^+ and WP2 hcr^-; and B. subtilis H17 Rec^+ and M45 Rec^-). The Committee noted that the battery of mutagenicity studies performed on Phosmet was limited, and recommended that additional studies be performed (e.g., mammalian cells in culture).

6. Metabolism data for phosmet in Long-Evans rats indicated that phosmet, which is a lipophilic molecule, was rapidly absorbed, metabolized, and excreted primarily in the urine. The major metabolites identified in urine and/or feces were phthalamic and phthalic acids. No unusual tissue localization of the compound occurred.

7. No teratogenic effects were reported for Phosmet in oral teratology studies in monkeys, rats and rabbits. The compound has no adverse reproductive performance effects in oral reproduction studies in rats and rabbits.

8. Sixteen structural analogues of Phosmet were identified following a computerized data base search using the Chemical Information System (CIS). No carcinogenicity or mutagenicity studies were performed on any of these analogues according to a National Library of Medicine database literature search. Phosmet is also structurally

similar to Folpet, a known mutagen and oncogen, producing intestinal tumors in mice. The oncogenicity of Folpet is thought to be due to conversion of its side chain to thiophosgene; this side chain is not present in Phosmet.

G. Classification of Oncogenic Potential:

The Committee concluded that the data presently available for Phosmet provided only limited evidence for oncogenicity in animals. The conclusion was based primarily on the following: 1) Phosmet produced a significantly elevated incidence of liver tumors (adenomas, and adenomas plus carcinomas combined) in male $B6C3F_1$ mice at the highest dose level tested. These were associated with a decrease in the time to tumor occurrence. 2) The chemical was associated only with positive dose-related trends for liver adenomas and carcinomas in female $B6C3F_1$ mice. 3) Phosmet was not oncogenic in a study conducted in male and female Charles River albino rats, but the study was inadequate in design and needs to be repeated. 4) Mutagenicity testing of Phosmet was conducted in a limited number of tests and the chemical was weakly mutagenic in only one of these. Additional mammalian cell mutagenicity studies are required. 5) Numerous structural analogues of Phosmet were identified for which no oncogenicity data were available. Phosmet is also structurally related to the oncogenic fungicide, Folpet, which causes intestinal tumors in mice. However, the oncogenicity of Folpet is thought to be related to conversion of its side chain to thiophosgene, and this side chain is not present in Phosmet.

Based on the above information and the criteria contained in the proposed EPA Guidelines (CFR, November 2, 1984), the Peer Review Committee classified Phosmet as a tentative Category C (possible human) carcinogen. That is, the Committee considered that Phosmet produced benign tumors of the liver only at the HDT in males, and trends for liver adenomas and carcinomas in females, in only one strain and species of experimental animal ($B6C3F_1$ mice) and in only one experiment. In addition, the chemical was questionably mutagenic (only one positive resulted occurred in a limited and inadequate battery of tests), and no positive correlations with respect to oncogenicity and mutagenicity could be made with known structural analogues. The Committee noted that there was insufficient evidence to consider the B_2 category for carcinogenicity, but agreed to reconsider all information after the results of a repeat two-year rat oncogenicity study and additional mutagenicity studies have been provided.

AMENDMENT TO PEER REVIEW MEETING ON PHOSMET

Subsequent to the Phosmet Peer Review meeting, one of the Committee members expressed some concern over whether the occurrence of Harderian gland adenomas and lymph node lymphomas in male mice could be attributed to dietary administration of the chemical. The issue was addressed by Mr. Greear who evaluated the data and concluded that the findings were not compound-related. His analysis of the problem is attached.

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

FROM: William B. Greear, M.P.H. William B. Greear 8/29/86
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C) ABK 8/28/86

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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