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

August 18, 1999

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Cancer Assessment Review Committee on Phosmet

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on Phosmet prepared by Linda Taylor. The meeting is scheduled for September 1, 1999 at 10:00 am in Room 813, CM2. The CARC meeting on CGA 184927 is moved to September 22, 1999. The main purpose of the Phosmet CARC meeting is to determine what type, if any, of cancer risk assessment is needed.

Addressees
K. Baetcke
L. Brennecke
L. Brunsman
W.Burnam
M.Copley
K. Dearfield
V. Dellarco
V. Dobozy
R. Hill
Y. Ioannou
N. McCarroll
E. Rinde
J. Rowland
J. Stewart
C. Swentzel
L. Taylor
Y. Woo

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

Office of Prevention, Pesticides
and
Toxic Substances

MEMORANDUM

SUBJECT: **PHOSMET**: Modified Data Package For Determining the Appropriate Means for Quantifying Human Cancer Risk.

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 8/17/99
Reregistration Branch I,
Health Effects Division (7509C)

THRU: Whang Phang, Ph.D. Whang Phang 8/17/99
Branch Senior Scientist
Reregistration Branch I
Health Effects Division (7509C)

TO: Sanjivani Diwan, Ph.D.
Executive Secretary, Cancer Assessment Review Committee
Health Effects Division (7509C)

Registrant: Gowan Company
Chemical: [N-(mercaptomethyl) phthalimide-S-(O,O-dimethylphosphorodithioate)]
Caswell No. 543
P.C. Code: 059201

Previously, the HED Carcinogenicity Peer Review Committee (CPRC) [Document No. 010998] classified Phosmet as a Group C - possible human carcinogen and recommended that for the purpose of risk characterization, the Reference Dose [RfD] approach be used for quantification of human risk [memo dated 5/26/94]. This decision was based on an increased incidence of liver tumors in male B6C3F1 mice at the high dose. The increase was statistically significant by pair-wise comparison, with a statistically significant trend, and there was an apparent early onset. Female mice had a significant dose-related trend for liver tumors and for mammary gland adenocarcinomas as well. There was no evidence for carcinogenicity in an acceptable study in rats. Phosmet was determined by the CPRC to be a potent direct-acting mutagen.

Since the CPRC assessment, the Agency has developed new guidelines for risk assessment with respect to carcinogenesis, and it was determined that a Q_1^* should be generated for Phosmet for use in risk assessment. The Q_1^* for Phosmet was calculated to be 0.358 $(mg/kg/day)^{-1}$, based on male mouse liver tumors combined. **The purpose of the meeting is to determine the appropriate means for quantifying human risk [i.e., Q^*_1 approach or RfD approach].**

Attached are the two previous HED Carcinogenicity Peer Review Committee documents on Phosmet, the DER for the B6C3F1 mouse carcinogenicity study, the Qualitative Analysis of the mouse study, data tables [ChEI and organ weight data] to supplement the mouse DER, Q^*_1 e:mail, and the DER for the metabolism study in rats.

ATTACHMENT I

010998

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

MAY 25 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Phosmet (2nd)

FROM: Marion P. Copley, D.V.M., Section Head
Review Section IV
Toxicology Branch I
Health Effects Division (7509C)
Marion Copley 5/24/94
and
Esther Rinde, Ph.D. E. Rinde
Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division (7509C)

TO: George LaRocca, PM #15
Insecticide Rodenticide Branch
Registration Division (7505C)
and
Larry Schnaubelt PM #72
Re-Registration Division (7508W)

THROUGH: Penelope A. Fenner-Crisp 5/24/94
Penelope Fenner-Crisp, Ph.D.
Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on November 17, 1993 and January 26, 1994, to discuss and evaluate the weight-of-the-evidence on Phosmet with particular reference to its carcinogenic potential. The CPRC agreed that Phosmet should be classified as Group C - possible human carcinogen and recommended that for the purpose of risk characterization the Reference Dose (RfD) approach should be used for quantification of human risk.

This decision was based on an increased incidence of liver tumors in male B6C3F1 mice at the high dose, that was statistically significant by pair-wise comparison, with a statistically significant trend and which also had an apparent early onset. Female mice had a significant dose-related trend for liver tumors, and for mammary gland adenocarcinomas, as well. There was no evidence for carcinogenicity in an acceptable study in rats. Phosmet was determined by the CPRC to be a potent, direct-acting mutagen.

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A. Individuals in Attendance at one or both meetings:

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

Penelope Fenner-Crisp

Reto Engler

William L. Burnam

Marcia Van Gemert

Karl Baetcke

Kerry Dearfield

Hugh Pettigrew

Esther Rinde

Elizabeth Doyle

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Marion Copley[1]

Bernice Fisher

Lucas Brennecke[2]
(PAI/Clement)

3. Other Attendees:

Diane Mandell (Clement)

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[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

B. Material Reviewed:

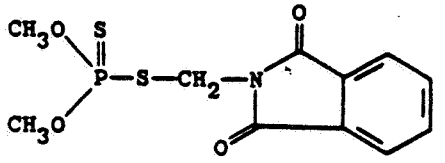
The material available for review consisted of DERs and other data summaries prepared by Marion Copley and statistical analyses prepared by Bernice Fisher. The material reviewed is attached to the file copy of this report.

C. Background Information

Phosmet [N-(mercaptomethyl) phthalimide S-(O,O-dimethyl phosphorodithioate] is a systematic, broad spectrum organophosphate insecticide and acaricide that is registered for use on a variety of crops and on domestic animals.

The Caswell (or Tox Chem) Number of Phosmet is 543.
The Chemical Abstract Registry Number (CAS No.) is 732-11-6.
The PC Code is 059201.

The structure of Phosmet is presented below:



The CPRC first met on June 30, 1986 to review the evidence for the carcinogenicity classification of Phosmet. The Committee concluded that the "data available for Phosmet provided only limited evidence for oncogenicity in animals," and classified Phosmet as a "tentative Category C (possible human) carcinogen." This was based on (1) the increased incidence of liver tumors (adenomas, and adenomas plus carcinomas combined) in male B6C3F1 mice at the highest dose tested (HDT); (2) the positive dose-related trends for liver adenomas and carcinomas in female B6C3F1 mice; (3) no oncogenic effects in an inadequate study conducted in male and female Charles River albino rats; (4) weakly positive results in one mutagenicity test; and (5) structural similarity to analogs with limited carcinogenicity data. 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 in not present in Phosmet." The CPRC agreed to reconsider all available information after the two-year rat study is repeated, and additional mutagenicity studies are provided.

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The Gowan Co. submitted new information regarding the Phosmet mouse cancer study to the Agency in a document entitled "Phosmet--Discussion Concerning Guideline Series 83-1 and 83-2 Studies" (dated August 12, 1993). This document presents the argument against the Agency's position that Phosmet is a Group C Carcinogen. The Agency's basis for this position in 1986 was a B6C3F1 mouse cancer study in which there was an apparent pair-wise increase in benign liver cell tumors in males but not females, with no pair-wise increase in malignant tumors observed in either sex. The Gowan document argues that since these findings (1) occurred in one sex only, and (2) were not substantiated by three other chronic studies (two 2-year rat studies and a 2-year dog study), they should not form the basis of our position. The document further argues that the incidence of benign tumors noted in male B6C3F1 mice is within the range of historical control data, both in-house, and the NTP data sets.

The present meeting of the CPRC was held to discuss and evaluate the following data: (1) the new rat oncogenicity study completed in 1991 by Ciba-Geigy Corporation (MRID No. 419164-01, Study No. T-13241), (2) the mouse study and newly submitted information as discussed by Gowan, and (3) six new genotoxicity studies conducted by the registrant. The first rat oncogenicity study, which was previously considered unacceptable by the CPRC on June 30, 1986, was not re-considered at this meeting.

D. Evaluation of Carcinogenicity Data

1. Rat Oncogenicity Study

Reference: RH-7592 Technical: 2-Year Chronic/Oncogenicity Study with R-1504 in Rats. MRID No. 419164-01, Study No. T-13241. Ciba-Geigy Corporation, Farmington, CT. Report issued April 15, 1991.

a. Experimental Design

Groups of Sprague-Dawley rats (Crl:CD(SD)BR) (60/sex/group, except 70/sex in controls) were fed diets containing Phosmet (95.2% purity) at doses of 0, 20, 40, and 200 ppm (equivalent dosages, males: 0, 1.1, 1.8 and 9.4 mg/kg/day, female: 0, 1.1, 2.1 and 10.9 mg/kg/day) for two years, and to 20/sex/group at 400 ppm (equivalent dosages, males: 23 mg/kg/day, female: 27 mg/kg/day) for 1 year. An interim sacrifice of 20/sex/group was conducted at 12 months; survivors were sacrificed at the termination of the study. The 40 ppm group inadvertently received 100 ppm for the first 6 weeks resulting in a time weighted average of 44.2 ppm in this group.

b. Discussion of Tumor Data

There were no significant compound-related tumors observed.

c. Non-neoplastic Lesions and Other Findings

The statistical evaluation of mortality did not indicate significant incremental changes with increasing doses of Phosmet in male or female rats. At 400 ppm in both sexes, body weight and body weight gain were decreased, though these decreases were not, for the most part, statistically significant.

An increased incidence of fatty change in male livers was observed at the 20 ppm dose and above. At 200 ppm and above, increases in the incidence of depressed hepatic foci (in males) and fatty liver change (in females) were noted; hyperkeratosis of the stomach (in males), and mineralization of the thyroid (in females) were also observed at this dose level.

Other systemic effects included decreased red blood cell (RBC) cholinesterase (ChE) levels in males at the mid and high dose levels (> 15 %) and borderline decreases in the low dose males; in males and females brain ChE activity was decreased (> 34 %) at 40 ppm and above; and at 400 ppm BUN in females was increased.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The 200 ppm dose was considered adequate for assessing the carcinogenic potential of Phosmet, based on decreased brain ChE activity in both sexes. Adequacy of dosing was supported by the non-neoplastic liver effects at doses ≥200 ppm.

2. Mouse Oncogenicity Study

Reference: T-10919: Two-Year Dietary Oncogenicity Study in Mice with Imidan Technical-Final Report. Stauffer Chemical Company. Accession No. 254608, 245609. Report issued May, 1984.

a. Experimental Design

Groups of B6C3F1 mice (50/sex/group) were fed diets containing Phosmet at doses of 0, 5, 25, or 100 ppm (male - 0, 1.0, 4 and 14 mg/kg/day; females - 0, 1.2, 5 and 18 mg/kg/day) for 2 years. An additional 10 animals/sex/group were treated and sacrificed at week 52; survivors were sacrificed at the termination of the study.

b. Discussion of Tumor Data

Male mice had increases in hepatocellular adenomas and combined adenoma/carcinoma that were statistically significant by pair-wise comparison of the HDT with controls, and a statistically significant increasing trend. Female mice had a significantly increasing trend in carcinoma, and combined adenoma/carcinoma (Tables 1 and 2).

At interim sacrifice, there was an increasing trend in the incidence of combined hepatocellular adenoma/carcinoma in male mice (Table 3). Females did not have liver tumors at this time.

Female mice also had a statistically significant increasing trend in the incidence of mammary gland adenocarcinomas (Table 4). This tumor was considered to be uncommon.

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Table 1.. Phosmet(Imidan) - B6C3F1 Male Mice, Liver Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	5	25	100
Liver Tumors				
Adenomas (%)	10/59 (17)	10[a]/60 (17)	12/60 (20)	21/60 (35)
p=	0.002**	0.437	0.245	0.008**
Carcinomas (%)	13/59 (22)	11/60 (18)	11[b]/60 (18)	14/60 (23)
p=	0.364	0.815(n)	0.843(n)	0.599
Both (%)	23/59 (39)	21/60 (35)	23/60 (38)	35/60 (58)
p=	0.002**	0.727(n)	0.585(n)	0.019*

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

a First adenoma observed at week 52, dose 5 ppm.
b First carcinoma observed at week 52, dose 25 ppm.

n Negative change from control.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ and if ** then $p<.01$.

Table 2. Phosmet(Imidan) - B6C3F1 Female Mice, Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	5	25	100
Liver Tumors				
Adenomas	5/49	4/50	5[a]/48	9/50
(%)	(10)	(8)	(10)	(18)
p=	0.061	0.487(n)	0.617	0.205
Carcinomas	5[b]/49	4/50	3/48	9/50
(%)	(10)	(8)	(6)	(18)
p=	0.046*	0.487(n)	0.369(n)	0.205
Both	10/49	8/50	8/48	18/50
(%)	(20)	(16)	(17)	(36)
p=	0.007**	0.379(n)	0.416(n)	0.066

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53 or were sacrificed at week 52.

a First adenoma observed at week 93, dose 25 ppm.
b First carcinoma observed at week 78, dose 0.

n Negative change from control.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then $p<.05$ and if ** then $p<.01$.

Table 3. Phosmet(Imidan) - *Interim Sacrifice*
B6C3F1 Male Mice, Liver Tumor Rates[+]
and Exact Trend Test and Fisher's Exact
Test Results (p values)

	Dose (ppm)			
	0	5	25	100
Liver Tumors				
Adenomas (%)	0/10	1/10 (10)	1/10 (10)	2/10 (20)
p=	0.0925	0.5000	0.5000	0.2368
Carcinomas (%)	0/10	0/10	1/10 (10)	1/10 (10)
p=	0.1306	1.000	0.5000	0.5000
Both (%)	0/10	1/10 (10)	2/10 (20)	3/10 (30)
p=	0.0436*	0.5000	0.2368	0.1053

[+] Number of tumor bearing animals/Number of animals examined.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then p<.05 and if ** then p<.01.

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Table 4. Phosmet(Imidan) -B6C3F1 Female Mice, Mammary Gland Adenocarcinoma Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	5	25	100
Mammary Gland Adenocarcinomas	1/49	0/50	1/48	5[a]/50
(%)	(2)	(0)	(2)	(10)
p=	0.007**	0.495(n)	0.747	0.107

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53 or were sacrificed at week 52.

a First tumor observed at week 92, dose 100 ppm.

n Negative change from control.

Note: Significance of trend denoted at Control.
Significance of pair-wise comparison with control denoted at Dose level.

If * then p<.05 and if ** then p<.01.

The CPRC had historical control data available from a single study in B6C3F1 mice conducted at Stauffer Chemical Company. Historical control data were also obtained from the National Toxicology Program (NTP) database. The NTP historical control data used in the last peer review document were obtained in 1986 and have since been updated. A summary of the tumor incidence and historical controls is tabulated in Table 5. Charles River data were also presented to demonstrate the highly variable nature of this tumor type in male mice.

Table 5. Historical Controls: Incidence of Hepatocellular Adenomas and Carcinomas in Male B6C3F1 Mice[a]

	Present Study		Historical Control Studies		
Liver Tumors	Phosmet HDT(%)	Phosmet Control(%)	Other study Stauffer Chem. Co.(%)[b]	New NTP Data(%)[c]	Charles River(%)
			Males		
Adenoma	35**, T**	17	42	26.4 (4-60)	17.2 (0-41.3)
Carcinoma	23	23	17	16.4 (3-29)	13.2 (4.2-24.6)
Total	58**, T**	38	52	35.2 (17-68)	Not Available
			Females		
Adenomas	18	10	18	12 (2-33)	7.1 (0-17.1)
Carcinomas	18 T*	10	6	6 (0-20)	2.4 (0-6.3)
Total	36 T**	20	22	16 (3-42)	Not Available

a Comparison historical control data in mean % (range).
b This data was obtained in 1985 and consisted of only one incomplete summary of control data.
c This data was obtained from the Gowan Co. (Document dated Aug. 12, 1993) and included all studies conducted from 1980-1987.

* $p < 0.05$ pair-wise comparison T = trend significance
** $p < 0.01$ " "

In male mice, the liver tumor incidences (adenomas, carcinomas and combined adenoma/carcinoma) exceeded the means but were all within the upper range of the NTP historical control data. The same was true for the adenoma and carcinomas relative to the Charles River historical control data (although carcinomas were near the top end reported in the latter). The incidence of combined adenoma/carcinoma in male mice (58%)[3], although within the range of 10 - 68% reported by NTP for 30 studies, exceeded the average of 36.2% and only 3 of the 30 NTP studies conducted from 1981-1984 had incidences $\geq$ 58%. The incidences of carcinoma and combined adenoma/carcinoma also exceeded the corresponding incidences in the other Stauffer study. In female mice, the liver tumor incidences (adenomas, carcinomas and combined adenoma/carcinoma) exceeded the means of the NTP data, and the incidence of carcinomas was near the top end of the range. The incidences of adenomas and carcinomas were also outside of the range of Charles River historical control data and the incidences of carcinomas and combined adenoma/carcinoma exceeded the corresponding incidences in the other Stauffer study.

It was further noted that the incidences of liver tumors in concurrent control mice of both sexes were near or slightly above the average of the ranges reported for historical controls. The increases found in treated animals could thus not be attributed to unusually low incidences in the concurrent controls.

In considering the incidences of liver tumors at the interim 1-year sacrifice in the male mice, the CPRC referred to interim data from 7 NTP studies[3] started between 1978 and 1979 in which the combined liver tumor incidence rates at 40-62 weeks were 0-6%. The combined liver tumor incidences at 52 weeks in the Phosmet study exceeded this range in all dose groups (10, 20, 30% at 5, 25, and 100 ppm, respectively), and there was a statistically significant positive trend.

The incidence of mammary tumors (10%) in female mice, while within the range 0-10% for 30 studies in the NTP database, exceeded the average of 1.5%; furthermore, only 1 out of these 30 studies had an incidence of 10%[3].

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[3]J.Haseman (personal communication to Kerry Dearfield).

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c. Non-neoplastic lesions and other findings

The statistical evaluation of mortality and body weight gain indicated no significant incremental changes with increasing doses of Phosmet in male or female mice. The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Clinical examination of the animals revealed a dose-response increase in the incidence of convulsions in males. At both the interim and terminal sacrifices, plasma ChE activity was inhibited about 50 % in males and females at the HDT. Additionally, brain ChE was depressed greater than 20 % in females at the HDT.

Treatment-related non-neoplastic lesions at the 12-month sacrifice included increased incidence of regenerative epithelial hyperplasia of the kidneys in HDT males and midzonal degenerative vacuolation of the liver, dilation of the uterus, and inflammation of the kidneys in HDT females.

At terminal sacrifice, there was an increased incidence of perivasculitis of the muscle, testicular atrophy, hyperplasia of the stomach mucosa, and degenerative vacuolation of individual liver cells in HDT males. Slight increases in midzonal degenerative vacuolation, necrotizing inflammation and necrosis of individual liver cells, myometrial atrophy of the uterus, and meningitis of the spinal cord in HDT females.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Dosing in this study was adequate for carcinogenicity testing, based on decreased plasma and brain ChE activity in both sexes, testicular changes in males, and liver changes in both sexes at the HDT.

e. New Information Regarding the Study

As described in Section C. (Background), a report dated August 12, 1993 was submitted to the Agency by the registrant, Gowan Company, arguing that the EPA failed to consider critical information in its evaluation of Phosmet. The report introduces new historical control data from NTP for liver tumors in B6C3F1 mice. These data include additional NTP studies conducted during the same time period as the studies given in the original NTP report. Gowan Company believes that these new NTP data are essential in evaluating the test material because they "confirm that the liver tumors observed in the Phosmet mouse study are

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toxicologically meaningless in light of the unpredictable and nearly universal incidence of spontaneous liver tumors in control B6C3F1 mice."

The CPRC considered these new data and noted that while the incidences of liver tumors (adenomas, carcinomas and combined adenoma/carcinoma) in the mouse study were within the new NTP historical control range, they exceeded the corresponding means, in both sexes. In female mice, the incidence of carcinomas was near the top end of the NTP range. The CPRC also took into account the incidence of combined adenoma/carcinoma in male mice at the interim sacrifice.

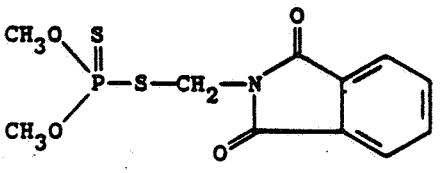
E. Additional Toxicological Data on Phosmet.

1. Structure-Activity Relationships

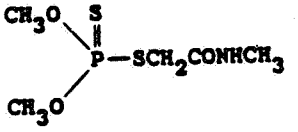
Phosmet contains the mercaptomethylphthalimide moiety as its primary structural configuration. A computer based search on the Chemical Information system (CIS) generated a list of 16 additional chemicals that also contained this moiety. Of these 16 chemicals only one, Folpet, is known to be carcinogenic or mutagenic, however it was not considered to be an appropriate structural analog for Phosmet. It was suggested (after the meeting) that a good structural analog for Phosmet would be Dimethoate, another organophosphate, which has been shown to be both carcinogenic and mutagenic. Dimethoate was associated with lymphatic tumors in male B6C3F1 mice and tumors of the skin and lymphatics in male Wistar rats.

Figure 1. Structures of Phosmet and Dimethoate

Phosmet



Dimethoate



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2. Genotoxicity

Summary of Previously-Available Data:

Phosmet was tested in a reversion assay using Escherichia coli strains B/r WP2 hcr$^+$ and WP2 hcr$^-$ and in a rec-assay with Bacillus subtillis strains H17 Rec$^+$ and M45 Rec$^-$ without metabolic activation. Phosmet was negative when tested at levels up to 20 µg dissolved in DMSO. Phosmet was tested at levels up to 5000 µg/plate in an Ames test using Salmonella typhimurium strains TA 100, TA 98, TA 1535, TA 1537 and TA 1538 and in E. coli strains WP hcr with and without metabolic activation. A positive response was obtained only in S. typhimurium strain TA 100 without metabolic activation. A dominant lethal test in the rabbit proved inconclusive.

Summary of New Data:

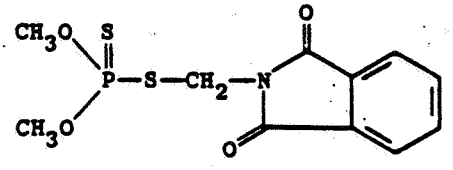
The following table summarizes the results of six new mutagenicity studies conducted and submitted on Imidan (Phosmet). All tests were graded Acceptable.

Study Title	Results
Salmonella typhimurium (TA100, TA1535) Reverse Mutation Study No. T-12819; MRID No. 00164884 March 3, 1986	Positive with and without activation
Mouse Lymphoma Forward Mutation Assay Study No. T12820; MRID No. 00164885 May 8, 1987	Positive with and without activation
Mouse Lymphoma Multiple Endpoint Test Cytogenetic Assay Study No. T 12821; MRID No. 00164886 July 13, 1987	Positive for structural chromosomal aberrations without metabolic activation Positive for SCE with and without metabolic activation
DNA Damage Assay in Human Fibroblast Study No. T 12823; MRID No. 00164887 May 21, 1987	Negative with and without metabolic activation
Morphological Transformation of BALB/3T3 Cells Study No. T-12822; MRID No. 00164888 August 12, 1986	Positive
Micronucleus Assay in Mouse Bone Marrow Study No. T86/756; MRID No. 401994-01 July 31, 1987	No clastogenic effect observed at 17 mg/kg in bone marrow cells 24, 48 or 72 hr after dosing. Dose level adequacy previously determined. Positive control (cyclophosphamide) established adequate sensitivity of test system.

Published literature also demonstrate the mutagenic activity of Phosmet in the Salmonella assay, gene mutation in mammalian cells and transformation in the SHE (Syrian hamster embryo) cells.

It should be noted that Formaldehyde, which is a probable metabolite of Phosmet, has been reported to be mutagenic in many systems (IARC Monographs on Evaluation of Carcinogenic Risks to Humans, Supplement 6, 1987). These include gene mutations in Salmonella, E.coli, yeast, Drosophila and in cultured mammalian cells without exogenous metabolic systems. Formaldehyde induces chromosome breaks, sister chromatid exchanges (SCE), structural aberrations, unscheduled DNA synthesis (UDS) and cell transformation in cultured mammalian cells without exogenous metabolic systems. In in vivo mouse micronucleus, structural aberration, mouse dominant lethal and SCE tests, results have been mainly negative (although there is an occasional positive report for aberrations and SCE).

The CPRC concluded that Phosmet is a very potent, direct-acting mutagen. This very potent in vitro activity may be attributed to the fact that Phosmet is expected to be a methylating agent, since it is a methyl ester of thiophosphoric acid. The poor correlation between carcinogenicity and mutagenicity of organophosphates may be due to rapid detoxification before they can reach their target sites[4]. Once an organophosphate loses one of its three ester groups, it is no longer an alkylating agent. The side chain in Phosmet is expected to undergo hydrolysis in acidic media (to formaldehyde and dimethyl thiophosphate) due to the presence of the methylene group between 2 heteroatoms (N & S); this may explain Phosmet's very weak carcinogenic activity despite its potent in vitro activity.



Phosmet.

[4]Woo, YT and Arcos, JC. Role of Structure-Activity Relationship Analysis in Evaluation of Pesticides for Potential Carcinogenicity. In: Carcinogenicity and Pesticides, NN Ragsdale, RE Menze, Eds. ACS Symposium Series 414. American Chemical Society, Wash. D.C. (1989).

F. Weight of the Evidence Considerations

The Committee considered the following observations regarding the toxicology of Phosmet for a weight-of-the-evidence determination of its carcinogenic potential:

1. Sprague-Dawley rats had no compound-related tumors in a study determined to have adequate dosing.

2. Phosmet produced a statistically significant elevated incidence of hepatocellular adenomas and combined adenomas/carcinomas in male B6C3F1 mice by pair-wise comparison between the HDT (100 ppm) and controls. The incidence of these tumors also occurred with a statistically significant increased trend.

   In female B6C3F1 mice there was a statistically significant increased trend for carcinomas and adenoma/carcinoma.

   At interim sacrifice, there was a statistically significant increasing trend in the incidence of combined hepatocellular adenoma/carcinoma in male mice. Females did not have liver tumors at this time.

   Female mice also had a statistically significant increasing trend in the incidence of mammary gland adenocarcinomas, a tumor type considered to be uncommon.

3. The NTP historical control database which was used in 1986 to evaluate Phosmet has since been updated. The CPRC noted that while the incidences of liver tumors (adenomas, carcinomas and combined adenoma/carcinoma) in the mouse study were within the new NTP historical control range, they exceeded the corresponding means, in both sexes. In female mice, the incidence of carcinomas was near the top end of the NTP range.

   The incidences of liver tumors in concurrent control mice of both sexes were near or slightly above the average of the ranges reported for historical controls. The increases found in treated animals could thus not be attributed to unusually low incidences in the concurrent controls.

   The incidence of mammary tumors (10%) in female mice, while within the range 0-10% in 30 studies, exceeded the average of 1.5%; furthermore, only 1 out of these 30 studies had an incidence of 10%.

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4. Phosmet was demonstrated to be a very potent, direct-acting mutagenic agent in in vitro genotoxicity tests. Formaldehyde, a probable metabolite of Phosmet, also had direct-acting in vitro activity. The poor correlation between the strength of the response of Phosmet in in vitro mutagenicity tests, compared to that obtained in the carcinogenicity studies, may be due to its rapid detoxification in vivo.

5. Phosmet is structurally related to Dimethoate, another organophosphate pesticide which has been shown to be both carcinogenic and mutagenic.

6. Carcinogenicity in animals -- Phosmet

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity based on the criteria used by the NTP[5], the Committee concludes that exposure to Phosmet resulted in a statistically significant increase in the incidence of hepatocellular adenomas and combined hepatocellular adenoma/carcinoma in male mice. There was also a statistically significant trend for these tumors in males and for hepatocellular carcinoma and combined adenoma/carcinoma and mammary adenocarcinoma in females, as well. Phosmet has been shown to be a direct-acting mutagen in many in vitro mutagenicity assays. There was no evidence for carcinogenicity of Phosmet in a rat study. The relevance of the tumor data to an evaluation of Phosmet's potential for human carcinogenicity is discussed elsewhere.

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[5]Arguments were presented for both "equivocal" and "some" evidence for the mouse, based on the NTP criteria for carcinogenicity:

> ***Some evidence*** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence. (***Clear evidence*** *of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such lesions to progress to malignancy.)*
>
> ***Equivocal evidence*** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical-related.

The majority opinion of the CPRC was that the animal evidence, using the NTP criteria as stated above, is "equivocal" for carcinogenic activity of Phosmet with studies showing a marginal increase in neoplasms in the mouse that may be chemically related. The overall consensus of the CPRC, based on the criteria contained in the EPA Guidelines in a weight-of-the-evidence determination, was that the evidence for Phosmet was "limited" and met the criteria for a Group C - possible human carcinogen. A discussion of this determination is provided elsewhere in this document (Section G).

G. Classification of Carcinogenic Potential:

The CPRC considered the criteria contained in the EPA's "Guidelines for Carcinogenic Risk Assessment" (FR51: 33992-34003, 1986) for classifying the weight of evidence for carcinogenicity.

There was much discussion on whether Phosmet should be given the classification of Group C or Group D. Arguments presented for a Group D classification were that liver tumors have a high background rate in the male B6C3F1 mice, the increases in males were considered to be marginal and occurred only at the high dose. The female liver tumor response had a statistically significant trend, but was not statistically significant by pair-wise comparison, and the response occurred only at high dose. The increases in both sexes were within the NTP historical control range. The increase in mammary gland adenocarcinomas in the females was also considered to be marginal.

The arguments for Group C were: increased incidence of liver tumors in male B6C3F1 mice at the high dose, which were statistically significant by pair-wise comparison, with a statistically significant trend and which also had an apparent early onset. Female mice had a significant dose-related trend for liver tumors. Although the incidences of these tumors were within the range of the NTP data base, they all were well above the means reported for these data. Also concurrent control incidences for liver tumors in both sexes were near or slightly above average (the increases found in treated animals could thus not be attributed to unusually low incidences in the concurrent controls). Further support for the Group C was that the incidences of tumors in male mice at the interim sacrifice exceeded the range of the NTP *interim* data base. Female mice also had a very significant trend ($p=0.007$) for mammary gland adenocarcinomas, as well. This tumor type is considered to be uncommon (10% incidence in the HDT vs 2% in concurrent controls); while the NTP data communicated to the CPRC indicated a range for this tumor of 0-10%, the average was 1.5%, and only one out of 30 studies had an incidence of 10%. Furthermore, Phosmet was determined by the CPRC to be a potent, direct-acting mutagen.

There was no evidence for carcinogenicity in an acceptable study in rats.

The consensus of the CPRC was that Phosmet should be classified as Group C - possible human carcinogen and recommended that for the purpose of risk characterization the Reference Dose (RfD) approach should be used for quantification of human risk.

ATTACHMENT II

#543

CASWELL FILE

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

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

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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

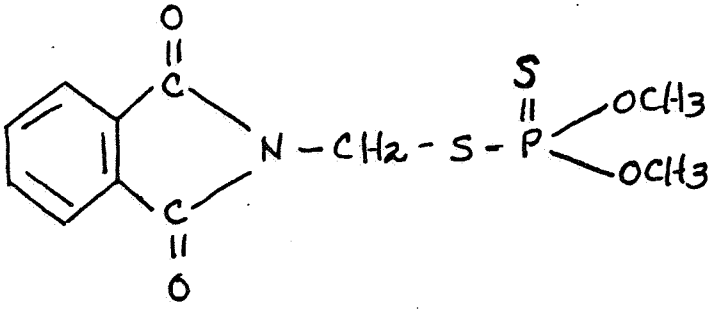
B. Material Reviewed:

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.

#13 7/23/86 sb

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 8/28/86
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.

88419:Greear:HED-06:KENCO:8/25/86:10/25/86:NeeCee:Lisa
R:88421:Greear:HED-06:KENCO:8/27/86:11/1/86:TAR:Lisa

ATTACHMENT III

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

005304

FEB 26 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imidan Technical (T-10719) - Qualitative Analysis of Mouse Oncogenicity Study

FROM: Bernice Fisher
Statistician, Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: David G. Van Ormer, Ph.D.
Toxicologist, Section II
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Bertram D. Litt, Leader, Statistical Staff
Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Summary:

Survival is not significantly affected by increasing doses of Imidan in either male or female mice. However, some early deaths with tumors are noted.

The liver tumor rates for combined number of adenomas and carcinomas, are significantly ($p < .01$) associated with increasing doses of Imidan for both sexes. The comparisons of control's liver tumor rates with the highest dosed Imidan groups support these findings with borderline results in both males ($p = .05$) and females ($p = .06$).

Background:

A 2-year dietary oncogenicity study in mice was conducted by Stauffer Chemical Company (April 1981 to May 1983). The mice, $B6C3F_1$-CRL-Br strain, were given concentrations of 0, 5, 25, and 100 ppm of Imidan Technical, respectively, for 24 months.

Sixty mice per sex were assigned by block randomization based upon weight distributions to each of the four treatment groups. At the end of 52 weeks, 10 animals were sacrificed in each of the groups.

Qualitative Analysis:

Survival analysis (see table 1A and B) indicated that mortality was not affected by increasing doses of Imidan. In females, the actual mortality trend was so obviously not dose related that further statistical analyses were not needed; while in males some additional statistical analysis was required. The Cochran-Armitage Trend test was applied first to male mortality for 81 to 104 weeks and then to the cumulative deaths that were tallied at the end of the study. No significant trends were found in either time period. In addition, the use of Fisher's Exact Test to compare all deaths in the control group with those in the highest dosed group in males for the two time periods mentioned above, did not show significant differences.

This Imidan study indicated that liver tumors alone were numerous enough to suggest statistical evidence of an effect with increasing doses . These occurrences in both males and females began only after 52 weeks of the study period and they were more or less equally distributed for adenomas and carcinomas (see table 2A and B for details). Most of the tumors were detected in the Final Kill data.

In males, adenomas were more prevalent with increasing doses of Imidan in comparison to the number of carcinomas. In females, both the adenomas and the carcinomas increased substantially with the increasing doses of Imidan during the time of the study. The liver tumor findings are shown in table 2A and B. Statistical tests were performed on the combined rates of adenomas and/or carcinomas.

The results of the Statistical Analysis By the Cochran-Armitage Dose-Adjusted Trend test for Summary results, and Peto's Prevalence Method for time of death adjusted dose-response, indicated that there is a significant dose-response trend ($p < .01$), for tumors associated with increasing doses of Imidan, in both males and females.

In the statistical comparisons of tumors in the controls versus the high dose group of Imidan, (by means of Fisher's Exact test) there is only borderline associations in both males and females,

p=.05 and .06, respectively. However in the evaluation of the Final Kill data, where most of the liver tumors were found, the females showed a significant (p = .027) increase in the comparison of the controls and the highest dose of Imidan by Fisher's Exact test. In addition, statistically significant (p = .036) differences were found in the number of liver tumors between the combined group of 0, 5, and 25 ppm of Imidan versus the highest dose of 100 ppm in the male mice and a borderline (p = .06) difference in the females.

Table 1. Imidan, Survival

A. Mortality,* Mice - Males

Time interval (weeks)	Dose (ppm) 0	5	25	100
0-28	1/60	0/60	0/60	0/60
29-52[a]	10/59	10/60	10/60	10/60
53-80	0/49	1/50	0/50	0/50
81-104	3/49	6/49	10/50	8/50
Total Deaths	4	7	10	8
104th week survivors	46	43	40	42

B. Mortality,* Mice - Females

Time interval (weeks)	Dose (ppm) 0	5	25	100
0-28	1/60	0/60	1/60	0/60
29-52[a]	11/59	10/60	11/59	10/60
53-80	1/48	1/50	1/48	0/50
81-104	11/47	14/49	8/47	12/50
Total Deaths	14	15	11	12
104th week survivors	36	35	39	38

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* Deaths/Animals at Risk
[a] Includes 10 animals sacrificed at week 52

Table 2. Imidan - Liver Tumors

A. Number of Mice - Males with Tumors

	Dose (ppm)							
Diagnosis	0	(%)	5	(%)	25	(%)	100	(%)
Adenoma	10	(17)	11	(18)	12	(20)	21	(35)
Adenoma and Carcinoma	3	(5)	-	(0)	2	(3)	6	(10)
Carcinoma	12	(20)	11	(18)	9	(15)	8	(13)
All Tumor-Bearing Animals	25	(42)	22	(37)	23	(38)	35	(58)
Animals Examined	59	(100)	60	(100)	60	(100)	60	(100)

B. Number of Mice - Females with Tumors

	Dose (ppm)							
Diagnosis	0	(%)	5	(%)	25	(%)	100	(%)
Adenoma	5	(10)	4	(8)	4	(8)	9	(18)
Adenoma and Carcinoma	1	(2)	-	(0)	-		2	(4)
Carcinoma	4	(8)	4	(8)	3	(6)	7	(14)
All Tumor-Bearing Animals	10	(21)	8	(16)	7	(15)	18	(36)
Animals Examined	48	(100)	50	(100)	48	(100)	50	(100)

Table 3. Imidan - Incidence* of Liver Tumors

A. Mice - Males

Dose (ppm)	Weeks	52[a]	53-104	FK	Total**
0		0/10	2/3	23/46	25/59
5		1/10	5/7	16/43	22/60
25		2/10	3/10	18/40	23/60
100		2/10	5/8	28/42	35/60
T		92.5	18.75	638.333	749.583
V		7207.53	11447.5	69051.4	87706.43
Z		1.090	0.175	2.429	2.531
p		0.14	0.43	7.60×10^{-3}	5.70×10^{-3}

B. Mice - Females

Dose (ppm)	Weeks	52[a]	53-104	FK	Total***
0		-	4/12	6/36	10/48
5		-	2/15	6/35	8/50
25		-	1/9	6/39	7/48
100		-	3/12	15/38	18/50
T			22.5	576.284	598.784
V			13327.8	41810.7	55138.50
Z			0.195	2.818	2.550
p			0.42	2.40×10^{-3}	5.39×10^{-3}

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[a] Sacrifice deaths only
* Deaths of Tumor-Bearing Animals/All Deaths
** Liver tumors did not appear in males previous to 52 weeks
*** liver tumors did not appear in females previous to 53 weeks

aTTACHMENT IV

CASWELL FILE

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

005304

JUL 15 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Imidan (Phosmet) Mouse Oncogenicity Study - Stauffer Report No. T-10719 EPA Registration No. 476-2178 (Accession Nos. 254608 and 254609)

TOX Chem. No. 543

FROM: William B. Greear, M.P.H. William B. Greear 6/13/86
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: George LaRocca, Product Manager #15
Insecticide-Rodenticide Branch
Registraiton Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D. ABK 7/1/86
Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Theodore M. Farber, Ph.D. 7/15/86
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Under a cover letter dated August 30, 1984, Ralph L. Riggs of the Stauffer Chemical Company has submitted the final report of the Imidan mouse oncogenicity study entitled "T-10719 Two-Year Dietary Oncogenicity Study in Mice with Imidan Technical Final Report." The study has been evaluated and it has been determined that under the conditions of the test, Imidan does not produce an oncogenic effect in $B_6C_3F_1$ mice when administered for a period of two years. The study has been designated a "Guideline" study.

Attachment

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005304

Reviewed by: William B. Greear, M.P.H.
Section VII: Toxicology Branch (TS-769C)
Assisted by: David G. Van Ormer, Ph.D.
Section II: Toxicology Branch (TS-769C)
Secondary Reviewer: Albin B. Kocialski, Ph.D.
Section VII: Toxicology branch (TS-769C)

DATA EVALUATION RECORD

Study Type: Oncogenicity - Mouse TOX CHEM No. 543

Accession Numbers: 254608, 254609 MRID No: Not available

Test Material: Imidan® Technical

Synonyms: Decemethion, ENT 25,705, Phthalophos, Prolate, R 1504, Imidan, Stauffer R 1504.

Study Number: Stauffer Report No. T-10719.

Sponsor: Stauffer Chemical Company
Richmond, CA 94804

Testing Facility: Stauffer Chemical Company
Environmental Health Center
Farmington, CT 06032

Title or Report: T-10719 Two-Year Dietary Oncogenicity Study in Mice with Imidan Technical-Final Report.

Authors: A.C. Katz, G.L. Sprague, D.W. Frank, J.C. Turnier, G.M. Zwicker and R.I. Freundenthal.

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Report Issued: May 1984.

Conclusions:

NOEL (ChE) < 5 ppm
LEL (ChE) = 5 ppm (inhibition of brain ChE in males and females)
NOEL (systemic) = 5 ppm
LEL (systemic) = 25 ppm (convulsions in males)

Oncogenicity: negative

Core Classification: Guideline

A. Materials:

1. Test compound - Imidan Technical Lot No. EHC-0139-37/WRC-4921-3131; 94.7%, described as a greyish-white crystalline material.

2. Test animals - Species: mouse; Strain: $B_6C_3F_1$-Crl-BR; Age: 6 weeks when dosing commenced; Mean weight: 22 to 23 g (males), 18 g (females); Source: Charles River Breeding Laboratories, Inc., Portage, Michigan.

B. Study Design:

1. Animal assignment - During a 3-week acclimatization period the animals were examined by a veterinarian. Then, the mice were assigned to treatment groups (60 mice/sex/group) so that group mean body weights would be similar at the time of assignment. Animal identification was by ear tag and by color coded labels affixed to each cage. The animals were assigned to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study-2 Years Male	Female
1 control	0	60	60
2 Low (LDT)	5	60	60
3 Mid (MDT)	25	60	60
4 High (HDT)	100	60	60

2. Animal maintenance - The animals were housed individually in wire mesh stainless steel cages in one room. Ventilation provided at least 15 air changes per hour. The temperature was maintained at 21 ± 3 °C and the relative humidity ranged from 40 to 60 percent. Twelve-hour per day illumination was provided by fluorescent lighting.

3. Diet preparation - Test diets were prepared by first dissolving the test material in Mazola® corn oil which was then premixed with basal feed (Purina Certified Rodent Chow #5002) using a 20-quart Hobart mixer. Each premix was blended with basal feed in a 3 ft^3 Patterson-Kelley twin shell blender. All blended diets contained 1 percent (wt/wt) added corn oil. Diets were stored at 4 °C for up to 3 weeks. Food jars in the animal room were replaced on a weekly basis. Stability of the 5 ppm test diet was determined at 7, 14, 21, and 28 days when stored at 4 and 60 °C and at ambient temperature. The concentration and homogeneity of the test material in the test diets were determined at approximately 3- to 6-week intervals throughout the study.

   Results - At 4 °C, no more than 2 percent of the test material was lost from the diet over a 28-day period. At ambient temperature, approximately 35 percent of the test material was lost from the diet over a 28-day period. At 60 °C, 87 percent of the test material was lost by day 7. During the experiment batches of the diets varied from 26 to 28 percent of the desired concentrations. The relative standard deviation for homogeneity ranged from 2.8 to 9.2 percent.

4. Statistics - Quantitative continuous variables such as body weights, food consumption, clinical laboratory values, and absolute and relative organ weights were analyzed by one-way analysis of variance and Dunnett's t-test. The critical level of significance was $p < 0.05$. The Fisher's Exact test was used to analyze incidence data on gross and microscopic findings. A level of significance of $p < 0.01$ was used to analyze the incidence of hepatic tumors. Tumor incidence was analyzed by specific site, individual tumor type, organ system and according to whether the tumor was benign or malignant.

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5. Quality assurance was conducted with inspections dates ranging from April 28, 1981 to November 10, 1983. The Quality Assurance Statement was signed by Patricia D. Royal.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for general appearance, behavior, mortality, and other signs of toxicity. The animals were also palpated once each week for the presence of tissue masses.

Results - Clinical observations in males show a dose-related trend in the incidence of convulsions, with incidence of 8, 11, 17, and 20 in the control-to-high dose groups, respectively. In females, the incidence of convulsions was 3, 10, 3, and 0 for the control-to-high dose groups, respectively. The authors state that the convulsions were observed in conjunction with handling during the second year of the study, and that a tendency to convulse is characteristic of mature male mice of the certain strains. The report also states that, "Although unestablished by the present study, Imidan treatment may lower the relative convulsive threshold or potentiate predisposing genetic and/or environmental factors" [leading to convulsions].

Survival was not affected by compound administration. Unscheduled deaths in males occurred at levels of 4, 7, 10, and 8 in the control-to-high dose groups, respectively. In females, unscheduled deaths occurred at levels of 14, 15, 11, and 12 in the control-to-high dose groups, respectively.

2. Body weight - The mice were weighed once a week for the first 12 weeks and then once every 2 weeks for the remainder of the study.

Results - Administration of the test material had no significant effect on body weight. After the first 3 months, body weights of mice in the high-dose group (particularly males) tended to be slightly greater than those of control mice. At termination, males in the high-dose group weighed approximately 8 percent more than controls and females in the high-dose group weighed 2 percent less than controls. The slight increase in body weight was not dose-related.

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3. Food consumption and compound intake - Food consumption data were provided weekly for the first 12 weeks and once every 2 weeks for the remainder of the study.

   Results - Mean weekly food consumption was reduced in all males in the treated groups and in females in the mid- and high-dose groups. The decrease in food consumption in females appeared to be dose-related. In the absence of concomitant weight reductions, this may be interpreted as an increase in food efficiency and/or reduced food wastage.

   Mean compound intake was estimated to be 1.2, 5, and 18 mg/kg/day for females and 1.0, 4, and 14 mg/kg/day for males in the low-, mid-, and high-dose groups, respectively.

4. Blood was collected from 10 mice/sex/group at the interim sacrifice (12 months) and at terminal sacrifice via severed cervical vessels while the mice were under sodium pentobarbital anesthesia. The CHECKED (X) parameters were examined.

   a. Hematology

X	Hematocrit (HCT)		Total plasma protein (TP)
X	Hemoglobin (HGB)	X	Leukocyte differental count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (M
X	Platelet count	X	Mean corpuscular volume (MCV)

   Results - At 12 months, there were no biologically significant effects on the hematological parameters measured that could be related to treatment. At termination, there was a decrease in the leukocyte count in mice in all female treated groups when compared to controls. However, a dose-response relationship was not apparent.

   b. Clinical chemistry

X	Cholinesterase - erythrocyte
X	Cholinesterase - plasma
X	Cholinesterase - brain

Results - At 12 months, plasma cholinesterase depressions of 11, 13, and 54 percent were observed in males in the low-, mid-, and high-dose groups, respectively, when compared to controls. In females, plasma cholinesterase was depressed 13 and 56 percent in the mid- and high-dose groups, respectively. Due to a problem with reagents, erythrocyte cholinesterase levels could not be determined in the treated animals. Brain cholinesterase was depressed 29, 22 and 31 percent in males in the low-, mid- and high-dose groups, respectively. In females, brain cholinesterase was depressed 28, 31 and 34% in the low- mid- and high-dose groups, respectively. At termination, plasma cholinesterase depression of 4, 10, and 47 percent was observed in males in the low-, mid- and high-dose groups, respectively. In females, plasma cholinesterase was depressed 7 and 52 percent in the mid- and high-dose groups, respectively. Erythocyte cholinesterase activity was comparable among the treatment and control groups in males and females. Brain cholinesterase activity in males was not affected by compound administration. In females, brain cholinesterase was depressed 14 and 22 percent in the mid- and high-dose groups when compared to controls.

5. Sacrifice and Pathology - At 12 months, 10 mice/sex/group were selected for sacrifice. All animals that died and that were sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the followed (XX) organs were weighed in mice that were sacrified.

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X		X	
Digestive system	Cardiovasc./Hemat.		Neurologic
Tongue	X Aorta (thoracic)	XX	Brain
Salivary gland (parotid)	XX Heart	X	Peripheral nerve (sciatic)
Salivary gland (submaxillary)	X Bone marrow (sternal)	X	Spinal cord (cervical and lumbar)
Esophagus	X Lymph mode (mesenteric)		
Stomach	X Spleen	X	Pituitary
Duodenum	Thymus		Eyes and Harderian glands
Jejunum	Urogenital		Glandular
Ileum	XX Kidneys	X	Adrenals
Cecum	X Urinary bladder		Lacrimal gland
Colon	XX Testes with epididymides	X	Mammary gland (inguinal)
Rectum	X Prostate	X	Thyroids
Liver	X Seminal vesicle and coagulating gland		Other
Gall bladder			Bone
Pancreas	X Ovaries		Skeletal muscle
Respiratory	X Uterus	X	Skin
Trachea	X Vagina	X	All gross lesions and masses
Lung		X	Tibiofemoral joint and attached muscle

Results

a. Organ weight - At 12 months, there was an increase in body weight and in the absolute weight of the kidneys and liver in females in the high-dose group. The relative weight of the brain, heart, and kidneys were decreased in females in the high-dose group. The relative weight of the liver was increased in males in the high-dose group. At termination, the absolute weight of the liver was increased in males in the high-dose group and in females in the mid- and high-dose groups. The relative weight of the liver was increased in females in the mid- and high-dose groups.

b. Gross pathology - In high-dose males, there was a slightly increased incidence of masses, nodules or cysts, and areas of focal discoloration/depression in the liver. There was an increase in the incidence of enlarged lymph nodes and spleens in males in the low- and mid-dose group.

c. Microscopic pathology

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d. Non-neoplastic - At 12 months, there was a slight increase in the incidence of regenerative epithelial hyperplasia of the kidneys in males in the high-dose group. In females, there was an increase in the incidence of midzonal degenerative vacuolation of the liver, dilation of the uterus and "inflammation" of the kidneys in females in the high-dose group. At termination, there was an increased incidence of perivasculitis of the muscle in males in the high-dose group (7/50 vs. 0/49 in controls). Testicular atrophy was slightly increased in high-dose males (19/50 vs. 11/49 in control). The incidence of hyperplasia of the stomach mucosa was slightly increased in high-dose males (10/48 vs. 5/49 in controls) and females (9/49 vs. 3/49 in controls). In high-dose males, there was an increased incidence of degenerative vacuolation of individual liver cells (16/50 vs. 3/49 in controls) and foci of vacuolated or clear liver cells (13/50 vs. 4/49 in controls). The liver changes were described as randomly scattered hepatocytes containing either single large cytoplasmic vacuoles or a cluster of small vacuoles imparting a foamy appearance, and randomly located clusters of foamy hepatocytes, some of which had poorly defined cytoplasmic vacuoles. High-dose females had slight changes in the liver including midzonal degenerative vacuolation (5/50 vs. 1/49 in controls), necrotizing inflammation (4/50 vs. 1/49 in controls) and necrosis of individual liver cells (4/50 vs. 1/49 in controls). High-dose females also exhibited slight increases in myometrial atrophy of the uterus (8/50 vs. 2/49 in controls) and meningitis of the spinal cord (18/50 vs. 12/49 in controls).

Neoplastic - The incidence of hepatocellular adenomas and hepatocellular carcinomas are tabulated on the following page for both the interim sacrifice (study days 0 to 365) and the terminal sacrifice (study days 366 to 736). Hepatocellular adenomas were described as "autonomous hepatocytic proliferation, lack of normal lobular architecture, and

Incidence of Hepatocellular Tumors

Males

Interim Sacrifice	Control	Low (5 ppm)	Mid (25 ppm)	High (100 ppm)
Hepatocellular adenoma	0/11(0%)	1/10(10%)	2/10(20%)	2/10(20%)
Hepatocellular carcinoma	0/11(0%)	0/10(0%)	1/10(10%)	1/10(10%)
Hepatocellular adenoma or carcinoma	0/11(0%)	1/10(10%)	2/10(20%)	3/10(30%)
Final and Interim Sacrifice	**Control**	**Low (5 ppm)**	**Mid (25 ppm)**	**High (100 ppm)**
Hepatocellular adenoma	13/60(22%)	10/60(17%)	14/60(23%)	27/60(45%)
Hepatocellular carcinoma	13/60(22%)	11/60(18%)	11/60(18%)	14/60(23%)
Hepatocellular adenoma or carcinoma	23/60(38%)	21/60(35%)	23/60(38%)	35/60(58%)

Females

Interim Sacrifice

No hepatocellular tumors were observed.

Final and Interim Sacrifice	Control	Low (5 ppm)	Mid (25 ppm)	High (100 ppm)
Hepatocellular adenoma	6/60(10%)	4/60(7%)	5/58(9%)	11/60(18%)
Hepatocellular carcinoma	5/60(8%)	4/60(7%)	3/58(5%)	9/60(15%)
Hepatocellular adenoma or carcinoma	10/60(17%)	8/60(13%)	8/58(14%)	18/60(30%)

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compression of adjacent liver cells." Liver tumor malignancy was characterized by "anaplasia, vascular invasion and trabecular formation." Liver tumors were not observed in females or control males that died or were sacrificed during the first year. During the first year, hepatocellular adenomas were found in one male in the low-dose group, two males in the mid-dose group and two males in the high-dose group. Hepatocellular carcinomas were found in one male in each of the mid- and high-dose groups. The time-to-tumor appearance was 545, 659, 651, and 617 days for females in the control-to-high-dose groups, respectively, and 610, 364, 364, and 364 days for males in the control-to-high-dose groups, respectively. Pulmonary metastasis of hepatocellular carcinoma occurred in 2, 4, 2, and 3 males in the control-to-high-dose groups, respectively. The number of early deaths that occurred in mice with hepatocellular tumors (excluding mice with hepatocellular tumors at the interim sacrifice) was 3, 4, 4 and 7 for males and 3, 2, 1 and 3 for females in the control-to-high-dose groups, respectively. In summary, the incidence of hepatocellular tumors was 23/60, 21/60, 23/60, and 35/60 in males in the control-to-high-dose groups, respectively. Hepatocellular tumors were observed in 10/60, 8/60, 8/58, and 18/60 females in the control-to-high-dose groups, respectively. Harderian gland adenomas occurred in 3/60(5%), 7/50 (14%), 4/52(8%), and 9/60(15%) males in the control-to-high-dose groups. Harderian gland adenocarcinomas occurred in 2/60 males in the low-dose group. The distribution of Harderian gland tumors did not appear to be related to treatment. The incidence of Harderian gland adenoma and carcinomas in historical control male $B_6C_3F_1$ mice has been reported to range from 0 to 12 percent and 0 to 2 percent, respectively (Goodman et al., 1985).

D. Discussion/Summary:

Survival and body weight gain were not significantly affected by administration of the test material. Clinical examination of the animals revealed an increased incidence of convulsions in males that exhibited a dose-response relationship. (Males in the mid- and high-dose groups were

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significantly affected). Mean weekly food consumption was slightly decreased in males in all treatment groups and in females in the mid- and high-dose groups. The hematologic parameters measured were not affected by administration of the test material. At 12 months, plasma cholinesterase was inhibited in males (54%) and females (56%) in the high-dose group. Brain cholinesterase was depressed 22 to 31 percent in treated males and 28 to 34 percent in treated females. At termination, plasma cholinesterase was inhibited in males (47%) and in females (52%) in the high-dose group. Brain cholinesterase was depressed in females (22%) in the high-dose group. At the 12-month sacrifice, the relative weight of the liver was increased in males in the high-dose group. At necropsy, there was an increase in the incidence of "masses, nodules or cysts" and areas of "focal discoloration/ depression" of the liver in males in the high-dose group. At terminal sacrifice, the absolute and relative weight of the liver was increased in females in the mid- and high-dose groups. The absolute weight of the liver was increased in males in the high-dose group. There was an increased incidence of degenerative vacuolation of individual liver cells and foci of vacuolated or clear cells in the liver of males in the high-dose group. Other findings in high-dose males included perivasculitis of muscle, hyperplasia of stomach mucosa and testicular atrophy. In high-dose females, there were also slight increases in the incidence of inflammation of the stomach and duodenum, myometrial atrophy of the uterus and midzonal degenerative vacuolation, necrotizing inflammation and individual cell necrosis of the liver.

Neoplastic findings show an increased incidence of hepatocellular adenomas and carcinomas in male and female mice in the high-dose group. There was no increase in the incidence of hepatocellular tumors in the low- and mid-dose groups when compared to controls. Statistical analysis of the data on the incidence of hepatocellular

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tumors in male and female mice administered the test material indicated borderline associations in both males and females of p = .05 and .06, respectively, using the Fisher's Exact test. It should be noted that the incidences used for analyzing liver tumor incidence were: 25/59, 22/60, 23/60, and 35/60 in the male control-to high-dose groups, respectively, and 10/48, 8/50, 7/48, and 18/50 in the female control-to-high dose groups, respectively (see attached memorandum of B. Fisher dated February 28, 1986). These incidences are not identical to the incidences reported in the study. Analysis of the data reported in the study would produce a lower p value when comparing the high-dose male group to controls.

A paper cited by the authors (Tarone, 1981) indicates that in 54 chronic studies conducted with the $B_6C_3F_1$ mouse at five different laboratories, the mean incidence of liver tumors was 32.1 percent for control males with a range of 7 to 58 percent. For females the range was 0 to 21 percent with a mean of 6.2 percent. The incidence of liver tumors in the $B_6C_3F_1$ mouse from the five laboratories are provided below:

Liver Tumor Incidence $B_6C_3F_1$ Mice at Five Laboratories

	Lab 1 N*=7	Lab 2 N*=7	Lab 3 N*=7	Lab 4 N*=22	Lab 5 N*=11
Male	40.1(24-58)	31.3(17-39)	25.0(16-39)	32.2(15-55)	2.4(7-45)
Female	9.7(2-21)	4.6(2-10)	7.3(0-13)	5.1(0-21)	4.8(0-17)

*N = Number of studies

The sponsor recently submitted data on the incidence of hepatocellular tumors in $B_6C_3F_1$ mice from one additional study that was conducted prior to switching to CD-1 strain mice. The cumulative mortality for male and female mice at 106 weeks was 34 and 40 percent, respectively. The data on the incidence of hepatocellular tumors in historical $B_6C_3F_1$ mice are provided below:

Incidence of Hepatocellular Tumors in $B_6C_3F_1$ Mice at Stauffer Chemical Co. Environmental Health Center

Tumor Type	Male	Female
Hepatocellular adenoma	25/60(42%)	9/60(15%)
Hepatocellular carcinoma	10/60(17%)	3/60(5%)
Hepatocellular adenoma or carcinoma	31/60(52%)	11/60(18%)

From the data compiled by Tarone, it is apparent that hepatocellular tumors occur with a high incidence in male control mice (mean of 32.1%). It is also apparent that the incidence is quite variable ranging from 7 percent to 58 percent. The incidence of hepatocellular tumors observed in high-dose males falls within the range observed for control males at Laboratory #1. When the incidence of hepatocellular tumors in high-dose males is compared to the incidence observed in 60 historical control male mice from the laboratory that conducted the study with phosmet, no significant difference is present. The incidence of hepatocellular tumors in high-dose males is only 6 percent greater than the incidence observed in the historical control mice.

In examining the data on the incidence of hepatocellular tumors in males and females, it is noted that there is not a good dose-response relationship for males or females. In addition, as the dose increases there is no significant increase in the incidence of malignant liver tumors occurring in the treatment groups. Pulmonary metastases occurred in 2, 4, 2, and 3 males in the control-to high-dose groups, respectively. This lack of a dose-response relationship would indicate that the degree of malignancy of the hepatocellur tumors failed to be increased by administration of the material. The time to first liver tumor is 545, 659, 651, and 617 days for females in the control-to-high-dose groups, respectively. The time to first liver tumor is 610, 364, 364, and 364 days for males in the control-to-high-dose groups, respectively. In females, there appears to be no decrease in the latency period. In males, there were 0, 1, 2, and 3 males observed with liver tumors at interim sacrifice (364 days), which indicates a decrease in the latency period. In light of the discussion presented above, the increase in incidence of hepatocellular tumors in high-dose males and the marginal increase in hepatocellular tumors in high-dose females are considered to be within biological variation.

The study was conducted with at least one dose level being tested at the maximum tolerated dose (MTD) as indicated by depression in cholinesterase activity in plasma and brain of high-dose males and females, an increase in microscopic pathological changes in the liver of high-dose males and females, and convulsions in males in the mid- and high-dose groups. (It was stated in a letter from R. L. Riggs dated August 30, 1984, that a 4-week range-finding study had been conducted. Males in the 150 ppm group exhibited decreases in mean food consumption and mean body weights. In addition, the absolute liver and kidney weights of males in the 150 ppm group were significantly lower than controls while the relative liver weights were increased.)

The no-observable effect levels (NOEL's) are set as follows:

NOEL (ChE) <5 ppm
LEL (ChE) = 5 ppm (inhibition of brain ChE in males and females)
NOEL (systemic) = 5 ppm
LEL (systemic) = 25 ppm (convulsions in males)

Oncogenicity: negative for hepatocellular tumors in male mice

Core Classification: Guideline

References

1. Goodman, D.G.; Boorman, G.A.; Strandberg, J.D. (1985) Selection and use of the $B_6C_3F_1$ mouse and F344 rat in long-term bioassays for carcinogenicity. In: Handbook of Carcinogen Testing.

2. Tarone, R; Chu, K.; Ward, J. (1981) Variability in the rates of some common naturally occurring tumors in Fisher 344 rats and (C57BL/6N X C3H/HeN)F_1($B_6C_3F_1$) mice. J.NCI 66(6):1175-1181.

ATTACHMENT V

Phosmet (8/18/99)

Page_____ is not included in this copy.

Pages 68 through 75 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

ATTACHMENT VI

From: Jeff Dawson on 07/13/99 01:15 PM

To: Whang Phang/DC/USEPA/US@EPA, Linda Taylor/DC/USEPA/US@EPA
cc:
Subject: Phomet Q1* FYI

---------- Forwarded by Jeff Dawson/DC/USEPA/US on 07/13/99 01:14 PM ----------

William Burnam
07/13/99 12:03 PM

To: Jeff Dawson/DC/USEPA/US@EPA
cc:
Subject: Phomet Q1* FYI

ok
---------- Forwarded by William Burnam/DC/USEPA/US on 07/13/99 12:02 PM ----------

Lori Brunsman
07/13/99 11:50 AM

To: Michael Metzger/DC/USEPA/US@EPA
cc: William Burnam/DC/USEPA/US@EPA
Subject: Phomet Q1* FYI

I checked the stats file and discovered that this study lasted 105 weeks. I re-ran the analysis using 105 weeks as the study duration, and the Q1* *does not* change.
---------- Forwarded by Lori Brunsman/DC/USEPA/US on 07/13/99 11:43 AM ----------

Lori Brunsman
07/13/99 11:40 AM

To: Michael Metzger/DC/USEPA/US@EPA
cc: William Burnam/DC/USEPA/US@EPA
Subject: Phomet Q1*

The Q1* for Phosmet (Imidan) is 3.58 X 10 E-1 based on male mouse liver tumors combined (tumor rates of 23/59, 21/60, 23/60, and 35/60 for 0, 5, 25, and 100 ppm dose groups, respectively). This assumes no mortality discrepancies among the dose groups and a 78-week study duration.

Lori

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ATTACHMENT VII

CASWELL FILE

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

000411

APR 3 1992

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Phosmet (Imidan) - Submission of a Metabolism Study in Rats in Compliance with Reregistration Data Requirements

Tox Chem No. 543
PC No. 059201
Project No. 1-1759
Submission No. S399599

FROM: William B. Greear, M.P.H. William B. Greear 3/30/92
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

TO: Brigid Lowery/Larry Schnaubelt, PM Team #72
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Marion P. Copley, D.V.M. Section Head Marion P. Copley 3/30/92
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)

I. CONCLUSIONS:

The submitted metabolism studies in rats (#T-13031; 8/16/89 and 1/29/90) are acceptable and together fulfill the data requirements for a Guideline Series 85-1 Metabolism study.

II. REQUESTED ACTION:

SRRD has requested that TB-I evaluate the following two metabolism studies on phosmet.

1. Fisher, G.D. T-13031: R-1504 (Imidan-R-1504) Metabolism Study in Rats: Recovery of Administered Dose. August 16, 1989 (MRID #412960-01).

2. Fisher, G.D. T-13031: R-1504 Metabolism Study in Rats: Biotransformation. January 29, 1990 (MRID #414257-01).

III. RESULTS

Strain: Sprague-Dawley (Crl. CD [SD] BRAF/Plus)
Route: Oral (gavage)

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Dose Levels: 1 and 25 mg/kg (single radiolabeled dose)
1 mg/kg (14-day repeat dose followed by 1 dose radiolabeled)

Results: Clearance at all dose levels was rapid with 68.9-82.6% in urine and 4.5-9.9% in feces. Tissues combined less than 1% of dose in all groups. Liver and whole blood contained the greatest activity. Peak blood levels were observed 0.5 hours after dosing. Two major urine metabolites were identified. Eleven minor metabolites were not identified.

Core classification: Minimum

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DOC92C130

FINAL

DATA EVALUATION REPORT

R-1504

Study Type: Metabolism in Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Author Karen Gan Date 2/13/92
Karen Gan

Reviewer Sanju Diwan Date 2/13/92
Sanju Diwan

QA/QC Manager Sharon Segal Date 2/13/92
Sharon Segal

Contract Number: 68D10075
Work Assignment Number: 1-10
Clement Number: 91-55
Project Officer: James Scott

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Guideline Series 85-1: Metabolism in Rats

EPA Reviewer: William Greear, M.P.H.
Review Section IV, Toxicology Branch I,
Health Effects Division

Signature: William B Greear
Date: 3/3/92

EPA Section Head: Marion Copley, DVM
Review Section IV, Toxicology Branch I,
Health Effects Division

Signature: Marion Copley
Date: 3/4/92

DATA EVALUATION REPORT

STUDY TYPE: Metabolism in Rats

EPA IDENTIFICATION NUMBER:

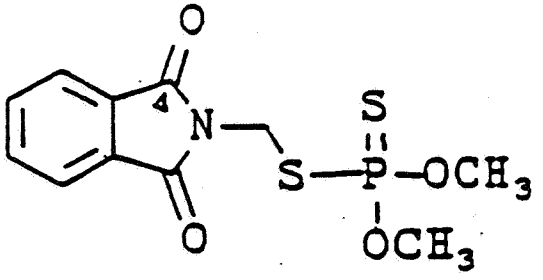
Tox. Chem. Number: 543

P.C. Number: 059201

MRID Number: 412960-01, 414257-01

TEST MATERIAL: R-1504 (purity >99.7%)

SYNONYMS: Phosmet; Imidan; (N-(mercaptomethyl)phthalimide-S-(O,O-dimethyl phosphorodithioate); ($C_{11}H_{12}NO_4PS_2$; MW = 317.3)



▵ denotes the position of the [^{14}C] label

SPONSOR: ICI Americas Inc., Wilmington, DE

TESTING FACILITY: CIBA-GEIGY Corporation
Environmental Health Center
Farmington, CT 06032

TITLE OF REPORTS:
1. Fisher, G.D. T-13031: R-1504 (IMIDAN-R-1504) Metabolism Study in Rats: Recovery of Administered Dose. August 16, 1989. 111 pp.

2. Fisher, G.D. T-13031: R-1504 Metabolism Study in Rats: Biotransformation. January 29, 1990. 79 pp.

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CONCLUSIONS: The absorption, distribution, metabolism, and excretion of R-1504 were studied in groups of male and female Sprague-Dawley rats administered a single oral gavage dose of 1 or 25 mg/kg [^{14}C]R-1504 or a 14-day repeated oral dosing of 1 mg/kg unlabeled R-1504 followed by a single dose of 1 mg/kg [^{14}C]-labeled R-1504 on day 15. The pharmacokinetics of [^{14}C]R-1504 were studied in rats administered a single oral dose of 1 or 25 mg/kg (males and females) of ^{14}C-labeled test material.

[^{14}C]R-1504 was rapidly absorbed, distributed, metabolized, and eliminated in rats for all dosing regimens. Most of the radioactivity was recovered within 24 hours in the urine (68.9-82.6% of the administered dose) and feces (4.5-9.9%) of all dose groups. There appeared to be a slight saturation of R-1504 in the high-dose group as indicated by the somewhat increased recovery of radioactivity in the feces of the 25-mg/kg animals (9.4-13.4% of dose) compared to the 1-mg/kg animals (single and repeated dosing) (6.3-8.0%). There was a wide distribution of R-1504, and the tissues contained low levels of radioactivity (<1% of the administered dose) in all dose groups; the highest activity was in the liver and whole blood and lowest activity in the fat and bone (on both a concentration and percent-of-dose basis). These data indicate that R-1504 and/or its metabolites do not bioaccumulate to an appreciable extent. The absorption of R-1504 appears to be rapid in rats because the peak blood levels of radioactivity occurred 0.5 hours after oral exposure to a single dose of 1 or 25 mg/kg R-1504. The metabolism of R-1504 appears to be relatively complete and rapid. The two major radioactive bands in the urine samples were identified as N-(methylsulfinyl-methyl)phthalamic acid (PaAMS[O]M) and N-(methylsulfonyl-methyl)phthalamic acid (PaAMS[O2]M); however, the 11 minor bands in the urine were not identified. Although nine radioactive components were characterized in feces, no attempt was made to identify these fecal metabolites. Furthermore, it was not determined if the parent compound was recovered in the urine or feces. Therefore, based on results, it is difficult to determine whether there were any remarkable sex-, dose-, or treatment-related differences in the absorption, distribution, metabolism, and elimination of [^{14}C]R-1504 in rats. The only sex-related differences observed in the urinary excretion of PaAMS(O)M and PaAMS(O2)M metabolites were as follows: the 1-mg/kg females (single and repeated dosing) had a higher recovery of PaAMS(O)M compared to males and all dosed males had a higher recovery of PaAMS(O2)M compared to females. These studies also showed that single oral administration of 1 and 25 mg/kg R-1504, as well as repeated dosing with 1 mg/kg/day, did not induce any apparent treatment-related clinical effects.

STUDY CLASSIFICATION: The study is core minimum, and satisfies the requirements set forth under Guideline 85-1 (and Addendum 7) for a metabolism study in rats. Although there were minor deficiencies in the study, they do not affect the overall study results and conclusions. The animals dosed repeatedly with 1 mg/kg R-1504 should probably have been observed for more than 4 days to obtain a complete recovery of the radioactivity. The analytical methods used failed to definitively identify the radioactive compounds characterized in the urine and fecal samples. Furthermore, the parent compound was not identified in the urine or feces.

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A. MATERIALS

1. Test Substance

The test material was described as an off-white crystalline solid (Lot Number M-736-F). The material was supplied by deGuigne Technical Center, ICI Americas, Inc. (Richmond, CA). The purity of the test material was >99.7%. Determinations were made by thin-layer chromatography (TLC) with toluene:ethyl acetate (9:1, v/v). The presence of contaminants or impurities was not reported.

The radiolabeled compound (Pathfinder Laboratories, St. Louis, MO; Lot Number 037F9214) had a purity of greater than 97.4%. R-1504 was labeled with [^{14}C] at the C-1 (carbonyl) position. The mean radiochemical purity was reported to be >97%. Specific activity was 34.3 mCi/mmol for the radiolabeled compound. Radioactive areas were visualized using autoradiography and counted using a Tracor Analytic Mark III Liquid Scintillation Counter (Searle Analytical, Inc., Des Plaines, IL) or a 2000CA Tricarb Liquid Scintillation Analyzer (Instruments Co., Downers Grove, IL).

2. Test Animals

Four- to five-week old male and female Sprague-Dawley rats (Crl.CD [SD] BRVAF/Plus) were obtained from Charles River Breeding Laboratories (Kingston, NY). A single oral dose or repeated dosing of R-1504 was administered to 5 males and 5 females. The male rats weighed 268-312 g and the female rats weighed 165-202 g at the time of the radiolabeled dosing of R-1504.

B. METHODS

1. Animals were quarantined for 2 weeks individually in the vivarium and examined by a veterinarian. They were then acclimatized for at least 4 days in individual glass metabolism cages. The diet (Purina Chow #5002, Purina Mills, Inc., St. Louis, MO) and tap water were given ad libitum except when food was withheld for 8-12 hours prior to dosing and 6 hours following dosing. Animals were dosed between 8 a.m. and 10 a.m.

2. Oral dosing solutions were prepared by a weighed amount of unlabeled and [^{14}C]-labeled R-1504 in ethanol and polyethylene glycol-200 (PEG-200) (3:1, v/v). The radiolabeled solutions were assayed by TLC for radioactive content. The solutions were administered by oral gavage at 2 mL/kg. The dose amount was determined by weighing the dosing syringe before and after dosing. The stability of the labeled and unlabeled dosing solutions was not reported. The intravenous exposure route was not used because the test material was insoluble in water and saline.

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Groups of 10 rats (5/sex) were given a single oral dose of 1 or 25 mg/kg [^{14}C]R-1504 or were given an oral dose of 1 mg/kg/day of unlabeled R-1504 for 14 days followed by a single administration of 1 mg/kg [^{14}C]R-1504 on day 15. All animals were observed for 4 days (96 hours) following the administration of the labeled R-1504.

3. The urine and feces were collected, over dry ice, from animals at 6, 12, 24, 36, 48, 72, and 96 hours after exposure to the labeled dose of R-1504. Excreta were retained at -20°C prior to analysis. The metabolism cages were rinsed with distilled water and the washings were collected together with the urine. Blood was collected; a weighed portion was analyzed for radioactivity and the remainder stored in heparin at 4°C. Plasma was separated from red blood cells by centrifuging a sample of whole blood, and the plasma was then counted for radioactivity. At 96 hours postexposure, all animals were sacrificed by anesthetization with ether and exsanguination until death by cardiac puncture. Necropsies were conducted and major tissues were removed, weighed, and stored at -20°C. Liquid scintillation counting was conducted in duplicate in the samples of urine, feces, G.I. contents, cage washing, tissue, and plasma, using either the Tracor Analytic Mark III Liquid Scintillation Counter (Searle Analytical Inc., Des Plaines, IL) or the 2000CA Tricarb Liquid Scintillation Analyzer (Instruments Co., Downers Grove, IL). Appropriate measures were taken to determine counting efficiencies and to minimize quenching. The limit of detection (LOD) of radioactivity was taken as twice the liquid scintillation counter background rate. To determine the oxidation efficiency, fecal and G.I. content samples were combusted in a Packard Oxidizer 306 (Packard Instruments, Sterling, VA) or a Harvey OX-300 Biological Oxidizer (R.J. Harvey Instruments Corp., Hillsdale, NJ). The expired air was not collected because a previous report by Ford et al. (1966) and an in-house preliminary study by Pomeroy and Fisher (1988) found no volatile materials exhaled after [^{14}C]R-1504 administration. No other details on these studies were provided.

4. For the pharmacokinetic study, blood samples (50 µL) from the tail vein of the single 1- and 25-mg/kg-dosed male rats were collected in capillary tubes at 10, 20, and 30 minutes, and 1, 2, 4, 6, 8, 11, 24, 48, 72, and 96 hours postexposure for radioactivity measurements. The pharmacokinetic parameters were estimated with ESTRIP data analysis software (Brown and Manno 1978). The area under the curve (AUC) was calculated using the trapezoidal rule. Statistical analysis was determined with STATPAK (Northwest Analytical, Inc., Portland, OR), one-way ANOVA, and Duncan's multiple range test.

5. For the biotransformation assay, the urines were pooled (0-96 hours) by dosing and sex groups, then were filtered (Millex HV 9.45 µm filter; Millipore Corp., Bedford, MA). The fecal samples were suspended in distilled water (1.5 mL/g), homogenized,

and pooled (0-96 hours). The pooled urine and fecal samples were stored at -15°C to -25°C.

To characterize metabolites in urine and feces, radioactive components were quantitated in quadruplicate using TLC plates developed in one of the following solvent systems: (A) acetonitrile-water (85:15, v/v), (B) pyridine-pentyl alcohol-water (7:7:6, by volume), (C) acetonitrile-0.1 M ammonium acetate (85:15, v/v), (D) acetonitrile-water (4:1, v/v), (E) methanol-water (3:1, v/v), or (F) toluene-ethyl acetate (9:1, v/v). The bands on the gel were visualized under UV light (254 nm) and detected for radioactivity by autoradiography (Kodak Diagnostic SB X-Ray Film, Rochester, NY) or TLC plate scanner (Ambis Systems, Automated Microbiology Systems, Inc., San Diego, CA).

To characterize the urinary metabolic pattern of R-1504, 1 mL of pooled urine was applied to a C-18 SPE column (40 m APD, 60A, Bakerbond SPE, J.T. Baker, Inc., Phillipsburg, NJ) that was conditioned with methanol and distilled water. The radioactive contents were quantitated in quadruplicate using TLC plates developed in solvent system A. In addition, to determine if glucuronide conjugates were excreted, pooled urine of the 25-mg/kg male rats was mixed with 0.1 M sodium acetate and 0.2 M glycine buffer (pH 10.4) and incubated with β-glucuronidase (Sigma Chemical Co., St. Louis, MO) for 30 minutes. A sham treatment was also conducted by omitting the glucuronidase. To isolate these metabolites, collected fractions of radioactivity were extracted from two C-18 SPE columns, in series, using methanol, then reduced with nitrogen, streaked on TLC plates, and developed in acetonitrile-water (85:15, v/v). The reference standards used to identify metabolites of R-1504 in rats were phthalamic acid (PAA), phthalic acid (PA), N-(methylsulfinyl-methyl) phthalimide (PiAMS[O]M), N-(methylsulfonyl-methyl) phthalimide (PiAMS[O2]M), N-(methylsulfinyl-methyl) phthalamic acid (PaAMS[O]M), and N-(methylsulfonyl-methyl) phthalamic acid (PaAMS[O2]M).

To identify the metabolites, only the pooled urine of the 25-mg/kg male rats was used. The major metabolites in the other dosing groups were identified by comparison with those of the high-dose group. About 8-9 mL of the pooled and filtered urine were diluted with acetonitrile (5:1, v/v). To extract by flash chromatography (Majors and Enzweiler 1988), a glass column (19 mm i.d., 45.7 cm effective length, Ace Glass, Inc., Vineland, NJ) containing silica gel 60 (particle size 0.04-0.063 mm; EM Reagents, MC/B Manufacturing Chemists, Inc., Cincinnati, OH) was preconditioned with acetonitrile-methanol (4:1, v/v). The metabolites were eluted at a rate of 1-1.5-inch column/minute with the acetonitrile-methanol solvent system. Fractions were collected in 2-inch intervals, analyzed for radioactivity, and stored at -15°C to -25°C. The fractions containing the major metabolites were concentrated under nitrogen and reconstituted with 0.05 M ammonium acetate. To further isolate these metabolites, samples were injected into the HPLC system A and resolved metabolites were

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collected. The major metabolites were eluted through HPLC system B using one of the following procedures: (1) HPLC system B eluent was applied to C-18 SPE column and extracted with methanol (for TLC band 3 metabolite), or (2) HPLC system B eluent was further purified using HPLC system C, and then fractions were collected, lyophilized (Freeze Dryer 18, Labconco Corp., Kansas City, MO), and reconstituted in methanol (used for TLC band 6 metabolite). HPLC system D and mass spectrometry (MS) were used to analyze isolated metabolites.

To characterize the fecal metabolic pattern of R-1504, the pooled fecal samples were extracted four times with methanol. The pellet was reconstituted in 1 mL of distilled water and the supernatants were assayed for radioactivity. The combined supernatants were concentrated to dryness and reconstituted in methanol to a volume of 5 mL. Using a 10-cc glass syringe barrel, 3 g of silica gel 60 were added and columns conditioned with 4 x 5 mL hexane, 4 x 5 mL methanol, and 4 x 5 mL ethyl acetate-methanol (3:1, v/v). Methanol extract of the pooled feces (1.5 mL) and ethyl acetate (4.5 mL) were added to the column and allowed to elute by gravity flow. The column was then dried under nitrogen, washed with hexane, and metabolites eluted with methanol. This procedure was done in triplicate. The three extracts were pooled, concentrated to dryness, reconstituted with 5 mL methanol, then concentrated under nitrogen to 1-2 mL. Using TLC system C, metabolites were applied to the gel in quadruplicate. The bands were visualized, isolated, and assayed for radioactivity. There was no methodology stated in the protocol for identifying the fecal metabolites.

6. Protocols: The methods followed the protocol.

C. REPORTED RESULTS

1. Elimination and Recovery: In the single and repeated oral dose studies, the mean total recoveries of radioactivity ranged from 83.6% to 99.2% of the administered dose after 96 hours (Table 1). Within 24 hours, 76-89% of the administered dose was recovered. The major route of elimination was the urine. Most of the recovery of radioactivity was in the urine within 24 hours postexposure. At 96 hours postexposure, 75-87.8% of the dose was measured in the urine. The feces was a minor elimination route of R-1504. After 12 hours postexposure, 1% or less of the administered dose was detected in the feces and 3-9% detected between 12 and 24 hours for all dosing groups. After 96 hours, 6-9% of the administered dose was measured in the feces for all groups, with the exception of the 25-mg/kg females with a recovery of 13%. There were no major sex-related differences in the elimination of R-1504, although the high-dose female (13.4% of administered dose) had slightly higher recovery of activity in the feces compared to the high-dose males (9.4%).

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2. Distribution: All tissues recovered less than 1% of the administered dose for all dosing groups, with the exception of the carcass (1.246-2.074%) after 4 days postexposure (Table 2). The whole blood and the liver had the highest radioactivity (0.181-0.322% and 0.100-0.171%, respectively, of the administered dose) for all groups. The fat and bone had the lowest tissue levels of radioactivity (0.001-0.002% and 0.000-0.002% of the administered dose, respectively) for all groups.

3. Pharmacokinetics: Radioactivity in the plasma and packed red blood cells of the male rats exposed to a single dose of R-1504 was highest at 0.5 hours after a single dose of 1 or 25 mg/kg [^{14}C]R-1504. The radioactivity in the plasma initially decreased rapidly during the first 6-8 hours postexposure, followed by a more gradual decrease for the remainder of the postexposure period. The packed red blood cells showed a similar biphasic decline in the concentration of radioactivity. The mean half-lives in the red blood cells for the initial decrease in radioactivity ranged from 0.2 to 6 hours (distribution), followed by a slower decrease of 41 to 1,543 hours (elimination) in the male rats exposed to a single dose of R-1504. There were no significant differences among the dosing groups or between the plasma and packed red blood cells. The AUCs for the plasma and red blood cells were 7 and 19 hour•µg equiv/g, respectively, following exposure to a single dose of 1 mg/kg R-1504 and 156 and 343 hour•µg equiv/g, respectively, following single exposure to 25 mg/kg. Therefore, R-1504 is rapidly distributed and eliminated following oral exposure as indicated by the biphasic decline in radioactivity in the blood.

4. Metabolism

Urinary Metabolites: To characterize the metabolite pattern, an average of 96.8% of the radioactivity in the pooled 0-96-hour urine was obtained. Two major radioactive bands (U3 and U6) and 12-14 minor bands (U1, U2, U4, U5, U7-U13) were isolated by TLC plates. The U3 band was 49.5-57.1% of the administered dose of the single and repeated 1-mg/kg females (65-66% of the total radioactivity recovered in urine) compared to 41.5-47% for the other groups (52-55%). Most of the radioactivity in the U3 band was represented by one metabolite in the single low-dose females. The U3 band was developed in TLC system D. Therefore, there appears to be a sex-related difference in the U3 band in the rats exposed to a single or repeated dosing of 1 mg/kg R-1504. The U6 band was 8.6-13% of the activity recovered in the urine of female rats (6.9-11.4% of the administered dose) and 20.2-26% of male rats (16.2-19.9% of the administered dose) for all dosing groups. The U6 band was developed in TLC systems A and E. Therefore, the male rats had a higher recovery of U6 band in the urine compared to the females for all dosing groups. In the females exposed to a single dose of R-1504, the U2 band appeared to be 2 distinct bands or metabolites in which U2a and U2b bands were 2.7% and 1.4%, respectively, of the administered dose in the 1-mg/kg females and

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4.0% and 7.4%, respectively, in the 25-mg/kg females. In the repeated-dose animals and the single-dose males, the U2 band could not be separated as 2 distinct bands. The U4 band was 5.2%-6.6% of the administered dose in the single-dosed animals and 2.1%-3.2% in the repeated-dosed animals. The other bands were less than 5%, usually less than or equal to 1%, of the radioactivity in the urine. In addition, the metabolite pattern was unaffected by β-glucuronidase treatment which indicates that conjugation of R-1504 and its metabolites are minimal.

The radioactive bands, isolated by TLC, from the urine samples were purified and identified by using the HPLC chromatogram. The urinary metabolite pattern from HPLC correlated well with the pattern using the TLC method. The two major urinary metabolites, U3 and U6 were identified as phthalamic derivatives, PaAMS(O)M and its sulfoxide, PaAMS(O2)M, respectively, based on HPLC and MS of the isolated metabolites compared to the reference standards. The U3 metabolite coeluted with PAA in the TLC system B, but had different R_f values in the TLC system C. U3 had the same retention time as PaAMS(O)M using HPLC system D. The mass spectra of U3 and PaAMS(O)M showed the ion at m/z 223, indicating that the ion had no exchangeable protons. However, U3 and its PaAMS(O)M reference was not apparent at m/z 241 (molecular weight of PaAMS[O]M). The authors concluded that the phthalamic acid, PaAMS(O)M, hydrolytically decomposed from the mass spectra to its corresponding phthalimide, PiAMS(O)M (m/z 223). Furthermore, the U6 metabolite and the reference for PaAMS(O2)M showed an ion at m/z 239 indicating that they were also decomposed to PiAMS(O2)M (molecular weight of 239). The TLC method indicated that U3 and U6 were not phthalamide derivatives, but phthalamic acid derivatives. The author reported that R-1504 metabolism involves thiophosphoryl hydrolysis, S-methylation, oxidation of sulfur to sulfoxide or sulfone, and hydrolysis of the phthalimide ring to the respective phthalamic acid. The proposed metabolic pathway for R-1504 in rats is presented in Figure 1.

Fecal Metabolites:

Approximately 85% of the 6-13% activity detected in the feces was used to characterize the fecal metabolites. Nine radioactive bands (F1-F9) from pooled feces were observed. The F7 band represented 7.1% of the administered dose in the 25-mg/kg females and represented only 3.2-5.1% in the other dosing groups. Other minor bands included F8, F9, and F6 which represented 1-2.4%, 0.6-1.5%, and 0.3-0.9% of the administered dose, respectively.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES

The author concluded that R-1504 is rapidly eliminated in the urine and feces of rats. Most of the recovery of the radioactivity occurred within 24 hours after exposure. In the urine, there was a slightly greater recovery of radioactivity in the single low-dose group compared

to the other dosing groups; however, urinary recoveries were similar by 96 hours (slightly lower in the repeated-dose group). There were no major sex- or dose-related differences in the rate and route of elimination. Four days following single and repeated oral dosing of R-1504, the distribution of radioactivity in the rat tissues was highest in the liver and whole blood and lowest in the bone and fat. Overall, most tissues showed low activities (<1% of dose). No major sex- or dose-related differences in the tissue distribution pattern were evident. The pharmacokinetic data indicated that distribution and elimination of R-1504 are rapid and occur in a biphasic decline. The pattern of metabolite radioactivity in the urine was similar for all dose groups--two major radioactive bands and 11 minor bands were found. The two major bands were identified and both were found to have a slight sex-related difference in the percentage of recovery. The feces contained nine radioactive bands; however, none of these bands were identified. There was one major radioactive band in the feces in which there appeared to be slightly higher radioactivity in the high-dose females compared to all the other dosing groups. The author reported that R-1504 in the expired air is not expected to be a route of elimination as indicated from previous literature, so no measurements were made. The author concluded that the results of this study are consistent with the absorption, distribution, and elimination findings from Ford et al. (1966).

Quality assurance statements and statements of compliance with Good Laboratory Practices that were signed, but not dated, were included for both studies.

E. CONCLUSIONS BASED ON REVIEWERS DISCUSSION AND INTERPRETATION OF DATA

The studies adequately described the absorption, distribution, metabolism, and excretion of [^{14}C]R-1504 in rats following single and repeated oral exposure. The data indicate that G.I. absorption of labeled R-1504 is nearly complete and that the urine is the primary elimination route, with the feces as a minor route. The slightly higher radioactivity in the feces of the 25-mg/kg animals compared to the single and repeated 1-mg/kg animals may be due to several possibilities: there may be slight saturation of the test material in rats, the test material was not absorbed by the intestine due to the larger dose, or biliary excretion occurred. It is possible that the lower recovery of radioactivity in the urine of the animals receiving a repeated dose of 1 mg/kg may have been due to experimental error. Absorption occurs readily since there is a rapid appearance of activity in the urine and feces within the first 24 hours. Furthermore, the short initial half-life of the radioactivity in the blood, as indicated by the pharmacokinetic data, suggests rapid distribution and elimination of R-1504. The low tissue levels of radioactivity demonstrate that bioaccumulation and retention of R-1504 and/or its metabolites is low. Recovery of the radioactivity is acceptable (83-97%) for all dose groups, although the animals from the repeated-dosing group should have been observed for more than 4 days. There are no major sex- or dose-

related differences in the absorption, distribution, metabolism, or excretion of R-1504.

The appropriate methods were used to characterize the urinary and fecal metabolites. Two major metabolites in urine were identified; however, the compounds of low activity were not determined by the methods used. There appeared to be a sex-related difference in the recovery of these two metabolites in the urine; the recovery of PaAMS(O)M was higher in females and the recovery of PaAMS(O2)M was higher in the males. The radioactivity compounds in the feces were characterized but no attempt was made to identify these metabolites since there was no procedure stated in the protocol. Furthermore, the authors were not able to identify if the parent compound was eliminated in the urine or feces.

The authors did not discuss the rationale for choosing the dose levels for this protocol. There was no discussion regarding the potential interference of the dosing vehicle to the kinetics of R-1504. The animals in the repeated dosing study should have been observed for more than 4 days to obtain a higher recovery in the urine and feces, although most of the activity had been recovered at 4 days.

References

Ford, I.M., Menn, J.J, and Meyding, G.D. 1966. Metabolism of N-(Mercaptomethyl)-phthalamide-carbonyl-C14-S-(O,O-dimethylphosphorodithioate) (Imidan-C14): Balance Study in the Rat. J Agric Food Chem 14(1): 83-86.

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TABLE 1. Mean Percent Recovery of Radioactivity 4 Days After Oral Administration of R-1504 to Rats

Dose Group	Sex	Percent of Administered Dose				
		Urine	Feces	Cage Wash	Tissues (+ carcass)	Total[a] Recovery
1 mg/kg (single)	Male[b]	86.9	6.7	0.8	3.2	97.6
	Female	87.8	8.0	1.1	2.3	99.2
25 mg/kg (single)	Male	80.2	9.4	1.5	2.8	94.0
	Female	79.7	13.4	1.8	2.6	97.4
1 mg/kg[c] (repeated)	Male	76.7	6.8	0.2	2.8	86.8
	Female	75.0	6.3	0.5	1.9	83.6

[a]Based on individual means. Expired air was not measured for radioactivity because previous data indicated that this was not an elimination route for R-1504.

[b]5 animals/sex

[c]Animals were given 1 mg/kg/day unlabeled R-1504 for 14 days and a single dose of 1 mg/kg [^{14}C]R-1504 on day 15.

Source: CBI Tables 2-14, CBI pp. 24-36 of Study 1.

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TABLE 2. Distribution of Radioactivity in Tissues of Rats 4 Days After Oral Administration of Phosmet

	ng equiv Phosmet/g of tissue in rats dosed at:					
	1 mg/kg (single)		25 mg/kg (single)		1 mg/kg (repeated)	
Tissue/Organ	Males	Females	Males	Females	Males	Females
Kidney	59 (0.057)[a]	49 (0.047)	1055 (0.049)	1257 (0.048)	46 (0.053)	38 (0.049)
Small Intestine	15 (0.027)	8 (0.016)	291 (0.019)	399 (0.033)	10 (0.018)	7 (0.019)
Large Intestine	16 (0.011)	12 (0.010)	506 (0.011)	1140 (0.038)	12 (0.012)	11 (0.015)
Gonads	13 (0.017)	<8 (0.001)	255 (0.009)	<282 (0.001)	9 (0.010)	<8 (0.001)
Brain	22 (0.015)	14 (0.013)	455 (0.012)	379 (0.015)	16 (0.011)	11 (0.012)
Heart	28 (0.011)	20 (0.009)	652 (0.009)	579 (0.009)	28 (0.012)	16 (0.008)
Stomach	51 (0.024)	31 (0.018)	626 (0.010)	553 (0.012)	26 (0.015)	28 (0.023)
Spleen	22 (0.006)	16 (0.005)	578 (0.005)	495 (0.006)	23 (0.005)	13 (0.004)
Lungs	31 (0.019)	20 (0.014)	756 (0.013)	624 (0.015)	23 (0.019)	18 (0.013)
Fat	<13 (0.002)	<13 (0.001)	<446 (0.002)	<518 (0.002)	<17 (0.001)	<12 (0.001)
Muscle	36 (0.004)	22 (0.002)	982 (0.004)	754 (0.007)	34 (0.003)	<16 (0.002)
Liver	32 (0.171)	15 (0.105)	607 (0.127)	463 (0.106)	26 (0.164)	15 (0.100)
Bone	10 (0.002)	5 (0.002)	36 (0.000)	23 (0.000)	4 (0.001)	3 (0.001)
G.I. Contents	-- (<0.03)	--(<0.019)	-- (<0.035)	-- (0.081)	-- (0.028)	-- (0.045)
Carcass	31 (2.074)	21 (1.470)	725 (1.818)	542 (1.426)	26 (1.893)	16 (1.246)
Whole Blood	83 (0.322)	53 (0.199)	1884 (0.278)	1598 (0.275)	68 (0.238)	43 (0.181)
Skin	21 (0.447)	20 (0.380)	527 (0.400)	689 (0.494)	14 (0.317)	10 (0.215)

[a]Each value represents the mean of five rats; values in parenthesis represent the mean percent of the dose; -- = not determined or not applicable.

Source: CBI Tables 9-14; CBI pp. 31-36 of Study 1.

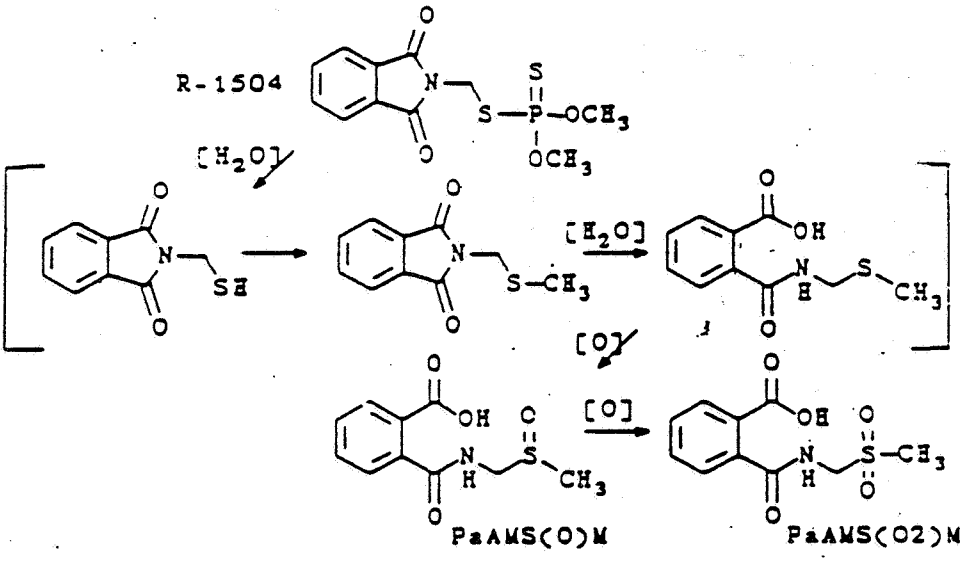

Figure 1. The proposed metabolic pathway of R-1504 following oral dosing in rats

Source: CBI Figure 17, CBI p. 38 of Study 2

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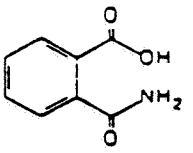
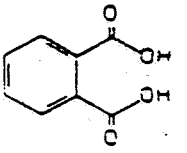
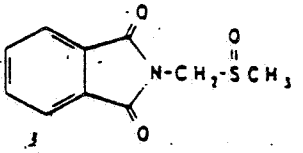
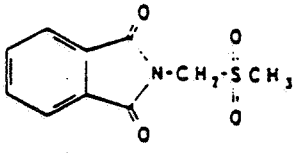
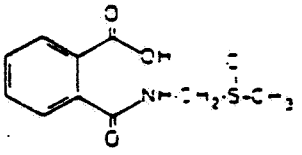
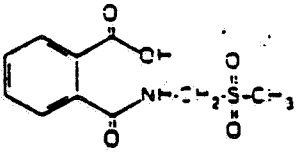
Abbreviation	Chemical Name	Structure
PAA	phthalamic acid	
PA	phthalic acid	
PiAMS(O)M	N-(methylsulfinyl-methyl)phthalimide	
PiAMS(O2)M	N-(methylsulfonyl-methyl)phthalimide	
PaAMS(O)M	N-(methylsulfinyl-methyl)phthalamic acid	
PaAMS(O2)M	N-(methylsulfonyl-methyl)phthalamic acid	

Table 3. The reference standards for identification of R-1504 Metabolites in Rats

Source: CBI Table 1, CBI p. 39 of Study 2.

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PHOSMET Additional data/information for the assessment of the high-dose level.

Clinical observations: The incidence of convulsions was increased in males in a dose-related manner [8, 11, 17, 20]. Convulsions were first observed in the control males at day 329, in the mid- and high-dose males at day 350, and in the low-dose males at day 364.

Body-weight data [see Table 1, below]: MALES: During the first week of the study, the high-dose males did not gain weight, while each of the other male groups gained 2 grams. At week 12, the high-dose males weighed slightly more than the control and other male groups. At study termination, the body weight of the high-dose males was 95% of the control value. Body-weight gains were comparable among the male groups throughout the study, after the initial week of treatment. FEMALES: Body weight and body-weight gains were comparable among the female groups throughout the study.

Table 1. Body Weight/Body-Weight Gain [grams]

Parameter/Time/Sex/Dose	0 ppm	5 ppm	25 ppm	100 ppm
MALES body weight				
0	22	22	23	23
1	24	24	25	23*
2	25	25	26	24
3	26	27	27	25*
12	31	31	31	32
104	38	39	37	36
FEMALES body weight				
0	18	18	18	18
1	19	20	19	19
2	20	21*	21	20
12	26	26	26	27*
104	41	38	41	40
MALES body-weight gain√				
0-1	2	2	2	0
1-2	1	1	1	1
2-3	1	2	1	1
3-4	1	0	0	2
0-6	6	7	6	5
0-12	9	9	8	9
0-14	10	10	9	10
0-104	16	17	14	18
FEMALES body-weight gain√				
0-1	1	2	1	1
1-2	1	1	2	1
2-3	1	1	1	1
3-4	1	0	0	1
0-6	5	5	6	6
0-12	8	8	8	9
0-14	8	9	9	11
0-104	23	20	23	22

√calculated by reviewer using data from Table 2 of the report; * $p<0.05$; data from Table 2 of the report [pages 32-45]

Cholinesterase: At the interim sacrifice, brain cholinesterase activity was significantly inhibited at all dose levels in both sexes, and the inhibition was dose-related in the females, but the low-dose males displayed a slightly greater inhibition than the mid-dose males [Table 2]. Because of technical difficulties, RBC cholinesterase measurements were not obtained at the interim sacrifice. Plasma cholinesterase activity was decreased in the mid-dose females and in both sexes at the high-dose level at the interim sacrifice. At the terminal sacrifice, plasma cholinesterase activity was significantly inhibited at the high-dose level in both sexes, and females at the mid- and high-dose levels displayed a dose-related inhibition in brain cholinesterase activity [Table 3]. The low-dose group of both sexes displayed the lowest RBC cholinesterase activity at termination.

Table 2. Cholinesterase [Interim]

Sex/Dose	0 ppm	5 ppm	25 ppm	100 ppm
MALES brain (IU/g protein) plasma (IU/L)	 63.1±3.8 7089±633	 44.9*±3.4 [29]√ 6316±1188	 49.1*±4.4 [22] 6187±828	 43.6*±2.4 [31] 3278*±674 [54]
FEMALES brain (IU/g protein) plasma (IU/L)	 68.4±5.4 8270±820	 49.0*±3.1 [28] 9133±1124	 47.0*±4.9 [31] 7175*±730 [13]	 45.1*±3.5 [34] 3675*±487 [56]

√ [% inhibition]; * $p<0.05$; data from Table 8 [pages 86-87] of the report

Table 3. Cholinesterase [terminal]

Sex/Dose	0 ppm	5 ppm	25 ppm	100 ppm
MALES brain (IU/g protein) RBC (IU/L) plasma (IU/L)	 52.6±3.4 7892±1390 7264±1898	 49.7±3.4 6532*±769 [17]√ 6992±1663	 52.8±8.2 8804±1176 6597±2371	 50.1±6.2 7704±1214 3828*±1870 [47]
FEMALES brain (IU/g protein) RBC (IU/L) plasma (IU/L)	 55.3±5.4 7664±767 8158±1119	 53.2±5.3 6606±928 [14] 8568±1597	 47.6*±4.1 [14] 8120±1497 7613±1386	 43.1*±6.7 [22] 7962±1035 3950*±781 [52]

√ [% inhibition]; * $p<0.05$; data from Table 8 [pages 88-89] of the report

Non-neoplastic lesions: Lesions observed at the interim sacrifice are listed in Tables 4 [males] and 5 [females]. The terminal sacrifice lesions are listed in Tables 6 [males] and 7 [females].

Table 4. Non-Neoplastic Lesions - MALES [Interim Sacrifice]

	0 ppm	5 ppm	25 ppm	100 ppm
Liver n=	**11**	**10**	**10**	**10**
degeneration, vac. centrilobular	3	1	4	2
focus of basophilic cells	0	1	0	0
focus of vacuol/clear cells	1	0	0	1
inflammation, pyogranulomatous	0	0	1	0
Kidney n=	**11**	**10**	**10**	**10**
degeneration, vacuolative, tubules	9	9	10	10
hyperplasia, regenerative, epithelial	2	1	2	4
inflammation, lymphocytic	2	6	3	1
inflammation, necrotizing	1	0	0	0
Stomach n=	**11**	**0**	**0**	**10**
cyst, squamous	0	-	-	2
hyperplasia, mucosal	0	-	-	2
inflammation, erosive	0	-	-	1

Pancreas n=	**11**	**0**	**0**	**10**
hyperplasia/hypertrophy, islets	1	-	-	3
hyperplasia, acinar	1	-	-	0
Testes n=	**11**	**0**	**0**	**10**
atrophy	2	-	-	1

data from Table 13 [pages 132-137] of the report

Table 5. Non-Neoplastic Lesions - FEMALES [**Interim** Sacrifice]

Organ/Lesion/Dose	0 ppm	5 ppm	25 ppm	100 ppm
Liver n=	**11**	**10**	**11**	**10**
area of vacuol/clear cells	0	0	1	2
area of eosinophilic cells	0	1	0	0
degeneration, vac. periportal	0	1	0	0
degeneration, vac. midzonal	2	2	0	6
degeneration, vac. panacinar	1	0	0	0
degeneration, vac. centrilobular	0	0	0	1
fibrosis, capsule	1	2	0	1
focus of basophilic cells	0	0	0	0
focus of vacuol/clear cells	1	2	2	0
inflammation	7	4	5	8
inflammation, necrotizing	0	0	1	0
necrosis	1	0	0	0
necrosis of individual cells	1	0	0	0
pericholangitis	0	0	0	1
pericholangitis, lymphocytic	1	1	0	1
pigment w/wout pigmented macrophages	0	0	1	0
Mammary Gland n=	**10**	**0**	**1**	**8**
inflammation, lymphocytic/lymphoid	0	0	0	1
Kidney n=	**11**	**10**	**11**	**10**
glomerulonephr, chronic, progress	0	0	0	1
hydronephrosis/dilated pelvis	1	0	0	0
hyperplasia, tubular, epithelial	1	1	1	2
inflammation	1	0	1	4
inflammation, lymphocytic/lymphoid aggregates	10	10	10	9
mineralization, medulla	0	2	1	0
mineralization, cortex	1	0	0	0
Stomach n=	**11**	**0**	**1**	**10**
inflammation	1	-	-	1
inflammation, erosive	1	-	-	0
Pancreas n=	**11**	**0**	**2**	**10**
hyperplasia/hypertrophy, islets	5	-	0	7
inflammation, lymphocytic	5	-	1	5
Spleen n=	**11**	**1**	**2**	**10**
hematopoiesis, extramedullary	1	0	0	0
hyperplasia, lymphoid	0	1	0	2
pigment w/wo pigmented macrophages	0	0	1	0
Uterus n=	**11**	**10**	**5**	**10**
atrophy, myometrial segment/ectasia	1	0	0	1
dilated glands w/wo attenuation	1	1	1	4
hyperplasia, cystic/endometrial	10	9	3	4
inflammation	1	1	0	0

data from Table 13 [pages 138-144] of the report

Table 6. Non-Neoplastic Lesions - MALES [Terminal Sacrifice]

Organ/Lesion/Dose	0 ppm	5 ppm	25 ppm	100 ppm
Liver n=	**49**	**50**	**50**	**50**
degeneration, vac. centrilobular	4	0	0	0
degeneration, vac. individual cells	3	1	2	16
focus of eosinophilic cells	6	4	2	5
focus of eosinophilic cells	4	2	0	8
focus of vacuol/clear cells	4	6	5	13
focus of mixed cells	1	1	2	1
hypertrophy/hyperplasia, Kupffer cell	0	2	0	2
hypertrophy, hepato, centrolobular	3	1	0	5
hypertrophy, vascular, portal	0	0	0	1
hypertrophy, hepato, periportal	0	1	0	0
hypertrophy	0	1	0	0
hyperplasia, reticuloendothelial	1	0	0	0
hyperplasia, lymphoid	0	0	1	0
hematopoiesis, extramedullary	2	0	0	0
inflammation, lymphocytic	1	4	0	1
inflammation, granulomatous	0	0	1	0
myositis, granulomatous	1	0	0	0
necrosis, centrolobular	0	0	0	1
necrosis, acinar	0	0	0	1
necrosis	5	6	2	1
necrosis of individual cells	0	0	0	1
thrombosis	1	0	0	1
atrophy of hepatic cords	0	1	0	0
hamertoma	0	0	0	1
Mammary Gland n=	**49**	**7**	**10**	**50**
adenitis	1	0	0	0
inflammation, pyogranulomatous	1	0	0	1
Kidney n=	**49**	**50**	**50**	**50**
degeneration, med vasc/hypertrophy	0	0	0	1
degeneration, vacuolative, tubules	47	47	44	45
fibrosis, interstitial	0	3	1	2
glomerulonephr, chronic, progress	0	3	2	3
hydronephrosis/dilated pelvis	1	2	1	2
hyperplasia, regenerative, epithelial	38	44	43	44
hyperplasia	0	0	1	0
inflammation, lymphocytic	37	19	24	23
inflammation, necrotizing	0	0	1	0
inflammation, interstitial	0	0	0	3
inflammation, suppurtive	0	1	1	1
mineralization, tubules	24	31	23	20
mineralization, vascular	0	1	0	0
vasculitis	0	0	0	1
necrosis	0	2	0	0
Stomach n=	**49**	**8**	**6**	**48**
hyperplasia, mucosal	5	1	1	10
inflammation, suppurative	1	0	0	1
inflammation, erosive	1	1	1	1
hyperplasia, mucinogenic epithelium	0	0	1	0
hyperplasia, squamous	0	0	0	1
edema	0	0	0	1
necrosis, glandular epithelium	0	0	0	1

Table 6. Non-Neoplastic Lesions - MALES [Terminal Sacrifice]

Organ/Lesion/Dose	0 ppm	5 ppm	25 ppm	100 ppm
Pancreas n=	**49**	**7**	**7**	**50**
atrophy/degeneration, islets	0	0	1	2
degeneration, chronic	0	0	0	3
hypertrophy, acinar	1	0	0	0
hyperplasia/hypertrophy, islets	23	3	3	23
inflammation, lymphocytic	6	0	0	4
infiltration, fatty	0	0	0	1
necrosis	0	1	1	0
Spleen n=	**49**	**20**	**16**	**50**
atrophy	0	0	2	1
ectasia, sinusoidal	0	0	0	1
hematosiderosis	0	0	0	1
hypoplasia, erythroid	0	0	0	1
hypoplasia, lymphoid	0	2	0	0
hematopoiesis, extramedullary	10	2	2	0
hyperplasia, lymphoid	3	6	1	7
lymphangiectasia	1	0	0	0
thrombosis	0	0	1	1
vasculitis	0	0	0	1
degeneration, vascular/hypertrophy	0	1	0	0
Testes n=	**49**	**9**	**10**	**50**
atrophy	11	4	5	19
Muscle n=	**49**	**7**	**10**	**50**
perivascilitis	0	0	0	7
inflammation, lymphocytic	2	0	0	1
inflammation, perineural	3	0	0	1
degeneration, muscle	0	0	1	0

data from Table 13 [pages 160-172] of the report

Table 7. Non-Neoplastic Lesions - FEMALES [Terminal Sacrifice]

Organ/Lesion/Dose	0 ppm	5 ppm	25 ppm	100 ppm
Liver n=	**49**	**50**	**48**	**50**
area of vacuol/clear cells	4	4	5	4
area of eosinophilic cells	0	1	1	0
area of mixed cells	1	0	1	1
area of basophilic cells	1	0	1	2
atrophy of hepatic cords	0	0	0	1
capsulitis	3	0	0	4
degeneration, vac. periportal	2	2	3	2
degeneration, vac. midzonal	1	3	1	5
degeneration, vac. panacinar	4	0	1	5
degeneration, vac. centrilobular	3	0	4	0
degeneration/hypertrophy, vascular	1	0	0	0
focus of eosinophilic cells	2	0	0	2
focus of vacuol/clear cells	0	4	6	0
focus of mixed cells	0	1	0	0
focus of basophilic cells	0	0	1	0
hematopoiesis, extramedullary	0	4	1	2
hyperplasia	2	0	0	1
hypertrophy	0	0	0	1
inflammation	36	32	41	37
inflammation, necrotizing	1	1	0	4
inflammation, granulomatous	1	1	0	0
inflammation, pyogranulomatous	0	0	0	1
increased mitotic figures	0	0	0	1
lymphoid aggregates	24	25	26	19
RE aggregates	0	1	0	0
necrosis	5	2	4	3
necrosis of induvidual cells	1	2	4	4
pericholangitis	1	1	0	1
pericholangitis, lymphocytic	14	9	17	14
telangiectasis	0	0	1	2
thrombosis	1	0	2	0
Mammary Gland n=	**45**	**15**	**11**	**49**
atrophy, follicular, adnexal	0	0	0	1
fibrosis	0	0	0	1
hyperplasia	2	1	0	1
inflammation	0	2	0	1
inflammation, lymphocytic/lymphoid aggregate	3	1	0	5
inflammation, ulcerative	0	1	0	0
pigment w/wo pigmented marcophages	1	0	0	0
Kidney n=	**49**	**50**	**48**	**50**
dilatation, tubules w/wo glom filtr	0	0	2	1
fibrosis, capsule	0	1	0	0
glomerulonephr, chronic, progress	4	0	2	5
glomerulonephropathy, membraneous	4	0	1	1
glomerulosclerosis	2	0	0	0
hydronephrosis/dilated pelvis	5	3	1	2
hyperplasia, tubular, epithelial	24	15	22	25
infarct	0	0	0	1
inflammation	13	12	4	10
inflammation, lymphocytic/lymphoid aggregates	45	45	43	46
mineralization, tubules	1	0	0	0
mineralization, cort/med junction	5	6	7	4
mineralization, medulla	1	4	6	5
mineralization, cortex	12	11	12	13
pigment, tubular epithelial cell	2	2	2	0
pigment w/wo pigmented macrophages	0	0	1	0
nephrosis	2	1	0	1

Table 7. Non-Neoplastic Lesions - FEMALES [Terminal Sacrifice]

Organ/Lesion/Dose	0 ppm	5 ppm	25 ppm	100 ppm
Stomach n=	**49**	**13**	**9**	**49**
atrophy, mucosal	0	0	0	2
edema	0	0	0	1
inflammation	3	2	1	9
inflammation, lymphocytic	1	0	0	0
inflammation, ulcerative	1	0	1	2
inflammation, erosive	0	1	1	3
Pancreas n=	**49**	**12**	**9**	**48**
atrophy	2	1	0	0
hyperplasia/hypertrophy, islets	24	8	3	30
inflammation	2	1	1	1
inflammation, lymphocytic	39	9	4	40
hyperplasia, acinar	1	0	0	0
degeneration	0	0	1	0
Spleen n=	**49**	**42**	**27**	**50**
capsulitis	0	1	0	1
depletion, lymphoid	1	0	0	1
fibrosis, capsule	0	1	0	1
hematopoiesis, extramedullary	11	8	7	8
hyperplasia, lymphoid	14	10	9	11
hyperplasia, reticuloendothelial	2	1	0	1
inflammation	2	2	0	0
necrosis	0	0	1	1
necrosis, follicular	0	0	0	1
pigment w/wo pigmented macrophage	0	0	0	1
Spinal cord n=	**49**	**15**	**9**	**50**
hemorrhage	0	0	1	2
hematopoiesis, extramedullary	11	6	0	12
hyperplasia, meninges	1	0	0	0
inflammation	0	1	0	0
inflammation, lymphocytic	0	0	0	1
meningitis, lymphocytic/lymphoid aggregates, meninges	12	2	2	18
Uterus n=	**49**	**41**	**26**	**50**
atrophy, myometrial segment/ectasia	2	4	2	8
dilated glands w/wo attenuation	4	2	2	5
edema	0	0	0	1
hyperplasia, cystic/endometrial	43	35	20	37
inflammation	6	9	3	2
inflammation, purulohemorrhagic	0	0	0	1
inflammation, lymphocytic	12	8	7	17
thrombosis	0	1	0	1

data from Table 13 [pages 173-186] of the report