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

SEP 11 1987

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Phosmet (Imidan) - Submission of a Mutagenicity (Micronucleus) Assay for Evaluation - Accession No. 401994-1 - EPA Registration No. 476-2178

TOX Chem. No. 543

FROM: William B. Greear, M.P.H. William B. Greear 9/2/87
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TO: Adam Heyward, PM Team 15
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THRU: Albin B. Kocialski, Ph.D. ABK 9/4/87
Section VII, Toxicology Branch
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and

Theodore M. Farber, Ph.D. 9/9/87
Chief, Toxicology Branch
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Under a cover letter dated May 11, 1987, M.E. Burt of Stauffer Chemical Company submitted a mutagenicity study for evaluation as specified in the Phosmet Registration Standard. The study is entitled "Phosmet: Report of a Micronucleus Test in the Mouse." The study was determined to be acceptable and the test material was reported not to be clastogenic. The Data Evaluation Record is attached.

Attachment

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EPA: 68-02-4225
DYNAMAC No. 318-B
July 31, 1987

DATA EVALUATION RECORD

PHOSMET/IMIDAN

Mutagenicity--Micronucleus Assay in Mouse Bone Marrow

STUDY IDENTIFICATION: Gibbs, J. A. and Mitchell, I. de G. Phosmet report of a micronucleus test in the mouse. (Unpublished study No. T86/756 prepared by Beecham Pharmaceuticals, Ingatestone, Essex, United Kingdom, for Stauffer Chemical Company, Richmond, CA; dated December 18, 1986.) Accession No. 401994-01.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-31-87

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1. CHEMICAL: Phosmet.

2. TEST MATERIAL: Phosmet was supplied as technical Prolate, lot No. 11694 (95.5% phosmet).

3. STUDY/ACTION TYPE: Mutagenicity--Micronucleus Assay in mouse bone marrow.

4. STUDY IDENTIFICATION: Gibbs, J. A. and Mitchell, I. de G. Phosmet report of a micronucleus test in the mouse. (Unpublished study No. T86/756 prepared by Beecham Pharmaceuticals, Ingatestone, Essex, United Kingdom, for Stauffer Chemical Company, Richmond, CA; dated December 18, 1986.) Accession No. 401994-01.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
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Signature: Brenda Worthy
Date: 7-31-87

Nancy E McCarroll, B.S.
Independent Reviewer
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Signature: Nancy E. McCarroll
Date: 7-31-87

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
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Signature: I. Cecil Felkner
Date: 7-31-87

Albin Kocialski, Ph.D.
W.B. Green EPA Reviewer and Section Head

Signature: W.B. Green 8/6/87
Date:

7. CONCLUSIONS:

A. Under the conditions of the micronucleus assay, phosmet, administered orally at 17 mg/kg to male and female mice, did not cause a clastogenic effect in the bone marrow cells assayed at 24, 48, or 72 hours after dosing. Based on the preliminary toxicity study and the cytotoxicity response noted in the assay, the dose selected was adequate. Cyclophosphamide (CP), the positive control, demonstrated the sensitivity of the assay to detect a clastogenic response.

B. The study is acceptable; phosmet is not clastogenic in this assay.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Phosmet with a purity of 95.5% was supplied to the laboratory as technical Prolate, lot No. 11694. The test material was suspended in 1% methylcellulose (MC), the vehicle control.

2. Test Animal: Six- to 12-week-old CD-1 mice of each sex were obtained from Charles River, Manston, Kent. Animals were group housed (6 or 7 mice/cage) according to sex and acclimated to laboratory conditions for 5 days. Environmental conditions were not reported. Food and water were available ad libitum. Animals were identified by earmarks. Prior to dosing, animals were weighed.

3. Preliminary Toxicity Study: Phosmet was administered as a suspension at 15, 20, or 30 mg/kg in 1% MC to six mice (three males and three females) in a single oral dose. Six mice also received the vehicle control. Animals were observed periodically for toxic signs until they were sacrificed. One animal per sex per group was sacrificed at 24, 48, or 72 hours after dosing, and femoral bone marrow cell smears were prepared. Smeared cells were examined for cytotoxicity as evidenced by an increase in the percentage of polychromatic erythrocytes (PCEs) to total erythrocytes. Five hundred PCEs per animal were scored.

4. Positive Control: CP at 74 mg/kg and dissolved in distilled water was used as the positive control.

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[1]Only items appropriate to this DER have been included.

5. Dose Groups/Compound Administration: It was not reported how animals were assigned to dose groups. Ten mice (five male and five female) per dose group/sacrifice interval were administered a single oral dose of the test material, vehicle, or positive control at a volume of 10 mL/kg. One extra animal per sex was dosed in the vehicle and positive control groups and two extra animals were included in the test material group to be used if necessary.

6. Micronucleus Assay:

   a. Animal Sacrifice/Bone Marrow Harvest: Twenty-four, 48, and 72 hours after the test material and vehicle control were administered or 24 hours after the positive control was administered, the animals were sacrificed by carbon dioxide asphyxiation and cervical dislocation. Bone marrow cells from both femurs were aspirated into 3 mL of fetal calf serum and mixed. Samples were centrifuged, the supernatants were discarded, and the pellets were resuspended in the remaining liquid.

   b. Slide Preparation: A drop of the suspension was spread onto a glass slide and air dried. Four slides per animal were stained using Wright's modification of Giemsa, then mounted and coded.

   c. Slide Analysis: One thousand PCEs per animal were scored for the number of micronucleated polychromatic erythrocytes (MPEs). Genetic activity was based on this data.

   Other parameters determined included the percentage of micronucleated mature erythrocytes and the percentage of PCEs in the total number of erythrocytes scored.

7. Statistical Analysis: If warranted, the data were analyzed by a one-sided Kolomogorov-Smirnov two-sample test.

8. Evaluation Criteria: A test material was considered positive if it caused a significant ($p < 0.005$) increase in MPE or if it caused a >3-fold increase in MPE over the vehicle control.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Preliminary Toxicity Study: In an acute toxicity study (Toxicology, Vol. 20, Section VI, pp. 1255, 1318-1322), the oral LD_{50} for phosmet in mice was reported to be 41.1 mg/kg. Based on the reported LD_{50}, the authors selected dose levels of 15, 30, and 45 mg/kg for the preliminary toxicity study; all animals in the 30- and 45-mg/kg dose groups died; therefore, the dosage

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level was reduced to 20 mg/kg. Death occurred in 1/6 mice (1 female) in the 20-mg/kg group; no deaths occurred at the 15-mg/kg dose level.

Slide-examination revealed cytotoxicity (decrease in the percent of PCEs to total erythrocytes) in the samples obtained from both male and female mice in the 15- and 20-mg/kg dose groups at the 48-hour interval. At 72 hours, cytotoxicity was observed in the male and female mice in the 15-mg/kg dose groups and in the males in the 20-mg/kg dose group. No cytotoxicity was noted in male or female mice in either dose group at the 24-hour sacrifice.

Results are presented in Table 1.

B. Micronucleus Assay: Based on the preliminary toxicity study, a dose of 17 mg/kg was selected for the micronucleus assay. No deaths occurred at the 24-, 48-, or 72-hour intervals. A slight decrease in the percent of PCEs was noted at the 24-hour interval for both male (11%) and female (6%) mice when compared to the vehicle control. No increase in the number of MPEs was observed in either sex at 24, 48, or 72 hours after dosing.

The positive control, CP (75 mg/kg), caused a >3-fold increase in MPEs, demonstrating the sensitivity of the test system to detect a clastogenic response.

Since there were no sex-related differences in the frequency of MPEs, the results were combined (see Table 2).

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that "Phosmet, at 17.0 mg/kg, did not give rise to a significant increase in the incidence of micronuclei in bone marrow polychromatic erythrocytes. Data were negative at all three sample times and in both sexes of CD-1 mice. Thus, phosmet showed no significant genetic activity in this in vivo assay."

B. A quality assurance statement was signed and dated December 18, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study authors interpreted the data correctly and that phosmet at 17 mg/kg did not cause an increase in MPEs in the bone marrow of male or female mice at 24, 48, or 72 hours after dosing. In the preliminary toxicity study, all animals dosed at 30 mg/kg died; a death occurred in the 20 mg/kg dose group with cytotoxic effects and cytotoxicity was noted in the 15 mg/kg dose group. The authors selected an intermediate dose of 17 mg/kg for the micronucleus assay. We assess that the 17 mg/kg dose was chosen

TABLE 1. Results of the Preliminary Toxicity Study in Mice Dosed Orally with Phosmet

Substance	Dose (mg/kg)	Harvest Interval (hour)	Three Animals per Sex per Group[a]	Mortality	Percent PCEs[b] per Group Males	Females
Vehicle Control						
1% Methylcellulose	10 mL/kg	24	2	0	31.49	34.51
		48	2	0	37.51	53.13
		72	2	0	42.77	38.26
Test Material						
Phosmet	15	24	2	0	41.43	34.99
		48	2	0	31.59 (16)[c]	36.58 (31
		72	2	0	34.89 (18)[c]	26.01 (32
	20	24	2	0	32.09	32.70
		48	2	0	30.98 (17)[c]	37.20 (30
		72	2	1	23.78 (44)[c]	--
	30		No animals survived.			

[a]One mouse/sex/group sacrificed at 24, 48, or 72 hours.

[b]PCEs--Polychromatic erythrocytes.

[c]Percent decrease in PCEs when compared to the vehicle controls; calculated by our reviewers.

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TABLE 2. Results from the Micronucleus Assay in Mice with Phosmet

Substance	Dose (mg/kg)	Harvest Interval (hour)	No. of Animals Analyzed[a]	No. of PCEs[b] Scored per Group	No. of MPEs[c] per Group	Percent MPEs per Group ± SD	Percent PCEs per Group ± SD
Vehicle Control							
1% Methylcellulose		24	10	10,000	9	0.09±0.10	47.94±5.3
		48	10	10,000	5	0.05±0.10	47.49±2.9
		72	10	10,000	12	0.12±0.10	44.27±4.9
Positive Control							
Cyclophosphamide	75	24	10	10,000	234	2.34±1.2[d]	39.87±3.7
Test Material							
Phosmet	17	24	10	10,000	11	0.11±0.10	43.80±4.5*
		48	10	10,000	9	0.09±0.10	48.99±4.2
		72	10	10,000	9	0.09±0.10	44.94±4.9

[a] Five male and five female mice/group; results combined by our reviewers.

[b] PCEs--Polychromatic erythrocytes.

[c] MPEs--Micronucleated polychromatic erythrocytes.

[d] Positive response >3-fold increase over the vehicle control.

* Cytotoxicity--slight decrease in PCEs (9% combined: 11% for males, 6% for females) when compared to vehicle controls.

because it would not cause death; however, it would elicit a cytotoxic effect which would demonstrate that the test material reached the target organ (bone marrow). Although the assay was performed with one dose only, it was adequate according to USEPA guidelines which state "For an initial assessment, one dose of the test substance may be used, that dose being the maximum tolerated dose or that producing some indication of cytotoxicity, e.g. a change in the ratio of polychromatic to normochromatic erythrocytes." Based on the preliminary toxicity data and the slight cytotoxic effect noted in the micronucleus assay, we concluded that the dose selected was adequate, and that repeating the assay would serve no useful purposes since the dose selected is within a narrow range approaching the MTD. Since a genotoxic response is not directly related to animal lethality but rather to reaching the target site, cytotoxicity provides adequate evidence for a sufficiently high test dose.

The positive control, CP, demonstrated the sensitivity of the assay to detect a clastogenic response.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-5.

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APPENDIX A

Materials and Methods

COMPOUND

Phosmet, supplied as Technical Prolate, lot no: 1169^, (95.5% phosmet).

All dose levels are stated in terms of 100% phosmet.

ANIMALS

Mouse: CDBS CD-1 (ICR) BR, Charles River (Manston, Kent), 6-12 weeks old.

Order No.: R100907.

MANAGEMENT

Animals were housed, according to sex, in polypropylene cages. Food and water were readily available (SDS Diet No. 1). The study was initiated after an acclimatisation period of approximately 5 days. Twenty-five animals were dosed in the preliminary test and 90 in the main test.

IDENTIFICATION

Animals were identified by earmarks throughout.

DOSE ROUTE

Oral by gavage.

DOSE VOLUME

The dose volume was 10 ml/kg.

PRELIMINARY SOLUBILITY AND TOXICITY

Phosmet was known to be soluble in water to only approximately 25 mg/litre (Royal Society of Chemistry Agrochemical Handbook 1983). Therefore, as in previous rodent tests, the vehicle chosen for this Study was 1% (w/v) methyl cellulose.

Originally it was planned that three males and three females would receive a single dose of phosmet at each of three concentrations:- 15.0, 30.0 and 45.0 mg/kg. The animals dosed at 15.0 mg/kg all survived. However, all animals dosed at 30.0 mg/kg died within one hour of dosing (as well as the single male dosed at 45.0 mg/kg). As a result, a further dose group (20.0 mg/kg) was set up instead of the top dose group (45.0 mg/kg).

In addition, three male and three female animals received the negative control 1% (w/v) methyl cellulose.

All animals were observed periodically until termination. One male and one female animal from each surviving group were killed at 24h, 48h or 72h after dosing and femoral bone marrow smears prepared. Slides were examined for evidence of specific bone marrow toxicity as judged by the number of polychromatic erythrocytes (as a percentage of the total erythrocytes scored).

No slides were prepared from animals which died before termination.

For the toxicity test, 500 polychromatic cells were scored (per animal).

MAIN MICRONUCLEUS TEST

SELECTED DOSE LEVEL

Phosmet, at 17.0 mg/kg, was selected as a suitable dose level to be used in the main test. This figure approximated the LD_5 value, estimated from the preliminary test results.

COMPOUND ADMINISTRATION

Phosmet was dosed as a suspension in 1% (w/v) methyl cellulose, orally by gavage, at a dose volume of 10 ml/kg. All animals received a single dose of the agent or controls.

CONTROLS

Negative control: 1% (w/v) methyl cellulose.

Positive control: Cyclophosphamide, dosed at 75.0 mg/kg in distilled water.

MAIN TEST DESIGN

Group	Treatment	No. of animals* for each sampling time (individual animal numbers in brackets)		
		Sample I (24h)	Sample II (48h)	Sample III (72h)
1	Negative control 1% methyl cellulose	6♂ (1-6) 6♀ (46-51)	6♂ (7-12) 6♀ (52-57)	6♂ (13-18) 6♀ (58-63)
2	Phosmet 17.0 mg/kg	7♂ (19-25) 7♀ (64-70)	7♂ (26-32) 7♀ (71-77)	7♂ (33-39) 7♀ (78-84)
3	Positive control Cyclophosphamide 75.0 mg/kg	6♂ (40-45) 6♀ (85-90)	———	———

* One spare animal was included in each cage, with the exception of the phosmet-treated group, where two spare animals were included.

TERMINATION

At the end of the test, animals were killed by carbon dioxide asphyxiation and cervical dislocation. Animals in Sample I were killed 24h after dosing, Sample II, 48h after dosing and Sample III, 72h after dosing. Femora were removed immediately after sacrifice and bone marrow cells extracted for the preparation of smears.

PREPARATION OF SLIDES FOR THE EXAMINATION OF MICRONUCLEI IN BONE MARROW ERYTHROCYTES

Animals were killed and both femora dissected out. Adherent tissue was removed from the bones and the epiphyses cut off. The bone marrow was washed out of each shaft with approximately 1 ml foetal calf serum into a 15 ml centrifuge tube containing 3 ml of serum and mixed well.

Each cell suspension was centrifuged at 1000 rpm for 5 minutes and the supernatant discarded. The cells were resuspended in the remaining liquid by gentle aspiration with a glass Pasteur pipette.

Smears were prepared by placing a small drop of the suspension on a clean, grease-free slide about 1-2 cm from the end. The spreading slide was placed at an angle of 45° to the slide and moved back to make contact with the drop. The film was spread by a rapid, smooth, forward movement of the spreader and then air-dried. Two to three slides per animal were prepared in the preliminary toxicity test and four slides per animal in the main test.

STAINING TECHNIQUE

Smears were stained on an automatic staining machine using Wright's modification of Giemsa. Slides were then cleared in CNP 30 for a few minutes and finally mounted in D.P.X.

SLIDE SCORING

Slides were coded by an independent observer to enable unbiased scoring. Slides were then selected according to technical suitability and scored for erythrocytes with or without micronuclei. All cells observed to contain micronuclei were referenced for slide position.

Polychromatic erythrocytes with and without micronuclei were scored up to a total of 1000 cells/animal. Results were recorded as cells with or without micronuclei and percentage micronucleated cells.

Mature erythrocytes were also examined and recorded as cells with or without micronuclei and percentage micronucleated cells.

In addition, the number of polychromatic cells as a percentage of the total number of cells scored, was recorded.

ANALYSIS OF RESULTS

Comparison between the negative control group and the treatment group was made for the following end-points:-

i) Micronucleated polychromatic cells as a percentage of the total number of polychromatic cells scored.

ii) Micronucleated mature cells as a percentage of the total number of mature cells scored.

iii) Polychromatic cells as a percentage of the total number of erythrocytes scored.

Genetic activity was assessed from the micronucleated polychromatic cell data only, the other two results serving as a check on the assay system (accessory data).

If the determination of stochastic significance had been necessary, a one-sided, Kolmogorov-Smirnov two sample test would have been used.

GENETICAL SIGNIFICANCE

To be considered genetically significant, results must have fulfilled two criteria:-

a) data must have been statistically significant with one sample significantly greater than the negative control at $p \leq 0.005$.

b) data must have been quantitatively significant defined here as exceeding the negative control by ≥ 3.0 fold.