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

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JAN 2 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

Subject: Review of Prometon Mutagenicity Supplements and Response to Waiver Request: PHASE 4 DATA CALL-IN. EPA ID # 080804

To: Thomas Luminello PM# 52
Reregistration Division
(H7508W)

EPA Record NO. S401683
Project No. 1-2288
Chemical: Prometon

TOX.CHEM NO:96

From: Vivian Williams, MS
Toxicology Section II
Toxicology Branch I
HED (H7509C)

V Williams 12/17/91

Thru: Joycelyn Stewart, Ph.D.
Section Chief
Toxicology Section II
Toxicology Branch I

12/14/91

and
Karl Baetcke, Ph.D.
Branch Chief
Toxicolcgy Branch I

Karl Baetcke 12/15/91

Action Requested:

Review the submitted supplemental data for three mutagenicity studies (Salmonella MRID# 419843-01; Micronucleus MRID# 419843-02; DNA Repair MRID# 419843-03) and respond to a waiver request for metabolism studies.

CONCLUSIONS:

Although certain deficiencies which were cited in the original mutagenicity review have been addressed in the supplemental data, the submission is still considered as inadequate since shortcomings continue to exist. In the micronucleus test, the selection of the highest dose level is not adequately explained and the positive control data is inappropriate. For the DNA repair test in rat hepatocytes, specific cytotoxicity data have not been provided for the highest dose selected for the assay.

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The deficiencies regarding characterization (purity and physical description) of the test chemical in the **Ames Salmonella Test** have been **satisfactorily addressed.**

The status of the micronucleus test and the DNA repair test remains as unacceptable.

If the necessary information cannot be provided which will allow us to upgrade these studies, a request could be initiated to have these studies repeated.

In regard to the registrant's waiver request for Guideline 85-1, (General Metabolism), it is understood that prometon is listed as a non-food use chemical which normally would not require oncogenicity and/or metabolism studies. However, this chemical has the unique quality of being a member of the triazine family and there is sufficient documentation that many triazines have tested positive for oncogenicity. Because of its structural relationship to other triazines such as simazine, propazine, atrazine and terbutryn, (all of which are recognized carcinogens), oncogenicity data are required for prometon as stated in 40 CFR 158.340, notes 9 & 21. Hence, based on the oncogenicity requirement, the metabolism study becomes a part of the required data set as stated in CFR 158.340, note 23. It should be acknowledged that the registrant has provided the required oncogenicity data and these data are currently under review within the OPP. It may be considered as appropriate for the OPP to delay a response to the waiver request until after the evaluation of the rat and mouse oncogenicity studies are finalized. If the conduct of these studies is deemed appropriate and the results reveal no oncogenicity, then it will be within the OPP's authority to grant a waiver since metabolism data would be of little support to negative oncogenicity data.

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Reviewed by: Vivian Williams, MS
Section II, Tox Branch I (H75090)
Secondary Reviewer: Irving Mauer, Ph.D.
Tox Branch I (H75090)
Date: November 21, 1991

DATA EVALUATION REPORT COVER

The Ames Salmonella Test

When the data were initially sent to the Agency for review, the preliminary toxicology data were not included in the submission for the Ames Assay. The highest concentration used in this study was 2500 ug/plate based on the response of tester strain TA-100 to concentrations of 5000 and 10,000 ug/plate. Cytotoxicity was noted at these levels by the absence of background growth at 10,000 ug and a severe reduction in background growth and the presence of many pinpoint colonies at 5000 ug; Vogel-Bonner E agar plates were used. This was indicative of a bactericidal effect on TA-100 at these concentrations. This bactericidal effect was also observed on Oxoid Nutrient agar #2 plates at these concentrations; there was failure of any cells from TA-100 inoculum to survive. The severe cytotoxicity noted at 5000 ug/plate and the slight decrease in the number of revertant and survival colonies at 2500 ug/plate (as shown in the submitted table on toxicity, survival, and solubility) was the basis for selecting 2500 ug as the high concentration.

The above information sufficiently provided the concentration at which cytotoxicity occurs. Although the supplemental information did not address the characterization of the test article material in terms of purity (active ingredient), or physical characteristics, this information was found in the registrant's oncogenicity (in mice) submission which listed the same batch number for the chemical.

Test Chemical: Prometon technical; description: white powder;
batch #: FL8411714; purity: 97-98.7%

Classification: Acceptable

The Micronucleus Test

This supplement addressed four specific areas which required clarification:

1) the utilization of rats (versus mice as stated in the guidelines);

2) the choice of 648 mg/kg as the highest dose level;

3) positive and negative controls, averages for number of polychromatic erythrocytes with micronuclei and P values;

4) the selection of test doses and sampling times.

Utilization of Rats vs Mice

According to the submitter, rats were utilized in this assay instead of mice because other triazine compounds when tested for oncogenicity, have given positive results in rats but not in mice. As a result, it was deemed appropriate by the submitter to conduct the micronucleus test using rats. After reviewing the current OPP database on some triazines such as simazine, atrazine, propazine, and terbutryn, it is confirmed that positive oncogenicity results have been obtained when rats were tested with these chemicals but negative results were obtained when mice were tested for oncogenicity. It is reiterated, however, that mice are utilized in most of the studies upon which the recommended protocol is based and is generally the preferred species for this type of test (Federal Register, Vol. 50, No.188, Friday, September 27, 1985) .

Selection of the Highest Dose Level

The supplement provided the procedure used for the oral range-finding/tolerability test which was run to determine the highest dose level for use in the micronucleus assay. In the first run of the range-finding test, two animals/sex/dose were administered a single dose of 5000, 1000, and 200 mg/kg of technical grade prometon in the food. At the highest dose level, 3/4 animals died; 2 died on day one after dosing and one died on day two post-dosing. All animals survived the other two dose levels. In the second step of the range finding study, a dose level of 3000 mg/kg was tested wherein all animals died; three died on day one after dosing and one died one day two. Two other dose levels of 1800 and 1080 mg/kg resulted in extreme mortality as well. When the dose level was further adjusted downward and administered at 648 mg/kg, all

animals survived. Although this information provides insight in terms of procedure, there is no indication whether 648 mg/kg was a clinical MTD or manifested target organ toxicity .

Positive and Negative Controls

In executing the micronucleus test, cyclophosphamide was used as the positive control. However, the dose level of 64 mg/kg appears to have been excessive for rats as evidenced by the submitter's in-house lab data which showed that it influences the development of erythrocytes and partially inhibits the formation of micronucleated polychromatic erythrocytes. Information was provided which showed that the ratio of polychromatic to normochromatic erythrocytes in cyclophosphamide-treated animals decreased relative to the negative controls:

		Ratio of PCE/NCE (mean)
24 hours	Neg controls CMC 0.5%	
	Females	0.8
	Males	0.7
	Pos controls Cyclophos. 64 mg/kg	
	Females	0.5
	Males	0.3

This is inappropriate positive control data.

Sampling Times

The rationale for dose selection was explained in the previously discussed range-finding/tolerability test. The rationale used for determining sampling times was explained by the following procedure in the supplement:

"In the first part of the study, the highest tolerated dose of the test substance (based on the results of the range-finding test) was administered and the animals of the appropriate treatment groups were sacrificed 16, 24 and 48 hours thereafter. In the second part of the study, three different doses of the test substance were administered. Because there was no increase in the number of micronuclei induced by the test substance in the first part of the study, the animals were sacrificed in the second part 24 hours after treatment."

The guidelines state that "other appropriate sampling times may be used in addition" but "if the most sensitive time interval is known and documented with data, only this time point need be sampled". The determination of sampling times was reasonable.

Overall, the registrant's rebuttal did not address the original reviewer's concerns for the micronucleus test.

Classification: Unacceptable

The DNA Repair Test

Although the percentages of viable cells were not provided in the initial submission, this information was presented in the supplemental report. Due to the toxic effect of the test substance at the highest concentrations of 5000, 2500, 1250 and 625 ug/ml, the number of viable cells was so small that no viability could be determined (see the supplement, page 4). At a concentration of 312.5 ug/ml, cell viability was 76%. Although the concentration of 400 ug/ml was selected as the highest level for this test, no hard data were provided which addressed cytotoxicity at this level. Based on the concentrations which were listed on the viability table (see supplement, page 4), 400 ug/ml was not a concentration that was tested.

Classification: Unacceptable