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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No. 7969-58, Poast® Herbicide. Mutagenicity Studies with 5-OH-MSO2 (Hydroxymetabolite MU-1), Accession Nos. 252349 and 252350.

TOX Chem. No. 72A and 584E

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THRU: Edwin R. Budd, Section Head
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Budd 2/19/85

2/19/85

Registrant: BASF Wyandotte Corporation
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Action Requested:

As a condition for the registration of the herbicide Poast®, BASF agreed to submit additional toxicology data using the most abundant hydroxymetabolite (5-OH-MSO2, also identified as MU-1). This present submission consists of two studies:

1. Mutagenicity of 5-OH-MSO2 After a Single Oral Administration to Chinese Hamsters (Cytogenetics, Bone Marrow Chromosome Analysis) Accession No. 252349.

2. Mutagenicity Evaluation of 5-OH-MSO2 in the CHO-HGPRT Forward Mutation Assay, Accession No. 252350.

Recommendations:

1. 5-OH-MSO2 is not mutagenic under the described conditions for the Bone Marrow Cytogenetics Assay with dosages up to 10,000 mg/kg body weight (highest dose tested).

Core Category: Acceptable according to current methodology.

22

479

2. 5-OH-MSO2 is not mutagenic in the CHO-HGPRT cell culture assay with or without metabolic activation at levels from 2.0 mg/ml through 10.0 mg/ml test article (highest dose tested)

Core Category: Acceptable according to current methodology.

DETAILED REVIEW OF STUDIES

Preface:

1. Bone Marrow Cytogenetics

Cytogenetics analysis is an indicator system for the evaluation of mutations induced by chemicals. It permits direct visual analysis of damaged chromosomes, including changes in numbers. For the bone marrow study, animals are challenged by single oral administration of a vehicle control, different doses of test chemical or a known chromosome damaging agent (in this case, cyclophosphamide) as a positive control. Treated animals are then sacrificed at time intervals, beginning two hours after injection with colchicine, a chemical that arrests cell division and facilitates the detection of abnormal chromosomes in metaphases. Bone marrow is extracted from the animals' femurs, chemically treated, applied to microscope slides and read directly through the microscope. Reading are usually made from around 100 metaphase cells. Results may show dose-related and/or time related increases in various categories of chromosome damage, including breaks, exchanges, increases or decreases in absolute numbers, etc. Comparisons between treated, negative (vehicle) controls and positive controls. Statistical significance is then determined.

2. CHO-HGPRT Forward Mutation Assay

The cell line in this assay is derived from the ovary of a female Chinese hamster (Cricetulus griseus). A number of other variants of this cell line are known, of which one, CHO-K1-BH4, was used for the study below. The assay for mutagenicity of a test chemical is as follows:

a) HGPRT stands for Hypoxanthine Guanine Phosphoribosyl Transferase, an enzyme for which a gene is located on the x chromosome of the cell. Since only one x chromosome is functional in the cell, a single mutation is capable of rendering the cell unable to pick up hypoxanthine, guanine or the (toxic) purine

TG (thioguanine). Under standard conditions, the cell is able to salvage hypoxanthine, guanine or TG. The uptake of TG however, lends to cell death. HGPRT mutants however, cannot take up TG, hypoxanthine or guanine but synthesize DNA by another metabolic pathway. If test chemical-tested cells are able to grow in the presence of TG, then they are considered to have mutated, either due to test chemical or spontaneously. Analysis of the data from S-9 activation vs. S-9 non-activation, generation of appropriate results with positive and negative controls, application of statistics and so on.

Detailed Review of Studies

Cytogenetic Investigation in Chinese Hamsters After a Single Oral Adminstration of 5-OH-MSO2. Bone Marrow Chromosome Analysis. BASF Aktiengesellischaft. Department of Toxicology Project No. 10M0179/8316. Dated January 30, 1984, 23 pages, 18 Tables. Accession No. 252349.

Protocol:

Chinese hamster, 7-13 weeks old, mean weights 25.5 g were administered 5-OH-MSO2 in 0.5% CMC (carboxymethyl cellulose) or vehicle (CMC) or 60 mg/kg cyclophosphamide (CP in aqueous solution), in single oral doses. Dosages ranged from 1,000 mg/kg 5-OH-MSO2 to 10,000 mg/kg to determine percentage of target dose. Observations were made for clinical signs or symptoms of toxicity. Animals were injected two hours prior to sacrifice with colcemid to arrest mitosis in metaphases in order to facilitate cytogenetic analysis. Sacrifices were made at 6, 24 and 48 hours after treatment. After sacrifice, femurs were removed for bone marrow extraction. Remains ere necropsied to determine if any gross changes occurred which might be related to treatment. (Table I presents the format of the study.) The extracted bone marrow was prepared by the method of W. Schmid and G. R. Staiger.

TABLE I STUDY DESIGN

Cytogenetic Assays in Bone Marrow from Treated Chinese Hamsters

Treatment (single Oral Admin.)		Bone Marrow Samples* 6 Hours	24 Hours	48 Hours
		Number of Animals		
Vehicle control - 0.5% CMC	** Males (M) Females (F)	0	0	0
1,000 mg/kg 5-OH-MSO2 in 0.5% CMC	M	0	5	0
	F	0	5	0
3,000 mg/kg 5-OH-MSO2 in 0.5% CMC	M	0	5	0
	F	0	5	0
10,000 mg/kg 5-OH-MSO2	M	5	5	5
	F	5	5	5
Positive Control 60 mg/kg cyclophosphamide in distilled water	M	0	5	0
	F	0	5	0

* Two hours prior to sacrifice, animals were intraperitoneally injected with 3.3 mg colcemid per kg body weight.

** Vehicle controls has 10 males and 10 females. All other groups had 5 each M and F.

Following applicaton of the bone marrow to microscope slides, staining was done with Giemsa dn Tritisol solution, clarified in xylene and embedded in Entallen. Generally, 100 metaphases with a 2n chromosome number from each male and female were examined. Analysis was made of structural chromosome aberrations an number of animals, number of animals with aberrant metaphases, including gaps, excluding gaps, exchanges, multiple aberrations, pulverisations, aneuploidy and polyploidy. The Fisher exact test was used to determine significance of difference between frequencies of a characteristic in two groups. The asymptotic U test of Mann-Whitney as modified by Wilcoxon. Statistical tests were determined at levels of 95% and 99%.

Results:

Analytical determination of test chemical actually administered showed 65-85% of the theoretical values, which is attributed to the use of carboxymethyl (CMC) cellulose suspension. Thus, for dose groups receiving 10,000 or 3,000 or 1,000 mg/kg 5-OH-MSO2 actual analytical values respectively were 86.4%, 64.8% and 78.2% of target dose.

All animals tolerated their respective orally administered dosages quite well. No clinical signs or symptoms attributable to treatment were seen. Gross necropsies showed no changes in organs attributable to treatment. No evidence of mutagenicity occurred in any group except in the positive control group which had received cyclophosphamide. Treatment with 5-OH-MSO2 did not result in the increase of aberrant metaphases or other chromosomal aberration frequency on comparison with vehicle controls.

Core Category: Acceptable according to current methodology.

Results:

Negative for mutagenicity under the conditions described. Highest dose tested is 10,000 mg/kg.

Mutagenicity Evaluation of 5-OH-MSO2 (metabolite MU-1) in the CHO HGPRT Forward Mutation Assay, Litton Bionetics. Dated October, 1983. Lawd Jueel 11, 3905 PE VEENEND AAL, The Netherlands. No. E9139. Accession No. 252350, 21 pages, 4 Tables, 1 Figure.

Protocol:

A preliminary cytotoxicity assay was performed using a range of concentrations from 0.01 mg/ml to 10.00 mg/ml of cell suspension with and without S-9 (rat liver S-9 microsomal

fraction) to determine a maximum concentration of test chemical which could be tolerated in the assay system. The pH range also had to be tested for most appropriate level. The most appropriate vehicle to solubilize the test chemical was also a factor in the system.

The assay itself was basically described in the Preface above. The clone of cells used in this assay was CHO-K1BH$_4$, derived from the hypodiploid cell line isolated from the ovary of the female Chinese hamster.

Through various cultivation procedures, the CHO-K1 cell system was derived, which led to the use of the percent CHO-K1-BH$_4$ subclone. This latter cell line is checked periodically for genetic stability. Generally, before doing an assay, selective cultivation is used to reduce the number of naturally occurring mutants. Dimethyl sulfoxide was used as a vehicle in these assays.

Negative Controls consisted of carrying cells unexposed through all of the test procedures without exposing them to test article.

Positive Controls - Treatment of cells with 5-Bromodloxyuridine (BrdU) which is highly mutagenic to CHO-K1 cells at 50 ug/ml was done as a positive control for non-activated studies (without S-9). For S-9 activated positive control, 3-methyl-cholomthrene (3-MCA) was used at a level of 5 ug/ml.

Assays - A modified assay of the procedure of Hsie et al was used. Briefly, 4x10^6 cells are exposed for 4 hours at 37° ± 2C° to the test chemical. Measured cell counts and volumes of culture media are then separated into samples for cytotoxicity assays and to select for mutant cell colonies. Under the assay conditions for the non-activation test, there was: one vehicle control culture, one positive control and 6-8 treatment level cultures. The expression of mutants was 6-7 days at which time reseeding of 2x10^5 cell sper 100 mm. dish for a total of 12 dishes in mutant selection medium. Following incubation for 7-10 days, colonies were alcohol-fixed, stained with Giemsa and counted for the number of TG resistant colonies (refer to Preface, #2) by visual inspection, excluding counts of 50 or less colonies.

The activation assay was performed independently from the non-activation study. It was similar to that above except for the addition of the S-9 liver homogenate fraction and for the use of 3-MCA and adjustments in pH, concentration of

Bovine fetal calf serum, nicotinamide dinucleotide phosphate (NADP, sodium salt), glucose-6-phosphate, $CaCl_2$, KCl and $MgCl_2$. S-9 homogenate was prepared from liver of Sprague-Dawley male rats treated with Aroclor 1254 was used as a source of microsomal enzymes for the activation procedures.

Results:

Because of the large number of parameters in this assay, a large number of control steps were taken. The assay itself is not difficult to perform, but the large number of possible variables must be accounted for.

Thus, relative survival to treatment was calculated, relative population growth, cloning efficiency, mutant frequency, culture medium, incubation times, temperatures, negative and positive control values, and so on were determined before the test article - treated cells cultures could be evaluated.

No dose response relationships were observed both with and without S-9 activation. Cell toxicity was not in evidence at concentrations of 6.0 mg/ml test article both with and without S-9 activation. Under the test conditions for assays with and without metabolic activation, the test article 5-OH-MSO2 at levels of 2.0 mg/ml through 10 mg/ml was not mutagenic. (Some cytotoxicity was observed at above 6.0 mg/ml.)

Conclusions:

5-OH-MSO2 is non-mutagenic in the CHO-HGPRT assay with and without metabolic activation.

Core Category: Acceptable according to current methodology.

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