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

JUL 9 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Mutagenicity Study Protocols for Hydroxymetabolites of POAST™ (BAS 9052 H) Reg. No. 7969-58.

Tox Chem No. 72A

FROM: Minnie Sochard, Ph.D. M. Sochard 6/22/84
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Hazard Evaluation Division (TS-769)

TO: Robert Taylor, Product Manager #25
Registration Division (TS-767)

THRU: Albin Kocialski, Acting Section Head ABK 7/2/84
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769) 7/18/84

Registrant: BASF Wyandotte Corp.
Parsippany, N.J.

Action Requested:

The registrant has submitted three protocols for proposed mutagenicity studies with the 5-OH-MSO_2 metabolite of POAST™. The protocols submitted for Toxicology Branch (TB) approval include (1) Gene mutation (CHO, HGPRT forward mutation, (2) Structural chromosome aberration (Chinese hamster bone marrow) and (3) Unscheduled DNA synthesis in rat liver primary cell cultures.

Recommendation:

TB finds the protocols acceptable for determination of the mutagenicity of the 5-OH-MSO2 hydroxymetabolite of POAST™.

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Commentary:

The following is excerpted from the TB review of POAST™, dated March 9, 1983 by E. Budd and M. Sochard pages 3 and 4:

RCB has deferred to TB as to whether or not the hydroxylated metabolites MU-2, MU-1 and MU need to be included in the tolerance regulation (see RCB review of 5/21/81 by E. Zager).

Studies with radioactive labeled BAS9052 H (active ingredient in POAST) show that in soybeans the hydroxymetabolites MU-1, MU-2 and MU account respectively for 23%, 8% and 12% of total recovered radioactive metabolites. Thus, individuals eating treated soybean products may be exposed to a total of 43% hydroxymetabolites.

TB believes that these hydroxylated metabolites, which obviously comprise a very large percentage of the residue on soybeans, should be included in the tolerance regulation. TB also believes this issue to be resolved in that the most recent tolerance statement, dated 2/1/83, adequately covers the parent compound and its metabolites.

TB is concerned about the potential toxicity of the hydroxylated metabolites MU, MU-1 and MU-2 since they comprise a very large percentage of the residue on soybean and toxicity studies using these metabolites as test materials are not available at the present time - except for acute oral LD_{50} studies recently submitted in accession #249241. Additional studies with these metabolites have been made a conditional requirement for registration of POAST™ Herbicide for use on soybeans (see #3 above). TB has recently met with BASF several times regarding this problem. As a result of these meetings and in consideration of additional information and rationale submitted by BASF in Supplement I (accession #249451), TB has requested additional toxicology data for the hydroxymetabolites of POAST™

Detailed Review of Protocols

Three protocols for evaluation of the mutagenicity of 5-OH-MSO2 are presented. Accession No. 25044. Two tests will be performed by Litton Bionetics. The third will be done at the Department of Toxicology, BASF aktienesgesellschaft in Germany. All three proposed protocols involve demonstration of mutagenic effects in mammalian cell cultures. The tests are to be done as follows:

1. Protocol for CHO HGPRT Forward Mutation Assay To be done by Litton Bionetics. No date given 12 pp. The assay involves determination of 5-OH-MSO2 (test article) to induce forward mutation at the HGPRT locus (Hypoxanthine Guanine Phosphoribosyl Transferase) of hypodiploid CHO cells (chinese hamster ovary cells of the CHO-K1-BH_4 cell line). The protocol is described by the registrant as in compliance with published EPA OTS guidelines (1982) (not OPP Guidelines) and with recommendations drafted by OECD (Organization of Economic Cooperation and Development) with the following exceptions:

   a. An independent repeat experiment for confirmation of results will not be done unless requested by the sponsor to do so, at additional cost.

   b. Two cultures per dose level will not be done unless requested (as for a, above).

   c. Treatment period with the test chemical will be 4 hours with and without metabolic activation, not 5 hours as suggested, in order to permit direct comparison with another assay standardized at a 4 hour incubation period (e.g., L51784 mouse lymphoma forward mutation).

   d. To provide a more adequate medium for the cloning and growth of induced $HGPRT^-$ mutants, whole bovine fetal calf serum will be used rather than dialyzed bovine calf serum.

2. Cytogenetic studies by chromosome analysis of the bone marrow of Chinese hamster after a single oral administration. BASF Toxicology Department, Germany, 14 pp. Dated June 30, 1983.

The protocol is based on procedures recommended by the OECD short-term toxicology group. Dosages of 10,000, 3,000 and 1,000 mg/kg of body weight of 5-OH-MSO2 were selected for single oral administration in 5 male and 5 female 7-13 week old Chinese hamsters at each dose level and sacrifice time. Dosages were selected with the HDT shown to be tolerated well by the animals without clinical symptoms. Cyclophosphamide will be used as a positive control. Bone marrow from femurs will be prepared by the method of Schmid and Staiger (1969). Analysis will be made of 100 metaphases per animal following appropriate staining of slide preparations. Structural chromosome aberrations, numerical aberrations and exchanges will be sought. Necropsies for gross pathological changes will be made on all animals at the end of the experiment. There is a discrepancy between the text and table 6. The text says that test groups will be sacrificed at 6, 24, and 48 hours after dosing while table 6 reveals that only the high dose will have three sacrifice intervals and the mid and low dose have only one sacrifice group.

3. Unscheduled DNA synthesis in rat liver primary cell cultures. No date, 11 pp. Litton Bionetics laboratories.

The protocol for this study is described as LBI protocol No. 447, taken from published guidelines of the Office of Toxic Substances, EPA (1982). The cells chosen as indicators will be hepatocytes from adult male Fischer 344 rats, 150-300 g. The use of hepatocytes eliminates the need for microsomal activation, since the cells already possess that metabolic capacity. The negative control will consist of either WME (standard culture medium) or DMSO (dimethyl sulfoxide) if the test article is not soluble in WME. The positive control will be 2-AAF (2-acetyl aminofluorine) unless the study sponsor specifies a different chemical control.

Conclusions:

All three of the protocols described above are acceptable for assessing the mutagenicity of the 5-OH-MSO2 metabolite of POAST™ according to current methodology and EPA guidelines with the following exceptions: In the CHO test, at least 2 cultures per dose level must be used. In the cytogenetic *in vivo* assay, the registrant should be aware that omitting the 6 and 48 hour sacrifices at the mid and low doses is risky. If effects are noted in the high dose at 6 or 48 hours but not at 24 hours, the study will be invalid.

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