



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

6-28-89
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of Mutagenicity Studies with Sencor
EPA Registration No. 3125-270
Acc. No. 251219; CASWELL No. 33D

TO: Robert J. Taylor, PM #25
Registration Division (TS-767)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) *RBJ 6/28/89*

FROM: John H.S. Chen, D.V.M. *John H.S. Chen*
Review Section #1
Toxicology Branch/HED (TS-769)

Action Requested:

Review and evaluation of the Mouse Bone Marrow Micronucleus Test with DIC-1468 and the Induction of Mitotic Gene Conversion in Sacchromyces cerevisiae with Sencor.

Recommendation:

The assays were not performed properly in accordance with the accepted procedure recommended for the Mouse micronucleus Test and the Induction of Mitotic Gene Conversion in Sacchromyces cerevisiae, and therefore, the results and their interpretations are unacceptable. The registrant should be apprised of the deficiencies reported in the following two studies:

1. Evaluation of DIC-1468 in the Mouse Bone Marrow Micronucleus Test Bayer AG Institute of Toxicology Report No. 10718, Acc. No. 251219 (Mobay Report No. 82361).
 2. Induction of Mitotic Gene Conversion in Sacchramyces cerevisiae with Sencor D. Siebert and E. Lemprele, Mutation Res. 22 (1974), 111-120, Acc. No. 251219 (Mobay Report No. 85884).
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**Study I: Evaluation of DIC-1468 (Sencor Active Ingredients)
in the Mouse Bone Marrow Micronucleus Test.**

Bayer AG Institute of Toxicology Report No. 10718,
Wuppertal-Elberfeld, October 3, 1982. Accession No.
251219 (Mobay Report No. 82361).

Procedure:

1. Animals

The study consisted of 40 NMRI (SPF Han) strain mice (8-12 weeks old, weighing 24-36 g) which were supplied by breeder and supplier F. Winkelmann, Borcheln. The animals were kept in groups of 3 mice, separated by sex in the Markrolon cages and divided into 4 groups (5 males and 5 females per experimental and control groups) for this study.

2. Route of Administration

The test compound suspended in aqueous Tylose solution (hydroxyethyl methyl cellulose) (2 X 200 and 2 X 400 mg/kg) was administered twice orally with a stomach tube at an interval of 24 hr. Concurrently, the positive control, Trenimon, (2 X 0.125 mg/kg) and the vehicle control were also given orally to the separate mouse groups.

3. Extraction of Bone Marrow

Fifty-two hours after dosing or 6 hours after the second application, the animals were sacrificed. The smears were prepared and produced according to Schmid (1975).

4. Evaluation of Criteria

One thousand polychromatic erythrocytes per animal were scored for the incidence of micronuclei. The following information were also recorded:

- (a) The number of micronucleated normochromatic erythrocytes in the optical field containing 1000 polychromatic erythrocytes (PCE);
- (b) The number of normochromatic erythrocytes in the optical field containing 1000 PCE;
- (c) The ratio of polychromatic erythrocytes to normochromatic erythrocytes.

5. Biometrics

The results were statistically evaluated with the NEMENIY non-parametric ranking test. A difference was considered statistically significant; if the error probability came to less than 5% ($P < 0.05$).

Results:

<u>Dose Levels</u>	<u>No. of PCE Examined</u>	<u>No. of NCE Per 100 PCE</u>	<u>No. of Cells W/Micronuclei</u>	
			<u>Per 1000 NCE</u>	<u>Per 1000 PCE</u>
Negative Control	10000	1122.2	0.8	0.6
DIC1468 2 X 200 mg/kg	10000	1186.8	1.8	1.1
DIC1468 2 X 400 mg/kg	10000	1400.3	1.2	1.5
Positive Control *(Trenimon) 2 X .125 mg/kg	10000	1664.4	1.1	54.5*

PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes

Summary of Findings: No significant increase in the frequency of micronucleated polychromatic erythrocytes was observed in the DIC-1468 treated groups when compared to the control group. The positive control, Trenimon (2 X 0.125 mg/kg), induced positive increase in the frequency of micronucleated polychromatic erythrocytes as expected. Therefore, the test compound, DIC-1468, is not a clastogenic agent at the dose levels tested in the mouse micronucleus assay.

Evaluation:

The assay procedure used for this study appear to be inadequate, not conducted according to the accepted procedures recommended by EPA Health Effects Test Guideline (EPA 560/6-82-001, 1982) and OECD Draft Guidelines (1981), and hence, the results and their interpretations are unacceptable. The following inadequacies in performing the mouse micronucleus test were noted:

1. Details of procedures used for dose determination in this study were not given. Only two doses (2 X 200 and 2 X 400 mg/kg) were used. At least three dose levels which cover a wide range from usage level up to the highest tolerable dose should be used.
2. Because the time course of micronuclei productin in polychromtaic erythrocytes from treated animal is proved to be varied with the individual chemical tested, single sampling time used in this study appears to be inadequate. Samples of bone marrow should be taken at three times starting not earlier than 12 hours after treatment with appropriate intervals following the first sample but not extending beyond 72 hours.

Study II: Induction of Mitotic Gene Conversion in Sacchramyces cerevisiae

D. Siebert and E. Lemperle, Mutation Res. 22 (1974), 111-120 (Mobay Report No. 85884).

Procedure:

The test compound, Sencor (Metribuzin), was tested for mutagenic effect on the diploid strain D4 of Sacchramyces cerevisiae heteroallelic at the two gene loci (ade 2 & trp 5) in a single does of 1000 ppm. The test system permitted the mitotic gene conversion in the yeast cells after 16-hour treatment with Sencor (pH 7) on a shaker at the temperature 25°C. The tryptophan-free medium required for 6 days at 25°C. The numbers of convertant per 10^6 surviving cells were determined. The significant difference was determined between the treated and control value. The experiment was performed 3 times and representative results were given.

Results:

Treatment with Sencor at 1000 ppm did not induce a significant increase in the conversion frequency (convertants/ 10^6 survivors) neither in the ade 2 nor in the trp 5 locus when compared to the control value (1.9-fold vlaue in the ade 2 locus and 0.7-fold value in the trp 5 locus). Therefore, non-induction of mitotic gene conversion was indicated by Sencor in the D4 yeast cells at the dose tested.

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Evaluation:

The assay used for this study appears to be inadequate, not conducted according to the accepted procedures of Mitotic Gene Conversion in Saccharomyces cerevisiae recommended by EPA Health Effects Test Guidelines (EPA 560/6-82-001), and hence, the results and their interpretations are unacceptable. The following inadequacies in performing the assay of mitotic gen conversion in Sacchramyces cerevisiae were noted:

1. This is a complied information with respect to the genetic effects of 32 herbicides found in the test system of mitotic gene conversion in Sacchramyces cerevisia. Results of the specific study with Sencor in the presence of metabolic activation from rat liver enzyme were not given.
2. Because the cytotoxicity study of the test compound on yeast D4 cells was not conducted, it is doubtful that the adequate exposure concentrations (upper or lower limits of the test compound) were selected for the study.
3. Since the genetic activity of yeast cells for the reaction of various mutagens are largely dependent on the pH treatment condition. The response of the test compound at pH 7 as well as around pH 4.5 should also be presented in this study.
4. The direct acting positive control (Hydrazine sulfate) and the positive control to ensure the efficacy of the activation system (2-Acetylaminofluorene) were missing in the report of this study.
5. Statistical evaluation of the test data was not included in the study.

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