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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: Azinphos-Methyl (Guthion): EPA ID Number 3125-108; Record No. 205829; TOX CHEM No. 374

From: John H.S. Chen, D.V.M.
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C)

To: Dennis Edwards, PM12
Insecticide-Rodenticide Branch
Registration Division (TS-767C)

Thru: Robert B. Taylor, Section Head
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Action Requested:

Review and assessment of the cytogenetic study with human lymphocyte cultures in vitro (Test Material: Azinphos-Methyl) BAYER AG Institute of Toxicology Report No. 94575.

Petitioner:

Mobay Corporation, A Bayer USA Inc. Company

Recommendation:

The Registrant should be apprised of the deficiency noted in this study without metabolic activation. However, the study under the activated system is considered acceptable. Positive response at 500 ug/ml with metabolic activation.

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84-2 In Vitro Cytogenetic Assay in Human Lymphocytes
Reviewed by: John H.S. Chen
Section I, Toxicology Branch (TS-769C)
Tertiary Reviewer: I. Mauer
Section VI, Toxicology Branch (TS-769C)
Reviewed by Section Head: R.B. Jaeger
Section I, Toxicology Branch (TS-769C)

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DATA EVALUATION REPORT

Study Type: Chromosomal Aberration (in-vitro) **TOX. CHEM. NO.:** 374

Accession No.: 403678-11 **MRID NO.:**

Test Material: Azinphos methyl (Batch No. B1582; 91.3% Purity)

Synonyms:

Study Number (s): 94575

Sponsor: Mobay Corporation

Testing Facility: BAYER AG Institute of Toxicology Laboratory, Germany

Title of Report: Cytogenetic Study with Human Lymphocyte Cultures In Vitro

Author(s): J.H. Thyssen

Report Issued: October 10, 1986

Conclusions:

Azinphos methyl (B1582) was clastogenic in cultured human lymphocytes at 500 ug/ml with metabolic activation. However, there was no clastogenic effect in this study at the concentration up to 100 ug/ml without metabolic activation and at the concentration up to 50 ug/ml with metabolic activation.

Concentrations tested: 1, 10 and 100 ug/ml without S9 mix;
5, 50 and 500 ug/ml with S9 mix.

Classification of Data: Acceptable for the study with S9 mix
Unacceptable for the study without S9 mix

(Deficiency: inadequate intermediate dose level used)

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Title of Study: Cytogenetic Study with Human Lymphocyte Cultures In Vitro to Evaluate for Harmful Effect on Chromosomes
BAYER AG Institute of Toxicology Laboratory Report No. 9575

I. Materials and Methods:

1. Test Materials

The solutions of Azinphos methyl (Batch No. E1582; 91.9% Purity) were made in DMSO. Solutions of Mitomycin-C and Cyclophosphamide in Hank's saline were prepared prior to use and served as positive controls.

2. Preparation of Human Lymphocyte Cultures (from 2 donors: 1 male and 1 female)

Human blood was drawn aseptically into sterile syringes that contained Liquemin. About 0.5 ml of heparinized blood were added to 9 ml of culture medium (chromosome medium B with phytohemagglutinin) in culture flasks to stimulate lymphocyte to divide. The culture flasks were incubated at 37°C for 48 hours.

3. Toxicity and Dose Determination

The concentrations used were based on a pilot test with cultures exposed to a wide range of test material (1 through 500 ug/ml). The top dose was determined by the limit of solubility of E1582 in DMSO and the toxicity of this solution to reduce the mitotic index. All cultures were incubated at 37°C for 2.5 hours.

4. The Metabolic Activation System

The metabolic activation system comprised rat liver enzymes and an energy-producing system. The enzymes were contained in a preparation of liver microsomes (S9 fraction) from six male rats treated previously with Aroclor 1254. The S9 fraction was added to a cofactor mixture to form the activation as described by Ames et al (Mutation Res., 31: 347-364, 1975). The composition of S9 mixture (per 100 ml) was: 871 mg MgCl; 410 mg KCl; 298.5 mg Glucose-6-phosphate; 525 mg NADP; 50 ml Phosphate buffer (100 mM).

5. Treatment of Cultures

Each 48-hour culture was exposed to the test compound for 24 hours at 37°C under the nonactivated test condition. In the case of assay with metabolic activation, freshly prepared S9 mix was added to the appropriate cultures with test material for an exposure of 2.5 hours at 37°C. After the exposure, cells were washed and then re-incubated in complete culture medium for futher 21.5 hours. Three hours before termination, cell division was arrested by the addition of colcemid (0.4 ug/ml) to each culture. The cells were swollen with hypotonic

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solution (0.56% KCl) at room temperature for 7 minutes, then fixed in ethanol:glacial acetic acid fixative (3:1 v/v), dropped onto clean slides and air-dried.

6. Staining and Scoring of Slides

Slides were stained with 5% Giemsa solution for subsequent scoring of chromosome aberration frequencies. Two hundread metaphases for each culture were scored for the test material, negative and positive controls. Morphological observations were scored as follows:

Chromatid gap	Chromatid deletion
Chromatid break	Interchange
Chromosome break	Intrachange
Chromsome fragment	Mutiple aberrations
	Polyploidy

7. Statistical Evaluation

A significant increase in the number of aberrant cells against the negative control (solvent) at least at 95% was determined. The one-sided correct Chi^2 test was used for the statistical evaluation.

II. Reported Results:

1. Preliminary Toxicity Test (Table 1 attached)

According to the results obtained from a pilot test for determining the highest doses in this study, 100 ug/ml in the experiment without S9 mix or 500 ug/ml in the experiment with S9 mix were selected. This selection was based on the mitotic index value obtained. An approximately 23.0% reduction ($P<0.01$) in mitotic index (reduced from 328 to 92) was apparent at 100 ug/ml (without S9 mix); 52.4% reduction ($P<0.01$) in mitotic index (reduced from 432 to 156) was observed at 500 ug/ml (with S9 mix).

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2. Main Cytogenetic Test (Tables 2, 3, 4 and 5 attached)

Summarized Incidence of Chromosomal Aberrations -gaps excluded

Treatment ug/ml	Total No. of Cells Examined	No. of Aberrations Examined	No of Aberrations Per Cell	Mitotic Index
W/O S9 Mix				
0 (Solvent)	200	5 (A,B)	0.025	328
1 (31582)	200	1 (A)	0.005	361
10 (")	200	6 (A,B,C)	0.03	401
100 (")	200	10 (A,C)	0.05	92**
0.1 (MMC)	200	50 (A,B,C,D,E)	0.25**	284*
W/S9 Mix				
0 (Solvent)	200	5 (A,D)	0.025	482
5 (31582)	200	5 (A)	0.025	440
50 (")	200	9 (A,B,D)	0.045	419*
500 (")	200	48 (A,B,D,E)	0.24**	156**
10 (Cycl)	200	43 (A,B,C,D)	0.215**	Toxic

* Significantly different from the control value P<0.05;
** Significantly different from the control value P<0.01;
MMC = Mitomycin-C; Cycl=Cyclophosphamide; (A)=Chromatid break;
(B) = Chromosome fragment;(C) = Chromatid deletion; (D) = Exchange;
(E) = Multiple aberraions.

Findings:

i. The positive controls, Mitomycin-C and Cyclophosphamide, induced significant positive responses (P<0.01) under the nonactivation and activation conditions respectively.

ii. Azinphos methyl (31582) demonstrated a statistically significant increase in the number of chromosomally damaged cells in the experiments with metabolic activation (P<0.01) at 500 ug/ml (a dose-related trend was also observed at the 50 and 500 ug/ml treatment groups). However, there was no clastogenic effect in vitro using human lymphocytes at the concentration up to 100 ug/ml without S9 mix and at the concentration up to 50 ug/ml with S9 mix.

iii. There was no significant increase in the numerical chromosome aberrations (i.e., Polyploidy) in the treatment groups when compared to the corresponding control group.

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III. Evaluation and Recommendation:

1. The positive controls, Mitomycin-C at 0.1 ug/ml and Cyclophosphamide at 10 ug/ml adequately demonstrated the sensitivity of the cultured lymphocyte system to detect a clastogenic effect.

2. The number of cells with chromosomal aberrations in the negative (solvent) control (2.5% of metaphases) was found within the acceptable range established by the testing laboratory.

3. The highest-dose levels of E1582 (100 ug/ml without S9 mix; 500 ug/ml with S9 mix) demonstrated cytotoxicity to dividing lymphocytes, resulting in reduction of mitotic index.

4. Although the preliminary assessment of cell cycle delay was not conducted in this study (Reference: EPA Health Effects Test Guidelines EPA 560/6-83-001), the single harvest time (21 hours post-treatment; 72 hour-total culture time) for cells exposed to the test material appeared adequate for the detection of chromosomal aberrations in cultured human lymphocytes.

5. In the experiments with metabolic activation, Azinphos methyl (E1582) was clastogenic in the cultured human lymphocytes at 500 ug/ml (at the toxic level). This result is considered acceptable. However, according to the acceptable procedure for performing the In Vivo Mammalian Bone Marrow Cytogenetic Assays (EPA Health Effect Test Guidelines 560.6/83-001), multiple concentrations of test material over a range adequate to define the response should be tested (i.e., one-third and one-tenth of the high dose should normally be used as the intermediate and low dose levels respectively). Therefore, a proper intermediate dose level of the test material should be included in this study.

6. Since the highest dose level (100 ug/ml) used in the experiments without metabolic activation demonstrated highly toxic effect to the human lymphocytes, the dose-selection for intermediate dose level is not considered adequate for this study. Therefore, the study under the non-activated system is unacceptable in the present form. However the study may be upgraded on the resolution of the reporting deficiency.

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Azinphos-methyl RIN: 7365-92

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