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10/20/1982

DATA EVALUATION RECORD

(1) CHEMICAL: FOSETYL-Al.

(2) FORMULATION: Technical (97.5 ± 0.5% purity).

(3) CITATION: Cordier, A. and Fournier, E. (1981) FOSETYL-Al (32 545 R.P., aluminum salt), Micronucleus test in the mouse by the oral route. (Unpublished report prepared by Rhone-Poulenc Industries, Centre Nicolas Grillet, Department of Toxicology, 13, Quai Jales Guesde, 94400 Vitry-sur-Seine, France.) Accession #247173-D

(4) REVIEWED BY:

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(6) TEST TYPE: Mutagenicity.

10/20/82

(7) CONCLUSION: The micronucleus test was well defined by the authors, and it clearly gave a positive response when animals were treated with triethylenemelamine (TEM) at 1 mg/kg p.o. FOSETYL-Al did not produce a mutagenic effect in the micronucleus test with CD-1 mice at doses ranging from 0.6 to 2.4 g/kg under the same conditions. The results were unambiguous, and therefore provide good evidence for the lack of mutagenicity.

Classification: Acceptable

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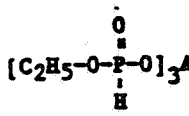
(8) MATERIALS AND METHODS:

1. The test compound, aluminum-tris-(o-ethylphosphonate) is a fungicide with the following structural formula:



The molecular weight was 354, and the compound was 97.5% + 0.5% pure. Impurities were [redacted]. The water solubility was 12% at 20° C, and the compound was a fine white powder.

2. The animal species was CD-1 mice ("C.O.B.S.") supplied by Charles River of France. Fifty males and 50 females, 7 weeks of age, were supplied for the study. The animals were housed individually in stainless steel cages with wire-mesh fronts and floors in groups of 70. Cage racks were located in rodent-dedicated, limited access areas, in environmentally controlled rooms (temperature was 22 ± 2° C, humidity was 55 ± 15%, illumination was 12-hour light/dark cycle, air exchange was 14 times/hour) from the acclimation period (4-7 days) until termination of the experiment.

Plastic-coated paper beneath the cages was changed at least twice a week. Powdered diet (VAR A04, certified by lot) and water were supplied ad libitum.

3. The dose levels selected were based on an LD_{50} study (MMA3257), which established the LD_{50} for the test compound in CD-1 mice as 4.8 g/kg. Healthy animals were weighed and randomized into treatment groups of five males and five females as described in Table 1:

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

TABLE 1. Dosing Regimen and Sacrifice Schedule

Group	Number of Doses	Dose Level (g/kg)	% LD_{50}	Sacrifice (hours after initial dose)	Marrow Slides Prepared
A	1	0.0	-	30	Yes
B	1	0.6	12.5%	30	No
C	1	1.2	25%	30	Yes
D	1	2.4	50%	30	Yes
E	1	3.6	75%	30	Yes
F	2	0.0	-	48	Yes
G	2	0.6	12.5%	48	No
H	2	1.2	25%	48	Yes
I	2	2.4	50%	48	Yes
J	2	3.6	75%	48	Yes

The test compound was suspended in a 10% aqueous solution of acacia, and volumes of 25 ml/kg were administered orally; control animals received the vehicle only. Animals were sacrificed by cervical dislocation at 30 hours after initial dose for groups A through E. Animal groups F through J received two doses with a 24-hour period between doses, and they were sacrificed 48 hours after the initial dose.

4. Bone marrow examination was conducted on the three highest dose levels (both single and repeated administration) at which animals survived. Both femurs were removed and the marrow was collected from each animal in fetal calf serum. This suspension was transferred to hemolysis tubes (one tube/animal) and centrifuged. Bone marrow slides were prepared, two per animal, then fixed in methanol, and stained with May-Grunwald-Giemsa.

   Slides from four animals/sex/group were examined, each slide by two investigators. Five hundred polychromatic erythrocytes/slide (1,000/mouse) and corresponding micronuclei were counted.

5. Normochromatic (EN), Polychromatic (X), and Nucleated (Y) erythrocytes were counted per slide, and the following ratios were determined:

$$A = \frac{X}{100\ EN} \qquad B = \frac{Y}{100\ EN}$$

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A decrease in the ratio A indicates a delay in, or inhibition of, erythroblast maturation, and a decrease in both ratios A and B indicates a cytotoxic effect.

6. The test groups were compared statistically using Kastenbaum and Bowman's test. An analysis of variance was used to compare the results of the cytotoxicity tests.

(9) REPORTED RESULTS:

All animals that were dosed at the highest level (3.6 g/kg, or 75% of the LD_{50}) and 10% of all midrange dose, single-exposure animals died. No increase in percentage of polychromatic erythrocytes with micronuclei or total number per 1,000 polychromatic erythrocytes examined was noted (see Table 2). Slight cytotoxicity was produced in groups D and I (2.4 g/kg single and repeated exposure). There was a delay in cell maturation for group H animals (1.2 g/kg repeated exposure).

A positive control experiment with 1 mg/kg triethylenemelamine (TEM) was conducted by the same method. TEM was highly mutagenic in the micronucleus test for animals at this level.

(10) DISCUSSION:

The micronucleus test was performed on FOSETYL-Al at doses that are generally recognized to be acceptable; i.e., the highest dose resulted in cytotoxicity and approached the LD_{50}. However, it is not clear whether the administered dose for animals receiving repeat administration was in fractions totaling the dose level listed or repeated administration at the level listed. The animal group sizes were adequate and both sexes were included. Also, the number of erythrocytes examined was large enough to obtain a statistically significant result. The experimental conditions, rationale for the assay, and criteria for judging mutagenicity were well defined, and the assay was performed as was specified in the protocol. Since the number of Howell-Jolly bodies (micronuclei) was not 2.5-fold higher than the spontaneous incidence (where $p = 0.05$), it is the opinion of this reviewer that this compound did not cause a mutagenic response, a conclusion also reached by the author. The only deficiency of any consequence was the absence of body weights for the CD-1 mice in the final report, although dosages for FOSETYL-Al had been calculated.

TABLE 2. Summary of the Results of the Cytotoxicity and Micronucleus Tests

Group	Dosage (g/kg)	Cytotoxicity Test: % Polychromatic RBC's with Micronuclei	Micronucleus Test: No. Polychromatic RBC's with Micronuclei/1,000 Polychromatic RBC's: Males	Females	Total	Cytotoxicity Ratios: A: No. Polychromatic RBC's/100 Orthochromatic RBC's	B: No. Nucleated RBC's/100 Orthochromatic RBC's
A	0.0	0.18	4	10	14	1.591	4.65
B	0.6	0.34	13	14	27	-	-
C	1.2	0.24	11	8	19	1.41	4.23
D	2.4	0.21	10	7	17	0.99**	3.18**
E	3.6	Died	-	-	-	-	-
F	0.0	0.23	11	7	18	2.19	5.19
G	0.6	0.13	4	6	10	-	-
H	1.2	0.19	8	7	15	1.63*	4.85
I	2.4	0.14	5	6	11	0.92**	2.75
J	3.6	Died	-	-	-	-	-

*Siguificant for 0.01.
**Significant for 0.001.

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