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DATA EVALUATION RECORD

10/20/1982

(1) CHEMICAL: FOSETYL-Al.

(2) FORMULATION: Aliette (80% wettable powder).

(3) CITATION: Cordier, A. and Fournier, E. (1981) FOSETYL-Al (32 545 R.P., aluminum salt). Aliette Formulation EXP 1659-B (wettable powder containing 80% FOSETYL-Al). 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 (Unpublished report prepared by Rhone-Poulenc Industries, Centre Nicolas Grillet, Department of Toxicology, 13, Quai Jales Guesde, 94400 Vitry-sur-Seine, France, Reference: C.R. Vitry/C.N.G. No. 21 198-E.) Accession # 247186-6

(4) REVIEWED BY:

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(6) TEST TYPE: Mutagenicity.

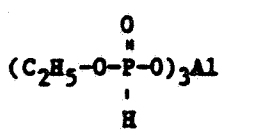
10/20/82

(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. Aliette did not produce a mutagenic effect in the micronucleus test with CD1 mice at doses ranging from 0.6 to 3.2 mg/kg under the same conditions. The results were unambiguous and, therefore, provide good evidence for the lack of mutagenicity.

Classification: Acceptable

(8) MATERIALS AND METHODS:

1. The test compound, Aliette Formulation EXP 1659-B, contains 80% FOSETYL-Al, aluminum-tris-(o-ethylphosphonate), and is an antifungal agent with the following structural formula:



mw = 345

The material tested was a wettable white powder from Lot OP-81-130 (sample 81-1).

2. The animal species was CD1 mice (C.O.B.S.) supplied by Charles River, France. Sixty males and sixty females, seven weeks of age were supplied for the study. The animals were housed in stainless steel cages with wire mesh fronts and floors. Cage racks were located in limited access areas, in environmentally controlled rooms (temperature was 22±2° C, humidity was 55±15%, illumination was 12 hr. light/dark cycle, air exchange was 14 times/hr) 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. Animal rations (UAR A 04 feed, meal form, certified by lot) and water were supplied ad libitum. Animals were identified by coat staining, and groups were identified by colored cage cards.

3. The dose levels selected were based on a previous micronucleus test with FOSETYL-Al. Normal animals were weighed and randomized into ten treatment groups as described in Table 1:

TABLE 1: Dosing Regimen and Sacrifice Schedule

Group	Number of Animals M	F	Number of Doses	Dose Level Aliette (g/kg)	Dose Level FOSETYL-Al (g/kg)	Sacrifice (hrs. post initial dose)	Marrow Slides prepared
A	5	5	1	0.0	0.00	30	Yes
B	5	5	1	0.6	0.48	30	No*
C	5	5	1	1.2	0.96	30	Yes
D	5	5	1	2.4	1.92	30	Yes
E	5	5	1	3.6	2.88	30	Yes
F	5	5	2	C.0	0.00	48	Yes
G	5	5	2	0.6	0.48	48	No*
H	5	5	2	1.2	0.96	48	Yes
I	5	5	2	2.4	1.92	48	Yes
J	5	5	2	3.6	2.88	48	Yes

* Slides were prepared only if 2 or more mice died in the highest dosage group.

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 post initial dose for groups A-E. Animal groups F-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 excised and the marrow was collected from each animal in fetal calf serum. The suspensions were transferred to hemolysis tubes (1 tube/animal) and centrifuged. Bone marrow slides were prepared, 2 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, or 8,000/group) and their corresponding micronuclei were counted.

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

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

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

The percentage of spontaneously occurring polychromatic erythrocytes with micronuclei was determined. On the basis of this criteria, mutagenic activity was determined as follows:

1.6%	- spontaneous incidence in CD1 mice.
4.0%	- negative
4.0% - 4.9%	- doubtful
5.0%	- positive
5.0% - 9.9%	- +
10.0% -19.9%	- ++
20.0%	- +++

6. The test groups were compared statistically using Kastenbaum and Bowman's test. A 2.5 fold increase in spontaneous incidence of polychromatic erythrocytes with micronuclei was considered significant for $P = 0.05$. An analysis of variance was used to compare the results of the cytotoxicity tests.

(9) REPORTED RESULTS:

No increase in the percentage of polychromatic erythrocytes with micronuclei or total number per 1,000 polychromatic erythrocytes examined was noted in treatment groups when compared to the control groups (see Table 2). Slight cytotoxicity was produced at the highest treatment dose tested with repeat administration. From data presented it is not clear to this reviewer if repeated administrations were fractionated dosages or 2 administrations at the dosages given, e.g., did group J receive 2 administrations of 3.6 g/kg or a total dosage of 3.6 g/kg?

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

(10) DISCUSSION:

The micronucleus test was performed on Aliette (Formulation EXP 1659-B) at dose ranges which are generally considered appropriate, i.e., the highest dose tested resulted in slight

cytotoxicity. 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 specified in the protocol. Since the number of 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 produce a mutagenic response, a conclusion reached by the author and supported by previous experiments with FOSETYL-Al, the active ingredient.

There were, however, some deficienices in this experiment. Although dosages for Aliette had been calculated, the body weights for the CD1 mice were not present in the final report. Also, at dosages of 0.6 and 1.2 g/kg, cytotoxicity ratios A and B were not reported in either of the two experimental trials (see Table 2). In addition, the % polychromatic RBC's with micronuclei was not reported from either assay at the 0.6 g/kg level. Therefore, the results of cytotoxicity and the micronucleus test are not complete and the reason for this data omission was not given.

5

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

Group	Dosage (g/kg) Aliette	Cytotoxicity Test: % Polychromatic RBC's with Micronuclei	Micronucleus Test: No. Polychromatic RBC's with Micronuclei/1,000 Polychromatic RBC's — Males (Total 4 per group)	Females	Total (8 per group)	Cytotoxicity Ratios: A: No. Polychromatic RBC's/100 Orthochromatic RBC's	B: No. Nucleated RBC's/100 Orthochromatic RBC's
A	0.0	0.05	3	1	4	1.14	5.87
B	0.6	-	4	5	8	-	-
C	1.2	0.10	2	3	5	-	-
D	2.4	0.06	3	4	7	1.14	4.48
E	3.6	0.09	3	2	5	-	-
TEM	1.0	3.74	158	141	299*	-	-
F	0.0	0.06	4	5	9	1.37	5.03
G	0.6	-	-	-	-	-	-
H	1.2	0.11	4	5	9	-	-
I	2.4	0.05	1	3	4	1.21	4.33
J	3.6	0.13	5	5	10	1.08	3.80*
TEM	1.0	4.04	131	191	323**	-	-

* Significant at p, 0.05

** Significant at p, 0.01