10-4-79

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: ~~September 26, 1979~~

SUBJECT: Roundup®, EPA Reg. No 524-308

FROM: Merry Lou Alexander MLA 9/27/79 Bdd 10/2/79 WSB 10.4.79
Toxicology Branch/HED (TS-769)

Caswell #661A

TO: Product Manager #25
RD (TS-767)

Action Type: Submission of new data for Glyphosate (98.4%) technical.

Summary

Glyphosate was not mutagenic in the following test systems:

1. Rec-assay in two strains of B. subtilis up to 2000 ug test material/disk;

2. Reverse mutation in five histidine-requiring strains of S. typhimuriam and one tryptophan-requiring strain of E. coli, with or without metabolic activation; and

3. Ames test in four strains of Salmonella, with or without metabolic activation.

One milligram glyphosate per plate was tolerated by Salmonella (TA 100) without toxic effects.

Review of Data

A. Microbial mutagenicity [Institute of Environmental Toxicology, Tokyo, Japan; July 20, 1978; Test material - CP67573 (Glyphosate)]

1. Rec-assay

Two strains of B. subtilis, recombination-wild (H17) and deficient (M45), were thawed and streaked separately onto a B-2 agar plate. A filter paper disk was soaked with 0.2 ml of a 10 mg/ml aqueous solution of test material, and placed so as to cover the starting parts of bacterial streaks. After overnight incubation at 37°C, the length of the inhibitory zone of each streak was measured. Kanamycin was negative control and mitomycin C was used as positive control.

Results: No inhibitory zone noted in either strain at doses of 20-2000 ug test material/disk. Positive control caused a marked difference (11 mm) in the length of the two inhibitory zones at 0.1 ug/disk. The negative control induced a slight (2 mm) difference at 10 ug/disk.

Classification: Core-minimum data

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2. Reverse mutation test with and without a liver metabolic activation system.

Five strains of S. typhimurium (TA1535, TA1537, TA1538, TA98, and TA100-all histidine auxotrophs) and tryptophan-requiring E. coli WP2 hcr were suspended in phosphate buffer. For S. typhimurium strains a solution of 0.5 mM L-histidine-0.5mM D-biotin was added to soft agar at a rate of 1/10 (v/v). For the E. coli strain a 0.5mM L-tryptophan was added at the same rate. The prepared agar was called "top agar".

A liver metabolic activation system was prepared from the livers of Sprague-Dawley male rats, taken on the fifth day following a single 500 mg/kg i.p. injection of Aroclor 1254. Livers were perfused with 0.15M KCl, homogenized, and centrifuged 10 minutes at 9000Xg. The components of 1.0 ml of the metabolic activaton system (S-9 mix) were as follows:

0.3 ml 9000Xg supernatant
8mM $MgCl_2$
33mM KCl
5mM glucose-6-phosphate
4mM $NADP^+$
100mM sodium phosphate (pH 7.4)

To 2 ml of the top agar were added 0.1 ml of a bacterial suspension, 0.1 ml of a test material solution, and, for metabolic activation, 0.5 ml of the S-9 mix. The mixture was poured onto the surface of a minimal agar plate (1.5% agar and 0.5% glucose) with modified Vogel-Bonner E medium. All plates were incubated at 37°C for 2 days after which the number of revertant colonies was counted. Compounds used as positive controls were AF-2; 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide; beta-propiolactone; 9-aminoacridine; 2-nitrofluorene; and 2-aminoanthracene.

Results: Test material induced no significant increase in the numbers of revertant colonies of any strain over control values, with or without the S-9 mix. Positive controls induced reverse mutations.

Classification: Core-minimum data

B. Salmonella mutagenicity assay (Monsanto Company, Environmental Health Laboratory; June 16, 1978; Test material-Glyphosate; Sample No. 04; Test No. LF-78-161)

The study design was after the methods and materials described by Ames et al. in Mutation Research (1975) Vol. 31, pp. 347-364, and as described in the standard protocol for Salmonella mutagenicity tests on file in the Monsanto Co. Environmental Health Laboratory.

The Salmonella strains used in plate incorporation assays were TA98, TA100, TA1535 and TA1537. The concentrations of 98.4% active test material tested were 0.1, 0.4, 1.0, 2.0, 10.0, 30.0, 100, and 1,000 ug/plate. Testing was conducted in the presence and absence of a mammalian activation system in both plate incorporation and spot tests. Positive controls were 2-acetamidofluorene, 2-aminoanthracene, 9-aminoacridine, benzo (a) pyrene, 4-nitroquinoline-N-oxide, $NaNO_2$, and tris(2,3-dibromopropyl)phosphate.

Results: Glyphosate was not mutagenic toward any Salmonella strains under the following test conditions:

(1) Spot test-at a maximum concentration of 1000 ug/plate using TA98, TA100, TA1535 and TA1537, with and without rat and mouse microsomal preparations.

(2) Plate incorporation test-at a maximum 1000 ug/plate in the presence and absence of rat mammalian activation system using TA98, TA100, TA1535 and TA1537.

(3) Toxicity test-at test compound concentrations of 0.03, 0.1, 1.0, 3.0, and 10 mg/plate using TA100. In the presence or absence of metabolic activation, a maximum 1.0 mg glyphosate/plate was tolerated by bacteria without toxic effects.

Classification: Core-minimum data.