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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Casewell No. 188AAA

DATE: December 30, 1982

SUBJECT: Goal 2E Herbicide: [A] Evaluation of Mouse Lymphoma Assay (Mammalian Cell Point Mutation), with oxyfluorofen technical, submitted July 20, 1982, Accession # 247909. [B] Response to Rebuttal Comments submitted by Rohm & Haas, dated November 12, 1982.

TO: Richard F. Mountfort, PM 23,
Registration Division (TS-767)

[A] Mouse Lymphoma Assay with Goal Technical (Data Evaluation Record).

Title: "Mutagenicity Evaluation of RH-2915 Technical in the Mouse Lymphoma Foreward Mutation Assay (Rohm and Haas Protocol No. 81P-438). Final Report. Rohm and Haas Report No. 82RC-37." [Submitted to Rohm and Haas Co., Toxicology Department, Philadelphia, PA, by Litton Bionetics, Inc., Kensington, MD, LBI Project No. 20989, June, 1982.]

Test Material: RH-2915, Technical (TD 81-306, lot # Z-3985) [aka, Goal Tech.], a brownish powder, dissolved in dimethyl-sulfoxide (DMSO) at 100 mg/ml as stock, from which final 1:100 dilutions into tissue culture medium were made for the assay (cloudy precipitation occurred at 62.5 ug/ml and above).

Procedures: Preliminary cytotoxicity testing indicated a moderate reduction in cell growth at 1000 ug/ml. Experimental trials were performed both in the absence of (one trial) as well

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as in the presence of (5 trials) a mammalian metabolic activation system provided by hepatic (microsomal) homogenates (S-9) from Aroclor 1254-treated Sprague-Dawley (one trial) or F-344 male rats (4 trials), plus appropriate cofactors, according to protocols developed by Clive and Spector (1975)*

In the (single) direct (non-activation) assay, replicate cell cultures were exposed to 5 concentrations of test compound, ranging from 62.5 thru 1000 ug/ml for 4 hours, followed by a compound-free growth and recovery (expression) period of two days, then cloned in agar containing bromodeoxyuridine or trifluorothymidine to select for TK mutants (TK-/TK-). After 10 days incubation, mutant colonies were counted, and the means of 3 plates per concentration compared to results of simultaneous solvent (DMSO) controls.

Activation assays (with S-9) were conducted in the same manner except for the addition of rat liver homogenate S-9 and cofactors during the 4-hour treatment period. In 5 trials with S-9, concentrations of Goal ranged between 1.95 and 50.0 ug/ml. The expression time was also extended to three days for the last (fifth) activation assay.

Suspension growth of cultures were monitored by cell counts during the recovery period, the relative cloning efficiencies and percent growth values (compared to solvent control) calculated, and induced mutant frequencies expressed as the ratio of mutant to viable colonies per 10,000 cells.

The direct alkylating mutagen, ethylmethane sulfonate (EMS), was the positive control for the non-activation study, while dimethylnitrosamine (DMN) served as positive control for the trials with S-9.

Results: In the single direct assay, none of the treatments (to 1000 ug/ml) induced a mutant frequency different from the solvent control, even at toxic doses (7.6% relative growth).

The presence of the activation mixture appeared to convert the test material to a more toxic form (by a factor of 30-40 times). In the first trial (with S-9 prepared from Sprague-Dawley rats), mutant frequencies ranged from 2.5 to 4.1 times above background at concentrations between 1.95 to 31.3 ug/ml (resulting in relative growth between 21% and 43%), but without a clear-cut dose-response. In the succeeding 4 trials to

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* Clive, D. and J.F.S. Spector. 1975. Laboratory procedures for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Res. 31:17-29.

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confirm these effects, (employing S-9 from F-344 rats), doses between 2.5 to 40.0 ug/ml induced erratic but definite increases in mutant frequencies 2 to 4 times greater than solvent controls at moderate to toxic ranges (relative growths from 60% to 2.5%), again without definitive dose responsiveness. Negative (solvent) control mutant frequencies in all trials were in the "normal" range (untreated/spontaneous background), while the positive control compounds (EMS, DMN) induced the expected responses greatly in excess of background (35X, and 7-20X, respectively). The report concluded that since the test material induced significant increases in 4 of 5 trials with S-9, it was therefore considered weakly mutagenic in the presence of metabolic activation.

Reviewer Evaluation:

Protocols employed for both the singular direct assay (non-activation), as well as repeat testing with metabolic activation (S-9 microsomes from Aroclor 1254-treated rats), are judged adequate to generate valid results (significant increases in mutant freguency at moderate to high toxicity), indicating Goal technical possesses weakly-positive induced mutagenic activity (2 to 4 times background) in the mouse lymphoma assay at the TK locus, but only in the presence of metabolic activation. The study is ACCEPTABLE.

NB: The following however, are noted from inspection of the data presented:

1. Test material was not characterized as to purity, presence and concentration of contaminants (impurities, etc.).

2. Test material was negative in the direct assay (without activation) well into the highly toxic range of concentrations, 1000 ug/ml (yielding only 7.6% relative growth).

3. All concentrations tested in the non-activation assay produced precipitation in culture.

4. Test material was more toxic in the presence of metabolic activation (S-9).

5. The presence of S-9 activation mixture prepared with F-344 microsomes increased background MF significantly (2.5-4.2X the value in the direct assay), compared to S-D microsomes (2-3X).

6. Test material was positive in 4 of 5 trials with metabolic activation, even at the lowest concentration employed, 1.95 ug/ml (Trial #1) (2.9 X solvent control).

7. In no trial demonstrating positive results was a clear-cut dose-response evident.

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[B] Response to R & H Rebuttal, dated November 12, 1982:

Issue (a): Determination of a "no-effect level".

Registrant: Determination of a NOEL is not an objective of this type of assay [in vitro mouse lymphoma].

Agency: Accepted. Although a dose-response relationship was not obtained in any trial in the presence of activation, the test material was positive in 4 of the 5 trials , even at the lowest concentration tested (1.95 ug/ml), which is a sufficient criterion to confirm a qualitative assessment.

Issue (b): Precipitation at all levels tested in the single direct assay (without activation).

Registrant: Although not tested below the level of precipitation [62.5 ug/ml], Goal technical was tested at 5 concentrations over more than a 10-fold concentration range [actually, 16-fold]; test material was in solution when added to culture medium.

Agency: Accepted. Although negative results below the 62.5 ug/ml concentration in the direct assay would be expected, it should be noted that no precipitation was reported at the highest concentration in the activation trials (50 ug/ml), presumably a non-precipitating level.

Issue (c): Presence of untreated (negative) controls.

Registrant: Untreated controls were present in trials with activation as shown in Tables 3 to 6 (of report).

Agency: Accepted. However, untreated (concurrent) controls should always be run with each experimental trial.

Issue (d): Erratic mutant frequencies of solvent controls.

Registrant: Mutant frequencies of solvent controls in these 6 trials ranged from 9.7 to 38.5 X 10^{-6}, compared to published values of 8 to 193 X 10^{-6} (e.g., Clive et al., Mutation Res. 59:61-108, 1979).

Agency: Accepted. However, a number of protocol changes appended to the report probably contributed to the variability in background frequencies. Although experience in performance of the lymphoma assay

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has consistently improved over the years, consistency is compromised by such dramatic protocol alterations as: (i) change in S-9 source ("Project Change No. 2"); (ii) "communication breakdown in the laboratory", compounded by a 7-month delay in effecting such ("Change No. 3"); (iii) adjustment in S-9 concentration ("Change No. 4"); and, (iv) inconsistent times for cloning counts ("Change. No. 5").

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