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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: Review of Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay of 5-OH-MSO_2

EPA No. 3F-2904/7969-58 Project No. 1086/1087
Record Number: 164167/164176 Caswell Number 72A

TO: Robert J. Taylor, PM Team #25
Registration Division (TS-767c)

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Budd 2/7/86

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The Registrant, BASF Wyandotte Corporation, submitted a Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay report which it had overlooked in a previous submission of toxicology data on 5-OH-MSO_2, a hydroxylated plant metabolite of NP-55 (Sethoxydim; Poast®).

The Toxicology Branch has reviewed the report and found it "Unacceptable" because it lacked an adequate description of the methods used, individual culture data were missing, and the study was not repeated with hepatocytes from a female rat. The report and review were critiqued by Irving Mauer, Geneticist, Toxicology Branch.

RAT PRIMARY HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY OF 5-OH-MSO_2

Litton Bionetics, Inc.; Report No. 20991; May, 1984; Accession No. 260544

PROTOCOL: Hepatocytes from an adult male Fischer 344 rat (150-300 g) were cultured as monolayers on plastic coverslips in culture dishes containing William's Medium E, supplemented with 5% fetal bovine serum 2 mM l-glutamine, 2.4 uM dexamethasone, 90 U/ml penicillin, 90 ug/ml streptomycin sulfate, and 140 ug/ml gentamicin, then maintained on WME. Cultures were dosed with fifteen serial dilutions (0.1-5000 ug/ml) of the test article dissolved in WME and 1% serum. Positive controls were dosed with 2-acetyl aminofluorene (2-AAF) at a dose of 0.10 ug/ml. Negative controls were dosed with an unspecified vehicle (probably water or WME). After 20-24 hours of exposure, trypan blue exclusion cell viability counts were made. Based on these findings, the six highest concentrations (100, 250, 500, 1000, 2500, and 5000 ug/ml) were selected for nuclear labeling.

UDS assays were performed by the Method of Williams (1977 and 1980). Prepared cultures were treated with 2.5 ml of WME containing 1% fetal bovine serum, 1 uCi/ml ^3H-thymidine, and dilutions of the test article. Five cultures were used for each test article concentration and each control. Two cultures each were used to assess cytotoxicity, and the remaining three cultures were prepared (including dipping in an emulsion of Kodak NTB2) for microscopic examination with an automatic counter. UDS was measured by counting the number of nuclear grains and subtracting the background levels to determine the net nuclear grain count.

RESULTS: The highest concentration used (5000 ug/ml) precipitated slightly, yet was not cytotoxic. The lower concentrations remained in solution. UDS in the cultures treated with the test article (measured as net nuclear grain counts) was similar to that seen in the negative control. This showed that the test article did not initiate UDS in male rat hepatocytes. The assay's sensitivity was substantiated by the significant increase in net nuclear grain counts in the positive cultures (12X that seen in the negative controls).

This study is UNACCEPTABLE. This report was lacking a description of the procedures actually used in performing this study. Instead, a laboratory SOP was appended which lacked specific information. There was no mention of the vehicle used in dosing the negative controls. The SOP stated that the positive controls were to be dosed at a concentration of 0.05 ug/ml, but the data table reported a concentration of 0.10 ug/ml. Mean values for each concentration/control were reported; there were no individual culture data. The assay should have been repeated using hepatocytes from a female rat to confirm the reported negative results. This study received Quality Assurance Review, yet these deficiencies were not detected.