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

JUN 6 1991

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Ally - UDS Assay

TO: Vickie Walters
Product Manager (25)
Registration Division (H7509C)

FROM: Linda L. Taylor, Ph.D. 5/28/91
Toxicology Branch II, Section II
Health Effects Division (H7509C)

THRU: K. Clark Swentzel 5/29/91
Toxicology Branch II, Section II Head
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. 6/5/91
Chief, Toxicology Branch/HFAS/HED (H7509C)

Registrant: duPont
Synonyms: T6376-74
Chemical: benzoic acid, 2-{{{{(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-, methyl ester
Project No.: 1-0957
Caswell No.: 419H
Identifying No.: 000352-00435
Record No.: not provided; Case 016737; Submission S392914
MRID No.: 417739-01
Action Requested: None specified.

Comment: As a condition of registration, a study to fulfill "Other genotoxic effects" was submitted by the Registrant. The study: "Assessment of IN T6376-74 in the In Vitro Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes" has been reviewed and the DER is appended. The study is summarized below.

Under the conditions of the assay, the test material met the criteria for a negative classification and was not shown to be genotoxic in the in vitro unscheduled DNA synthesis assay in rat hepatocytes at concentration levels of 0.5 to 2500ug test material. The study is classified unacceptable, pending submission of data/information to support the contention that the limit of solubility was reached for the test material.

This study does not satisfy the guideline requirements (84-2) for mutagenicity, Category III, Other genotoxic effects, but it may be upgraded with the submission of the required data.

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REVIEWED BY: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 5/12/91
TOX. BRANCH II, SECTION II, (H7509C)
SECONDARY REVIEWER: JOHN CHEN, DVM not J. Chen 5/21/91
TOX. BRANCH II, SECTION I, (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: MUTAGENICITY - UDS ASSAY TOX. CHEM. NO.: 419H

MRID NO.: 417739-01

TEST MATERIAL: T6376-74

SYNONYMS: DUPONT ALLY HERBICIDE; BENZOIC ACID METHYL-2-[[[[(4-METHOXY-6-METHYL-1,3,5TRIAZIN-2-YL)AMINO]CARBONYL]AMINO]SULFONYL]-, METHYL ESTER; METSULFURON METHYL

STUDY NUMBER: MEDICAL RESEARCH PROJECT NUMBER: 4581-834; HLR 574-90

SPONSOR: DUPONT; AGRICULTURAL PRODUCTS

TESTING FACILITY: HASKELL LABORATORY FOR TOXICOLOGY AND INDUSTRIAL MEDICINE

TITLE OF REPORT: ASSESSMENT OF IN T6376-74 IN THE IN VITRO UNSCHEDULED DNA SYNTHESIS ASSAY IN PRIMARY RAT HEPATOCYTES

AUTHORS: DR VINCENT

REPORT ISSUED: NOVEMBER 16, 1990

QUALITY ASSURANCE: A QUALITY ASSURANCE STATEMENT WAS PROVIDED.

CONCLUSION: UNDER THE CONDITIONS OF THE STUDY, THE TEST MATERIAL MET THE CRITERIA FOR A NEGATIVE CLASSIFICATION AND WAS NOT SHOWN TO BE GENOTOXIC IN THE IN VITRO UNSCHEDULED DNA SYNTHESIS ASSAY IN RAT HEPATOCYTES AT CONCENTRATION LEVELS OF 0.5 TO 2500 UG TEST MATERIAL (CONCENTRATION IN THE MEDIUM, WHICH WAS 1% OF THE TREATMENT MEDIUM).

CLASSIFICATION: THIS STUDY IS CLASSIFIED UNACCEPTABLE, PENDING SUBMISSION OF DATA/INFORMATION TO SUPPORT THE CONTENTION THAT THE LIMIT OF SOLUBILITY WAS REACHED FOR THE TEST MATERIAL.

THIS STUDY DOES NOT SATISFY THE GUIDELINE REQUIREMENTS (84-2) FOR MUTAGENICITY, CATEGORY III-OTHER GENOTOXIC EFFECTS, BUT MAY BE UPGRADED WITH THE SUBMISSION OF THE REQUIRED DATA.

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A. MATERIALS

1. TEST MATERIAL: T6376-74; DESCRIPTION: WHITE SOLID; BATCH #: LOT # NOT PROVIDED; MEDICAL RESEARCH # 4581-834; PURITY: 98.8%; SOLVENT: DIMETHYL SULFOXIDE (DMSO).

2. CONTROL MATERIAL: NEGATIVE: DMSO; POSITIVE: 2-ACETYLAMINOFLUORENE (2-AAF) (ALDRICH, MILWAUKEE, WI) PREPARED IN DMSO.

3. TEST ORGANISM: (A) SPECIES: RAT-MALES; STRAIN: CRL: CD®BR; AGE: APPROXIMATELY 8 WEEKS OLD; BODY WEIGHT: NOT PROVIDED; SOURCE: CHARLES RIVER (LOCATION NOT PROVIDED). RATS WERE FED PURINA® CERTIFIED RODENT CHOW #5002 AND WATER AD LIBITUM. (B) PRIMARY RAT HEPATOCYTES: OBTAINED FROM THE LIVERS OF THE RATS DESCRIBED ABOVE. LIVERS WERE PERFUSED WITH A STERILE COLLAGENASE SOLUTION [WILLIAMS' MEDIUM E (WME) CONTAINING L-GLUTAMINE (292 MG/L, GENTAMICIN (50 UG/ML), 10 MM HYDROXYPIPERAZINE-N-2-ETHANE SULFONIC ACID (HEPES), AND COLLAGENASE (100 UNTIS/ML)] AND EXCISED. THE ISOLATED HEPATOCYTES WERE COMBED OUT OF THE PERFUSED LIVER AND COLLECTED BY CENTRIFUGATION. CELLS WERE RESUSPENDED IN COLD HANKS' BALANCED SALT SOLUTION (HBSS), FILTERED, AND MIXED WITH AN EQUAL VOLUME OF COLD PERCOLL® WORKING SOLUTION (90% PERCOLL®, 10% 10X-HBSS), AND THE MIXTURE WAS CENTRIFUGED [KREAMER, BL, ET AL., IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY 22, 201-211 (1986)]. THE HEPATOCYTES WERE WASHED TWICE AND RESUSPENDED IN COLD HEPATOCYTE PLATING MEDIUM (HPM) CONTAINING WME, L-GLUTAMINE (292 MG/L), GENTAMICIN (50 UG/ML), HEPES (10 MM), AND FETAL BOVINE SERUM (10% V/V).

VIABILITY AND DENSITY OF HEPATOCYTE SUSPENSIONS WERE ASSESSED. HEPATOCYTE VIABILITY WAS REPORTED AS 96% IN TRIAL 1 AND 93% IN TRIAL 2. CHAMBER SLIDES (MILES LABORATORIES, 2 CHAMBERS/SLIDE) CONTAINING HPM WERE INOCULATED WITH 5×10^5 VIABLE HEPATOCYTES/CHAMBER, AND CELLS WERE ALLOWED TO ATTACH TO THE SURFACE OF THE SLIDES IN AN INCUBATOR (5% ± 1% CO_2, 37° ± 1.5°, AND > 90% RELATIVE HUMIDITY). FOUR CULTURES WERE PREPARED FOR EACH TREATMENT LEVEL AND IN EACH TRIAL, 2 SLIDES (4 CULTURES) WERE PREPARED FOR EACH TREATMENT LEVEL.

4. CYTOTOXICITY: A CYTOTOXICITY TEST WAS PERFORMED BY MEASURING THE AMOUNT OF LACTATE DEHYDROGENASE (LDH) THAT HAS LEAKED FROM THE CELLS INTO THE CULTURE MEDIUM IN ORDER TO DETERMINE THE HIGHEST CONCENTRATION OF THE TEST MATERIAL FOR THE UDS ASSAY. THERE WAS NO SIGNIFICANT ELEVATION OF LDH ACTIVITY FOUND AT THE CONCENTRATIONS TESTED (0.5-2500 UG/ML; TABLE 1).

5. TEST MATERIAL CONCENTRATIONS: THE TEST MATERIAL WAS DISSOLVED IN DMSO, WHICH WAS CHOSEN BASED ON INFORMATION GIVEN IN THE SAMPLE EVALUATION FORM SUPPLIED BY THE SPONSOR AND RESULTS OF SOLUBILITY TESTING. THE LIMIT OF THE TEST MATERIAL SOLUBILITY IN DMSO WAS SAID TO BE 250 MG/ML; ADDITION OF A 250 MG/ML STOCK SOLUTION TO CULTURE MEDIUM AT APPROXIMATELY 1% (V/V) RESULTED IN HEAVY PRECIPITATION. THE PRECIPITATE DISSOLVED WHEN THE MIXTURE WAS STIRRED FOR 5 MINUTES. THE CONCENTRATIONS IN THE MEDIUM WERE 0.5, 5, 10, 50, 100, 500, 1000, AND 2500 UG TEST MATERIAL/ML AND 0.02 AND 0.2 UG POSITIVE CONTROL/ML. NOTE: NO DOCUMENTATION WAS PROVIDED TO SUPPORT THE STATEMENT REGARDING THE LIMIT OF SOLUBILITY.

6. CONTROL ARTICLES: NEGATIVE - DMSO. ADDED TO THE CULTURE MEDIUM AT APPROXIMATELY 1% (V/V). POSITIVE - 2-AAF. PREPARED IN DMSO. TEST MEDIUM CONCENTRATIONS OF 0.1 UM (0.02 UG/ML) AND 1 UM (0.2 UG/ML) 2-AAF WERE

INCLUDED IN EACH TRIAL TO ESTABLISH THAT THE HEPATOCYTES WERE CAPABLE OF UDS.

7. HOMOGENEITY, CONCENTRATION, AND STABILITY: MIXTURES OF TEST AND CONTROL ARTICLES WERE NOT ANALYZED FOR UNIFORMITY, CONCENTRATION, OR STABILITY. IN THE ABSENCE OF VISIBLE EVIDENCE TO THE CONTRARY, THE PROCEDURES USED WERE BELIEVED TO RESULT IN MIXTURES THAT WERE UNIFORM, STABLE, AND OF CALCULATED CONCENTRATION AT THE TIME OF TREATMENT. STABILITY WAS ASSUMED FOR BOTH THE TEST AND CONTROL ARTICLES, AND TREATMENT MEDIUM WAS NOT ASSAYED FOR STABILITY OR CONCENTRATION OF TEST AND CONTROL ARTICLES, SINCE THIS ASSESSMENT WAS NOT CONSIDERED NECESSARY TO ACHIEVE THE OBJECTIVES OF THE STUDY.

B. TEST PERFORMANCE

STOCK SOLUTIONS AND DILUTIONS OF BOTH THE TEST ARTICLE AND 2-AAF WERE MADE IN DMSO IMMEDIATELY PRIOR TO USE. TREATMENT MEDIA WERE MADE BY MIXING APPROPRIATE DILUTIONS OF THE SOLUTIONS WITH WME CONTAINING L-GLUTAMINE (292 MG/L) GENTAMICIN (50 UG/ML), HEPES (10 MM), AND [METHYL-^{3}H]-THYMIDINE (NEW ENGLAND NUCLEAR, 82.4 CI/MMOL, 5 UCI/ML) TO A FINAL CONCENTRATION OF APPROXIMATELY 1%. HBM WAS REMOVED AND REPLACED WITH TREATMENT MEDIUM, AND THE CULTURES WERE INCUBATED FOR APPROXIMATELY 18 HOURS.

AUTORADIOGRAPHY - SLIDES WERE MOUNTED INTO PLASTIC GRIPS, RINSED IN WME, AND DIPPED IN 1% SODIUM CITRATE SOLUTION FOR 7 MINUTES TO SWELL THE NUCLEI. CELLS WERE FIXED WITH 3 CHANGES OF ETHANOL: GLACIAL ACETIC ACID (3:1) FOR 30 MINUTES/CHANGE, RINSED IN WATER, AND DRIED WITH ABSOLUTE ETHANOL.

SLIDES WERE DIPPED INTO KODAK® NTB-2 AUTORADIOGRAPHIC EMULSION, DRIED FOR 2 HOURS, AND STORED IN DESICCATED SLIDE BOXES AT -20° C FOR 4 TO 5 DAYS TO EXPOSE THE EMULSION. SLIDES WERE DEVELOPED AND STAINED WITH METHYL-GREEN PYRONIN Y. ALL OF THE ABOVE STEPS WERE PERFORMED IN DARKNESS, EXCEPT FOR STAINING.

SCORING OF UDS: SCORING WAS PERFORMED BLIND. CELLS IN THE CULTURES THAT HAD ALL OF THE FOLLOWING ATTRIBUTES WERE SCORED FOR UDS: (1) NORMAL NUCLEAR MORPHOLOGY; (2) APPARENT CYTOPLASM, EVIDENT EITHER BY TRITIUM LABELING AND/OR BY PINK COUNTER STAIN; (3) FREE OF DEBRIS AND STAINING ARTIFACTS; AND (4) ONE NUCLEUS.

SILVER GRAINS WERE COUNTED. IN EACH CULTURE, 25 CELLS (100 TOTAL NUCLEI/TREATMENT LEVEL) MEETING THE ABOVE CRITERIA WERE SCORED INDIVIDUALLY BY MEASURING THE AREA OF THE SILVER GRAINS OVER THE NUCLEUS AND THE AREA OF THE GRAINS IN 2 OR MORE NUCLEUS-SIZED REGIONS IN THE CYTOPLASM IMMEDIATELY ADJACENT TO THE NUCLEUS. THE HIGHEST CYTOPLASM VALUE WAS RECORDED.

C. ANALYSIS OF DATA

1. DATA CALCULATIONS: AREAS OF THE GRAINS WERE CONVERTED TO GRAIN COUNTS USING A FACTOR (MEAN RATIO OF MANUALLY-COUNTED GRAINS TO AREAS OF THE SAME GRAINS FROM 3 NUCLEUS-SIZED PATCHES OF THE SLIDE) DETERMINED FOR EACH SLIDE. NUCLEAR OR CYTOPLASMIC AREAS WERE THEN MULTIPLIED BY THE FACTOR TO DERIVE NUCLEAR OR CYTOPLASMIC GRAIN COUNTS. CYTOPLASMIC GRAIN COUNTS WERE

SUBTRACTED FROM THE NUCLEAR GRAIN COUNTS TO DETERMINE THE NET NUCLEAR GRAINS (NNG) OF EACH CELL. THE MEAN NNG AND STANDARD ERROR OF THE MEAN NNG WERE CALCULATED FOR EACH SLIDE. MEAN NUCLEAR GRAINS, MEAN CYTOPLASMIC GRAINS, MEDIAN NNG, AND PERCENT OF CELLS IN REPAIR (CELLS WITH 5 OR MORE NNG) WERE ALSO CALCULATED FOR EACH SLIDE. THE MEAN NNG FROM ALL SLIDES OF THE SAME TEST CONCENTRATION WERE AVERAGED TO DETERMINE THE UDS RESPONSE FOR THAT TREATMENT IN EACH TRIAL. ALL DATA CALCULATIONS AND GRAPHICS WERE PERFORMED USING VALIDATED COMPUTER SOFTWARE (RS1®, BBN SOFTWARE PRODUCTS CORPORATION).

2. STATISTICAL METHODS: INCLUDED THE CALCULATION OF WITHIN-TRIAL MEANS AND ATTENDANT STANDARD ERROR OF THE MEAN FOR EACH TREATMENT LEVEL.

3. CRITERIA FOR POSITIVE AND NEGATIVE RESULTS: THE TEST ARTICLE WAS CONSIDERED POSITIVE IF (1) THE AVERAGE UDS RESPONSE FOR ANY TEST ARTICLE CONCENTRATION FROM BOTH TRIALS IS +5 NNG OR MORE AND THIS INCREASE IS AT LEAST 3 STANDARD DEVIATIONS ABOVE THE CONTROL RESPONSE; AND (2) THERE IS A POSITIVE CORRELATION BETWEEN INCREASING CONCENTRATIONS OF TEST ARTICLE AND THE AVERAGE UDS RESPONSE IN THE ABSENCE OF CYTOTOXICITY. THE TEST ARTICLE WAS CONSIDERED NEGATIVE IF (1) THE AVERAGE UDS RESPONSE FOR ALL CONCENTRATIONS OF THE TEST ARTICLE FROM BOTH TRIALS IS LESS THAN 0 NNG; AND (2) THERE IS NO EVIDENCE FOR A CONCENTRATION-RELATED EFFECT OF THE TEST ARTICLE.

4. CRITERIA FOR A VALID ASSAY

NO CRITERIA WERE PROVIDED WITH REGARD TO VALIDITY OF THE ASSAY.

D. RESULTS

RESULTS OF THE CYTOTOXICITY EVALUATION WERE PRESENTED IN TABLE 1, COPY APPENDED. CYTOTOXICITY WAS NOT APPARENT IN EITH TRIAL AT ANY DOSE LEVEL.

THE RESULTS OF THE UDS ASSAY WERE PRESENTED IN TABLES 2 AND 3, COPIES APPENDED. UDS WAS NOT OBSERVED IN EITHER TRIAL AS A RESULT OF TREATMENT OF PRIMARY RAT HEPATOCYTES WITH THE TEST MATERIAL AT CONCENTRATIONS UP TO 2500 UG/ML. IT WAS STATED THAT THIS SOLUTION PRODUCED THE LIMIT OF TEST MATERIAL SOLUBILITY WHEN COMPRISING 1% OF THE TREATMENT MEDIUM (2500 UG/ML).

UNDER THE CONDITIONS OF THE ASSAY, THE TEST MATERIAL WAS NEGATIVE FOR UDS IN PRIMARY RAT HEPATOCYTES.

E. CONCLUSION

THE POSITIVE CONTROL COMPOUND, 2-AAF, INDUCED A SIGNIFICANT INCREASE IN THE MEAN NET NUCLEAR GRAINS PER CELL WHEN COMPARED TO THAT OF THE CORRESPONDING CONTROL. THE RESULTS INDICATED THAT THE CELL POPULATION EMPLOYED IN THE IN VITRO RAT HEPATOCYTE UDS ASSAY WAS ADEQUATE FOR THE DETECTION OF UDS IN RAT HEPATOCYTES. THE NUCLEAR LABELING IN THE NEGATIVE (SOLVENT) CONTROL WAS FOUND WITHIN THE NORMAL RANGE OF NET NUCLEAR GRAIN COUNT (-8.8 TO -11.2) PER NUCLEUS FOR PERFORMING A RAT HEPATOCYTE UDS ASSAY AS RECOMMENDED BY MITCHELL, ET AL. [MUTATION RES. 123, 363-410 (1983)].

THE HIGHEST CONCENTRATION (2500 UG/ML) EMPLOYED WAS NOT A CYTOTOXIC LEVEL, BUT WAS STATED TO BE THE LIMIT OF SOLUBILITY. NO DETAILED RESULTS OF THE

SOLUBILITY TEST FOR THE TEST MATERIAL WERE GIVEN TO CONFIRM THIS. THE INFORMATION ON THE MAXIMUM SOLUBILITY OF THE TEST MATERIAL IN OTHER APPROPRIATE SOLVENTS (E.G., ETHANOL OR ACETONE) WERE NOT PROVIDED EITHER. HOWEVER, UNDER THE CONDITIONS OF THE STUDY, THE TEST MATERIAL MET THE CRITERIA FOR A NEGATIVE CLASSIFICATION AND WAS NOT SHOWN TO BE GENOTOXIC IN THE IN VITRO UNSCHEDULED DNA SYNTHESIS ASSAY IN RAT HEPATOCYTES.

THIS STUDY IS CLASSIFIED UNACCEPTABLE, PENDING SUBMISSION OF DATA/INFORMATION TO SUPPORT THE CONTENTION THAT THE LIMIT OF SOLUBILITY WAS REACHED FOR THE TEST MATERIAL. THIS STUDY DOES NOT SATISFY THE GUIDELINE REQUIREMENTS (84-2) FOR MUTAGENICITY, CATEGORY III-OTHER GENOTOXIC EFFECTS, BUT MAY BE UPGRADED WITH THE SUBMISSION OF REQUIRED DATA.

METSULFURON-METHYL

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