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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

10/12/82

MEMORANDUM

TO: R. Mountfort, PM #23
Herbicides-Fungicides Branch
Registration Division (TS-767)

THRU: Orville E. Paynter, Ph.D.
Chief
Toxicology Branch/HED (TS-769)

SUBJECT: Dacthal - Miscellaneous Toxicity Data
EPA Reg. No. 677-166

CASWELL #: ~~382~~ 382

CAS Registry Number: 1861-32-1

Sponsor: Diamond Shamrock
Cleveland Ohio

INTRODUCTION:

The Diamond Shamrock Corporation is submitting toxicity data in connection with a ground water survey in Suffolk Country, Long Island. Residues of Dacthal and/or its metabolites have been detected in ground water supplies at levels ranging from 0.3 to 891 ppb calculated as Dacthal (e.g., see the E. Regelman review of 1/5/82). This aquifer constitutes the only source of potable water in the area. The data are being submitted simultaneously to the Office of Drinking water (ODW).

DISCUSSION:

The toxicity of Dacthal has been defined for tolerance purposes (see the review of PP #7E1968, 8/24/77, D. Ritter). The ADI has bet established at 0.5 mg/kg/day based on a NOEL of 1,000 ppm in a rat two year feeding study. Tolerances for

residues of Dacthal and its metabolites (calculated as Dacthal; diemthyl- tetrachloroterephthalate, monomethyltetrachloroterephthalate and tetrachloroterephthalic acid) have been established under 40 CFR 180.185 on numerous racs. The theoretical Maximum Residues Contribution (TMRC) from these sources occupies about 2% of the ADI (review of PP #9E2185, 3/24/82, W. Dykstra).

Toxicology Branch has calculated that a 1 ppm level in drinking would add 2 mg/day to the human diet or about 6% of the ADI (memo to J. Melone, W. L. Burnam, 3/19/82).

REGULATORY CONSIDERATIONS:

Food additive tolerances for pesticide residues in potable water have been regulated pursuant to 21 CFR §193. However, the Agency has recently proposed in the Federal Register (6/15/82) to discontinue establishing potable water tolerances and to delete those subparts of 21 CFR §193 which establish potable water tolerances as an uncessary burden on applicants for registration of products which may be used in potable water supplies. We have confirmed that this Rule will soon become Final (telecon with Dr. Reto Engler, RD, 8/31/82). Toxicity and residue data requirements would remain the same.

One provision of the above Proposal would require that OPP consult with the ODW when pesticide residues in potable water are anticipated.

REVIEW OF TOXICITY DATA:

1. Study: 90 day rat dietary administration

Laboratory: International Research and Development Corp. (IRDC)

Study Number: 293-044

Accession Number: 247493

Material Tested: DTX-76-0010; DAC 1209 (Tetrachloroterephthalic acid).

Animals: Charles River CD Rats 15/sex/group.

METHODS:

75 rats of each sex weighing 123 - 181 grams were assembled into one of five groups recieving 0, 50, 500, 1,000 or 1,000 ppm DAC 1209 in the diet for ninety days.

General observations were made daily, and detailed observations were made weekly for mortality, appearance and behavior.

Body weights and food consumption were recorded weekly.

Ophthalmic examinations using slit lamp and 1% Tropicamide were conducted initially and at termination.

Blood and urine samples were obtained at 1, 2 and 3 months. The same five animals per group were used throughout the study.

Hematological paramaters measured included Hemoglobin, Hematocrit, Red cells, White cells and differentials counts.

Serum Chemistry parameters measured included BUN, SGOT, SGPT, Alkaline Phosphatase, Na^+, K^+, Ca^{++}, Zn^{++} and Cl^-.

Urinalyses included volume, pH, Specific Gravity, color and appearance, and the presence albumin, glucose, bilirubin and formed elements.

Animals were killed at termination and gross necropsy was conducted. Selected organs and tissues from each surviving rat were reserved in 10% buffered formalin. The following tissues from all control and all 10,00 ppm animals were prepared fro histopathologic examination and evaluated microscopically: (*-organ weights obtained for all animals).

adrenals *
aorta
brain*
eye
heart*
large intestine
small intestine
  (3 levels)
kidneys*
liver*
lung
mesenteric lymph node
spleen*
pancreas
pituitary*
prostate/uterus
sternum (bone marrow)
skin (mammary gland)
spinal cord
stomach
testis/ovary*
thymus
thyroid*
urinary bladder
nerve (with muscle)
femur
tibia

RESULTS:

Mortality: 13 of 15 50 ppm males and 14 of 14 10,000 ppm females survived. Cause of death was not reported.

Body weights and food consumption were not affected by treatment.

No changes in appearance or behavior were reported.

Eyes changes were not seen.

Hematology: No compound-related effects were noted.

Serum Chemistry: No compound-related effects were noted.

Urinalysis was negative for compound effects.

Gross necropsy of all purposefuly terminated animals was negative for compound-related effects.

Organ weights: Apparent decreases in thyroid/body weight and pituitary/body weight ratios were found, upon Student's "t" test analysis, to be insignificant.

Microscopic examination of representative tissues and organs from the control and 10,00 ppm animals did not reveal compound related effects.

CONCLUSIONS:

The no effect level for this rat ninety day feeding study using teterchloro-terephthalic acid is greater than 10,000 ppm in the diet.

CORE RATING:

Minimum Data. Only five animals per group were sampled for labortatory analysis. This could have seriously compromised the study had adverse effects been noted. Not repairable.

002230

2. Mutagenicity Tests (Reviewed by Dr. John Chen)

Study A. Activity of DTX-77-0003 in the Salmonella Microsomal Assay for Bacterial Mutagenicity.

Procedure:

The mutagenic response of the test compound dissolved in DMSO at five concentrations (1, 10, 33.3, 100, and 333 ug per plate) were performed by the plate-incorporation method of the Salmonella/Microsomal Mutagenicity Test in the presence or absence of the mammalian metabolic activation from rat liver enzymes. Five histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538) were used in the histidine-reversion assay. The in-vitro mammalian metabolic activation system consisted of liver homogenate fraction "S-9" from arocolor 1254 treated Sprague-Dawley rats according to the preparation procedures described by Ames (one ml of S-9 fraction contained microsomes from 250 mg of wet liver)
and combined with the NADPH-dependent
microsomal-enzyme system (8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mM Na_2PO_4, pH 7.4). Mutations were quantified on triplicate for each strain by counting the number of his^+ revertant colonies that had grown after 48 hrs. incubation at 37°C on a minimum medium containing trace amount of histidine (5×10^{-4} M). If the compound was mutagenic, it would demonstrate at least 2X the number of colonies at the negative control and also exhibited a dose-response effect. The toxic level of test compound (100 ug/ml) which produced an approximation close to 50% reduction in growth rate of the bacteria was recorded but no toxicity was observed on the minimum agar assay plate at 333 ug of test compound per plate. The number of revertant colonies per plate on both negative and strain specific controls was also presented.

Results and Conclusion:

The compound, DTX-77-003, failed to induce a significant increase in the number of revertant colonies over the negative control culture in the Ames reversion assay either with or without the metabolic activation from rat liver enzymes and thus, the test compound was not mutagenic at the dose levels tested, 1 through 333 ug/plate. The strain specific controls apparently gave the expected positive results.

Evaluation:

The evaluation of the mutagenicity of DTX-77-0003 in the Salmonella/Microsomal Mutagencicity Test cannot be accomplished without the following supplemental information accompying the test report:

1. The bacterial cell suspensions of Ames tester strains at the late exponential phase of growth must be standardized prior to testing. The bacteriological procedures used for standardizing the bacterial cell suspensions of Salmonella tester strains to a desired density of viable cells per milliliter should be included in the report.

2. Individual numerical data for checking the tester strain genotypes are required. The specific procedures used in this study to confirm the histidine requirement, deep-rough character, ultraviolet sensitivity as well as the presence of R factor conferring ampicillin resistance of tester strains were not presented with the report.

3. The source of nutrient broth must be identified since a considerable variation has been found in the extent of growth of Ames tester strain in overnight nurient broth between laboratories in recent years. This appears to result from variability in the nutritional quality of the medium.

B. Activity of DTX-77-0004 in the Dominant Lethal Assay in Rodents for Mutagenicity.

Procedure:

Three groups of adult male Sprague-Dawley rats (225-260 g), 10 per group, were given a single treatment with DTX-77-0004 at the three dose levels (316, 31.6 & 3.16 mg/kg). Positive control, TEM (0.5 mg/kg) and negative control, corn oil (2 ml/kg) were run concurrently. Each male rat was mated two untreated virgin Sprague-Dawley female rats for 8 consecutive weeks to cover the total spermatogenic cycle. Fourteen days from the mid-week of mating, the females were sacrificed by CO_2 asphyxiation. Ovaries were examined for number of corpora lutea, and utrine horns for the number of fetal deaths and total implantations. Nine parameters with the appropriate statistical evaluation (FDA developed specification) for each study were used to determine the dominant lethal effect of the test compound: 1)fertility index; 2)total implantations; 3)total corpora lutea; 4)preimplantations losses; 5)dead implants; 6)proportion of females with one or more dead implants; 7)proportion of females with two or more dead implants; 8)dead implants/total implants; 9)live implants/pregnant females.

Results:

The test compound, DTX-77-0004, showed no difference ($p>0.05$) from the negative control in all parameters except in two instances: the average numbers of pre-implantation losses and live implantations per pregnant female were significantly different than the negative controls ($p<0.05$), but, these values were well within the range of values for the negative controls in this test. No dose response was demonstrable in any parameter at any dose level. The positive control, TEM (0.5 mg/kg) exhibited its usual severe genetic damages between weeks 2 to 5 by showing greater pre-implantation losses, more females with dead implants - both one or more and two or more per female - lower total implants and lower total live implants. The fertility indices were within normal values for this species and strain of rodents.

Conclusion:

The test compound, DTX-77-0004, did not induce dominant lethals when administered to male rats in a single treatment with the three dose levels (316, 31.6 and 3.16 mg/kg) prior to mating.

Evaluation:

Since the total numbers of implanations for females mated with negative control males are shown in the accepted range and the sample size designed for the study is based on the recommended procedure for dominant lethal assay, this assay appears to have been conducted in a manner adequate to generate valid results. The negative response as judged by the 9 parameters for the dominant lethal effect of test compound is acceptable. However, the dominant lethal assay is relatively (as compared to the translocation and the germ cell specific locus assays) insensitive test, measures only clastogenic events and would not detect nonclastogenic agents.

Study C: Activity of DTX-77-0005 in a Test for Differential Inhibition of Repair Deficient and Repair Competent Strains of Salmonella typhimurium: The Repair Test.

Procedure:

Overnight cultures of *Samonella typhimurium* TA1538 (repair deficient strain) and TA1978 (repair proficient strain) were prepared in nutrient broth at 37 C. One-tenth ml of the overnight culture was added to 2 ml of molten agar (0.6% NACl, 100mM histidine and 0.05 mM biotin) in a screwcapped tube, mixed and the contents poured over a base plate of Spizzen's minimum medium. After solidification of the overlay, the test compound dissolved in DMSO (1 mg/ml) was tested undiluted at 20, 10, and 2 ul per plate in the presence or absence of metabolic activation from rat liver enzymes. The in-vitro mammalian metabolic activation system consisted of liver homogenate fraction "S-9" from Arocolor 1254 treated Sprague Dawley rats according to the preparation procedure described by Ames (1 ml of S-9 fraction contained microsomes from 250 mg of wet liver) and combined with the NADPH-depent microsomal-enzyme system (8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP and 100 mN Na_2PO_4, pH 7.4). Plates were incubated overnight at 37 C. and the zone of growth inhibition was measured and compared for strain TA1538 and strain TA1978. If the compound was a DNA modifying agent, significant difference in the size (diameter) of the zone inhibition between TA1538 and TA1978 would be demonstrated. One negative and two positive controls were run concurrently with the test compound: the solvent DMSO (50 ul); the direct mutagen/carcinogen, 4-nitroquinoline-N-oxide and the procacinogen, 6 aminochrysene.

Results and Conclusion:

The compound, DTX-77-0005, tested in the assay (20, 10, and 5 ug/plate) showed no growth inhibition either in the strain TA1538 or the strain TA1978 in the presence or absenc of metabolic activation from rat liver enzymes, exhibited no differential cell killing between the repair deficient and the repair proficient strain of organisms and was not a DNA-modifying agent.

Evaluation:

The results of this study appear to be inadequate to support the conclusion drawn in the report. There are a number of reporting deficiencies which cast doubt that an adequate assay has been performed.

1. Because of the poorly diffusible property of the test compound, it did not yield interpretable results (i.e., no test results) by the spot test assay. To overcome this effect, the following critical steps for performing a valid tet must be considered:

A. Since the test compound at the maximum dose (20 ug/plate) generated "no test" results, the spot test assay should be repeated using more of the test agent per disk if possible.

B. If the spot test assay is apparently unable to evaluate the difference of growth inhibition between the repair deficient strain and the repair proficient strain of *Salmonella typhimurium*, the test compound should be further evaluated by using modified liquid suspension procedure described by Rosenkranz and his coworkers (1980). In this procedure, portions of the test agent are added liquid cultures and survivors are enumerated.

2. It is not stated whether both of the bacterial cell suspension of tester strains (TA1978 & TA1538) were standardized to a equally desired density of viable cells prior to testing. In evaluating the defferential inhibition of these bacterial strains, the sensititivity of the assay is largely dependent on the number of organisms seeded in the agar overlay.

3. Since the correct response of these two bacterial strains of *Samonella typhimurium* to chloramphinicol (30 ug/plate) was not indicated in the assay ("no test" result), it is questionable that the integrity of test cultures was properly maintained in performing the assay. The assay should be repeated using a new set of the identified cultures with specific genotypes (rfa mutation with and without excision repair function).

Study D. Activity of DTX-77-0006 in the in Vivo Cytogenetic Assay in Rodents for Mutagenicity.

Procedure:

Eight-to-ten-week-old, randomized bred male Sprague-Dawley rats (225-260 g) were injected intraperitoneally with single doses of the test compound dissolved in corn oil (316, 31.6 and 3.16 mg/kg). Twenty-four hours after treatment with the test compound, animals were given a single IP treatment of 4 mg/kg colchicine and sacrificed six hours later by CO_2 asphyxiation. In each animal, bone marrow cells were flushed from one or both tibia with HBSS, swollen in hypotonic KCl (0.075%) and fixed twice in methanol: glacial acid (3:1). Chromosome slides were prepared by ignition drying and stained with giemsa after the fixed cells were refrigerated overnight and resuspended to opalescene in fixative. The concurrent negative control compound, corn oil and the positive control compound, TEM (0.25 mg/kg and 05 mg/kg) were also administered via IP injection.

At least 50 metaphase plates for each animals were examined and scored for chromosome breaks and gaps, structural rearrangement, ploidy, fragmentation and pulverlization. The number of chromosomes per metaphase plate was counted and the mitotic index calculated on each animal. A positive response in the in-vivo cytogenetic assay was determined by the significantly higher difference between the treated and negative control animals (five male animals per group).

Results:

No no significant difference in the induced chromosomal aberrations observed between the negative control and treated groups at the dose levels tested. Percentages breaks, gaps and mitotic index demonstrated in negative control and positive control group were adequate to qualify the validity of the cytogenetic assay.

Conclusion:

DTX-77-0006 did not induce chromosomal alterations in bone marrow cells of male rats (Sprague-Dawley) when administered vai IP injection.

Evaluation:

The design procedure of the study is not conducted according to the guideline established by the Ad Hoc Committee on chromosome methodologies in mutagen testing of the Mutagen Society (1972), and hence the results and their interpretations are considered to be invalid. The following inadequacies in performing the bone marrow cytogenetic assay are:

1. In acute assay (single dose), the bone marrow samples were not taken at 6, 24, and 48 hrs. after the acute administration of test compound according to the accepted procedure for in Vivo cytogenetic assay in order to detect chromosomal aberrations in cells that have been delayed in their progression through the mitotic cycle.

2. There is no subchronic studies for the test agent. Both acute (single dose) and subchronic (five consecutive doses 24 hours apart) studies in bone marrow cytogenetic assay are required to provide more definitive results of the test agent.

3. Only a single sex of Sprague-Dawley rats is used. Ideally, when both sexes are examined in this assay, four rats per sex per dose should be used.

4. It is not stated whether the acute toxicity information (i.e., LD_{50}) was determined statistically by probit analysis (Finney, 1971).

5. The procedure used for preparing chromosome slides from bone marrow cells are not clear to assure the top quality of chromosome spread for aberration analysis.

DCR 10/12/82

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