Rev. 4/14/82 Toxicology Branch/HED Review 003770

APR 20 1984

Caswell No(s).: 299

To: Taylor / Walters

Registration No(s).: 524-119, 524-151, 524-306

Pesticide Petition No(s).:

Chemical(s): diallate, Avadex EC

Requested Action(s): Review of studies relating to Registration Standard.

Recommendation: The amounts of time assigned for filling data gaps should be reviewed.

Inert(s) cleared 180.1001:

% of ADI occupied: Existing: Resulting:

Resulting % increase in TMRC:

Data considered in setting the ADI:

Attached (?): ADI printout: YES/NO ; TOX "one-liner": YES/NO ; DER: YES/NO

Existing regulatory actions against registration:

RPAR status:

New Data: See attached reviews. Note that a NOEL was not found in the 3-months feeding study and that the acute toxicity studies confirms the need for a 3-months rat neurotoxicity study.

~~Data gaps~~ Data Gaps: 90 days feeding studies in two species (unless chronic studies are submitted), teratology, acute inhalation, DNA-Damage, and 3-months rat neurotoxicity.

~~Comments~~:

Reviewer: Thomas Edwards 4-9-84

Date: APR 20 1984

Section Head: [illegible] 4/13/84 [illegible] 4/20/84

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Three-months feeding, male and female hamsters

Accession Number: 251863 (I & II)

MRID Number:

Sponsor: Monsanto

Contracting Lab: Monsanto Environmental Health Lab. No. ML-80-253

Date: 3-22-83

Test Material: diallate technical, 97.1 %

Monsanto abstract:

A 90 day study was conducted with 30 hamsters per sex exposed to Diallate at target dietary concentrations of 0, 600, 2,000 or 6,000 ppm. No deaths or abnormal clinical signs attributable to Diallate exposure occurred for either sex at any of the three treatment levels. Body weight depression was noted for 11 out of 13 weeks among 6,000 ppm males. Both sexes at 6,000 ppm developed signs of minimal hypochronic anemia with responding bone marrow. Renal pigmentation occurred for both sexes at 6,000 ppm. Hepatic changes were found with increasing severity as dosage increased for both sexes at all three exposure levels. Therefore, a no-effect level was not established for either sex during this 90 day study.

Conclusions:

I have read the report of this study and agree with the above abstract and with the attached review by Richard C. Dirks of Monsanto.

NOEL: (90 day feeding, hamsters): less than 600 ppm (LDT).

Core Classification:

"Minimum"

TOX/HED:kh:TEdwards:3-19-84:card #1

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Dermal sensitization, guinea pigs

Accession Number: 251863 (III)

MRID Number:

Sponsor: Montsano

Contracting Lab: Bio/dynamics Inc., No. 4267-83

Date: 10-7-83

Test Material: diallate, Avadex EC

Protocol and Results: See attached review by Timothy J. Long of Montsano.

Conclusions:

Montsano concluded that "under the conditions of this study, diallate exhibited no potential to produce dermal irritation after repeated exposures and no potential to produce dermal sensitization in guinea pigs."

I have read the report and concur in this conclusion.

Core Classification:

Minimum

TOX/HED:kh:TEdwards:3-19-84:card #1

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Three months feeding of hybrid hamsters for in vivo cytogenetic assays.

Accession Number: 251863 (IV 1)

MRID Number:

Sponsor: Monsanto

Contracting Lab: Monsanto Co., Environmental Health Lab., No. ML-81-123

Date: 11-9-82

Test Material: Diallate, Technical, 97.1 %

Protocol:

"Diallate technical (lot no. NBP 1992001) was fed ad libitum for three months to groups of male and female F1D Alexander hybrid strain of syrian golden hamaters at dietary concentrations of 0, 600, 2000, and 6000 ppm. This study was conducted concurrently with the Three-Month Feeding Study of Diallate with Hamsters (ML-80-253). Animals assigned to test groups for cytogenetic analyses are listed below.

Group	male	Female	
Negative Control	14	14	
600 ppm (Diallate)	10	10	Half of animals in each group were sacrificed at week 6/7 and the other half at week 13/14 (study termination)
2000 ppm	10	10	
6000 ppm	14	14	
Positive Control (TEM)**	10	10	

**triethylenemelamine, injected i.p. 24 hr before sacrifice

Clinical observations were made twice daily. Body weights and food consumption were recored weekly for all animals assigned to both the full three-month feeding study (ML-80-253) and this in vivo cytogenetic phase of the three month feeding study (ML-81-123).

For the full three month feeding study, diet analyses, clinical chemistry, gross necropsy and histopathology evaluations were performed and will be summarized separately."

"Animals assigned for cytogenetic analyses (see preceding tabulation) were injected intraperitoneally with colochicine to block bone marrow cells in mitosis and sacrificed two hours later. An additional group of animals (positive control group) were fed diet without diallate and received a single intraperitoneal injection of triethylenemelamine (1.0 mg/kg/body weight) 24 hours before sacrifice.

Bone marrow cell isolation and fixation were performed at the Environmental Health Laboratory. Subsequenlty, staff at SRI Interonational prepared slides and microscopically exmained cells for evidence of cytogenetic (chromosomal) damage.

Results:

Hepatic histopathy (liver cell pigmentation and degeneration, and sinusoidal cell proliferation) was found at all treatment levels. Severity of effects increased with dosage level in both sexes.

Body weight was depressed without decreased food consumption for the 6000 ppm group.

Statistically significant decreases in red blood cell parameters were observed for all treatment groups and were dose related.

"Mildly increased bone marrow activity was evident in both males and females in both analysis periods. Male Group 3 had elevated reticulocyte counts at week 7($p \leq .05$) and elevations at week 14 that did not reach statistical significance. Females had elevated reticulocyte counts at week 7 (not statistically significant) that remained elevated at week 14 ($p \leq .05$)."

Conclusions:

Bone marrow cells were satisfactorily prepared isolated and fixed for subsequent analysis by cytogenetists at SRI International.

Core Classification:

Acceptable

TOXICOLOGY BRANCH
DATA REVEIW

Study Type: Evaluation of in vivo Cytogenics in Syrian golden hamster

Accession Number: 251863 (IV 2)

MRID Number:

Sponsor: Monsanto

Contracting Lab: SRI International, No. LSC-3433

Date: April, 1982

Test Material: diallate, technical, 97.1%

Protocol:

Syrian hamster bone marrow cells received from Monsanto Co., Environmental Health Lab. were evaluated. The preparation of these cells at Montsano is described in the preceding review, ML-81-123 (Acc No. 251863, Tab IV 1).

The Cytological evalvation and statistical analyses was performed at SRI International as follows.

Cell Fixation

"Upon receipt at SRI, the tubes were centrifuged at 700 rpm for 10 minutes and the supernatants were discarded. The cell pellets were then resuspended in 1 ml of chilled fixative, and the tubes were vortexed. An additional 4 ml of fixative was added to each tube and mixed. After the tubes had stood at room temperature for 30 minutes, they were again centrifuged for 10 minutes at 700 rpm, the supernatants were discarded, and the fixation process was repeated one more time. After the final centrifugation, the cell pellets were resuspended in a volume of Carnoy's fixative that would result in an appropriate final cell density."

Slide Preparation and Staining

"Clean labeled slides were dipped in distilled water using tongs. Aliquots of the cell suspensions were dropped onto the slides using a Pasteur pipette. The slides were then passed over a flame from an alcohol lamp and allowed to dry inside a fume hood.

"Cells on slides were stained in a freshly prepared 7% Giemsa stain (Gurr R66 Giemsa, M/15 Sorensen's buffer, pH 6.8) for 5-20 minutes at room temperature. The stained slides were rinsed in distilled water, air dried, and dipped in xylene; coverslips were mounted using Depex mounting medium."

Cytogenetic Analysis

"Slides from the 6/7 week and 13/14 week sacrifices were coded and analyzed separately. The coded slides from each sacrifice were divied into two groups with one-half of the animals being represented in each group. The two groups contained approximately equal numbers of male and female animals from each treatment group (i.e., 600, 2000, and 6000 ppm Diallate, negative and positive controls). Two cytogeneticists then analyzed separately the coded slided for each animal within each of the two groups. Thus, four cytogeneticists analyzing the slides for each sacrifice, with two cytogeneticists analyzing the slides for each animal.

Slides were evaluated for mitotic index, or MI (based on 1000 cells per coded sample), and for chromosomal aberrations (based on a maximum of 30 cells per coded sample). Chromosomal aberrations (based on a maximum of 30 cells per coded sample). Chromosomal aberrations were classified according to Savage's (1975) scheme. If enough cells (60) were analyzed from each of five animals in the 6000-ppm Diallate sacrifice groups, the chromosome preparation from the two additional animals in each of these groups were not evaluated."

Results:

In-life effects have been presented (ML-81-123). Increased bone marrow reticulocyte counts were noted.

"There were no differences between the mitotic indices of bone marrow cells from control and treated groups at either the 6 to 7 and 13 week sacrifices. These data suggest that treatment did not affect the proliferation of bone marrow cells in the intact animals. For both the 6 to 7 and 13 to 14 week sacrifices, the mean number of aberrations per cell per animal and proportion of aberrant cells per animal were comparable for all diallate treated groups and the negative control group. The degree of cytogenetic damage observed in the treated animals was very low and compatible with normal background levels. At each sacrifice, the positive control article, triethylenemelamine, induced statistically significant elevations in both the percentage of aberrant cells and the frequency of abberations per cell.

Conclusion

"Diallate did not induce any adverse cytogenetic effects, including chromosomal damage, in bone marrow cells when fed to Syrian golden hamsters for 13 weeks up to a dietary concentration of 6000 ppm, a level which caused slight signs of subchronic toxicity."

I have read the study report and concur in the conclusion.

Core Classification:

Acceptable

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute oral toxicity, rat

Accession Number: 251863 (V 1)

MRID Number:

Sponsor: Monsanto

Contracting Lab: Monsanto Co., Environmental Health Lab., No. 820002

Date: 11-9-82

Test Material: diallate, Avadex EC

Protocol:

"AVADEX EC, lot XLB-176, received as an amber liquid, was used to assess its potential toxicity by ingestion. Young adult Sprague-Dawley albino rats (Crl: CD® (SD)BR), (Registered trademark of Charles River Breeding Laboratories, Wilmington, MA), obtained from Portage, MI, weighing 234-256 grams (males) and 167-183 grams (females) on the day of dosing were fasted overnight prior to test material administration. Each animal was identified by ear tag and bar coded cage card. Groups of five male and five female rats were randomly selected for geometrically spaced dosage levels. The test material was administered as received by oral intubation. The volume administered was adjusted according to body weight and the dosage to be given.

"Following dosing, each rat was individually housed and provided food and water ad libitum. Clinical observations were made three times within the first eight hours after dosing and twice daily (morning and afternoon) thereafter until sacrifice, with the exception of one day when observations were only recorded once. Body weights were recorded on days 0 (day of dosing), 7, 14, 21, and 28. After 29 days on test, the suriving animals were sacrificed. Necropsies were performed on all animals. The acute oral LD50 for each sex and for the combined sexes was calculated using the method of Finney (1971)."

Results:

"Acute oral LD50 values for AVADEX EC were calculated to be:

LD50 (Both sexes): 1045 mg/kg
95% Confidence Limits: 851 and 1245 mg/kg
Slope: 6.8
95% Confidence Limits: 3.4 and 10.1

LD50 (Male rats): 1256 mg/kg
95% Confidence Limits: 961 and 1642 mg/kg
Slope: 10.0
95% Confidence Limits: 2.7 and 17.3

LD50 (Female rats): 865 mg/kg
95% Confidence Limits: 417 and 1149 mg/kg
Slope: 6.6
95% Confidence Limits: 1.2 and 12.1 "

"Neurotoxicity was suggested by the frequent occurrence of involuntary head jerking, circling motions, and tremors. All three of these signs of intoxication occurred in 30 animals, and at least one was observed in 34 rats. Fourteen of the 16 rats that had none of these effects were prostrate by the second day after dosing (and dead by day three), which is probably why these clinical signs were generally observed in decreasing numbers of animals as the dosages increased. (Prostration was mostly observed in animals at the three highest dosages.) With one exception, head jerking, circling motions, and tremors were observed beginning on the first or second day after dosing. The duration of these effects was related to the dosage administered; at the lowest dosage, six of the eight survivors appeared normal by termination, and the only other incidents of clinical recovery were in two male rats at the second lowest dosage. Other signs of toxicity to the nervous system included convulsions and a tendency to move backward rather than in the normal forward direction. Convulsions occurred in three rats on the second day after dosing, and all three affected animals were dead by the following day. A tendency to move backward was observed in seven rats. Five of these animals also substantially lost the use of their hind limbs. By the fifth day after dosing, this loss of use had occurred in six rats. Approximately 10 days later, five of these animals had begun to regain some mobility in their hind legs. In four animals, this roughly (within five days) coincided with the onset of the tendency to move backward (although this effect had also been observed in one of these rats before the loss of use of the hind limbs had occurred). At about the same time, these

animals also began to consistently circle when placed on the floor, an effect that had previously been observed only sporadically and had been absent in three of these rats for approximately one week. The remaining animals that moved backward included a rat that had not regained notable mobility of the hind limbs by termination and one rat that died nine days after dosing. By the second day after dosing, the hind limbs of seven rats were splayed sideways, and six of these rats subsequently died. It appears that this effect was similar to the loss of use of the hind limbs, with the exceptions of earlier onset and, in most cases, subsequent death. Other frequently observed clinical abnormalities included salivation, lethargy, and ptosis which were common occurrences in animals at all dosages. Urine with a brown and/or green coloration was observed in six decedents. Abnormal respiration (dyspnea, audible breathing, or tachypnea) occurred in 12 rats, but in most instances was considered to be a secondary effect of intoxication. Body weight loss during the first week on test occurred in each animal that lost use of the hind limbs and survived for at least seven days after dosing. All survivors gained weight throughout the remainder of the study."

NEUROTOXICITY was indicated by results at all dosage levels.

Conclusions:

The acute oral LD50,s are shown above under results.

LD50 (female rats): 865 mg/kg (95% confidence limits, 417-1149 mg/kg).

Slope: (female rats): 6.6 (95% Confidence limits 1.2-12.1).

Acute oral Toxicity Category: II

A neurotoxicity 90 day study in rats is needed. A dosage must be high enough to produce neurotoxic effect.

Core Classification:

Guideline

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute dermal Toxicity, rabbit

Accession Number: 251863 (V 2)

MRID Number:

Sponsor: Monsanto

Contracting Lab: Monsanto Co., Environmental Heatlh Lab., No. 820003

Date: 11-9-82

Teat Material: Diallate, Avadex EC

Monsanto Summary:

"No daeths followed a dermal application of 5,000 milligrams per kilogram (mg/kg) of AVADEX® EC herbcide to the shaved and abraded dorsal surface of albino rabbits of both sexes. Therefore, the acute dermal LD50 of this material is considered to be in excess of 5,000 mg/kg. Clinical abnormalities suggesting toxicity to the nervous system, occurred in each animal and included circling motions, involuntary head movements, tremors of the hind limbs, and ataxia."

Conclusions:

I have read the report and agree with the above summary. The report is attached because of the neurotoxic effects reported, which support the need for further neurotoxicity investigation.

Acute dermal Toxicity Category: III

Core Classification:

Guideline

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Primary eye irritation, rabbit

Accession Number: 251863 (V 3)

MRID Number:

Sponsor: Monsanto

Contracting Lab: Monsanto Co., Environmental Health Lab., No. 820005

Date: 11-9-82

Test Material: diallate, Avadex EC

Protocol:

"A Volume of 0.1 ml of test material, as received, was instilled into the conjunctival sac of the right eye of each test animal by gently pulling the lower eyelid away from the eyeball to form a cup. The lids were then held together for approximately one second." Treated eyes of six animals were not rivsed. "Three animals schedule to have their eyes rinsed were restrained for approximately 25 seconds, after which physiological saline. Following exposure, all animals were returned to their cages. The test material was administered to two animals without the prior use of a topical anesthetic. After without the prior use of a topical anesthetic. After vocalization occurred in the second rabbit, proparacaine hydrochloride (0.5%) was administered to both eyes of these two animals. This same anesthetic was applied to both eyes of the remaining seven rabbits approximately 30 seconds prior to test material administration.

Each animal was individually housed and provided food and water ad libitum. Observations for signs of irritation were conducted on the first, second, and third days after dosing. Additional observations for signs of irritation in individual animals were conducted as outlined in Table 2. The untreated eye of each animal served as a negative control. As considered necessary for evaluation of potential corneal involvement, sterile fluorescein sodium was applied to the eyes. After approximately 10 seconds, the excess fluorescein was flushed from the eyes with physiological saline, and an ultraviolet lamp was used to determine whether staining persisted."

"The method of Draize (1944) was used for the scoring of eye irritation. The average of the Draize scores for 24, 48, and 72 hours was calculated for each animals and then averaged over the six animals with unwashed eyes. A separate average was calculated in a similar manner for the three animals with washed eyes.

Results:

1. Unrinsed eyes

   Corneal opacity cleared by fourth day.

   Irritation cleared by tenth day.

   Primary irritation category: II

2. Rinsed eyes

   Irritation cleared by second day

Core Classification:

Guideline

TOX/HED:kh:TEdward:3-19-84:card #1

TOXICOLOGY BRANCH
DATA REVEIW

Study Type: Primary skin irritation, rabbit

Accession Number: 251863 (V 4)

MIRD NUMBER:

Sponsor: Monsanto

Contracting Lab: Monsanto Co., Environmental Health Lab., No. 820004

Date: 11-9-82

Test Material: diallate, Avadex EC

Protocol:

"AVADEX EC, lot XLB-176, received as an amber liquid,was used to assess potential skin irritation resulting from dermal contact with the product. Six (6) young adult New Zealand white rabbits (Isf: (NZW), Isaac's Farm, Litchfield, Illinois) weighing 2.16-3.06 kilograms on the day of exposure were randomly assigned to this study. Each animal was identified by ear tag and bar coded cage card. The skin on the dorsal surface of each animal was shaved with an electric clipper prior to the aministration of the test material. A volume of 0.5 ml of the test material, as received, was applied to each of two intact and two abraded sites on each animal. The abrasions, made with a hypodermic needle, were sufficiently deep to penetrate the stratum corneum, but not deep to penetrate the stratum corneum, but not deep enough to produce bleeding. The test material was applied to the skin under one inch square gauze patches and held in contact with the skin by means of an occlusive wrap of latex rubber secured by bandaging and elastic tape. The occlusive wrap and guaze patches were removed after approximately of the animals.

Each animal was individually housed and provided food and water ad libitum. The initial (day one) observation for skin irritation was made approximately one hour after the removal of the wappings. Dermal irritation was scored by the method of Draize (1944), and results were recorded on first, third, and sixth days after topical application. The male rabbit that had irritation evident on the sixth day after exposure was also examined for signs of irritation on the seventh, eighth, and ninth days after dosing. The Primary Irritation Index was calculated by averaging the mean score at 24 and 72 hours."

Results:

The Primary Irritation Index for this group of animals is 1.4 on a scale of 0.0 to 8.0. As defined by Draize, all skin irritation had subsided by the ninth day after test material administration. By the sixth day after exposure, epidermal desquamation had occurred on all four sites of all six rabbits. In each animal, this effect was still evident the last time irritation scores were recored in that rabbit. Since desquamation is not considered to represent primary irritation by the method of Draize, no further observations for signs of irritation were conducted after the examination when all erythema and edema had subsided."

Conclusions:

"A slight degree of irritation resulted when 0.5 millilters of undiluted AVADEX EC was held in continuous 24-hour contact with intact and abraded rabbit skin. The Primary Irritation Index was 1.4 on a scale of 8.0."

Primary dermal Irritation Category: IV

Core Classification:

Guideline