UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D C. 20460

004419

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Trifluralin, Response to PD 4

TO: Richard Mountfort, PM 12
Registration Division (TS-767)

FROM: Stephanie P. April, Ph.D. Stephanie P. April 4/25/85
Review Section III
Toxicology Branch
HED (TS-769)

THRU: Clint Skinner, Ph.D., Section Head Clint Skinner 4-25-85
Review Section III
HED (TS-769)

Theodore M. Farber, Ph.D., Chief
Toxicology Branch
HED (TS-769)

Compound: Trifluralin Tox. Chem: 889

Registration No. 1471-35 Registrant: Elanco

Accession No.: 255295
255296

Action Requested: Review Reproduction study in rats, Interim Report and a teratology study in rats and two teratology studies in rabbits which were submitted in response to the PD 4.

Conclusion: The reproduction study was unacceptable as it was an interim report. The rat teratology study was core minimum as was the second rabbit teratology study. The first rabbit teratology study was inconclusive due to a low rate of fertility. These studies indicate that trifluralin is not a teratogen.

1 of 9

004419

Reproduction

DATA EVALUATION REPORT

Compound: Trifluralin

Compound Numbers: Caswell Number 889

Citation: A Generation Study in Charles River CD Rats Given Trifluralin in the Diet.

Review by: Stephanie April, Ph.D. SPA 4/25/85
Section III
Toxicology Branch, HED

Core Classification: Unacceptable at this time.

Conclusion: There is not yet adequate data to review or comment upon it. The expected date of study termination is anticipated in September 1986.

Teratology:

DATA EVALUATION REPORT I

Compound: Trifluralin

Compound Numbers: Caswell Number 889

Citation: Markham, J. K. and Byrd R. D., A Teratology Study of Trifluralin (EL-152, Compound 36352) Administered Orally to Charles River CD Rats, Study No. R08484, Tox. Br., Lilly Research Lab; November, 1984.

Reviewed by: Stephanie P. April, Ph.D. SPA 4/25/85
Review Section III
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HED (TS-769)

Conclusion: The maternal toxicity NOEL for trifluralin in this study based upon a treatment related decrease in body weight gain and a lowered food consumption at 1000 and 475 mg/kg/day was 225 mg/kg/day. The teratology NOEL was > 1000 mg/kg/day. The fetotoxicity NOEL was 475 mg/kg/day based upon a decreased mean fetal body weight at 1000 mg/kg/day.

Classification: Core minimum

Materials:

Test Material: Trifluralin (EL-152, compound 36352); α, α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine, Lot Number 00554AP2 (Technical), 96.7% purity.

2

Animals: Rat, Crl:COBS CD (SD)BR, 220.8 ± 1.5 g

Methods: Groups of 25 female rats received 0, 100, 225, 475 and 1000 mg/kg/day by oral gavage from gestation day 6 to day 15, of a suspension (w/v) of trifluralin in 10% aqueous acacia.

The parameters studied included maternal survival, toxic clinical signs, maternal body weight gain and food consumption as well as reproductive and fetal parameters. In the category of the latter two are included number of corpora lutea, number of implantations, proportion of live fetuses, proportion of dead fetuses, proportion of resorptions, sex ratic, proportion of fetal runts, proportions of normal fetuses, and proportion of fetuses with developmental variations or abnormalities.

Termination was on day 20 of gestation and a subsequent necropsy was conducted on the maternal carcasses.

Results:

Actual Dosages: Trifluralin concentrations of the suspensions were found to be not significantly different from the theoretical concentrations as well as being homogeneous and stable for at least four hours in the vehicle.

Maternal Survival and Signs of Toxicity

Trifluralin had no effect on the maternal survival nor were there any significant signs of clinical toxicity. The test material was eliminated through the urinary tract as indicated by yellow colored urine. There was alopoecia at the upper doses probably indicative of dose related stress.

Necropsy of the maternal carcasses revealed that trifluralin and/or its metabolites localize in adipose tissue from observations of yellow abdominal fat tissue. Other observations were a single instance of splenomegaly, hydroureter at one dose level and several apparent hydronephroses in control as well as all treated groups.

Maternal Body Weight Gain and Food Consumption:

There was a depressed body weight gain and a lowered food consumption in the 475 and 1000 mg/kg/day groups throughout the treatment period which was reversible upon removal of the trifuralin dosing. The lower doses experienced these same effects on days 6-10 and which reversed on days 11-15.

Reproduction Parameters:

All groups had a similar fertility of 92.8%. There was no difference in the number of gravid rats, corpora lutea and implantations among all the groups. The ratio of live/dead fetuses was not different than that found in the control group.

Fetal Parameters

There were very few structural malformations excluding the runts (2 at 225 mg/kg/day and 2 at 475 mg/kg/day).

There was no difference amongst the experimental groups (0-475 mg/kg/day) in the number of runts. The number of runts at 1000 mg/kg/day was greater but this difference was not found to be significant and there was maternal toxicity at this level. The number of runt animals was not dose response related but rather scattered throughout the groups.

The sex ratio was not changed from control with trifluralin treatment. There was no significant variation found in the sex ratios, members of normal, variant or abnormal fetuses in this study.

The fetal weight in the litters for the dams given 1000 mg/kg/day was significantly depressed compared to the control group.

004419

Compound: Trifluralin

Compound Number: Caswell 889

Citation: A Teratology Study (1) of Trifluralin (EL-152, Compound 36352) Administered Orally to Dutch Belted Rabbits), Study B 02283, J. K. Markham and R. A. Byrd, Lilly Research Laboratories, Greenfield, Indiana November, 1984.

Review by: Stephanie P. April, Ph.D. SPA
Review Section III
Toxicology Branch
HED (TS-769)

Conclusion: Inconclusive; the experiment was repeated to obtain adequate fertility to yield enough fetuses to evaluate the teratogenic potential.

Classification: Core Supplementary, abnormally low fertility in all groups.

Material Tested: Trifluralin (EL-152, compound 36352),α, α,α trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine, 96,7% pure, lot number 00554AP2

Materials: Virgin female Dutch Belted rabbits from Langshaw Farms (Augusta, Michigan) weighing 2.63 ± 0.03 kg were used. These were randomly distributed into five groups, 21 per group. These rabbits were prophylactically inoculated against coccidiosis with sulfaquinoxaline.

Methods: On gestation day 0, the female rabbits received 20 i.u./kg of chorionic gonadotrophin. In three hours the rabbits were artificially inseminated with sperm from three untreated Dutch Belted male rabbits (Langshaw Farms, Augusta, Michigan).

After acclimation and insemination, the does were administered the test material by gavage at doses of 0, 100, 225, 300; 500 and 800 mg/kg/day on gestation days 6-15. The test material is administered at 10 ml/kg of 0, 1.00, 2.25, 5.00 and 8% trifluralin suspended in 10% acacia in water. Maternal survival, signs of toxicity, and food consumption were observed, while body weight was measured and recorded on gestation days 0, 6, 13, 19, 24 and 28. Gross necropsy and gravid uterine weight were completed at termination for the maternal carcasses.

Aborted fetuses were examined macroscopically and the maternal animal was evaluated for intrauterine parameters.

Results:

Fetal parameters

The total number of fetuses examined externally, viscerally and for skeletal anomalies per group is indicated below:

| Dose (mg/kg/day) | Number of Fetuses | Fetuses with Structural Malformations |
|---|---|---|
| 0 | 58 | 1 |
| 100 | 107 | 2 |
| 225 | 87 | 1 |
| 500 | 43 | 3 |
| 800 | 15 | 0 |

The structural malformations (3) at 500 mg/kg/day were cardiomegaly associated with wavy ribs. The other structural malformations were short snout, single nares, absent philtrum, incisor fusion, absent incisors, hydrocephaly and craniostenosis. There were developmental anomalies as well as the above mentioned structural malformations all of which were scattered throughout the experimental groups with no trend. The sex ratios were varied with no trend. At the high dose 800 mg/kg/day, there was an elevated proportion of male fetal runts and depressed male fetal weights.

Discussion:

The maternal NOEL in this study, 225 mg/kg/day, was borne out in the surviving dams by anorexia and cachexia which resulted in abortion and fetal loss in utero. At levels where maternal toxicity occurred there was embryo/fetal toxicity as indicated by fetal growth retardation, increased number of fetal runts or prenatal mortality (i.e., only 15 fetuses at 800 mg/kg/day compared to 43 to 107 in lower dose groups). One litter at 500 mg/kg/day had three cases of cardiomegaly. The no effect level was 225 mg/kg/day and the cardiac toxicity is not considered to be a direct effect due to its clustering in one litter.

This experiment was repeated to compensate for the overall low fertility of the animals.

004419

DATA EVALUATION RECORD III

Compound: Trifluralin

Compound Number: Caswell Number 889

Citation: Markham, J.K. and Byrd, R.A., A Teratology Study (II) of Trifluralin (EL-152, Compound 36352) Administered Orally to Dutch Betted Rabbits, Study B01784, Toxicology Division, Lilly Research Laboratories, November, 1984.

Reviewed by: Stephanie P. April, Ph.D. SPA 4/25/85
Review Section III
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Conclusion: The NOEL for maternal toxicity was 100 mg/kg/day and the NOEL for fetal/embryo toxicity was 225/mg/kg/day. The fetal embryo toxicity was only developmental and not found in litters from dams who did not experience trifluralin toxicity. This is not considered to be a teratogenic effect. Trifluralin is not considered to be a teratogen from these two rabbit studies.

Classification: Core minimum.

Materials:

Test Material: Trifluralin (EL-152, compound 36352); α, α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine, 96.7% purity; Lot Number 00554AP2.

Animals: Virgin female Dutch Betted rabbits from Longshaw-Farms (Augusta, Michigan).

Methods: Trifluralin was administered by gavage to artificially inseminated rabbits. There were groups of 25 animals inseminated at the following dose levels: 0, 100, 225 and 500 mg/kg/day from day 6 of gestation to day 28. Reproductive and fetal parameters (external, visceral and skeletal) were assessed.

Results:

Dose from Assays:

Uniform suspensions of trifluralin in aqueous acacia were administered. These were assayed to be between 91 to 96% of the theoretical concentration.

Maternal Survival and Signs of Toxicity.

| | deaths | abortions |
|---|---|---|
| 0 | 0 | 0 |
| 100 | 0 | 1 (no anorexia) |
| 225 | 0 | 4 |
| 500 | 2 | 5 |

The above events occurred after day 12 of gestation and were preceded by anorexia and cachexia. These animals were found to have fatty livers, trichobezoar, test article unabsorbed from the stomach and empty g.i. tracts. These same signs of toxicity were found in the survivors at these dose levels. The same orange urine, yellow fat and orange pilage with dermal irritation as was seen in the previous experiment were also observed with a dose related intensity in this study.

II. Maternal Body Weight Gain and Food Consumption

These parameters were marked depressed in those animals that died or were aborted at 225 and 500 mg/kg/day. For animals at 100 mg/kg/day, the depressed body weight gain in the previous group was reversible in the post-treatment period, in the 225 mg/kg/day group, but not in the 500 mg/kg/day group.

There was no difference in numbers of corpora lutea and in implantations in any of the groups. At the high dose there was a decreased number of live fetuses and increased resorption.

III. Fetal Parameters

The following fetuses were examined:

| Dose (mg/kg/day) | Number of Fetuses |
|---|---|
| 0 | 85 |
| 100 | 83 |
| 225 | 70 |
| 500 | 21 |

Cardiomegaly occurred in two female runts in one litter from dam #1714 in conjunction with nonpigmented skin, spade ribs, edema, hypoplastic thymus and lungs. The historical control rabbits of the same species and source in this laboratory have had a low incidence of these fetal terata. There were structured malformations in the nonrunt fetuses as indicated below:

| Dose (mg/kg/day) | Number of Malformations |
|---|---|
| 0 | 3 |
| 100 | 0 |
| 225 | 3 |
| 500 | 0 |

The isolated malformations in the treated groups include omphalocile, edema and hydrocephaly. Although, the number of fetuses and litters examined at 500 mg/g was approximately 1/4 that of the number of control fetuses (21 versus 85 fetuses and 5 versus 15 litters) a significant increase in hypoplastic thymus, cardiomegaly and hypoplastic lungs was noted in this group. This may be considered fetoxicity. These developmental effects were all found in the same litter as were other developmental skeletal problems. Fetal toxicity at 500 mg/kg/day included depressed fetal weight and increased number of fetal runts. The male sex ratio was unaffected by trifluralin.

Discussion:

As in the previous rabbit teratology study, the maternal deaths and abortions were associated with the nutritionally compromised does with trifluralin, inducing anorexia and cachexia. In this study the NOEL for these effects was lower than in the first study, the LEL being 225, not 500 mg/kg/day for death and abortion with a NOEL for maternal toxicity being 100 mg/kg/day. In this experiment as in the previous one, the dam involved at 500 mg/kg/day consumed the least food of the group and lost the most weight, having no maternal weight gain. In this experiment the litter consisted of 2 fetuses, both runts with several developmental abnormalities. The cardiomegaly, i.e., ventricular hypertrophy, and hypoplastic thymus and lungs, was only in conjunction with moderate to severe maternal toxicity.