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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: 19-MAY-1998

SUBJECT: PP#6F04772. **Fluroxypyr** in/on Barley, Oats, Wheat. **HED Hazard Assessment and Revisions**
DP Barcode: D232215 Chemical #: 128959, 128968
PRAT Case # : 288019 Submission #: S510931
Class: Herbicide

FROM: Myron Ottley, Ph.D. and William Dykstra, Ph.D.
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THROUGH: Melba Morrow, D.V.M. Branch Senior Scientist
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TO: Jess Rowland, M.S.
Executive Secretary
Hazard Identification Committee
Health Effects Division (7509C)

ISSUES/CONCLUSIONS

1. Upon re-evaluation of the developmental toxicity study in rabbits (MRID 44080319, attached), it is confirmed that there was a significant increase in post-implantation loss at the high dose level (250 mg/kg/day), and that this loss was largely due to early resorptions. This finding suggests that a single treatment of fluroxypyr can

produce an adverse effect, and validates use of this study in assessing acute endpoints.

2. At the Risk SARC held on 23-April-1998, it was determined that while it was appropriate to apply an additional 3X uncertainty factor to cover FQPA concerns (females 13+) in Acute Dietary exposure, it was unnecessary to add the 3X factor to the RfD as was recommended by the Hazard ID Committee (cf. HED Doc. No. 012464, 28-Jan-1998), because the developmental endpoint from which the 3X factor stems, applies only to females 13+ and not the general population. It is recommended, therefore, that the official Hazard ID report be amended to reflect this change.

3. On page six of the Hazard ID Committee report (cf. HED Doc. No. 012464, 28-Jan-1998) under the section "Chonic Dietary Risk Assessment", it was stated that although this study shows increased susceptibility to developing offspring, the FQPA factor should be 3X and not 10X. Reevaluation of the study does not change the conclusion that 3X is the appropriate factor. The Risk SARC also concurred with this conclusion.

cc: PP#6F04772, M.S. Ottley, W. Dykstra, M. Morrow, O. Odiott

FLUROXYPYR METHYLHEPTYL ESTER **Developmental Study OPPTS 870.3700 (§83-3(a))**

EPA Reviewer: Myron S. Ottley, Ph.D. Myron S. Ottley _____, Date 5/13/98
Team 2, Registration Action Branch I (7509C)
EPA Secondary Reviewer: William Dykstra, Ph.D. William Dykstra _____, Date 5/13/98
Team 2, Registration Action Branch I (7509C)

DATA EVALUATION RECORD--
Supplemental

STUDY TYPE: Prenatal Developmental Study - rabbit; OPPTS 870.3700 §83-3(*b*)

DP BARCODE: D232215 SUBMISSION CODE:S510931
P.C. CODE: 128959, 128968 TOX. CHEM. NO.:

TEST MATERIAL (PURITY): fluroxypyr methylheptyl ester (95.8% a.i.)

SYNONYMS: None

CITATION: Tesh, JM; Ross, FW; Wightman TJ. 02-February-1984. Dowco 433. Effects of oral administration upon pregnancy in the rabbit. Life Science Research Eye, Suffolk England. Report No. 84/DCC006/025, 02-February-1984. MRID 44080319. Unpublished.

SPONSOR: Dow Chemical Europe

EXECUTIVE SUMMARY:

This DER is a supplement to the original DER (HED Doc. 006688), and contains information not included in the original document.

In a developmental toxicity study (MRID 44080319) fluroxypyr methylheptyl ester (95.8% a.i.) was administered to 29 female New Zealand White rabbits/dose by gavage at dose levels of 0, 25, 100, 250 or 400 mg/kg/day from days 6 through 19 of gestation.

There were no treatment-related effects in mortality, clinical signs, body weight, food consumption, or cesarean parameters at the three lower dose levels. The highest dose level (400 mg/kg/day) caused severe maternal toxicity, resulting in the termination of that group, and exclusion of those results from the study. **The maternal LOEL is >250 mg/kg/day. The maternal NOEL is 250 mg/kg/day (HDT).**

Developmental toxicity was observed in the form of increased post-implantation loss at the high-dose level, due to increases in early and late resorptions. This increase (20.9% vs. 11.1% in controls) was determined by HED to be statistically significant. In addition, the increase is just outside the range of historical values (1.0% - 20.5%), and it is well above the historical mean

(10.5%), thus strengthening the case for its toxicological significance. Also observed at the HDT (250 mg/kg/day) was a 6% decrease (7.9 vs. 8.4 in controls) in live fetuses/litter. **The developmental LOEL is 250 mg/kg/day, based on post-implantation loss. The developmental NOEL is 100 mg/kg/day.**

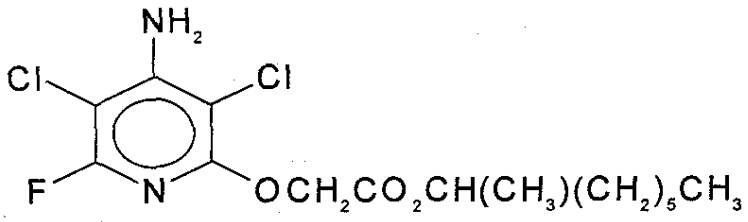
The developmental toxicity study in the rabbit is classified acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3(b) in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Dow 433
   Description: To be provided by sponsor
   Lot/Batch #: J.3199; 433-T-0683-16
   Purity: To be provided by sponsor
   CAS #:



Fluroxypyr 1-methylheptyl ester

2. Vehicle: Carboxymethyl cellulose 0.6% w/v
   Lot/Batch #: Not provided
   Purity: Not provided

3. Test animals: Species: Rabbit
   Strain: New Zealand White
   Age at mating: 18 - 24 wks
   Weight at mating: 1.56 - 4.65 g
   Source: C & J Morton Ltd., Essex, England

Housing: singly in galvanized steel cages
Diet: Beta Rabbit Standard diet ad libitum
Water: tap, ad libitum
Environmental conditions:
Temperature: 64 - 69 °F
Humidity: 72 ± 14%
Air changes: 17 - 20/hr
Photoperiod: 14 hrs dark/ 10 hrs light
Acclimation period (P): three weeks minimum.

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 28-Jun-1983; end: 15-Dec-1983

2. Mating: females were artificially inseminated with pooled semen from New Zealand White bucks of established fertility. Following insemination, each female was injected i.v. with 25 i.u. luteinizing hormone to ensure successful ovulation. The day of insemination was designated Day 0 of gestation.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was random to four treatment groups in order of insemination so that females inseminated on one day were evenly distributed among groups.

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	29
Low (LDT)	25	29
Mid (MDT)	100	29
High**	400	5
High (HDT)	250	29

** High dose terminated after treatment of one batch of animals, on day nine, with sponsor's concurrence, in view of the unlikely survival of animals until the end of dosing.

4. Dose selection rationale:

In a preliminary study, four pregnant rabbits/group were administered Dow 433 suspended in carboxymethyl cellulose by oral gavage at dosage levels of 0, 300, and 1000 mg/kg/day. Severe toxicity was observed at the high dose level, including 50% mortality, which resulted in termination of that dose level and introduction of a 500 mg/kg/day dose level. Toxicity in the 500 mg/kg/day group included increased respiration rate, muscular weakness, and unsteadiness/incoordination. In addition, mean values for postimplantation loss, and fetal and placental weights, were depressed in this group; one maternal death occurred but was not considered by the investigators to be treatment related. Based on these results, it was concluded that the HDT for the principal study should be in the 300 - 500 mg/kg/day range. The study directors therefore chose 0, 25, 100 and 400 mg/kg/day as the dose levels to be used. However, shortly after commencement of dosing at 400 mg/kg/day (gd 9), severe toxicity was observed which prompted the immediate replacement of this treatment group with a 250 mg/kg/day group. RAB1 does not consider the time lag for the replacement group to compromise the integrity of the findings.

5. Dosage preparation and analysis *Each* pregnant female received by oral gavage a single daily dose of vehicle (0.6% carboxymethyl cellulose) or Dowco 433 suspended in the vehicle on days six through 19 of gestation. Dosage suspensions were prepared daily. The volume administered daily was based on the animal's body weight on the day administered.

Results - Homogeneity Analysis: Not provided

Stability Analysis: Not provided

Concentration Analysis: Not provided.

These data were to be provided by the Sponsor, and are not part of the study report.

6. Dosage administration: All doses were administered once daily by gavage, on gestation days six through 19 in a volume of 5 ml/kg of body weight/day. Dosing was based on the body weight on the day of treatment.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for mortality or clinical signs daily. Body weights were recorded daily; food consumption data collection was not specified. Dams were sacrificed on day 29 of gestation. Each animal was examined macroscopically for evidence of disease or adverse reaction to treatment, and specimens of tissues considered abnormal were retained in an appropriate fixative. The reproductive tract, including ovaries, was dissected out and the following recorded: number of corpora lutea/ovary; number of implantation sites (Salewski staining was used in apparently non-pregnant animals to discover evidence of implantation sites); number of resorption sites, classified as early or late; number and distribution of live and dead fetuses.

2. Fetal Evaluations - The fetuses were examined in the following manner:
External Examination included fetal weights, placental weights, external abnormalities of each fetus and placenta.
Internal examination: all fetuses were killed by subcutaneous injection of pentobarbitone sodium. The neck, thoracic and abdominal cavities of all fetuses from each litter were dissected, the contents examined and sex recorded. Following examination, the fetuses were eviscerated prior to fixation in industrial methylated spirit.
Skeletal Examination Eviscerated fetuses were processed using a modification of the Dawson Alizarin staining technique, and skeletons were examined.

D. DATA ANALYSIS

1. Statistical analyses: The following procedures were utilized in examination of the numerical data: multiple t-test or t-test used for body weights, body weight change, fetal weight, placental weight and litter size; Mann-Whitney U-test for corpora lutea count, implantation count and resorption count; Chi-square test, Fischer's Exact Probability test or Mann-Whitney U-test for pre-implantation loss and post-implantation loss.

2. Historical control data: Historical control data were not provided to allow comparison

with concurrent controls.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: The following observations were reported: The 400 mg/kg/day group exhibited significant toxicity such as increased respiratory rate, ataxia and muscular weakness to the degree that animals were terminated on gd 9 and a lower dose level (250 mg/kg/day) was initiated. Lower dose levels showed no toxicity greater than controls.

2. Body Weight - Body weight data are summarized in Table 2 and as follows: Body weight gains among the control group and the treatment groups (25 mg/kg/day or LDT; 100 mg/kg/day or MDT; 250 mg/kg/day or HDT) were similar.

TABLE 2. Maternal Body Weight Gain (g)[a]

Interval	Dose in mg/kg/day (# of Dams)			
	Control (N)	LDT (N)	MDT (N)	HDT (N)
Pretreatment: Days 0 - 6	0.10 (25)	0.09 (24)	0.12 (24)	0.18 (24)
Treatment: Days 6 - 18	0.20 (25)	0.19 (24)	0.17 (24)	0.12 (24)
Posttreatment: Days 18 - 28	0.15 (25)	0.08 (24)	0.19 (24)	0.12 (24)

a Data extracted from report no. 84/0CC006/025, Table 2.

3. Food Consumption - Food consumption data were not presented or reported on.

4. Gross Pathology - Gross pathology data are summarized as follows: At necropsy on gd 29, no macroscopic changes in maternal condition were observed that could be attributed to treatment with Dowco 433. Kidney weights were measured and showed no treatment-related effects.

5. Cesarean Section Data - Data are as follows: *[Describe findings]*; as summarized in Table 3. ***[Some form of this table is MANDATORY; data should be presented as both fetal and litter incidences]***

TABLE 3 Cesarean Section Observations[a]

Observation	Dose (mg/kg/day)			
	0	LDT	MDT	HDT
# Animals Assigned (Mated)	29	29	29	29
# Animals Pregnant Pregnancy Rate (%)	26	25	25	25
# Nonpregnant				
Maternal Wastage # Died # Died Pregnant # Died Nonpregnant # Aborted # Premature Delivery	3 1	1 1	1	1
Total # Corpora Lutea Corpora Lutea/Dam	11.5 ± 3.5	11.2 + 2.4	10.7 ± 2.4	12.4 ± 2.7
Total # Implantations Implantations/Dam	Not reported 9.4 ± 2.0	Not reported 9.1 ± 2.9	Not reported 9.0 ± 2.3	not reported 9.6 ± 3.0
Total # Litters	26	25	25	25
Total # Live Fetuses Live Fetuses/Dam	218 8.4	203 8.1	195 7.8	190 7.6
Total # Dead Fetuses Dead Fetuses/Dam	Not reported	not reported	not reported	not reported
Total # Resorptions Early Late Resorptions/Dam Early Late Litters with Total Resorptions	Not reported 1.0 ± 1.0 0.2 ± 0.5 0.8 ± 0.9 0	Not reported 1.0 ± 1.0 0.3 ± 0.5 0.7 ± 0.8 0	Not reported 1.2 ± 1.1 0.2 ± 0.4 1.0 ± 1.0 1	not reported 2.0 ± 1.4 1.4 ± 1.2 0.6 ± 0.8 1
Mean Fetal Weight (g) Males Females	Not reported	Not reported	Not reported	not reported
Sex Ratio (% Male)	46	53	51	50
Preimplantation Loss (%)	18.4	18.7	16.0	22.9
Postimplantation Loss (%)	11.1	11.0	13.8	20.9

a Data extracted from report no. 84/0CC006/025, table 4.)

B. DEVELOPMENTAL TOXICITY

1. External Examination - No external findings were reported.

2. Visceral Examination - The data presented show an increase in "gall bladder variants" at the high dose level, compared with controls (table 4b) Independent analysis by HED shows this finding to be statistically significant. No other apparent treatment-related effects were reported.

3. Skeletal Examination - No skeletal findings of toxicological importance were observed (see Table 4c).

TABLE 4a. External Examinations[a]

Observations+	Dose (mg/kg/day)			
	0	LDT	MDT	HDT
#Fetuses(litters) examined	205 (25)	194 (24)	194 (24)	189 (24)
#Fetuses(litters) affected				
Findings: None repported				

a Data extracted from report no. 84/0CC006/025)
b Fetal (litter) incidence

TABLE 4b. Visceral Examinations[a]

Observations	Dose (mg/kg/day)			
	0	LDT	MDT	HDT
#Fetuses(litters) examined	205 (25)	194 (24)	194 (24)	189 (24)
#Fetuses(litters) affected				
gall bladder variants	26.2 (20)[b]	23.2 (10)	22.2 (18)	43.4 (21)

a Data extracted from report no. 84/0CC006/025, table 5.)
b Fetal (litter) incidence

TABLE 4c. Skeletal Examinations[a]

Observations+	Dose (mg/kg/day)			
	0	LDT	MDT	HDT

#Fetuses(litters) examined	205 (25)	194 (24)	194 (24)	189 (24)
#Fetuses(litters) affected				
Number with heads of long bones unossified	37.8 (22)[b]	47.9 (18)	50.5 (23)	51.3 (21)

\+ Some observations may be grouped together

a Data extracted from report no. 84/0CC006/025, table 6.)

b Fetal (litter) incidence

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The investigators have concluded that within the parameters of the study, there was no "adverse effect upon the progress and outcome of pregnancy. Fetal morphogenisis and growth were similarly unaffected by treatment." As such, the maternal and developmental NOEL would be 250 mg/kg/day, the highest dose level used in this study.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: No maternal effects were observed at the highest dose level used (250 mg/kg/day).
2. DEVELOPMENTAL TOXICITY:
   a. Deaths/Resorptions: An increase in post-implantation loss was observed at the high dose level (20.9%) vs. controls (11.1%). These values are derived from all the animals, including those with total litter loss. (The author's comparisons exclude animals with total litter loss, resulting in a lower incidence (18.2%) of post-implantation loss.)
   The original HED DER states that this difference is statistically significant, as "determined independently". Therefore, while details concerning the degreee of significance, etc. are missing, there is no reason at this time for HED to challenge its own analysis. The historical mean for this endpoint, derived from 71 studies, is 10%, with the range being 1.0 - 20.5%). Thus, the value for the current study is about twice the historical mean, and is just outside the historical range, supporting the conclusion that the effect is real.
   b. Altered Growth: No significantly altered patterns of growth were observed. Upon further examination, the gall bladder anomalies observed in fetuses were not of toxicological significance.
   c. Developmental Variations: None observed
   d. Malformations: No malformations were observed.

C. STUDY DEFICIENCIES

1. Test substance stability data were not provided, nor homogeneity of test suspensions.
2. Food consumption data were not provided.

Normally, these deficiencies would lead to a supplementary classification, but since this test substance data is readily available from other studies submitted, and since credible endpoints were identified with LOELs and NOELs, the HAZID committee concluded that an **Acceptable** classification is appropriate.