Reviewed By: Brian Dementi, Ph.D. Brian Dementi 1/12/88
Section VI, Toxicology Branch (TS-769C)
Secondary Reviewer: Judith Hauswirth, Ph.D. Judith W. Hauswirth 1/12/88
Section VI, Toxicology Branch (TS-769C)

006688

DATA EVALUATION REPORT

Study Type: Teratology

TOX Chem. No.: 463-0

Accession No.: 402445-09

MRID No.: 7G3541

Test Material: Dowco 433

Synonyms: Fluroxypyr; 4-amino-3,5-dichloro-6-fluoro-2-pyridinyl oxyacetic acid

Study Number(s): DWC 369/370/83107

Sponsor: Dow Chemical, Midland, MI

Testing Facility: Huntingdon Research Centre, Cambridgeshire, England

Title of Report: Effect of Dowco 433 on Pregnancy of the Rat

Author(s): Bottomley, A.M.; Mayfield, R.; Clark, R.; Offer, J.M.

Reported Issued: March 31, 1983

Conclusions:

1. Parental Animals

Clinical signs of toxicity including salivation and brown facial staining were observed at the two highest doses, i.e., at 250 and 500 mg/kg/day. The NOEL = 125 mg/kg/day for clinical signs of toxicity. One animal died in the high-dose group which may have been treatment related. There were no deaths in any other dose group or in the control group. Dosing did not affect any of the following parameters: food consumption, body weight gain, live young, embryonic deaths, implants, corpora lutea, preimplantation loss, postimplantation loss, litter weight, or mean fetal weight.

Mean kidney weight for animals in the high-dose group was increased by 10 percent, which was statistically significant. For this parameter, NOEL = 250 mg/kg/day. Renal pelvic dilatation was observed with increased incidence at the highest dose. For this parameter, NOEL = 250 mg/kg/day.

2. Fetal Malformations and Anomalies

The test material did not induce any increases in fetal malformations at any dose tested. There were no dose-related fetal visceral anomalies. There may have been an increase in fetal skeletal anomalies in the high-dose group, but the exclusion of three nonrepresentative litters from the calculations (one from the control and two from the high-dose group) as discussed, yielded more uniform findings across the groups. There was evidence to suggest an increase in the high-dose group in the percentage of fetuses exhibiting reduced skeletal ossification; which is indicative of maternal and/or developmental toxicity at the high dose. NOEL = 250 mg/kg/day.

The testing of Dowco 433 did not produce evidence of a teratogenic effect.

Classification: Core-Guideline

A. Materials:

1. Test Compound: Dowco 433; Description: off-white powder; Lot No.: 433T-1082-5; Purity: 99%; Contaminants: not provided.

2. Test Animals: Species: rat; Strain: CrL COBS CD (SD) Br; Age: 8 to 9 weeks; Weight: 174 to 210 g; Source: Charles River UK Ltd., Margate, Kent, England.

B. Study Design

The study design was based in part upon the results of a preliminary study in pregnant rats employing 6 animals/dose group in which Dowco 433 was administered via intragastric intubation at dosage levels of 0, 250, 500, and 1000 mg/kg/day. The agent was administered suspended in 1 percent methyl cellulose. In this preliminary study, adverse effects associated with the highest dose included deaths among one-third of the group, increased salivation, retardation of mean body weight and increased mean kidney weight. No deaths occurred at the lower doses. However, clinical signs at the two lower doses were described as similar to those seen at 1000 mg/kg/day, but the responses were of reduced magnitude. Kidney weight was "overtly" higher for the 500 mg/kg/day group but increases were only "suggested" at 250 mg/kg/day.

There were no pronounced indications of dose-related effects on litter parameters.

In view of the described findings for the preliminary study, dosages chosen for the primary teratology study were 0, 125, 250, and 500 mg/kg/day.

Animal Assignment

Pregnant rats were assigned 25/group to the following test groups:

Test Group	Treatment Level mg/kg/day	Vehicle	Concentration of Suspension % w/v	Dosage Volume mL/100 g
A	0	1% methyl cellulose	0	1
B	125	" " "	1.25	1
C	250	" " "	2.50	1
D	500	" " "	5.00	1

Each pregnant female rat received by intragastric intubation a single daily dose of vehicle (1% methylcellulose) or Dowco 433 suspended in 1 percent methylcellulose on days 6 through 19 of gestation.

Experimental Procedures - (The following is paraphrased or quoted from pages 17 and 18 of the submitted study.)

1. Parental Animals

All animals were handled daily and observed for clinical signs and mortality. All animals were weighed on the following days of gestation: 1, 3, 6, 10, 14, 17, and 20. Food consumption was measured "from 'weighday to weighday'."

2. Litter Evaluations

"On Day 20 of pregnancy the animals were killed by CO_2 asphyxiation, dissected, and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The kidneys of all females were weighed and preserved in buffered neutral formalin for subsequent histological investigation where required. The ovaries and uteri were examined immediately to determine:

(a) number of corpora lutea
(b) number and distribution of live young
(c) number and distribution of embryonic/foetal deaths
(d) individual foetal weight from which the total litter weight and mean foetal weight was calculated
(e) foetal abnormalities

"Embryonic/foetal deaths were classified as:

Early: only placenta visible at termination.
Late: both placental and embryonic remnants visible at termination.

"Uteri or individual uterine horns without visible implantations were immersed in a 10% solution of ammonium sulphide to reveal evidence of embryonic death at very early stages of implantation.

"Live young were examined externally and weighed. Half the foetuses in each litter were preserved in Bouin's solution for subsequent free-hand sectioning to discover visceral abnormalities (Wilson technique); the remainder were fixed in 74 OP industrial methylated spirit for subsequent macroscopic examination, evisceration and determination of sex prior to clearing and alizarin staining (modified Dawson technique) for skeletal examination. Young showing suspected abnormalities were processed by the more appropriate technique for clarification of initial observations.

130

4

"All foetuses were uniquely identified to allow correlation of initial with subsequent findings.

"Structural deviations were classified as:

"Malformations: rare and/or probably lethal, e.g. exencephaly, anury.

"Anomalies: minor differences from 'normal' that are detected relatively frequently either by free-hand sectioning, e.g. increased renal pelvic cavitation, or at skeletal examination, e.g., bipartite centrum.

"Variants: alternative structures occurring regularly in the control population are classified as variants. These may be permanent structures, e.g. an extra pair of ribs or they may be transient stages of development, e.g. unossified sternebra(e)."

Statistical Analysis - (The following is paraphrased from page 19 of the submitted study.)

Non-parametric methods of statistical analysis were performed routinely on litter data, using the litter as the basic sample unit.

For assessing intergroup differences in mean kidney weight of dams, analysis of co-variance was employed.

Quality Assurance

The study was confirmed by Audrey M. Bottomley, Deputy Head of Department of Reproductive Toxicology as having been conducted in compliance with Good Laboratory Practice regulations as set forth in Part 58 of Title 21 of the Code of Federal Regulations, "with the exception of possible minor items" not considered essential to the validity of the study.

Results

A. Preliminary Study

At the highest dose, 1000 mg/kg/day, notable effects included deaths of two animals of six in the group, transient clinical signs (salivation and brown facial staining) following dosing, increased mean kidney weight and reduced mean body weight gain. Among animals of the mid-dose group, the same clinical signs evident at the high dose were noted, but were of reduced magnitude. Mean body

weight gain for the group was slightly greater than that of the control. Mean kidney weight was also elevated in this group. There were no deaths in this group. At the low dose, clinical signs were still observed, but were less dramatic than at either of the higher doses. A slight mean kidney weight increase was apparent at this dose. There were no deaths at the low dose.

Among litter parameters, there were no remarkable findings except the report of two malformed fetuses, ". . . but no association with treatment was indicated." (p. 25). Reviewer cannot find in the report any description of the malformations or dose group(s) in which they occurred.

B. Primary Teratology Study

1. Parental Animals

Consistent with recorded observations in the preliminary study, clinical signs of toxicity included salivation and the appearance of brown facial staining. These clinical signs were evident in essentially all animals of the two highest dose groups (pp. 50 to 51). At the low dose, only two dams of the group of 25 were reported as exhibiting such clinical signs (p. 49). One animal in the high dose group died, which may have been treatment-related. There were no deaths in any of the other groups. Neither food consumption (pp. 29 and 35) nor body weight increases (pp. 30 and 36) were observed to be influenced by dosing with Dowco 433. Pregnancy rate was not inhibited by dosing (p. 38).

As summarized in tabular form on p. 38 and supported by independent calculations of certain of the mean values, there were no remarkable effects of dosing identified with respect to the following group mean litter data: live young, embryonic deaths, implants, corpora lutea, preimplantation loss, postimplantation loss, litter weight or mean fetal weight. One dam each from control and low dose groups experienced total resorptions, however, there is no reason to conclude that these bore any relationship to circumstances of testing.

Since increased kidney weight was a finding in the preliminary study, this effect was of particular concern in this complete study. Accordingly, as determined at terminal autopsy, mean kidney weight in the high-dose group was elevated (increased) to about 110 percent of control values, an increase

which was statistically significant at $p < 0.01$ by analysis of covariance. At the lower doses there were minor, < 1 percent, increases in kidney weights which were not statistically significant. However, the study authors report that there was a significant trend for increased kidney weight across all doses ($p < 0.05$) (p. 37). This reviewer does not consider the lower two doses as having a meaningful effect on the kidney on the basis of kidney weight increases. The effect at the high dose should be viewed as real and as identifying LOEL = 500 mg/kg/day and NOEL = 250 mg/kg/day.

Renal pelvic dilatation was observed at incidences of 0/25, 1/25, 1/25, and 4/25 at the respective dose levels of 0, 125, 250, and 500 mg/kg/day. This reviewer considers the finding at the highest dose to be of toxicological significance, but not the low incidences of 1/25 observed at the mid and low dose levels. Hence, LOEL = 500 mg/kg/day for this parameter.

2. Fetal Malformations and Anomalies

Examinations of fetuses for malformations revealed the following incidences per dose group: control - one, low dose - none, mid-dose - two, and high-dose - one. The malformations in question were characterized as follows. From the control group, the one fetus in question exhibited hydrocephaly and bilateral microphthalmia with retinal dysplasia. In the mid-dose group, one fetus exhibited left microphthalmia and the other interventricular septal defect and displaced left adrenal gland and inferior vena cava. The one malformed fetus in the high-dose group displayed inappropriate positioning (retro-esophageal) of the right subclavian artery.

This reviewer does not consider these few randomly distributed malformations as providing evidence of a potential for Dowco 433 to induce malformations in the developing fetus.

Examinations of fetuses for visceral anomalies did not disclose any findings which could establish an effect of dosing with Dowco 433. Hemorrhage at various loci, particularly intra-abdominal, appears as a fairly frequent anomaly. A count of the incidence of such effects in the various groups revealed the following: control - 8, low dose - 3, mid-dose - 15, high dose - 7. This reviewer does not consider these findings as demonstrating any adverse effect of the agent.

~~133~~

7

Examination of fetuses for skeletal anomalies revealed a higher incidence of such anomalies in the high-dose group as compared to the control. The percentages of fetuses affected in the various groups were: control - 19.4, low dose - 18.0, mid dose - 13.4, and high dose - 27.1 (p. 40). The study authors acknowledge that the high dose incidence of 27.1 percent not only exceeds the concurrent control incidence, but the historical control range for recent studies as well. The authors argue, however, that this finding is principally attributable to the undue influence on this parameter by two litters in the high-dose group (p. 27). When these plus one litter similarly affected in the control group are excluded from consideration, a recalculation revealed the following incidences (percent): control - 14.9, low dose - 18.0, mid-dose - 13.4, and high dose - 18.9. The study authors point out the greater uniformity of data across the groups shown with the recalculations. This reviewer agrees with the latter observation. If these recalculated percentages are valid, an effect of dosing has not been identified. However, the justification in eliminating the two sets of pups from the high-dose group should be examined. In one of these litters (dam 79), there was but one fetus constituting the litter. In the other litter (dam 86), of 12 fetuses in the litter, 6 were examined for skeletal anomalies. All six, or 100 percent, exhibited anomalies. Thus in one case the dam did not produce the normal number of pups and in the other, while providing a normal number, all pups examined exhibited skeletal anomalies. The fact that 100 percent were anomalous is suggestive of maternal ill health or toxicity rather than specific fetotoxic effects, since such large percentages were not affected in litters from other dams. This amounts to speculation, however. A conclusion that this does not provide evidence of a potential for Dowco 433 to cause fetal anomalies is partially supported by the finding of one such dam (No. 13) in the control group, where 100 percent of the pups examined were likewise anomalous. In the absence of more serious findings in terms of malformations and/or skeletal anomalies, this reviewer concurs with the opinion that skeletal anomalies do not constitute a remarkable finding in the high-dose group and certainly not in the lower dose groups.

In Appendix 11, which presents individual fetal malformations and anomalies, an effect frequently described under skeletal anomalies is reduced ossification at various skeletal sites. There are no striking, dose-related, anomalies evident for any particular skeletal site, but when reduced ossification is tallied for all skeletal sites the following numbers are obtained:

	Reduced Ossification At Any Center		
	(A)	(B)	
Dose Group	Fetuses Affected (%)	Litters Affected	(A)/(B)
Control	7 (6.5)	6	1.17
Low	10 (8.3)	7	1.43
Mid	10 (7.3)	7	1.43
High	15 (12.9)	8	1.88

The increases in the frequency of ossification evident at the high dose by the various modes of expression is suggestive of compound induced developmental toxicity and/or maternal effects. NOEL = 250 mg/kg/day.