2,4-D/Tox

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 3,C,2,b data call-in on 2,4-D. Teratology of 2,4-D and 2,4 Dichlorophenol with respect to PP# 3E2876 related action.
CASWELL No. 315.

TO: Richard Mountford, PM #23
Herbicide Branch/RD (TS-767)

Lois Rossi, PM #61
SRB/RD (TS-767)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

FROM: Henry W. Spencer, Ph.D.
Review Section #1
Toxicology Branch/HED (TS-769)

Conclusions and Recommendations:

1. The range-finding and teratology studies are sufficient to indicate that 2,4-D and 2,4-Dichlorophenol are not teratogenic at up to 75 mg/kg and 750 mg/kg, respectively.

2. Toxicology Branch considers the studies adequate to indicate a fetotoxic effect for 2,4-D at 75 mg/kg (LEL) and a NOEL of 25 mg/kg. Delayed ossification is the fetotoxic effect.

3. Toxicology Branch considers the 2,4-Dichlorophenol teratology studies adequate to indicate an LEL of 750 mg/kg and a NOEL of 350 mg/kg for delayed ossification. A teratogenic effect is not demonstrated in the study.

4. Toxicology Branch recommends that these studies be added to the data base to support the registrations of 2,4-D.

5. It is noted that generally a maternally toxic dose should be attained in a teratology study. However, in these cases 2,4-D has been previously tested by a registrant, Dow Chemical Co. which demonstrated a slightly maternal toxic effect at 87.5 mg/kg in rodents. These data were run in a manner to indicate a NOEL for only fetotoxicity.

As a result of previous studies in other species and strains of rodents, the Toxicology Branch continues to consider 2,4-D to be a teratogenic agent. These previous studies are in no way negated by the lack of a teratogenic finding in these current studies since they were run at much higher dosage levels.

Summary of Toxicity Studies Reviewed

I. 2,4-D acid

a. Range finding study of 2,4-D for maternal toxicity:
(HDT) = 250 mg/kg
LEL for maternal toxicity = 150 mg/kg
NOEL for maternal toxicity = 100 mg/kg for reduction in feed consumption and body weight loss.

b. Teratology study of 2,4-D

LEL for maternal toxicity NOT found
NOEL for maternal toxicity = 75 mg/kg = (HDT)
LEL for fetotoxicity (delayed ossif.) = 75 mg/kg
NOEL for fetoxicity = 25 mg/kg
NOT teratogenic at up to 75 mg/kg (HDT)

II. 2,4-Dichorophenol

a. Range finding study with 2,4-Dichlorophenol:

Phase III LEL = 750 mg/kg (LDT)

Phase II LEL = 400 mg/kg (500 mg/kg = HDT)
NOEL = 300 mg/kg for mucous membrane effects (maternal)

Phase I LEL = 75 mg/kg (HDT = 150 mg/kg)
NOEL = 25 mg/kg for mucous membrane effects (maternal)

b. Teratology Study with 2,4-Dichlorophenol Maternal Toxicity LEL = 200 mg/kg (LDT) equivocal wt. gain effects.

Fetotoxicity LEL = 750 mg/kg
NOEL = 350 mg/kg as delayed ossification.
Not teratogenic at up to 750 mg/kg (HDT)

Study:

Range-finding teratology study in Fischer 344 rats with 2,4-Dichlorophenoxy acetic acid by WIL Research Labs. Inc. dated May 17, 1983. WIL - 22002., Acc. No. 251032.

Material Tested:

2,4-Dichlorophenoxyacetic acid (Technical) from ITT Research Institute, Chicago, Ill. on March 15, 1982, 97.5% purity.

Animal Tested:

60 virgin, sexually mature female, Fischer 344 rats from Charles River Labs, Portage Michigan were used.

Methods:

After a 25 day quarantine, the females were bred if body wts. were greater than 170 g. Evidence of gestation day 0 was the finding of sperm from a vaginal smear. 10 rats were assigned to each dosage level. Dosages of 0, 75, 100, 150, 200 and 250 mg/kg were tested. The test material was mixed in corn oil and dosed at a volume of 4 ml/kg by gavage on days 6-15 of gestation.

Clinical observations were made daily on days 0-16 of gestation. Body wts. were also recorded on days 0-16 of gestation.

On day 16 of gestation the dams were sacrificed. The usual parameters were noted and included: number of corpora lutea formed, determination of viable and non viable fetuses and implantation status.

Statistical significance of changes was tested by using programs on a Digital Computer. Methods were obtained from: BMPD-79 Biomedical Computer Programs, Univ. of Calif. Press, Berkely, Calif. 1979. PP. 612, 780, 781.

Results:

A statistically significant reduction in body wt. gain was noted for groups at (1) 150 mg/kg on days 15 and 16 of gestation and (2) 200 mg/kg and 250 mg/kg on days 10 through 16 of gestation. Mean body wt. changes were not statistically different from controls at 75 and 100 mg/kg throughout the study while significant changes were noted at 150 mg/kg and above.

Feed consumed on a mean group basis was found to be significantly reduced at 150 mg/kg and above.

Dams treated in the study did not abort. However, 1/10 dam in the 200 mg/kg group and 3/10 dams in the 250 mg/kg group died during treatment. Total resorptions occurred in 2/8 pregnant females at 150 mg/kg and in 8/8 and 7/7 pregnant dams at 200 mg/kg and 250 mg/kg respectively.

A significant reduction in viable fetuses is seen at 150 mg/kg where 6.4 fetuses per litter were produced when compared to 9.1/litter in controls. No viable fetuses were found at 200 and 250 mg/kg. No effect on the number of viable fetuses at 100 mg/kg and below was seen. The LEL for resorptions, increased post implantation losses and implantation site effects is 150 mg/kg. A threshold NOEL is 100 mg/kg for fetotoxic and or lethal effects noted. It is based on the slight increase in litters displaying early resorptions even though no increase in numbers of litters effected was seen.

Liver-to-body wt. ratios and kidney wts./body wt. ratios in dams were increased above 150 mg/kg.

	Ratio Liver/bwt.	Right/Ratio Kidney/bwt.	Left/Ratio Kidney/bwt.
Control	.0436	347	359
75 mg/kg	.0412	361	370
100	.0414	372	367
150	.0440	374	369
200	.0473	384	386
250	.0524	394	399

Histopathological findings were reported as negative.

Conclusions:

This reviewer considers an LEL for maternal toxicity based on a reduction in feed consumption as 150 mg/kg. A NOEL is 100 mg/kg. The study is to determine a NOEL for maternal toxicity but is of limited value because the evaluation only lasted for 16 days and not the 21 days as would be the case in the teratology study.

Core:

Supplementary data.

Study:

A range finding Teratology Study/s in Fischer 344 rats with 2,4-Dichlorophenol. Phase I, II, III by WIL Research Labs. Inc. WIL-81133 dated March 31, 1983 for the Industry Task Force on 2,4-D Research Data. Acc. No. 251029.

Material Tested:

2,4-Dichlorophenol purity - approximately 99.2%. I.D. No.: AGR 182992.

Animal Tested:

Fischer 344 virgin female rats from Charles River Labs, Portage, Mich.

Methods:

After a quarantine period and an acceptable body wt. of 170 g or greater, animals were bred. Groups of ten animals were gavaged with 0, 750, or 1000 mg/kg in Phase III; 0, 200, 300, 400, or 500 mg/kg in Phase II; 0, 25, 75, or 50 mg/kg in Phase I. The test material was suspended in corn oil and dosed in a volume or 4 ml/kg.

Dosages were given on days 6 through 15 of gestation. Gestation day 0 was determined with the finding of sperm by a vaginal smear. Feed, Purina® rodent chow, and tap water were provided ad libitum. Temperature, humidity, and photoperiods were 72 ± 3° F, 40% and 12 hr. respectively throughtout the study.

Clinical observations were made daily on days 0-16 of the study. Body wts. were recorded daily and calculated for 3 day intervals. Food and water intake values were recorded for the above intervals. Uterine and ovarian examination allowed for counting corpora lutea, implantations and locations, number of viable fetuses, and resorptions. Organ wts. of livers and kidneys were recorded.

Statistical evaluation was made using appropriate programs for a Digital Computer with significance for 2-tailed tests set at $p < .05$. The Mann-Whitney U-test was used to compare early and late resorptions, dead fetuses and post implantation losses. Other parameters were analyzed by a one way ANOVA and Dunnett's test.

Results:

Phase III, (0, 750, 1000 mg/kg). Body wts. of treated dams were statistically reduced only on the last few days at 750 mg/kg and by day 9 at 1000 mg/kg. Food intake was significantly depressed at 750 mg/kg and markedly depressed at 1000 mg/kg after day 6 of study. No females aborted. However, 7/10 females and 1/10 at 1000 mg/kg and 750 mg/kg respectively died during the study. All dams dying were pregnant. One of 2 pregnant dams at 1000 mg/kg totally resorbed its embryos. Viable fetuses were reduced only at the highest dosage when compared to controls because one of two dams completely resorbed.

Implantation sites were not reduced in treated dams. However, early resorptions were increased at the HDT. No significant changes in liver and kidney were noted at 750 mg/kg when compared to controls. A slight increase in kidney wt./body wt. ratios was seen, ($p < .05$), but so few (3) dams were available for analysis, that the signficance of this value is questionable. Red staining of the urogenital area was noted at 1000 mg/kg. Mucous membrane effects were also noted at 750 mg/kg.

Phase II: (0, 200, 300, 400, 500 mg/kg). All treated females exhibited a slight but non-significant reduction in weight gain values. Feed consumption was also only slightly reduced when compared to controls.

No females aborted in the study and none died prior to completion of the study. Total resorption of fetuses did not occur in treated dams. Only at 300 mg/kg was the number of viable fetuses reduced, but is considered to be only a spurious non-treatment effect since the effect was not seen at other dosage levels. Slight, nonsignificant increases in early resorptions and post implant loses in both groups treated with 400 and 500 mg/kg are noted. Only a relative organ wt. increase is noted in the liver of dams at the HDT (500 mg/kg).

Dried red discharges (LEL = 400 mg/kg, NOEL = 300 mg/kg) were increased over controls at 400 and 500 mg/kg. Some occurrances of these dried discharges appeared to be the result of cleaning other areas of the body. An LEL of 400 mg/kg is set for the dams using mucous membrane red discharges with a NOEL of 300 mg/kg.

Phase I (0, 25, 75, 150 mg/kg)

Body wt. gains of treated dams were not different from controls during gestation. Values for feed intake per animal in each group were similar. No females died or aborted in the study. Group pregnancy rates were not significantly different within the study. Controls exhibited an unusually low number of implantation sites, and a subsequent lower number of viable fetuses than seen in the other treatment groups. Relative organ wts. were not different when comparing groups.

Clinical observations included urogenital staining in 3 and 4 30% and 50% of treated dams in groups respectively, exhibiting the effect. Red vaginal discharge was occassionally observed in groups at 25, 75, and 150 mg/kg with 20%, 30% and 10% females involved respectively.

There were no teratogenic or fetotoxic effects at doses up to and including 150 mg/kg (HDT). However, effects on the mucous membranes in the dams were evident at 75 mg/kg with no observable effects at 25 mg/kg.

The dried red discharge on the nares was noted in all groups with substantial increase in response seen at 75 and 150 mg/kg compared to controls.

These data (e.g. range-finding studies) are considered Core: Supplementary.

Study:

Teratology study in Fischer 344 rats with 2,4-Dichlorophenoxy acetic acid by WIL Research Labs. Inc. dated March 3, 1983. Lab No. WIL - 81135, for the Industry Task Force on 2,4-D Research Data. Acc. No. 251031.

Material Tested:

2,4-D acid, Technical grade from ITT Research Institute, Chicago, Ill. 97.5% 2,4-D acid and related chlorophenoxy compounds, 1.41% water, and low ppb levels of the 2, 7 dichlor; monochloro; 1, 3, 8 and 1, 3, 6, 8 chlorinated-p-dioxins as contaminants.

Animal Tested:

Virgin, Fischer 344 rats from Charles River, Labs., Portage, Michigan.

Methods:

After acclimation and quarantine for a period of 1 week, test animals were individually caged, given Purina® rodent chow #5002 and municipal water ad libitum. Fresh air was at 72° ± 3° F with 40% relative humidity and a 12 hr. light-darkness cycle. At breeding each female must have weighed greater than 170 g. Cohabitation with males was recorded and evidence of sperm by vaginal smear represented day 0 of gestation. Thirty five females were placed in each test group by randomized block determination.

The test material was suspended in corn oil and given in a dosage volume of 4 ml/kg by gavage so that groups were given either corn oil, 8, 25, or 75 mg/kg of the 2,4-D acid on days 6-15 of gestation. Clinical observations were recorded on days 0-20 of gestation. Observations were made 2X daily. Body wts. of dams were recorded on days 0, 6, 10, 12, 15 and 20 of gestation. At termination of the study, corpora lutea were enumerated, and fetuses were recorded as viable or nonviable. Resorptions were reported as early or late, and implantation sites were enumerated. Ammonium sulfide staining was performed on nongravid uteri.

Fetal Observations:

Individual body wts. and crown to rump lengths were determined for each fetus. External examination findings were recorded. Approximately one-half the number of fetuses born were fixed in Bouins solution for Wilson sectioning. The other 50% of fetuses were fixed in 95% alcohol and prepared for skeletal examination by the Alizarin Red S dye method of Dawson.

Statistical Examination: Excerpted from study report.

Two-tailed tests were limited at the 5% p-value. "All statistical tests were performed by a Digital Computer with appropriate programming as referenced below.

1. The fetal sex ratios were compared by the Chi-square test[7] with Yates correction factor.

2. The number of litters with malformations were compared by Fisher's Exact Test[7].

3. The number of early and late resorptions, dead fetuses and post-implantation losses were compared by the Mann-Whitney U-test[7].

4. Mean number of corpora lutea, total implantations, viable fetuses, mean fetal and maternal body weight at each interval and maternal body weight gain were analyzed by a one-way analysis of variance, and Dunnett's test.[8]

References for statistical tests above:

7. BMPD - 79 Biomedical Computer Programs, Univ. of California Press, Berkeley, California, 1979. pp. 612, 780-781.

8. Steel, R.G.D. and Torrie, J.H., Principles and Procedures of Statistics, McGraw Hill, New York, New York, 1960 pp. 106, 107, 114."

Results:

Pregnancy status of rats treated with 2,4-D:

	Number	Nongravid	Pregnant	% Pregnant
Controls	34	5	30	85
8 mg/kg	34	5	30	85
25 mg/kg	35	7	28	80
75 mg/kg	35	8	27	77

One control and one group 1 (8 mg/kg) female delivered early. Treated animal pregnancy rates were not significantly different from controls.

Mean weight gains in the dams were slowed in the 6-10 day period and only slightly in the 10-12 day period in the group four (75 mg/kg) females. These changes are only equivocal.

Data presented in the report indicate that no significant differences were apparent between treated and control groups when comparing the mean number of viable fetuses per litter for controls (9.0) vs. 9.3 in 75 mg/kg group litters (HDT). No increased numbers of born-dead fetuses are noted.

Fetal Parameters:

Fetal weights were not significantly different in the 75 mg/kg (HDT) group when compared to controls. In addition, crown-rump lengths were similar in all tested groups. Group 4 (75 mg/kg) early and later resorptions were not increased over control values.

External malformations occurred in a sporadic fashion. Cleft palate was seen in only 1 fetus of 1 litter at the HDT. One fetus in one litter of control exhibited exencephaly. One fetus in one litter at the HDT exhibited incomplete twining. Micropthalmia and/or anopthalmia occurred in 1 fetus in each of two litters in control and 8 mg/kg groups, respectively; while 1 fetus in 1 litter at HDT dose was also found with opthalmic abnormality.

Visceral abnormalites in the 75 mg/kg group were limited to one fetus in one litter as kidney and/or ureter missing, one fetus with hydrocephaly, and one fetus with ovary and/or uterus absent. No significance to these incidences is noted.

Skeletal anomalies were limited to occurrances of severely malaligned sternebrae with one fetus in one litter at 25 mg/kg, one fetus in each of two litters at 75 mg/kg. In addition, 1 vertebral anomally was noted in the control litters.

Developmental variations (feto toxicity) were seen to be slightly increased only at the HDT. These variations at the HDT included an increase in: 7th cervical rib to 4 occurrances in 3 litters, vs 0 in control; 14th rudimentary rib to 4 in 3 litters, vs 0 in controls; reduced ossification increased to 6 in 5 litters vs 2 in 1 control litter and; 15 fetuses in 10 litters with malaligned sternebrae vs 7 in 7 litters in controls. A slight increase in unossified sternebrae #5 and #6 was noted in 73 fetuses/22 litters (3.31 litter) at the HDT while 62 fetuses in 24 litters (2.58 aver/litter) exhibited the effects in controls.

Conclusion:

Toxicology Branch considers the study to exhibit slight fetotoxicity expressed as delayed ossification: LEL = 75 mg/kg
NOEL = 25 mg/kg

The study does not indicate a teratogenic effect at the 75 mg/kg (HDT). Core: Minimum.

Study:

A teratology study in Fischer 344 Rats with 2,4-Dichlorophenol by WIL Research Labs Inc. Project No. WIL - 81134 dated March 31, 1983, for Industry Task Force on 2,4-D Research Data., Acc. No. 251030.

Material Tested:

AGR 182992, 99.2% of 2,4-Dichlorophenol.

Animal Tested:

Fischer 344 sexually mature, virgin, female rats from Charles River Labs, Portage Michigan.

Methods:

After a quarantine period of approximately 2 months 203 females were bred. Positive sperm findings on daily vaginal smears determined day 0 of gestation. Females with positive smears were randomly placed into 4 groups of 34 animals each. The groups were gavaged on days 6-15 of gestation with either 0, 200, 375 or 750 mg/kg of the test material suspended in corn oil at 4 ml/kg. Feed and water were supplied ad lib throughout the study. Photoperiods were 12 hrs., temperature was maintained at 72 ± 3° F with a relative humidity of 40%. Clinical observations of the individual dams were recorded daily through day 20 of gestation. Body wts. were recorded on days 0, 6, 10, 12, 15 and 20 of the study period. Uterine examination provided implantation sites, resorptions, post-implantation losses, and numbers of viable fetuses. Examination of the fetuses provided numbers of external malformations, anomalies and, crown-rump measurements. Visceral examination of approximately half the fetuses was carried out and the remaining fetuses were fixed in alcohol, stained and cleared for skeletal examination using Alizarin Red S.

Statistical evaluation of the parameters were carried out by the Laboratory as presented below: excerpted from study report.

"All statistical tests have been performed by a Digital Computer with appropriate programming as referenced below.

1. The fetal sex ratios were compared by the Chi-square test[6] with Yates' correction factor.

2. The number of litters with malformations were compared by Fisher's Exact Test[6].

3. The number of early and late resorptions, dead fetuses and post-implantation losses were compared by the Mann-Whitney U-test[6].

4. Mean number of corpora lutea, total implantations, viable fetuses, mean fetal and maternal body weight at each interval and maternal body weight gain were analyzed by a one-way analysis of variance, and Dunnett's test[7].

References to Statistical Tests in Submission:

6. BMPD - 79 Biomedical Computer Programs, University of California Press, Berkeley, California, 1979. pp. 612, 780, 781.

7. Steel, R. G. D. and Torrie, J. H., Principles and Procedures of Statistics, McGraw Hill, New York, New York, 1960. pp. 106, 107, 114."

Results:

Mean body wts. of only group 4 (750 mg/kg) were significantly reduced throughout the study following day 6 of gestation. However, mean body wt. changes within the 3 day-time intervals of 12-15 days were significantly reduced in all treated groups compared to controls ($p < .01$). Only 4/34 dams at the (HDT), 750 mg/kg, died during the study. These 4 dead females were also gravid. Pregnancy rates were similiar in all groups. Total resorptions of litters were not increased compared to controls. Sex ratios of pups were similar in all groups. Mean numbers of viable pups per litter were reduced in all treated groups compared to controls but were not significantly reduceds. Early resorptions were increased on a litter basis in group 4 to 1.2 ± 2.7 S.D. compared to 0.8 ± 0.9 S.D. in controls. Postimplantation losses were also slightly increased to 1.4 ± 0.9 S.D. and late resorptions were increased to 0.2 ± 0.4 S.D. compared to none in control. The values taken together suggest an embryotoxic effect at 750 mg/kg but were all nonsignificant. Developmental effects such as exencephaly, micro/or/anopthalmia or cleft palate, occurred in test groups at rates which were not dissimilar to those occurances noted in controls. Skeletal effects such as malaligned sternebrae were not significantly different from controls. Development variations, with the exception of occurances of unossified sternebrae #1 - #4, and reduced ossification of vertebral arches, were not signficantly increased when compared to controls.

Conclusion:

Fetotoxicity expressed as delayed or unossified bones exhibited an LEL of 750 mg/kg and a NOEL of 375 mg/kg. The test material was not teratogenic under the conditions of the study.

Maternal toxicity expressed as reduced body wt. gain was equivocal in all test groups except the HDT, which produced death in 4/34 as well as significant reductions in body wt. gains.

Core: Minimum.

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