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

008186

DEC 11 1990

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: 2,4-D BEE, 2,4-D TIPA, and 2,4-D IPA Developmental Toxicity Studies

TO: Ms. Judith H. Coombs, PM 74
OSRD (H7509C)

FROM: Karen E. Whitby, Ph.D. 12/4/90
Section, II
Toxicology Branch II/HED (H7509C)

THRU: E. Clark Swentzel 12/6/90
Section Head
Toxicology Branch II/(HED) (H7509C)

and

Kercia van Gemert, Ph.D. 12/6/90
Chief, Toxicology Branch II/(HED) (H7509C)

EPA ID No. 030002-2 (2,4-D TIPA/Caswell No. 315AE)
EPA Record No. 250,213 (2,4-D BEE/Caswell No. 315AI)
HED Project No. 0-1707 (2,4-D IPA/Caswell No. 315D)

Data Evaluation Reports for the subject developmental toxicity studies are attached.

Action Requested

Review rat teratology studies (§ 83-3) for 2,4-D BEE, TIPA, and IPA which are flagged for immediate review in the Registration Standard.

Study Findings & Conclusions

1) A Range-Finding Study to Evaluate the Toxicity of 2-Butoxyethyl Ester of 2,4-D in the Pregnant Rat. (ERID No. 136271-04)

Two test articles were administered at 0, 73, 145, and 218 mg/kg. Evaluation of fetal toxicity consisted of recording the number of live fetuses on day 16 at the time of cesarean. 2,4-D BEE produced fetotoxicity at 145 and 218 mg/kg as

1/29

4) A Teratogenicity Study in Rats with 2,4-D Triisopropanolamine (MRID No. 415271-02)

2,4-D TIPA was evaluated for maternal and developmental toxicity at 0, 32.5, 100, and 325 mg/kg. Maternal toxicity was evident at 32.5 mg/kg by decreased bodyweight gain days 6-9. At 325 mg/kg there was increased maternal mortality, decreased bodyweight gain, decreased corrected bodyweight gain, and decreased absolute liver weight. Developmental toxicity was observed at 100 mg/kg and above by increased skeletal malformations/variations.

Maternal NOEL = not determined
Maternal LOEL = 32.5 mg/kg (LDT)
Developmental Toxicity NOEL = 32.5 mg/kg
Developmental Toxicity LOEL = 100 mg/kg

Core Classification: Core Minimum Data

5) A Range-Finding Study to Evaluate the Toxicity of 2,4-D Isopropylamine Salt in the Pregnant Rat. (MRID No. 415271-06)

The test article was administered at 0, 63, 127, and 190 mg/kg. Evaluation of fetal toxicity consisted of recording the number of live fetuses on day 16 at the time of cesareans. There were significant reductions in maternal bodyweight gain and corrected maternal bodyweight gain at the high-dose. There was also a 77.1% increase in postimplantation loss.

Maternal NOEL = 127 mg/kg
Maternal LOEL = 190 mg/kg (HDT)
Developmental Toxicity NOEL = not assessed
Developmental Toxicity LOEL = not assessed

Core Classification: Supplementary Data

6) A Teratogenicity Study in Rats with 2,4-D Isopropylamine. (MRID No. 415271-03)

2,4-D IPA was evaluated for maternal and developmental toxicity at 0, 22, 65, and 190 mg/kg. Maternal toxicity was evident at 190 mg/kg by decreased bodyweight gain days 6-9, and decreased food consumption 6-11. There was mild developmental toxicity at 190 mg/kg indicated by skeletal variations.

Maternal NOEL = 65 mg/kg
Maternal LOEL = 190 mg/kg (HDT)
Developmental Toxicity NOEL = 65 mg/kg
Developmental Toxicity LOEL $\geq$ 190 mg/kg

Core Classification: Core Minimum Data

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GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D. K-Wh 11/16/90
Toxicologist, Review Section II, Toxicology Branch II/HED (H7509C)

Secondary Review by: K. Clark Swentzel K. Clark Swentzel 11/17/90
Section Head, Review Section II, Toxicology Branch II/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification Nos.: EPA MRID (Accession) No. 415271-03 /-06
EPA ID No. 030025-1
EPA Record No. 268,213
Caswell No. 315U
HED Project No. 0-1707

Test Material: 2,4-D Isopropylamine Salt

Synonyms: (2,4-D IPA)

Sponsor: The Dow Chemical Co., 1803 Toxicology Research Laboratory 1803 Building, Midland, MI 48674

Study Num Nos.: HET M-004725-011 (The Dow Chemical Co.,)
Project No. 89-3405 (Bio/dynamics, Inc.)

Testing Facility: Bio/dynamics, Inc., East Millstone, New Jersey 08873

Title of Report: A Teratogenicity Study in Rats with 2,4-D Isopropylamine

Author(s): Raymond E. Schroeder

Report Issued: May 7, 1990

Conclusions:

2,4-D IPA was evaluated for maternal and developmental toxicity at 0, 22, 65, and 190 mg/kg. Maternal toxicity was evident at 190 mg/kg by decreased bodyweight gain days 6-9, and decreased food consumption 6-11. There was mild developmental toxicity at 190 mg/kg indicated by skeletal variations. Although the incidence is not high in any of the studies, it is worthy of note that the 2,4-D TIPA (MRID No. 413271-02) 2,4-D BEE (MRID No. 413271-01) and the current study have detected ocular effects (anophthalmia, microphthalmia, and folded retina) that did

not occur at a high incidence. In addition, all studies found variations of the ureters (distended and/or tortuous) and dilated renal pelvises. In the current study dilated renal pelvis was noted in the mothers as well as the fetuses. It was also noted once in the mothers of each group in the 2,4-D TIFA main study (MRID No. 419271-02). It is not clear whether these effects are related to treatment or are a spontaneous occurrence.

Maternal NOEL = 65 mg/kg
Maternal LOEL = 190 mg/kg (HDT)

Developmental Toxicity NOEL = 65 mg/kg
Developmental Toxicity LOEL ≥ 190 mg/kg

Core Classification: Core Minimum Data

RANGE-FINDING STUDY

The main developmental toxicity study was preceded by a pilot study, which was performed to determine the maternal and embryotoxic potential of 2,4-D Isopropylamine Salt in the pregnant rat. Evaluation of developmental toxicity consisted of recording the number of live fetuses on day 16 at the time of cesareans. Both of these studies were performed by Bio/Dynamics.

Study Title: A Range-Finding Study to Evaluate the Toxicity of 2,4-D Isopropylamine Salt in the Pregnant Rat

Study No.: HRLS No.415271-06
Study ID: DOW K-004725-010 and DOW K-004725-010 (The Dow Chemical Co.) both appear on the title page
Project No. 89-2466 (Bio/Dynamics, Inc.)

Date of Report: March 20, 1990

Date of Study: (Live Portion) July 10, 1989 - August 9, 1989

Author: Raymond E. Schneider

Materials and Methods

2,4-D Isopropylamine Salt (Lot No. ASR 27646), purity 80.2% (the amount of test article added to the vehicle was corrected for purity) was mixed as a CD® (Sprague-Dawley derived) 9 rats (10/group) by gavage at dose levels of 0 (vehicle: distilled, deionized water), 61, 127, and 190 mg/kg. Test article was administered on days 6-19 of gestation. Dams were euthanized on day 16 of gestation; postmortem examination included weighing of the gravid uterus, kidneys, and liver; recording the number of corpora lutea and uterine implants (no. live, dead, and resorbed).

Results

There was no mortality in this study. Days 6-9 and 6-16 of gestation the mean bodyweight gain of the 190 mg/kg group was -50.3 and -28.3% relative to control, respectively. The difference was only significant days 6-16. The corrected maternal bodyweight gain days 6-16 of the 190 mg/kg group was significantly lower than control (-46.1%). The incidence of successful gestation was 0, 70, 100, and 100% among the 0, 61, 127, and 190 mg/kg groups. Two 2 low- and 3 mid-dose group animals were determined to be not pregnant. There was an increase (125%) in the number of resorptions in the high-dose group relative to the control that was reported to be not significant. As such, there was an increase (77.1%) in the postimplantation loss that was also reported to be not significant.

Conclusions

The dosage levels of 2,4-D IPA for the main developmental toxicity study were 0, 22, 65, and 190 mg/kg. Based upon the treatment related effects observed in this study, the dosages selected for the main study appeared to be appropriate.

Core Classification: Supplementary Data (Range-Finding Study)

Main Study

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 50.2%
Description: Dark Brown Liquid
Lot No.: AGR 276461

Vehicle(s): Identity: Distilled, deionized water

Test Animal(s): Species: Rat
Strain: CD® (Sprague-Dawley derived)
Source: Charles River Laboratories, Inc., Portage, MI 49081
Age at Initiation of Mating: ♀ 63 days
♂ approx. 43 weeks
Weight of ♀ on day 0: (mean) 200 g
(range) 167 - 254 g

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Isopropylamine Salt when administered by gavage to rats on gestation days 6 through 15, inclusive.

Mating

Females were placed with males nightly in a 1:1 ratio. Animals not utilized in the study that remained from the original population were sacrificed and discarded after all study groups had been filled and the last mated females within study groups had initiated dosing.

Group Assignment:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	30
Low Dose	22	30
Mid Dose	65	30
High Dose	190	30

Dosing:

All doses were in a volume of 5 ml/kg of body weight/day prepared once prior to initiation of treatment in a volume adequate for the duration of the study. When the dosing solutions were prepared, the amount of test article added to the vehicle was adjusted to correct for the

purity of the test article. The dosing solutions were analyzed for concentration, homogeneity, and stability. Concentrations of the test chemical ranged from 98.5 to 102.1% of the target concentration at the beginning of dosing. Upon the conclusion of the dosing period, the range of concentration was 100.1 to 110.4%. The coefficient of variation was within 3%, indicating homogeneity. The dosing solution was found to be stable for at least 51 days (± 10% of the day 0 concentration). Initial dose volumes were based on gestation day 6 bodyweights and were adjusted for each animal during the remaining portion of treatment to the most recent bodyweight.

Observations

Maternal

The animals were checked for mortality or abnormal condition twice daily. In addition, each female was given a detailed physical examination on days 0, 6-18, and 20 of gestation. Dams were sacrificed on day 20 of gestation by overdose of carbon dioxide inhalation. Examinations at necropsy consisted of: the external surface, all orifices, the cranial cavity, exposed, external surface of the spinal cord and exposed surfaces of the brain, nasal cavity and paranasal sinuses, the thoracic, abdominal, and pelvic cavities, viscera, kidney and liver weights cervical tissues and organs.

Maternal bodyweights were recorded on days 0, 6, 9, 12, 16, and 20 of gestation. Food consumption was recorded on days 0-6, 6-11, 11-16, and 16-20.

Reproductive

The intact uterus including ovaries was removed and weighed. The number and location of the following was recorded for each uterine horn: live fetuses, dead fetuses, late resorptions, early resorptions, and implantation sites. The ovaries were dissected free and the number of corpora lutea were recorded. If uterine implantations were not evident, the uterus was stained with ammonium sulfide, according to the method of Salewski, 1964.

Fetal

The fetuses were examined in the following manner: each fetus was individually identified, weighed, sexed externally, and examined for gross external malformations/variations. Approximately one-half of the fetuses of each litter (every other fetus within the litter) were evaluated for visceral malformations/variations via a modified Staples, 1974 technique. Fetuses designated to undergo the visceral exam were decapitated and the heads were fixed in Bouin's for subsequent examination. The fetuses were secured under a dissecting scope, and the thoracic and abdominal cavities were examined. Upon completion of this, the fetuses were eviscerated and individually placed in 70% ethanol. The heads were serially transverse sectioned using a razor

blade after fixation was complete. The sections were ob... malformations of the palate, eyes, and brain via dissection...

The remaining fetuses in each litter were euthanized v... inhalation of ether. The fetuses were eviscerated (internally sexed), and stained with Alizarin Red S via a procedure (Crary, 1962) modified by Dawson. Skeletal specimens were evaluated under a dissecting scope for ossification malformations and variations.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

The interval data (mean bodyweights, mean weight change, gravid uter... weight, day 20 corrected bodyweight, mean food consumption, mean o... weight, and reproduction data) were analyzed by the following proc... Bartlett's test was performed to determine if groups had equal variance. If so, a standard one way ANOVA using the ... distribution to assess significance was performed. If w... ted, Dunnett's test was used to determine which means were significantly different from the control. When necessary, the Kruskal-Wallis test ... was used as a nonparametric test of equality of means. If differences were found, a ranked rank test (Dunn) was used to determine which groups differed from the control. A test for trend in the dose levels was also performed. For parametric data, standard regression test ... with a test for trend and lack of fit were used. For nonparametric data, Jonckheere's test for monotonic trend was used. All ratios were transformed via the arc sine transformation before analysis.

The incidence data (litters with resorptions, mortality rates, pregnancy rates, fetuses with malformations/variations, and litters with fetuses with malformations/variations) were analyzed as follows. First, a standard chi-square analysis was performed to determine if the proportion of incidences differed between the groups tested. Each treatment group was compared to the control using a two Fisher Exact test. The significance level was corrected via the Bonferroni inequality. Jonckheere's test for linear trend in the dose groups was performed. If any one cell had an expected value less than 5, the chi-square and Jonckheere's tests were not reported. When this occurred, only the Fisher Exact test (corrected via Bonferroni inequality) was performed and reported.

Compliance

A signed statement of no data confidentiality claims was provided.

A signed statement of compliance with EPA GLP's was provide...

A signed Flagging Statement was provided.

A signed Quality Assurance Statement was provided.

C. Results

Mortality

There was no mortality in the control, low-, or mid-dose groups. In the high dose group one female died on day 7 of gestation (after one day of treatment).

Clinical Observations

The incidence of excessive salivation was noted at least once during the treatment period for the 0, 22, 66, and 190 mg/kg groups were 0, 0, 16.7, and 500, respectively. Treatment alopecia was noted in 0, 16, 3.3, and 20% of the animals in the 0, 22, 66, and 190 mg/kg groups, respectively.

Bodyweight

The investigators supplied the following data:

Table 1 Bodyweight Gains (grams)*

Group (mg/kg):	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains[1] During P.	Entire[2]
0	21	39	82	142.0	-19.5	70.4
22	31	55	59	145.0	-30.1	69.9
66	31	51	58	140.4	-19.6	69.4
190	31	40	96	135.6	-18.7	68.9

[1] corrected bodyweight gain for dosing period = bodyweight gain for dosing period minus gravid uterus weight.
[2] corrected bodyweight gain for entire gestation period = bodyweight gain for entire gestation period minus gravid uterus weight.
[Note of the above values 1 to calculated by the reviewer from individual animal data]

* Data extracted from (study number MRL A-004788-011 Appendices C, D, etc.) 8 pp. 12-67)

Mean bodyweight gain for the pre- and post-treatment periods of gestation were comparable between the treated and control groups. Days 6-8 of gestation the mean weight gain of the 190 mg/kg group was significantly lower than the control (-37.1g).

Feed Consumption

The investigators supplied the following data:

Table 9 Feed Consumption Data (g/kg/day)[a]

Groups: (mg/kg)	Days 0-6	Days 6-11	Days 11-16	Days 16-20	Days 0-20
0	114	102	103	96	414
22	111	101	100	96	406
65	115	102	102	96	414
169	112	93*	103	99	408

[a] Data extracted from (study number MRC K-004726-011 appendix F pg. C8-73)
* Significantly different from control ($p \leq 0.01$)
(some of the above values were calculated by the reviewer from individual animal data)

The only significant finding for the feed consumption data was in the 169 mg/kg group days 6-11, where the amount consumed was -5.8% relative to the control.

Organ Weight Data

There were no significant changes in the absolute and relative liver and kidney weights.

Gross Pathological Examination

Gross pathological examination did not reveal any treatment related effects. Most of the observations noted were similar among the groups. The incidence of dilated renal pelvis was 1/19, 2/20, 2/20, and 2/53 in the 0, 22, 65, and 169 mg/kg groups, respectively.

Treatment Related Observations

Table 3 Observed Toxicity Observations*

Dose (mg/kg):	0	22	65	190
Animals Assigned	30	30	30	30
Animals Mated/Inseminated	29	27	26	26
Pregnancy Rate (%)	26.7	80.0	93.3	96.7
Maternal Deaths				
Fetal	0	0	0	1
Fetal/pregnant	0	0	0	1/26
Post Dosing	1	3	2	3
Aborted	0	0	0	0
Premature Delivery	0	0	0	0
Total Corpora Lutea	432	407	409	361
Corpora Lutea/Dam	14.9	15.1	14.6	15.0
	±1.7	±3.1	±2.0	±1.9
Total Implantations	389	363	391	324
Implantations/Dam	13.9	14.2	14.0	13.5
	±3.4	±3.6	±3.1	±2.7
Total Live Fetuses	370	355	364	302
Live Fetuses/Dam	13.1	13.9	13.0	12.6
	±3.7	±3.7	±2.5	±3.1
Resn Rt. M/Litter	6.7	7.8	8.9	6.6
Resn Rt. F/Litter	6.4	6.3	7.1	6.2
Total Resorptions	23	10	27	22
Resorptions/Dam	0.8	0.7	1.0	0.9
	±0.9	±0.6	±0.9	±0.9
Total Dead Fetuses	0	0	0	0
Mean Fetal Weight (gm)	3.51	3.54	3.45	3.37
♂ Fetuses	3.57	3.60	3.51	3.44
	±0.2	±0.2	±0.2	±0.2
♀ Fetuses	3.45	3.47	3.40	3.29
	±0.2	±0.2	±0.2	±0.2
Preimplantation Loss(%)	6.3	9.3	6.4	9.9
Postimplantation Loss(%)	5.7	4.7	7.4	5.6
Sex Ratio (total ♂/total ♀)	1.1	1.1	0.8	1.0

* Data extracted from (study number MRL-6041715-011 appendix G pp. 71-78)

One female in the high dose group was noted to have one fecus upon staining of the uterus with ammonium sulfi[illegible] and was included in calculation of the pregnancy rate; however [illegible] data for this female were excluded from the calculation of the implantation data.

Statistical evaluation of the corpora lutea and uterine implantation data did not reveal any differences between the control and treated groups. In the high dose group there was one female that totally resorbed a litter of 4. Omission of this data point results in a postimplantation loss of 3.5%. There was a slight treatment related decrease (-4.4% for the 190 mg/kg group relative to the control) in male fetal bodyweights.

2. Developmental Toxicity

Four fetuses with external malformations in the high dose group were processed for both visceral and skeletal malformations. These fetuses were not decapitated and the visceral evaluation was restricted to microdissection and examination of the tissues in the thoracic, abdominal and pelvic cavities.

One fetus in the 65 mg/kg group had a hindlimb flexure and a constriction at the base of the tail. The 2 fetuses in the 190 mg/kg group with omphalocele and exencephaly were from the same litter. No external variations were noted in any of the groups, except for one fetus in the 65 mg/kg group that had shiny skin and was very small.

Table 6 External Examinations

Observations*	Control	Low Dose	Mid Dose	High Dose
External Malformations[a] groups(litters) examined	380(29)	369(27)	364(28)	302(23)
Filarentous Tail	0(0)	0(0)	0(0)	1(1)
Constriction of Tail	0(0)	0(0)	1(1)	0(0)
Hindlimb Flexure	0(0)	0(0)	1(1)	0(0)
Omphalocele (abdominal viscera protruding)	0(0)	0(0)	0(0)	1(1)
Exencephaly (brain tissue protruding)	0(0)	0(0)	0(0)	1(1)
Total External Malformations				
fetal incidence N(%)	0(0)	0(0)	1(0.3)	3(1.0)
litter incidence N(%)	0(0)	0(0)	1(3.6)	2(8.7)

* some observations may be grouped together
[a] fetal (litter) incidence
Data taken from study number MRT E-004725-011 appendix L p. 230

The most common visceral malformation observed was folded retina. This was observed only in one litter of the control group. Therefore, given the sporadic nature of the observed malformations which were not treatment related it would appear that treatment of pregnant rats with 22, 65, and 190 mg/kg of 2,4-D Isopropylamine salt did not result in visceral ~~malformations~~.

Table 5 Visceral Examinations

Observations[a]	Control	Low Dose	Mid Dose	High Dose
Visceral Malformations[b]				
Group(litters) examined	199(29)	191(27)	189(28)	197(23)
Retina(e) - folded	3(1)	1(1)	1(1)	0(0)
Anophthalmia	0(0)	0(0)	0(0)	1(1)
Total Visceral Variations				
fetal incidence N(%)	3(1.5)	1(0.5)	1(0.5)	1(0.6)
litter incidence N(%)	1(3.4)	1(3.7)	1(3.6)	1(4.3)
Visceral Variations[b]				
Group(litters) examined	199(29)	191(27)	189(28)	197(23)
Kidney(s) - distended renal pelvis (papilla present)	0(0)	2(2)	3(2)	1(1)
Ureter(s) - tortuous	8(4)	11(6)	9(2)	3(1)
Ureter(s) - distended	3(3)	0(0)	0(2)	1(1)
Total Visceral Variations				
fetal incidence N(%)	8(4.0)	11(5.8)	13(6.9)	3(1.5)
litter incidence N(%)	5(17.2)	6(22.2)	4(14.3)	2(8.7)

[a] some observations may be grouped together
[b] fetal (litter) incidence
Data taken from study number MRI K-004725-011 appendix N pp. 288-292

The most common visceral variation was distended and/or tortuous ureters. The fetal incidence for this variation did not indicate a treatment related effect.

Table 6 Skeletal Examinations

Observations[a]	Control	Low Dose	Mid Dose	High Dose
Skeletal Malformations[b]				
Group(litters) examined	182(29)	174(27)	176(28)	148(23)
Kole in Cranium	0(0)	0(0)	0(0)	1(1)
Reduced Number of Cervical Vertebra(ae)	0(0)	0(0)	1(1)	0(0)
Cervical Transverse Process(es) - misshapen	0(0)	0(0)	1(1)	0(0)
Presence of Cervical Rib(s)	0(0)	0(0)	0(0)	1(1)
Skeletal Variations[b]				
Group(litters) examined	182(29)	174(27)	176(28)	148(23)

Table 6 Skeletal Examinations - cont'd

Observations*		Control	Low Dose	Mid Dose	High Dose
Squamosal(s) - inc. oss.		9(6)	19(12)	15(7)	20(10)
Malar(s) -inc. oss.		7(6)	15(9)	10(5)	12(8)
Parietal(s) - inc. oss.		3(2)	5(3)	11(6)	8(4)
Frontal(s) - inc.oss.		1(1)	1(1)	3(3)	4(2)
Nasal(s) - inc. oss.		1(1)	4(3)	6(3)	8(4)
Cervical Transverse Process(es) - inc. oss.		4(3)	12(7)	10(7)	17(11)
Ossification Adj. to 7th Cervical Vertebra(ae)		3(2)	2(2)	2(2)	6(5)
Rib(s) - Rudimentary		0(0)	1(1)	0(0)	2(2)
Rib(s) - 1st Lumbar Rudimentary		7(5)	5(4)	8(7)	15(6)
3rd Sternebra - inc. oss.		11(5)	8(5)	4(3)	11(6)
Total Skeletal Variations					
Fetal incidence	N(%)	153(82.2)	143(82.2)	150(89.8)	138(86.5)
Litter incidence	N(%)	28(96.6)	27(100)	28(100)	23(100)

* some observations may be grouped together
° fetal (litter) incidence
Data taken from study number MRT R-034720-611 appendix N pp. 283-351

Skeletal malformations were seen in the one mid-dose and one high-dose fetus noted externally with tail defects. The mid-dose fetus had a constriction at the base of the tail and did not have ossified vertebral elements posterior to the third lumbar vertebra. The high-dose fetus had a filamentous tail and no ossified vertebral elements posterior to the second caudal vertebra. A hole in the cranium in the area of the frontals and parietals was noted in the high-dose fetus with exencephaly. The incidence of fetuses with at least one skeletal variation was 100% in each of the treatment groups and 96.6% in the control. There was a slight treatment related increase in the incidence of incompletely ossified squamosal(s), nasal(s), and cervical transverse process(es). In addition, there was a slightly increased incidence of ossification adjacent to the 7th cervical vertebra(ae). These effects are most prominent at the high-dose.

D. Discussion/Conclusions

a. Maternal Toxicity:

In the 180 mg/kg group there was one death on day 7 of gestation. Other than this there were no deaths in this study. Days 6-9 of gestation there was a significant decrease in maternal bodyweight at 180 mg/kg. There was a significant decrease in food consumption for this group days 6-11 of gestation. Also at 180 mg/kg, excessive

salivation was noted in 50% of the animals.

b. Developmental Toxicity:

i. Deaths/Resorptions:
There were no fetal deaths. The number of resorptions per litter was comparable among the treatment groups.

ii. Altered Growth:
There was a slight reduction in the mean body weight of viable fetuses and of male fetuses at 190 mg/kg.

iii. Developmental Anomalies:
At 190 mg/kg, there was a slight increase in the incidence of incompletely ossified squamosal(s), nasal(s), and cervical transverse process(es). In addition, there was a slight increased incidence of ossification adjacent to the 7th cervical vertebra(ae).

iv. Malformations:
The distribution and incidence of the malformations noted in this study do not indicate a treatment related effect.

2. Study Deficiencies:

The title page for this report has two study ID #'s MRT K-004725-011 and FDRL K-004725-011. More recent historical control data than 1982-1987 would be helpful as this study was conducted in late 1989.

3. Study Classification: Core Minimum Data.

This study satisfies the guideline requirements (83-3) for a developmental toxicity study.

Maternal NOEL = 65 mg/kg
Maternal LOEL = 190 mg/kg (MDT) based upon decreased bodyweight gain days 6-9 and decreased food consumption days 6-11.

Developmental Toxicity NOEL = 65 mg/kg
Developmental Toxicity LOEL = 190 mg/kg based upon slight increases in skeletal variations.

4. Risk Assessment: None at this time.

RIN 2465-01

2,4-D DER

Page_____ is not included in this copy.

Pages 17 through 44 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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GUIDELINE: 83-3

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DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No. 415271-01
EPA ID No. 030025-1
EPA Record No. 260,213
Caswell No. 315AI
HED Project No. 0-1707

Test Material: 2-Butoxyethyl Ester of 2,4-D

Synonyms: 2,4-D BEE

Sponsor: The Dow Chemical Co., The Toxicology Research Lab., 1803 Building, Midland MI

Study Number(s): MRT K-007722-017 (The Dow Chemical Co.)
Project No. 89-3467 (Bio/Dynamics)

Testing Facility: Bio/dynamics, Inc., East Millstone, New Jersey 08875

Title of Report: A Teratogenicity Study in Rats With 2-Butoxyethyl Ester of 2,4-D

Author(s): Raymond E. Schroeder

Report Issued: May 9, 1980

Dates of Laboratory Phase: December 6, 1979 to January 11, 1980

Conclusions: Maternal toxicity was evidenced at 185 mg/kg by significant effects on body weight gain, food consumption, and hematology. Developmental toxicity was observed at 75 mg/kg and above as skeletal variations.

Maternal NOEL = 75 mg/kg
Maternal LOEL = 185 mg/kg
Developmental Toxicity NOEL = 25 mg/kg
Developmental Toxicity LOEL = 75 mg/kg

Core Classification: Minimum

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: 2,4-D Butoxyethyl Ester (2,4-D BEE), AGR276426
Purity: 95.6%
Description: dark brown liquid
Lot No.: AGR 276426
Contaminant: none provided

Vehicle( ): Mazola® Corn Oil
Purity: 100%
Description: clear yellow liquid
Lot No. SEP 26 90S
Contaminant: none provided

Test Animal(s): Species: Rat
Strain: CD® (Sprague-Dawley derived)
Source: Charles River Laboratories, Inc.
Portage, MI 49081
Age at Initiation of Mating: ♀ 57 days/♂ 48 weeks
♀ Weight: Range on Day 0, 169-275 g (mean = 218 g)

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Butoxyethyl Ester when administered by gavage to rats on gestation days 6 through 15, inclusive.

Mating

Females were mated with males nightly in a 1:1 ratio. Animals not utilized in the study that remained from the original population were sacrificed and discarded after all study groups had been filled and the last mated females within study groups had initiated dosing.

Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	30
Low Dose	25	30
Mid Dose	75	30
High Dose	150	30

Dosing:

The control group received the vehicle (corn oil) by gavage. All doses were administered in a volume of 5 ml/kg, and were prepared once prior to initiation of treatment with sufficient quantity prepared to accommodate the entire dosing regimen. The volume of test article used for the preparation of the dosing solutions was adjusted to compensate for the purity. The dosing solutions were analyzed for concentration, homogeneity, and stability. Initial dose volumes were derived from day 6 body weights and were adjusted for each animal during the remaining portion of the treatment period to the most recent body weights.

Observations

Animals were checked twice daily for signs of obvious pharmacologic or toxicologic effects and mortality/morbidity. Females were given an additional detailed exam on gestation days 0, 6-15, and 20. Dams were sacrificed on day 20 of gestation. Animals were weighed on days 0, 6, 9, 12, 16, and 20. Food consumption was recorded days 0-6, 6-11, 11-16, and 16-20. Bodyweight was used to calculate the grams of food consumed/kg of bodyweight/day.

A gross post mortem exam was performed. Examinations at sacrifice consisted of: the external surface, all orifices, the cranial cavity, carcass, the external surface of the spinal cord, and sectioned surfaces of the brain, nasal cavity and paranasal sinuses; thoracic, abdominal and pelvic cavities, viscera, cervical tissues and organs, and kidney and liver weights. The intact uterus and ovaries were removed from the abdominal cavity, weighed and then number and location of live fetuses, dead fetuses, late resorptions, early resorptions, corpora lutea, and implantation sites were recorded. In the event implants were not evident, the uterus was stained with ammonium sulfide. Females found to have implants when observed in this manner were included as pregnant; the number of foci noted for these females were not included in the calculation of uterine implantation data.

The fetuses were examined in the following manner. Each fetus was individually identified, weighed, sexed, and examined for external malformations/variations (including palatal defect).

Approximately one half of the fetuses in each litter were examined for visceral defects using a technique similar to that of Staples (Staples, 1974), shortly after removal from the uterus. The fetuses were decapitated, and the heads were fixed in Bouin's for subsequent exam by razor blade sectioning. Upon completion of the exam of fetal tissues in the thoracic and abdominal cavities under a dissecting scope, the viscera were removed and placed in 70% ethanol for fixation.

The other half of the fetuses were euthanized with ether, eviscerated (internally sexed), and processed for staining by Alizarin Red S (Crary, 1962) via a method modified by Dio/dynamics.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

Mean maternal bodyweight, bodyweight change, gravid uterus weight, day 20 corrected weights, net maternal bodyweight change, mean food consumption, mean organ weight data (absolute and relative to corrected day 20 bodyweight), and reproduction data were statistically evaluated as follows. First, Bartlett's test was performed to determine if the groups had equal variance. If the variances were equal, parametric tests (standard one way ANOVA using the F distribution) were used; if not, nonparametric procedures were used. Finally, Dunnett's test was used to determine which means (when appropriate) were significantly different from the control. The Kruskal-Wallis test was used to determine which treatments differed from control for testing the equality of means by a nonparametric procedure.

The incidence of litters with resorption sites, mortality rates, pregnancy rates, and incidences of malformations/variations was first analyzed by a standard chi-square to determine if the proportion of incidences differed between the groups tested. Next, each treatment group was compared to the control group using a 2X2 Fisher Exact test; the significance level was corrected via the Bonferroni inequality to assure an overall test of the stated significance level. Finally, Armitage's test for linear trend in the dosage groups was performed. If any one cell had an expected value less than 5, the chi-square and Armitage's tests were not reported. In this instance, only the Fisher Exact test (corrected via Bonferroni inequality) was performed and reported.

Compliance

A signed Statement of No Confidentiality Claims was provided.

A signed Good Laboratory Practice Statement (EPA GLP's) was provided.

A signed Flagging Statement was provided.

A signed Quality Assurance Statement was provided.

C. Results

Maternal Toxicity

Mortality

There was no mortality in the control or treated groups.

Clinical Observations

Excessive salivation was seen with increased incidence, particularly among the animals at the mid- and high-dose groups. The incidences of females with excessive salivation noted at one or more intervals during the treatment period for the control, low-, mid-, and high-dose groups were 0%, 6.7% (2/30), 33.3% (10/30), and 56.7% (17/30), respectively.

One non-pregnant female in the high dose group was noted to exhibit a pale, emaciation, decreased food consumption, and poor condition on day 11 of gestation (6 days after initiation of treatment). Gross postmortem observation did not reveal any lesions that explained the deterioration of the animal.

Bodyweight

The investigators supplied the following data:

Table 1: Bodyweight Gains (grams)[a]

Group mg/kg:	Days 0-6	Days 6-16	Days 16-20	Days 0-20	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
0	37	64	60	169	-14.0	91.2
25	42	60	72	174	-20.2	93.3
75	37	62	70	169	-16.0	89.7
185	36	56*	68	160	-29.5	84.6

[1] = corrected bodyweight gain for dosing period = bodyweight gain days 6-16 minus gravid uterus weight.
[2] = corrected bodyweight gain for entire gestation period = body weight gain days 0-20 minus gravid uterus weight.
[a] = Data extracted from Report MRI 7-007723-017 Appendices C, D, and E (pp. 57-71)
* Significantly different from control ($p \leq 0.05$).
[Some of the above values were calculated by the reviewer from individual animal data]

Food Consumption

The investigators supplied the following data:

Table 2: Mean Maternal Food Consumption (g/kg/day)[a]

Group mg/kg:	Days 0-6	Days 6-11	Days 11-16	Days 16-20	Days 6-20	Days 0-20
0	101	87	94	92	273	374
25	103	82	90	93	271	378
75	106	83	92	94	269	375
185	106	80**	90	92	261	367

[a] Data extracted from Report NET K-007722-017 Appendix F (pp.72-76).

** Significantly different from control ($p \leq 0.01$).

Hematology Data

The majority of the hematological parameters (Table 3) were comparable within the groups, except for the RBC count, which was significantly lower than control (-4.4%). This was also observed in the dose-range finding study. In addition, an increase in the reticulocyte count (447.4% was noted in the high dose group relative to the control, which was statistically significant.

Table 3 Mean Hematology Values[a]

mg/kg	0	25	75	185
Hgb (g/dl)	13.0	12.9	12.7	12.7
Hct (%)	38	38	38	35
RBC (mil/ul)	9.72	9.69	9.55	9.47*
Plat (100 T/ul)	13.29	11.94	12.73	12.41
WBC (thous/ul)	12.1	12.1	13.3	12.4
Retic (% RBC)	1.9	2.2	2.1	2.6**

[a] Data taken from Report NET K-007722-017 Appendix J (p.100)

* Significantly different from control ($p \leq 0.05$)
** Significantly different from control ($p \leq 0.01$).

Organ Weight Data

Absolute and relative organ bodyweights (corrected to day 20 gestation bodyweight) were similar among treated and control

Gross Pathological Observations

Gross pathological findings did not indicate an adverse treatment related effect.

Cesarean Section Observations

Table 4: Cesarean Section Observations[a]

Dose (mg/kg):	0	25	75	185
#Animals Assigned	30	30	30	30
#Animals Mated/Inseminated	26	28	30	27
Pregnancy Rate (%)	86.7	93.3[b]	100	90.0
Maternal Wastage				
#Died	0	0	0	0
#Died/pregnant	0	0	0	0
#Non pregnant	4	2	0	3
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	420	435	483	424
Corpora Lutea/dam	16.2 ±2.8	16.1 ±2.0	16.1 ±2.0	15.7 ±3.0
Total Implantation	390	399	451	397
Implantations/Dam	14.9 ±3.4	14.8 ±1.8	15.0 ±2.0	14.7 ±2.1
Total Live Fetuses	363	379	431	375
Live Fetuses/Dam	13.9 ±3.4	14.0 ±1.9	14.4 ±1.9	13.9 ±2.9
Total Resorptions	26	20	20	22
Resorptions/Dam	1.0 ±1.0	0.7 ±0.9	0.7 ±1.0	0.8 0.9
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (gm)	3.53 ±0.34	3.46 ±0.19	3.62 ±0.33	3.44 ±0.23
Males	3.68 ±0.31	3.75 ±0.18	3.72 ±0.20	3.55 ±0.26
Females	3.43 ±0.33	3.53 ±0.20	3.53 ±0.24	3.33 ±0.22
Preimplantation Loss(%)	9.0	7.8	6.4	6.0
Postimplantation Loss(%)	7.6	5.0	4.3	5.4
Sex Ratio (total ♂/total ♀)	0.8	1.1	0.9	1.0

[a] Data extracted from Report NET K-007128-617 Appendix G (pp.

77-81)

[b] One female was noted to have one focus upon staining of the uterus with ammonium sulfide and is included in calculation of pregnancy rate, but the data for this female was excluded from calculation of uterine implantation data.
<<Some of the above values were calculated by the reviewer from the individual animal data.>>

There was no significant treatment related effect on the parameters investigated.

2. Developmental Toxicity

External Malformations

Table 8: External Examinations[a]

Observations	0 mg/kg	25 mg/kg	75 mg/kg	185 mg/kg
fetuses(litters) examined	362(26)	379(27)	431(30)	375(27)
malformations fetuses(litters) affected	0	1(1)	0	1(1)
variations fetuses(litters) affected	1(1)	0	0	1(1)

[a] Data extracted from Report MRT K-007722-017 Appendix G (pp. 77-81)

The incidences noted in the above table did not differ statistically between the control and treated groups. The fetus in the low-dose group that was malformed did not have normal sized eye bulges (upon visceral exam, right-sided microphthalmia and left sided anophthalmia was observed (see visceral examinations below)). The malformations noted in the fetus in the high-dose group included: filamentous tail; abnormal curvatures of the spine; misshapen jaw; and defect of the urethral/anal opening (upon visceral exam this same fetus was noted to have fused and ectopic kidneys).

A fetus in the control group had a shiny appearance, was quite small for the litter (1.5 g), and had a constriction on the genital tubercle. The fetus in the high dose group with multiple external malformations also had a shiny appearance, and was quite small for the litter (1.6 g). The shiny appearance noted in these fetuses was attributed to the thin translucent skin of these developmentally retarded fetuses.

Visceral Examination

Table 6: Visceral Examinations[a,b]

Observations	0 mg/kg	25 mg/kg	75 mg/kg	185 mg/kg
Visceral Malformations				
#pups(litters) examined	188(26)	196(27)	226(30)	195(27)
Retina(e) - folded	0	1(1)	0	0
Micro ophthalmia	0	1(1)	0	1(1)
Anophthalmia	0	1(1)	0	0
Distension of the Lateral Cerebral Ventricle(s)	0	0	0	1(1)
Distension of the Third Cerebral Ventricle	0	0	0	1(1)
Abn. Pattern of Aortic Arch Vessels	0	0	1(1)	0
Kidney - fused	0	0	0	1(1)
Kidney - ectopic	0	0	0	1(1)
Total Visceral Malform.				
fetal incidence N(%)	0	2(1.0)	1(0.4)	3(1.5)
litter incidence N(%)	0	2(7.4)	1(3.3)	3(11.1)
Visceral Variations				
#pups(litters) examined	188(26)	196(27)	226(30)	195(27)
Kidney(s) - Distended Renal Pelvis (Papilla Present)	0	2(2)	1(1)	1(1)
Ureter(s) - Tortuous	2(1)	2(2)	1(1)	4(4)
Ureter(s) - Distended	2(1)	2(2)	2(1)	1(1)
Total Visceral Variations				
fetal incidence N(%)	4(2.1)	3(1.5)	3(1.3)	5(2.6)
litter incidence N(%)	2(7.7)	3(11.1)	1(3.3)	4(14.8)

[a] fetal (litter) incidence

[b] Data from Report MRI N-001738-017 Appendix N (pp. 293-296)

The incidences of the visceral malformations for the treated groups did not differ statistically from the controls.

With the exception of the ocular malformations (anophthalmia and microphthalmia) discussed above in the section on external malformations, the only other malformation noted in the low-dose group was a folded retina. This was observed in a fetus of a

different litter, than the one exhibiting the other ocular defects.

In the high dose group, the 3 fetuses exhibiting visceral malformations are of different litters.

There were no statistically significant treatment related differences found for the visceral variations noted in the above table. Distended and/or tortuous ureters were the most commonly seen visceral variation in this study. Renal variations noted in the above table found by this laboratory in CD rats during 12/30-1/15/87, were such that: 0/163 fetuses (from 22 litters) exhibited distended renal pelvis only, 3/163 fetuses (in 2/22 litters) exhibited distended renal pelvis and distended ureter, and 3/163 fetuses (in 1/22 litters) exhibited distended or tortuous ureter. The historical control data has been appended.

Skeletal Examinations

The incidences of skeletal malformations in the treated groups did not differ statistically from the control when the analyses were performed on the litter or fetal basis. In the mid-dose group, one fetus was observed to have a cervical rib, another fetus in a different litter was found to have wavy ribs. These findings were not noted in the high-dose group. With the exception of the fused cervical transverse process, all skeletal malformations noted in the high-dose group occurred in one fetus. This was the same fetus observed to have numerous malformations during the external exam (shiny appearance, filamentous tail, misshapen lower jaw, and a defect of the urethral/anal opening). Data for this fetus appears in the results of both the visceral and skeletal findings (although not stated, it is apparent that the fetus was not decapitated, and the head fixed in Bouin's and subsequently sectioned, as was standard procedure following the visceral exam). The historical control data for the CD rat, from Bio/dynamics during 1982-1987 Appendix G (p. 446) indicates that of 4,470 fetuses (527 litters) in 23 study groups, indicates that 27 fetuses in 21 litters with malformed fetuses were observed to have wavy ribs. Similarly, the historical incidence for cervical rib was 1 fetus.

Because of the numerous skeletal variations which were observed in this study, only those that occurred with notable frequency are reported in the table.

Table 2: Skeletal Examinations

Observations	0 mg/kg	25 mg/kg	75 mg/kg	185 mg/kg
Skeletal Malformations				
#pups(litters) examined	174(25)	183(27)	205(30)	181(27)
Mandible - fused	0	0	0	1(1)
Cervical Rib(s)	0	0	1(1)	0
Cervical Transverse Process - fused	0	0	0	1(1)
Thoracic Transverse Process - fused	0	0	0	1(1)
Thoracic Vert. 6 Corresp. Ribs - absent	0	0	0	1(1)
Lumbar Vert. - absent	0	0	0	1(1)
Sacral Vert. - absent	0	0	0	1(1)
Caudal Vert. - absent	0	0	0	1(1)
Ribs - Wavy	0	0	1(1)	0
Ilia - misaligned	0	0	0	1(1)
total skeletal malformations				
Fetal Incidence N(%)	0	0	2(1.0)	2(1.1)
Litter Incidence N(%)	0	0	2(6.7)	2(7.4)
Skeletal Variations				
#pups(litters) examined	174(25)	183(27)	205(30)	181(27)
Incompletely Ossified				
Interparietal	20(13)	43(19)	49(16)	45(19)
Supraoccipital	14(6)	20(10)	27(11)	28(12)
Squamosal(s)	7(7)	9(6)	19(13)	26(14)
Malar(s)	4(3)	5(4)	17(8)	9(6)
Maxilla	0	2(2)	8(4)	6(4)
Nasal(s)	2(2)	4(4)	10(5)	5(4)
Parietal(s)	5(4)	6(4)	9(5)	8(7)
4th Sternebra	12(6)	9(7)	21(9)	20(11)
Not Ossified				
Hyoid	39(19)	36(17)	48(19)	51(23)
5th Sternebra	53(20)	32(17)	47(18)	67(23)
Presence of 14th rib pairs	1(1)	1(1)	0	4(3)
Rib(s) - short	3(3)	2(2)	4(3)	10(5)
Rib(s) - rudimentary	2(1)	1(1)	3(3)	8(5)
Rib(s) - 1st lumbar rudimentary	13(10)	16(10)	42(21)	45(15)
Rib(s) - 14th unilateral	2(1)	1(1)	4(3)	8(6)

Table 7: Skeletal Examinations - cont'd

Observations*	0 mg/kg	25 mg/kg	75 mg/kg	185 mg/kg
total skeletal variations				
fetal incidence N(%)	138(79.3)	133(72.7)	167(81.5)	102(89.5)**
litter incidence N(%)	25(100)	27(100)	29(96.7)	27(100)

* some observation may be grouped together
a fetal [litter] incidence
* fetuses with at least one ossification variation
** significantly different from control (P≤0.05).
b Data from Report HRT K-007722-017 Appendix G (pp. 321-401)

D. Discussion/Conclusions

a. Maternal Toxicity:

There was no mortality as a result of the administration of 2,4-D BEE. The daily observation of the animals found an increased incidence of excessive salivation among the mid- and high-dose animals. In the high-dose group the mean weight gains for the day 6-9 and 6-16 intervals were -33.36 and -19.00 respectively. Furthermore, during days 6-16 of gestation the mean weight gain for the high-dose group was lower than control (+12.50). This difference was statistically significant. Using the corrected day 20 body weights for the 185 mg/kg dose group, the mean weight gain for the interval during days 6-20 was lower than control (-10.5%). However this difference was not statistically significant. Days 6-11 of gestation, the mean food consumption for the high dose group was significantly lower than control (-8.0%). Otherwise there were no significant effects on food consumption. The statistical examination of the hematology data indicated that there was a significant reduction (-4.4%) in the RBC count and a significant increase in the reticulocytes in the 185 mg/kg group when compared to the control.

By 2,
significant treatment related effects were found in the dose range finding study (HRD # 418-71-04) at 145 and 218 mg/kg, on maternal body weight gain, red blood cell counts, hemoglobin, & hematocrit.

b. Developmental Toxicity:

i. Deaths/Resorptions:
The mean number of uterine implantations, viable fetuses, and resorptions were similar among control and treated groups.

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ii. Altered Growth:
No treatment related effects were noted

iii. Developmental Variations:
There was a statistically significant increase in the fetal incidence of total skeletal variations at the 125 mg/kg level, as compared to the control. Dose related trends were observed for the following incompletely ossified bones: supraoccipital, squamosal, maxilla, and the 4th sternebra. There was a dose related increase in the incidence of rudimentary rib(s), and unilateral 14th ribs.

iv. Malformations:
There were a few sporadic malformations noted in the visceral and skeletal exams. In the visceral exam, microphthalmia was seen in one low-dose and one high-dose fetus. A folded retina, and anophthalmia were each observed in one low-dose fetus. One fetus at the high dose group was noted to have fused and ectopic kidneys. Due to the sporadic nature of the malformations observed, they were not interpreted as being related to treatment.

E. Study Deficiencies:

No deficiencies were noted in this study. As the study was conducted in late 1989 to early 1990 it would be helpful to have historical control data more recent than 1982-1987.

F. Core Classification: Core Minimum Data.

This study satisfies the guideline requirements (83-3) for a developmental toxicity study.

Maternal NOEL = 75 mg/kg
Maternal LOEL = 188 mg/kg based upon the following significant effects: decreased bodyweight gain days 6-16, decreased food consumption days 6-11, decreased red blood cells, and increased reticulocytes.

Developmental Toxicity NOEL = 25 mg/kg
Developmental Toxicity LOEL = 75 mg/kg based upon dose related skeletal variations (incompletely ossified interparietal, supraoccipital, squamosal(s), maxilla(s), and 4th sternebra; the presence of 14th rib pairs, short rib(s), rudimentary first lumbar rib(s), and unilateral 14th rib(s).

G. Risk Assessment:

None at this time.

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RIN 2465-01

2,4-D DER

Page_____ is not included in this copy.

Pages 59 through 86 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

GUIDELINE: 83-3

Primary Review by: Karen B. Whitby, Ph.D. K-87 12/4/90
Toxicologist, Review Section II, Toxicology Branch II/HED
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DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rat
Guideline: 83-3

EPA Identification Nos.: EPA MRID (Accession) No. 415271-02/-05
EPA ID No. 030625-1
EPA Record No. 268,213
Caswell No. 315AZ
HED Project No. 0-1707

Test Material: 2,4-D Triisopropanolamine Salt

Synonyms: (2,4-D-TIPA)

Testing: The Dow Chemical Co., The Toxicology Research Laboratory
1803 Building, Midland, MI 48674

Study Completed: DER# X-000066-013 (The Dow Chemical Co.,)
Project No. 89-3463 (Bio/dynamics, Inc.)

Testing Facility: Bio/Dynamics, Inc., East Millstone, New Jersey
08875

Title of Report: A Teratogenicity Study in Rats with 2,4-D
Triisopropanolamine

Author(s): Raymond E. Schroeder

Report Issued: May 25, 1990

Conclusions:

2,4-D TIPA was evaluated for maternal and developmental toxicity at 0, 33.9, 100, and 338 mg/kg. Maternal toxicity was evident at 33.9 mg/kg by statistically significant decreased bodyweight gain days 6-9 of gestation. A maternal NOEL was not achieved. Developmental toxicity was observed at 100 mg/kg and above by skeletal malformations/variations. At 338 mg/kg postimplantation loss was three times that of control.

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Maternal NOEL = not determined
Maternal LOEL = 32.5 mg/kg

Developmental Toxicity NOEL = 32.5 mg/kg
Developmental Toxicity LOEL = 100 mg/kg

Core Classification: Core Minimum Data

RANGE-FINDING STUDY

The main developmental toxicity study was preceded by a pilot study, which was performed to determine the maternal and embryotoxic potential of 2,4-D Triisopropanolamine Salt in the pregnant rat. Evaluation of fetal toxicity consisted of recording the number of live fetuses on day 16 at the time of cesareans. The study was conducted in two phases. The first phase evaluated 0, 95, 190, and 280 mg/kg with 10 mated rats/group, and did not establish embryo/fetal toxicity. The second phase, utilizing a second shipment of animals, evaluated 10 mated animals/group receiving 0, 280, 375, and 465 mg/kg. Both of these studies were conducted by Bio/dynamics.

Study Title: A Range-Finding Study to Evaluate the Toxicity of 2,4-D Triisopropanolamine Salt in the Pregnant Rat

Study No.: MRID No. 418271-05
Study ID: DER K-000846-011 (Dow Chemical Co.)
Project No. 89-3464 (Bio/dynamics)
89-3464A

Date of Report: April 10, 1990

Date of Study: (Live Portion of 89-3464) July 10 - 29, 1989
(Live Portion of 89-3464A) August 26 - September 15, 1989)

Author: Raymond E. Schroeder

Material and Methods:

Triisopropanolamine (TIPA) Salt (Lot No. AGR 276429, purity (the amount of test article added to the vehicle was corrected for purity)) was administered to mated CD® (Sprague-Dawley derived) 9 rats (10/group) by gavage at dose levels of 0 (vehicle: distilled, deionized water), 95, 190, 280 mg/kg (study 89-3464); and 0, 280, 375, and 465 mg/kg (study 89-3464A). Test article was administered on gestation days 6-15. Dams were sacrificed on day 16 of gestation; postmortem examination included weighing of the gravid uterus, kidneys, and liver; recording the number of corpora lutea and uterine implants (no. live, dead, and resorbed).

Results:

89-3464
There was no mortality in this study. In the 280 mg/kg group there was a significant reduction in bodyweight gain (-58.3%) relative to the control, days 6-9. Days 6-16 bodyweight gain for this group was -11.6% compared to control. The corrected bodyweight gain (days 6-16) was -16.4% relative to the control

for the group receiving 280 mg/kg (not statistically significant). Mean food consumption days 6-11 of gestation was -10.5% for the 280 mg/kg group relative to the control. The incidence of excessive salivation was 0, 50, 90, and 100% among the 0, 95, 190, and 280 mg/kg levels, respectively. The corpora lutea and implantation data did not reveal any treatment related differences.

89-3464A
The 465 mg/kg group had 30% mortality (1 ♀ died after three days of treatment, 2 ♀ died after four days of treatment). At necropsy these females were found to have yellow staining of the skin/fur in the ano-genital region, and 2 had a slight dilatation of the renal pelvis. Mean weight gain was reduced in a dose related manner during the dosing period. Bodyweight gain was significantly reduced at 375 and 465 mg/kg days 6-9, 12-16, and 6-16. There was a dose-related decrease in uterine weight which was significant only at 465 mg/kg. Corrected bodyweight gain was significantly reduced at 375 and 465 mg/kg. Food consumption was significantly depressed in the 375 and 465 mg/kg groups days 6-11 and 11-16. Daily observation of the animals found an increased incidence of salivation and stiffness of the limbs in all treated groups. Corpora lutea and implantation data revealed a nonsignificant decrease in the mean number of viable fetuses, an increase in the number of resorption sites, and a higher mean resorption/implant ratio at 375 and 465 mg/kg.

Conclusions

The dosage levels of 2,4-D TIPA chosen for the main developmental toxicity study were 0, 32.5, 100, and 325 mg/kg. Based upon the treatment related effects observed in this study, the dosages selected for the main study appeared to be appropriate.

Core classification: Supplementary data (Range-Finding Study)

MAIN STUDY

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 72.2%
Description: Black Liquid
Lot No.: AGR 276428

Vehicle(s): Identity: Distilled, deionized water

Test Animals: Species: Rat
Strain: CD® (Sprague-Dawley derived)
Source: Charles River Laboratories, Inc., Portage, MI 49081
Age at Initiation of Mating: ♀ approx. 11 weeks ♂ approx. 14 weeks
Weight of ♀ on day 0: (mean) 230.6 g
(range) 183 - 271 g

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Triisopropanolamine Salt when administered by gavage to rats on gestation days 6 through 15, inclusive.

Mating

Females were placed with males nightly in a 1:1 ratio. Animals not utilized in the study that remained from the original population were sacrificed and discarded after all study groups had been filled and the last mated females within study groups had initiated dosing.

Group Data Summary:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	30
Low Dose	32.5	30
Mid Dose	100.0	30
High Dose	325.0	30

Dosing:

All doses were in a volume of 5 ml/kg of body weight/day prepared once prior to initiation of treatment in a volume adequate for the duration of the study. When the dosing solutions were

prepared, the amount of test article added to the vehicle was adjusted to correct for the purity of the test article. The dosing solutions were analyzed for concentration, homogeneity, and stability. Concentrations of the test chemical ranged from 110.2 to 114.9% of the target concentration at the beginning of dosing. Upon the conclusion of the dosing period, the range of concentration was 108.1 to 110.4%. The coefficient of variation was 1.2% or less, indicating homogeneity. The dosing solution was found to be stable for at least 34 days (± 10% of the day 0 concentration). Initial dose volumes were based on gestation day 6 bodyweights and were adjusted for each animal during the remaining portion of treatment to the most recent bodyweight.

Observation

Maternal

The animals were checked for mortality or abnormal condition twice daily. In addition, each female was given a detailed physical examination on days 0, 6-15, and 20 of gestation. Dams were sacrificed on day 20 of gestation by overdose of carbon dioxide inhalation. Examinations at sacrifice consisted of: the external surface, all orifices, the cranial cavity, carcass, external surface of the spinal cord and sectioned surfaces of the brain, nasal cavity and paranasal sinuses, the thoracic, abdominal, and pelvic cavities, viscera, kidney and liver weights cervical tissues and organs.

Maternal bodyweights were recorded on days 0, 6, 9, 12, 16, and 20 of gestation. Feed consumption was recorded on days 0-6, 6-11, 11-16, and 16-20.

Reproductive

The intact uterus including ovaries was removed and weighed. The number and location of the following was recorded for each uterine horn: live fetuses, dead fetuses, late resorptions, early resorptions, and implantation sites. The ovaries were dissected free and the number of corpora lutea were recorded. If uterine implantations were not evident, the uterus was stained with ammonium sulfide, according to the method of Salewski, 1964.

Fetal

The fetuses were examined in the following manner: each fetus was individually identified, weighed, sexed externally, and examined for gross external malformations/variations. Approximately one-half of the fetuses of each litter (every other fetus within the litter) were evaluated for visceral malformations/variations via a modified Staples, 1974 technique. Fetuses designated to undergo the visceral exam were decapitated and the heads were fixed in Bouin's for subsequent examination. The fetuses were

secured under a dissecting scope, and the thoracic and abdominal cavities were examined. Upon completion of this, the fetuses were eviscerated and individually fixed in 70% ethanol. The heads were serially transverse sectioned using a razor blade after fixation was complete. The sections were observed for malformations of the palate, eyes, and brain via dissecting scope.

The remaining fetuses in each litter were euthanized via inhalation of ether. The fetuses were eviscerated (internally sexed), and stained with Alizarin Red S via a procedure (Crary, 1962) modified by Dio/dynamics. Skeletal specimens were evaluated under a dissecting scope for ossification malformations and variations.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

The interval data (mean bodyweights, mean bodyweight change, gravid uterus weight, day 20 corrected bodyweight, mean feed consumption, mean organ weight, and reproduction data) were analysed by the following procedures. Bartlett's test was performed to determine if groups had equal variance. If so, a standard one way ANOVA using the F distribution to assess significance was performed. If warranted, Dunnett's test was used to determine which means were significantly different from the control. When necessary, the Kruskal-Wallis test was used as a nonparametric test of equality of means. If differences were found, a summed rank test (Dunn) was used to determine which groups differed from the control. A test for trend in the dose levels was also performed. For parametric data, standard regression techniques with a test for trend and lack of fit were used. For nonparametric data, Jonckheere's test for monotonic trend was used. All ratios were transformed via the arc sine transformation before analysis.

The incidence data (litters with resorptions, mortality rates, pregnancy rates, fetuses with malformations/variations, and litters with fetuses with malformations/variations) were analysed as follows. First, a standard chi-square analysis was performed to determine if the proportion of incidences differed between the groups tested. Each treatment group was compared to the control using a 2X2 Fisher Exact Test. The significance level was corrected via the Bonferroni inequality. Armitage's test for linear trend in the dose groups was performed. If any one cell had an expected value less than 5, the chi-square and Armitage's tests were not reported. When this occurred, only the Fisher Exact test (corrected via Bonferroni inequality) was performed and reported.

Compliance

A signed Statement of No Data Confidentiality Claims was provided.

A signed Statement of compliance with EPA GLP's was provided.

A signed Flagging Statement was provided.

A signed Quality Assurance Statement was provided.

C. Results

Mortality

In the 0, 32.5, and 100.0 mg/kg groups no mortality occurred. In the group receiving 325 mg/kg, four females died (13.3% mortality) or were sacrificed in the moribund condition during the treatment period. All of the animals that died or were sacrificed in the moribund condition were pregnant and were noted with stiffness of the limbs at least once.

Clinical Observations

"stiffness of the limbs" was seen at one or more intervals during the treatment period in 26.7% of the animals (8/30) in the 325 mg/kg group. This was first observed in three animals on day 6 of treatment. The majority of animals exhibiting this finding did not do so until day 8. One female was still noticed to have "stiffness of the limbs" on day 20 prior to sacrifice. This finding was not observed in the 0, 32.5, and 100 mg/kg groups.

Excessive salivation was also seen among the 325 mg/kg group. Ten animals were noted with this finding during the treatment period. This finding was not observed in the 0, 32.5, and 100 mg/kg groups.

Bodyweight

The investigators supplied the following data:

Table 1 Bodyweight Gains (grams)[a]

Group (mg/kg):	Pri. to Dosing Period	Dosing Period	Post Dosing Period	Entire Gestation Period	Corrected Body Weight Gains Dosing P.[b]	Entire[c]
0	29	50	63	141.4	-25.6	65.5
32.5	29	45	58	132.5	-25.6	61.0
100.0	30	44	60	134.0	-20.9	61.0
325.0	27	29	52	109.9	-26.5	52.6

[1] corrected bodyweight gain for dosing period = bodyweight gain for dosing period minus gravid uterus weight.
[2] corrected bodyweight gain for entire gestation period = bodyweight gain for entire gestation period minus gravid uterus weight.
* significantly different form control (p≤0.01)
[Some of the above values were calculated by the reviewer from individual animal data]

[6] Data extracted from (study number HET K-008866-012 Appendices C, D, and E pp. 64-79)

There were significant reductions in bodyweight gain days 6-9 in the low-, mid-, and high-dose groups -40, -30, and -130%, respectively, relati e to the control. When maternal bodyweight gain was calculated over the entire gestation period, there was a -6.3, -5.2, and -22.3% change relative to the control for the 32.5, 100, and 325 mg/kg groups respectively (these differences were not analysed statistically). The corrected maternal bodyweight gain for the entire gestation period indicated changes of -6.9, -6.0, and -19.7% relative to the control, for the 32.5, 100, and 325 mg/kg groups respectively (these differenc s were not analysed statistically).

On day 9 of gestation, after receiving three days of treatment, a female in the high-dose group was sacrificed moribund. This female lost 53 g days 6-9. Another female i the same gr up who underwent convulsions on day 7 subsequently died on day 9, after having lost 35 g days 6-9. A female in the high-dose group was sacrificed moribund on day 12 of gestation; this female lost 36 g during days 6-12. Also in the 325 mg/kg group, a female lost 29 g days 6-9, then died on day 11.

Food Consumption

The investigators supplied the following data:

Table 2 Food Consumption Data (g/kg/day)[6]

Group: (mg/kg)	Days 6-6	Days 6-11	Days 11-16	Days 16-20	Days 6-20
0	101	90	92	89	366
32.5	96	89	92	89	365
100.0	104	91	93	90	370
325.0	101	87**	90*	91	363

[6] Data extracted from (study number HET K-008866-012 appendix F pp. 80-84)
* significantly different from control (p≤0.05)
** significantly different from control (p≤0.01)

[Some of the above values were calculated by the reviewer from individual animal data]

There were no statistically significant differences in food consumption between the 0, 32.5, and the 100 mg/kg groups. During the treatment period, mean food consumption for the 325 mg/kg group was significantly less than the control group (-25.6 and -13.0%, days 6-11 and 11-16, respectively).

Organ Weight Data

Mean absolute kidney weights were comparable between the control and treated groups. Mean relative kidney weights were slightly increased in a dose-related manner. Mean absolute liver weights were comparable between the 0 and 32.5 mg/kg groups. At the 100 and 325 mg/kg levels, a significant decrease in the mean absolute liver weight was seen relative to the control. However, since the mean relative liver to bodyweight ratio for these groups were comparable to the control data, the differences were probably due to the lower final bodyweight.

Table 3 Terminal Organ and Bodyweights and Organ/Bodyweight Ratios

mg/kg	0	32.5	100	325
Terminal Bodyweight (g)	290.2	282.3	276.2*	277.0
Kidney Wt. Absolute (g)	1.926	1.885	1.852	1.845
Relative (X 1000)	6.66	6.69	6.72	6.81
Liver Wt. Absolute (g)	15.389	14.726	14.408*	14.507*
Relative (X 1000)	5.31	5.22	5.22	5.26

* Significantly different from control ($p \leq 0.05$)
@ Data taken from Report HLT K-000066-012 Appendix J (p. 120)

Organ Weight Observations

There were no significant adverse pathological effects as a result of treatment. Ten percent of the animals in the high dose group were found to have abnormal contents in their uterine cavities (i.e. both horns filled with a black fluid (2 animals)

or one horn filled with a red material (1 animal)]. This observation was not made in the control or mid-dose animals, but was made once in the low-dose animals. Dilated renal pelvis was an observation made once in each treatment group.

Cesarean Section Observations

Table 4 Cesarean Section Observations[a]

Dose (mg/kg):	0	32.2	100	325
#Animals Assigned	30	30	30	30
#Animals Mated/Inseminated	30	27	28	28
Pregnancy Rate (%)	100	90	93	93
Maternal Wastage				
#Died	0	0	0	4
#Died/pregnant	0	0	0	4/28
#Non pregnant	6	3	2	2
#Aborted	0	[illegible]	0	0
#Premature Delivery	0	[illegible]	0	0
Total Corpora Lutea	472	406	441	370
Corpora Lutea/dam	15.7 ±1.3	15.0 ±1.6	15.8 ±2.5	15.4 ±1.4
Total Implantations	446	374	411	334
Implantations/Dam	14.9 ±1.9	13.9 ±2.5	14.7 ±1.4	13.9 ±3.5
Total Live Fetuses	422	352	382	272
Live Fetuses/Dam	14.1 ±2.1	13.0 ±2.7	13.6 ±1.6	11.3 ±5.0
Mean no. ♂/litter	7.9	6.6	6.7	5.1**
Mean no. ♀/litter	6.2	6.4	7.0	6.2
Total Resorptions	24	22	29	62
Resorptions/Dam	0.8 ±1.2	0.8 ±1.1	1.0 ±1.0	2.6 ±4.6
Total Dead Fetuses	0	0	0	0
Mean Fetal Weight (gm)	3.42	3.40	3.35	2.81
♂ fetuses	3.48 ±0.2	3.52 ±0.2	3.45 ±0.4	2.95** ±0.5
♀ fetuses	3.32 ±0.2	3.31 ±0.2	3.27 ±0.4	2.75** ±0.5
Preimplantation Loss(%)	5.4	3.3	5.5	9.6
Postimplantation Loss(%)	5.3	6.0	6.9	16.8
Sex Ratio (Total ♂/Total ♀)	1.3	1.0	1.0	0.8

[a] Data extracted from (study number HET K-008866-012 appendix G pp. 85-89)

** Significantly different from control ($p \leq 0.0$)

Although the number of viable fetuses in the 325 mg/kg group was lower than the study control (-19.9%) the difference was not statistically significant (the decrease was -20.4% relative to the historical control). Although the preimplantation loss for the 325 mg/kg group is increased (77.8%) relative to the study control, the value is not as vastly different when compared to the historical control (18.5%). The mean number of resorption sites per dam was also increased for the 325 mg/kg group (225% relative to the study control, and 271% relative to the historical control). One female in the 325 mg/kg group totally resorbed a litter of 18. Another female in this group resorbed 13 of 14 implantations, while another dam resorbed 9 of 15 implants. These findings apparently account for the dose-related increase in post-implantation loss (13.2, 30.2, and 217% were the increases for the 32.5, 100, and 325 mg/kg groups respectively, relative to the study control, and 33.3, 53.3, and 273.3% relative to the historical control). The mean number of males per litter statistically indicated a linear response related to the dose levels (the number of males/litter was -16.5, -15.2, and -35.4% respectively for the 32.5, 100, and 325 mg/kg levels relative to the control). Mean fetal weight at 325 mg/kg was -17.8% of the study control and -19.9% of the historical control for litters; -15.2% of the study control and -18.1% of the historical control for males; and -17.2% of the study control and -19.6% of the historical control for females.

2. Developmental Toxicity

Table 5 External Examinations

Observations[+]	Control	Low Dose	Mid Dose	High Dose
External Malformations[a] #pups(litters) examined	422(30)	352(27)	382(28)	272(23)
Edematous	0(0)	1(1)	0(0)	1(1)
Filamentous tail	0(0)	0(0)	0(0)	2(2)
Exencephaly	0(0)	1(1)	0(0)	0(0)
Protruding tongue	0(0)	1(1)	0(0)	0(0)
Eye bulge(s) - absent	0(0)	2(2)	0(0)	2(2)
Eye bulge(s) - small	0(0)	0(0)	0(0)	1(1)
Total External Malformations				
Fetal Incidence N(%)	0(0)	2(0.6)	0(0)	5(1.8)*
Litter Incidence N(%)	0(0)	2(7.4)	0(0)	5(21.7)*
External Variations[a] #pups(litters) examined	422(30)	352(27)	382(28)	272(23)
Shiny (glassy) appearance	0(0)	3(1)	4(3)	2(1)

Total External Variations				
Fetal Incidence N(%)	0(0)	3(0.9)	4(1.0)	2(0.7)
Litter Incidence N(%)	0(0)	1(3.7)	3(10.7)	1(4.3)

* some observations may be grouped together
[a] fetal (litter) incidence
[c] Significantly different from control ($p \leq 0.05$)
Data taken from study number MRT K-008866-012 appendix L pp. 250-251

The incidence of external malformations at the 325 mg/kg level was significantly different from control when evaluated on the fetal and litter basis. The edema, bulging eyes, and protruding tongue were observed in one fetus of the low-dose group.

The only external variation noted was shiny skin. This is attributed to the thin translucent skin of developmentally retarded fetuses. The finding was not dose-related and therefore, not attributed to treatment.

Table 6 Visceral Examinations

Observations*	Control	Low Dose	Mid Dose	High Dose
Visceral Malformations[a]				
Groups(litters) examined	219(30)	183(27)	199(28)	144(23)
Anophthalmia	0(0)	1(1)	0(0)	2(2)
Microphthalmia	0(0)	0(0)	0(0)	2(2)
Retina(s) - folded	0(0)	0(0)	0(0)	2(1)
Lateral Cerebral Ventricle(s) - distended	0(0)	0(0)	0(0)	1(1)
Aortic Arch Malformation	0(0)	0(0)	0(0)	1(1)
Cardiac Interventricular Septal Defect	0(0)	0(0)	0(0)	1(1)
Total Visceral Malformations				
Fetal Incidence N(%)	0(0)	1(0.5)	0(0)	7(4.9)[c]
Litter Incidence N(%)	0(0)	1(3.7)	0(0)	6(26.1)[c]
Visceral Variations[a]				
Groups(litters) examined	219(30)	183(27)	199(28)	144(23)
Distended Renal Pelvis (papilla present)	2(2)	3(3)	0(0)	1(1)
Ureter(s) - distended	4(4)	1(1)	2(2)	4(3)
Ureter(s) - tortuous	7(4)	11(9)	3(2)	4(4)
Total Visceral Variations				
Fetal Incidence N(%)	10(4.6)	12(6.6)	5(2.5)	9(6.3)
Litter Incidence N(%	7(23.3)	9(33.3)	3(10.7)	8(34.8)

[a] some observations may be grouped together
[b] fetal (litter) incidence
[c] significantly different from control ($p \leq 0.05$)
Data taken from study number HET K-008866-012 appendix H pp. 290-314

There were no visceral malformations in the 0, and 100 mg/kg group. The only visceral malformation in the 32.5 mg/kg group was anophthalmia. Malformations of the eye (micro- and anophthalmia) were seen in four 325 mg/kg fetuses of four different litters. The cardiac defects were observed in the high-dose fetus found to have edema of the cervical and thoracic regions during external examinations.

Tortuous and/or distended ureters were the most commonly observed visceral variations. The incidence of this and the other visceral variations were relatively comparable to the control.

Table 7 Skeletal Examinations

Observations*	Control	Low Dose	Mid Dose	High Dose
Skeletal Malformations[a]				
Groups(litters) examin	203(30)	170(27)	183(28)	131(23)
Hole in Cranium	0(0)	1(1)	0(0)	0(0)
Premaxilla(e) - fused	0(0)	1(1)	0(0)	0(0)
Exoccipital(s) - misshapen	0(0)	1(1)	0(0)	0(0)
Cervical Transverse Process(es) - misshapen	0(0)	1(1)	0(0)	0(0)
Malposition in # of Cervical Vertebrae	0(0)	0(0)	0(0)	1(1)
Presence of Cervical Rib(s)	0(0)	0(0)	0(0)	3(1)
Thoracic Transverse Process(es) - fused	0(0)	0(0)	1(1)	1(1)
Transverse Process(es) - small and misshapen	0(0)	0(0)	1(1)	0(0)
Thoracic Centrum(a) - split & misshapen	0(0)	0(0)	1(1)	0(0)
Thoracic Transverse Process(es) - absent	0(0)	0(0)	0(0)	1(1)
Lumbar Centra - fused	0(0)	1(1)	0(0)	0(0)
Lumbar Centrum(a) - split & misshapen	0(0)	0(0)	1(1)	0(0)
Malposition in # of Lumbar Vertebra(e)	0(0)	0(0)	0(0)	4(2)
Sacral Vertebra(e) - absent	0(0)	0(0)	0(0)	1(1)
Caudal Vertebra(e) - absent	0(0)	0(0)	0(0)	1(1)
Rib(s) - wavy	0(0)	0(0)	5(4)	5(4)
Ribs) - fused	0(0)	0(0)	0(0)	5(4)
Scapula(e) - bent	0(0)	0(0)	0(0)	3(3)
Radius(i) - bent	0(0)	0(0)	0(0)	2(2)
Ulna - thickened and misshapen	0(0)	0(0)	0(0)	1(1)
Femur - bent	0(0)	0(0)	0(0)	2(1)
Pelvi - misaligned	0(0)	0(0)	0(0)	1(1)
Total Skeletal Malformations				
Total Incidence N(%)	0(0)	2(1.2)	7(3.8)*	16(12.2)*
Litter Incidence N(%)	0(0)	2(7.4)	5(17.9)	9(39.1)*
Skeletal Variations[a]				
Groups(litters) examined	203(30)	170(27)	183(28)	131(23)
Rib(s) - 14th, ossified	9(5)	1(1)	10(7)	13(7)
Xiphoid - ossified	3(3)	1(1)	5(4)	5(5)
Premaxilla - incompletely ossified	0(0)	0(0)	2(2)	6(3)
Cervical Transverse Process(es) - incompletely ossified	9(7)	17(12)	21(12)	50(16)

Table 7 Skeletal Examinations - cont'd

Observations*	Control	Low Dose	Mid Dose	High Dose
Sacral Transverse Process(es) - inc. oss.	5(3)	9(5)	7(6)	10(6)
Oss. Adj. to 7th Cervical Vertebra(ae)	2(1)	1(1)	1(1)	10(6)
Thoracic Centrum(a) - not oss.	2(2)	1(1)	4(3)	6(4)
Thoracic Centrum(a) - split	0(0)	0(0)	0(0)	3(3)
Sacral Centrum(a) - not oss.	0(0)	0(0)	0(0)	2(2)
Sacral Centrum(a) - inc. oss.	0(0)	1(1)	1(1)	3(1)
Caudal Centrum(a) - not oss.	2(1)	1(1)	6(4)	14(8)
2nd Sternebra - not oss.	4(3)	1(1)	3(2)	5(4)
3rd Sternebra - inc. oss.	4(2)	1(1)	8(6)	7(6)
3rd Sternebra - not oss.	1(1)	0(0)	0(0)	3(3)
4th Sternebra - inc. oss.	18(11)	9(8)	25(15)	40(17)
4th Sternebra - not oss	1(1)	0(0)	3(2)	6(3)
5th Sternebra - not oss.	60(23)	46(19)	68(24)	104(22)
6th Sternebra - not oss.	24(12)	10(6)	29(14)	49(16)
Sternebra(ae) - misshapen	0(0)	0(0)	0(0)	2(2)
Rib(s) - 1st Lumbar Rudimentary	3(2)	4(4)	7(6)	11(6)
Ribs - thickened	0(0)	0(0)	5(4)	12(7)
Metacarpal(s) - inc.oss.	4(4)	1(1)	6(6)	11(6)
Metatarsal(s) - inc.oss.	1(1)	2(1)	6(4)	11(7)
Scapula(e) - inc. oss.	1(1)	3(1)	1(1)	5(4)
Total Skeletal Variations				
Fetal Incidence N(%)	179(88.2)	139(81.8)	152(83.1)	127(96.9)**
Litter Incidence N(%)	30(100)	27(100)	28(100)	23(100)

* some observations may be grouped together
@ fetal (litter) incidence
* Significantly different from control ($p \leq 0.05$)
** Significantly different from control ($p \leq 0.01$)
Data taken from study number NST R-000566-012 appendix N pp. 315-418

The evaluation of the fetal specimens revealed sporadic malformations and numerous skeletal variations. Therefore, for the most part, only those that occur in a treatment related manner are included in table 7. The incidence of litters with at least one skeletal variation was 100% in each of the groups. Skeletal variations appear with some frequency in the vertebral transverse processes, sternebrae, and ribs. These effects for the most part are dose related, the lowest dose at which the incidence begins to increase is 100 mg/kg.

D. Discussion/Conclusions

a. Maternal Toxicity:

Mortality in this study was 13.3% at the high dose (325 mg/kg). "Stiffness of the limbs" was seen at one or more intervals during the treatment period in 96.7% of the animals (29/30) in the 325 mg/kg group. There was a significant decrease in terminal and corrected bodyweight of the 100 mg/kg females. There was a significant decrease in the absolute liver weight of the 100 and 325 mg/kg dams. Bodyweight gain was significantly reduced at 32.5 mg/kg and above, days 6-9. Bodyweight gain was significantly reduced through the dosing period at 325 mg/kg. Food consumption was also significantly reduced days 6-11 and 11-16 in this group. Food consumption days 0-20 of gestation in the 325 mg/kg group was -6.8% relative to the control.

b. Developmental Toxicity:

i. Deaths/Resorptions:
Preimplantation loss in the 325 mg/kg group was increased 77.8% relative to the control. There was a nonsignificant increase in the number of resorptions/dam at 325 mg/kg. Postimplantation loss was increased in a dose-related manner (13.2, 30.2, and 31.7%, respectively for the 32.5, 100, and 325 mg/kg groups. There was a significant decrease in the number of males per litter at 325 mg/kg. This effect was treatment related.

ii. Altered Growth:
Fetal weights at 325 mg/kg were significantly reduced for males and females (not for litters).

iii. Developmental Anomalies:
The incidence of fetuses with at least one ossification variation was significantly higher than control at 325 mg/kg. The incidence of litters containing at least one fetus with an ossification variation was 100% for the control and each treated group. At 100 mg/kg there was an increased incidence (dose-related) of incomplete ossification of the cervical and sacral vertebral transverse processes, metatarsals, and the 3rd and 4th sternebrae. In addition, at this dose, the incidence of unossified caudal centra, rudimentary first lumbar ribs, and thickened ribs was increased.

In general, the incidence of these effects increases at 325 mg/kg with additional findings (unossified caudal centra, unossified sacral and cervical vertebral transverse processes, unossified 4th, 5th, and/or 6th sternebra, and incompletely ossified metacarpals).

iv. Malformations:
There was a significant increase in the fetal incidence of skeletal malformations at 100 mg/kg. For the 325 mg/kg group there was a significant increase in the incidence of skeletal malformations on the fetal and the litter basis. The skeletal malformations seen most frequently in this study were wavy ribs (increased incidence at 100 and 325 mg/kg), fused ribs, and defects of the vertebrae. The registrant has supplied a reference to support their opinion that these malformations are frequently associated with maternal toxicity. There was a significant increase at the 325 mg/kg level in the fetal and litter incidence of visceral malformations.

E. Study Conclusion:

A maternal NOEL was not attained in this study. More recent historical control data than 1983-1987 would be helpful as this study was conducted in late 1989.

F. Data Classification: Core Minimum Data.

This study satisfies the guideline requirements (83-3) for a developmental toxicity study.

Maternal NOEL = Not determined
Maternal LOEL = 32.5 mg/kg based upon decreased bodyweight gain during days 6-9

Developmental Toxicity NOEL = 32.5 mg/kg
Developmental Toxicity LOEL = 100 mg/kg based upon a significant increase in the fetal incidence of skeletal malformations (wavy ribs) and variations of select bones (vertebral transverse processes, caudal centra, sternebrae, rudimentary ribs, thickened ribs, and metatarsals)

G. Risk Assessment: None at this time.

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