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TRIFLOXYSTROBIN-Prenatal Developmental Toxicity-Rat OPPTS 870.3700;OPP §83-3a

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DATA EVALUATION RECORD

Study Type: Teratology - Prenatal Developmental Toxicity
Species: Rat; Guideline: OPPTS 870.3700; OPP §83-3a

EPA ID No.s: EPA MRID No. 44496708
EPA Pesticide Chemical Code 129112
EPA DP Barcode D243979
EPA Submission No. S538757

Test Material: CGA-279202 TECHNICAL
Synonyms: Trifloxystrobin

Citation: Khalil, S. (1995): CGA-279202 Technical, Rat Oral Teratogenicity, EPA Guideline No. 83-3, Short/Long-term Toxicology, Novartis Crop Protection for Novartis Crop Protection, Inc., Test No. 943042, Novartis Nexus Number 772-95, March 6, 1995 (Unpublished Study); EPA MRID Number 44496708.

Executive Summary: In a developmental study (MRID# 44496708), groups of 24 Tif: RAI f (SPF), hybrids of RII/1 x RII/2 albino rats from Animal Production, WST-455, CIBA-GEIGY Ltd., 4332 Stein, Switzerland received CGA-279202 Technical (Trifloxystrobin; Purity: 96.4%; Batch No.: P.405009) in a 0.5% w/w aqueous solution of sodium carboxymethylcellulose at either 0, 10, 100 or 300 mg/kg/day by oral gavage from gestation days 6 through 15, inclusive. Maternal observations and measurements included daily mortality, cage-side observations, body weights and food consumption determined on days 6, 11, 16 and 21 post coitum. The maternal animals were sacrificed on gestation day 21 and subject to a gross necropsy, the uterine contents were examined and the fetuses were examined for external, visceral or skeletal anomalies by standard techniques.

Maternal systemic toxicity was seen as reduced body weights in the 1000 mg/kg/day group on gestation days 16 and 21. There were also decreased body weight gains in the 1000 mg/kg/day group during the dosing period ($p<0.01$; gestation days 6-16), the dosing period plus post dosing period (gestation days 6-21), the entire gestation period (days 0-21) and in the 100 and 1000

mg/kg/day groups for the corrected body weight gain for the dosing period plus post dosing period ($p<0.05$ for 100 mg/kg/day and $p<0.01$ for the 1000 mg/kg/day groups). There was increased body weight gains in the 100 mg/kg/day and 1000 mg/kg/day groups in the postdosing period (gestation days 16-21) an indication of a rebound effect. There was reduced food consumption in the 100 mg/kg/day and 1000 mg/kg/day groups during the dosing period ($p<0.05$ for the 100 mg/kg/day and $p<0.01$ for the 1000 mg/kg/day groups). No biologically relevant effects were noted in food efficiency data.
The Maternal Toxicity NOAEL was 10 mg/kg/day and the Maternal Toxicity LOAEL was 100 mg/kg/day based on reduced body weight gains and food consumption.

No developmental toxicity was observed at dose levels tested.
The Developmental Toxicity NOAEL is equal to or greater than 1000 mg/kg/day and the Developmental Toxicity LOAEL is greater than 1000 mg/kg/day.

This study is classified as Acceptable-Guideline and satisfies the guideline requirements (OPPTS 870.3700, OPP §83-3a) for a teratology study in rats.

Compliance: A signed and dated STATEMENT OF NO CONFIDENTIALITY CLAIMS, a CERTIFICATION OF GOOD LABORATORY PRACTICES along with a Certification of GLP and Verification of the Report and Statement of Compliance with Good Laboratory Practices, a FLAGGING STATEMENT (according to the study authors the study neither meets nor exceeds the applicable criteria), and a Quality Assurance Statement was provided.

The design and conduct of this study was in accordance with the following guidelines (scanned from page 16 of the study report):

OECD Chemical Test Guideline No. 414 "Teratogenicity", May 1981
U.S. EPA FIFRA 83-3 "Teratogenicity study", November 1982
U.S. EPA TSCA 798.4900 "Developmental toxicity study", August 1987
Japan MAFF 59 NohSan No. 4200, "Teratogenicity Study" January 1985
European Communities Commission Directive 87/302/EEC, "Teratogenicity Study Rodent and Non-Rodent", OJ No L133/24 November 18, 1987.

The study was performed in accordance with "Good Laboratory Practice (GLP) in Switzerland, Procedures and Principles", Swiss Federal Department of the Interior and Intercantonal office for the Control of Medicaments, March 1986. The Swiss GLP Regulations are based on the OECD Principles of Good Laboratory Practice (Council Decision 81/30, adopted May 1981), and are compatible with U.S. EPA 40 CFR 160 (FIFRA), August 1989; U.S. EPA 40 CFR 792 (TSCA), August 1989; and Japan MAFF 59 NohSan No. 3850, August 1984.

THIS REVIEW CONTAINS TEXT INFORMATION SCANNED FROM THE STUDY REPORT BY THE REVIEWER INTO ELECTRONIC FORMAT (USED IN MATERIALS AND METHODS, STUDY DESIGN AND CONCLUSIONS-INVESTIGATORS SUMMARY SECTIONS).

A. Materials and Methods

Test Compound: CGA 279202 Technical (Trifloxystrobin)
Purity: 96.4%
Description: light brown powder
Batch No.: P.405009
Stored at room temperature

Vehicle(s): 0.5% (w/w) aqueous solution of sodium carboxymethylcellulose: CMC, Pharmacopeia quality, high viscosity (HERCULES POWDER Company, Product No. 7HF)

Test Animal(s):
Species: Rat, albino
Strain: Tif: RAI f (SPF), hybrids of RII/1 x RII/2
Source: Animal Production, WST-455, CIBA-GEIGY Ltd., 4332 Stein, Switzerland
Age: Minimum 8 weeks
Body Weight: 201.5-202.1 g an day 0 p.c.
Additional information: This stock is an outbred cross between two genetically stable inbred Sprague- Dawley derived strains, used for all study types at this facility; it has high fecundity, and extensive historical data are available.

B. Study Design

This study was designed to assess the developmental toxicity potential of CGA-279202 (Trifloxystrobin) when administered by oral gavage on gestation days 6 through 15, inclusive.

From page 17 of the study report:

Time Schedule

Start Date of Mating: August 23, 1994
Start Date of Dosing: August 29, 1994

Start Date of Necropsy: September 13, 1994
Experimental End Date: December 14, 1994

Mating Procedure (scanned from page 20 of the study report)

Nulliparous females were mated overnight with males of the same stock and proven fertility at an initial ratio of three females to one male in mating cages. Each cage is divided into two parts by a guillotine door, separating the sexes until 3 a.m. on the mating day, when the door opens automatically. Three to six hours later, successful mating is assessed by the presence of a vaginal plug or of spermatozoa in a vaginal smear. This day is designated as day 0 (of pregnancy) = day 0 post.coitum (p.c.). Pregnant females were removed from the mating cages and the procedure repeated for remaining females until sufficient dams were produced.

Appendix 12 [of the study report] identifies the animals used for mating in this study.

Acclimation under test conditions was for at least seven days, between delivery from animal production (WST-455, in-house) and the first treatment on day 6 post coitum.

The mated females were allocated to experimental and control groups using a method of randomization by weight stratification as shown in Appendix 13 [of the study report]. After allocation to groups, the females were individually identified by a color code on the tail (i.e. a dash-dot code, painted with a felt-tipped waterproof marker) and placed one per cage in Macrolon cages. The cages were identified by a colored label according to the dose group. Each cage label also showed the test number, test substance code, animal number (=cage number), dose level (mg/kg), dose volume (ml/kg), and dates of treatment and necropsy.

Animal Husbandry (scanned from page 19 of the study report)

The study was conducted under optimal hygienic conditions (OHC). The animals were housed individually in Macrolon cages with wire mesh tops and standardized granulated soft wood bedding material (Societe Parisienne des Sciures Pantin, Paris, France), with the following environmental conditions:

Temperature (°C):	22 ± 3
Relative Humidity (%):	50 ± 20
Ventilation:	about 16 air changes/hour
Light Cycle:	12 hours of light per day (6 a.m. - 6 p.m.)

Neither insecticides nor other chemicals were applied in the animal room the exception of the disinfectant, BRADOPHEN (TM) Limited, Basle, Switzerland).

Pelleted, certified standard feed (Nafag No. 890, Tox; Nafag, Naehr- und Futzermittel AG, Gossau, Switzerland) was provided ad libitum; all batches of feed were analyzed for composition and contaminant levels. Tap water was provided ad libitum in plastic bottles; the water quality is routinely checked to standard specifications.

...Appendix 2 [of the study report] provides ...quality specifications

of diet and water.

Group Arrangement: (scanned from page 20 of the study report)

Group	Female and Cage Numbers	Color of Cage Labels
1: Control	1 - 24	blue
2: Low Dose	25 - 48	green
3: Mid Dose	49 - 72	yellow
4: High Dose	73 - 96	red

Dose Administration: (scanned from page 21 of the study report)

The following dose levels were selected based on the results of a previous rangefinding study no. 943040 [wrong number in text] in pregnant rat [discussed below]:
Low Dose: 10 mg/kg body weight
Mid Dose: 100 mg/kg body weight
High Dose: 1000 mg/kg body weight

Citation: FitzGerald. (1993): CGA-279202 Technical, Rangefinding Rat Oral Teratogenicity, EPA Guideline No. 83-3, Toxicology Services, Reproductive Toxicology, Novartis Crop Protection for Novartis Crop Protection, Inc., Test No. 943340, Novartis Nexus Number 632-93, June 9, 1993 (Unpublished Study); EPA MRID Number 44496706.

This study used CGA 279202 (Batch Number: HZ-13/16-18) administered to four groups of seven mated 2 month old virgin female albino rats (Tif: RAI f (SPF), hybrids of RII/1 x RII/2) at dose levels of 0, 10, 100 and 1000 mg/kg/day (in sodium carboxymethylcellulose) from gestation days 6 through 15, inclusive. No treatment related effects were noted in clinical signs, body weights and body weight gains, post-implantation loss, fetal weights, mean gravid uterine weights, carcass weights, maternal necropsy or external fetal observations. There was a slight reduction in food consumption at 1000 mg/kg/day.

Preparation Dates:	fresh every day
Preparation Method:	Test substance-vehicle mixtures were prepared with a high-speed homogenizer (Polytron PT6000, Kinematica AG, 6014 Littau, Switzerland). Homogeneity of the mixtures during administration was maintained with a magnetic stirrer.
Administration Schedule:	Daily from day 6 to day 15 of gestation.
Administration Route:	Intragastrically by gavage. The oral route was used because it is a potential route of human exposure.
Administration Volume:	10 ml mixture/kg actual body weight
Test Substance Content:	0, 1, 10 and 100 mg/ml mixture

In order to permit determination of content, homogeneity and stability of the test substance under the actual conditions of administration during the study, samples of test substance-vehicle mixtures were taken on the date(s) designated below, once before and once after dosing. The samples from before dosing were taken from the top, middle and bottom of the container; the samples from after dosing were taken from the middle of the container.

Samples were taken in duplicate. Together with 10 ml of vehicle and approximately 2.0 g of test substance they were transported frozen to the analytical laboratory for analysis.

Date(s) of Sampling: September 1 and 6, 1994
The results of these analyses are given in Appendix 1 [of the study report].

From the data available in Appendix 1, page 100 of the study report, the mean concentrations for homogeneity in samples were 85.7, 81.8 and 94.0% for the first analysis and 93.8, 85.2 and 87.5% for the second analysis of the nominal concentrations for the 1, 10 and 100 mg/ml solutions, respectively, the homogeneity ranged from -9% to +7% for the first analysis and -8% to +6% for the second analysis of the mean concentrations and that the compound was stable at room temperature in 0.5% CMC/distilled water solution for at least 2 hours.

Observations (scanned from pages 22-26 of the study report)

Maternal Observations and Measurements

The following were recorded:

Mortality:	daily
Cage-side Observations:	daily
Body Weight:	daily
Feed Consumption:	days 6, 11, 16 and 21

Mean daily feed consumption per animal was calculated according to the following formula:

$$\frac{\text{feed consumption (g) per period}}{\text{days per period}}$$

Dams were killed on day 21 by carbon dioxide inhalation, and fetuses removed by hysterectomy.

Dams found dead or electively sacrificed before scheduled necropsy were subjected to macroscopic pathological examination, including examination of the uterine content.

The following were recorded at necropsy:

-Macroscopic pathological examination of the main organs of the thoracic and abdominal cavities, in particular the genitals
-Number of corpora lutea in each ovary

-Weight of the uterus including contents
-Uterine contents:
In dams at scheduled necropsy
-number and location of live and dead fetuses
-number and location of early and late (embryonic/fetal) losses
-total postimplantation loss (dead + early + late)
In dams sacrificed or dying before scheduled necropsy
-number and location of implantation sites

Classification of Uterine Findings

Early Resorption: any implantation site without visible fetal remains: the embryo may be visible; placental remains may or may not be present, occasionally only placenta is present

Late Resorption: implantation site with fetal remains visible (usually head, hands and feet can be seen)

Implantation Site: used to describe implantations in animals dying or killed before scheduled sacrifice, when it is not possible to establish whether and what form of losses have occurred

Uterine horns showing no implantation sites were placed into an aqueous solution of ammonium sulfide to visualize possible hemorrhagic alterations of such sites, according to the method of Salewski [1] {reference provided}.

Fetal Examination and Measurements

Following removal from the uterus, the fetuses were numbered, sexed (on the basis of ano-genital distance), externally examined and weighed. They were then killed by subcutaneous injection of an appropriate barbiturate anesthetic in the scruff of the neck and processed for visceral or skeletal examination.

Fetuses were assigned to either visceral or skeletal evaluation at an approximate 1:1 ratio within each litter, independent of sex (starting with skeletal). In the case of gross external anomaly or malformation, fetuses were allocated to one technique depending on the type and incidence of finding.

Fetal External Examination

In the fetal external examination, special attention was paid to possible alterations in the following body regions:

Body surface (e.g. generalized or localized edema, hemorrhage)
Head (e.g. cranioschisis, encephalocele, cleft palate)
Trunk (e.g. rachischisis, atresia of a body orifice, omphalocele)
Extremities (e.g. deformed, limb position anomaly, kinked tail)

Visceral Examination

The viscera of approximately half of the fetuses per litter were fixed whole in Bouin's solution for at least two weeks and then micro-dissected as follows: limbs, tail and skin are removed, leaving the cranial skin in situ. The fetus is placed ventral surface up on a cork board and the head cut between upper and lower jaws downwards in a line towards the ears. After removal of the tongue, the head is sectioned transversely (perpendicular to the palate) through eyes and brain, including a central lenticular section [1]. The trunk is cut, just penetrating the body wall, down both sides in a line from shoulder blade to hind limb, along a line across the diaphragm region, and from jaw to diaphragm along the line of the sternum (penetrating the sternum). The body walls and ribs are peeled back and pinned down to reveal the abdominal and thoracic organs. Heart and kidneys are examined by slicing.

Visceral examination included, in particular, morphology and position of the following organs and organ systems:

-Skin	
-Central Nervous System:	brain (olfactory bulbs, cerebrum, lateral and medial ventricles), spinal cord
-Eyes:	lens, vitreous, retina
-Body Cavities:	thorax and abdomen, including diaphragm
-Respiratory System:	nasal cavity (nasal septum, turbinates, choanae), trachea, bronchi, lungs, pleura
-Digestive System:	oral cavity, palate, tongue, esophagus, stomach, intestine, rectum, liver, peritoneum
-Endocrine System:	thyroid, pancreas, adrenals, thymus, pituitary
-Circulatory System:	spleen, pericardium, heart (atria, ventricles, septae), major vessels
-Excretory System:	kidneys (renal papillae, renal pelvis), ureters, urinary bladder
-Genital System:	testes, epididymides, vas deferens, seminal vesicles; ovaries, oviducts, uterus

Skeletal Examination

Skeletal assessment in approximately half of the fetuses per litter was done according to the staining technique of Dawson [2]{reference provided}; after clearing with potassium hydroxide and staining with alizarin red S, the specimens were stored in glycerol.

Routine investigation of these fetuses included the following skeletal elements:

-Facial bones:	nasal, premaxillary, maxillary and zygomatic bones, mandibula
-Cranial bones:	frontal, parietal, interparietal, occipital and exoccipital bones, fontanel
-Sternum:	sternebrae 1 to 6
-Shoulder girdle:	scapula and clavicle
-Forelimbs:	humerus, ulna, radius, metacarpals 2 to 5, proximal and distal phalanges of anterior digits 1 to 5 (except proximal phalanx 1: not present)
-Pelvic girdle:	ilium, ischium, pubis
-Hindlimbs:	femur, tibia, fibula, calcaneus, metatarsals 1 to 5, proximal and distal phalanges of posterior digits 1 to 5 (except proximal phalanx 1: not present)
-Ribs:	anteroposterior 1 to 13
-Spinal column:	cervical vertebral centers and arches 1 to 7 thoracic vertebral centers and arches 1 to 13 lumbar vertebral centers and arches 1 to 6 sacral vertebral centers and arches 1 to 4

Classification of Fetal observations [note: these are not Agency definitions, this is according to the investigators]

Malformation:	Very rare, permanent structural change that may adversely affect fetal survival, development or function.
Anomaly:	Rare, slight to moderate, permanent or reversible structural change that is not considered to impair fetal survival, development or function.
Variation:	Relatively frequent, transient structural deviation from normal development that is considered not to have any detrimental effect on fetal survival, development or function. Variations occur regularly in control fetuses.
Incidental:	Finding of no experimental relevance, e.g. due to processing (hemorrhage, mottled lung).

Some structural alterations may not fit into a general classification scheme. Such changes may be classified as malformations, anomalies or variations even if they do not strictly fulfill all criteria of a specific class of observation.

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

Statistical analysis (scanned from pages 27-28 of the study report)

Data Collection and Reporting

Data were collected by hand and on a Digital Equipment Corporation (DEC) VAX computer with SCC Reprotoxicology System software (Scientific Computer Consultants Inc., Ringwood, NJ 07456, USA; customized for CIBA-GEIGY Reproduction Toxicology Stein by SCC). Validation Certification of the SCC Reprotoxicology System was issued by Weinberg Associates Inc., Boothwyn, PA 19061, U.S.A. (Project Code 91041, December 1991).

The SCC Reprotoxicology System is protocol driven and allows authorized personnel to create a study protocol, including related work schedules, diets and dosages. The system prompts for appropriate data input (feed consumption, body weight, dosing, clinical signs, C-section data, and fetal visceral and skeletal observations), and checks that input is reasonable and complete. Weight data are input directly from balances to the on-line database. The system allows loading of proper historical data and produces data tables with statistical analyses on request.

This report, consisting of text, figures, and formatted SCC tables, was produced with LEX-WP and LEX-GRAPH software (Ace Microsystems Ltd., London W5 4EH, England) running on a DEC VAX computer.

Statistical Analyses

Statistical analysis of continuous data (e.g. body weight, feed consumption) was performed using the Analysis of Variance Procedure (ANOVA) [3] {reference provided} followed by Dunnett's t-Test [4] {reference provided} in case of a significant result in the ANOVA.

Categorical data (e.g. Malformation counts) were examined using Chi-Square test [5] {reference provided} followed by Fisher's Exact Test [6] {reference provided} in case of a significant result in the Chi-Square test.

Non-parametric data (e.g. mean percent affected fetuses/litter) were analyzed using the Kruskal-Wallis nonparametric analysis of variance test [7] {reference provided} followed by Mann-Whitney U-test [8] {reference provided}.
In all summary tables with statistics, the p value for the blocking test (ANOVA, Chi-square or Kruskal-Wallis) is given in the control column. P values for subsequent comparisons against controls (Dunnett's, Fisher's Exact or Mann-Whitney U) are given in the appropriate group column, if the blocking test is significant.

Statistical analyses are performed to draw attention to distinctive values. The responsible scientist may consider statistically Significant values lying within the historical control range as not relevant, and may also comment on values which are not statistically significant but which differ substantially from the expected normal values.

The statistics used are indicated by footnotes in the tables; no statistics are performed when the 'number of observations is insufficient (normally n<2).

Censoring of Data

Positively mated females which were not pregnant are excluded from summary tables for body weight, body weight gain, and feed consumption during gestation.

Other information: (scanned from page 29 of the study report)

Deviations from the Protocol

There were no circumstances that could affected the quality and/or integrity of the data.

There was one amendment to the protocol, effective from August 9, 1994 (prior to experimental start date), as follows:

Protocol page 6, 1106.01 Test Substance:

Change: Batch Number: 943042 to Batch Number: P.405009

[This amendment did not affect the outcome of the study]

References (from page 30 of the study report)

[1] Wilson J.G., in: Teratology: Principles and Techniques. Wilson J.G. and Warkany J., The University of Chicago Press, Chicago and London, 1965, pp. 251-278.

[2] Dawson A.B., Stain Tech. 1, 123-124, 1926.

[3] Winer B.J., Statistical Principles in Experimental Design. McGraw-Hill, New York, 2nd edition, 1971

[4] Dunnett C.W., J. Am. Stat. Assoc. 50, 1096-1121, 1955

[5] Gad S. and Weil C.S., Statistics and Experimental Design for Toxicologists. The Telford Press, Caldwell, New Jersey, 1986, p. 57

[6] Dixon W.J., Fisher's Exact Probability, in: BMDP Statistical Software, University of California Press, 1981, p. 663

[7] Kruskal W.H. and Wallis W.A., J. Am. Stat. Assoc. 47., 583-621, 1952

[8] Mann H.B. and Whitney D.R., Ann. Math. Stat. 18, 50-60, 1947

NOTE FROM THE REVIEWER: THE PROTOCOL DESCRIBED ABOVE IN THE MATERIALS AND METHODS SECTION IS ACCEPTABLE TO FULFILL THE INFORMATION SUGGESTED BY THE GUIDELINE OPPTS 870.3700; OPP §83-3a.

B. Results

Maternal Toxicity:

Mortality

No animals were reported to have died.

Clinical Observations

No treatment observations were noted.

Body Weight

The following Tables I and II present selected mean maternal body weights and mean maternal body weight gains (from Tables 2,3 and 7, pages 33-39, 49-50 and Appendix 4, page 110 of the study report):

Table I: Body Weights (mean grams±sd)

Day	0	6	16	21	
Control	202.1 ±9.5	231.4 ±10.9	300.2 ±19.5	375.6 ±32.4	
10 mg/kg/day	201.5 ±8.4	229.4 ±10.6	294.9 ±15.3	365.7 ±28.7	(98.2)[1] (97.4)
100 mg/kg/day	201.5 ±8.2	230.4 ±9.6	296.3 ±13.7	373.3 ±19.0	(98.7) (99.4)
1000 mg/kg/day	202.1 ±10.1	229.8 ±11.9	284.2** ±14.9	362.7 ±20.5	(94.7) (96.6)
Historical Control	**200.2**	**230.0**	**298.7**	**374.4**	

[1] = percent of control

Table II: Body Weight Gains (mean grams±sd)

Days	0-6	6-16	16-21	6-21[1]	0-21	C6-21[2]
Control	29.3 ±6.3	68.8 ±11.2	75.4 ±16.3	144.2 ±12.0	173.5 ±27.1	46.5
10 mg/kg/day	27.9 ±5.3	65.5 (95.2)[3] ±9.8	70.8 (94.5) ±16.6	136.3 (94.5)	164.2 (94.6) ±24.5	44.4 (95.5) ±12.1
100 mg/kg/day	29.0 ±7.4	65.9 (95.8) ±6.9	77.0 (↑) ±10.5	142.9 (92.3)	171.8 (98.9) ±16.5	38.2* (82.2) ±9.1
1000 mg/kg/day	27.7 ±6.8	54.4** (79.1) ±10.2	78.5 (86.5) ±12.9	132.9 (86.5) ±9.3	160.5 (92.5) ±20.1	31.4** (67.5)

*** = p < 0.05; ** = p < 0.01 by ANOVA + Dunnett test**
[1] = calculated by the reviewer from mean body weights; [2] = net weight change from gestation day 6 = carcass weight minus day 6 body weight; [3] = percent of control

Food Consumption

The following Tables III and IV present the provided food consumption data (from Table 4, page 38) and food efficiency data (calculated by the reviewer from mean data):

Table III: Food Consumption Data (mean grams/animal/day±sd)

Days	0-6	6-11	11-16	16-21
Control	23.0 ±2.0	25.0 ±2.3	27.1 ±2.4	27.7 ±2.0
10 mg/kg/day	22.4 ±1.9	24.4 ±2.0	26.4 ±2.3	26.8 ±2.5
100 mg/kg/day	22.5 ±2.1	23.1* ±2.4	25.0* ±2.1	27.5 ±2.1
1000 mg/kg/day	22.1	17.6**	22.9**	28.8

	±3.1	±2.1	±2.7	±2.3

Table IV: Food Efficiency Data (%)

Days	0-6	6-16	16-21	6-21	0-21
Control	21.2	26.4	54.4	36.1	32.3
10 mg/kg/day	20.7	25.8	52.8	35.1	31.4
100 mg/kg/day	21.5	27.4	56.0	37.8	33.5
1000 mg/kg/day	20.9	26.9	54.5	38.4	33.5

There were reduced body weights in the high dose group on gestation days 16 and 21. There were also decreased body weight gains in the high dose group during the dosing period ($p<0.01$; gestation days 6-16), the dosing period plus post dosing period (gestation days 6-21), the entire gestation period (days 0-21) and in the mid and high dose groups for the corrected body weight gain for the dosing period plus post dosing period ($p<0.05$ for mid dose and $p<0.01$ for the high dose groups). There was increased body weight gains in the mid and high dose groups in the postdosing period (gestation days 16-21) an indication of a rebound effect. There was reduced food consumption in the mid and high dose groups during the dosing period ($p<0.05$ for the mid and $p<0.01$ for the high dose groups). No biologically relevant effects were noted in food efficiency data.

Gross Pathological Observations

The investigators supplied group summary and individual animal data; no treatment related effects were noted during maternal postmortem examinations.

Cesarean Section Observations

The following Table V presents cesarean section observation data (from Tables 5 and 6, pages 40-44 and Appendices 5 and 6, pages 111-113).

Table V: Cesarean Section Observations

Dose(mg/kg/day):	Control	10	100	1000	HC[1]	
#Animals Assigned	24	24	24	24		
#Animals Mated/Inseminated	24	24	24	24	773	
#Animals Pregnant	23	22	20	22	735	
Pregnancy Rate (%)	95.8	91.7	83.3	91.7	95.1	
Maternal Wastage						
#Died	0	0	0	0	0	0
#Died/pregnant	0	0	0	0		
#Non pregnant	1	2	4	2		
#Died/nonpregnant	0	0	0	0		
#Aborted	0	0	0	0	0	
#Premature Delivery	0	0	0	0	2	
#Total litter loss	0	0	0	0	3	
Total live litters exam.	23	22	20	22	729	
Total Corpora Lutea	380	360	332	372	12354	
Corpora Lutea/dam	16.5±2.6	16.4±2.9	16.6±1.3	16.9±2.1	16.9±2.5	
Total Implantations	331	305	312	331	10833	
Implantations/Dam	14.4±4.0	13.9±4.0	15.6±1.4	15.0±2.1	14.8±2.7	
Total Live Fetuses	306	281	290	302	10234	
Live Fetuses/Dam	13.3±4.1	12.8±4.0	14.5±1.7	13.7±2.8	14.0±3.0	
Total Resorptions	25	24	22	29	575	
Early	25	24	22	28	566	
Late	0	0	0	1	9	
Resorptions/Dam	1.1±0.9	1.1±1.2	1.1±1.2	1.3±1.5	0.8±1.3	
Total Dead Fetuses	0	0	0	0	1	
Dead Fetuses/Dam	0	0	0	0		
Mean Fetal Weight (gm)	5.4±0.3	5.3±0.5	5.5±0.3	5.3±0.3	5.6	
Preimplantation Loss(%)	13.8±21.8	13.4±25.1	6.0±5.4	10.3±12.7	12.0±14.0	
Postimplantation Loss(%)	8.2±9.8	7.9±8.3	7.0±7.6	9.1±11.1	5.5±10.3	
Sex Ratio (% Male)	45.1	49.8	46.9	54.0	50.0	

[1] = HC = Historical Control

No treatment related effects were noted in the above data.

2. Developmental Toxicity

The following Tables VI, VII and VIII present the external, visceral and skeletal observation data, respectively (from Tables 9-13, pages 50-83 and Appendices 7-11, pages 114-124).

Table VI: External Examinations

Dose (mg/kg/day):	**Control**	**10**	**100**	**1000**	**HC[1]**
#pups/litters examined	**306/23**	**281/22**	**290/20**	**302/22**	10257/730
Observations					
Umbilical hernia	1/1	0/0	0/0	0/0	3/3
Generalized edema	0/0	1/1	0/0	0/0	6/6

[1] = HC = Historical Control

No treatment related effects were noted in the above data.

Table VII: Visceral Examinations

Dose (mg/kg/day):	**Control**	**10**	**100**	**1000**	**HC[1]**
#pups/litters examined	**149/23**	**135/22**	**139/20**	**146/22**	4793/725
Observations					
Umbilical hernia	1/1	0/0	0/0	0/0	3/3
Enlarged thymus	3/3	3/1	3/3	11*/7	13/13
Pulmonary hypoplasia	0/0	1/1	0/0	0/0	1/1
Accessory lobulet-liver	1/1	2/2	1/1	2/2	30/29
Renal pelvic dilation	3/3	1/1	0/0	1/1	2/2

[1] = HC = Historical Control

No treatment related effects were noted in the above data.

Table VIII: Skeletal Examinations

Dose (mg/kg/day):	**Control**	**10**	**100**	**1000**	**HC[1]**
#pups/litters examined	**157/23**	**146/22**	**151/20**	**156/22**	5426/728
Observations					
Sternebra(e) fused #1&2	6/4	7/5	4/3	4/3	54/46
asymmetrically shaped					
#1	0/0	5*/2	1/1	1/1	7/7
#2	2/2	0/0	0/0	1/1	7/7
#4	2/2	0/0	0/0	0/0	19/19
#5	7/7	2/2	2/2	3/3	43/42
#6	3/2	2/2	3/3	2/2	21/20
reduced #1	5/4	2/2	1/1	3/3	10/10
absent ossification					

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#2	0/0	1/1	0/0	0/0	2/2

continued

Table VIII: Skeletal Examinations continued

Dose(mg/kg/day):	Control	10	100	1000	HC[1]
#pups/litters examined	157/23	146/22	151/20	156/22	5426/728
Observations					
Cranial bones:					
irregular ossification: occipital	0/0	5/2	2/2	1/1	11/11
Metacarpals:					
#5 poor ossific.	0/0	1/1	0/0	0/0	6/6
Cervical vertebral arch additional	0/0	0/0	0/0	1/1	
Thoracic vertebral centers:					
bipartite	0/0	4/3	1/1	2/2	14/14
dumbbell-shaped	2/2	8/7	3/3	6/6	102/91
Hind limb:					
calcaneus ossification					
absent	143/23	139/21	133/19	151/22	4991/7224
poor	0/0	2/2	3/2	0/0	104/92
Metatarsals:					
#1 absent ossific.	13/9	18/8	15/9	20/9	420/225
poor ossific.	4/4	4/3	2/2	2/2	83/69
Cervical vertebral centers:					
absent ossification	156/23	146/22	147/20	154/22	5110/707
bipartite	10/8	4/4	9/6	12/9	552/332
poor ossification	26/13	28/19		25/16	27/15
dumbbell-shaped	2/2	0/0	1/1	2/2	813/4280 144/111
Ribs #13:					
shortened	4/4	6/4	6/4	8/4	352/210
absent ossification	0/0	1/1	0/0	1/1	26/20
Anterior digit:					
#1 distal phalanx absent ossific.	0/0	1/1	0/0	1/1	10/10
#2 proximal phalanx absent ossific.	5/4	10/3	4/3	5/4	111/80
poor ossific.	1/1	2/2	3/3	2/2	59/53
distal phalanx absent ossific.	0/0	1/1	0/0	0/0	10/10
#3 proximal phalanx absent ossific.	0/0	1/1	0/0	0/0	9/9
#4 proximal phalanx absent ossific.	0/0	2/1	0/0	0/0	15/14
distal phalanx absent ossific.	1/1	0/0	0/0	0/0	4/4

Table VIII: Skeletal Examinations continued

Dose(mg/kg/day):	**Control**	**10**	**100**	**1000**	HC[1]
#pups/litters examined	**157/23**	**146/22**	**151/20**	**156/22**	5426/728
Observations					
Anterior digit continued:					
#5 proximal phalanx					
absent ossific.	12/8	14/6	12/8	10/7	261/170
poor ossific.	5/4	4/3	3/3	9/5	116/97
distal phalanx					
absent ossific.	4/3	1/1	3/2	0/0	57/46
poor ossific.	1/1	0/0	0/0	0/0	15/12
Posterior digit:					
#1 distal phalanx					
absent ossific.	0/0	1/1	0/0	1/1	11/10
poor ossific.	0/0	4/4	0/0	0/0	4/4
#2 proximal phalanx					
absent ossific.	54/18		50/15	48/14	41/14 1218/452
poor ossific.	16/11	7/6	5/5	7/7	279/204
distal phalanx					
poor ossific.	0/0	4/1	0/0	1/1	4/4
#3 proximal phalanx					
absent ossific.	33/13		38/13	32/13	33/13 816/353
poor ossific.	7/5	8/4	8/5	8/5	198/151
distal phalanx					
poor ossific.	0/0	4/1	0/0	1/1	3/3
#4 proximal phalanx					
absent ossific.	32/12		36/12	30/12	30/13 800/353
poor ossific.	5/5	8/5	6/6	6/5	192/159
distal phalanx					
poor ossific.	0/0	4/1	0/0	1/1	4/4
#5 proximal phalanx					
absent ossific.	87/21		66/17	71/16	65/20 2111/577
poor ossific.	16/11	8/7	19/13	17/12	452/315
distal phalanx					
absent ossific.	0/0	1/1	0/0	1/1	7/7
poor ossific.	0/0	4/1	0/0	0/0	5/5

[1] = HC = Historical Control; * = p < 0.05; ** = p < 0.01 by Chi square + Fishers exact test

There was an increase in fetal and litter incidence of

enlarged thymus in the high dose group, however, the biological relevance of this observation is unclear and it occurs at the limit dose. This finding should be checked in other studies.

D. Discussion/Conclusions

I. Investigators' Conclusions (from page 15 of the study report)

In this study, CGA 279202 Technical was tested for its embryotoxic, fetotoxic, and teratogenic potential in rats. The test substance was administered by gavage in an aqueous solution of carboxymethylcellulose (0.5% w/w) at daily doses of 0, 10, 100 and 1000 mg/kg body weight to 24 mated rats per group from day 6 through day 15 post-coitum (=p.c.) inclusive. Dams were killed on day 21 p.c. and fetuses removed by cesarean section for examination.

Maternal Data

There were no treatment-related clinical signs or mortalities. There was a treatment-related reduction of body weight gain and body weight at 1000 mg/kg and reduction in feed consumption at 100 and 1000 mg/kg during the dosing period.

Reproduction and Cesarean Section Data

Post-implantation losses and number of live fetuses per litter were not affected by treatment.

Necropsies did not reveal any pathological findings.

Fetal Examination

Mean fetal body weights were not affected by treatment.

The overall incidence of external, visceral and skeletal malformations, anomalies, and variations were not affected by treatment. Increased fetal incidence of enlarged thymus was seen at 1000 mg/kg.

Conclusion

Maternal toxicity (reduced body weight parameters) was seen in the 1000 mg/kg group and reduced feed consumption was seen in both the -100 and 1000 mg/kg groups.

The no observed effect level (NOEL) of CGA 279202 Technical for rat dams in this study was 10 mg/kg body weight/day, and for fetuses was 100 mg/kg. There was no evidence for embryotoxic or teratogenic potential.

II. Reviewer's conclusions

a. Maternal Toxicity:

There were reduced body weights in the 1000 mg/kg/day group on gestation days 16 and 21. There were also decreased body weight gains in the 1000 mg/kg/day group during the dosing period ($p<0.01$; gestation days 6-16), the dosing period plus post dosing period (gestation days 6-21), the entire gestation period (days 0-21) and in the 100 and 1000 mg/kg/day groups for the corrected body weight gain for the dosing period plus post dosing period ($p<0.05$ for 100 mg/kg/day and $p<0.01$ for the 1000 mg/kg/day groups). There was increased body weight gains in the 100 mg/kg/day and 1000 mg/kg/day groups in the postdosing period (gestation days 16-21) an indication of a rebound effect. There was reduced food consumption in the 100 mg/kg/day and 1000 mg/kg/day groups during the dosing period ($p<0.05$ for the 100 mg/kg/day and $p<0.01$ for the 1000 mg/kg/day groups). No biologically relevant effects were noted in food efficiency data.

b. Developmental Toxicity:

i. Deaths/Resorptions:

No treatment related effects were noted.

ii. Altered Growth:

No treatment related effects were noted.

iii. Developmental Anomalies:

No treatment related effects were noted. There was an increase in fetal and litter incidence of enlarged thymus in the high dose group, however, the biological relevance of this observation is unclear and it occurs at the limit dose (1000 mg/kg/day). It may be an indication of an immune response and should be checked in other studies with this chemical.

iv. Malformations:

No treatment related effects were noted.

E. Study Deficiencies: No relevant study deficiencies were noted that would have affected the outcome of the study.

F. Classification: This study is classified as Acceptable-Guideline and satisfies the guideline requirements (OPPTS 870.3700, OPP §83-3a) for a teratology study in rats.

Maternal Toxicity NOAEL = 10 mg/kg/day

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Maternal Toxicity LOAEL = 100 mg/kg/day
Developmental Toxicity NOAEL ≥ 1000 mg/kg/day
Developmental Toxicity LOAEL > 1000 mg/kg/day

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SignOff Date:	**8/3/99**
DP Barcode:	**D243979**
HED DOC Number:	**013599**
Toxicology Branch:	**TOX2**