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

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MAR - 2 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

Subject: PP#9E2145 - Gibberellic Acid (A_3) Rat Teratology and Three Mutagenicity Studies in Support of an Exemption from Tolerances for Gibberellic Acid (Gibberellin A_3)

TOX Chem No. 467

FROM: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII
Toxicology Branch
Hazard Evaluation Division (TS-769C) ABK 3/1/88

TO: Hoyt Jamerson, PM Team 43
Minor Uses and Emergency Response Section
Registration Division (TS-767C) 3/1/88

The Toxicology Branch has received and reviewed several studies in support of an exemption from tolerances for Gibberellic Acid (A_3). The studies are listed below along with their classification or acceptability. The Data Evaluation Records (DERs) for each study reviewed are attached.

- Teratology Study in Rats (MRID No. 401552-01)

  Classification: Supplementary. The study can be upgraded pending the submission of maternal and fetal individual data. A significantly increased incidence of rib rudiments was reported for the high-dose group. The lack of individual data, however, precluded a comprehensive assessment of the biological or toxicological significance of these findings. In addition, since no data on corpora lutea were available, we were not able to assess possible effects on preimplantation losses. The effect levels for developmental toxicity were not determined due to the above deficiencies.

- Salmonella/Mammalian Microsome Mutagenicity Test (Ames Test) of Gibberellic Acid (MRID No. 402616-01)

  Classification: Acceptable. Negative in Ames testing up to limit doses (5000 and 10,000 ug/plate).

- Gibberellic Acid (A_3); In Vitro Cytogenetic Assay Measuring Sister Chromatid Exchange in Chinese Hamster Ovary Cells (MRID No. 402616-02)

  Classification: Unacceptable. No justification was provided for the use of 2700 ug/mL, a nontoxic concentration, as the highest dose tested. A repeat study incorporating toxicity testing at least up to limit doses is required to confirm the reported negative in this single experiment.

- Evaluation of Gibberellin A_3 (Acid Gibberellic) in Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay (MRID No. 402616-03)

  Classification: Acceptable. Gibberellin A_3 was negative for induction of UDS up to concentrations at the limit of solubility.

Attachments

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Primary Reviewer: William Woodrow, Ph.D.
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: Irving Mauer, Ph.D.
Section VI, Toxicology Branch (TS-769C)

3/1/88

DATA EVALUATION RECORD

1. Subject: Salmonella/Mammalian Microsome Mutagenicity Test (Ames Test) of Gibberellic Acid

2. Test Material: Gibberellic Acid, Lot No. 84-526-CD, Technical Grade, 90% Pure.

3. EPA File No.: 9E2145; TB Project No. 7-0913

4. Accession No.: 402616-01

5. Sponsor: Abbott Laboratories
Chemical and Agricultural Products Division
North Chicago, IL 60064

6. Testing Facility: Abbott Laboratories
Pharmaceutical Products
Research and Development
Abbott Park, IL 60064

7. Report No./Date: Project No. T87-109, June 5, 1987

8. Author: M.S. Diehl

9. Toxicity Category: N/A

10. Classification:

Acceptable. Negative in Ames testing up to limit doses (5000 and 10,000 ug/plate).

3

11. Materials and Methods:

Histidine deficient (auxotrophs) of Salmonella typhimurium, TA-1535, TA-1537, TA-1538, TA-98, and TA-100 were employed. Aroclor 1254-induced rat liver microsomes (the S9 fraction) Lot No. 07415 plus generating cofactors were used in metabolic activation testing.

Vogel-Bonner Medium E agar plates in triplicate were used, with top agar containing L-histidine and biotin.

Positive control chemicals included N-methyl-N-nitro-N-nitrosoguanidine (MNNG), 9-aminoacridine (9AA), 2-nitrofluorine (NF), and 2-aminoanthracene (2-AA).

Positive control compounds and test material were dissolved in DMSO; DMSO was used as the negative vehicle control.

The numbers of colonies from triplicate treatment petri plates were averaged, and compared to numbers of colonies on vehicle control plates.

Three tests were conducted. The first test used all five S. typhimurium strains tested separately using triplicate plating, with and without S9 microsome activation, and exposed to 1, 5, 10, 50, 100, 500, 1000, or 2000 ug gibberellic acid per plate, 50 ug DMSO negative control per plate, and the following positive control chemicals, according to strain.

Strain	Nonactivated + Control		Activated + Control
TA-1535	N-methyl-N'-nitro-N-nitrosoguanidine	10 ug	2-aminoanthracene 2.5 ug
TA-1537	9-aminoacridine	50 ug	2-aminoanthracene 2.5 ug
TA-1538	2-nitrofluorene	10 ug	2-aminoanthracene 2.5 ug
TA-98	2-nitrofluorene	10 ug	2-aminoanthracene 2.5 ug
TA-100	N-methyl-N'-nitro-N-nitrosoguanidine	10 ug	2-aminoanthracene 2.5 ug

The second test was similar to the first test except 5000 and 10,000 ug/plate gibberellic acid concentrations were also included. Negative and positive control testing remained identical to the first test.

4

A third test used only strain TA-1537 tested against the same negative and positive control conditions and 1, 10, 100, 1000, or 10,000 ug/plate gibberellic acid.

12. Results:

a. Gibberellic acid did not demonstrate mutagenic potential in Ames testing under any of the conditions tested, (refer to tables 1, 2, and 3).

b. Triplicate plates were employed to insure test consistency. Although specific reference was made to establishing a bacterial cell MTD, gibberellic acid was tested at 5000 ug and above, which is considered the "limit" test concentration, with negative results.

c. The results shown in test number 2 largely did not reproduce the test results found at similar concentrations for test number 1; however, these findings did not affect the final testing evaluation.

d. Number 3 test with strain TA-1537 was conducted because both positive controls for TA-1537 in test 1 and the activation series in test 2, did not show any colony increase. Test number 2 showed a positive control increase only for the nonactivated series. In test number 3, the positive controls did show significant increases for both nonactivated and activated test conditions.

13. Conclusions:

Gibberellic acid did not demonstrate mutagenic potential when tested by the Ames Salmonella/microsome mutagenicity test.

14. Toxicity Category: N/A

15. Classification: Acceptable

5

Gibberellins toxicology review

Page _____ is not included in this copy.

Pages 6 through 8 are not included in this copy.

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 product 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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Primary Reviewer: William Woodrow, Ph.D.
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: Irving Mauer, Ph.D.
Section VI, Toxicology Branch (TS-769C)

3/1/88

DATA EVALUATION RECORD

1. Subject: Gibberellin Acid (A_3); Lot #84-526-CD; List Code 33690 in an In Vitro Cytogenetic Assay Measuring Sister Chromatid Exchange in Chinese Hamster Ovary Cells

2. Test Material: Gibberellic Acid (A_3), Technical Grade, 90% Pure. (Concentrations used in study not corrected for purity.)

3. EPA File No.: 9E2145; TB Project No. 7-0913

4. Accession No.: 402616-02

5. Sponsor: Abbott Laboratories
Chemical and Agricultural Products Division
North Chicago, IL 60064

6. Testing Facility: Hazleton Biotechnologies Company
5516 Nicholson Lane, Suite 400
Kensington, MD 20895

7. Report No./Date: HB Project No. 20990, March 1986

8. Author: James L. Ivett

9. Toxicity Category: N/A

10. Classification:

Unacceptable. Highest dose tested was nontoxic and soluble. Repeat study required incorporating toxicity testing up to limit doses.

11. Materials and Methods:

Chinese Hamster Ovary (CHO) cells were grown in McCoy's 5a tissue culture medium supplemented with 10% fetal calf serum (FCS), 1% L-glutamine, and 1% streptomycin and penicillin.

Positive control agents were Mitomycin C (MMC, 5 and 10 ng/mL) for the nonactivated positive control and cyclophosphamide (CP, 1.5 and 2.0 ug/mL) for the metabolic activation positive controls.

The hepatic mixed function oxidase S9 fractions used for the metabolic activation assays were prepared from male Sprague-Dawley rats treated with Aroclor 1254. 1.5 mg/mL NADP and 2.7 mg/mL isocitric acid were added to make the active S9 mixture.

Sister Chromatid Exchange Assay Without Activation

Cultures were established approximately 24 hours prior to treatment. The cultures were then dosed with the test article until approximately 2.5 hours prior to harvesting the cells. Ten um 5-bromo-2'-deoxyuridine (BrdUrd) was added 2 hours after the initial exposure of the cells to the test article, and the cultures were reincubated for an additional 23 hours (BrdUrd is added to sensitize the cell DNA, so that following treatment with the fluorescence-plus-Giemsa (FGP) technique, sister chromatid exchanges along the length of chromosomes and chromatids may be readily visualized).

Following the additional 23 hours incubation, the cells were washed with buffered saline and fresh medium, BrdUrd and Colcemid (0.1 ug/mL) were added (the Colcemid was added to arrest cell division and accumulate cells in metaphase). Cultures were harvested, slides prepared and stained by the FPG technique.

Sister Chromatid Exchange Assay With Metabolic Activation

CHO cells were incubated at 37 °C for 2 hours in the presence of test material and the S9 fraction in the tissue culture medium without FCS. Cells were then washed free of test material with buffered saline. Complete McCoy's 5a medium (with FCS) with 10 um BrdUrd was added to the cultures, which were incubated an additional 23 hours, at which time 0.1 ug/mL Colcemid was added to accumulate metaphase cells. Cultures were harvested, slides prepared and stained with the fluorescence-plus-Giemsa stain.

10

Fifty cells per dose were read from each of the top four doses from which sufficient M2 metaphase cells were available. Fifty cells were read from each of the negative and solvent controls, and at least 20 cells were read from one dose level of the positive control.

A positive finding for SCE must meet one of the following two criteria:

a. A twofold increase: Approximately a doubling in SCE frequency over the "background" (solvent and negative control) levels at one or more doses.

b. Dose response: A positive assessment may be made in the absence of a doubling if there was a statistically significant increase at a minimum of three doses and evidence for a positive dose response.

In some cases, statistically significant increases were observed with neither a doubling nor a dose response. These results were assessed according to repeatability, magnitude of response, and the proportion of the dose levels affected.

A Student's t-test was employed for statistical analysis to compare SCE frequencies in the tested cultures with the negative and solvent controls.

12. Results:

Gibberellic Acid$_3$ (GA_3) was soluble at 270 mg/mL in DMSO. When diluted 1:100 with culture medium (2700 ug/mL), a clear solution was obtained.

A (GA_3) final concentration of 2700 ug/mL was the highest concentration for the test. One-half log series of doses from 90 through 2700 ug/mL was tested in the SCE assay.

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Sister Chromatid Exchange in Chinese Hamster Ovary Cells

a. Without Metabolic Activation

Treatment	SCEs/ Cell ± S.E.	Cell Cycle Stages (%)				% Increase Over Solvent Control
		M1	M1+	M2	M2+	
Controls						
Negative: McCoy's 5a	9.20 ± 0.41	0	5	82	13	
Solvent: DMSO 10 uL/mL	10.54 ± 0.53	1	12	87	0	
Positive: Mitomycin C 5.0 ng/mL	24.25 ± 1.17	0	3	92	5	130
Test Compound (GA_3)						
90.0 ug/mL	9.44 ± 0.41	2	27	71	0	
270.0 ug/mL	9.40 ± 0.45	2	11	87	0	
900.0 ug/mL	10.18 ± 0.45	3	20	77	0	
2700.0 ug/mL	9.70 ± 0.46	2	21	77	0	

b. With Metabolic Activation

Treatment	SCEs/ Cell ± S.E.	Cell Cycle Stages (%)				% Increase Over Solvent Control
		M1	M1+	M2	M2+	
Controls						
Negative: McCoy's 5a	9.94 ± 0.43	1	9	77	13	
Solvent: DMSO 10 uL/mL	9.36 ± 0.41	1	6	87	6	
Positive: Cyclophosphamide 1.5 ug/mL	31.15 ± 0.98	1	3	87	9	233

12

b. With Metabolic Activation (cont'd)

Test Compound (GA_3)

Treatment	SCEs/ Cell ± S.E.	Cell Cycle Stages (%)				% Increase Over Solvent Control
		M1	M1+	M2	M2+	
90.0 ug/mL	9.98 ± 0.46	1	3	82	14	7
270.0 ug/mL	9.94 ± 0.45	0	9	78	13	6
900.0 ug/mL	9.72 ± 0.45	0	9	80	11	4
2700.0 ug/mL	10.06 ± 0.51	0	5	86	9	7

13. Conclusions:

a. No increases in SCEs were found when GA_3 Lot #84-526-CD Code 33690 was tested in CHO cells, without metabolic activation.

b. A very slight percent increase in SCE occurred when GA_3 was tested in CHO cells with metabolic activation, compared to solvent controls. The GA_3 concentrations used were 90.0, 270.0, 900.0, and 2700 ug/mL. The slight SCE increase was observed at all dose levels tested; however, the percent increases over solvent controls were not statistically significant.

c. GA_3 Lot #84-526-CD Code 33690 did not demonstrate mutagenic potential when tested in CHO cells using the SCE test.

14. Classification:

UNACCEPTABLE. No justification is provided for the use of 2700 ug/mL, a nontoxic concentration, as the highest dose tested. A repeat study incorporating toxicity testing at least up to limit doses is required to confirm the reported negative in this single experiment.

13

Primary Reviewer: William Woodrow, Ph.D.
Section VII, Toxicology Branch (TS-769C)
Secondary Reviewer: Irving Mauer, Ph.D. 3/1/88
Section VI, Toxicology Branch (TS-769C)

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DATA EVALUATION RECORD

1. Subject: Evaluation of Gibberellin A_3 (Acid Gibberellic) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

2. Test Material: Gibberellin A_3, Lot No. 84-526-CD, List Code 33690, 90.0% Pure.

3. EPA File No.: 9E2145; TB Project No. 7-0913

4. Accession No.: 402616-03

5. Sponsor: Abbott Laboratories
Chemical and Agricultural Products Division
North Chicago, IL 60064

6. Testing Facility: Hazleton Biotechnologies Company
5516 Nicholson Lane, Suite 400
Kensington, MD 20895

7. Report No./Date: HBC Project No. 20991, May 1986

8. Author: Maria A. Cifone

9. Toxicity Category: N/A

10. Classification:

Acceptable. Gibberellin A_3 was negative for induction of UDS up to concentrations at the limit of solubility.

14

11. Materials and Methods:

A Fischer 344 male rat served as the source of hepatocyte cells for the present in vitro rat hepatocyte Unscheduled DNA Synthesis (UDS) Assay for testing Gibberellin A_3. The rat liver was perfused about 4 minutes in situ with Hanks balanced salts (Ca^{++}-Mg^{++}-free) containing 0.5 mM ethylene-glycol-bis (β-aminoethyl ether)-N,N-tetraacetic acid and HEPES buffer at pH 7.0. Williams' Medium E (WME) with 50 to 100 units/mL of collagenase was then perfused through the liver for about 10 minutes. Hepatocytes were obtained by mechanical dispersion of excised liver tissue.

Hepatocytes were allowed to attach to plastic coverslips in 3 mL WME for a period of 1.5 to 2 hours at 37 °C in a 5% CO_2 atmosphere. The WME was supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 2.4 uM dexamethasone, 90 U/mL penicillin, 90 ug/mL streptomycin sulfate, and 140 ug/mL gentamicin.

Negative controls consisted of assay procedures performed on cells exposed only to the test material solvent (ethanol). A stock solution of test material was prepared in ethanol at 125.8 mg/mL. Test concentrations were then prepared by making 1:100 dilutions of the stock solution into WME growth medium containing 1% fetal bovine serum, to obtain the final desired concentrations of the test material.

A single positive control consisted of 2-acetyl-amino-fluorene (2-AAF) at 4.48×10^{-7} M (0.010 ug/mL).

Following the cell attachment period (1.5-2 hours), unattached cells were removed and cultures refed with WME. UDS assays were initiated within 3 hours by replacing the media with 2.5 mL WME/petri plate containing 1% fetal bovine serum, 1 uCi/mL ^{3}H-thymidine (20 Ci/mMole), and the test material. Each treatment, including positive and negative controls, was performed on five cultures, two of which were used for cytotoxicity measurements. Treatment was for 18 to 19 hours, and was terminated by washing cells 2X with WME. Three of the cultures from each treatment were washed 2X with WME containing 1 mM thymidine, and treated as follows.

Cultures were fixed in 1:1 acetic acid-ethanol and dried. The coverslips were then mounted on glass slides (cells up), dipped in Kodak NTB2 photographic emulsion and dried, then into light-tight boxes for 6 to 10 days. After development in Kodak D19, slides were fixed, and stained with Williams' modified hematoxylin and eosin.

15

The test material would be considered positive in the UDS assay at concentrations that caused:

1. Mean net nuclear grain count increase to at least 6 grains/nucleus, and/or

2. An increase in the percent of nuclei having six or more net grains to at least 10 percent of the analyzed population, after subtraction of the concurrent negative controls, and/or

3. The percent of nuclei with 20 or more grains reaches or exceeds 2 percent of the analyzed population.

UDS Mechanism

Fresh hepatocytes from rat livers will attach to a culture surface and continue to metabolize for several days without undergoing cell division. Only a small percentage of the cells enter S phase (replicative DNA synthesis). Thus, if ^{3}H-thymidine is added to the culture medium, little or no label is normally incorporated into nuclear DNA. However, the addition of test material which interacts with DNA often stimulates a repair response such that the altered DNA part is excised, and the missing portion replaced by DNA synthesis. This synthesis of DNA by nondividing cells is known as UDS and can be measured by determining the amount of ^{3}H-thymidine incorporation by grain counting.

12. Results:

TABLE 1

Gibberellin A3 (Acid Gibberellic) SOLVENT: Ethanol

Test Condition	Concentration	UDS* Grains/ Nucleus	Avg.† % Nuclei with ≥ 6 grains	Avg.† % Nuclei with ≥ 20 grains	Survival†† at 21 hour, %
Solvent Control (Ethanol)	1%	0.39	0.0	0.0	100.0
Positive Control (2-AAF)	0.1 ug/mL	9.82	72.7	8.7	84.4
Test Material:					
Gibberellin A3	1260 ug/mL	0.69	0.0	0.0	85.2
Gibberellin A3	1000 ug/mL	0.44	0.0	0.0	84.7
Gibberellin A3	602 ug/mL	0.29	0.0	0.0	86.0
Gibberellin A3	500 ug/mL	0.35	0.0	0.0	91.0
Gibberellin A3	250 ug/mL	0.39	0.0	0.0	95.2
Gibberellin A3	100 ug/mL	0.36	0.0	0.0	ND
Gibberellin A3	50 ug/mL	0.33	0.0	0.0	ND

*UDS = Average of net nuclear grain counts on triplicate coverslips (150 total cells).
†Average values for triplicate coverslips.
††Survival = Number of viable cells per unit area relative to the solvent control x 100.
2-AAF = 2-acetyl aminofluorene.
ND = Not determined.
Mean cytoplasmic grain count for solvent controls = 2.47.

17

13. Conclusions:

Table 1, above, shows data at concentrations of 1260, 1000, 602, or 500 ug/mL Gibberellin A_3/mL (1260 and above = limit of solubility).

None of the criteria used to show UDS were approached by Gibberellin A_3 treatment at any dose and no dose-related response was observed. Positive control (2-AAF) results show a significant increase in nuclear grain counts.

Gibberellin A_3 did not demonstrate any mutagenic potential when tested in the UDS assay.

14. Classification:

Acceptable. Negative for induction of UDS up to concentrations at the limit of test article solubility (1260 ug/mL).

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EPA: 68-02-4225
DYNAMAC No. 332-A
January 26, 1988

DATA EVALUATION RECORD

GIBBERELLIC ACID

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Lehrer, S. B. Evaluation of the effects of orally administered gibberellic acid on the embryonic and fetal development of the rat (Segment II, TFR). (Unpublished study No. TA86-014 by Abbott Laboratories, North Chicago, IL; dated September 17, 1986.) Accession No. 401552-01.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Rachel Fehrenbacher for

Date: 1-26-88

19

1. CHEMICAL: Gibberellic acid.

2. TEST MATERIAL: Gibberellic acid was from lot No. 86-178-CD and had a purity of 93.39%.

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: Lehrer, S. B. Evaluation of the effects of orally administered gibberellic acid on the embryonic and fetal development of the rat (Segment II, TFR). (Unpublished study No. TA86-014 by Abbott Laboratories, North Chicago, IL; dated September 17, 1986.) Accession No. 401552-01.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 1-26-88

Guillermo Millicovsky, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: G Millicovsky
Date: 1-26-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 1-26-88

William B. Greear, M.P.H.
EPA Reviewer

Signature: William B Greear
Date: 2/1/88

Albin Kocialski, Ph.D.
EPA Section Head

Signature: A. Kocialski
Date: 3/1/88

20

7. CONCLUSIONS:

A. Based on the submission of summarized data, exposure of pregnant rats to 10, 100, and 1000 mg/kg/day gibberellic acid did not cause maternal toxicity.

A significantly increased incidence of rib rudiments was reported for the high-dose group. The lack of individual data, however, precluded a comprehensive assessment of the biological or toxicological significance of these findings. In addition, since no data on corpora lutea were available, we were not able to assess possible effects on preimplantation losses. The effect levels for developmental toxicity were not determined due to the above deficiencies.

B. The study is classified Core Supplementary, but can be upgraded pending the submission of maternal and fetal individual data.

Items 8-10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: Gibberellic acid from lot No. 86-178-CD was 93.39% pure. The test material was suspended weekly in 0.2% hydroxypropylmethylcellulose at appropriate concentrations to yield doses of 0 (control), 10, 100, and 1000 mg/kg when administered at 10 mL/kg. The preparations were refrigerated when not in use. Once during the study, samples of the formulations were analyzed to determine the concentrations of the test material. After the study was completed, a sample of the bulk material was reassayed. Stability assays were conducted on test material formulations that were stored at 5°C for 1 week.

2. Animals and Experimental Design: Timed-pregnant Crl:CD(SD)BR rats were obtained from Charles River Breeding Laboratories before gestation day (GD) 6. The animals were individually housed in a room that was regulated at 72±5°F. Temperature and humidity were monitored daily (ranges were not reported) and the animals were exposed to light for 14 hours/day. Purina Certified Rodent Chow No. 5002 and tapwater were provided ad libitum.

Prior to dosing on GD 6, 24 rats were randomly assigned to each of the four dose groups. The appropriate test suspensions were administered daily by gavage on GD 6 through 15. Volumes administered were based on the most recently recorded

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[1]Only items appropriate to this DER have been included.

21

individual body weights. On GD 20, the dams were killed and their litters were delivered by cesarean section.

3. Observations and Measurements: During the dosing period, the females were examined for behavioral and physical alterations 1 to 2 and 24 hours postdosing. After the dosing period, the animals were examined once per day. Body weights were recorded on GDs 6, 9, 12, 15, and 20. Food consumption was recorded for the periods of GD 6-9, 9-12, and 12-15.

   The numbers and uterine positions of live, dead, and macerated fetuses and resorptions were recorded. All fetuses were removed, weighed, sexed, and grossly examined for external alterations. Approximately one-half of the fetuses in each litter were fixed in Bouin's solution and examined for visceral alterations using Barrow and Taylor's modification of Wilson's technique. The remaining fetuses were stained with alizarin red S and examined for skeletal alterations.

4. Statistical Methods: Body weights, food consumption, and implantation data were evaluated using a one-way analysis of variance; three elementary contrasts were used to identify significant differences from the control group. Proportions of implantation types, sex ratios, fetal body weights, and fetal abnormalities were analyzed using a jackknife technique (to adjust for a litter effect) and t-tests. The Bonferroni inequality equation was used to obtain an overall level of significance of 0.05. The proportions of implantations and fetal abnormalities were also analyzed for trend using linear regression on arc-sine transformed data that was weighted for litter size. Incidences of dams with at least two nonviable implants were analyzed using Fisher's exact test for a 2 x 4 contingency table and with Armitage's test for linear trend.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

Test Material Analyses: Pre- and poststudy assays of the bulk material indicated that the test material contained 93.39 and 93.2% active ingredient, respectively. Assays of the test suspensions yielded 88, 85.6, and 89.42% theoretical concentrations for the low-, mid-, and high-dose preparations, respectively. Stability assays conducted on samples stored for 1 week at 5°C indicated that all samples contained approximately 95% of the initial concentrations.

Maternal Data: No animals died during the study. The study report stated that one female in each treatment group was not pregnant; the data from these animals were not included in the evaluation. Loose stools were observed in one female each in the control and the mid-dose groups and three females in the high-dose group. These findings were noted for only 1 day in the control and mid-dose dams, but for at least 2 days in the high-dose females.

22

Individual data were not presented; however, summarized body weight (Table 1) and food consumption (Table 2) results were comparable for all groups.

Developmental Data: Summarized data for 23 litters per treatment group were reported. As shown in Table 3, the numbers of implantations, live fetuses, postimplantation losses, fetal weights, and fetal sex distribution were comparable for all groups.

Except for rib rudiments, fetal examinations revealed comparable findings in all groups. Rib rudiments were noted only in the high-dose group but resulted in a significant dose-related increasing trend (Table 4). Neither the anatomical position of these findings in individual fetuses nor the frequency among the litters were reported. Since these data were processed through several statistical steps, the actual number of affected fetuses could not be determined by the reviewers.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded that "Gibberellic acid was devoid of maternal or embryotoxic effects and was not teratogenic when administered to pregnant rats at dosages as great as 1000 mg/kg/day."

B. A quality assurance statement was signed and dated October 13, 1986.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material: The results of chemical analyses indicated that the dosing solutions were accurately prepared. In addition, the storage procedures ensured the stability of the test material in the dose solutions under conditions of this study.

Maternal Data: All animals survived and the clinical observations did not suggest any adverse effects associated with the test material. We assess that summarized mean body weights and weight gains were comparable for all groups.

Developmental Data: Based on the summarized data, we assess that the pregnancy rates, implantation data, litter size, fetal sex distribution, and fetal weights were comparable for all groups. Since corpora lutea data were not reported, we were not able to determine if any effects on preimplantation loss occurred. Fetal examination findings were comparable for all groups; however, a significant increase in the incidence of rib rudiments was noted in the high-dose group. The absence of individual data precluded our assessment of the anatomical position(s) of the rib rudiments (i.e., cervical or thoracic), frequency among the litters, or the actual number of affected fetuses. The biological significance,

23

TABLE 1. Mean (± S.D.) Maternal Body Weights of Rats[a] Dosed with Gibberellic Acid

Dose Level (mg/kg)	Body Weight (g) on Gestation Day		
	6	15	20
0	230±12	283±16	347±25
10	226±12	281±15	347±21
100	221±16	276±21	341±25
1000	230±17	288±20	348±26

Dose Level (mg/kg)	Body Weight Gain (g) on Gestation Days	
	6-15	6-20
0	54± 9	118±17
10	55±10	121±17
100	55± 6	121±13
1000	57±10	118±17

[a]Twenty-three females per group.

24

TABLE 2. Mean (± S.D.) Maternal Food Consumption of Rats[a] Dosed with Gibberellic Acid

Dose Level (mg/kg)	Food Consumption (g/rat/interval) on Gestation Days		
	6-9	9-12	12-15
0	85± 6	87± 8	86±10
10	82±11	83±12	85± 8
100	82± 8	83± 9	82± 8
1000	84± 8	87± 9	88±11

[a]Twenty-three females per group.

25

TABLE 3. Mean (± S.E.) Litter Data[a] of Rats Dosed with Gibberellic Acid

Dose Level (mg/kg)	Implantations	Live Fetuses	Post-implantation Loss (%)	Fetal Weight (g)
0	12.4±0.5	11.8±0.5	4.9±1.6	3.67±0.11
10	13.2±0.4	12.6±0.5	4.6±1.7	3.62±0.06
100	12.9±0.3	12.4±0.4	4.0±1.0	3.60±0.07
1000	11.8±0.6	11.1±0.6	5.5±1.1	3.67±0.08

[a]Twenty-three litters per treatment group.

TABLE 4. Jackknifed Mean (± S.E.) Incidence of Abnormalities per Litter[a] of Fetuses from Rats Dosed with Gibberellic Acid

Dose Level (mg/kg)	Umbilical Hernia	General Edema	Hydro-nephrosis	Rib Rudiments	Rudimentary 13th Rib
0	0	0	0	0*	2.2±1.6
10	0	0	1.4±1.0	0	1.4±1.0
100	0	0	0.7±0.7	0	6.2±3.2
1000	0.4±0.4	0.4±0.4	0.8±0.8	1.6±1.1	2.4±1.7

Dose Level (mg/kg)	Wavy Ribs	Reduced Ossifi-cation of Skull	Malaligned Sternebra(e)
0	0	10.8±2.9	0.7±0.7
10	2.1±1.1	9.7±3.3	1.4±1.0
100	0	4.1±2.1	1.4±1.0
1000	0	9.3±3.2	0.8±0.8

[a]Twenty-three litters per treatment group.

*Significantly increasing trend with dose ($p \leq 0.05$).

27

if any, of the increased incidence of rib rudiments, therefore, cannot be ascertained.

B. We agree with the study author's conclusions that gibberellic acid administered at doses up to 1000 mg/kg/day did not induce maternal toxicity. However, a definitive assessment of the developmental toxic potential of the test material was not possible because of the absence of individual fetal data.

In addition to the lack of all maternal and fetal individual data, the following deficiencies were noted:

1. Corpora lutea were not reported.

2. Fetuses were not individually identified.

3. Environmental conditions were not reported.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 9-12; Appendix B, Protocol, CBI pp. 17-23.

APPENDIX A

Materials and Methods

Gibberellins toxicology review

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