3/13/95

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

MAR 13 1995

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Reregistration of Gibberellins (GA_3 and GA_4A_7)

FROM: Sheryl K. Reilly, Ph.D., Biologist
Biopesticides and Pollution Prevention Division
(7501W)

TO: Denise L. Greenway, Regulatory Action Leader
Robert F. Torla, Team Leader, Team 112
Biopesticides and Pollution Prevention Division (7501W)

Action Requested: Review of two subchronic oral toxicity studies for Gibberellins GA_3 (MRID 416175-01) and GA_4A_7 (MRID 416166-01).

Conclusions: In the first subchronic dietary study, rats of both sexes were fed diets containing gibberellic acid (GA_3, purity 88.5%) at concentrations of 0, 1,000, 10,000, or 50,000 ppm for 13 weeks. A group of control animals and high-dose animals were fed regular rodent diet for an additional 4-week recovery period. The consumption of test material was 53-117, 550-1178, or 2994-5786 mg/kg/day (males) and 67-130, 730-1283, or 3872-6241 mg/kg/day (females). The only treatment-related clinical sign of toxicity was a low incidence of soft stools in both sexes receiving the highest dose. Very slightly decreased body weight gains were observed in mid-dose males and high dose animals of both sexes. Slightly increased total food consumption in all treated groups were observed. Evidence suggestive of a compound-related effect on kidney function included significantly increased blood urea nitrogen levels (BUN) and increased relative kidney weights in female rats in the high-dose group. BUN values and kidney weights were comparable to controls at the end of a 4-week recovery period, indicating reversibility of renal effects. Other effects observed in high-dose males included decreased globulin levels at termination of the study and decreased glucose levels ($p \leq 0.05$) at the end of the 4-week recovery period. Increased relative liver weights were observed in males at 50,000 ppm and in females at 10,000 ppm and 50,000 ppm. At the end of the recovery period, increased relative liver weights were still evident in females (11%), but not in males. In the absence of clinical chemistry correlates and gross and microscopic hepatic abnormalities, the liver weight changes are considered compensatory

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rather than a toxic effect of the test material. **Under the conditions of this study, the NOEL is 10,000 ppm; the LOEL is 50,000 ppm, based on the occurrence of soft stools in both sexes, and increased BUN levels, liver and kidney weights in females.** This study is classified as **Core-Minimum**, because it generally satisfies the guideline requirement for a subchronic dietary toxicity study in rodents.

In the second subchronic dietary study, rats were fed diets containing 0, 1,000, 10,000 or 25,000 ppm gibberellins A_4A_7 (purity 85.5%) for 13 weeks. Additional groups of 10 control and 10 high-dose animals were held for a 4-week recovery period. The 25,000 ppm exposure group was fed a diet containing 50,000 ppm during the first 14 days of the study; however, due to low weight gain and clinical signs the dosage was reduced for the duration of the study. The average calculated doses for the 1,000, 10,000 and 25,000 ppm groups, the latter corrected for the exposure to 50,000 ppm, were 67, 704 and 2238 mg/kg/day for males and 85, 814 and 2403 mg/kg/day for females. No treatment-related effects were observed at the 1,000 or 10,000 ppm dietary levels. One male in the 25,000 ppm treatment group died during the third week of the study. Compared to controls, treatment of rats of both sexes at 25,000 ppm had significant effects on clinical signs, food consumption (during the first 5 weeks of the study), body weight, organ-to-body weight ratios, hematology, clinical chemistry and gross and microscopic pathology. Clinical signs in the high-dose groups included hunched posture, thin, rough hair coat, a bloody crust on the nose and urine stains. Body weights for males and females were significantly lower than controls throughout the study, including the recovery phase. There was a marked increase in body weights when the treatment was reduced from 50,000 ppm to 25,000 ppm after 14 days, and by the end of the recovery period at week 17, body weights were within 91% of control groups for both sexes.

Males had significantly decreased hemoglobin and hematocrit values and significantly increased total bilirubin, cholesterol and alkaline phosphatase values. Females had significantly lower total protein, albumin and calcium values and significantly higher globulin, total bilirubin, cholesterol and alkaline phosphatase values. Both sexes had significantly increased relative brain weights and males had significantly increased relative kidney and testis weights. Gross pathological changes in the kidneys (rough surfaces and depressed areas in the cortex) were present in both sexes. Histologically, chronic tubular nephritis, tubular dilation, and focal loss of nephrons were observed. Histological changes in the liver were marginal in incidence and severity. **Under the conditions of this study, the NOEL is 10,000 ppm; the LOEL is 25,000 ppm based on alterations in clinical chemistry, decreased food consumption, decreased body weights, increases in relative organ weights (brain, kidney and testis), and gross and histopathological changes in the kidney.**

This study is classified as **Core-Minimum** because it generally satisfies the guideline requirement for a subchronic dietary toxicity study in rodents.

DATA EVALUATION REPORT

GIBBERELLIC ACID (GA_4A_7)

Study Type: SUBCHRONIC FEEDING – RAT (82-1).

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
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Task Order No. 94-40B

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Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

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Biopesticides and Pollution Prevention Division

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Feeding – Rat (82-1 or 152-20)

TOX. CHEM. NO: Not reported

P.C.CODE.: 073801

MRID NO.: 416166-01

TEST MATERIAL: Gibberellins A_4A_7

SYNONYMS: Gibberellic Acid (GA_4A_7), ProGibb® A_4A_7

STUDY NUMBER: HLA 6161-114

SPONSOR: Abbott Laboratories, Chemical and Agricultural Products Division, North Chicago, IL

TESTING FACILITY: Hazleton Laboratories America, Inc., 3301 Kinsman Boulevard, Madison, WI 53704

TITLE OF REPORT: 13-Week Dietary Toxicity Study with Gibberellins A_4A_7 in Rats

AUTHOR: Karen M. MacKenzie, Ph.D.

REPORT ISSUED: July 10, 1990 (study completion date)

EXECUTIVE SUMMARY: Groups of 10 male and 10 female Crl:CD®BR rats were fed diets containing 0, 1,000, 10,000 or 25,000 ppm gibberellins A_4A_7 (purity 85.5%) for 13 weeks. Additional groups of 10 control and 10 high-dose animals were held for a 4-week recovery period. The 25,000 ppm exposure group was fed a diet containing 50,000 ppm during the first 14 days of the study; however, due to low weight gain and clinical signs the dosage was reduced for the duration of the study. The average calculated doses for the 1,000, 10,000 and 25,000 ppm groups, the latter corrected for the exposure to 50,000 ppm, were 67, 704 and 2238 mg/kg/day for males and 85, 814 and 2403 mg/kg/day for females. No treatment-related effects were observed at the 1,000 or 10,000 ppm dietary levels. One male in the 25,000 ppm treatment group died during the third week of the study. Compared to respective control groups, treatment of males and females at 25,000 ppm had significant effects on clinical signs, food consumption (during the first 5 weeks of the study), body weight, organ-to-body weight ratios, hematology, clinical chemistry and gross and microscopic pathology. Clinical signs in the high-dose groups included hunched posture, thin, rough hair coat, a bloody

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crust on the nose and urine stains. Body weights for males and females were significantly lower than controls throughout the study, including the recovery phase. There was a marked increase in body weights when the treatment was reduced from 50,000 ppm to 25,000 ppm after 14 days, and by the end of the recovery period at week 17, body weights were within 91% of control groups for both sexes.

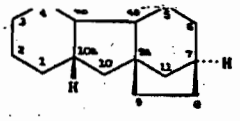
Males had significantly decreased hemoglobin and hematocrit values and significantly increased total bilirubin, cholesterol and alkaline phosphatase values. Females had significantly lower total protein, albumin and calcium values and significantly higher globulin, total bilirubin, cholesterol and alkaline phosphatase values. Both sexes had significantly increased relative brain weights and males had significantly increased relative kidney and testis weights. Gross pathological changes in the kidneys (rough surfaces and depressed areas in the cortex) were present in both sexes. Histologically, chronic tubular nephritis, tubular dilation, and focal loss of nephrons were observed. Histological changes in the liver were marginal in incidence and severity. **Under the conditions of this study, the NOEL is 10,000 ppm; the LOEL is 25,000 ppm based on alterations in clinical chemistry, decreased food consumption, decreased body weights, increases in relative organ weights (brain, kidney and testis), and gross and histopathological changes in the kidney.**

This study is classified as **Core-Minimum** because it generally satisfies the guideline requirement for a subchronic dietary toxicity study in rodents.

A. MATERIALS

1. Test material: Gibberellins A_4A_7

Description: white powder
Lot/Batch No.: 21-018 CD
Purity: 85.5% a.i. (technical grade)
Stability of compound: stable at 25°C for at least 3 years
CAS No.: not available
Structure: Gibberellins A_4A_7 are diterpenoid acids based on the gibberellane skeleton containing the gibbane nucleus (shown).



2. Vehicle and/or positive control

Dry test material was mixed with feed; therefore, no vehicle was required. A positive control was not included.

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3. Test animals

Species: rat
Strain: Crl:CD®BR
Age and weight at study initiation: 43 days; 192-243 g (males), 136-159 g (females)
Source: Charles River Laboratories, Inc., Portage, Michigan
Housing: individually in suspended, stainless steel, screen-bottom cages
Environmental conditions:
Temperature: 22°C (16-25.5°C; the temperature was out of range on 6 occasions)
Humidity: 50% (24-69%; the humidity was out of range on 2 occasions)
Air changes: not reported
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 15 days

B. STUDY DESIGN

1. Animal assignment

Animals were assigned to the 4 test groups in Table 1 using a computer-generated randomization. Doses were calculated by the reviewer (See Section C.3.b.)

TABLE 1. STUDY DESIGN

Dose Group	Conc. in Diet (ppm)	Dose (mg/kg/day)[a]		No. of Animals	
		male	female	male	female
1 Control	0	0	0	20	20
2 Low (LDT)	1,000	67	85	10	10
3 Mid (MDT)	10,000	704	814	10	10
4 High (HDT)[b]	50,000/25,000	2,238	2,403	20	20

[a] Based on nominal dietary concentrations and calculated from weekly food consumption data.
[b] High dose reduced to 25,000 ppm after 14 days due to low weight gain and clinical signs.

Dose selection rationale: The doses used in this study were determined by the sponsor; no details were provided. Ten animals/sex in the control and high dose groups, designated as recovery animals, were observed for 4 weeks post-treatment for toxic effects. Beginning on day 15, the dose level for the high dose group was changed from 50,000 to 25,000 ppm due to clinical signs and low weight gain. Thereafter, it is referred to as the 25,000 ppm treatment group.

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2. Diet preparation and analysis

Diet was prepared weekly by mixing appropriate amounts of test substance with the rat chow feed. Diets were stored refrigerated in covered containers (temperature not stated). Homogeneity, stability, and diet concentrations were tested by the sponsor. Analysis was by high performance liquid chromatography. For homogeneity analysis, 10 g duplicate samples were taken from the top, bottom, and two opposing sides of each dose mix and sent to the sponsor for analysis.

For stability analysis, five sets of duplicate samples were taken from all test material dietary concentrations during week 0. One set was taken on the day of mixing; one set (except the 25,000 ppm dose level) was stored for 3 days refrigerated followed by 9 days of storage in animal room conditions; one set was stored 7 days refrigerated plus 7 days in animal room conditions; two sets were stored below 0°C for 2 or 7 weeks prior to analysis.

All diets were sampled for test material concentrations by taking duplicate samples during the first 14 weeks of the study. During weeks 1, 2, and 3, samples were collected on the day of preparation (Friday) and sent to the sponsor on the following Monday or Tuesday. During the remaining weeks, all diets were prepared, sampled, and sent to the sponsor one week before being fed to the animals.

Results –

a. Homogeneity analysis – The diets were homogenous (the relative standard deviation of the mean total gibberellins A_4A_7 concentration for all four portions from each batch was <2%).

b. Stability analysis – Changes in the gibberellin A_4 or A_7 content under the various storage conditions (i.e., storage at 0°C for 7 weeks and at animal room conditions for up to 9 days) was negligible.

c. Concentration analysis – All samples had total gibberellin A_4 plus A_7 contents within a 90-110% range.

3. Diet

Animals were fed a standard laboratory diet of Certified Rodent Chow® #5002 (Purina Mills, Inc.) and watered *ad libitum.*

4. Statistics

A standard, 1-way ANOVA was used to analyze body weights, weekly and cumulative body weight gains, food consumption, clinical chemistry and hematology values, organ weights, organ-to-body weight percentages, and organ-to-brain weight ratios. Group comparisons found to be statistically significant at the 5.0% two-tailed probability level were flagged. Statistically significant differences cited are based on comparisons with the control group.

5. Signed GLP and quality assurance statements (dated 7/10/90) were present.

C. METHODS AND RESULTS

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. A physical examination was performed pretest and weekly thereafter.

Results – There was one death during the treatment period - a male in the high-dose group died during week 3. Observations for this animal during the week prior to death included hunched posture, thin, rough hair coat, and a bloody crust on the nose. These effects were present in other males in the high-dose group when the dose was 50,000 ppm. Urine-stained tails were observed in 11 males and 2 females in the high-dose groups; these observations were not present at the end of the 4-week recovery period.

2. Body weight

Animals were weighed once before initiation of treatment, on the first day of treatment, weekly thereafter, and on the day of necropsy (after overnight fasting).

Results – After 13 weeks of treatment, the body weights of male and female rats in the 1,000 and 10,000 ppm treatment groups were similar or slightly lower (within 5%) of their respective control groups (Table 2). Body weights for the high-dose treatment group were significantly lower than controls throughout the study, including the recovery phase. There was a marked increase in body weights when the treatment was reduced from 50,000 ppm to 25,000 ppm after two weeks; at week 13, body weights were 84% (males) and 89% (females) of controls, and at week 17, body weights were 91% of control groups for both sexes.

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TABLE 2. GROUP MEAN BODY WEIGHTS (G) AT WEEKLY INTERVALS

Week of Study	Treatment Group/Exposure Level (ppm)							
	males				females			
	1 (0)	2 (1,000)	3 (10,000)	4 (25,000)	1 (0)	2 (1,000)	3 (10,000)	4 (25,000)
0	228.0	225.3	225.0	219.6	146.0	146.8	151.6	149.4
1	288.6	288.4	284.4	221.5*a	168.4	169.6	173.4	151.4*a
2	336.6	338.7	332.9	229.0*a	188.9	188.9	191.4	165.1*a
3	381.8	380.3	374.2	292.2*	205.3	205.4	209.0	185.9*
4	411.6	409.5	399.7	329.8*	216.4	214.1	210.9	195.6*
5	443.2	435.0	426.5	365.2*	229.6	225.4	230.8	205.9*
6	463.1	457.8	451.5	386.7*	238.7	236.8	241.0	213.6*
7	491.3	476.5	472.2	410.7*	247.9	244.1	249.6	224.3*
8	515.0	501.5	490.7	426.1*	250.1	245.3	251.4	222.5*
9	538.9	527.7	511.5	449.8*	262.6	257.9	262.3	234.2*
10	548.7	537.0	523.3	459.8*	266.2	261.2	267.0	236.5*
11	564.6	552.4	537.9	472.8*	272.9	267.3	273.5	243.8*
12	577.5	561.1	551.0	487.0*	278.6	272.5	280.0	247.8*
13	588.7	574.5	562.3	496.4*	282.1	278.0	283.3	252.1*
14	571.0	ND	ND	502.1*	279.6	ND	ND	249.7*
15	589.7	ND	ND	527.1*	288.2	ND	ND	257.8*
16	598.5	ND	ND	540.5*	294.2	ND	ND	263.9*
17	615.8	ND	ND	560.6*	303.5	ND	ND	275.0*

Data taken from Tables 3 and 4, MRID No. 416166-01.
ND indicates no data.
[a] Concentration in diet was 50,000 ppm during first 14 days; 25,000 ppm thereafter.
* Statistically significant ($p \leq 0.05$) compared with control group.

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3. Food consumption and compound intake

Food consumption for each animal was determined weekly during the treatment and recovery phase; food consumption was also reported as weekly means for each exposure group. Compound intake and food efficiency were not reported by the author of the study. Compound intake (mg/kg/day) values were calculated as time-weighted averages by the reviewer as follows: compound intake = [food consumption (mg/day) x concentration in feed (%)] divided by body weight (kg).
Results -

a. Food consumption - Group mean weekly food consumptions for males and females are shown in Table 3. Food consumptions were significantly lower for males in the high-dose group for weeks 1 through 3 and for females in the high-dose group for weeks 1, 2, 4, and 5.

b. Compound consumption (time weighted average) - The estimated compound intake decreased over the course of the study (Table 4). Daily, time-weighted averages were 67, 704, and 2,238 mg/kg/day for males in the 1,000, 10,000 and 25,000 ppm treatment groups; corresponding values for females were 85, 814 and 2,403 mg/kg/day.

4. Ophthalmoscopic examination

Eyes were examined before initiation of treatment and during week 13 before terminal necropsy; recovery animals were examined during week 17.

Results - No treatment-related changes were noted at the 13- and 17-week examinations.

TABLE 3. GROUP MEAN FOOD CONSUMPTION (G/ANIMAL/WEEK) AT WEEKLY INTERVALS

Week of Study	Treatment Group/Exposure Level (ppm)							
	males				females			
	1 (0)	2 (1,000)	3 (10,000)	4 (25,000)	1 (0)	2 (1,000)	3 (10,000)	4 (25,000)
1	185.5	184.3	185.7	129.2*[a]	120.5	125.8	122.0	87.4*[a]
2	187.6	193.5	197.1	150.4*[a]	129.2	128.3	123.2	109.7*[a]
3	189.8	190.6	198.5	178.8*	126.7	125.4	126.6	108.3
4	195.3	199.4	202.1	199.5	133.8	131.6	133.9	119.9*
5	193.6	196.2	199.9	200.2	134.5	137.3	129.4	122.0*
6	193.3	188.0	201.0	197.6	133.8	136.8	133.8	124.5
7	196.8	189.5	201.4	191.2	127.4	131.8	128.5	120.3
8	194.3	198.7	197.5	183.3	117.1	117.3	119.5	115.3
9	202.3	200.9	203.1	197.2	129.4	142.4	122.2	123.1
10	196.6	192.8	200.0	187.5	124.5	135.5	130.1	118.5
11	191.6	197.8	200.1	185.9	128.0	125.8	126.7	119.0
12	193.9	188.7	202.8	189.1	124.3	130.6	130.5	120.8
13	188.2	191.9	191.4	182.2	118.3	121.6	127.1	115.0
14	156.4	ND	ND	160.3	95.0	ND	ND	101.1
15	192.6	ND	ND	185.9	129.8	ND	ND	122.8
16	187.6	ND	ND	183.7	128.0	ND	ND	128.7
17	184.0	ND	ND	177.1	118.5	ND	ND	114.0

Data taken from Tables 9 and 10, MRID No. 416166-01.
ND indicates no data.
[a] These groups received 50,000 ppm during first 14 days of study.
* Statistically significant ($p \leq 0.05$) compared with control values.

TABLE 4. ESTIMATED COMPOUND INTAKE (MG/KG/DAY)[a]

Week of Study	Treatment Group/Exposure Level (ppm)					
	males			females		
	1,000	10,000	25,000	1,000	10,000	25,000
1	117	1179	4,202[b]	122	1160	4179[b]
2	96	990	4,850[b]	108	1015	5176[b]
3	80	852	2,789	95	945	2343
4	75	772	2,438	92	915	2303
5	68	714	2,168	92	877	2228
6	62	673	1,932	87	828	2160
7	60	637	1,766	80	762	2011
8	60	598	1,594	69	684	1836
9	57	591	1,653	83	694	1976
10	52	559	1,489	75	709	1807
11	53	546	1,444	69	678	1797
12	49	539	1,428	70	682	1770
13	49	496	1,336	64	648	1657
Average	67	704	2,238	85	814	2403

[a] Calculated from data in Tables 2 and 3 as: Compound intake = [food consumption (mg/day) x concentration in feed (%)] ÷ body weight (kg). Body weights previous to the week of food consumption were used for calculation.

[b] These values were based on a dietary concentration of 50,000 ppm during first 14 days of study.

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5. Blood was collected for hematology and clinical analysis from all surviving animals during weeks 1 and 13 (all treatment groups) and from the recovery animals during week 17 (control and 25,000 ppm treatment groups). The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit(HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
X	Blood clotting measurements (Prothrombin time)		

*Required for subchronic studies.

Results – Hemoglobin and hematocrit were slightly lower in males and females in the high-dose group (Table 6). The difference was statistically significant ($p \leq 0.05$) in males. Other statistically significant differences in clinical hematology values were: higher hemoglobin in males given 1,000 ppm; slightly higher mean corpuscular hemoglobin (MCH) in females given 10,000 ppm; slightly higher mean corpuscular hemoglobin concentration (MCHC) in males given 1,000 ppm and females given 10,000 ppm; slightly lower absolute lymphocyte count in males given 10,000 ppm; and higher absolute monocyte count in males given 1,000 ppm. The changes in the low- and mid-dose groups did not appear to be dose-related. There were no significant differences in hematology values between the control and high-dose groups following the 4-week recovery period (data not shown).

TABLE 5. CLINICAL HEMATOLOGY PARAMETERS FOR MALE AND FEMALE RATS FED GIBBERELLINS A_4A_7 FOR 13 WEEKS

Parameter	Males Treatment group (ppm)[a]				Females Treatment group (ppm)[a]			
	0	1,000	10,000	25,000	0	1,000	10,000	25,000
Hemoglobin (g/dL)	14.5	15.3*	14.7	14.0*	14.1	14.3	14.4	13.3
Hematocrit (%)	53.2	54.7	53.2	50.9*	51.7	51.5	51.3	48.8
MCHC (%)	27.2	27.9*	27.6	27.4	27.2	27.7	28.1*	27.3
MCH (%)	15.0	15.2	15.1	15.1	16.0	16.5	16.8*	15.8
Lymphocytes (E3/UL)	7.2	6.0	5.4*	7.3	3.5	3.1	3.2	4.6
Monocytes (E3/UL)	0.0	0.1*	0.0	0.0	0.0	0.0	0.0	0.0

Data taken from Tables 15 and 16, MRID No. 416166-01.

[a] Based on 19-20 animals of each sex in the 0 and 25,000 ppm treatment groups and 10 animals of each sex in the 1,000 and 10,000 ppm treatment groups.
MCHC = Mean corpuscular hemoglobin concentration.
MCH = Mean corpuscular hemoglobin.

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b. Clinical chemistry

	Electrolytes
X	Calcium*
X	Chloride*
	Magnesium*
X	Phosphorus*
X	Potassium*
X	Sodium*

	Enzymes
X	Alkaline phosphatase (ALK)
	Creatinine phosphokinase*
	Lactic acid dehydrogenase (LDH)*
X	Serum alanine aminotransferase (also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*

	Other
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Cholesterol*
X	Globulins
X	Glucose*
X	Total bilirubin
X	Total serum protein (TP)*

* Required for subchronic studies.

Results – Treatment with the test material caused the following statistically significant ($p \leq 0.05$) effects on clinical chemistry parameters: lower total protein, albumin, and calcium in females in the high-dose group; mildly higher globulin in females in the high-dose group; mildly higher total bilirubin in males and females in the high-dose group; and higher cholesterol and alkaline phosphatase in males and females in the high-dose group (Table 6. Females in the 10,000 ppm treatment group had a lower albumin value. The lower total protein and albumin in females in the high-dose group were not reversible during the recovery period (data not shown). Males had a higher creatinine value than controls after the recovery period, but the same parameter was not different than controls at 14 weeks. Blood magnesium, creatinine phosphokinase and lactic acid dehydrogenase were not measured.

TABLE 6. CLINICAL CHEMISTRY PARAMETERS
FOR MALE AND FEMALE RATS FED GIBBERELLINS A_4A_7 FOR 13 WEEKS

Parameter	Males Treatment group (ppm)[a]				Females Treatment group (ppm)[a]			
	0	1,000	10,000	25,000	0	1,000	10,000	25,000
Total protein (g/dl)	7.0	6.9	7.0	7.0	7.3	7.2	7.0	6.8*
Albumin (g/dl)	4.4	4.4	4.4	4.4	5.2	5.0	4.7*	4.4*
Calcium (mg/dl)	10.1	10.0	10.0	10.2	10.4	10.3	10.1	10.0*
Globulin (g/dl)	2.5	2.5	2.6	2.6	2.2	2.2	2.3	2.4*
Total bilirubin (mg/dl)	0.2	0.2	0.2	0.2*	0.2	0.2	0.2	0.2*
Cholesterol (mg/dl)	59	56	65	90*	71	71	73	85*
Alkaline phosphatase (IU/l)	96	109	95	124*	61	63	61	87*

Data taken from Tables 21 and 22, MRID No. 416166-01.
[a] Based on 19-20 animals of each sex in the 0 and 25,000 ppm treatment groups and 10 animals of each sex in the 1,000 and 10,000 ppm treatment groups.

6. Urinalysis

Urinalysis is not required for subchronic studies and was not performed.

7. Sacrifice and pathology

The animal that died and those sacrificed on schedule were subjected to gross pathological examinations. All organs/tissues from the animal that died and the nonrecovery animals in the control and high dose groups were examined microscopically. In addition, macroscopic lesions, lungs, liver, and kidneys from the low- and mid-dose groups were examined microscopically. Kidneys and liver of the treated recovery animals were also examined microscopically. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

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Digestive system		**Cardiovasc./Hemat.**		**Neurologic**	
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	X	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen	X	eye (optic n.)*
X	Jejunum*	X	Thymus*	**Glandular**	
X	Ileum*	**Urogenital**		XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*+	X	Parathyroids*++
XX	Liver*+	X	Epididymides	X	Thyroids*++
	Gall Bladder*	X	Prostate	**Other**	
X	Pancreas*	X	Seminal vesicle	X	Bone*
Respiratory		XX	Ovaries*+	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin*
X	Lung*			X	All gross lesions and masses*

* Required for subchronic and chronic studies.
+ Organ weight required in subchronic and chronic studies.
++Organ weight required for non-rodent studies.

Results –

a. Organ weight – Males in the 25,000 ppm treatment group had lower terminal body weights and statistically significantly higher organ-to-body weight percentage values for brain, left and right kidney, and left testis (Table 7). The brain-to-body weight percentage for females in this treatment group was also significantly higher. There were no differences between the control and any dose group in absolute organ weights. After the 4-week recovery period, males and females administered the highest dose had lower terminal body weights and correspondingly lower absolute organ weight values for liver (males only) and left and right kidneys (females only) (absolute organ weight data not shown). These animals had higher organ-to-body weight percentage values for brain (both sexes), liver (females only), and right ovary.

TABLE 7. BODY WEIGHTS AND SELECTED ORGAN-TO-BODY WEIGHT RATIOS

Parameter	Males Treatment group (ppm)[a]				Females Treatment group (ppm)[a]			
	0	1,000	10,000	25,000	0	1,000	10,000	25,000
End of treatment period (13 weeks)								
Mean terminal body weight (g)	556.1	542.2	525.2	456.8*	254.7	255.4	260.8	228.9
Organ-to-body weight (%)								
Brain	0.391	0.399	0.406	0.462*	0.782	0.761	0.753	0.871*
Left kidney	0.328	0.344	0.340	0.381*	0.369	0.388	0.366	0.369
Right kidney	0.332	0.342	0.328	0.377*	0.368	0.403	0.359	0.370
Left testis	0.332	0.336	0.338	0.406*				
Right ovary					0.026	0.025	0.021	0.025
Liver	2.82	2.87	2.99	3.16	2.85	2.69	2.80	3.06
End of recovery period (17 weeks)								
Mean terminal body weight (g)	571.8	ND	ND	518.7*	276.1	ND	ND	246.5*
Organ-to-body weight (%)		ND	ND			ND	ND	
Brain	0.377			0.422*	0.720			0.810*
Left kidney	0.342			0.358	0.363			0.362
Right kidney	0.348			0.368	0.364			0.363
Left testis	0.318			0.368				
Right ovary					0.022			0.026*
Liver	2.89			2.77	2.54			2.80*

Data taken from Tables 25 and 26 (terminal body weights) and 27 and 28 (organ-to-body-weight percentages), MRID No. 416166-01 (Note: Some of the data in Tables 27 and 28 were difficult to read; values were rounded off to three figures).
ND indicates no data.
[a] 10 animals per treatment group.
* Statistically significant ($p \leq 0.05$).

b. Gross pathology – Animals in the 25,000 ppm treatment group had a high incidence of kidney changes described as a diffusely rough surface or depressed foci/areas in the cortex. The animal that died had no gross macroscopic lesions.

c. Microscopic pathology –

1) Non-neoplastic – Animals in the 25,000 ppm treatment group with macroscopic kidney lesions had a high incidence of chronic or chronic active tubulo-interstitial nephritis with tubular dilation and focal loss of nephrons (Table 8). These animals also had a high incidence of hepatocellular vacuolization with some associated hepatocellular degeneration. These changes were greater in males than in females and were present in the male that died during week 3 of the study. After the 4-week recovery period, the kidneys from the 25,000 treatment groups had evidence of chronic or chronic active inflammation, cortical fibrosis/scarring, and tubular dilation; these changes were less severe in both

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sexes when compared with the changes at 14 weeks. There was no evidence of hepatocellular degeneration and only two incidences of hepatocellular vacuolization.

2) Neoplastic – Neoplastic lesions were not observed in any of the treated or control groups.

TABLE 8. MICROSCOPIC PATHOLOGY INCIDENCE SUMMARY

Organ/Lesion	Males Treatment group (ppm)[a]				Females Treatment group (ppm)[a]			
	0	1,000	10,000	25,000	0	1,000	10,000	25,000
End of treatment period (13 weeks)								
Liver								
Hepatocellular degeneration	0	0	0	3	0	0	0	0
Hepatocellular vacuolization	0	0	1	8	0	0	1	2
Kidney								
Inflammation	0	0	0	8	0	0	0	4
Fibrosis/scarring (cortical)	0	0	0	9	0	1	0	7
Cysts	0	0	0	5	0	0	0	3
Atrophy	0	0	2	2	0	0	0	7
Tubular dilation	0	0	0	10	0	0	0	2
End of recovery period (17 weeks)								
Liver								
Hepatocellular degeneration	0	0	0	0	0	0	0	0
Hepatocellular vacuolization	1	0	0	0	0	0	0	1
Kidney								
Inflammation	0	0	0	7	0	0	0	5
Fibrosis/scarring (cortical)	1	0	0	8	0	0	0	10
Cysts	1	0	0	3	0	0	0	0
Atrophy	7	0	0	2	0	0	0	0
Tubular dilation	0	0	0	9	0	0	0	2

Data taken from Table 33, MRID No. 416166-01.
[a] 10 animals per treatment group.

D. DISCUSSION

Male and female Crl:CD®BR rats were fed diets containing 0, 1,000, 10,000 or 25,000 ppm gibberellins A_4A_7 for 13 weeks followed by a 4-week recovery period. The high-dose concentration was 50,000 ppm at the start of the test period but was lowered to 25,000 ppm after 14 days due to low weight gain in both males and females compared to respective control groups. One male rat in this treatment group died during the third week of the study, presumably due to exposure to 50,000 during the first 14 days of the

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study. Clinical signs in the 25,000 ppm exposure group included hunched posture, thin, rough hair coat, a bloody crust on the nose and urine stains. Body weights for the groups consuming 1,000 and 10,000 ppm in the diet were unaffected, but body weights for males and females in the high-dose treatment group were significantly lower than controls throughout the study, including the recovery phase. There was a marked increase in body weights when the treatment was reduced from 50,000 ppm to 25,000 ppm after 14 days, and by the end of the recovery period at week 17, body weights were within 91% of control groups for both sexes. It should be noted that as the rats grew during the course of the study, the estimated dose (mg/kg/day) received decreased from weeks 1-13 because the concentration of test substance in the diet remained the same..

Treatment-related changes in blood and clinical chemistry parameters were observed only in the highest exposure group. In male rats, the following blood parameters were statistically altered: lower hemoglobin and hematocrit and higher total bilirubin, cholesterol and alkaline phosphatase. Females had statistically significant lower total protein, albumin, and calcium and statistically significantly higher globulin, total bilirubin, cholesterol and alkaline phosphatase. Females in the 10,000 ppm treatment group also had a lower albumin value.

According to the study author, the effects of the highest dose on hemoglobin, hematocrit, total protein, and albumin were small and probably associated with the test material effect on body weight and possibly food consumption. Statistically significant alterations in blood parameters in the other treatment groups were mild, and because they were not present in the high-dose groups, they should not be considered treatment related. Furthermore, even when statistically different from control values, most parameters were within the normal range for this strain of rat. However, no historical data were provided in the submission.

Organ weight data showed increases in relative but not absolute organ weights for both sexes for brain and, in males, the kidney and testis. Following the recovery period, relative organ weights were higher for brain (both sexes) and female liver and ovary.

The kidney appeared to be the target organ for gibberellins A_4A_7 as shown by the increased relative weight (in males), gross and histological changes, and biochemical changes (total protein, albumin, and calcium in females). Treatment-related gross pathological changes were limited to the kidneys of the 25,000 ppm exposure groups and included a rough surface and depressed foci/areas in the cortex. Histopathology examinations of these treatment groups (and the dead male rat) revealed chronic tubular nephritis with tubular dilation and focal loss of nephrons. Liver changes in the high exposure groups were observed primarily in males and included hepatocellular degeneration and vacuolization; total bilirubin, cholesterol, and alkaline phosphatase were also higher in the 25,000 ppm treatment groups. However, these histological changes were not accompanied by absolute or relative liver weight changes during the treatment period. The liver effects were considered by the author to be marginal in incidence and severity. No treatment-related microscopic changes were observed in other organs or in any organs of the low- and mid-dose groups.

Thirteen-week exposure of male and female rats to dietary gibberellins A_4A_7 at

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concentrations as high as 10,000 ppm did not produce any biologically significant signs of treatment-related toxicity. Therefore, this exposure is considered the NOEL for this study. The LOEL for this study is 25,000 ppm, based on decreased weight gain, organ-to-body weight ratio changes, changes in hematology and clinical chemistry values, and kidney and liver lesions.

E. STUDY DEFICIENCIES

A deficiency in this study was that the doses of test compound were not increased or adjusted to the increasing weight of the rats, resulting in decreasing doses from week 1 to week 13. In addition, the high-dose male and female treatment groups were administered 50,000 ppm in the diet during the first 14 days of the study and 25,000 ppm thereafter. The short treatment period with 50,000 ppm may have shortened the onset and increased the severity of toxic effects observed in the 25,000 ppm treatment groups.

Other deficiencies in the study was the clinical chemistry parameter lactic acid dehydrogenase the lack of calculations of compound intake (expressed as mg/kg/day). In addition, some of the data in the appendices were difficult to read and data on analyses of compound concentration in the diet, appear to have partially been left out (see pp. 452-454, Appendix G),

These deficiencies do not invalidate the study.

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82-1 Subchronic Feeding in the Rodent and Nonrodent

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 10 rodents or 4 nonrodents/sex/group (3 test groups and control group).
3. ✓ Dosing duration daily for 90-days or 5 days/week for 13 weeks.
4. ✓ Doses tested include signs of toxicity at high dose but no lethality in nonrodents or a limit dose if nontoxic (1000 mg/kg).
5. ✓ Doses tested include a NOEL.
6. • ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
7. ✓ Individual daily observations.
8. ✓ Individual body weights.
9. ✓ Individual or cage food consumption.
10. • ✓ Opthalmoscopic examination (at least pretest and at term) control and high dose.
11. ✓ Clinical pathology data of 12 & 13 at termination for rodents, before, monthly or midway and at termination for nonrodents.
12. ✓ Hematology.
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - • ✓ Differential count
    - ✓ Platelet count (or clotting measure)
13. ___ Clinical chemistry.
    - ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - • ___ Creatinine kinase
    - ___ Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - ✓ Cholesterol
    - • ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - • ✓ Potassium
    - ✓ Sodium
    - • ✓ Chloride
14. • ___ Urinalysis, only when indicated by expected or observed activity. As scheduled in 11.
    - ___ Blood
    - ___ Protein
    - ___ Ketone bodies
    - ___ Appearance
    - ___ Glucose
    - ___ Total bilirubin
    - • ___ Urobilirubin
    - ___ Sediment
    - ___ Specific gravity (osmolality)
    - • ___ Volume
15. ✓ Individual necropsy of all animals.
16. ✓ Histopathology of the following tissues performed on all nonrodents and rodents, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.

Criteria marked with a • are supplemental and may not be required for every study.

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✓ aorta	✓ jejunum	✓ peripheral nerve
✓ eyes	✓ bone marrow	✓ kidneys†
✓ caecum	✓ liver†	✓ esophagus
✓ colon	✓ lung† not weighed	✓ ovaries†
✓ duodenum	✓ lymph nodes	___ oviduct
✓ brain†	✓ stomach	✓ pancreas
✓ skin	✓ mammary gland	✓ rectum
✓ heart† not weighed	✓ spleen† not weighed	✓ spinal cord (3x)
✓ testes†	✓ musculature	✓ thyroid / parathyroids
✓ pituitary	✓ epididymis	✓ salivary glands
✓ ileum	✓ adrenals†	✓ thymus
✓ trachea	✓ uterus	✓ urinary bladder

† organs to be weighed

Criteria marked with a • are supplemental and may not be required for every study.