

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
GIBBERELLINS

Chemical Code # 310, Tolerance # 224
SB 950 # 265

January 29, 1998, revised July 21, 1999

I. DATA GAP STATUS

Subchronic, rat	No data gap, no adverse effect ^a
Chronic toxicity, rat:	Data gap, no study on file ^a
Chronic toxicity, dog:	Data gap, no study on file ^a
Oncogenicity, rat:	Data gap, no study on file ^a
Oncogenicity, mouse:	Data gap, no study on file ^a
Reproduction, rat:	Data gap, no study on file ^a
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, possible adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

^a Gibberellins have been classified as "biochemicals" for purposes to toxicological data requirements by US EPA. See discussion below. No further studies are required at this time to satisfy SB950 data requirements for gibberellins.

Toxicology one-liners are attached.

All record numbers for the above study types through 166958 (Document No. 224-113) were examined. This includes all relevant studies indexed by DPR as of March 8, 1999.

In the 1-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

File name:T990721

Prepared by J. Gee, January 29, 1998; revised by Gee, 7/21/99

Note: Gibberellins are a class of naturally occurring plant hormones, first isolated from the fungus, *Fusarium*, which affect plant growth, plant maturation and seed germination. The class consists of C₂₀ and C₁₉ terpenes. The term "gibberellic acids" includes the acid (GA₃), the related isomers GA₄ and GA₇, and the potassium salt. In December, 1995, US EPA issued a Reregistration Eligibility Document (RED) for gibberellic acid, EPA-738-F-96-005. For purposes of toxicity testing, gibberellins have been classified by US EPA as biochemicals, hence requiring a more limited battery of studies (See Code of Federal Regulations, 40 CFR § 158.690 (c), July 1, 1997). Those required in Tier I include a 90-day feeding study, developmental toxicity study (1 sp) and a genotoxicity battery. Listed below are summaries of those relevant studies on file at DPR as of 1/26/98, indicating that gibberellins are relatively nontoxic to mammalian species. With the possible exception of the induction of chromosomal aberrations, gibberellins were negative in genotoxicity assays.

[Three studies were upgraded 7/21/99 to acceptable status, Gee 7/21/99]

The studies listed below satisfy the current data requirements for biochemicals. No further studies as mandated by SB950 are required at this time. (Gee, 1/26/98)

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

CHRONIC, RAT

No study on file.

002 914834 Brief summary of a study in rats conducted in 1957 - 1959. Rats were given 1 g/kg by gavage, 6 days per week for 18 months. There were 6/sex/group. A number of deaths were due to broncho-pneumonia. Unacceptable. Insufficient information for evaluation. No worksheet. (Gee, 1/28/98)

SUBCHRONIC TOXICITY, RAT

** 224-071 114118 "13-week dietary toxicity study with gibberellins A4A7 in rats." (K. M. MacKenzie, Hazleton Laboratories America, WI, HLA 6161-114, July 10, 1990) Gibberellins A₄A₇, lot 21-018 CD, 85.5%, was tested by feeding in the diet to CrI:CD[®]BR albino rats, 20 per sex per group for controls and high dose and ten per sex for low- and mid-dose groups. Ten/sex of the controls and high dose were continued on the study for a 4-week recovery period. Doses were 0 (diet), 1000, 10,000 or 50,000/25,000 ppm. The high dose was reduced on day 15, based primarily on the significantly lower body weight gain in males and females. Body weights, food consumption, hematology, clinical chemistry, ophthalmology, and histopathology were conducted on acceptable parameters. No urinalysis was performed. There was, however, no indication from clinical chemistry or microscopic examination that this deficiency invalidated the study. There were a few treatment-related responses, typically of mild degree. Body weight and weight gain were lower in the high dose animals. There were some changes in clinical chemistry/hematology, some of which recovered following cessation of treatment. These included lower hemoglobin and hematocrit, slightly elevated alkaline phosphatase, and cholesterol. The liver and kidney were target organs for microscopic

changes. Kidney: chronic inflammation, cortical fibrosis, tubular dilatation and tubular atrophy. Liver: Centrolobular vacuolization and degeneration. Effects in males were greater than in females. No adverse effects. NOEL = 10,000 ppm. Acceptable. (Gee, 1/22/98)

** 224-060 091769 “ A subchronic (3 month) oral toxicity study in the rat with gibberellic acid (GA 3) via dietary admixture.” (C. S. Auletta, Bio/dynamics, East Millstone, NJ, Project 89-3472, 8/15/90) Gibberellic acid, GA3, lot 28-310-CD, 88.5%, was fed to Sprague-Dawley CD® rats at 0 (feed), 1000, 10000 or 50000 ppm for 13 weeks followed by a 4-week recovery period for 10/sex in the controls and high-dose groups. There were 10/sex/group for the terminal sacrifice after the 13 weeks of exposure. Adequate parameters were measured for hematology, clinical chemistry, ophthalmology, urinalysis, necropsy and histopathology. Results at the high dose showed some increase in soft stools, slightly lower body weight without statistical significance, increase in BUN - especially in females, increase in kidney and liver organ weights (absolute and organ/body weight ratio) with less difference after 4-week recovery, and no histopathology related to treatment. The study did not identify any adverse effect. NOEL = 10,000 ppm (soft stools, elevated BUN, increased kidney and liver weights). Acceptable. (Gee, 1/26/98)

CHRONIC TOXICITY, DOG

No study on file.

ONCOGENICITY, RAT

No study on file.

REPRODUCTION, RAT

No study on file.

TERATOLOGY, RAT

** 224-059, -073 091767, 114088 “Evaluation of the effects of orally administered gibberellic acid on the embryonic and fetal development of the rat (Segment II, TFR).” (S. B. Lehrer, Abbott Laboratories, TA86-014, 9/17/86) Gibberellic acid, GA₃, 93.4%, was tested by gavage with CrI:CD®(SD)BR rats at 0 (0.2%hydroxypropylmethylcellulose), 10, 100 or 1000 mg/kg/day, 10 ml/kg, days 6 - 15 of gestation. There were 24 per group. No treatment-related deaths occurred. Approximately half of the fetuses were given visceral examinations and half, skeletal exams. There was no effect on body weight or food consumption with treatment. No developmental toxicity was reported. Maternal = Developmental NOEL ≥ 1000 mg/kg/day. No adverse effects. Acceptable. Individual data were in the supplemental submission in -073, #114088. (Gee, 1/23/98)

TERATOLOGY, RABBIT

** 224-072 114119 "Teratology study with gibberellins A₄A₇ (GA₄A₇) in rabbits." (S. M. Henwood, Hazleton Laboratories America, HLA 6161-111, 6/12/89) Gibberellins A₄A₇ was used to treat Hra:(NZW)SPF rabbits, 18 per group, by oral gavage with 0 (0.2% hydroxypropylmethylcellulose), 100, 300 or 1000 mg/kg/day from day 7 through 19 of gestation. All live fetuses were given an examination for visceral and skeletal changes. Treatment at 1000 mg/kg resulted in significant maternal toxicity: 14 of 18 either were found dead, were sacrificed moribund or were sacrificed after aborting (4). Only 4/18 survived to yield live fetuses at terminal sacrifice. Clinical signs were also noted at the high dose: few or no feces, prostration, hypoactivity and unsteady gait. The percentage of early resorptions was increased compared with controls: 7.06% for controls, 21.07% for the high dose. There was no apparent effect on fetal body weight at any dose nor were there treatment-related malformations/variations at any dose. No adverse effect. Maternal NOEL = developmental NOEL = 300 mg/kg/day. Note that the fetal effects (abortion, resorption) occurred in the presence of significant maternal toxicity. Acceptable. (Gee, 1/23/98)

GENE MUTATION

** 224-101 153796 "Falgro technical, bacterial mutation assay" (J. Kitching, Study Director, Huntingdon Research Centre, 5/15/95) Falgro technical, 91% gibberellic acid, batch 4015/94, was used with and without male rat liver activations. Concentrations were 0 (DMSO), 50, 150, 500, 1500 or 5000 ug/plate, triplicate plates per concentration in two trials. Tested by the plate incorporation assay with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100. Positive controls were functional. No evidence of mutagenicity was reported under conditions of the assays. Acceptable. (Gee, 12/15/97)

** 224-085 132754 "Mutagenicity test on gibberellic acid A₄A₇ in the L5178Y TK +/- mouse lymphoma forward mutation assay with an independent repeat." (M. A. Cifone, Hazleton Washington, 15393-0-431, April 6, 1994) Gibberellic acid A₄A₇, lot 21-018-CD, 87.3%, was tested with mouse lymphoma TK +/- cells with and without rat liver activation for 4 hours. Two successful trials for each condition were performed. Five concentrations per trial were processed for mutation frequency determination. Concentrations without S9 ranged from 218 to 1570 µg/ml. With activation, concentrations ranged from 218 to 1530 µg/ml. Higher concentrations were tested but were stated to be too toxic for processing beyond the expression period. Ethanol was the vehicle control, methyl methanesulfonate the positive control without activation and methylcholanthrene the positive control with activation. The mutation frequency was determined with triplicate plates from a single culture per concentration after a two-day expression period. 5-Trifluorothymidine was used to select for mutants. Since there was no induction of mutations to TK -/-, the colonies were not sized. No mutagenic effect was found under the conditions of the assay. Acceptable. (Gee, 12/18/97)

** 224-059, -113 091763, 166954 "Salmonella/mammalian microsome mutagenicity test (Ames test) of gibberellic acid." (M. S. Diehl, Abbott Laboratories, T87-109, June 5, 1987) Gibberellic acid, 90%, lot 84-526-CD, was tested with *Salmonella typhimurim* strains TA1535, TA1537, TA1538, TA98 and TA100 with and without rat liver activation. Levels tested were 0 (DMSO), 1, 5, 10, 50, 100, 500, 1000, 2000, 5000 and 10,000 ug/plate. Appropriate positive controls were included and were functional. Triplicate plates per concentration per two trials. Results were reported as the average of the triplicate plates with no individual plate counts.

The study was evaluated as unacceptable but upgradeable with submission of individual plate counts and comments on cytotoxicity at 5000 and 10,000 ug/plate. (Gee, 12/17/97) Record 166954 contains the individual plate counts and a statement regarding the lack of evidence of cytotoxicity. This supplemental submission upgrades the study to acceptable status. (Gee, 7/21/99).

** 224-070 114116 "Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) with a confirmatory assay." (T. E. Lawlor, Microbiological Associates, T8201.501014, September 21, 1988) Gibberellin A₄A₇, 90%, was tested with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 at concentrations of 0 (DMSO), 667, 1000, 3333, 6667 or 10000 ug/plate, triplicate plates, two trials, with and without rat liver S9 activation. The background lawn of some strains was reduced at the highest concentration. No increase in revertants was reported. Negative for mutagenicity under the conditions of the assays. Acceptable. (Gee, 12/22/97)

** 224-101 153800 "Falgro technical (Gibberellic acid technical), mammalian cell mutation assay." (K. Adams, study director, Huntingdon Research Centre, May 23, 1995) Mouse lymphoma L5178Y cells were exposed *in vitro* to Falgro technical, 91%, batch 4015/94, at 0 (DMSO), 300, 600, 1250, 2500, 3750 or 5000 µg/ml for three hours in the absence and presence Aroclor 1254 male rat liver S9 preparation. There were two cultures per concentration with two trials and three plates per culture for mutation frequency. Ethyl methane sulphonate and 20-methylcholanthrene were functional as the positive controls. There was little cytotoxicity up to and including 5000 µg/ml. There was no increase in mutation frequency with treatment under the conditions of the test. No adverse effect. Acceptable. (Gee, 12/16/97)

224-032 042841 "Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals." (McCann, J., E. Choi, E. Yamasaki and B. N. Ames, *Proc. Nat. Acad. Sci.* 72: 5135 - 5139, Dec. 1975) Gibberellic acid (no purity stated) was one of a large number of chemicals tested with *Salmonella* strains for mutagenicity. Results were negative with TA100 with and without PCB induced rat liver activation. Concentrations tested were 0, 5000 and 20,000 µg/plate, according to a letter from D. Maron dated October 28, 1985, attached to the publication. Unacceptable (summary data only). (Gee, 12/17/97)

-113 166952 Response to the above review of record 942841 stating that the study was a peer reviewed publication and the individual data are not available. The study remains unacceptable. No worksheet. (Gee, 7/21/99)

CHROMOSOME EFFECTS

** **224-085 132755** "Mutagenicity test on gibberellic acid A₄A₇ measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without metabolic activation with a confirmatory assay with multiple harvests." (H. Murli, Hazleton Washington, 15393-0-437Z, August 17, 1994) Gibberellic acid A₄A₇ was tested with Chinese hamster ovary cells with and without rat liver S9 activation. Each assay had duplicate cultures per concentration with 100 cells per culture analyzed for chromosomal aberrations. Harvest times without activation were 24 or 48 hours. In one assay, treatment with the test material was for only 6 hours with continued incubation and harvest at 24 hours. With activation, harvest times were 12, 24 or 48 hours in two separate trials. Positive controls were mitomycin C without activation and

cyclophosphamide with activation. Concentrations were 0 (ethanol), 262, 655, 1310, 1970 or 2620 µg/ml. Cytotoxicity was seen at 1970 and 2620 µg/ml in terms of cell debris, percent of confluency and mitotic index. Chromosomal aberrations were increased in percentage at 1970 and 2620 µg/ml at 24 hours (2 assays), at 1970 µg/ml at 48 hours but not at 2620 µg/ml when CHO were treated for 6 hours followed by incubation until the 24-hour harvest. Results with activated cultures were negative at all concentrations tested in one assay but positive in a second assay at 2620 µg/ml with a suggestion of a concentration-related increase at lower concentrations. Because of the lack of reproducibility with S9 activation, the results for a positive effect are less clear. **Possible adverse effect:** A reproducible increase in chromosomal aberrations was found without activation at concentrations with cytotoxicity. Acceptable. (Gee, 12/19/97)

224-101 153801 “Falgro technical (gibberellic acid technical), metaphase chromosome analysis of human lymphocytes cultured *in vitro*.” (K. Adams, Study Director, Huntingdon Research Centre, May 23, 1995) Gibberellic acid, 91%, batch 4015/94, was used to treat purified lymphocytes from the pooled blood of approximately 10 male subjects. Lymphocytes were stimulated for 48 hours with phytohemagglutinin before treatment. Concentrations used ranged from 9.8 µg/ml to 5000 µg/ml for three hours in the presence of rat liver S9 activation and for 18 hours without activation. There were two cultures per concentration with 4 slides prepared from each culture. A total of 100 cells per culture were scored for aberrations and 1000 cells were evaluated for the mitotic index. There was a repeat trial with S9 activation but data from only one trial were reported. **Possible adverse effect:** Gibberellic acid was clastogenic at 2500 µg/ml without activation and at 4500 µg/ml with S9 activation. Not all treatment levels were evaluated - only three per series. Unacceptable, possibly upgradeable with submission of the data from the other trial or an explanation why the data were not included. (Gee, 12/16/97)

** 224-059, -113 091762, 166956 “Gibberellin A3 lot # 84-526-CD list code 33690 in an in vitro cytogenetic assay measuring sister chromatid exchange in Chinese hamster ovary cells.” (J. L. Ivett, Hazleton Biotechnologies, Project No. 20990, March, 1986; response, 1/21/99) Chinese hamster ovary cells were incubated with gibberellin A3 (90%), lot #84-526-CD, with and without rat liver activation. Concentrations scored were 0 (DMSO), 90, 270 and 900 µg/ml and 2.7 mg/ml. Without S9, cells were exposed for approximately 25 hours to gibberellin A3 and with S9, for 2 hours followed by further incubation until harvest with most cells in M2. Mitotic cells were harvested by mitotic shake-off, fixed and stained. Cultures were observed for confluence of the monolayer but results were not reported. Fifty (50) cells per concentration were scored for sister chromatid exchanges. No evidence for induction of SCEs was reported. The study was evaluated as unacceptable due to the lack of comment on cytotoxicity but possibly upgradeable. (Gee, 12/17/97). A response dated 1/21/99 was submitted in record #166956. No comments on the confluency of the cultures was included although the original report stated this was observed. The basis of the response was the solubility of the gibberellin A3 and the statement that a precipitate would complicate the dosing and interfere with scoring of the SCEs. Despite the continued deficiency in a statement about confluency, the study has been upgraded to acceptable status with no induction of SCEs noted under the conditions of the study. (Gee, 7/21 99)

** 224-070 114115 “Micronucleus cytogenetic assay in mice.” (D. L. Putman, Microbiological Associates, Project T8201.122, October 24, 1988) Gibberellin A₄A₇ was tested with ICR mice for micronuclei formation. Five/sex/dose/sacrifice time were treated with 0 (corn oil), 120, 600 or 1200 mg/kg with 0.5 mg/kg triethylenemelamine as the positive control. Mice

were sacrificed at 24, 48 or 72 hours for control and treated groups and 24 hours only for positive control. Bone marrow preparations were analyzed for polychromatic erythrocytes with micronuclei, 1000 per animal. The ratio of polychromatic erythrocytes to total erythrocytes was determined. The positive control (TEM) was functional. Gibberellin did not increase the frequency of micronucleated polychromatic erythrocytes at any dose. Negative for genotoxicity under the conditions of the assay. Acceptable. (Gee, 12/19/97)

DNA, OTHER

** 224-059, -113 091768, 166958 "Evaluation of gibberellic A3 (acid gibberellic) in the rat primary hepatocyte unscheduled DNA synthesis assay" (M. A. Cifone, Hazleton Biotechnologies, No. 20991, May, 1986, amended July, 1986; response dated 1/22/99) Gibberellic A3, 90%, lot 84-526-CD, was tested with primary Fischer 344 rat hepatocytes at concentrations of 0 (ethanol), 50, 100, 250, 500, 602, 1000 or 1260 ug/ml for 18 hours. The highest soluble concentration was 1260 ug/ml. Fifty cells were analyzed from each of three coverslips per concentration for net nuclear grain counts. Data reported as the average net nuclear grains per 150 cells with no individual data included. Survival at 21 hours as measured by trypan blue dye exclusion ranged from 84 to 100% (control). Positive control was 2-acetyl aminofluorene which was functional. No evidence of unscheduled DNA synthesis as a result of treatment. The study was evaluated as unacceptable but upgradeable with submission of more detailed results of the net nuclear grain counts. (Gee, 12/17/97). Record 166958 contained the response consisting of 2 pages of the results for each of the three slides per concentration with the relevant data. With this submission, the study was upgraded to acceptable status with no evidence for the induction of unscheduled DNA synthesis under the conditions of the study. (Gee, 7/21/99)

** 224-070 114117 "Unscheduled DNA synthesis assay in rat primary hepatocytes with a confirmatory assay." (R. D. Curren, Microbiological Associates, T8201.380009, October 21, 1988) Gibberellin A₄A₇ was tested with primary male rat hepatocytes for the induction of unscheduled DNA synthesis as measured by autoradiography. Treatment was for 18 - 20 hours at concentrations ranging from 0.5 to 1500 µg/ml with cultures evaluated from 0.5 to 50 µg/ml in the first assay and 5 to 500 µg/ml in the second assay. Triplicate cultures per concentration with 50 cells per culture evaluated for UDS. Cytotoxicity was measured by release of lactic acid dehydrogenase. Only cultures with satisfactory morphology were evaluated. No evidence for the induction of UDS was found. Acceptable. (Gee, 12/22/97)

NEUROTOXICITY

Not required at this time.