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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: TILT Fungicide; Petition Nos. 4F3007 (Pecans), 4E3026 (Bananas), 4F3074 (Rice, Wheat, Barley, Rye), 4G3075 (Rice, Wheat, Barley, Rye); Caswell No. 323EE.

TO: Henry Jacoby
Product Manager #21
Registration Division (TS-767C)

THRU: Christine F. Chaisson, Ph.D. C.F. Chaisson 2/1/85
Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769C)

FROM: Alan C. Katz, M.S., D.A.B.T. Alan C Katz 2/8/85
Toxicology Branch
Hazard Evaluation Division (TS-769C)

The Toxicology Branch has conducted a preliminary review of the 90-day rat feeding study with triazole alanine (THS 2212; Bayer AG Institute of Toxicology, Wuppertal-Elberfeld, Report no. 12397; EPA Accession Nos. 072207, and 073114), including the histopathological data which was submitted to this Agency by CIBA-GEIGY Corporation on November 29, 1984. The following deficiencies have been noted:

1) Except for the histopathology section, the final report is not signed by responsible personnel and does not contain a Quality Assurance statement by the sponsor or contractor.

2) Clinical observations are not presented for individual animals or summarized according to sex/dose group.

3) Results of ophthalmological examinations are not presented.

4) Sufficient data to establish purity of the test substance and homogeneity, stability and concentration in the diet are not presented.

The sponsor must address the deficiencies cited above in order for the Toxicology Branch to complete its evaluation of this study.

1753

004276

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: CGA 64 250, Technical (Banner/Tilt; Propiconazole)
Petition No. 4F3007; 100-617
Sponsor: CIBA-GEIGY Corporation
Accession No.: 072206
Caswell No.: 323EE

TO: Henry Jacoby
Product Manager (21)
Registration Division (TS-767)

THRU: Christine F. Chaisson, Ph.D.
Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: Alan C. Katz, M.S., D.A.B.T.
Toxicology Branch
Hazard Evaluation Division (TS-769)

Toxicology Branch reviews of the following studies with CGA 64 250 are presented below:

1. L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenicity Test.
2. Saccharomyces Cerevisiae D7/Mammalian-Microsome Mutagenicity Test In Vitro.
3. Point Mutation Assay with Mouse Lymphoma Cells; Host-Mediated Assay.
4. Chromosome Studies in Male Germinal Epithelium (Mouse Spematogonia).
5. Chromosome Studies in Male Germinal Epithelium (Mouse Spermatocytes).
6. Autoradiographic DNA Repair Test on Human Fibroblasts.
7. Autoradiographic DNA Repair Test on Rat Hepatocytes.
8. Sister Chromatid Exchange Study (Chinese Hamster).
9. BALB/3T3 Cell Transformation Assay.
10. Two-Generation Study in Rats.

Test Material:

CGA 64 250 Technical is a triazole derivative fungicide. 004276
Its chemical name is 1[[2(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole.

Conclusions/Recommendations:

Overall, the experiments cited in this review for detection of gene mutations, chromosomal aberrations, primary DNA damage and cell transformation, in addition to previously reviewed studies with CGA 64 250 Technical (i.e., Accession No. 244272: Salmonella/Mammalian Microsome Mutagenicity test, Dominant Lethal Study in Mice and Nucleus Anomaly Test in Chinese Hamsters), appear to represent a reasonably comprehensive and balanced battery of tests for mutagenic effects. With the exception of the BALB/3T3 Cell Transformation Assay, the reports of the short-term tests included in this review were considered unacceptable, pending submission of additional data as specified in the attached individual summaries.

The 2-generation study included in the present review is assigned a Supplementary core-classification. The data developed in this study may be used in evaluating results of a repeat 2-generation study which was initiated March, 1983 at ToxiGenics.

1. CGA 64250; L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenicity Test in Vitro; CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 811516. 8/10/82.

004276

Background/Methods:

CGA 64250 (Batch No. Op. 103119; 90.7% a.i.) was tested for mutagenic properties in mouse lymphoma cells (L5178Y/TK$^{+/-}$) in vitro. The test system is designed to detect the induction of forward point mutations in mammalian cells. Cytotoxicity test were performed to provide a basis for selection of the test substance concentration range. The mutagenicity experiments were conducted with the test substance in DMSO at concentrations of 7.81, 15.62, 31.25, 62.50 and 125.00 ug/ml, with and without microsomal activation. Ethylmethane sulfonate (0.5 ul/ml) and dimethylnitrosamine (0.5 ul/ml) were used as positive controls for the non-activated and activated cultures, respectively.

Details of the procedures used in this assay, excerpted from the study report submitted by CIBA-GEIGY, are presented in Appendix 1.

Results:

Incubation of cells with CGA 64250 at concentrations of 7.81, 15.62, 31.25, 62.50 or 125.00 ug/ml, in the presence or absence of metabolic activation, resulted in no apparent dose-related trend with respect to mutation frequency. Calculated mutation factors (mutation frequency of treated cultures divided by mutation frequency of the controls) were as follows:

CGA 64250 Conc. (ug/ml)	Mutation Factor	
	Without Activation	With Activation
7.81	1.41	1.20
15.62	1.22	1.20
31.25	1.48	1.55
62.50	1.51	1.36
125.00	1.42	1.65
Positive Control	4.29	2.72

Conclusions/Recommendations:

Results of the study, as reported to this Agency, are unacceptable. The Toxicology Branch requests submission of additional data. The summary tables of mean values, without indicating individual data, standard deviation or standard error, are inadequate. Appropriate statistical analyses should be performed on the mutagenicity data obtained in this assay.

2. CGA 64 250; Saccharomyces Cerevisiae D^7/Mammalian-Microsome Mutagenicity Test In Vitro; CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 811558. 8/19/82.

Background/Methods:

CGA 64 250 (Batch No. Op. 103119, 90.7% a.i.) was tested for mutagenic properties in yeast cells, using the D^7 strain of Saccharomyces cerevisiae. The tests were performed with and without activation (by rat liver microsomes and co-factors), with CGA 64 250 concentrations of 10, 30, 90 and 270 ug/ml.

The test system is designed to detect induction of mitotic crossing-over, mitotic gene conversion, chromosome loss, deletion and point mutation. Materials and methods used, excerpted from the study report submission, are presented in Appendix 2.

Results:

Results were as follows*:

Concentration	Colony forming units X 10^6/ml	Adenine-dependent Cells X 10^3/ml	Convertants per ml	Revertants per ml
0 (DMSO control)	8.20(9.41)	8(6)	292(208)	40(22)
10 ug/ml	7.82(8.34)	6(2)	258(212)	60(36)
30 ug/ml	4.66(10.29)	6(6)	146(178)	40(10)
90 ug/ml	0.16(5.19)	0(1)	52(90)	0(8)
270 ug/ml	0.01(0.90)	0(2)	6(42)	0(0)
Positive control 4-nitroquinoline-N-oxide, 0.2 ug/ml	1.08(--)	52(--)	1136(--)	100(--)
Cyclophosphamide, 800 ug/ml	--(10.79)	--(108)	--(3162)	--(756)

*Note: In the data presented above, the first values in each set represent results of tests without microsomal activation and the second values (in parentheses) are with activation. An inhibitory effect on the growth of yeast cells, especially at concentrations of 30, 90 and 270 ug/ml, was noted.

004276

Conclusions/Recommendations:

The data submitted are considered unacceptable. The Toxicology Branch requests additional data.

1) Individual values should be presented for each plate. Standard deviation and/or standard error should be calculated for each mean value.

2) A table of statistics for the assay with the activation system should be submitted (i.e., Table 3 of the study report is missing).

004276

3. CGA 64 250; Point Mutation Assay with Mouse Lymphoma Cells (Host-Mediated Assay); CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 811513. 8/10/82.

Background/Metods:

CGA 64 250 (Batch No. Op 103119) was tested for mutagenic effects on mouse lymphoma cells (L 5178Y) in a host-mediated assay system. This test system is designed to detect the induction of forward point mutations in mammalian cells.

Male mice (DBA/Bom/SPF) were dosed orally with 496 mg CGA 64250/kg body weight. Details of the procedures, excerpted from the study report submission, are presented in Appendix 3.

The dose level was selected based on a toxicity test in which 5 groups of 6 mice (DBA/Bom/SPF), injected ip with 10^6 L5178Y cells in 1 ml of medium without serum, were dosed 3 days later with CGA 64 250 in 2% carboxymethylcellulose by gavage at levels of 0 (vehicle control) to 496 mg/kg. Mice given the highest dose of CGA 64 250 showed no decrease in the number of target cells when sacrificed 3 days after administration of the test substance. According to the study report, this dose corresponds to "1/3 of the LD_{50} of the substance in this species." The study report does not specify whether the animals were fasted prior to dosing.

Results:

The results of this study are summarized as follows:

	Antimetabolites					
	Cytosine-arabinoside		Methotrexate		Thymidine	
	MF	MFF	MF	MFF	MF	MFF
Control	0	--	5.85	--	4.81	--
CGA 64 250	0	0	3.54	0.61	2.56	0.53

MF = Mutant frequency per 100,000 cells.
MFF= Mutant frequency factor (MF of CGA 64 250-treated group/ MF control).

Individual data were not reported.

004276

Conclusion/Recommendations:

This study is considered unacceptable.

1) Concurrent positive control data should be presented.

2) The Toxicology Branch requests submission of the individual data used in calculating the mean values presented in the summary table. Standard deviation and/or standard error should be calculated for each mean value.

3) Additional data should be submitted to demonstrate that the maximum tolerated dose for male mice was used. Data for this strain should be cited, if available.

4) Data must be provided to demonstrate that the test substance, administered by gavage, was available for exposure to the ip-injected target cells.

4. CGA 64 250; Chromosome Studies in Male Germinal Epithelium; CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 81761. 8/31/82.

004276

Background/Methods:

CGA 64 250 (Batch No. Op 103119, 90.7% a.i.) was tested for mutagenic effects on mouse spermatogonia.

Male mice (NMRI-derived) were dosed by gavage with 0 (vehicle), 166 or 498 mg CGA 64 250 (in 0.5% aqueous sodium carboxymethylcellulose) per kg once daily for 5 consecutive days. Each of the treated groups was comprised of 15 animals and the control group was comprised of 12 animals. One day after administration of the last dose of CGA 64 250, the mice were injected with colcemide, 10 mg/kg i.p., and sacrificed 3 hours later. Testes were removed and processed. Metaphase figures of spermatogonia (100 from each of 8 animals in the control and low dose groups and 7 in the high dose group) were examined for aberrations.

Details of the procedures used in this assay, excerpted from the study report submitted by CIBA-GEIGY, are presented in Appendix 4.

Results:

Seven of the 15 high dose (496 mg/kg) animals died following the first application. There was no indication in the study report with respect to whether these animals were necropsied.

Metaphase Aberrations in Mouse Spermatogonia

	Control	CGA 64 250	
		166 mg/kg	498 mg/kg
Number of Animals Examined	8	8	7
Number of Animals Affected/ (Percent of Metaphases*) with:			
- Chromatid breaks	0	1/(1)	1/(2)
- Chromatid gaps	2/(1); 1/(2)	1/(1)	2/(1)
- Isochromatid gaps	0	1/(1)	0
- Aneuploidy	1/(1)	2/(1)	2/(1)
- Polyploidy	3/(1); 1/(2); 1/(3); 2/(4); 1/(6)	5/(2)	1/(1); 2/(2); 2/(5); 1/(7)
- Aneuploid Polyploidy	0	3/(1)	0

*Percent of aberrant metaphases in each specifically affected animal.

004276

Conclusions/Recommendations:

The study report, as submitted, is considered unacceptable.

1) The death of 7 of 15 high dose animals after the first dose, with no other deaths in this group following subsequent doses, suggests that the animals may have been misdosed. If possible, further information (e.g., clinical or necropsy observations) should be submitted so that conclusions may be drawn as to the likely cause of death.

2) Concurrent positive control data must be provided.

3) A brief explanation should be provided with reference to the finding that slides from only 7 of the 8 high dose animals were considered "scorable."

5. CGA 64 250; Chromosome Studies in Male Germinal Epithelium; CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 811512. 8/13/82.

Background/Methods:

CGA 64 250 (Batch No. Op. 103119; 90.7% a.i.) was tested for mutagenic effects on mouse spermatocytes. Male mice (NMRI-derived) were dosed by gavage with 0(vehicle), 166 or 498 mg CGA 64 250 (in 0.5% aqueous sodium carboxymethylcellulose) per kg body weight on days 0, 2, 3, 5 and 9. Each of the treated groups was comprised of 15 animals and the control group was comprised of 12 animals. On day 12, the animals were injected with colcemide, 10 mg/kg i.p., and sacrificed 3 hours later. The testes of 10 animals in each of the treated groups and 12 animals in the control group were removed and processed. Metaphase figures of 100 primary and 100 secondary spermatocytes from 8 animals of each group were examined for aberrations.

Details of the procedures used in this assay, excerpted from the study report submitted by CIBA-GEIGY, are presented in Appendix 5.

Results:

One aberration (a fragment)/100 primary spermatocytes in metaphase was found in 1 of 8 animals in the high dose group, and 1 aberration (a break)/100 primary spermatocytes was found in 1 of 8 animals in the low dose group. No metaphase pattern abnormalities were found in primary spermatocytes of any of the 8 control animals or in secondary spermatocytes of any of the treated or control animals.

Conclusions/Recommendations:

Under the conditions of this study, there was no evidence found that CGA 64 250 is mutagenic in mouse spermatocytes when administered orally at levels up to and including 498 mg/kg. However, the study report, as submitted, is considered unacceptable.

1) Rationale for selection of the high dose level (498 mg/kg) should be explained.

2) Concurrent positive control data must be provided.

3) A copy of the protocol, as well as records of any deviations from the protocol, should be submitted.

4) Presence or absence of clinical signs of toxicity, especially at the high dose level, should be established.

5) Since the testes of only some of the treated mice were examined microscopically, the selection criteria for determining which mice would be examined should be explained.

6. CGA 64 250; Autoradiographic DNA Repair Test on Human Fibroblasts; CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 811655. 8/12/82.

004276

Background/Method:

CGA 64 250 (lot number/purity not specified) was tested for DNA-damaging properties on human fibroblasts in vitro. Cytotoxicity tests were performed to provide a basis for selection of the test substance concentration range. The experiment was conducted using concentrations of 0, 0.0768, 0.384, 1.92 and 9.60 nl/ml. Two negative controls were used: 1 containing the vehicle, and 1 untreated. 4-nitroquinoline-N-oxide (5uM) was used as a positive control.

^{3}H-thymidine was added to treated and control cultures of human fibroblasts. Autoradiographs were prepared and 4 slides (50 cells/slide) were scored (silver gains counted) for each treated and control group. Details of the procedures used in this assay, excerpted from the study report submitted by CIBA-GEIGY, are presented in Appendix 6.

Results:

Individual data were not reported. Mean values are presented in the following table:

Treatment	Concentration	Silver grains/nucleus
Culture medium, control	- -	1.85
Vehicle, control	- -	1.89
4NQO, positive control	5 uM	30.98
CGA 64 250	0.0768 nl/ml	1.58
	0.384 nl/ml	1.43
	1.92 nl/ml	1.34
	9.60 nl/ml	1.14

Conclusions/Recommendations:

No evidence of CGA 64 250-induced DNA damage was found. This study is considered unacceptable, pending submission of additional data.

1) A full description of the test substance (e.g., lot number, batch number, purity) must be provided.

2) Individual data is required. The number of grains counted must be presented for each of 4 slides per treatment group.

3) Standard deviation and/or standard error should be calculated for each mean value of grains/nucleus.

4) Data for background count levels should be presented.

5) Concentrations of test material must be expressed on a weight/volume basis (e.g., ug/ml).

004276

7. CGA 64 250; Autoradiographic DNA Repair Test on Rat Hepatocytes; CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 811514. 8/12/82.

Background/Methods:

CGA 64 250 (lot number/purity not specified) was tested for DNA-damaging properties on rat hepatocytes in vitro. Cytotoxicity tests were performed to provide a basis for selection of the test substance concentration range. The experiment was conducted using concentrations of 0, 0.69, 3.44, 17.2 and 86 nl/ml. Two negative control groups were used; one was untreated and the other contained the vehicle (DMSO). Dimethylnitrosamine (100 mM) was used as a positive control. Freshly isolated hepatocytes from a male rat (Tif:RAIF(SPF), weighing 245g) were cultivated in Williams' Medium E containing 10% fetal bovine serum.

^{3}H-thymidine was added to treated and control cultures of rat hepatocytes. Autoradiographs were prepared and 3 slides, (50 cells/slide) were scored (silver grains counted) for each treated and control group.

Details of the procedures used in this assay, excerpted from the study report submitted by CIBA-GEIGY, are presented in Appendix 7.

Results:

Individual data were not reported. Mean values are presented in the following table:

Treatment	Concentration	Silver grains/nucleus
Culture medium, control	- -	4.40
Vehicle, Control	- -	4.99
DMN, positive control	100 mM	30.93
CGA 64 250	0.69 nl/ml	4.59
	3.44 nl/ml	4.96
	17.2 nl/ml	4.41
	86.0 nl/ml	5.36

004276

Conclusions/Recommendations:

No evidence of CGA 64 250-induced DNA damage was found. This study is considered unacceptable, pending submission of additional data.

1) A full description of the test substance (e.g., lot number, batch number, purity) must be provided.

2) Individual data is required. Number of grains counted must be presented for each of 3 slides per treatment group.

3) Standard deviation and/or standard error should be calculated for each mean value of grains/nucleus.

4) Data for background count levels in cell-free areas should be presented.

5) The concentration of the test material must be expressed in terms of weight/volume (e.g., ng/ml).

004276

8. CGA 64 250; Sister Chromatid Exchange Study; CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 811515. 10/18/82.

Background/Methods:

CGA 64 250; (Batch No. Op. 103119; 90.7% a.i.) was tested for mutagenic properties in Chinese hamster bone marrow cells in vivo. Two hours after subcutaneous neck implantation of 45 mg of 5-bromodeoxyuridine (BUdR), the test substance was administered via gavage to 4 animals/sex/group at levels of 0, 255, 510 or 1020 mg in arachid oil per kg body weight. Dose volume was 20 ml/kg. Control animals were dosed with the arachid oil vehicle. The following day, the animals were injected with 10 mg colcemide/kg i.p. and sacrificed 2 hours later by cervical dislocation. Bone marrow was extracted, suspended in 1% sodium citrate, and centrifuged, resuspended and processed onto slides.

The slides of 2 animals/sex/group were examined microscopically. For each animal, 25 metaphase patterns of the second cell cycle with BUdR substitution were examined and SCE's counted. Details of the procedures used in this assay, excerpted from the study report submitted by CIBA-GEIGY, are presented in Appendix 8.

Results:

Data for males and females (combined) are summarized as follows:

Treatment	Concentration	SCE's per cell (Mean ± SD)
Arachid Oil (Control	- -	4.62 ± 2.75
DMBA (positive Control)	100 mg/kg	11.57* ± 7.03
CGA 64 250	255 mg/kg	4.82 ± 2.65
	510 mg/kg	4.78 ± 2.25
	1020 mg/kg	4.76 ± 2.13

* Significantly different from control value, $p<0.01$.

Conclusions/Recommendations:

CGA 64 250 did not induce sister chromatid exchange in Chinese hamsters after oral administration at levels up to and including 1020 mg/kg. This study is considered unacceptable pending submission of additional information.

1) The Toxicology Branch requests additional data to establish that the high dose level in this study, 1020 mg/kg, represents the maximum tolerated dose of CGA 64 250 in the Chinese hamster.

2) Data should be provided to establish the bioavailability of the test compound in the bone marrow.

3) Justification should be presented for use of less than 5 animals/sex in this assay.

004276

9. CGA 64 250; BALB/3T3 Cell Transformation Assay; CIBA-GEIGY Limited, Basle, Switzerland; Experiment No. 790806. 8/10/82.

Background/Methods:

Technical CGA 64 250 (Batch No. Op. 103119; 90.7% a.i.) was tested for transformation-inducing properties in mammalian fibroblasts in vitro. Concentrations of 1.16, 2.31, 4.63, 9.25 and 18.50 ug/ml DMSO (1% in Eagle's Minimum Essential medium containing 10% fetal bovine serum) were used. The highest dose level was calculated to produce a 25% reduction in colony-forming ability, based on a preliminary in vitro toxicity test. Two negative controls were used: 1 untreated and 1 vehicle-treated. Two positive control groups were treated with methylcholanthrene at concentrations of 1.5 or 3.0 ug/ml. Fifteen replicate dishes were used in each of the treated and control groups.

Details of the procedures used in this assay, excerpted from the study report submitted by CIBA-GEIGY, are presented in Appendix 9.

Results:

Results of this study may be summarized as follows:

Group		Transformation Frequency*
Vehicle control		0.88
Untreated control		2.15
Methylcholanthrene,	1.5 ug/ml	23.03**
Methylcholanthrene,	3.0 ug/ml	32.29**
CGA 64 250	1.16 ug/ml	0
	2.31 ug/ml	0
	4.63 ug/ml	0
	9.25 ug/ml	0.90
	18.50 ug/ml	0.64

*Transformation frequency = # of transformed cells/ 10^4 surviving cells.
**Significantly different from vehicle and untreated control values, $p<0.05$.

Conclusions:

Under the conditions of this experiment, CGA 64 250 did not cause a measurable increase in transformation of BALB/3T3 cells. Results of this study are considered acceptable.

004276

10. CGA 64 250; 2-Generation Study in Rats; CIBA-GEIGY Limited, Sisseln, Switzerland and Basle, Switzerland; Experiment No. 790010. 6/29/81.

Background/Methods:

This study was conducted to evaluate the effects of CGA 64 250 technical (Batch No. P.4-6, 91.9% a.i.) on the growth and the reproductive performance of 2 generations in the albino rat (Tif:RAIf[SPF]). The test material was administered at concentrations of 0, 400, 2000 or 5000 ppm in the diet. The F_0 (parental) and F_1 generation rats were fed treated diet continuously for approximately 150-160 days, including a 12-day mating period, and were sacrificed after weaning of the F_1 and F_2 generations, respectively. During the mating period for the F_0 and F_1 animals, each of 10 males (F_0) or 12 males (F_1) were housed with 2 females. All animals were necropsied at termination of dosing, and tissues from a selected number of F_1 adults and F_1 and F_2 weanlings were processed and examined microscopically.

Details of the procedures used in this study, excerpted from the report submitted by CIBA-GEIGY, are presented in Appendix 10.

Results:

Due to the perinatal deaths of all of the F_0 females treated at 5000 ppm, this dose group was discontinued. According to the study report, the high dose F_0 males were terminated "after weaning of the F_1 pups "(evidently referring to the F_1 generation in the 0, 400 and 2000 ppm groups). Data with respect to litter sizes, sex ratios and duration of pregnancy for the 5000 ppm group were not submitted. Reduced body weight gain occurred in the F_0, F_1 and F_2 animals at the 2000 ppm concentration, and in the F_1 and F_2 animals at 400 ppm. The F_0 mating ratio (number of females mated/total number of females) of the 5000 ppm group was slightly lower than the control value.

Group	Mating ratio (%)
F_0 females	
Control	80
400 ppm	90
2000 ppm	90
5000 ppm	65
F_1 females	
Control	75
400 ppm	100
2000 ppm	96

Compared to control values, the fertility indices of the F_o and F_1 generations of all CGA 64 250 treated groups revealed no apparent adverse effect. The investigators reported that, for the control and 5000 ppm groups of F_o females, parturition was delayed and the fertility indices were "unusually low." The implantation rate among the F_o females in the 5000 ppm group was also reported to be significantly reduced. No statistical results or historical control data were presented to support these findings.

Parturition data may be summarized as follows:

Group	Mean duration of pregnancy (days)
F_o females	
Control	21.8
400 ppm	21.4
2000 ppm	21.6
5000 ppm	22.0

The mean duration of pregnancy for the F_1 females was not presented in the study report. However, the investigators reported that parturition for these animals "was not disturbed and the normal range of duration of pregnancy (21-22 days) was maintained in all females." The fertility indices are tabulated below:

Group	Fertility Index (%)*
F_o	
Control	60.0
400 ppm	70.0
2000 ppm	75.0
5000 ppm	60.0
F_1	
Control	66.7
400 ppm	100.0
2000 ppm	91.7

*Fertility Index (for mating ratio 1 male :2 females) :(Number of females with implantation sites/number of males) X50.

Implantation rates for the F_o females are presented in the following table:

004276

Group	Total no. of females with implantation sites	No. of implantations Mean	S.D.
Control	12	14.6	1.5
400 ppm	14	14.4	1.4
2000 ppm	15	14.2	1.2
5000 ppm	12	12.3	2.5

Relative (organ: body) liver weights were found to be significantly increased in the 2000 ppm male and female F_1 adults, the 400 and 2000 ppm female F_1 weanlings, and the 2000 ppm male and female F_2 weanlings. Neither individual nor mean relative organ weight data were presented in the study report.

Histopathologic evaluation revealed a treatement-related, slight hypertropy of centrilobular hepatocytes in male and female F_1 adults in the 2000 ppm concentration groups.

Discussion:

Apparently due to the death of all of the females caused by CGA 64 250 at the highest dietary concentration (5000 ppm), and/or the treatment-related effects observed in the animals given the lowest concentration (400 ppm), a second 2-generation reproduction study in rats was conducted. The repeat study was initiated March, 1983 at ToxiGenics.

Conclusions:

Administration of CGA 64 250 at a dietary concentration of 5000 ppm caused the death of all F_0 females in that treatment group. Mating ratios and implantation rates were also reduced in the 5000 ppm group. No other reproductive parameters appeared to be affected by treatment with CGA 64 250. Dose-related reductions in body weight gain and increased relative liver weights were evident for groups treated at all concentrations of CGA 64 250, including 400 ppm. At a dietary concentration of 2000 ppm, the test substance caused hypertrophy of centrilobular hepatocytes in F_1 adults.

This study is considered a supplementary study. It provides data which may be useful in evaluating the results of the repeat 2-generation study which was initiated in March, 1983.

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Pages 22 through 52 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 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.

✓ FIFRA registration data.

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