Reading

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

APR 30 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: 100-ART, Tilt™ 3.6E; 100-ARI, CGA-64250; New Ciba-Geigy Fungicide CASWELL #323EE Acc.#244271-2

FROM: William Dykstra, Toxicologist
Toxicology Branch, HED (TS-769) WHD 4/30/81

TO: Henry Jacoby (21)
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Recommendations:

1) The registration of Tilt™ 3.6E (100-ART) can be toxicologically supported. The label signal word and precautionary labeling are acceptable.

2) The registration of technical CGA-64250 (100-ARI) can be toxicologically supported.

3) The submitted toxicology studies are acceptable as Core-Minimum Data.

Review:

A. Tilt 3.6E Fungicide

1. Rat Acute Oral Toxicity (Stillmeadow Project No. 1663-80; June 23, 1980)

Test Material: CGA-64250 3.6E FL 80040

Groups of 5M + 5F BLU(SD) rats were given oral doses of 964, 1150, 1390, 1670, 2000 and 5020 mg/kg of test material. Observation was for 14 days.

Results: LD_{50} = 1510 (1270-1800) mg/kg - males
LD_{50} = 1100 (798-1520) mg/kg - females
LD_{50} = 1310 (1130-1520) mg/kg - both sexes

Toxic Signs: Salivation, constricted pupils, piloerection, decreased activity, polyuria, lethargy, ptosis, lacrimation, hematuria, diarrhea, exophthalmos, difficult and labored breathing, ataxia.

Body Weight: Most survivors gained weight during 14-day period.

Necropsy: Corneal opacity, discoloration of the stomach and intestines, urinary bladder empty, intestines distended with gas, discoloration of liver and spleen.

Toxicity Category III: Caution

Classification: Core-Minimum Data

2. Rabbit Acute Dermal Toxicity with CGA 64250 3.6E FL 800410 (Stillmeadow Project No. 1664-80; May 15, 1980)

One group of 5 male + 5 female NZW albino rabbits received a dermal application of 5010 mg/kg of test material on the skin (the exposure areas of two males and three females were abraded) under an impervious cuff for 24 hours. Observation was for 14 days.

Results: No deaths, LD_{50} > 5010 mg/kg

Dermal Irritation: Erythema and edema were present throughout the study and averaged 1.98 and 2.13 respectively for the entire study.

Body Weight: Survivors gained weight.

Necropsy: No gross abnormalities.

Toxicity Category III: Caution

Classification: Core-Minimum Data

3. Rabbit Eye Irritation with CGA 64250 3.6E FL 800410 (Stillmeadow Project No. 1665-80; April 30, 1980)

0.1 ml of test material was instilled in one eye of each of nine NZW rabbits with the untreated eye serving as control. Three of the treated eyes were each washed with room temperature deionized water for one minute beginning thirty seconds after treatment. The treated eyes of all animals were examined and evaluated for irritation of 1, 24, 48, and 72 hours and at 4, 7, 10, and 13 days after treatment.

The corneas of all treated eyes were re-examined immediately after the 1 and 24 hour observations with 0.2% fluorescein.

Unwashed Eyes: Corneal opacity in 6/6 eyes reversible by day 7. A maximum average irritation score of 25.5 was obtained for unwashed eyes at 24 hours after treatment.

Washed Eyes: Corneal opacity in 2/3 washed eyes reversible by day 7. A maximum average irritation score of 19.0 was obtained for washed eyes at one hour after treatment.

Conclusion: Moderately irritating.

Toxicity Category II: Warning

Classification: Core-Minimum Data

4. Rabbit Primary Skin Irritation Test with CGA-64250 3.6E FL 800410 (Stillmeadow Project No. 1666-80; April 25, 1980)

0.5 ml of test material was applied to two intact and two abraded skin sites on the fur clipped trunks of 6 NZW rabbits (3M + 3F) for 24 hours under an impervious cuff. Observation at 24 and 72 hours.

Results: P.I. = 4.60

Conclusion: Moderately irritating.

Toxicity Category III: Caution

Classification: Core-Minimum Data

5. 4-Hour Acute Aerosol Inhalation Toxicity Study in Rats of CGA-64250 3.6E (Toxigenics Report 420-0212; June 10, 1980)

One group of 10 albino king rats, 5M + 5F, were exposed at a time-weighed average analytical concentration of 2.45 mg/L for 4 hours. Observation for 14 days.

Results: Two female rats died on day 3; LC_{50} > 2.45 mg/L.

Toxic Signs: Nasal discharge, salivation, gasping, lacrimation, prostation.

Necropsy: Focal tan depressions of the lung were noted in one rat.

Toxicity Category III: Caution

Classification: Core-Minimum Data

6. Guinea Pig Sensitization with CGA-64250 3.6E FL 800410 (Stillmeadow Project No. 1667-80; June 27, 1980)

A sensitization study was conducted on twenty male albino guinea pigs to determine if the test material produced a sensitizing reaction. There were ten males per each of two treatment groups. Positive control animals were treated with ten applications of a 0.05% w/v solution of 2,4-dinitro-chlorobenzene in ethanol for 6 hours per day under an occlusive patch. The test group animals were treated with a 1.0% v/v of the test material in ethanol for 6 hour day under an occlusive wrap for 10 applications. The animals were treated on days 2, 4, 7, 9, 11, 14, 16, 18, 22, 24 and 38. A marked increase in positive skin reactions after the day 38 treatment (final treatment) above those observed after the day 2 treatment (initial treatment) was indicative of a sensitizing reaction.

Results: A sensitizing reaction in guinea pigs was produced by 2,4-dinitrochlorobenzene (day 2 score = 0.7 vs day 38 score = 3.1).

A sensitizing reaction in guinea pigs was produced by the test material (day 2 score = 0.3 vs day 38 score = 0.9).

Conclusion: The test material is a skin sensitizing agent.

Classification: Core-Minimum Data

B. CGA-64250 Technical

1. Acute Oral LD_{50} in the Rat of Technical CGA-64250 (Ciba-Geigy Ltd. Project No. 785244; December 7, 1978)

Groups of 5M + 5F healthy random bred rats of the Tif:Raif (SPF) strain were gavaged with single oral doses of 500, 1000, 3000, and 4000 mg/kg of test material. Observations were for 14 days.

Results: LD_{50} = 1517 (958-2291) mg/kg (both sexes)

Toxic Signs: Sedation, dyspnea, ruffled fur, curved body position.

Body Weight: Survivors gained weight.

Necropsy: No gross pathology was seen.

Toxicity Category III: Caution

Classification: Core-Minimum Data

2. Acute Oral LD_{50} in Mouse of Technical CGA-64250 (Ciba-Geigy Ltd. Report No. 785243; May 7, 1979)

   Groups of 5M + 5F Tif:MAG (SPF) strain mice were gavaged with single oral doses of 800, 1500, 2500 and 3000 mg/kg of test material. Observations were for 14 days.

   Results: LD_{50} = 1490 (1138-1875) mg/kg (both sexes)

   Toxic Signs: Sedation, dyspnea, ruffled fur, clipped body position.

   Body Weight: Survivors gained weight.

   Necropsy: No gross pathology was seen.

   Toxicity Category III: Caution

   Classification: Core-Minimum Data

3. Acute Oral LD_{50} in the Chinese Hamster of Technical CGA-64250 (Ciba-Geigy Ltd. Report No. 790146; April 20, 1979)

   Groups of 5M + 5F Chinese hamsters were gavaged with a single oral dose of 1000, 3000, 4500 and 6000 mg/kg of test material. Observations were for 14 days.

   Results: LD_{50} = 3006 (2152-3943) mg/kg (both sexes)

   Toxic Signs: Sedation, dyspnea, exopthalmos, ruffled fur, curved body position, ataxia.

   Body Weight: Survivors gained weight.

   Necropsy: No gross pathology was seen.

   Toxicity Category III: Caution

   Classification: Core-Minimum Data

4. Acute Oral LD_{50} in the Rabbit of CGA-64250 (Ciba-Geigy Ltd. Report No. 785247; November 2, 1978)

   Groups of 6M + 6F rabbits were gavaged with single oral doses of 600, 1000, 2150 and 3590 mg/kg of test material. Observations for 14 days.

   Results: LD_{50} = 1344 (1062-1710) mg/kg (both sexes)

   Toxic Signs: Sedation, dyspnea, ruffled fur, ataxia, tremors, convulsions, curved body position.

   Body Weight: Survivors gained weight.

   Necropsy: No gross pathology was seen.

   Toxicity Category III: Caution

   Classification: Core-Minimum Data

5. Acute Intraperitoneal LD_{50} in Rat of Technical CGA-64250 (Ciba-Geigy Ltd. Report No. 785246; March 13, 1979)

Groups of 5M + 5F Tif:Raif (SPF) rats received single intraperitoneal doses of 200, 300, 500, 1000 and 1500 mg/kg. Observation was for 14 days.

Results: LD_{50} = 508 (381-653) mg/kg (both sexes)

Toxic Signs: Sedation, dyspnea, ruffled fur, curved body position.

Body Weight: Survivors gained weight.

Necropsy: No gross pathology was seen.

Toxicity Category II: Warning

Classification: Core-Minimum Data

6. Acute Dermal LD_{50} in the Rat of Technical CGA-64250 (Ciba-Geigy Ltd. Report No. 785245; June 22, 1979)

Groups of 5M + 5F Tif-Raif (SPF) rats received dermal applications of 3000 and 4000 mg/kg of test material on the intact skin of the fur clipped trunk under an impervious cuff for 24 hours.

Results: No deaths, LD_{50} > 4000 mg/kg (both sexes)

Toxic Signs: Dyspnea, ruffled fur, curved body position.

Body Weight: Survivors gained weight.

Necropsy: Unremarkable.

Toxicity Category III: Caution

Classification: Core-Minimum Data

(a) Abraded skin not tested.

7. Eye Irritation in the Rabbit after Single Application of Technical CGA-64250 (Ciba-Geigy, Ltd. Report No. 785248; October 26, 1978)

0.1 ml of test material was inserted into the conjunctival sac of the left eye of 6 rabbits. The right eye was not treated and served as untreated control. In 3 of the 6 rabbits approximately 30 seconds after treatment, the treated eye was flushed with 10 ml of physiological saline. The eye irritation was appraised with a slit-lamp on day 1, 2, 3, 4 and 7 and scored according to Draize.

Results: Unwashed Eye: P.I. = 2.4/110; corneal opacity in 2/3 eyes reversible within 72 hours.

Washed Eye: P.I. = 0.4/110

No corneal opacity; conjunctivitis reversible within 48 hours.

Toxicity Category II: Warning

Classification: Core-Minimum Data

8. Skin Irritation in the Rabbit after Single Application of Technical CGA-64250 (Ciba-Geigy, Ltd. Report No. 785249; October 29, 1978)

0.5 ml of test material was applied to intact and abraded skin sites on the backs of 6 rabbits under an impervious cuff for 24 hours. Observations at daily intervals for 7 days.

Results: P.I. = 1.4

Slight erythema and edema which lasted in 3 rabbits up to 4 days. No irritation at day 7.

Toxicity Category IV: Caution

Classification: Core-Minimum Data

9. Acute Aerosol Inhalation Toxicity in the Rat of CGA-64250 EC 250 (A-609713) (Ciba-Geigy, Report No. 79074; June 21, 1979)

Groups of 10M + 10F Tif:Raif (SPF) rats were exposed for 4 hours to aerosols of 454, 924 and 1296 mg/m^3 of test material. Observation was for 14 days. 85% of airborne particles were smaller than 7 um in diameter.

Results: LC_{50} = 1264 (1075-1650) mg/m^3 (both sexes)

Toxic Signs: Dyspnea, ruffled fur, curved body position.

Body Weight: Survivors gained weight.

Necropsy: Hemorrhages in the lungs.

Toxicity Category II: Warning

Classification: Core-Minimum Data

10. Skin Sensitizing (contact allergenic) Effect in Guinea Pigs of Technical CGA-64250 (Ciba-Geigy, Ltd. Report No. 785250; February 8, 1979)

The test was performed on groups of 10 male and 10 female guinea pigs, one control group (20 animals) and one experimental group (20 animals). During the induction period the animals received one injection every second day (except weekends) to a total of 10 intracutaneous injections of a freshly prepared 0.1% dilution of CGA-64250 in propylene glycol. One control group was treated with the vehicle alone.

Fourteen days after the last sensitizing injection, a challenge injection of 0.1 ml of a freshly prepared 0.1% dilution of CGA-64250 in propylene glycol was administered into the skin of the left flank.

Twenty-four hours after each injection during the first week of the induction period and 24 hours after the challenge injection the reactions were scored. Ten days after the intracutaneous challenge injection a subirritant dose of the test compound was applied epicutaneously under occlusive dressings which were left in place for 24 hours.

Results: Under the experimental conditions employed, no differences between the test group and the vehicle treated controls were seen, after either epidermal or intradermal challenge application of CGA-64250.

Conclusion: CGA-64250 was found to be devoid of skin sensitizing (contact allergenic) potential in albino guinea pigs.

Classification: Core-Minimum Data

11. Salmonella/Mammalian Microsome Mutagenicity Test with CGA-64250 (Ciba-Geigy, Ltd.; Report No. 7812577; January 4, 1979)

CGA-64250 was tested for mutagenic effects on histidine-auxotrophic mutants of Salmonella typhimurium (TA-98, TA-100, TA-1535, TA-1537).

The tests were performed with the following concentrations of test material with and without microsomal activation: 25, 75, 225, 675 and 2025 ug/0.1 ml. Positive controls were tested.

Results: In the experiments performed with and without microsomal activation, no mutagenic effect was noted with the various concentrations of CGA-64250.

Classification: Core-Minimum Data

12. Dominant Lethal Study with CGA-64250 in the Mouse (Ciba-Geigy, Ltd. Report No. 790034; October 31, 1979)

The test material was administered orally by intubation as single doses of 165 and 495 mg/kg to groups of twenty male albino mice (Tif:MAG F (SPF) which were then each mated to two untreated females from the same strain over a period of six weeks. At the end of each week the females were replaced by new ones. The experiment was done to evaluate any cytotoxic or mutagenic effects on the male germinal cells as expressed by the loss of pre-implantation zygotes as well as by the rate of deaths of post-implantation stages of embryonic development.

Results: The females mated to males which had been treated with the compound did not differ significantly from the females mated to controls, neither in mating ratio nor in the number of implantations and embryonic deaths (resorptions).

Conclusion: No evidence of dominant lethal effect was observed in the progency of male mice treated with CGA-64250.

Classification: Core-Minimum Data

13. Nucleus Anomaly Test in Somatic Interphase Nuclei with CGA-64250 in the Chinese Hamsters (Ciba-Geigy, Ltd. Report No. 79-0805; September 17, 1979)

CGA-64250 was administered by gavage at dosages of 251, 502, and 1004 mg/kg in 20 ml/kg PEG 400 to groups of 6 male and 6 female animals each. Treatment consisted of one daily application on 2 consecutive days. The animals were sacrificed 24 hours after the second application. From the bone marrow smears were made.

Results: The bone marrow smears from animals treated with various doses of CGA-64250 showed no significant difference from the control. The incidence of bone marrow cells with anomalies of nuclei corresponds to the frequency observed in the control group. By contrast, a positive control experiment with cyclophosphamide (128 mg/kg), yeilded 8.92% cells with anomalies of nuclei. This is significantly different from the controls treated with the vehicle (PEG 400) alone.

Conclusion: No evidence of mutagenic effects obtained in Chinese hamsters treated with CGA-64250.

Classification: Core-Minimum Data

14. Report on CGA-64250. Teratology Study in Rats (Ciba-Geigy Ltd. Report No. 790011; September 10, 1979)

Groups of 25 bred Sprague-Dawley derived (Tif:Raif (SPF) rats were gavaged orally from day 6 to day 15 of gestation with 0 (vehicle control), 30, 100 and 300 mg/kg of CGA-64250.

During the period of treatment, general condition, weight gain and symptomatology were checked daily. Food consumption was noted on days 6, 11, 16 and 21 of pregnancy.

Dams were killed by CO_2 asphyxiation and fetuses removed on day 21 of gestation.

Following assessment of the dams' organs, especially of the ovaries and uterus (mucosa and contents, including amniotic fluid and placentae as well as abortions and resorption sites), the fetuses were removed, sexed, and subjected to careful external inspection. They were then weighed individually and submitted to the following procedures:

1. Examination of the viscera according to the slicing techniques of Wilson: one third of the live fetuses.

2. Skeletal assessment in two thirds of the fetuses following clearing in potassium hydroxide and staining with Alizarin Red S.

   Uterus horns without any visible implantation sites were placed into a solution of ammonium sulfide to visualize possible hemorrhagic alterations of implantation sites.

   Statistical analyses of the data were performed.

Results:

During the period of treatment, body weight gain and food consumption were markedly reduced in the 300 mg/kg group. Three dams of the high-dose group died while on test. Females nos. 86 and 87 on day 19 and female no. 90 on day 20. Female no. 77 could not be treated on two consecutive days of pregnancy (12 + 13) because the esophagus was filled up with bedding material.

The overall implantation rates as well as the resorption rates (embryolethality and/or fetotolethality) were comparable for all groups.

The sex ratios were not significantly changed in the experimental groups by comparison with the vehicle control.

The mean distribution of live fetuses and fetal and/or embryonal deaths within the uterine horns were comparable for all groups.

The average body weight of the live fetuses were comparable for all groups.

The gross examination of the fetuses did not reveal any malformation among the fetuses of the experimental groups and the vehicle control. By applying the slicing technique, one out of the 45 male fetuses examined in the 100 mg/kg dose group showed internal hydrocephaly. The latter type of malformation was also recorded to occur in a fetus of the historical control.

Apart from the hydrocephalus, no other malformations of viscera were found among fetuses of the treated and control group. The skeletal assessment revealed an increase in the number of not yet ossified phalangeal nuclei of fore and hind limbs as well as calcaneus in the 300 mg/kg dose group. One instance of irregularly ossified sternum was recorded each for the 100 mg/kg dose group and the vehicle control. This type of skeletal anomaly was also found in the historical control.

Conclusion:

CGA-64250 was not teratogenic at gavage doses up to 300 mg/kg during days 6-15 of gestation. The NOEL for fetotoxicity is 100 mg/kg and the fetotoxic effect at 300 mg/kg was ossification retardation.

Classification: Core-Minimum Data

15. Report on CGA-64250 Technical. Teratology Study in Rabbits (Ciba-Geigy Ltd. Project No. 790009; September 10, 1979)

Groups of 20 bred Chinchilla rabbits were gavaged daily with dosages of 0 (vehicle control), 30, 90 and 180 mg/kg during days 6-18 of gestation. During the period of treatment, general condition, weight gain and symptomatology were checked daily. Food consumption was noted on days 6, 11, 15, 19, 24 and 28 of pregnancy. Dams were killed by cervical dislocation and fetuses removed by cesarean section on day 28 of pregnancy.

Following assessment of the dams' organs, especially of the ovaries (corpora lutea counted) and uterus (mucosa fluid and placentae as well as abortions and resorption sites), the fetuses were removed and subjected to careful external inspection. They were then weighed individually and submitted to following procedures:

1) Assessment of the situs of the body cavities (thorax, abdomen, pelvis); sex was noted.

2) Examination of the cephalic viscera according to the slicing technique of Wilson.

3) Skeletal assessment of the fetal trunks (including limbs) following clearing in potassium hydroxide and staining with Alizarin Red S according to the technique of Dawson.

Statistical analyses of the data were performed.

Results:

At the three doses employed food consumption was reduced during the period of treatment, predominantly at the intermediate and high dose. Sedation was recorded to occur in the high-dose group during the first 3 days of treatment. Spontaneous litters due to erroneous mating of dams before the initiation of the experiment occurred in animals nos. 10, 14 and 20 of the 90 mg/kg dose group and animal no. 7 of the vehicle control. Female no. 4 of the 30 mg/kg dose group was excluded from the experiment because of diarrhea observed one day before the initiation of treatment. Females nos. 2 and 7 of this dose group developed diarrhea while on test.

The mean number of corpora lutea and implantation sites as well as the ratios of corpora lutea/implantation sites were comparable for all groups.

The rates of embryolethality and fetolethality (resorptions) were not increased by comparison with the vehicle control.

The sex ratios of the experimental groups were not changed significantly.

The average weights of the live fetuses were comparable for all groups.

The mean distribution of live fetuses and embryonal and/or fetal deaths within the uterine horns was comparable for all groups.

Among the live fetuses of the 180 mg/kg dose group one male fetus showed cleft palate among the 52 male fetuses and 123 fetuses of this group. Although this malformation may not be considered compound-related; the historical control data has no fetuses with cleft palate of 928 examined.

The examination of the fetal heads by the slicing technique did not reveal any malformations in either the experimental groups or vehicle control. The skeletal assessment did not reveal any deviation from the vehicle control which may be attributed to treatment of the dams with the test material.

Irregular ossification of the 6th sternebrae was observed in one out of 121 fetuses of the vehicle control as well as one out of 928 fetuses of the historical controls.

Conclusion:

CGA-64250 is considered not teratogenic at gavage dosages up to 180 mg/kg during days 6-18 of gestation. The fetotoxic NOEL is also 180 mg/kg.

Classification: Core-Minimum Data

16. CGA-64250 Technical. Three Months Toxicity Study on Rats (Ciba-Geigy, Ltd. Report No. 790014; August 30, 1979)

Groups of 20M and 20F Tif(RAIF) SPF rat, approximately four weeks of age and housed in groups of 5 in Macrolon cage, were fed dietary levels of 0 (controls), 240, 1200 and 6000 ppm of CGA-64250 technical for 3 months. Food and water was available ad libitum. The rats were observed daily for symptoms and mortality and weekly for body weight and food consumption. Eye examinations and a hearing test were made by observation prior to dosing and after the treatment period. Hematologic, blood chemistry and urinalysis measurements were carried out by standard methods on 20 males and 20 females per group from the control and three test groups at 4, 8 and 13 weeks. At the end of the 90 days toxicity study all control and treated rats were anesthetized with ether and bled. The total weight of each animal was then determined. Complete necropsy was performed and the following organs were weighed: brain, heart, liver, kidneys, adrenals and gonads. Tissue portions of brain (cerebrum, cerebellum, brainstem), spinal cord, eye, pituitary, salivary gland, heart, thymus, thyroid, lungs, trachea, spleen, bone marrow, lymph nodes, sciatic nerve, esophagus, stomach, small intestine, large intestine, adrenals, pancreas, liver, kidneys, urinary bladder, ovaries or testes, prostate or uterus, skin (mammary area) and skeletal muscle were fixed in 10% neutral formalin. The fixed tissue samples were embedded in paramat and cut at 3-5 um. The routine stain was hematoxylin and eosin. Sections from liver and spleen were stained for iron by Perl's method. Additional frozen sections from liver were specifically stained for fat with Sudan III. Sections from the brain and spinal cord were stained with Luxol-fast blue/cresylviolet.

Statistical analyses of the data were performed.

Results:

No clinical symptoms and no signs of local and/or systemic toxicity were observed. No animals died during the 3 months of the experiment. The mean food consumption of all treated male and female rats was comparable to the controls. The body weight gain of all male and females at 6000 ppm was significantly decreased from weeks 2-13. The body weight gain of the females of the 1200 ppm group were significantly decreased from weeks 9-13 when compared to the control.

The body weight gain of the 240 ppm females was significantly decreased at weeks 4, 7, 11 and 12 when compared to the control.

Ophthalmic inspection made prior to dosing and after the treatment period revealed no evidence of a reaction to treatment. Auditory perception made prior to dosing and after the treatment period revealed no loss of the hearing ability. The observed hematological findings between treated rats and controls were generally unremarkable and considered within the normal limits of the control values. Some statistically significant intergroup differences were observed in the study, however, these changes were minimal and considered the result of normal individual variations in these parameters.

Erythrocyte count, hematocrit and hemoglobin concentration were found to be significantly lower in the female rats of the high-dose group at week 13.

With respect to blood chemistries, no obvious changes related to the treatment were observed between treated rats and controls. The findings were considered within the normal limits of the controls. The only changes of note were a increase in alkaline phosphatase activity in the high-dose female rats at week 13 and an increase the gramma-glutamyl transpeptodase activity in male and female rats of the high-dose groups at weeks 4, 8, and 13.

The findings in the urine were unremarkable. Individual rats revealed some degree of proteinuria including those of the control group. This finding is considered normal in laboratory rats.

There were no gross anatomical changes which could be related to the administration of CGA-64250. In the spleen of all female rats from the 6000 ppm group an increase hemosiderosis was observed.

The terminal body weights of all male and female rats of the 6000 ppm group and the terminal body weight of the females of the mid and low-dose were significantly decreased when compared to controls.

The organ weights, organ to body weight and organ to brain weight ratios were changed acccordingly.

Conclusion:

Although there was some statistically significant reduction (2-5%) in body weight gain during the same weeks of the study in the low-dose females, the slight decrease in body weight gain in this group is not considered biologically significant.

The NOEL for the study is considered 240 ppm. The LEL is 1200 ppm and the effect is reduced body weight gain in females.

Classification: Core-Minimum Data

17. CGA-64250; 3-Month Toxicity Study on Dogs (Ciba-Geigy, Ltd., August 9, 1979)

Groups of 4M and 4F pure-bred Beagle dogs were daily administered in the diet dosages of 0 (control), 50, 250 and 1250 ppm of CGA-64250 technical for 3 months.

The initial age of the dogs was 19-28 weeks and body weights ranged from 7.9-13.0 kg for males and 6.0-11.6 kg for females. The dogs were housed in kennels equipped with underfloor heating.

The floor temperature was 23°C and the room temperature was 20°C. The dogs were exposed to 10 hours light/day and for 8 hours of this time music was broadcasted. Two dogs were kept in each kennel. Each dog was fastened with a chain during the feeding period. Thereafter indoor or outdoor runs were available for the dogs.

Food was ad libitum but not in excess of 350 gm/day/animal. Water was available at all times.

Daily observations were made for mortality and symptoms of local and/or systemic toxicity. Auditory perception (calling the dog) was performed weekly. Ophthalmoscopy was performed pretest and after 3-months. After external examination, the media and the fundus were examined with an ophthalmoscope.

Hematologic, blood chemistry and urinalysis measurements were carried out by standard methods on each dog from the control and three test groups before treatment commenced and at 4, 8 and 13 experimental weeks.

At the end of the 90 days toxicity study all control and treated dogs were anesthetized with intraveous injection of T61 (Hoechst) and bled. The total weight of each animal was then determined.

Complete necropsy was performed and the following organs were weighed: heart, liver, kidneys, adrenals, thyroid, pituitary, gonads, brain and spleen.

Tissue portions of brain (cerebrum, cerebellum, brainstem), spinal cord (2 sections), eye, pituitary, salivary gland, heart, thymus, thyroid, lungs with mainstem bronchi, trachea, aorta, urinary bladder, esophagus, stomach, small intestine (duodenum, ileum, jejunum), large intestine (cecum, colon), adrenals, pancreas, liver, kidneys, ovaries and uterus or testes and prostate, skin, skeletal muscle, sciatic nerve, gall bladder and mammary gland were fixed in 10% neutral formalin.

The fixed tissue samples were embedded in paramat and cut at 3-5 um. The routine stain was hematoxylin and eosin. Sections from liver and spleen were stained for iron by the Perl method. Sections from the brain and spinal cord were stained with Luxol fast blue/cresylviolet.

Additional frozen sections from liver, kidneys and adrenal glands were sepcifically stained for fat with Sudan III.

Statistical analyses of the data were performed.

Results:

Some animals of all groups showed slight to moderate diarrhea during the whole study. There was no mortality during the study. The food consumption of all treated animals was comparable to that of the controls, with the exception of the high-dose females, which consumed temporarily less food when compared with other treated and control dogs. The body weight gain of all animlas was similar. Pretest, and after 3 months, no findings related to treatment were recorded during the eye examination. No impairment of the auditory perception was found. At 4, 8 and 13 weeks, no hematological changes were observed between treated animals and controls which could be related to the substance administration. In the assessment of blood chemistry values, the findings were unremarkable and comparable to those of the controls.

The findings in the urine were unremarkable. No statistically significant differences between control and treated groups of dogs were noted in organ weights. Necropsy showed that in 3/8 dogs (13, 14, 15M) from the highest dosage group (1250 ppm), slightly granular surface in the pyloric and propyloric part of the stomach was noted. Apart from this finding no gross anatomical changes were seen neither in treated nor in control dogs. Microscopically, in 3 out of 8 dogs from the highest dose-group (13, 14, 15M) and 1 out of 8 dogs from the 250 ppm group (25F) in the pyloric part of the stomach slightly increased amount of lymphoid follicles in the mucous membrane was seen. These histological findings are considered compound-related.

Conclusion:

The NOEL for the 3-month dog feeding study is considered to be 50 ppm. The LEL is 250 ppm and the effect is lymphoid follicles in the mucous membrane in the pyloric part of the stomach in 1 of 8 dogs.

Classification: Core-Minimum Data

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