(10-13-1982)

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

002664

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Timothy A. Gardner
Registration Division (TS-767)

THRU: William Butler, Section Head #3
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: PP#2F2707/2H5355; Larvadex (Cyromazine) in eggs, meat, poultry, fat and meat byproducts of poultry. CASWELL #167B

Petitioner: Ciba-Geigy
Greensboro, North Carolina

Action Requested:

Permanent tolerance for residues of cyromazine and its principle metabolite, melamine (1,3,5-triazine-2,4,6-triamine) in eggs, meat, fat and meat byproducts of poultry at 0.4 ppm.

Recommendation and Conclusions:

Based upon the existing toxicity data base, the requested permanent tolerances can be supported.

Data to Set Tolerances:

Study No. 382-081, a two year feeding and oncogenicity study in the rat can be used as a basis for the ADI. The NOEL of 30 ppm (1.5 mg/kg) in this study and a safety factor of 100 yields an ADI of 0.015 mg/kg/day and an MPI of 0.90 mg/day (60 kg).

A previous TOX approved action, 9G2230, produced a TMRC of 0.0334 mg/day ~~(1.5 kg)~~ which ~~occupied 3.71% of the ADI~~ and contained some of the same RAC's as requested in the current action.

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The present action increases the percentage of the ADI occupied from 3.71% to 5.61% and the TMRC from 0.0334 mg/day (1.5 kg) to 0.0505 mg/day (1.5 kg). The printout is attached.

Data Gaps:

There are no data gaps for Larvadex at this time.

RPAR State:

N/A

Formulations:

I. Larvadex

Cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) - 95%

Impurities: Total - 5%

[redacted]

The proposed use is 900 lbs of Larvadex premix in poultry feed for direct consumption by 20 million chickens over a two-year period.

II. Larvadex Premix

Cyromazine Technical, 95% a.i. - 0.32%

[redacted]

Summary of Data Reviewed Previously:

Oral LD_{50} rat - 3387 mg/kg
Oral LD_{50} mouse - 2029 mg/kg
Oral LD_{50} rabbit - 1467 mg/kg
Acute dermal LD_{50} rat >3100 mg/kg
Sensitization, guinea pig - non-sensitizing
Ames - negative
90 day rat feeding NOEL 30 ppm
Teratology rat - NOEL teratology >600 mg/kg
NOEL-fetotoxicity 300 mg/kg, LEL = 600 mg/kg

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Formulations:

I. Larvadex

Cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) - 95%

Inerts: Total - 5%

[redacted]

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II. Larvadex Premix

Cyromazine Technical, 95% a.i. - 0.32%

[redacted]

Review of New Studies from Present Submission:

A. Subchronic

(1) CGA-72662 Technical, Acute Oral Dose Range in Dogs, Hazelton, Vienna, Va., No. 483-179, Accession No. 070919, August 20, 1979.

Material Tested: CGA-72662 Technical, white powder 96.3% pure.

Material and Method:

Six (3/sex) beagle dogs weighing 5.7 to 7.5 kg (female) and 10.3 kg (male) were housed individually and given food and water ad libitum. These dogs were randomly divided into 3 groups (I-III) of one male and one female each. Group I received untreated diet for 4 weeks. Group II were fed 3000 ppm CGA-72662 in the diet for two weeks followed by two weeks of diet containing 5000 ppm. Group III were given 3000 ppm (equivalent dose) in a capsule administered for 2 days once a day. Due to emesis the dosages were

fractionated for days 3, 4, 5 and administered 3, 4 and 6 hours apart respectively. The Group III female died on day 6 and the Group III male received only 1500 ppm (capsule). On day seven the Group III male was fed control diet then placed on 2000 ppm diet for weeks 2, 3 and 4.

Results:

The Group III female dogs at the 3000 ppm of body weight dose level was found dead on Day 6. All other dogs survived until termination (Day 29) of the study.

Group I (Control Group) dogs had mucoidal, soft, and scant stools.

The Group II dogs (especially the female) had sclera injection and lacrimation after four days of treatment. Among the Group II animals a few cases of mucoidal, soft, and less stool than normal were noted. The female of Group II had a sore on the right side of her neck from Day 15 until termination.

Emesis containing the test material was observed in both Group III dogs following administration of each capsule.

At approximately one hour after dosing, mydriasis was noted in both Group III dogs. Vasodilation, sclera injection, eye lacrimation, abnormal salivation, mucoid stool, and diarrhea were observed before and after capsule dosing on Days 4, 5, and 6. From Day 7 through Day 11, the Group III male appeared inactive; its eyes were puffy; and the nictitating membrane of its left eye was inflammed. After Day 11, the Group III male was observed to have sclera injection and scant stools (3 of the 18 days) which was sometimes soft.

Except for the Group II male, body weight gains were comparable between the control and treated groups. At Week ', food consumption values were slightly lower in the treated groups when compared to the control group. Consequently decreased food consumption was noted in the Group II animals during the remainder of the study, possibly attributable to the increase in dose level at Week 2. Beginning with Week 2, food consumption values consistently increased in the Group III male. This may reflect the change ~~from oral~~ administration ~~to dietary administration in this group.~~

No gross pathology findings were observed in four of the animals surviving the four-week exposure period. The Group III female was noted as having a sore on its neck: This female that died on Day 6 had a darkened thymus, lungs, spleen and kidney. The inner surface of the left ventricle of the heart had darkened areas. The rugae of the stomach (pyloric end), the jejunum, and the ileum were all reddened.

Conclusion:

CGA-73662 Technical administered by capsule has a maximum tolerated dose between 1500 and 3000 mg/kg. The maximum tolerated dose for CGA-73662 in the diet is greater than 5000 ppm.

Classification: Supplementary, inconsistent dosing of insufficient number of animals.

(2) CGA-72662 Technical, Subchronic in Dogs, Hazelton, Vienna, Va., No. 483-180, Accession No. 070919, October 22, 1980.

Material tested: CGA-72662 Technical, a white powder, Batch FC 790733, 96.3% pure.

Material and Method:

Fifty-six healthy young adult beagles (twenty-eight per sex) from twenty-eight to thirty-five weeks of age at initiation of treatment and were individually housed were given Wayne® Dog Food and water ad libitum.

Healthy dogs selected for study use were randomly assigned to the following groups using a table of ten thousand random digits.

Group	Number of Animals		Dosage Level
	Males	Females	ppm
1 (Control)	8	8	0
2 (Low)	6	6	30
3 (Mid)	6	6	300
4 (High)	8	8	3000

The body weights for males ranged from 7.9 to 14.6 kg and for female 6.1 to 11.1 kg. The test material was mixed fresh weekly with the diet at the appropriate concentrations.

Observations of all of the dogs were recorded twice daily for mortality and morbundity and once daily for appearance, behavior, appetite, elimination, and signs of toxic and pharmacologic effects. Body weights were recorded weekly beginning one week prior to the initiation of treatment. Food consumptions were recorded twice weekly and presented as weekly food consumptions.

"The following clinical laboratory studies were performed on all dogs initially (Week 0); at Weeks 4, 8, 13, 17, 21, and 26; and at Week 30 for the recovery animals.

Hematology: hematocrit (HCT), hemoglobin (HGB), erythrocyte count (RBC), total (WBC) and differential leukocyte counts, platelet count (PLATELET), and prothrobin time (PT).

Clinical Chemistry: total cholesterol (T CHOL), blood urea nitrogen (BUN), serum glutamic pyruvic transaminase (SGPT), serum glutamix oxaloacetic transaminase (SGOT), lactate dehydrogenase (LDH), alkaline phosphatase (ALK PHOS), total protein (T PROT), albumin (ALBUMIN), globulin (GLOBULIN), albumin/globulin ratio (ALB/GLOB RATIO), glucose (GLUCOSE), potassium (POTAS), calcium (CALCIUM), direct bilirubin (D BILI), total bilirubin (T BILI), and protein electrophoresis (SEL).

Urinalysis: appearance (APPEAR), specific gravity (SP GR), protein (PROTEIN), ph (PH), glucose (GLUCOSE), bilirubin (BILI), acetone (KETONES), urobilinogen (UROBIL), reducing substances (RED SUBS), and microscopic examination of the sediment."

Blood samples were collected from the jugular veins of fasted dogs for these clinical chemistry and hemotology tests.

Ophthalmologic examinations were performed on all dogs prior to treatment and during Week 12 and 26 using a slit lamp, an indirect ophthalmologic and a mydriatic agent.

Gross Pathology: The remaining surviving dogs from the control and each test group, besides those randomly chosen for recovery group, were sacrificed after twenty-six weeks on study by exsanguination while under the influence of Surital® anesthesia and necropsied. The four males and four females recovery animals were sacrificed and necropsied four weeks later. Necropsies were also performed on all dogs which died or were sacrificed moribund during the study."

"Organ Weights: The following organs were weighed from each dog and the organ/body weight ratios determined - brain (including brain stem), pituitary, thyroids, heart, liver, spleen, kidneys, adrenals, and testes with epididymides (males) or ovaries (females)."

"Tissue Preservation: The following tissues from each dogs were preserved in 10% neutral buffered formalin - brain (entire), pituitary, spinal cord (cervical), lungs (with bronchi), heart, thoracic aorta, liver, gall bladder, spleen, kidneys, adrenals, stomach, pancreas, small intestine (three sections), large intestine (colon and cecum), cervical and mesenteric lymph nodes, urinary bladder, ovaries and uterus (females) or prostate (males), mammary gland with skin, nerve with muscle, bone marrow (femur), and any unusual lesions."

Results:

One low-dose male was found dead during Week 16 and one high-dose male was sacrificed moribund during Week 14. No premortem clinical signs were observed in the low-dose dog. Clinical signs observed in the high-dose dog were indicative of respiratory distress.

Losses in body weight or decreased rates of body weight gain were noted for the high-dose males and females at all treatment and recovery intervals evaluated. These changes were considered compound-related. The mean weekly food consumption values for the high-dose males were less than the respective male control values. These changes were not as evident in the female dogs, and in most instances, the female low- and mid-dose values were lower than those of the high-dose dogs. Total mean weekly food consumption of the high-dose males and females at both the treatment and recovery intervals were lower than the respective control values; however, as the mean weekly food consumption values, the changes were not as evident in the female dogs.

A treatment-related decrease occurred in the red cell mass of the high-dose males, with a similar but less pronounced decrease in the high-dose females. The changes were characterized by significantly decreased hematocrit and hemoglobin values for the males at mid-dose and high-dose at all treatment intervals when compared to the respective pretreatment or the control values. A stepwise progressive decrease in the hematocrit and hemoglobin values of the compound-treated males is positively correlated to increase in dose levels. Post-recovery hematocrit and hemoglobin values of the high-dose males approached the male control values obtained at the same interval. Decreased mean total

cholesterol values at all of the compound-treatment intervals for the high-dose males followed by a near return to control values at the recovery interval suggests a treatment-related phenomenon The mean serum glutamic oxaloacetic transaminase values of the high-dose males were significantly increased at five of the six compound-treatment intervals followed by a return to control values during the recovery period. This finding is also suggestive of a treatment-related effect. No other changes were noted in the clinical laboratory studies which were indicative of a CGA-72662 Technical treatment-related phenomenon.

Slight but consistent increases were noted for the relative brain, heart, and liver weights of the high-dose males and females sacrificed at Weeks 26 and 30 and the ovary weights of the high-dose female sacrificed at Week 26 and 30 when compared to the decreased body weight gains noted for the high-dose dogs.

Conclusion:

No distinct treatment-related findings were observed with respect to clinical signs, ophthalmologic examinations, or gross and microscopic pathology.

The no-effect level of CGA-72662 Technical, when administered in the diet to beagle dogs for six months, was considered to be 30 ppm.

Classification: Core-Minimum.

B. Teratology

1) CGA-72662, Pilot Teratology in Rats, IRDC, Mattawas, Michigan, No. 382-069, Accession No. 070920, August 7, 1979.

Material Tested:

CGA-72662 Technical 96.3% as a cream-colored powder, Batch No. FL-781149.

Materials and Methods:

"Untreated, sexually mature, virgin female and proven male (30 per sex) Charles River CD® COBS® rats were used in this pilot study to determine dosage levels of CGA-72662 Technical for a teratology study. These rats were approximately 12 weeks of age at the time of mating and had been acclimated in the

aboratory for two weeks prior to study initiation. All rats ere individually housed, except during mating and maintained in temperature-, humidity- and light-controlled environment. urina® Rodent Laboratory Chow® #5001 and tap water were vailable ad libitum.

Mating was initiated on April 30, 1979 and the last uterine xamination was performed on May 23, 1979.

One female rat and one male rat of the same strain were laced together for mating. The occurrence of copulation was etermined by daily inspection for a copulatory plug or by vaginal nspection for sperm. The day that evidence of mating was detected as designated day 0 of gestation and the female was returned to n individual cage.

Mated females were consecutively assigned to control groups nd treatment groups.

CGA-72662 Technical was suspended daily in 1% weight/volume queous carboxymethyl cellulose at concentrations to permit the dministration of dosage levels of 300, 600, 1000, 1500 and 2500 g/kg/day at a constant volume of 10 ml/kg/day.

The test article was administered orally by gavage as a ingle daily dose on days 6 through 19 of gestation. The control roup received the vehicle only, 1% weight/volume aqueous arboxymethyl cellulose, on a comparable regimen at a volume of 0 ml/kg/day. Individual dosages were determined from individual ody weights recorded on gestation day 6.

Prior to treatment, the females were observed daily for ortality and overt changes in appearance and behavior while they ere observed daily for mortality and clinical signs of toxicity n days 6 through 20 of gestation.

On gestation day 20, all surviving females were sacrificed y CO_2 inhalation. Immediately following sacrifice, the number nd location of viable and nonviable fetuses, early and late esorptions and the number of total implantations and corpora utea were recorded. The abdominal and thoracic cavities of the ams were examined and the carcasses were discarded."

sults:

There were no significant differences in behavior due to CGA-662 Technical at 300 mg/kg/day compared to control. At 600, 00and 1500 mg/kg/day there was increased oral discharge alivation) matting of hair around the mouth as well as matting hair in the abdcmen and anogenital area. Two rats at 1500 /kg/day and all the rats at 2500 mg/kg/day died. The cause of ath was not determined at necropsy.

The maternal body weight gains were decreased in treated oups: i.e., slightly at 300 mg/kg/day and severely at 1000 or 00 mg/kg/day.

There was no significant difference in treated animals (300, 0, and 1000 mg/kg/day) from controls with respect to the number viable fetuses, early or late resorptions, post implantation ss, total implantations or corpora lutea. In the 1500 mg/kg/day sage group there was decrease in the mean numbers of viable tses, late resorptions and post implantation loss. These fferences may not be significant since they can be attributed one dam who had only late resorptions and a total of four plantations.

nclusion:

There were no teratological effect in rats in this study. 1500 mg/kg/day at which dosage the maternal toxicity was vere enough (decrease in maternal weight gain) to consider at amount of test material as excessive for teratology testing.

assification: Core-Minimum.

2) CGA-72662, Pilot Teratology in Rabbits, IRDC, No. 382-071, vember 14, 1979.

terial Tested:

A cream-colored powder, CGA-72662 Technical, 96.3%, Accession . 070920, ARS No. 1330/79, Batch No. FL-781149/SL99.

Materials and Methods:

Sexually mature virgin female Dutch Belted rabbits were used to determine dosage levels of CGA-72662 Technical. Following acclimatization these rabbits were approximately six months of age and were inseminated at this time. "The rabbits were individually housed and maintained in a temperature-, humidity- and light-controlled environment. Purina® Rabbit Chow® Checkers® 5301 and tap water were available ad libitum."

Three proven male rabbits of the same strain were selected as semen donors. Semen was collected by means of an artificial vagina and the ejaculate from those with a 50% or greater motility was used for insemination. "The ejaculate was diluted with 4 ml of 0.9% Sodium Chloride for injection, U.S.P. at 35°C. Using an insemination pipette the semen was injected and ovulation was induced within one hour."

"On gestation day 28, all surviving females were sacrificed by injection of an overdose of sodium pentobarbital into the marginal ear vein. Immediately following sacrifice, the location of viable and non-viable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities of the dams were examined and the carcasses discarded."

The rabbits were randomly assigned by a computer-generated program to five treatment groups and one control group of five rabbits each.

CGA-72662 Technical was suspended daily in 1% weight/volume aqueous (distilled water) carboxymethyl cellulose at concentrations to permit the administration of dosage levels of 50, 150, 300, 600 and 1200 mg/kg/day at a constant volume of 3 ml/kg.

"The test article was administered orally by gavage as a single daily dose on days 6 through 27 of gestation. The control group received the vehicle only, 1% weight/volume aqueous (distilled water) carboxymethyl cellulose, on a comparable regimen at a volume of 3 ml/kg. Individual dosages were determined from individual body weights recorded on gestation day 6."

"Individual maternal body weights were recorded on gestation days, 0, 6, 12, 18, 24 and 2 "

esults:

There was no difference in behavior and appearance in the
imals receiving 50 mg/kg/day as compared to the control animals.
ie animals receiving 300, 600, and 1200 mg/kg/day had matted
iogenital areas and matted hair around the nose. Several maternal
eaths occurred in all treated groups as follows: 3 at 150
j/kg/day; 4 at 300 mg/kg/day; and all animals at 600 and 1200
j/kg/day. There was a mean maternal weight loss in all treated
roups as well as the controls.

All treated groups showed decreased corpora lutea as compared
o controls. At 50 mg/kg/day there was a slight reduction in
iable fetuses and total implantations where as these were
oderately decreased in the 150 mg/kg/day group. The 300 mg/kg/day
ingle surviving animal showed a slight decrease in total
mplantations.

onclusion:

The doses used in this study caused maternal toxicity
recluding an adequate assessment in this range-finding experiment.

lassification: Core-Minimum.

3) CGA-72662 Technical, Teratology in Rabbits, IRDC
32-072/072a, Accession No. 070920, May 7, 1981.

aterial Tested:

Two shipments of CGA-72662 Technical 96.3% were received:

(1) a cream-colored powder ARS No. 1330/79, Batch No. FL-781149,
nd;

(2) a white powder FL-781149.

aterials and Methods:

Dutch Belted sexually mature, virgin, female rabbits (129),
pproximately 7 to 8 months old were inseminated artificially
ith the semen of 11 proven male rabbits in two experiments.
ie individually housed animals were kept at constant temperatures,
umidity and on/off light cycyles with ad libitum Purina Rabbit
ow and water. The female rabbits selected for experiment I
ere healthy with parasite free stools while those animals used
or experiment II had to be treated with sulfa-water prior to
est since coccidia species were found in their stools. The
nsemination day was day 0 of gestation.

In experiment I CGA-72662 technical suspended in carboxymethyl cellulose was administered in a constant volume of 1 ml/kg at each of the following dosage levels: 25, 50 and 75 mg/kg/day. The CGA-72662 was administered in the same fashion in experiment II at dosage levels of 10, 30 and 60 mg/kg/day. These doses were given daily by gavage from days 6 through 27 of gestation.

The dams were observed for mortality, appearance, behavior and toxicity. During gestation any females was died were gross necropsied as were the fetus which were also fixed. Maternal body weights were taken on gestation days 0, 6, 12, 18. 24 and 28.

On day 28 all females remaining were sacrificed and the uteri and fetuses removed and examined. The parameters recorded were uterine weight, fetal weight, number and location of viable and nonviable fetuses, early and late resorptions, total number of implantations and corpora lutea. The fetuses were examined for external and visceral malformations as well as skeletal examinations.

Results:

In Experiment I, the following is a summary of the maternal deaths on test.

Dosage (mg/kg/day)	No. of deaths	Day of gestation
Control	0	-
25	1	12
50	2	26, 27
75	4	18, 21, 24, 24

Of the seven deaths, two were from heart failure, one from pneumonitis-pleuritis and four of undetermined cause. Two of these animals (dead during treatment) had first aborted. In addition to these mortalities three other animals aborted on test and were sacrificed. This later group consisted of: one at 50 mg/kg/day on day 27 and two at 75 mg/kg/day on days 25 and 27.

Some blood found underneath two cages of treated days at 75 mg/kg/day was associated with postimplantation loss in the litters.

In Experiment 2, the following is a summary of the maternal deaths on test:

Dosage (mg/kg/day)	No. of deaths	Day of gestation
0	0	
10	0	
30	0	
60	2	10, 11

The cause of death was apparently severe lung congestion and edema. Four dams, two in each of the two high dose groups aborted. Three of these dams had congested lungs while the other abortion was attributed to a perforated esophagus from poor intubation technique.

Necropsy reports revealed no dose-related differences in congested lungs, foci on the lungs, pitted kidneys or hydroceles on the oviducts.

In Experiment II, there was a slight decrease in weight gain in the 10 mg/kg/day group as compared with controls. A dose-related change in mean maternal adjusted body weight value was observed in the 30 and 60 mg/kg/day groups.

In Experiment I, all control and treated animal groups had a decreased pregnancy rate when compared to historical controls. There was no differences in the mean number of corpora lutea, fetal sex distribution or mean fetal body weight at 25, 50 and 75 mg/kg/day. The mean postimplantation loss at 25 and 50 mg/kg/day was not significant. At 75 mg/kg/day there was a slight decrease in the mean number of total implantations and a moderate increase in mean postimplantation loss comparable to the significant decrease in the mean number of viable fetuses.

In Experiment II, there were no statistically significant differences in the mean numbers of corpora lutea, total implantations, postimplantations loss, viable fetuses or fetal sex distribution in any of the groups.

Although there was a slight decrease in mean fetal body weight at 10, 30 and 60 mg/kg/day groups compared to control groups this finding was only found to be statistically significant in the 30 mg/kg/day group. This finding probably does not indicate an embryotoxic effect since the range of values was large within each group and the mean value of the treated groups was based on a greater number of fetuses. The other values for the treated groups fall within the historical range and there was no dose-dependent relationship of this effect.

In Experiment I, there were no fetal morphological observations in 11 control litters and 8 litters at 50 mg/kg/day. At 25 mg/kg/day there was one fetus in one litter out of 8 litters with fused sternebrae. Two fetuses in one litter out of four litters had fetal anasarca in the 75 mg/kg/day group. The fused sternebrae is within the range found in the historical controls. Although fetal anasarca was not found in the submitted controls it is not unusual for this species. At 75 mg/kg/day the following are findings in slightly increased percentages as compared to controls: 27 presacral vertebrae, talus unossified and sternebrae #5 or #6 unossified.

In Experiment II anomalies were observed in 10, 30 and 60 mg/kg/day groups. These malformations were significant only $p < 0.05$ level in the 60 mg/kg/day group. The anomalies which mostly single occurrences include: hydrocephaly, carpal flexure, fused sternebrae, malformed sternebrae, malformed nasals, premaxilla and jugals, forked thoracic rib, cartaracts, and fetal anasarca.

Discussion:

In these two experiments there was 100% survival in the control groups and at 10 and 30 mg/kg/day. There were no significant maternal weight losses in Experiment I and only a slight loss in maternal weight gain in Experiment II.

In Experiment I, the pregnancy rate in all groups including control was reduced probably due to errors in insemination technique. At the high dose 75 mg/kg/day there was a decrease in the number of implantations, increase in postimplantation loss and decrease in number of viable fetuses. The only malformations were fused sternebrae and fetal anasarca.

Experiment II had no significant differences in number of corpora lutea, total implantations, viable fetuses or fetal sex distribution. While there were more malformations in this study, they were single occurrences or seen in the historical control data. Although the malformations increased, their low frequency of occurrence is not typical of a teratogenic effect.

Conclusion:

Oral administration of 75 mg/kg/day of CGA-72662 Technical or less to pregnant Dutch Belted rabbits did not produce a teratogenic response. The teratology NOEL is >75 mg/kg/day.

Classification: Core Minimum.

C. Reproduction Studies

1) CGA-72662, Two Generation Reproduction in Rats, IRDC. #382-086, Accession No. 070920/070921, December 1, 1981.

Material Tested: Coarse cream-colored powder CGA-72662, FL 790733/SL116

Materials and Methods: (as described in the submission.)

Weanling male and female Sprague-Dawley COBS® CD® rats were assigned temporary animal numbers and housed individually in galvanized hanging wire-mesh cages for acclimatization.

"During the 15-day acclimation period, the rats were provided with basal laboratory diet of Purina Rodent Chow® and tap water available ad libitum. The animals were maintained in a room controlled for temperature, humidity and photoperiod (12 hr/light/12 hr dark cycle). Throughout the acclimation period, the rats were observed for changes in appearance and behavior.

The F_0 rats were mated once to produce the F_1 litters. Rats from the F_1 litters underwent a selection process to obtain rats for the breeding of the next generation. The selected F_1 rats were mated once to produce the F_2 litters."

Healthy F_1 parental animals were selected in a manner that avoided brother sister matings.

"According to the Sponsor's request, the highest dosage level, 4000 ppm, was reduced to 3000 ppm four weeks after the study initiated because of increased toxicity in the 4000 ppm group. All other dosage levels remained the same and thereafter, CGA-72662 was administered in the diet to males and females at varying concentrations to achieve dosage levels of 0, 30, 1000 and 3000 ppm. The dosage levels correspond to the study groups designated as control, T-I, T-II and T-III; respectively". Each study group had 15 males and 30 females.

"The parental rats and pups were observed twice each day for signs of overt toxicity, changes in general appearance and behavior and mortality. The nesting and nursing behavior of the females was also observed. Detailed observations were recorded weekly on the parental rats and the pups after weaning. Examinations for gross deformaties of the pups were conducted at birth and on lactation days 4, 7, 14 and 21."

Individual body weights were recorded weekly for adult rats from initiation of the F_0 and F_1 generations until mating. Males were weighed monthly after the mating period and just prior to sacrifice. Females were weighed prior to sacrifice. As specified by the protocol, weights were not measured during gestation and lactation. Pups were weighed individually on lactation days 0, 4, 7, 14 and 21 and individually by sex on lactation days 4 and 21.

"After the F_0 animals had been fed the test material for 100 days, each male was randomly mated with two females from the same group to produce F_1 generation. Fifteen male and thirty female weanlings were randomly chosen from each dosage group to become F_1 parents. After selection of the F_1 parents five weanlings per sex were randomly chosen from each group for a complete gross necropsy. Tissues were saved from each animal for a complete histopathologic examination. Due to the 30-day mating period, selection of the pups for necropsy could not be conducted at weaning (lactation day 21). Therefore, selection was done after all deliveries were complete resulting in some pups being approximately 30 days of age at necropsy. A protocol amendment was issued to clarify this procedure which does not affect the validity of the study.

After 100 days of dietary administration of the test article, the F_0 generation was mated, and upon weaning of the F_1 pups, was sacrificed. At 30 days of age, the F_1 generation was selected from the F_1 pups and the remaining pups were sacrificed. After 120 days of dietary administration of the test article, the F_1 generation was mated, and upon weaning of the F_2 pups, both the F_1 generation and the F_2 pups were sacrificed.

"The following organs were trimmed free of fat and connective tissue and weighed:

brain (including stem)
heart
kidneys
liver
testes

In addition, the epididymis was weighed for males of the F_0 and F_1 generations. Organ weights were not measured for animals dying spontaneously or sacrificed in extremis.

From all animals which were to be examined histopathologically, the organs and tissues were collected and fixed in neutral buffered formalin. Hematoxylin and eosin stained paraffin sections were prepared from them and microscopically examined by an IRDC staff pathologists.

From males of hte F_0 and F_1 generations, the tests and epididymis were also examined microscopically.

All F_0 and F_1 adult males were tested for spermatogenesis at sacrifice by examination of the epididymis for the presence of mature sperm. Upon sacrifice, the testes and epididymides were removed. Each epididymis was trimmed from the testis; testes were weighed.

An incision was made in the tail of one epididymis and a sample of semen was removed. The semen was mixed with 0.9% physiological saline on a glass slide. The slide was examined microscopically at 400x magnification. The testes of all F_0 and F_1 males were saved for future histopathologic examination."

Results:

There were no significant treatment-related observations in general appearance and behavior of the F_0 parental animals. There were no deaths in the first 28 weeks of study.

There were no treatment-related deaths in the F_1 parental animals. There were no significant differences from control in any group of the F_1 parental animals. Slight differencs in treated as control were (1) limb hair loss in females and (2) increased swollen areas in the ventral and lateral neck in 3 males at 1000 ppm, 2 males at 3000 ppm and in females at 30 and 1000 ppm (this was reversible) and characteristic of viral infections.

There was a decreased mean body weight only in the F_0 parental generation male CGA-72662 1000 and 3000 ppm groups of 7.5% and 17.5% respectively. These body weight decreases progressed to a statistically significant difference on weeks 15 (before mating) and 28. This same pattern of body weight loss was found in the females.

There was no significant mean body weight decrease difference from control in the F_1 generation from week 28 to 55 at 30 or 1000 ppm CGA-72662. In the male 3000 ppm group there was a moderate decrease in mean body weight from week 28 through 55 with a statistical significance ($p < 0.01$) on weeks 28, 43 and 55.

The female 1000 and 3000 ppm groups had a dose-related decrease in mean body weight that was statistically significant ($p < 0.01$) on weeks 28, 43 and 55. In the 30 ppm group the body weights were an average of 4% less than the control and the only study week 43 statistically significant. The food consumption was not different in the 30 ppm groups, slightly decreased in the 1000 ppm groups and moderately decreased in the 3000 ppm groups of males and females compared to controls. The same pattern or food consumption was found in the F_1 generation.

The F_0 generation males and females has no treatment-related decrease in fertility. Although there was an apparent decrease in male and female fertility at 30 ppm the lack of this effect at 1000 and 3000 ppm strongly suggests that this is not treatment-related.

There was no treatment-related effect on parturition and length of gestation in the F_0 and F_1 generations.

The number of viable pups per total born and mean litter size was similar in all groups of the F_0 generation. In the F_1 generation the 30 ppm and 1000 ppm groups were not different from control in these parameters. There was a statistically significant decrease in the number of viable pups and mean number of pups per litter in the 3000 ppm group.

The pup survival to weaning in the F_1 generation was not affected at 30 and 1000 ppm but was very slightly reduced on lactation day 4 in the 3000 ppm group. The F_1 generation was not affected except at 3000 ppm where the number of viable pups was reduced.

The pup weight at birth reduction was only statistically significant at 3000 ppm in the F_0 and F_1 generations.

The pup growth in the F_0 generation during lactation was comparable to control at 30 ppm. At 1000 ppm the growth was not different for 7 days of lactation but slightly reduced on day 14 and 21 (statistically significant in the males). The mean pup body weight on days 4 to 21 in the male and females were statistically significantly reduced.

In the F_1 generation the 30 ppm groups were not different from control. At 1000 ppm there was a slight lowering of mean body weight at day 4 adn 7 (where it is statistically significant) and reversing to levels similar to control at days 14 and 21 in males and females. A statistically significant reduction in mean pup growth on days 4, 7 and 21 with a level comparable to control at day 14 were reported in the 3000 ppm groups.

The behavior, appearance, spermatogenesis (morphology, motility and frequency of cytoplasmic droplets) were comparable in the treated groups to the control groups.

There were no treatment-related gross or microscopic lesions found in the F_0, F_1, F_1 weanlings and F_2 weanlings in this study.

Discussion:

In relation to the behavior, appearance of mortality of the F_0 and F_1 generations, there was no significant difference between CGA-72662 treatment and control. Only one F_1 1000 ppm female died or unrelated causes from all the groups.

The changes in the parental mean body weight of F_0 and F_1 generations were not CGA-72662 related. The decreased food consumption with increased doses which was statistically significant is indication of a toxic response to treatment, i.e. decrease in body weight at 1000 and 3000 ppm. At 30 ppm there was a very slight weight loss in the females but not in the males which was not treatment-related.

There was no alteration of reproduction performance from CGA-72662. The male fertility at 3000 ppm F_0 was decreased as was the male and female fertility in the F_1 generation at 3000 ppm. The lack of consistency across the groups precludes the effect being dose related. There was no effect on the process of parturition or the length of gestation. The pup viability at birth and mean litter size were comparable to control in all groups except the F_2 litters at 3000 ppm. These affected litters maintained a survival time through lactation comparable to control.

A treatment effect on mean pup weight at birth at 3000 ppm is indicated. All F_1 and F_2 litters had appearance and behavior comparable to control.

There was no treatment effect of CGA-72662 on mean absolute or relative organ weights correlative to morphological microscopic examination.

Conclusion:

CGA-72662 in oral doses of 1000 to 3000 ppm decreased both parental and pup body weight. The reproductive NOEL is 1000 ppm. the oral toxicity NOEL is 30 ppm.

Classification: Core Minimum.

D. Mutagenicity

1) CGA-72662, Dominant Lethal Study in Mice, Ciba-Geigy, Basle, Switzerland, #790033, March 17, 1981.

Material Tested:

CGA-72662, Accession No. 070921 Technical, Batch #3 in PEG400 vehicle (0.2 ml/lo g of body weight).

Materials and Methods:

Single oral doses of the test material were administered to groups of 20 male albino mice (Tif: MAG f[SPF]) in doses of 226- and 678 mg/kg. The controls and females were untreated. Eight hours after dosing each male was caged with [illegible] females which were substituted for 2 new females each week for 6 weeks or the period of sperm maturation. Day 0 of gestation was counted from observation of a vaginal plug.

General condition and symptomatology were checked. An autopsy of the female and examination of the progeny were used to obtain a total number of implantations (to indicate losses), number of matings and pregnancies or embryonic deaths.

Results:

No difference between the females mated to treated males and those mated to the untreated males in mating ratio, number of implantations and resorptions.

Discussion:

Dr. Irving Mauer noted the following points. The doses used should been derived from an oral LD_{50} in the same strain of mice and expressed as a fraction of the LD_{50} used.

To obtain a comprehensive assay a positive control should be run concurrently. This assay uses multiple dosing over five days during eight weeks of mating. Unless these NMRI mice have such different a spermatogenic cycle this should be different. The route of administration should more correctly be parental, dermal or inhalation in order to obtain results comparable to human exposure.

Conclusion:

There was no evidence of a dominant lethal effect in progeny of male mice treated with CGA-72662.

Classification: Core Minimum.

2) CGA-72662, Nucleus Anomaly Test in Chinese Hamster, Ciba-Geigy, Basle, Switzerland, #79-1347, Accession No. 070921, February 6, 1980.

Material Tested: CGA-72662 P3 Technical 98.9% in EMC 0.7%

Materials and Methods:

Equal numbers of male (22-30 gm) and female (20-29 gm) (Cricetulus griseus) fed standard diet (NAFAG No. 924) and tap water ad libitum were used. They were maintained 23-24°C, humidity 35-50% and 12 hour illumination cycles. It was reported that 3 animals per sex were treated once a day for 2 consecutive days and sacrificed 24 hours after the last dose. It was stated that

They received 2000, 4000 and 8000 mg/kg in 20 ml/kg 0.7% CMC solution; but it was not indicated whether this was a total dose or per day. The positive control cyclophosphamide (Endoxan) was administered at 128 mg/kg in 20 ml/kg 7% CMC solution. The negative control was given as 20 ml 0.7% CMC solution.

Homogeneous mixtures of bone marrow from the shafts two femurs from each animal spread onto slides to air dry, then stained and mounted in Evkitt. One thousand bone marrow cells per animal were scored for the following anomalies: single jolly bodies, fragments of nuclei in the erythrocytes, micronuclei in leucopoietic cells and polyploid cells. The Chi square test was used to assess the statistical significance.

Results:

Four deaths occurred in the study two in the negative control after the first application and two in the high dose after the second application. The dosed groups were similar to the control in the percentage of anomalies. The positive control displayed 9.17 or 9.2% anomalies yielding of highly significant difference from cotnrol ($p < 0.05$).

Stephanie P. April 10/13/83
Stephanie P. April, Ph.D.
Review Section III
Toxicology Branch/HED (TS-769)

TS-769:th:TOX/HED:SPApril:3-30-83:card misc. 3

NO CFR Number [illegible] 4/11/83

EL change not recorded -20-

File last updated 3/14/83

ACCEPTABLE DAILY INTAKE DATA

002664

Rat, older	NOEL	S.F.	ADI	MPI
mg/kg	ppm		mg/kg/day	mg/day(60kg)
1.500	30.[illegible]0	100	0.[illegible]150	0.[illegible]000

Unpublished, Tox Approved [illegible]

Crop	Tolerance	Food Factor	mg/day(1.5kg)
Poultry(123)	0.2[illegible]0	2.[illegible]	[illegible]00363
Eggs(54)	0.[illegible]0	2.77	0.[illegible]031
Cattle(2[illegible])	0.[illegible]00	7.[illegible]	0.[illegible]107[illegible]
Hogs([illegible]9)	0.100	3.[illegible]	0.00[illegible]15
Sheep(145)	0.[illegible]00	[illegible].15	0.0[illegible]029

MPI	TMRC	% ADI
0.9000 mg/day(60kg)	0.0334 mg/day(1.5kg)	3.71

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Current Action 2F2707/2H5355

Crop	Tolerance	Food Factor	mg/day(1.5kg)
Poultry(128)	0.200	2.[illegible]	0.[illegible]0883
Eggs(54)	0.200	2.77	0.00531

MPI	TMRC	% ADI
0.9000 mg/day(60kg)	0.[illegible]505 mg/day(1.5kg)	5.61

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DRAFT

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