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

003310

MAY 21 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Reg.#100-607; Ridomil 2E; PP#1F2500; 1H5299; Metalaxyl: [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester] and its metabolites containing the 2,6-dimethlaniline moiety each expressed as metalaxyl in or on the following raw agricultural commodities: broccoli, cabbage, cauliflower, cotton, cucumbers, lettuce, melons, onions, potatoes, soybeans, spinach, tomatoes and wheat. CASWELL#375AA Accession#070012-016

FROM: William Dykstra, Toxicologist
Toxicology Branch, HED (TS-769) WHD YDC 5/18/81

TO: Henry Jacoby (21)
Registration Division (TS-767)
and
Residue Chemistry Branch
Hazard Evaluation Division (TS-769)

Recommendations:

1) In the 2-year rat feeding study, the non-neoplastic microscopic pathology summary tables (Tables 11A-11D) and the neoplastic microscopic pathology summary tables (Tables 12A-12C) list only the number of animals examined and not the number of various organs examined for the number of animals examined. The petitioner is required to resubmit the report of pathology tables indicating the number of organs examined for the number of animals examined microscopically. As reported in the summary tables, every organ designated for every animal was examined microscopically. The individual animal pathology sheets do not demonstrate this.

   Additionally, the determination of the MTD for the study is required to be detailed. The required information may require a new revised report.

2) The rabbit teratology study is acceptable as Core-Minimum Data. CGA 48-988 technical was considered not teratogenic or fetotoxic at gavage doses up to 20 mg/kg during days 6-18 of gestation.

3) The multi-generation rat reproduction study is acceptable as Core-Minimum Data. The NOEL for reproductive parameters is considered to be 1250 ppm.

4) The 6-month dog study is acceptable as Core-Minimum Data. The NOEL is considered to be 250 ppm.

5) The following studies are presently lacking and are required to be submitted within a reasonable period of time:

   a) oncogenicity -second species
   b) mutagenicity - additional multi-test evidence

Section F: Proposed Pesticide Tolerances

The petitioner requests tolerances for combined residues of the fungicide, metalaxyl [N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester] and its metabolites containing the 2,6-dimethylaniline moiety, each expressed as metalaxyl, in or on the following raw agricultural commodities:

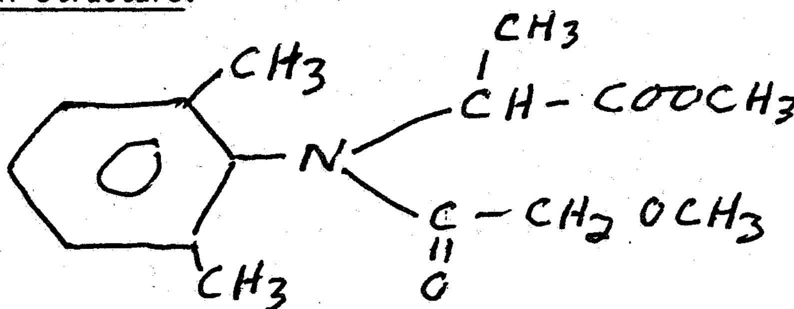
Spinach at 10.0 ppm
Soybean forage and fodder at 7.0 ppm
Wheat forage and straw at 2.0 ppm
Tomatoes at 1.0 ppm
Dry bulb onions at 1.0 ppm
Kidney of cattle, goats, hogs, horses, poultry and sheep at 1.0 ppm
Broccoli at 0.6 ppm
Cabbage at 0.6 ppm
Cauliflower at 0.6 ppm
Cucumbers at 0.5 ppm
Head lettuce at 0.5 ppm
Potatoes at 0.5 ppm
Soybean grain at 0.5 ppm
Melons at 0.3 ppm
Liver of cattle, goats, hogs, horses, poultry and sheep at 0.3 ppm
Wheat grain at 0.2 ppm
Cottonseed at 0.1 ppm
Eggs and meat of poultry excluding liver and kidney at 0.05 ppm
Meat, fat, and meat by-products excluding liver and kidney at 0.05 ppm
Milk at 0.02 ppm

Proposed Food Additive Tolerances

The petitioner requests feed additive tolerances for combined residues of the fungicide, metalaxyl[N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester] and its metabolites containing the 2,6-dimethylaniline moiety, each as expressed as metalaxyl, in or on the following processed foods:

Dry tomatoes pomace at 16.0 ppm
Wet tomato pomace at 4.0 ppm
Processed tomato products at 3.0 ppm
Soybean hulls at 1.0 ppm
Soybean meal at 1.0 ppm
Soybean soapstock at 1.0 ppm

Chemical Structure:



Review:

1) Previously Submitted Toxicity Studies.

Toxicity Studies on Ridomil 2E

a) Acute Oral LD_{50} in Rats: 2342 mg/kg for males and 1520 mg/kg for female, 1980 mg/kg combined; Category III.

b) Acute Dermal LD_{50} in Rabbits: 3571 mg/kg; Category III.

c) Primary Eye Irritation in Rabbits: Corneal opacity and conjunctival irritation persisting through 7 days; Category I.

d) Primary Skin Irritation: Draize score 0.5; Category IV.

e) An Acute Inhalation Study in Rats: Was not acceptable because the LD_{50} was not determined. However, no deaths at 3.38 mg/L for 6M & 6F rats; Category III.

Toxicity Studies on Technical Metalaxyl

a) Acute Oral LD_{50} in Rats: 669 mg/kg; Category III.

b) Acute Dermal LD_{50} in Rabbits: Greater than 6000 mg/kg; Category III.

c) Acute Dermal LD_{50} in Rats: 3170 mg/kg; Category III.

d) Skin Irritation in Rabbits: Draize Index = 0.1/8, mild skin irritant; Category IV.

e) Primary Eye Irritation in Rabbits: Corneal involvement, completely clearly in 3 days; Category II.

f) Skin Sensitization in Guinea Pigs: Negative

g) 3-Month Dietary Study in Rats: NOEL = 250 ppm

h) 90-Day Dietary Study in Dogs: NOEL = 250 ppm

i) Teratology Study in Rats: Not teratogenic at doses up to 120 mg/kg.

j) Salmonella/Mammalian Microsome Mutagenicity Study: Not mutagenic.

k) Mouse Dominant Lethal Mutagenic Study: Not mutagenic.

l) 21-Day Subchronic Dermal in Rabbits: NOEL = 1000 mg/kg

2. Toxicology Studies Submitted with this Petition.

A. Teratology Study in Rabbits with CGA 48 988 Technical (Ciba-Geigy, Ltd., Project#784868; December 6, 1978)

The Chinchilla rabbits were obtained from a closed SPF breeding colony (WIGA, Sulzfeld, Germany). The females were 4 to 5 months of age and their initial body weight was 3.0 kg. Groups of 20 females were mated with males of proven fertility. The day on which mating was observed was designated as "Day 0" of pregnancy. Throughout the experiment the mated females were kept one to a cage in a separate animal housing area at a temperature of 21 $\pm$ 1°C and a humidity of 60% $\pm$ 5%. The room was illuminated for 10 hours daily. The standard diet fed was Nafag No. 814. Tap water was available at all times.

The compound was given at doses of 5, 10 and 20 mg/kg orally by intubation from Day 6 until Day 18 of pregnancy, inclusive. As vehicle the 2% aqueous solution of sodium carboxymethylcellulose was applied. The amount of fluid administered was 4 ml/kg of body weight.

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 Caesarean section on day 28 of pregnancy.

Following assessment of the dams' organs, especially of the ovaries (corpora lutea counted) 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 proccedures:

1) Assessment of the situs of the body cavities (thorax, abdomen, pelvis).

2) Examination of the cephalic viscera according to the slicing technique of Wilson: the heads of the fetuses were fixed in a mixture of trichloroacetic acid and formol and preserved in 90% ethyl alcohol.

3) Skeletal assessment of the thoracic and abdominal parts of the fetuses (including limbs) following clearing in potassium hydroxide and staining with alizarine red S according to the technique of Dawson.

Statistical analysis of the data were performed.

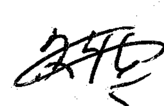
Results:

During the period of treatment dam food consumption was found to be slightly reduced at the 10 mg/kg dose and markedly reduced at the 20 mg/kg dose. The average body weight gain appeared to be slightly reduced in both these dose groups, in addition.

The mean numbers of corpora lutea and/or implantations were comparable for all groups.

The rates of embryo and/or fetolethality (resorptions) were not significantly altered when compared with the vehicle control.

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



The gross examination and/or visceral examination of the fetuses revealed a few malformations in the 10 mg/kg and 20 mg/kg dose groups, as well as the historical control. The few instances of malformations observed in the 10 mg/kg dose group (one agenesis of left kidney and ureter, one hypoplasia of kidneys) and the 20 mg/kg dose group (one agenesis of right kidney and ureter) were considered to be of a spontaneous origin and not related to treatment.

Renal malformations of these types have been found in the historical controls of the breed of rabbits used in the present study.

No skeletal malformations were present in any of the groups.

The skeletal assessment did not reveal any increase in the number of unossified skeletal elements in comparison to the vehicle control. Three instances of skeletal anomaly were noted in the historical control.

Conclusion:

CGA 48 988 technical was not considered teratogenic in rabbits at gavage doses up to 20 mg/kg.

The NOEL for fetotoxicity is considered to be also 20 mg/kg.

Classification: Core-Minimum Data

B. CGA 48 988: Toxicity and Oncogencity in Dietary Administration to Rats for Two Years (Life Science Research Report No. 80/CIA009/315; September 23, 1980)

In this 2-year rat feeding study, the non-neoplastic microscopic pathology summary tables (Tables 11A-11D) and the neoplastic summary tables (Tables 12A-12C) list only the number of animals examined and not the number of various organs examined for the number of animals examined. The petitioner is required to resubmit the report of pathology tables indicating the number of organs examined for the number of animals examined. As reported, every organ designated for every animal was examined microscopically. The individual animal pathology sheets do not demonstrate this.

Additionally, the determination of the MTD for the study is required to be detailed.

After the petitioner has satisfactorily addressed the above issues, the study will be reviewed. This required information may require the submission of a new, revised report.

C. Effect of CGA 48-988 Technical on Reproductive Function of Multiple Generations in the Rat (Huntingdon Research Center; Test No. 770023; CBG 181/80254; May 23, 1980)

Four week old, SPF rats of the CrL:COBS CD (SD) BR strain were obtained from Charles River UK Ltd. Following a period of acclimatization (ca 7 days) they were allocated to 4 groups giving approximately equal initial group mean body weights and were then ear marked to give individual identification within the cage. The experimental design was as follows:

Group	Treatment	No. of Rats Male	Female
1	Control	25	25
2	CGA 48-988, 50 ppm	25	25
3	CGA 48-988, 250 ppm	25	255
4	CGA 48-988, 1250 ppm	25	25

The test compound was administered in the diet and fresh batches of diet were mixed weekly. Control animals received untreated powdered diet (Spratt's Laboratory Diet No. 2 -low-fat).

Food and tap water were available at all times.

Animals room controls were set to maintain temperature and relative humidity at 20 ± 2°C and 55 ± 10%, respectively, with 13 air changes per hour. Natural lighting in the room was supplemented by artificial light between 8 a.m. and 8 p.m.

During the pre-mating periods, rats of the F0 and F1B generations were housed five to a cage and rats of the F2B generation were housed four to a cage in suspended galvanized metal cages, equipped with solid sides and back, wire mesh front, floor and tap. Cages of males were interpersed amongst those holding females to promote the development of regular estrous cycles.

During the mating period, rats of the F0 and F1B generations were housed on the basis of one male to one female and rats of the F2B generation were housed one male to two females in plastic breeding cages.

At the end of the mating periods, the males were returned to their original cages and the females were caged individually prior to sacrifice at day 20 of gestation or for the birth and rearing of young, as appropriate.

Throughout the study each cage was identified by a label recording the study schedule number, animal number(s), details of treatment and the name of the study supervisor.

Animals of the F0 generation were maintained on their respective diets for 91 days prior to mating. The animals were then mated on a one male to one female basis for a period of 20 days. Resulting litters (F1A) were reared to 21 days post partum, sacrificed, examined macrosopically and then discarded.

Shortly following (approximately 10 days) the weaning of the F1A litters, the F0 generation were re-mated for a period of 20 days. Resulting litters (F1B) were reared to 21 days post partum when 25 males and 25 females were selected from each group to form the basis of the F1B generation.

It had been intended that five F0 females per group would be killed on day 20 of gestation for examination of effects on late embryonic/fetal development. However, because of an anticipated low pregnancy rate, judged from the appearance of the vaginal smears during the mating period, no day 20 sacrifice was made in order to safeguard the selection of the F1B generation in terms of maintaining as wide a genetic pool as possible.

Following the selection of the F1B generation, 15 F0 females per group were re-mated for a period of 20 days and these were sacrificed on day 20 of gestation for examination of effects on late embryonic/fetal development.

Parent (F0) animals not required for the F0 to F1C mate and surplus F1B pups were sacrificed and examined macroscopically after selection of the F1B generation.

Parent F0 males from the re-mate (F0 to F1C) were sacrificed and examined macroscopically when the majority of the re-mated F0 females had been sacrificed.

The F1B animals selected at weaning were maintained on their respective diets to an age of at least 90 days and were then mated on a one male to one female basis for a period of 20 days. Resulting F2A litters were reared to 21 days post partum, sacrificed, examined macroscopically and then discarded.

Shortly following (approximately 10 days) the weaning of the F2A litters, the F1B generation were re-mated for 20 days. Ten dams in each group were allocated for examination of effects on late embryonic/fetal development having first ensured that selection of the F2B generation was guaranteed by choosing 14 pregnant dams in each group to rear their litters. F1B parents were sacrificed and examined macroscopically after weaning of the litters.

At weaning, 12 male and 12 female pups from each group (derived from as many litters as possible) were selected and maintained on their respective diets for at least 90 days, after which time they were subjected to detailed macroscopic examination and organ weight analysis. Representative tissues were retained in fixature but histopathological examination was not performed.

A further 12 male and 12 female pups from each group were retained from as many litters as possible at weaning of the F2B generation. These animals were reared on their respective diets to an age of least 90 days when they were mated on a one male to two females basis for a period of 20 days. Resulting litters (F3A) were reared to 21 days post partum, sacrificed, examined macroscopically and discarded.

Shortly following the weaning of the F3A litters the F2B animals were re-mated for a period of 20 days. Resulting litters (F3B) were reared to 21 days post partum when 10 males and 10 females were selected from each group for detailed macroscopic examination and organ weight analysis. Representative tissues were retained in fixature but histopathological examination was restricted to the control and high dosage groups.

Parent F2B animals were sacrificed and examined macroscopically.

All animals were regularly handled and examined for obvious changes or signs of reaction to treatment.

Any rat that showed signs of debility or intoxication was isolated and/or killed to prevent cannibalism or autolytic degeneration. All rats found dead, or killed in extremis, were examined macroscopically to establish, if possible the cause of death.

Food intake of rats was recorded weekly during the first pre-mating phase of each generation. Food conversion ratios were calculated.

Water intake was measured during the first or second and penultimate weeks of the pre-mating phase.

The weight of each rat of the F0 generation was determined initially and subsequently at weekly intervals. Animals of all subsequent generations were weighed at birth, 4, 8, 12 and 21 days post partum and subsequently at weekly intervals.

Additionally, females were weighed on alternate days from the beginning of the mating period until the occurrence of a positive indication of mating (i.e. sperm or plug); thereafter, they were weighed on presumed days 0, 7, 14, 17 and 20 of gestation. Weights of pregnant animals without positive indication of mating were calculated retrospectively from birth (assuming a 22 day gestation period) or from sacrifice. Dams allowed to litter were weighed on days 0, 7, 14 and 21 post partum.

Pregnancy rate was determined as the percentage of surviving paired females that became pregnant.

Vaginal smears taken during the mating period enabled the number of animals that mated on specific days to be determined. From this it was possible:

(i) to select animals for day 20 sacrifice, where appropriate
(ii) to determine whether or not pregnancy was interrupted after mating
(iii) to detect marked anomalies of the estrous cycle
(iv) to determine the median pre-coital period per group

On day 20 of pregnancy the animals selected for examination of effects on late embryonic/fetal development were killed by CO_2 asphyxiation, dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs; the ovaries and uteri were examined immediately to determine:

(a) number of corpora lutea
(b) number and distribution of live young
(c) number and distribution of embyronic/fetal deaths
(d) litter weight, from which the mean pup weight was calculated
(e) fetal abnormalities

Embryonic/fetal deaths were classified as:

Early: only placenta visible at termination
Late: both placental and embryonic remnants visible at termination

Uteri or individual uterine horns without visible implantations were immersed in a 10% solution of ammonium sulphide to reveal evidence of embryonic death at very early stages of implantation.

Live young were examined externally and weighed. Half the pups in each litter were preserved in Bouins solution for subsequent free-hand sectionings to discover visceral abnormalities (Wilson technique); the remainder were fixed in 740P industrial methylated spirit for subsequent macroscopic examination, evisceration and determination of sex prior to clearing and alizarin staining (modified Dawson technique) for skeletal examination. Young showing suspected abnormalities were processed by the more appropriate technique for clarification of initial observations.

Where necessary pups were uniquely identified to allow correlation of initial with subsequent findings.

Structural deviations were classified as:

Major malformations: Rare and/or probably lethal, e.g. exencephaly, anury.

Minor anomalies: Minor differences from "normal" that are detected relatively frequently either by free-hand sectioning, e.g. increased renal pelvic caviation, or at skeletal examination, e.g. bipartite centrum.

Variants: Alternative structures occurring regularly in the control population are classified as variant. These may be permanent structures, e.g. an extra pair of ribs, or they may be transient stages of development, e.g. unossified sternebrae.

For those animals allowed to litter, the young were counted, individually identified within the litter by toe amputation, sexed, weighed and examined for external abnormalities as soon as possible after parturition. Keeping nest disturbance to a minimum all litters were examined daily for dead and/or abnormal young. The pups were also weighed on days 4, 8, 12 and 21 post partum.

If possible, dead young, except those excessively cannibalised, were autopsied.

Surplus pups were sacrificed on or after day 21 post partum and examined externally and internally for abnormalities; sex was confirmed by gonadal inspection.

The above observation allowed determination at birth, 4, 8, 12 and 21 days post partum of:

(a) Mean values at birth were determined both for the total number of young and the number of viable young; at 4, 8, 12 and 21 days post partum for the number of viable young.

(b) Litter and mean pup weight

Liver weight was calculated from the individual pup weights.

(c) Pup mortality

Pup mortality rates were calculated as % losses within individual litters at birth and cumulatively on days 4, 8, 12 and 21. Group mean values were calculated from individual litter percentages.

(d) Abnormalities

Where indicated, young were preserved for further examination of suspect organs.

(e) Pups selected for rearing were chosen from litters weaned as close to the median weaning data as possible for the study or group, but paying attention to retaining as wide a genetic pool as possible. Within each litter, young were selected as close as possible to the median weaning of each respective sex in the litter. Brother and sister matings were avoided for second and subsequent generations.

The selected rats of the F2B and F3B generations were killed by carbon dioxide asphyxiation. The macroscopic appearance of the tissues was noted, and the following organs from all animals were dissected free of fat and weighed:

adrenals
brain
heart
kidneys
liver
lungs
ovaries
pituitary
spleen
testes
thymus
thyroid
uterus

Samples of the following tissues from all animals were preserved in buffered 10% formalin (except eyes, which were preserved in Davidson's fixative):

adrenals
aorta*
bone marrow
brain (medullary, cerebellar and cortical sections)
coecum
duodenum
eye
heart
ileum
jejunum*
kidneys
liver
lungs
lymph nodes (cervical and mesenteric)
mammary gland*
mid-colon*
oesophagus*
ovaries
pancreas
pituitary
prostate*
salivary gland
sciatic nerve*
second eye*
seminal vesicle
skeletal muscle*
skin*
spleen
stomach (glandular and non-glandular)
testes
thymus
thyroids
tongue*
trachea*
urinary bladder
uterus

Any other tissue showing macroscopic abnormality.

*These tissues were not precessed further.

The routine stain used was haematoxylin and eosin with further sections of liver and kidney stained with Oil Red O.

No histopathological examination was performed on the F2B generation.

Histopathological examination was restricted to the control and high dosage groups for the F3B generation. A duplicate set of slides was prepared and both sets of slides were stored in HRC Archives, one set to be available to the sponsor upon request.

In assessing litter parameters from birth to weaning, or at day 20 sacrifice (other than litter or fetal weights or abnormal values), group mean values were calculated from observations in two ways:

Mean A: Includes all valid data from surviving animals that provided evidence of pregnancy including those subsequently losing the entire litter, i.e. showing total resorption at day 20 sacrifice or failing to wean any young, as appropriate.

Mean B: Includes valid data from any animals with viable young at day 20 sacrifice or at weaning, as appropriate.

Mean B has more meaning when group size is low in which case the mean values would be unduly influenced by the presence of one or two animals totally losing their litters. Mean A is a more accurate index when several litters are completely last.

For litter and mean fetal weights and abnormality values only Mean B values or the equivalent were calculated.

In assessing results, for each litter sacrificed at day 20 of pregnancy, pre-implantation loss was calculated as a percentage from the formula:

$$\frac{\text{(No. of corpora lutea - No. of implantation)}}{\text{No. of corpora lutea}} \times 100$$

Post-implantation loss was similarly calculated from the formula:

$$\frac{\text{(No. of implantations - No. of live young)}}{\text{No. of implantations}} \times 100$$

For all values expressed as a percentage or ratio, values were first calculated within the litter and the group values derived as a mean of individual litter percentages.

Statistical analyses were then performed using the litter as the basic sample unit.

Non-parametric tests, (Jonckheers and Kruskal Wallis) were employed routinely for mean values of litter size, cumulative loss (pup mortality), litter weight, mean pup weight and the incidence of anomalous offspring as these values rarely follow a 'normal' distribution.

Analysis of co-variance (or variance) followed by the method of LSDs or William's test was used for assessing intergroup differences in mean bodyweight change, mean food consumption, mean water consumption and organ weights.

Results:

Throughout the three generations, there were no consistent dosage-related affects on adult animals in respect of signs of reaction, mortalities, food consumption, water consumption, bodyweight change, food conversion ratios, mating performance, pregnancy rate, duration of gestation or findings at terminal autopsy.

In the F1B generation only, mean weight gain of males showed a slight, apparently dosage-related retardation during the first nine weeks after selection.

Data obtained from the teratology sacrifices of both the F0 and the F1B females did not indicate any adverse effect of treatment with CGA 48-988 in respect of:

(a) mean litter parameters, as assessed by pre- and post-implantation loss, litter size, litter and mean fetal weights;

(b) embryonic and fetal development, as assessed by the incidence of major malformations, minor anomalies and skeletal variants.

Over the three generations, mean litter parameters, as assessed by the incidence of total litter loss, litter size, cumulative pup mortality, litter and mean pup weights, findings at terminal autopsy and the incidence of abnormalities provided no evidence of adverse treatment-related effects.

In the organ weight analysis of F2B animals sacrificed at approximately 17 weeks of age, the only statistically significant difference from control values considered, possibly, to be related to treatment was the increased mean liver weight of females which received CGA 48-988, 1250 ppm.

In the organ weight analysis of F3B weanling animals, none of the intergroup differences from control values were considered to be related to treatment with CGA 48-988.

Microscopic examination of tissues from selected F3B pups from the control and CGA 48-988, 1250 ppm groups showed no changes that were considered to be related to treatment with CGA 48-988.

Conclusion:

The NOEL for reproductive parameters and teratologic parameters is considered to be 1250 ppm.

Classification: Core-Minimum Data

D. 6-Month Chronic Oral Toxicity Study with CGA 48-988 Technical in Beagle Dogs (Elars Bioresearch; Project No. 1545; January 10, 1981)

Fifty-six healthy dogs (28 males, 28 females), 6-8 months old at initiation of dosing, were randomly assigned to four treatment groups: Group I (control), 8M + 8F; Group II (50 ppm), 6M + 6F; Group III (250 ppm), 6M + 6F; and Group IV (1,000 ppm), 8M + 8F. Two males and two females each from Group I and IV were randomly selected as recovery dogs.

The test material was administered orally by offering the feed mixtures ad libitum for 180 days. After at least 180 days of dosing, the 48 non-recovery dogs were killed and necropsied. The eight recovery dogs (4 control and 4 Group IV) were fed untreated diets for an additional four weeks before being killed and necropsied. Terminal body weights were obtained on all dogs prior to necropsy.

All dogs were observed daily for general appearance, behavior, elimination, and signs of toxic or pharmacologic effects.

Each dog was weighed one week prior to study initiation, weekly thereafter, and prior to termination. Mean weekly weight gains were computed and compared for statistically significant differences among groups.

Daily feed consumption was measured twice weekly.

Ophthalmic examinations were performed on all dogs prior to the beginning of dosing and at approximately 180 days on study.

Blood was collected from each dog one week before dosing and afterwards at 30 day intervals until termination of dosing.

Recovery dogs had blood samples collected approximately four weeks after termination of dosing. Hematology and clinical chemistry determinations were made from the collected blood samples for each dog. Individual hematology and clinical chemistry determinations were recorded and mean group determinations were calculated so that groups could be compared for statistically significant differences.

Hematology parameters included:

RBC
Hematocrit
Hemoglobin
Platelets
Activated partial thromboplastin time (APTT)
Protime
WBC
Segmented neutrophils
Band neutrophils
Lymphocytes
Monocytes
Eosinophils
Basophils
Methemoglobin
Heinz bodies
Reticulocyte count (performed only if evidence of anemia was observed)

Clinical chemistry parameters included:

Total protein
Albumin
Alkaline phosphatase
SGOT
SGPT
LDH
Glucose
BUN
Total bilirubin
Cholesterol
Calcium
Potassium
Sodium

Urine was collected from each dog one week prior to dosing and afterwards at 60 day intervals until termination of dosing. Urine was collected from recovery dogs prior to necropsy.

Selected urinalysis determinations were made for each dog and recorded. Also, group means and standard deviations for pH and specific gravity were calculated.

Urinalysis parameters included:

protein concentration
specific concentration
pH determination
Ketones
Bilirubin
Urobilinogen
Microscopic elements examination

A complete necropsy was performed on all dogs, except for the recovery dogs, after at least 180 days of test material administration. Recovery dogs were killed and necropsied after four weeks on untreated dog chow. Each dog was fasted overnight, anesthetized with sodium pentobarbital, injected intravenously, and exsanguinated by cardiac puncture prior to necropsy.

The following tissues were trimmed in a uniform manner, weighe! and weights recorded: liver, kidneys, heart, brain, spleen, gonads, adrenals, thyroids (with parathyroids) and pituitary gland. Organ/body weight ratios and organ/brain weight ratios were calculated. Additionally, mean organ weights and weight ratios were calculated for each group and compared statistically.

Selected tissues were removed from each animal and fixed in 10% buffered formalin solution (eyes and testes in Bouin's solution). The tissues were trimmed, embedded in paraffin, sectioned at 4-5 microns, affixed to glass slides, stained with hematoxylin and eosin, and submitted to Westpath Laboratories, Inc., Fort Collins, Colorado, for histopathologic evaluation.

The following tissues and organs were subjected to histopathologic examination:

Adrenal gland
Aorta
Bone marrow
Brain; cerebrum
cerebellum, pons
cecum
colon
esophagus
eyes with optic nerve
gall bladder
heart
kidney
liver
lung with main stem bronchi
lymph node; cervical,
  mesenteric
mammary gland
muscle
ovaries
pancreas
Peripheral nerve (sciatic)
Pituitary gland
Prostate
Salivary gland
submaxillary
skeletal muscle
skin
small intestines; duodenum,
  jejunum, ileum
spinal cord (2 levels)
spleen
stomach; cardia, fundus, pylorus
testes with epididymides
thymus
thyroid (with parathyroid)
trachea
urinary bladder
uterus; corpus + cervix

Statistical Analyses of the data were performed.

Results:

No dosage-related trends were noted in daily observations. Histiocytomas occurred on the paws of several dogs during the study; these occurred at all dosages and usually disappeared spontaneously. One of these lesions was still present at necropsy, and a section was submitted for histopathological evaluation.

No statistically significant differences were observed in body weights or weight gains.

Weekly feed consumption calculations showed no significant differences among groups and no trends were detected.

No abnormalities or changes were observed in the ophthalmic examinations and the Schirmer tear test results between the predose and terminal examinations.

Hematology parameters obtained predose showed no statistically significant differences among groups. All parameters were within normal limits for all dogs.

Red blood cell count, hematocrit, and hemoglobin count were significantly decreased for Group IV males compared with control males at 60, 90, and 180 days. All values were, however, within normal limits and no clinical signs of anemia were observed.

All clinical chemistry parameters from the predose bleeding were within normal limits, and no statistically significant differences among groups were detected.

The most consistent clinical chemistry findings were in alkaline phosphatase levels. Alkaline phosphatase levels in Group IV (1000 ppm) male and female dogs were statistically significantly higher than in controls at 120, 150, and 180 day bleedings.

Alkaline phosphatase levels typically decrease with age, as occurred in Groups I (control), II (50 ppm), and III (250 ppm). Alkaline phosphatase levels for Group IV, however, remained relatively constant over time.

During the recovery period, alkaline phosphatase levels for Group IV dogs, both males and females decreased. Although Group I dogs still showed lower levels than Group IV, the values between groups were much closer than those previosuly observed.

Semi-monthly urinalysis parameters were all within normal limits, and no dosage related trends were detected.

The liver/brain weight ratios of Group IV (1000 ppm) females were significantly increased compared with control. Absolute liver weights and liver/body weight ratios of female dogs showed a trend of increased liver weight with increased dosage; absolute liver weights and liver/body weight ratios showed a similar trend in male dogs. These trends were not statistically significant in either sex. The liver/brain weight ratios of male dogs did not show a clear trend, although male dogs of the control group showed lover liver/brain weight ratios than any treatment group. No other trends were detected in organ weights or ratios.

No treatment-related lesions were seen at gross necropsy and histopathological examination of the tissues.

Conclusion:

The NOEL is considered to be 250 ppm in the diet of dogs for 6-months. The LEL is 1000 ppm and the effects consisted of increased alkaline phosphatase and liver/brain weight ratio.

Classification: Core-Minimum Data

3. Formulation to be used is Ridomil 2E (EPA Reg.#100-607); Inerts are cleared under 180.1001.

4. No permanent tolerances have been established.

PROPOSED TOLERANCES

CROP	TOLERANCE
Broccoli	.60
Tomatoes	1.00
Soybeans	.50
Onions (Dry Bulb)	1.00
Onions; Green	5.00
Potatoes	.50
Melons	.30
Cabbage; Sauerkraut	.60
Cauliflower	.60
Cottonseed	.10
Lettuce	.50
Spinach	10.00
Wheat	.20
Cucumbers; Inc Pickle	.50
Liver	.30
Kidney	1.00
Milk & Diary Product	.02
Eggs	.05
Meat; Inc Poultry	.05

Conclusions and Recommendations:

The proposed tolerances are not toxicologically supported until items #1 and 5 of the recommendations are satisfactorily addressed by the petitioner.

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