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

TXR# 0051643

PREVENTION, PESTICIDES AND

OFFICE OF
TOXIC SUBSTANCES

Date: 6/30/2003

MEMORANDUM

SUBJECT: CYROMAZINE (PC Code: 121301)
Revised Executive Summaries and DERs of Cyromazine
DP Barcode: D284318
Submission Code:S590374
CAS#: 66215-27-8
PC Code:121301
Trade Name: Larvadex Trigard, CGA-72662
Class: Insecticide

FROM: Waheeda Mani Tehseen, Ph.D. (Toxicologist) 6/30/20
Registration Action Branch 3
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THROUGH: Stephen C. Dapson, Ph.D., Branch Senior Scientist
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08/04/2003

TO: Product Manager: Robert Forrest
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Following revised Executive Summaries and/or DERs of Cyromazine are attached

i) 00103202
ii) 00115735
iii) 00115736
iv) 00149656
v) 41442101
vi) 00103193
vii) 00103197
viii) 40168601
ix) 00135432
x) 00135433
xi) 00027488
xii) 00150471

i) MRID No 00103202 or 00115735.

STUDY TYPE: Combined Chronic Toxicity/Carcinogenicity Study in Rats

EXECUTIVE SUMMARY:

A 24-month chronic toxicity/oncogenicity study in Charles River Sprague-Dawley rats was conducted by IRDC, Mattawan, Michigan, and issued June 30, 1982, for Ciba-Geigy Corporation, Greensboro, North Carloina (IRDC Study No. 382-081).

The study design allocated groups of 60 rats per sex to dose levels of 0, 30, 300 and 3000 ppm (0, 1.5, 15, 150 mg/kg/day) of Cyromazine (95.5% pure cyromazine - Larvadex technical). An additional 10 animals per sex in the control and 3000 ppm groups were designated for interim sacrifice at one year.

The statistical evaluation of mortality provided by the registrant indicated no significant incremental charges with increasing doses of Cyromazine in male or female rats (41% survival in HDT vs. 42% survival in controls). Survival in males and females were excellent in all groups after 24 months. Female rats had decreased body weight gain at Cyromazine doses of 300 and 3000 ppm, as did males at doses of 3000 ppm. Body weight gains were reduced by 22% in the high-dose males and 33% in females, and in the mid-dose females body weight gain was reduced by about 10% from week 49 until termination. Food consumption was also decreased at these dose levels; however food efficiency was unchanged from control.

Cystic hyperplasia of the mammary gland was observed only in 1/9 control female rat at the interim sacrifice. At the 24-month sacrifice, cystic hyperplasia was found in 0/55, 1/59, 5/56, and 4/59 rats in the 0, 30, 300, and 3000 ppm groups, respectively.

Female rats showed a number of different neoplasms of the mammary glands. There was no increase in the incidence of benign tumors of the mammary gland due to cyromazine treatment. Although the number of animals with the malignant tumors in the mammary gland was slightly greater in the highest dose group (150 mg/kg/day) as compared to the control females, this difference was not statistically significant and there was an absence of dose response. Neither the adenomas nor the fibroadenomas nor the combination of adenomas, fibroadenomas and adenocarcinaomas showed a significant trend ($p<0.01$) and pair-wise comparison ($p<0.05$). Moreover the incidence of mammary tumors in treated rats did not exceed the historical control data, and the tumors were not considered treatment related (HED CPRC, Jan. 6, 1995, TXR No. 0011384).

Based on statistical analysis, there was a slight increased incidence of renal interstitial cell tumors in high dose males (6/57 or 11%) compared to controls (1/60 or 2%), however, these lesions were within the historical control range and the lesions were not considered treatment related.

Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing may have been excessive for assessing the carcinogenic potential of Cyromazine in rats, based on depressions in mean body weight gain of 22% in males, and 33% in females at doses of

3000 ppm. This rather large depression in body weight gain was not, however, accompanied by increases in mortality or signs of toxicity. Morever, the body weight gain seemed to be reversible, increasing towards control levels during the four-week post treatment period.

The systemic toxicity LOAEL is 300 ppm (15 mg/kg/day) based on decreased body weight and the systemic toxicity NOAEL is 30 ppm (1.5 mg/kg/day).

This study is **acceptable-guideline** and satisfies the requirement for a combined chronic/carcinogenicity study (OPPTS # 870.4300 / 83-5) in rat.

ii) MRID No. 00115736.

STUDY TYPE: Carcinogenicity Study in Mouse

EXECUTIVE SUMMARY

A 24-month chronic toxicity/oncogenicity study in Charles River CD-1 mice was conducted by IRDC, Mattawan, Michigan, and issued June 30, 1982, for Ciba-Geigy Corporation, Greensboro, North Carolina (IRDC Study No. 382-082).

The study design allocated groups of 60 mice per sex to dose levels of 0, 50, 1000 and 3000 ppm (0, 7.5, 150, 450 mg/kg/day) of Cyromazine. An additional 8 animals per sex per dose were designated for interim sacrifice at one year.

Male mice had decreased body weight gains from 8 weeks (high-dose, 450 mg/kg/day) onward and 19 weeks onward (mid-dose, 150 mg/kg/day). The body weights of the males were consistently 5-9% less than controls. Female weights were unaffected. Hematological parameters and behavioral signs were not affected by the test substance. There was no increase in incidence of non-neoplastic lesions as compared to controls. Relative liver weights were increased at terminal sacrifice in the high-dose (450 mg/kg/day) males and these animals also had a slightly increased incidence of liver masses. Cystic hyperplasia of the mammary gland occurred, however the incidence did not increase with higher cyromazine doses.

Malignant lymphomas (lymphatic lymphomas and histiocytic lymphomas) were found in both control and treated groups in both sexes. However, two independent pathology labs have reviewed these data and both determined that these tumors did not appear to be treatment-related.

Liver neoplasms (hepatocellular carcinoma/adenomas, hemangiosarcomas and hemangiomas) also were seen in both sexes in treated and control animals but there was no apparent dose-response relationship.

Hemangiosarcoma of the spleen is a frequently observed tumor type in mice and was seen in a few male and female treated mice with no strong dose-response relationship.

Female mice showed various mammary tumors (adenocarcinomas and adenocanthomas). However, the incidence of mammary tumors is similar to the control groups and there is no clear dose-response relationship. Mammary gland neoplasms (adenocarcinomas) were slightly increased in high-dose females (6/57 or 4%) as compared to controls (2/56 or 11%), however, the difference was not statistically different than controls. Hence, there was no statistically significant increase in tumors in the treated groups; there were no statistical trends and no dose response (HED CPRC, Jan. 6, 1995, TXR No. 0011384).

The historical control data for female albino mice conducted at a contract laboratory showed that the incidence of adencarcinomas in the mouse study slightly exceed the range of historical controls but not concurrent controls. Reliable historical control data could not be located for mammary gland adenoacanthomas in the female albino mouse.

Adequacy of Dosing for Assessment of Carcinogenic Potential

Body weight gains in female mice were comparable to controls and there were no indications of toxicity reported. The study report stated that "there is suggestion of a possibly slightly increased mortality" in high dose females; based on this the dosing in the female mouse was considered "marginally adequate"(HED CPRC, Jan. 6, 1995, TXR No. 0011384).

The dosing in male mice was considered adequate, based on body weight gain reductions (12% at the mid dose, 23% at the high dose) relative to controls.

The systemic toxicity LOAEL is 1000 ppm (150 mg/kg/day) based on decreased body weight and the systemic toxicity NOAEL is 50 ppm (7.5 mg/kg/day).

This study is **acceptable-guideline** and does satisfy the requirement for a carcinogenicity study (OPPTS # 870.4200 / 83-2) in mouse.

iii) MRID no. 00103193.

STUDY TYPE: Chronic (6 months) Oral Toxicity in Dogs (feeding);

EXECUTIVE SUMMARY:

In this study (MRID No. 00103193, TXR No. 002664) groups of male and female beagle dogs (3/sex/dose at 30 and 300 ppm and 4/sex/dose at 3000 ppm and control) were fed diets containing cyromazine at 0, 30, 300 or 3000 ppm (0, 0.75, 7.5 or 75 mg/kg/day, respectively) for 6 months.

All dogs were housed individually in controlled environments. All dogs were observed twice daily for mortality and morbundity and once daily for appearance, behavior, appetite, elimination, and signs of toxicity. Food consumption were recorded twice weekly. Hematology, clinical chemistry, and urinalysis were performed on all dogs at various intervals during the treatment period and for

recovery animals at week 30. Ophthalmologic examinations were performed on all dogs prior to treatment and during week 12 and 26. Selected surviving animals were sacrificed for gross and microscopic pathology.

No treatment-related effects were observed in survival, clinical signs, food consumption, body weights, ophthalmologic parameters and gross and microscopic pathology. Mean body weight and body weight gain values were minimally decreased in males at the highest dose, and in females at all dose levels. Mean food consumption values of the high dose animals were also slightly decreased. These changes were slight, hence may not be considered treatment related.

Pronounced treatment-related effects on hematological parameters, were manifested in males and females as decreases in hematocrit and hemoglobin levels at 3000 ppm. Decreased mean cholesterol and serum glutamic oxaloacetic transaminase (SGOT) values were noted at high dose (3000 ppm) during the treatment time period, however, the values reached normal during the recovery period. Slight increases were noted for the relative brain, heart, and liver weights of the high dose (3000 ppm) males and females and the ovary weights of the high dose females.

The systemic toxicity NOAEL was 300 ppm (7.5 mg/kg/day) and the systemic toxicity LOAEL was 3000 ppm (75 mg/kg/day) based on decreases in hematological parameters (hematocrit and hemoglobin), body weights, and slight increases in brain, heart and liver weights.

This chronic toxicity study is classified **acceptable-guideline** and does satisfy the guideline requirement for a chronic oral study (82-1b) in dogs.

iv) MRID no. 00135432.

STUDY TYPE: Subchronic (13 Weeks) Oral Toxicity in dogs (feeding);

EXECUTIVE SUMMARY:

In this study (MRID no. 00135432. TXR no. 002687) groups of male and female Beagle Dogs (4M/4F in each low and medium dose and 6M/6F in high dose and control) were fed diets containing cyromazine at 0, 30, 300, 1000 or 3000 ppm (0, 0.75, 7.5, 25, and 75, respectively) for 13 weeks.

All animals were observed for general signs of toxicity and mortality daily. Ophthalmoscopic parameters were observed in all animals on 8, 12 and 17 weeks (withdrawal dogs only). Food consumption and body weights were recorded weekly. Hematology, clinical chemistry, and urinalysis were performed on weeks 4, 7, 12 and 17 (withdrawal dogs only). All animals were sacrificed for gross and microscopic pathology.

No treatment-related effects were observed in survival, clinical signs, ophthalmologic parameters hematology, clinical chemistry, urinalysis, gross necroscopy, and microscopic pathology. Males showed significant increases in relative liver weights at 1000 and 3000 ppm.. Females did not show

this effect in the liver. Kidney weights were increased significantly at 3000 ppm in both sexes. There was no clear dose related effect on body weights.

The NOAEL was 300 ppm (7.5 mg/kg/day) and the LOAEL was 1000 ppm (25 mg/kg/day) based on alteration in liver weights in males.

This subchronic toxicity study is classified **acceptable-guideline** and does satisfy the guideline requirement for a subchronic oral study (82-1b) in dogs.

v) MRID no. 00135433.

STUDY TYPE: Subchronic (13 Weeks) Oral Toxicity in Rats (feeding);

EXECUTIVE SUMMARY:

In this study (MRID No. 00135433. TXR No. 002687) groups of male and female Albino rats (20/sex/dose) were fed diets containing cyromazine at 0, 30, 300, 1000 or 3000 ppm (0, 3, 30, 100, or 300 mg/kg/day, respectively) for 13 weeks.

All animals were observed for general signs of toxicity and mortality twice daily. Ophthalmoscopic exams were conducted pre-treatment and at terminal. Food consumption and body weight were recorded weekly. Hematology, clinical chemistry, and urinalysis were performed on days 29, 61 and 89. All animals were sacrificed for gross and microscopic pathology.

No treatment-related effects were observed in survival, clinical signs, ophthalmologic parameters, hematology, clinical chemistry, urinalysis, and microscopic pathology. Males showed increased relative heart and testes weights at 1000 and 3000 ppm, however, absolute weights of these organs were not changed. Animals showed decreases in body weights at the highest dose, however, this decrease was associated with a decreased food consumption.

Males showed highly significant ($P < 0.01$) decreases in absolute and relative liver weights at 300, 1000 and 3000 ppm. Males also showed slight decreases in liver weights values at 30 ppm.. The females did not demonstrate any clear compound-related effects on liver weight.

The NOAEL was 30 ppm (3 mg/kg/day) and the LOAEL was 300 ppm (30 mg/kg/day) based on alteration in liver weights in males.

This subchronic toxicity study is classified **acceptable-guideline** and does satisfy the guideline requirement for a subchronic oral study (82-1a) in rats.

vi) **MRID no. 41442101**

STUDY TYPE: Metabolism and Pharmacokinetics in rat;

EXECUTIVE SUMMARY:

In a metabolism study (MRID no. 41442101), the disposition and metabolism of the insect growth regulator ^{14}C-cyromazine (97.2% and 99%) was investigated in male and female CD rats. The animals were treated with a low oral dose (3 mg/kg), a low intravenous dose (3 mg/kg), repeated low oral doses (3mg/kg) and a high dose (300 mg/kg) to determine the absorption, distribution, and excretion of ^{14}C- cyromazine. One percent of carboxymethylcelluslose plus 4mg/ml Hi Sil 233 was used as a vehicle. Urine and feces were collected at 4, 8, 12, 24, 36, and 48 hours following oral or iv doses. After sacrifice, bone (femur), brain, fat (urogenital), ovaries, testes, heart, liver, kidneys, lungs, muscle (thigh), spleen, uterus, and residual carcass were analysed of radioactivity.

Comparison of oral and i.v. disposition data demonstrated that cyromazine was well absorbed after oral administration. In those rats dosed orally with 3 mg/kg ^{14}C-cyromazine, 82-86% of the dose was excreted in urine, while approximately 86% of a dose of ^{14}C-cyromazine was excreted in urine in those rats dosed i.v. at this same dose level. Thus, the absorption of ^{14}C-cyromazine appeared complete.

Excretion was also rapid at the low dose, with the majority of radioactivity eliminated in the urine by 24 hours. No apparent differences were observed between dosing regimens in the total urinary radioactivity eliminated (between 82 and 91%) when cage washes and rinses were taken into consideration. At the high dose, however, there was an apparent delay (urinary excretion was not complete until 48 hours) in elimination of ^{14}C-cyromazine derived radioactivity, suggesting either some type of renal pathology or a shift in biotransformation kinetics of cyromazine.

Fecal elimination (3-8%) of ^{14}C-cyromazine was a minor route. Fecal elimination of ^{14}C-cyromazine derived radioactivity was equivalent between dose groups and sexes, with the exception that in the high dose males, a greater percentage of radioactivity was apparently eliminated by this route. The similarity between the intravenous and oral dose groups in fecal elimination of ^{14}C-cyromazine derived radioactivity supports the conclusion that the fecal radioactivity arises from biliary excretion of the test material in rats.

Residual ^{14}C-cyromazine was minimal in all dose groups, with non-detectable levels in all tissues examined except liver, red blood cells, and carcass.

In this rat metabolism study, urinary and fecal metabolites of ^{14}C-cyromazine were isolated and identified by TLC, HPLC, and GC/MS. The major compounds were the N-dealkylated product melamine, hydroxycyromazine, and unmetabolized cyromazine identified by GC/MS, while tentative identification was given to methylcyromazine, based upon co-chromatography with TLC and HPLC. A following proposed pathway for cyromazine biotransformation was given based upon these data.

↗ methycyromazine

Cyromazine → melamine

↘ hydroxycromazine

Core Classification: minimum

This study satisfies the guideline requirements (85-1) for a metabolism study in rats.

vii) MRID 40168601

STUDY TYPE: Dermal Absorption in Rats

EXECUTIVE SUMMARY:

In a dermal absorption study (MRID 40168601), male rats received dermal application of a "simulated" 75W formulation of ^{14}C-Cyromazine in aqueous solution at doses of 0.10, 1.0 and 10 mg/rat. Four animals per dose were exposed for durations of 2, 4, 10 and 24 hours. A second set of rats (4 per dose) also received dermal doses of 0.10, 1.0 and 10 mg/rat for 10 and 24 exposure periods followed by a 48 hour "depletion period". Mean recoveries of applied radioactivity from all dose groups ranged from 77 to 120%. Table 1 summarizes mean percent absorption at each time period for rats terminated immediately after exposure and percent absorption for animals followed for 48 hours post-exposure.

Table 1. Mean Percent Absorption - No Post-Exposure Period

Dose Level (mg/rat)	Mean % of Dose Absorbed (no post-exposure period)				Mean % Dose Absorbed (48 h post exposure period)	
	2 hours	4 hour	10 hours	24 hours	10 hours	24 hours
0.1	2.86	5.25	7.01	6.02	12.61	14.44
1	6.18	2.67	4.54	2.26	7.80	9.81
10	2.00	0.57	0.74	2.38	10.98	8.50

Mean absorption based on blood, urinary/fecal excretion, and carcass, ranged from 0.6 to 7% for animals sacrificed at the end of the exposure periods. For animals exposed for 10 and 24 hours and followed for 48 hours post-exposure, mean absorption ranged from 8 to 14.5%. Total radioactivity absorbed generally decreased as dose increased indicating saturation of absorption with increasing dose. Amounts remaining in/on the skin at termination ranged from 4.5% (10 mg dose/2 h exposure) to 24% (0.1 mg dose/24 hour exposure). The majority of the absorbed radioactivity was found in the urine and carcass. Most of the unabsorbed radioactivity was found in the skin washes from each dose/duration.

There were no deficiencies observed in the study.

The study is classified **Acceptable** and satisfies the guideline requirement 870.7600 (85-3) for a dermal absorption study.

viii) MRID No. 00149656.

STUDY TYPE: Dermal Absorption in Rats

EXECUTIVE SUMMARY:

In a dermal absorption study (Accession No. 257488), a formulation of "simulated" 75W formulation of ^{14}C-Cyromazine in aqueous solution was administered to 36 male Harlan Sprague-Dawley albino rats (3 rats per dose group per time period). ^{14}C-Cyromazine was dermally applied at doses of 0.1, 1.0, and 100 mg/rat to a 10 cm^2 area on the upper back portion of the rats. Animals were exposed for durations of 1, 2, 4, and 10 hours followed by skin wash and termination. Table 1 summarizes mean percent absorption and the mean percent remaining in/on the skin after skin wash at each time period for all doses.

Table 1 - Mean Percent Absorption

Dose Level	Mean Percentage of Dose Absorbed & In/On Skin							
	1 hour		2 hours		4 hours		10 hours	
	absorbed	skin*	absorbed	skin	absorbed	skin	absorbed	skin
0.01 mg/cm^2 (0.1 mg/rat)	7.72	29.14	4.47	34.69	8.13	30.14	9.99	34.69
0.1 mg/cm^2 (1.0 mg/rat)	3.48	18.50	4.84	25.87	6.57	20.04	11.43	27.37
10 mg/cm^2 (100 mg/rat)	2.23	7.64	11.72	26.29	3.27	6.37	7.08	15.21

* amount of radioactivity in/on skin after skin wash

Mean total recoveries of applied radioactivity from all dose groups ranged from 85 to 101%. Mean absorption based on blood, urinary/fecal excretion, and carcass, ranged from 2% to 11%. Total radioactivity absorbed generally increased with increasing exposure time but decreased with increasing dose indicating saturation of penetration with increasing dose. The majority of the absorbed radioactivity was found in the urine and carcass. Most of the unabsorbed radioactivity was found in the skin washes from each dose/duration (35-90%). However, based on measurements of skin absorption, a significant amount of radioactive dose was also found in the skin itself (9-40%). Mean absorption with inclusion of radioactivity in dissolved skin ranged from 10 to 45%. The ratio of the amount of radioactive dose in the skin wash to the radioactivity in the skin itself decreased with time indicating penetration into the subsurface of the skin with time after treatment.

There were no deficiencies observed in the study.

The study is classified **Acceptable** and satisfies the guideline requirement 870.7600 (85-3) for a dermal absorption study.

ix) MRID No. 00027488.

STUDY TYPE: Prenatal Developmental Toxicity in Rat (gavage); OPPTS 870.3700 [§83-3]

EXECUTIVE SUMMARY:

The developmental toxicity range finding study (MRID no: 00027488; IRDC no: 382-069 & TXR no: 003873 - August 7, 1979) was adequate to establish dosing levels for this primary study. In this range finding rat study, technical Cyromazine (CGA - 72662 technical) was administered to 30 animals by gavage on gestation days 6-20. Dose levels were 0, 300, 600, 1000, 1500 mg/kg/day. Severe toxicity was observed at 2500 mg/kg/day. At 1500 mg/kg/day significant reduction in maternal weight gains and clinical signs were observed. Reported results at 300, 600 and 1000 mg/kg/day supported the use of these dose levels and below for the primary prenatal developmental toxicity study.

In the primary prenatal developmental toxicity study in COBS® CD® rats (25/group) (MRID no: 0002748; TXR no: 003873 - IRDC no: 382-070), technical Cyromazine (CGA - 72662 technical) was administered by gavage in aqueous 1.0% carboxymethylcellulose at a dose volume of 10 ml/kg on gestation days 6-19. Dose levels were 0, 100, 300, or 600 mg/kg/day.
All animals were housed in individual cages. Animals were observed daily for mortality and clinical signs of toxicity. Individual maternal body weights were recorded on gestation days 0, 6, 9, 12, 16 and 20. No data were provided for food consumption. On gestation day 20, all dams were sacrificed . Following sacrifice, the uterus was weighed prior to removal of fetuses. The number and location of viable and nonviable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for morphological changes. All fetuses were individually weighed and examined for malformations and variations. Fetuses were examined for visceral and skeletal examination after fixation.

At 300 and 600 mg/kg/day dose levels, animals showed red nasal discharge and increase in matting and staining of the anogenital haricoat. At the lowest dose only rats showed clear oral discharge on 2 separate days. The mid dose (300 mg/kg/day) group showed a slight reduction in overall maternal body weight gain (0-20 days), whereas the high dose (600 mg/kg/day) group showed a reduction in both overall maternal body weight gain and body weight gain during the dosing periods (days 6-19). All of the dams survived. There were no statistically significant differences in mean numbers of viable fetuses, late or early resorptions, postimplantation loss, total implantations, corpora lutea or fetal sex distribution in any treatment group compared to control. At the highest dose level, a significant increase in skeletal variations (unossified sternebrae) was observed in fetuses.

For maternal toxicity, the NOAEL was 100 mg/kg/day, and the LOAEL was 300 mg/kg/day, based on increased incidences of clinical observations (red or clear nasal discharge) and decreased body weight gain. For developmental toxicity, the NOAEL was 300 mg/kg/day, and the LOAEL was 600 mg/kg/day, based on increased incidence of minor skeletal variations.

The developmental toxicity study in the rat is classified **acceptable-guideline** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 a) in rat.

x) MRID no. 00150471.

STUDY TYPE: Prenatal Developmental Toxicity in Rabbit (gavage); OPPTS 870.3700 [§83-3b]

EXECUTIVE SUMMARY:

In a prenatal developmental toxicity study (MRID no.00150471, Accession no. 256348 and TXR no. 004263) in New Zealand White rabbits (16/group- artificially inseminated females), 95.2% Cyromazine was administered by gavage at doses of 0, 5, 10, 30, or 60 mg/kg/day on GD 7-19. The test substance was delivered in 0.5% aqueous carboxymethylcellulose at a dose volume of 1 ml/kg.

All animals were observed daily for moribundity and mortality and clinical signs of toxicity. Females which aborted during the experimental period were sacrificed and necropsied on that day. A gross necropsy was performed on the females which died during the course of the study. Maternal body weights were recorded on gestation days 0, 7, 10, 14, 20, 24, and 29. Mean gravid uterine weights were determined for each female at the scheduled cesarean section. Individual food consumption was recorded daily from days 0 through 29 of gestation. On gestation day 29, all surviving females were sacrificed by an iv injection of T-61 euthanasia solution. Following sacrifice, the uterus was weighed prior to removal of fetuses. The number and location of viable and nonviable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. All fetuses were individually weighed and examined for malformations and variations. Fetuses were then examined for visceral and skeletal examination after fixation.

Three animals died, one each in the vehicle control, 30 mg/kg and 60 mg/kg groups. The two treated animals died due to intubation errors and not due to treatment. A low pregnancy rate noted in the 10 mg/kg group (38.9%) restricted the extent of information that could have been obtained from this group. Only 7 litters with viable fetuses were obtained from this group with a limited number of fetuses (45). At 5 and 10 mg/kg/day doses, cyromazine did not produce toxic symptoms or any significant changes in food consumption, body weight, and reproductive status in pregnant rats. Three dams with only resorprtions were found at the 30 mg/kg level and one each in the vehicle, 0 and 5 mg/kg groups. The number of corpora lutea was similar between the treated and control groups except for the 10 mg/kg group which had a slightly higher number of corpora lutea. However, these maternal toxicities were not considered treatment related. No compound related changes in preimplantation loss were observed. However, the incidences of postimplantation loss was slightly increased in the two high dosage levels. Significant decreases in the number of female fetuses/dam were noted in the lower doses of 5, 10, and 30 mg/kg, but not in the higher dose of 60 mg/kg group. No significant changes were observed in fetal weights at any dose level. Slight decreases in fetal weight were noted only in the mid doses of 10 and 30 mg/kg groups, but apparently resulted from a larger litter size associated with these two dosage levels. The only treatment related effects observed in pregnant rabbits were decreased body weight gain and food consumption at 30 and 60 mg/kg/day.

For developmental toxicity, several malformations were noted in this study, but none of them were

clearly related to the treatment. Cyclopia [1(1) fetus (litter) each in the 10 and 30 mg/kg groups] and diaphagrmatic hernia [1(1) and 3(2) fetuses (litters) in the 10 and 30 mg/kg groups] were observed in the 10 and 30 mg/kg groups. These malformations were not dose related and were not observed in the group of 60 mg/kg. Hydrocephaly was noted in the three highest dose groups (10, 30, and 60 mg/kg/day). However, the incidences of hydrocephaly were comparable to the historical controls. According to the developmental toxicity risk assessment screening committee (TXR numbers 009774 and 006877; Sept. 1992 and Oct. 1992, respectively) these developmental effects were likely due to chance and were not considered treatment-related. This was based on the conclusion that the incidence was within historical control range for the strains utilized. Moreover, these developmental effects were not consistently reproducible across various developmental studies, and clearly not dose-related. Hence, no clear evidence of developmental toxicity in study was noted.

For maternal toxicity, the NOAEL was 10 mg/kg/day and the LOAEL was 30 mg/kg/day, based on decreased body weight gain and food consumption. For developmental toxicity, the NOAEL was ≥ 60 mg/kg/day (HDT); a LOAEL was not established.

The developmental toxicity study in the rat is classified **acceptable-guideline** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 b) in rabbit.

xi) MRID 00103197.

STUDY TYPE: Two-Generation Reproductive and fertility effects (feeding-rat);

EXECUTIVE SUMMARY:

In a two-generation reproduction study (MRID No. 00103197, TXR No. 002664, IRDC, Study No. 382-086) in Sprague-Dawley rats COBS®CD® rats (15 males and 30 females/group), 95.3% cyromazine was administered at dietary levels of 30, 1000, or 3000 ppm (1.5, 50, or 150 mg/kg/day).

The F0 rats were mated once to produce the F1 litters. F1 rats were randomly selected to mate to produce the F2 litters. Animals were observed for toxicity, behavioral changes, mortality, clinical signs, body weight, food consumption, pregnancy rate, litter size and weight, pup weight, gross pathology, organ weights, and histopathology.

No significant treatment-related effects were observed in general appearance, behavioral changes, parturition and length of gestation in the animals.

There was a significant decrease in parental mean body weights in the F_0 and F_1 generation at 1000 and 3000 ppm. The decreased body weights which ranged for high dose (3000 ppm) males from 14.5% for the F_1 to 17.5% for the F_0, and for high dose females from 17.0% for the F_0 to 20.0% for the F_1, these values represent average differences observed during weeks 1 through 55 of the study, excluding the gestation and lactation days. At 30 ppm there was a very slight weight loss in the females (4%) but not in the males. The effect was not considered treatment related. Food consumption data followed a similar pattern. The corresponding significant decrease in food

consumption of F_0 and F_1 generations at 1000 ppm (slightly decreased) and 3000 ppm group (moderately decreased) was a clear indication of a response to the treatment. No effect was noted on pup survival rates in all groups and generations. Fertility rates were unaffected by treatment. No differences in mean litter size were noted. The difference between the group mean pup weights at birth and on postnatal days 7, 14, and 21 for the high dose and control groups in each generation was consistently statistically significant (p,0.05 or p>0.01). The pup weights in the high dose (3000 ppm) group averaged 10 to 20 % less than that of the control group from birth to weaning.

The parental systemic toxicity NOAEL was 1000 ppm (50 mg/kg/day), and the parental systemic toxicity LOAEL was 3000 ppm (150 mg/kg/day), based on decreased body weights that were associated with decreased food efficiency. The offspring systemic / developmental toxicity NOAEL was 1000 ppm (50 mg/kg/day) and the offspring systemic/developmental toxicity LOAEL was 3000 ppm (150 mg/kg/day), based on decreased body weights at birth and through weaning. The reproductive toxicity NOAEL is ≥ 3000 ppm (150 mg/kg/day - HDT). The reproductive toxicity LOAEL was not acheived.

The reproductive study in the rat is classified **acceptable-guideline** and does satisfy the guideline requirement for a two-generation reproductive study (OPPTS 870.3800, §83-4) in rat.

Note: See the supplementary documents - (TXR numbers 009774, Developmental peer review, July 1992)

EPA Reviewers: Waheeda Mani Tehseen, Ph. D.
OPP-HED Registration Action Branch 3 (7509C)

Date 9/14/2002

EPA Secondary Reviewer: Steve Dapson, Ph. D.
OPP-HED Registration Action Branch 3 (7509C)

Date 09/13/2002

DATA EVALUATION RECORD (DER)

This executive summary is an upgrade to previously written DER (MRID - 41442101 & TXR - 008223)

STUDY TYPE: Metabolism and Pharmacokinetics in rat; OPPTS 870.7485, §85-1

DP BARCODE: D284318 SUBMISSION CODE: S590374

P.C. CODE: 121301

TEST MATERIAL (PURITY): ^{14}C-Cyromazine (97.2% - 99%)

SYNONYMS: N-cyclopropyl-1,3,5-triazine-2,4,6-triamine; Trigard

CITATION: Thomas M. Capps. (1989).Laboratory Project no. ABR-89108, "Characerization and Identification of ^{14}C-Cyromazine and Metabolites in Rats;" Laboratory Project no.HLA 6117-160, "Metabolism of ^{14}C-Cyromazine in Rats. ABR-89108: January 1990; HLA 6117-160, October 4, 1989. MRID no. 41442101

SPONSOR: Ciba-Geigy Corporation, Agricultural Division, 410 Swing Road, Greensboro, NC 27419.

EXECUTIVE SUMMARY:

In a metabolism study (MRID no. 41442101), the disposition and metabolism of the insect growth regulator ^{14}C-cyromazine (97.2% and 99%) was investigated in male and female CD rats. The animals were treated with a low oral dose (3 mg/kg), a low intravenous dose (3 mg/kg), repeated low oral doses (3mg/kg) and a high dose (300 mg/kg) to determine the absorption, distribution, and excretion of ^{14}C- cyromazine. One percent of carboxymethylcelluslose plus 4mg/ml Hi Sil 233 was used as a vehicle. Urine and feces were collected at 4, 8, 12, 24, 36, and 48 hours following oral or iv doses. After sacrifice, bone (femur), brain, fat (urogenital), ovaries, testes, heart, liver, kidneys, lungs, muscle (thigh), spleen, uterus, and residual carcass were analysed of radioactivity.

Comparison of oral and i.v. disposition data demonstrated that cyromazine was well absorbed

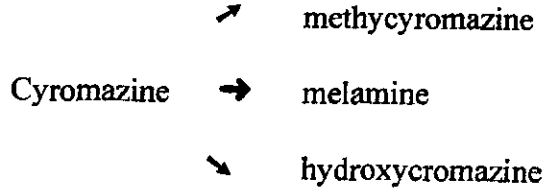
after oral administration. In those rats dosed orally with 3 mg/kg ^{14}C-cyromazine, 82-86% of the dose was excreted in urine, while approximately 86% of a dose of ^{14}C-cyromazine was excreted in urine in those rats dosed i.v. at this same dose level. Thus, the absorption of ^{14}C-cyromazine appeared complete.

Excretion was also rapid at the low dose, with the majority of radioactivity eliminated in the urine by 24 hours. No apparent differences were observed between dosing regimens in the total urinary radioactivity eliminated (between 82 and 91%) when cage washes and rinses were taken into consideration. At the high dose, however, there was an apparent delay (urinary excretion was not complete until 48 hours) in elimination of ^{14}C-cyromazine derived radioactivity, suggesting either some type of renal pathology or a shift in biotransformation kinetics of cyromazine.

Fecal elimination (3-8%) of ^{14}C-cyromazine was a minor route. Fecal elimination of ^{14}C-cyromazine derived radioactivity was equivalent between dose groups and sexes, with the exception that in the high dose males, a greater percentage of radioactivity was apparently eliminated by this route. The similarity between the intravenous and oral dose groups in fecal elimination of ^{14}C-cyromazine derived radioactivity supports the conclusion that the fecal radioactivity arises from biliary excretion of the test material in rats.

Residual ^{14}C-cyromazine was minimal in all dose groups, with non-detectable levels in all tissues examined except liver, red blood cells, and carcass.

In this rat metabolism study, urinary and fecal metabolites of ^{14}C-cyromazine were isolated and identified by TLC, HPLC, and GC/MS. The major compounds were the N-dealkylated product melamine, hydroxycyromazine, and unmetabolized cyromazine identified by GC/MS, while tentative identification was given to methylcyromazine, based upon co-chromatography with TLC and HPLC. A following proposed pathway for cyromazine biotransformation was given based upon these data.



Core Classification: minimum

This study satisfies the guideline requirements (85-1) for a metabolism study in rats.

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Data Evaluation Report

Study type: Metabolism (85-1) Tox. Chem. No.: 167B

EPA identification numbers:
EPA MRID number: 414421-01
EPA identifying number: 100-654
EPA record numbers: 262, 735
Caswell number: 167B
HED project number: 0-1076
Laboratory Project numbers: ABR-89108; HLA 6117-160

Test material: ^{14}C-Cyromazine

Synonyms: N-cyclopropyl-1,3,5-triazine-2,4,6-triamine; Trigard

Testing Facilities:
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Metabolism Department
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Sponsor: Ciba-Geigy Corporation
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Title of reports: Laboratory Project no. ABR-89108, Characerization and Identification of ^{14}C-Cyromazine and Metabolites in Rats; Laboratory Project no.HLA 6117-160,Metabolism of ^{14}C-Cyromazine in Rats.

Author(s): Thomas M. Capps

Reports issued: ABR-89108: January 1990; HLA 6117-160, October 4, 1989

Conclusions:
In study # HLA 6117-160, the disposition and metabolism of ^{14}C-cyromazine was investigated in male and female rats at a low oral dose (3 mg/kg), a low intravenous dose (3 mg/kg), repeated

low oral doses (3mg/kg) and a high dose (300 mg/kg). Comparison of oral and i.v. disposition data demonstrated that cyromazine was well absorbed after oral administration. Excretion was also rapid at the low dose, with the majority of radioactivity eliminated in the urine by 24 hours. No apparent differences were observed between dosing regimens in the total urinary radioactivity eliminated when cage washes and rinses were taken into consideration. At the high dose, however, there was an apparent delay in elimination of ^{14}C-cyromazine derived radioactivity, suggesting either some type of renal pathology or a shift in biotransformation kinetics of cyromazine.

Fecal elimination of ^{14}C-cyromazine derived radioactivity was equivalent between dose groups and sexes, with the exception that in the high dose males, a greater percentage of radioactivity was apparently eliminated by this route. The origin of fecal radioactivity from administration of ^{14}C-cyromazine was apparently biliary in nature, as an equivalent percentage of ^{14}C-cyromazine derived radioactivity was eliminated by both orally dosed and intravenously dosed rats.

Residual ^{14}C-cyromazine derived radioactivity was minimal in all dose groups, with non-detectable levels in all tissues examined except liver, red blood cells, and carcass.

In study # ABR-89108, urinary and fecal metabolites of ^{14}C-cyromazine were isolated and identified by TLC, HPLC, and GC/MS. The N-dealkylated product melamine, hydroxycyromazine, and unmetabolized cyromazine were definitively identified by GC/MS, while tentative identification was given to methylcyromazine, based upon co-chromatography with TLC and HPLC. A proposed pathway for cyromazine biotransformation was given based upon these data.

Core Classification: minimum

This study satisfies the guideline requirements (85-1) for a metabolism study in rats.

I. MATERIALS

A. Test Material:

Metabolism Studies (# HLA-6117-160):

^{14}C-Cyromazine, CGA-72662
Lot no: CL-XVIII-78 (3 mg/kg dose)
CL-XVIII-77 (300 mg/kg dose)
Radiochemical Purity: 97.2% (3.0 mg/kg dose)
97.7% (300 mg/kg dose)
Specific Activity: 9.8 µCi/mg (3.0 mg/kg dose)
0.8 µCi/mg (300 mg/kg dose)
Unlabelled Cyromazine, S83-0379-1
Chemical Purity: 96.3% (cyromazine standard)
98.8% (3.0 mg/kg dose)
99.0% (300 mg/kg dose)

Metabolite Characterization and Identification (# ABR-89108):
^{14}C-Cyromazine, CGA-72662
Radiochemical Purity: 97.2% (3.0 mg/kg dose)
97.7% (300 mg/kg dose)

B. Vehicle: 1% carboxymethylcellulose plus 4mg/ml Hi Sil 233

Test Animals: Species: rat
Strain: Crl:CD(SD)BR
Source: Charles River Laboratories (Portage, MI facility)
Weight: males, 191-276g; females, 164-216g.

II. METHODS

A. Study Design

1) Metabolism Study

The bioavailability and disposition of ^{14}C-Cyromazine was assessed in male and female rats following both oral and intravenous administration of the test compound. Rats received either a single oral dose of 3 or 300 mg/kg, a single intravenous dose of 3 mg/kg, or 14 repeated daily doses of unlabelled test material at 3 mg/kg followed by a single radiolabelled dose. Dose groups were as follows:

Group	Dose (mg/kg)	Dose Route	Number of Animals Male	Female
1	0 (vehicle control)	IV [a]	1	1
2	3	IV [a]	5	5
3	3	Oral[a]	5	5
4	3	Oral[b]	5	5
5	300	Oral[a]	5	5
6	0 (vehicle control)	Oral[a]	1	1
7	0 (vehicle control)	Oral[c]	1	1
8	0 (vehicle control)	Oral[a]	1	1

[a]single dose
[b]14 daily unlabelled doses followed by one radiolabelled dose.
[c]group 7 was dosed with the vehicle each time group 4 received a dose.

2) Metabolite Characterization and Identification Study

Major urinary metabolites of cyromazine were characterized and identified in this study. In addition, urinary metabolites were compared with those found in fecal extracts, and from these data a major metabolic pathway for biotrans formation of cyromazine in rats was proposed.

Metabolites in rat urine were resolved using either normal phase or reversed phase TLC. Cyromazine and metabolites were isolated from rat urine and characterized by o-chromatography on TLC and HPLC. GC/MS or LC/MS of isolated metabolites was employed to confirm structural identity.

C. Experimental

1) Metabolism Study

a. Animal Husbandry

Animals were acclimated to the laboratory environment for 7 days (groups 4 and 7) or 8 days (all other groups) before study initiation. Animals were examined for health abnormalities during acclimation and at least twice daily for moribundity and mortality. Animals were given food (Purina Certified Rodent Chow #5002) and water *ad libitum*, except for an overnight fast prior to dosing and approximately 4 hours post dosing. Animals were housed in a temperature (72 ± 3 °F), humidity (50 ± 20%) and light (12 hour light/dark) controlled room during the entire course of the study.

b. Dosing

Groups 1 and 2 were administered cyromazine in deionized water *via* intravenous injection into the tail vein at a dose volume of 2 ml/kg, while groups 3 through 8 received cyromazine in 1% carboxymethylcellulose *via*

gavage with a disposable syringe and a steel ball tipped needle at a dose volume of 5 ml/kg. Radiolabeled dose was determined by weighing the dosing syringe before and after dosing.

Body weights were recorded for all animals at randomization and study initiation. Body weights in groups 4 and 7 were recorded daily. During the study period, animals were observed twice daily for mortality and moribundity.

Comment: The type of cage used for collection of urine and feces was not mentioned in this study. In addition, no preliminary study was mentioned which examined possible excretion of test compound as volatiles.

c. Sample Collection and Analysis

Urine was collected on ice at 4, 8, 12, 24, 36, and 48 hours following oral or intravenous administration of test material. Feces were collected at the same times as urine. Cages of treated animals were rinsed with tap water at 24 hours following dosing. Following the last collection time point, cages were rinsed with 1% trisodium phosphate solution and the wash was collected. Urine and feces samples were stored in a freezer before and after analysis.

At sacrifice, rats were anesthetized with halothane and approximately 2 to 5 ml of blood was collected *via* cardiac puncture. After sacrifice, the following tissues were collected, weighed, and saved for analysis of radioactivity: bone (femur), brain, fat (urogenital), ovaries, testes, heart, liver, kidneys, lungs, muscle (thigh), spleen, uterus, and residual carcass.

Small tissue samples (bone, heart, spleen, ovaries, and uterus) were split into two portions and analyzed for radioactivity by direct combustion. Remaining tissues were homogenized and aliquots of approximately 2g were analyzed in duplicate by combustion. Carcasses were ground using a Hobart meat grinder and Cuisinart food processor, and duplicate aliquots of approximately 0.5g were analyzed by combustion. Plasma was analyzed for radioactivity by liquid scintillation counting, while aliquots of blood cells (approximately 0.5g) were analyzed by combustion. Fecal samples were homogenized in 2 volumes of water and duplicate 0.5g samples analyzed by combustion. Duplicate aliquots of urine, cage rinse, and cage wash samples were analyzed by liquid scintillation counting.

d. Statistics

According to the registrant, statistical procedures were limited to simple expressions of variation such as mean and standard deviation.

2) Metabolite Characterization and Identification Study

According to the registrant (page 14), high dose urine gave essentially the same metabolic profile as low dose urine. Thus, because of the greater mass per volume of high dose urine, this dose was chosen for isolation and ident-

ification of metabolites.

Whole urine, urine components, and fecal extracts were analyzed by both normal and reversed phase TLC. Normal phase TLC consisted of 3 solvent systems, identified as follows:

SS1: chloroform: methanol: formic acid: water (75:20:4:2)
SS2: ethyl acetate: ethanol: ammonium hydroxide (80: 15: 5)
SS3: toluene: p-dioxane: methanol: ammonium hydroxide (40: 40: 30: 10)

Reversed phase TLC solvent system consisted of 100% water.

Urine and feces were prepared for TLC through application of urine or feces to a Sephadex A-25 anion exchange column followed by a methanol rinse. Rinse from the urine wash was concentrated to a suitable volume for TLC, while the feces rinse was concentrated to approximately 2ml and then applied to Bio-Rad AG50W-8X cation exchange column. The extract was eluted with 1% NH_4OH in 3:1 water:methanol. This eluate was then concentrated to a suitable volume for TLC application.

Following TLC, plates were scraped at the major areas of radioactivity, then sonicated three times with approximately 10ml methanol. The supernatants from each extraction were pooled and concentrated to dryness.

Whole urine or fecal extract was also subjected to HPLC with both UV and radiochemical detection. Five different procedures were employed for complete metabolite separation and identification, as listed on pages 17-18 of the registrant report. Electron impact mass spectrometry and Thermospray LC/MS were employed for comfirmation of metabolite idenitity and structure.

Standards of cyromazine (96.3%), methylcyromazine (97.8%), hydroxcyromazine (93 and 99%), and melamine (99%) were employed for comparison to unknown radioactive zones on TLC and for mass spectral analysis.

D. Compliance

A signed statement of no data confidentiality claims was provided with both studies.

A signed statement of GLP compliance (40 CFR 160.35) was provided with both studies.

A signed statement of quality assurance was provided with both studies.

III. RESULTS

1) Metabolism Study

The stability of the dose solution for the repeated low-dose study was confirmed by comparative analysis of the dose solution and freshly prepared cyromazine

standard using HPLC. It was stated in the report (page 75) that stability of the low dose solution was verified by the consistent retention times observed between the low dose solution and the cyromazine standard. While consistent retention times are desirable, information regarding the concentration of test article in the dose solution over time would be more appropriate in judging stability of the dose solution.

Verification of dose for rats in the 3 and 300 mg/kg dose groups was presented by comparison of predose and postdose aliquots. Recovery of radiolabel from urine and tissues ranged from 95-103%.

a. Absorption

In those rats dosed orally with 3 mg/kg ^{14}C-cyromazine, 82-86% of the dose was excreted in urine, while approximately 86% of a dose of ^{14}C-cyromazine was excreted in urine in those rats dosed i.v. at this same dose level. Thus, the absorption of ^{14}C-cyromazine appeared complete.

b. Distribution

Analysis of tissue and blood levels of ^{14}C-cyromazine derived radioactivity 7 days following a 3 mg/kg intravenous dose showed undetectable levels of radioactivity in all tissues examined except liver and residual carcass for both male and female rats. Liver levels of ^{14}C-cyromazine derived radioactivity in males ranged from < 0.01 % of the dose to 0.03% (mean value < 0.01%), while levels in residual carcass ranged from 0.002-0.24% of the dose (mean value 0.15%). Liver and carcass values for female rats in this dose group were similar.

The distribution of ^{14}C-cyromazine derived radioactivity in rats given an oral dose of 3 mg/kg ^{14}C-cyromazine was similar to that observed in intravenously dosed rats. Mean liver levels of ^{14}C-cyromazine derived radioactivity were 0.03% of the dose in males, and 0.02% in females. Mean residual carcass levels were 0.16 and 0.13% of the dose in males and females, respectively. In both male and female rats in this dose group, some residual ^{14}C-cyromazine derived radioactivity was observed in red blood cells in a few rats, but amounted to < 0.01% of the dose of ^{14}C-cyromazine.

In rats given 14 repeated oral doses of cyromazine followed by a single dose of ^{14}C-cyromazine, distribution of ^{14}C-cyromazine derived radioactivity showed a similar pattern as that observed in the single dose oral and i.v. studies. The only exception to this was found in female rats in this dose group, where the mean level of ^{14}C-cyromazine derived radioactivity in the carcass was 0.58% of the dose.

Rats in the high dose oral group (300 mg/kg ^{14}C-cyromazine) showed similar residual levels of ^{14}C-cyromazine derived radioactivity in blood and liver, but slightly higher levels of radioactivity in the carcass (mean values of 0.3 and 0.2% of the dose in males and females, respectively) when compared to the single low dose oral groups.

c. Excretion

The excretion of ^{14}C-cyromazine in urine and feces at both 3 and 300 mg/kg is summarized in the following Table:

Excretion of ^{14}C-Cyromazine Derived Radioactivity in Male and Female Rats[a]

	LDM	LDF	IVM	IVF	PCM	PCF	HDM	HDF
urine (+cage wash, rinse, wipe)	82.4± 18.6	86.4± 4.9	86.5± 5.0	86.5± 7.5	91.9± 7.0	90.1± 3.0	83.5± 6.1	86.4± 2.2
feces	4.07± 3.5	3.77± 1.9	5.16± 3.7	6.43± 5.2	3.31± 1.5	2.69± 1.0	7.52± 3.3	6.36± 2.7
carcass (mean)	0.19	0.15	0.16	0.13	0.18	0.6	0.3	0.2
Total	86.5	90.2	91.7	93.0	95.3	93.3	91.3	92.9

Abbreviations are: LD, low dose (3 mg/kg); IV, intravenous dose (3mg/kg); PC, pre-conditioned dose (3mg/kg x 14days); HD, high dose (300 mg/kg).
[a]data represent the mean percent dose excreted at 168 hours post-dosing

With the exception of the males in the low dose oral group, >90% of a given dose of ^{14}C-cyromazine was excreted within 5 days. Examination of the time course of urinary excretion in all dose groups (Figure 1) shows that urinary excretion in males and females was essentially complete in all dose groups by 24 hours, with the exception of the high dose groups, where urinary excretion was not complete until 48 hours.

Fecal elimination of ^{14}C-cyromazine derived radioactivity was a minor route of excretion, representing between 3 and 8% of a given dose in all dose groups. Excretion in the single low dose groups and repeated low dose groups appeared similar (between 3-4% of a given dose), while fecal excretion in the intravenous and high dose groups was somewhat higher (between 5-7% of a given dose). The similarity between the intravenous and oral dose groups in fecal elimination of ^{14}C-cyromazine derived radioactivity supports the conclusion that the fecal radioactivity arises from biliary excretion of the test material. Although recovery of test material was >90% in most cases, the possibility of excretion as volatiles or $^{14}CO_2$ in minor amounts was apparently not explored in this study.

2) Metabolite Characterization and Identification Study

a. Preparative TLC of urine

Rat urine from the high dose groups was separated into 5 zones by preparative TLC. Radioactivity from zone 1 was further resolved by reversed phase TLC into at least 3 components. These components accounted for no more than 0.4% of total urinary radioactivity.

Radioactivity in zone 2 corresponded to the melamine standard. This was confirmed by isolation of zone 2 radioactivity and analysis by GC/MS (Figure 14, page 50 of report, and Figure 16, page 52 of report).

TLC zone 3 was separated into at least 4 components by TLC SS2. The major component of this separation was tentatively identified as hydroxycyromazine, based upon comparison to the percentage isolated from HPLC separation (Table VI, page 30 of report). A minor component of prep TLC zone 3 accounted for 1.1% of total urinary radioactivity, and was tentatively identified as methylcyromazine, based also on comparison to percentages isolated by HPLC (Table VI, page 30 of report). The major component of zone 3 was confirmed as hydroxycyromazine by GC/MS (Figure 14, page 50 of report, and Figure 17, page 53 of report).

Urinary radioactivity in zone 4 was separated into at least 4 additional components using TLC SS2. No component in this zone accounted for more than 1.6% of total urinary radioactivity, and no component of this zone was identified.

Zone 5 of prep TLC represented the largest percentage of total urinary radioactivity (Table III, page 25-26 of report). Isolation of this zone and analysis by GC/MS confirmed the identity of this radioactivity as cyromazine (Figure 14, page 50 of report, and Figure 15, page 51 of report).

b. HPLC of urine

Urinary metabolites of cyromazine were also separated and characterized by HPLC. In this system, urine could be separated into five clusters. Cluster 1 corresponded to the metabolite melamine, based on the retention time of the authentic standard and analysis by GC/MS (Figure 16, page 52 of report).

Cluster 2 retention time did not correspond to the retention times of any of the authentic metabolite standards of cyromazine, or to parent compound. This peak accounted for an average of 7% of total radioactivity in urine in all of the low dose groups, and approximately 3% of total radioactivity in the high dose groups.

Retention time of cluster 3 on HPLC corresponded to the authentic standard of melamine, Cluster 4 to that of hydroxycyromazine, and Cluster 5 to the parent compound, cyromazine.

Quantitation of urinary metabolites by TLC and HPLC is presented in the following Table:

TLC and HPLC Quantitation of Urinary Metabolites of Cyromazine
(values expressed as percent of urinary radioactivity)

	LDM	LDF	IVM	IVF	PCM	PCF	HDM	HDF
HPLC cluster 1 (methylcyromazine)	2.5	2.6	2.4	3.5	1.8	1.8	---	---
TLC zone 3	19.3	15.7	17.6	16.0	19.6	17.0	8.8	8.9

(methyl+hydroxy+ other)								
HPLC cluster 2 (unidentified)	7.6	8.7	6.3	7.3	7.3	6.9	3.2	2.1
TLC zone 3 (methyl+hydroxy)								
HPLC cluster 3 (melamine)	7.6	9.2	6.4	8.6	7.8	12.1	4.3	2.8
TLC zone 2 (melamine)	8.5	12.3	6.3	7.5	5.5	7.3	5.3	5.0

(continued)

HPLC cluster 4 (hydroxycyromazine)	16.3	10.9	8.2	6.8	9.2	4.6	6.1	5.9
TLC zone 3 (methyl+ hydroxy)								
HPLC cluster 5 (cyromazine)	63.2	65.4	71.5	70.8	70.6	69.8	83.6	83.3
TLC zone 5 (cyromazine)	55.8	59.5	67.0	68.1	66.8	70.6	80.7	81.6

Cluster 1

There was no apparent difference in the percentage of urinary radioactivity identified as methylcyromazine when comparing results from i.v. vs oral exposure to cyromazine. Repeated oral dosing resulted in an apparent decrease in the percentage of urinary radioactivity, as indicated by results of HPLC analysis. Because TLC zone 3 was a mixture of metabolites, the effects of different dosing regimens were not apparent.

Cluster 2

Radioactivity in this cluster was not identified by HPLC. This cluster of radioactivity was also a component of TLC zone 3. Because the radioactivity in TLC zone 3 could be further resolved into components identified as methylcyromazine and hydroxycyrom- azine, it is conceivable that some of the unidentified HPLC radioactivity could be stereoisomers of identified metabolites (e.g., methylcyromazine) or other polar metabolites of cyromazine. This becomes more apparent when the radioactivity in HPLC zones 1, 2, and 4 is added and compared to TLC radioactivity in zone 3 (see below).

	LDM	LDF	IVM	IVF	PCM	PCF	HDM	HDF
HPLC cluster 1+2+4	26.4	22.2	16.9	17.6	18.3	13.3	9.3	8.0
TLC zone 3	19.3	15.7	17.6	16.0	19.6	17.0	8.8	8.9

Cluster 3

Cluster 3 was identified as melamine. No apparent effects of i.v. or repeated dosing was observed on formation of this metabolite. However, at the high dose, an apparent decrease in formation of this metaboltie was observed in both male and female rats relative to the low dose exposure. A decrease in the percentage of this metabolite could be observed in the repeated oral dose groups vs the low dose groups when evaluating TLC data; however, this effect was not observed from results of HPLC analysis.

Cluster 4

From HPLC analysis, there was an apparent decrease in formation of hydroxycyromazine in the repeated oral dose and high dose groups relative to the low dose groups.

Cluster 5

Increasing the oral dose of cyromazine resulted in an apparent increase in excretion of unchanged cyromazine, as seen both by HPLC and TLC analysis.

Overall, the most consistent effect observed in this study is a decrease in formation of the methylated and hydroxylated products of cyromazine as well as the N-dealkylated product melamine upon increasing the oral dose from 3 to 300 mg/kg. The apparent alteration in the pattern of cyromazine biotransformation may have some relationship to the altered pattern of urinary elimination observed at the high oral dose of cyromazine. However, the lack of statistical analysis and statement of recoveries of metabolites during sample preparation make a definitive analysis difficult.

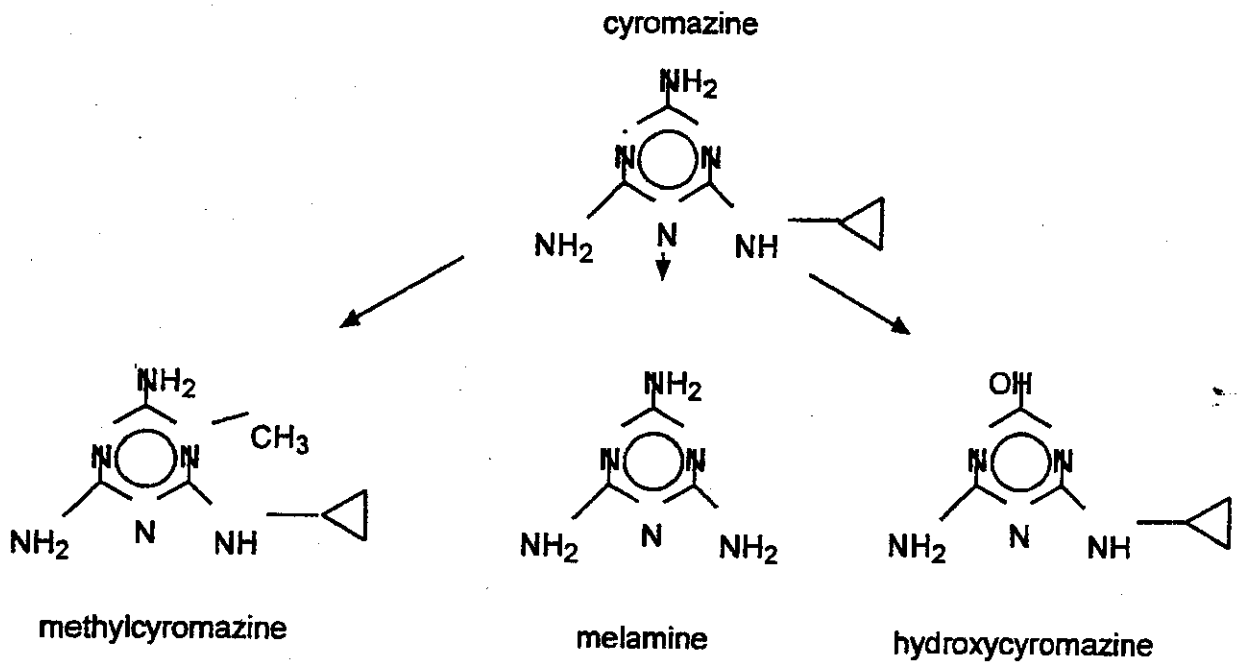
c. TLC of fecal extracts

TLC quantitation of fecal extracts of cyromazine dosed rats is presented in Table VII, page 32 of the registrant report. Metabolites in feces were identified by co-chromatography with authentic standard metabolites of cyromazine. A summary of the findings of this aspect of the study is given here.

Metabolites identified in feces were melamine, cyromazine, hydroxycyromazine, and methylcyromazine. Methyl- and hydroxycyromazine were not distinguishable by TLC. Unmetabolized parent compound accounted for between 68-76% of fecal radioactivity in all dose groups. Melamine accounted for an average of 7%, 7.6%, 6.1%, and 4.7% of fecal radioactivity in the low oral dose, intravenous dose, repeated oral dose, and high dose groups, respectively. Radioactivity comprising the mixture of methyl- and hydroxycyromazine accounted for an average of 12.5%, 7.5%, 5.5%, and 4.7% in the low oral dose, intravenous dose, repeated oral dose, and high dose groups, respectively.

It should be noted that male rats in the low oral dose group had what appeared to be greater percentages of melamine and the methyl- and hydroxylated metabolites of cyromazine in feces when compared to female rats. This was also apparent when comparing metabolite percentages as a percentage of the total dose (Table VII, pages 32 and 33 of registrant report). However, urinary analysis did not show any sex difference in the percentage of these metabolites. While the sex difference in percentage of fecal metabolites found at the low oral dose may be real, it is not known whether some losses occurred for specific fecal metabolites during extraction and analysis.

GC/MS analysis confirmed the identity of parent compound (cyromazine) and the metabolites melamine and hydroxycyromazine in urine of male and female rats. TLC analysis supported the existence of the methyl- and hydroxy metabolites of cyromazine. Other minor metabolites are inferred based upon the small amounts (approximately 6%) of unidentified urinary radioactivity. Based on the results of this study, the proposed pathway of cyromazine biotransformation in rats is as follows:



IV. CONCLUSIONS

In this study, the disposition and metabolism of the insect growth regulator cyromazine was investigated in male and female rats. Data was presented in study # HLA 6117-160 demonstrating the absorption, distribution, and excretion of ^{14}C- cyromazine in male and female rats at a low oral dose (3 mg/kg), a low intravenous dose (3 mg/kg), repeated low oral doses (3mg/kg) and a high dose (300 mg/kg).

Comparison of oral and i.v. disposition data demonstrated that cyromazine was well absorbed after oral administration. Excretion was also rapid at the low dose, with the majority of radioactivity eliminated in the urine by 24 hours. No apparent differences were observed between dosing regimens in the total urinary radioactivity eliminated when cage washes and rinses were taken into consideration. At the high dose, however, there was an apparent delay in elimination of ^{14}C-cyromazine derived radioactivity, suggesting either some type of renal pathology or a shift in biotransformation kinetics of cyromazine. The available evidence indicates that the latter possibility may be the case, as decreases in formation of melamine, methyl- cyromazine, and hydroxycyromazine were observed in high dose rats compared to low dose rats. This possibility deserves further investigation.

Fecal elimination of ^{14}C-cyromazine derived radioactivity was equivalent between dose groups and sexes, with the exception that in the high dose males, a greater percentage of radioactivity was apparently eliminated by this route. The origin of fecal radioactivity from administration of ^{14}C-cyromazine was apparently biliary in nature, as an equivalent percentage

of ^{14}C-cyromazine derived radioactivity was eliminated by both orally dosed and intravenously dosed rats.

Residual ^{14}C-cyromazine derived radioactivity was minimal in all dose groups, with non-detectable levels in all tissues examined except liver, red blood cells, and carcass. However, only one time point was examined for tissue radioactivity in this study, and thus potential accumulation of ^{14}C-cyromazine derived radioactivity could not be demonstrated.

In study # ABR-89108, urinary and fecal metabolites of ^{14}C-cyromazine were isolated and identified by TLC, HPLC, and GC/MS. The N-dealkylated product melamine, hydroxycyromazine, and unmetabolized cyromazine were definitively identified by GC/MS, while tentative identification was given to methylcyromazine, based upon co-chromatography with TLC and HPLC. While some of the *in vivo* metabolites of cyromazine were identified in rats, the kinetics of these biotransformations was not studied. Given the altered urinary excretion seen at the 300 mg/kg dose and the apparent shift in percentage of cyromazine metabolites at this same dose, this avenue of cyromazine biotransformation should be explored.

Core Classification: minimum

This study satisfies the guideline requirements (85-1) for a metabolism study in rats.

Supplement to Tox. Document 0006110, review for MRID 40168601, dermal absorption study in rats. This supplement corrects errors in dermal absorption summary tables included in the original review, and provides a revised executive summary that reflects the corrected dermal absorption data. Revised summary tables are attached.

EPA Reviewer: Becky Daiss Becky Daiss, Date 8/14/02
Branch (7509C)

EPA Secondary Reviewer: Robert Zendzian _____, Date 8/14/02
Branch (7509C)

8/19/2002

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Dermal Absorption in Rats

OPP Number: 85-2
DP BARCODE: D284318
PC CODE: 121301

OPPTS Number: 870.7600
SUBMISSION CODE: S590374
TOX CHEM NO: 167B

TEST MATERIAL (PURITY): "simulated" ^{14}C- Cyromazine - 75W formulated product in an aqueous suspension.

CHEMICAL NAME: Cyromazine

CITATION: Murphy, T.G.; Brown, K.; Doornheim, D. Dermal absorption of cyromazine in rats (special metabolism study in rats) Ciba-Geigy. Report Number: M7-329-11A, 329950, ABR-86069, 1987/2/19 MRID 40168601 Unpublished

SPONSOR: CIBA GEIGY Corporation, Agricultural Division, P.O. Box 18300, Greensboro, NC, 27419

EXECUTIVE SUMMARY:

In a dermal absorption study (MRID 40168601), male rats received dermal application of a "simulated" 75W formulation of ^{14}C-Cyromazine in aqueous solution at doses of 0.10, 1.0 and 10 mg/rat. Four animals per dose were exposed for durations of 2, 4, 10 and 24 hours. A second set of rats (4 per dose) also received dermal doses of 0.10, 1.0 and 10 mg/rat for 10 and 24 exposure periods followed by a 48 hour "depletion period". Mean recoveries of applied radioactivity from all dose groups ranged from 77 to 120%. Table 1 summarizes mean percent absorption at each time period for rats terminated immediately after exposure and percent absorption for animals followed for 48 hours post-exposure.

Table 1. *Mean Percent Absorption - No Post-Exposure Period*

Dose Level (mg/rat)	Mean % of Dose Absorbed (no post-exposure period)				Mean % Dose Absorbed (48 h post exposure period)	
	2 hours	4 hour	10 hours	24 hours	10 hours	24 hours
0.1	2.86	5.25	7.01	6.02	12.61	14.44
1	6.18	2.67	4.54	2.26	7.80	9.81
10	2.00	0.57	0.74	2.38	10.98	8.50

Mean absorption based on blood, urinary/fecal excretion, and carcass, ranged from 0.6 to 7% for animals sacrificed at the end of the exposure periods. For animals exposed for 10 and 24 hours and followed for 48 hours post-exposure, mean absorption ranged from 8 to 14.5%. Total radioactivity absorbed generally decreased as dose increased indicating saturation of absorption with increasing dose. Amounts remaining in/on the skin at termination ranged from 4.5% (10 mg dose/2 h exposure) to 24% (0.1 mg dose/24 hour exposure). The majority of the absorbed radioactivity was found in the urine and carcass. Most of the unabsorbed radioactivity was found in the skin washes from each dose/duration.

There were no deficiencies observed in the study.

The study is classified **Acceptable** and satisfies the guideline requirement 870.7600 (85-3) for a dermal absorption study.

Cyromazine **Dermal Absorption in Rats**

Revised 2/25/02

Table I: Percent of Applied Dose Absorbed, Unabsorbed and Remaining on/in Skin (4 animals per dose)*

	Dose (mg/rat)	2 h	4 h	10 h	24 h
Absorbed[a]	0.1	2.86	5.25	7.01	6.02
	1	6.18	2.67	4.54	2.26
	10	2.00+	0.57	0.74	2.38
Skin[b]	0.1	22.59	19.02	18.64	23.92
	1	9.92	13.58	12.00	21.25
	10	4.46	6.29	8.59	9.25
Absorbed+ Skin	0.1	25.45	24.27	25.65	29.94
	1	16.1	16.25	16.54	23.51
	10	6.46	6.86	9.33	11.63
Unabsorbed[c]	0.1	75.35	64.4	69.86	62.27
	1	83.3	86.00	81.36	75.60
	10	80.86	70.76	67.42	77.39
Total C^{14}	0.1	100.80	88.67	95.51	92.21
	1	100.40	102.25	97.90	99.11
	10	87.32	77.53	76.75	89.02

Animals treated for period specified below followed by a skin wash and a "depletion period" of 48 hours.

	Dose (mg/rat)	10 hours	24 hours
Absorbed[a]	0.1	12.61	14.44
	1	7.80	9.81
	10	10.98	8.50
Skin[b]	0.1	14.81	7.24
	1	8.40	11.49
	10	3.15	5.86
Absorbed + Skin	0.1	27.42	21.68
	1	16.20	21.30
	10	14.13	14.36
Unabsorbed[e]	0.1	92.77	79.89
	1	85.81	81.97
	10	80.73	85.72
Total C^{14}	0.1	120.12	101.57
	1	102.01	103.27
	10	94.86	100.08

a = blood, urine, feces, carcass
b = Skin I, Skin II
c = bandage, bridge rinse, paper rinse, paper, soap rinse, water rinse, gauze A&B
d = blood, urines at 10, 34, & 58 hours, feces at 10, 34, & 58 hours, carcass, cage wash I & II
e = bandage, bridge rinse, paper rinse, paper, soap rinse I & II, water rinse I & II, gauze A, B, C, & D
+ = mean of 3 animals, no sample for feces for one animal
* = Data extracted from Study No. ABAR-86069, Tables III, IV, V, IV & VII

REVIEWEI

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

AUG 14 1987

006110

OFFICE OF
PESTICIDES AND TOXIC SUBSTANC

MEMORANDUM

SUBJECT: Review of a dermal absorption study in rats with Cyromazine.
EPA ID # 100-632; EPA Record # 194838; EPA Accession # 40168601; Caswell #167B; Tox Branch Project 7-0683.

TO: Richard King, PM #17
Herbicide - Fungicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 8/10/87
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Robert P. Zendzian, Ph.D. 5/11/87
Senior Pharmacologist
and
Quang Q. Bui, Ph.D., D.A.B.T. 5/13/87
Acting Section Head, Review Section V
and
Theodore M. Farber, Ph.D., D.A.B.T. 5/14/87
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: CIBA-GEIGY Corporation
Agricultural Division
P.O. Box 18300
Greensboro, North Carolina 27419

Action Requested: Toxicology review of dermal absorption of cyromazine in rats.

Recommendations:

From data presented in this study, radiolabeled Cyromazine (as a "simulated 75W formulation of ^{14}C-Cyromazine") is apparently rapidly absorbed into the skin (no peak discernable) in an inverse dose related manner. The absorption into the skin is followed by a slower release into the body. The main route of excretion is apparently in the urine. The investigators state that "decreasi excretion values 24-48 hours after exposure (maximum 24 hours), means further absorption does not occur", however, there is no evidence that the compound is sequested in the skin "permanently". This study is Acceptable.

006110

Primary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 8/10/87
Review Section V, Toxicology Branch/HED (TS-769C)

Dermal Absorption Secondary Reviewer: Robert P. Zendzian, Ph.D. 8/11/87
Senior Pharmacologist, Toxicology Branch/HED (TS-769C)

Section Head Sign Off: Quang Q. Bui, Ph.D., D.A.B.T. 8/13/87
Acting Section Head, Review Section V, Toxicology Branch/HED (TS-796C)

I. Study Type: Dermal Absorption
Guideline §85-3

Study Title: Dermal Absorption of Cyromazine in Rats
(Special Metabolism Study)

EPA Identification Numbers: EPA Identifying No. 100-632
EPA Accession No. 40168601
EPA Record No. 194838
Shaughnessy No. 121301
Caswell No. 167B
Tox Branch Project No. 7-0683
Document No.

Sponsor: CIBA-GEIGY Corporation
Agricultural Division
P.O. Box 18300
Greensboro, N.C. 27419

Testing Laboratory: CIBA-GEIGY Corporation
P.O. Box 18300
Greensboro, N.C. 27419

Study Number(s): Study (Report) No. ABR-86069
Experiment No. M7-329-11A
Project No. 329950

Study Date(s): February 19, 1987

Study Author(s): Tamara G. Murphy
K. Brown
D. Doornheim

Test Material: Cyromazine (also known as Trigard® and CGA-72662)
N-cyclopropyl-1,3,5-triazine-2,4,6-triamine

Test material is a "simulated" ^{14}C-Cyromazine-75-W formulated product in an aqueous suspension

Vehicle: Aqueous Suspension in deionized water

Dose(s): 0.10, 1.0 or 10 mg/rat

Test Animal: Male Harlan Sprague-Dawley Albino Rats
from Harlan Sprague-Dawley
Madison, Wisconsin

This study was designed to determine the potential dermal absorption of a "simulated 75W formulation of ^{14}C-Cyromazine" in the albino rat.

II. Materials and Methods: A copy of the "materials and methods" section from the investigators report is appended. The following comments and highlights on these "materials and methods" are noted:

The test substance was "Cyromazine uniformly labeled with ^{14}C in the triazine ring..." (see attached Figure 1 from the investigators report) with "...a specific activity of 10.0 uCi/mg for the low and middose levels and 1.00 uCi/mg for the high dose level". The investigators further stated that the "Radioactive purity as determined by TLC analysis was 99%".

Preparation for dermal application of the test compound involved shaving each rat of its dorsal hair approximately 20-24 hours prior to dosing. The area was then washed with acetone. A defined area of 10 sq.cm.(4.0 cm by 2.5 cm) was used. This area was selected to minimize possible oral ingestion of the test compound and further, the rear legs of the animals were "shackled" to prevent scratching of this area.

The investigators employed dermally either 0.10, 1.0 or 10.0 mg/rat of the test compound for exposure periods of 2, 4, 10 and 24 hours. A second set of rats was also used, dermally exposed to the test compound for 10 or 24 hours, washed with soap and water, and then monitored for dose elimination over an additional 48 hour period.

Dose preparation is described in detail in the attached "materials and methods".

This study used 4 male rats per time point with sacrifices at 2, 4, 10, 24, 58 and 72 hours after treatment. Under "study design" it was stated the low dose was 0.05 mg/rat whereas in other parts of the study the low dose was stated as 0.10 mg/rat.

Application procedure is also outlined in the attached "materials and methods". The area of application was covered with a non-occlusive skin cover after allowing the area to air dry for 5-10 minutes after application.

After application, all 2, 4, 10, 24 hour animals were housed in separate metal metabolism cages with "free access" to food and water. At the specified sacrifice time point the animal was anesthetized with ethyl ether, the non-occlusive cover removed, separated into individual parts and each part was placed into individual glass jars containing 50 ml of acetone. The treated skin of the animal was washed first with a liquid detergent and water mixture (20 ml:1 liter) and then with deionized water, both times using sterile guaze squares. Each rinse or component of the cover was brought up to a total volume of 100 ml with

acetone. Two samples of skin were removed, "Skin I" consisting of dosed area and "Skin II" consisting of area covered by the non occlusive skin cover. These skin samples were solubilized and brought up to a volume of 100 ml in toluene (see procedure in attached "materials and methods"). Urine and feces were collected at each time interval along with a cage wash using acetone and water. Animal carcasses were also analyzed for "all recoverable radioactivity". Expired air was not collected.

For the 58 and 72 hour animals, the non occlusive skin cover was removed at 10 and 24 hours, respectively. The treatment of the non occlusive covering and skin washings occured as described previously for the 2, 4, 10 and 24 hour animals. After the skin washing the animals remained in clean metal metabolism cages with urine and feces collection at 10, 34, 58 or 24, 48 and 72 hours (for 58 and 72 hour animals, respectively). Again expired air was not collected.

The radioassay and calculation procedures are described in detail in the attached "materials and methods".

A signed statement relating to confidentiality was included.

A signed statement relating to GLP's was included.

No statement relating to Quality Assurance was provided.

III. Results

The investigators determined the dose variability with standard deviations (dpms/rat) of ±19.5, ±16.0 and ±14.9 for the low, mid and high dose respectively. They attributed this variability to "settlement of the powder formulation".

A. 2 Hour Animals

Table I presents data extracted from the investigators' report. The ^{14}C label recoveries were 100.80, 100.40 and 87.32 percent for the low, mid and high doses, respectively. The percent of the radiolabel "absorbed" (blood, urine, feces, carcass and cage wash) was 3.27, 6.55 and 2.10 percent for the low, mid and high doses, respectively. It was noted that the main route of excretion was by the urine. The cage wash was included in the absorbed count since it represents radiolabel that has passed through the animal (the dosed area is covered). The amount of radioactivity in (or on) the skin was 22.59, 9.92 and 4.46 percent for the low, mid and high doses, respectively. The "absorbed" plus "skin" totals were 25.86, 16.47 and 6.56 for the low, mid and high doses, respectively. The quantity of radiolabel absorbed by the animals was inversely related to dose. The unabsorbed totals (consisting of bandage rinse, bridge rinse, paper rinse, paper, soap rinse, water rinse and gauze A & B) was 74.94, 83.94 and 80.76 for the low, mid and high doses, respectively. It was noted that large quantities were removed by the soap rinse.

B. 4 Hour Animals

From Table I the ^{14}C label recoveries were 88.67, 102.25 and 77.53 percent for the low, mid and high doses, respectively. The percent of radiolabel absorbed was 7.33, 3.53 and 0.81 percent for the low, mid and high doses, respectively. The main route of excretion was again the urine. The radioactivity in (or on) the skin was 19.02, 13.58 and 6.29 percent for the low, mid and high doses, respectively. The "absorbed" plus "skin" totals were 26.35, 17.11 and 7.10 percent for the low, mid and high doses, respectively. The quantity of radiolabel absorbed by the animals was again inversely related to dose. The unabsorbed totals were 62.32, 85.14 and 70.43 percent for the low, mid and high doses, respectively. It was again noted that large quantities were removed by the soap rinse.

C. 10 Hour Animals

Table I shows ^{14}C label recoveries of 95.51, 97.90 and 76.75 percent for the low, mid and high doses, respectively. The values for radiolabel absorbed were 7.57, 5.06 and 0.84 percent for the low, mid and high doses, respectively. The main route of excretion was again the urine. The radioactivity in (or on) the skin was 18.64, 12.00 and 8.59 for the low, mid and high doses, respectively The "absorbed" plus "skin" totals were 26.21, 17.06, and 9.43 for the low, mid and high doses, respectively. The quantity of radiolabel absorbed by the animals was again inversely related to dose. The unabsorbed totals were 69.30, 80.84 and 67.32 percent for the low, mid and high doses, respectively. Again large quantities of the radiolabel were removed by the soap rinse.

C. 24 Hour Animals

Table I shows ^{14}C label recoveries of 92.21, 99.11 and 89.02 percent for the low, mid and high doses, respectively. The values for radiolabel absorbed were 6.87, 2.78 and 2.63 percent for the low, mid and high doses, respectively. The main route of excretion was again the urine. The radioactivity in (or on) the skin was 23.92, 21.25 and 9.25 for the low, mid and high doses, respectively The mid dose was apparently sequestered to a greater extent at 24 hours than that observed at previous time points, although all doses tended towards higher "skin" levels. The "absorbed" plus "skin" totals were 30.79, 24.03, and 11.88 for the low, mid and high doses, respectively. The quantity of radiolabel absorbed by the animals was again inversely related to dose. The investigat stated that "The rate of absorption of ^{14}C-cyromazine is inversely related to dose level." This is apparently supported by the data provided. The unabsorbed totals were 61.42, 75.08 and 77.14 percer for the low, mid and high doses, respectively. Again large quantities of the radiolabel were removed by the soap rinse.

D. 10 Hour Treated Animals with "Depletion" Period

Four animals per dose were treated with radiolabeled cyromazine for 10 hours, the application area was washed and the animals were followed for a 48 hour "depletion" period. Table I presents the results of these measurements. The ^{14}C label recoveries for low, mid and high dose groups were 120.12, 102.01 and 94.86 percent, respectively (low dose total ^{14}C recovery was high as were the Cage Wash I and II levels when compared to the other dose groups). The values for radiolabel absorbed for the low, mid and high dose groups were 30.08, 8.82 and 11.56 percent, respectively. The main route of excretion was again the urine. The radioactivity in (or on) the skin was 14.81, 8.40 and 3.15 percent for the low, mid and high dose groups respectively. The "absorbed" plus "skin" totals were 44.89, 17.22 and 14.71 percent for the low, mid and high doses, respectively. The quantity of radiolabel absorbed by the animals was again inversely related to dose. The unabsorbed totals were 75.23, 84.79 and 80.14 for the low, mid and high dose groups, respectively. Large quantities of radiolabel were removed during the first soap rinse.

E. 24 Hour Treated Animals with "Depletion" Period

Four animals per dose were exposed to radiolabeled cyromazine for 24 hours, the area of application was washed and the animals were followed for a 48 hour "depletion" period. Table I presents the results of these measurements. The ^{14}C label recoveries for low, mid and high dose groups were 101.57, 103.27 and 100.08 percent, respectively. The values for radiolabel absorbed for the low, mid and high dose groups were 16.07, 12.45 and 9.10 percent, respectively. The main route of excretion was again the urine. The radioactivity measured in (or on) the skin was 7.24, 11.49 and 5.86 percent for the low, mid and high dose groups, respectively. The "absorbed" plus "skin" totals were 23.31, 23.94 and 14.96 percent for the low, mid and high doses, respectively. The unabsorbed totals for the low, mid and high dose groups were 78.26, 79.33 and 85.12 percent, respectively. Large quantities of the radiolabel were removed from the skin during the first soap rinse.

IV. Conclusions

From data presented in this study, radiolabeled Cyromazine (as a "simulated 75W formulation of ^{14}C-Cyromazine") is apparently rapidly absorbed into the skin (no peak discernable) in an inverse dose related manner. The absorption into the skin is followed by a slower release into the body. The main route of excretion is apparently in the urine. The investigators state that "decreasing excretion values 24-48 hours after exposure (maximum 24 hours), means further absorption does not occur", however, there is no evidence that the compound is sequested in the skin "permanently".

V. Classification: Acceptable.

0061

Table I: Percent of Applied Dose Absorbed, Unabsorbed and Remainir on (or in) Skin (4 animals per dose point)†

	Dose (mg/rat)	Hours: 2	4	10	24
Absorbed[a]	0.10	3.27	7.33	7.57	6.87
	1.00	6.55	3.53	5.06	2.78
	10.0	2.10+	0.81	0.84	2.63
Skin[b]	0.10	22.59	19.02	18.64	23.92
	1.00	9.92	13.58	12.00	21.25
	10.0	4.46	6.29	8.59	9.25
Absorbed + Skin	0.10	25.86	26.35	26.21	30.79
	1.00	16.47	17.11	17.06	24.03
	10.0	6.56	7.10	9.43	11.88
Unabsorbed[c]	0.10	74.94	62.92	69.30	61.42
	1.00	83.94	85.14	80.84	75.08
	10.0	80.26	70.43	67.32	77.14
Total C^{14}	0.10	100.80	88.67	95.51	92.21
	1.00	100.40	102.25	97.90	99.11
	10.0	87.32	77.53	76.75	89.02

Animals treated for period specified below followed by a skin wash and a "depletion period" of 48 hours.

	Dose (mg/rat)	Treatment Period 10 hours	24 hours
Absorbed[d]	0.10	30.08	16.07
	1.00	8.82	12.45
	10.0	11.56	9.10
Skin[b]	0.10	14.81	7.24
	1.00	8.40	11.49
	10.0	3.15	5.86
Absorbed + Skin	0.10	44.89	23.31
	1.00	17.22	23.94
	10.0	14.71	14.96
Unabsorbed[e]	0.10	75.23	78.26
	1.00	84.79	79.33
	10.0	80.14	85.12
Total C^{14}	0.10	120.12	101.57
	1.00	102.01	103.27
	10.0	94.86	100.08

[a] = blood, urine, feces, carcass, cage wash; [b] = Skin I, Skin II;
[c] = bandage, bridge rinse, paper rinse, paper, soap rinse, water rinse, guaze A & B; [d] = blood, urines at 10, 34 & 58 hours, feces at 10, 34 & 58 hours, carcass, cage wash I & II.
[e] = bandage, bridge rinse, paper rinse, paper, soap rinse I & II, water rinse I & II, guaze A, B, C & D.
+ = mean of 3 animals, no sample for feces for one animal
† = Data extracted from [illegible]

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00611

MATERIALS AND METHODS

This study was conducted according to Biochemistry Protocol 8-86 and the conditions used are described below.

Compound: Cyromazine uniformly labeled with ^{14}C in the triazine ring had a specific activity of 10.0 µCi/mg for the low and middose levels and 1.00 µCi/mg for the high dose level. Radioactive purity as determined by TLC analysis was 99%.

DERMAL APPLICATION

The dorsal hair of all rats utilized in this study was shaved approximately 20-24 hours prior to dosing and the area washed with acetone. A 10 square centimeter area, 4.0 by 2.5, was marked before the dose was applied. The treated area on the upper back of each rat was selected to minimize possible oral ingestion of the compound. The rear legs of the rats were shackled with a short length of stainless steel jewelers chain to prevent scratching of the treated area.

RADIOACTIVE DOSE PREPARATION

^{14}C-Cyromazine was dermally applied at a level of either 0.10 mg/rat, 1.0 mg/rat or 10.0 mg/rat. This study involved rats dosed dermally at three levels and for exposure periods of 2, 4, 10 and 24 hours. A second set of male rats dermally exposed for either 10 or 24 hours were washed with soap and water and monitored for dose elimination over an additional 48-hour period. Rats utilized in this study were male Harlan Sprague-Dawley (Madison, Wisconsin) albino rats weighing 200-250 grams.

The low dose was prepared by dry mixing thoroughly 10 mg of ^{14}C-cyromazine and 3.33 mg of blank formulation (WP) then suspending the mixture with 5.0 ml of deionized water. The middose was prepared by dry mixing thoroughly 100 mg of ^{14}C-cyromazine and 0.33 mg of blank formulant (WP). The mixture was suspended in 5.0 ml of deionized water. The resultant mixtures simulated a typical 75WP formulation.

The high dose was prepared by dry mixing thoroughly 266 mg of blank formulation (WP), 720 mg of unlabelled cyromazine and 80 mg of ^{14}C-cyromazine then suspending the mixture in 8 ml of deionized water. The resultant mixture contained 10% ^{14}C-cyromazine and 90% of unlabelled cyromazine to give a representative 75WP formulation with a ratio of cyromazine to formulant of 3.5:1 (800 mg:266 mg).

STUDY DESIGN

This study utilized four male rats per time point. The treated animals were sacrificed 2, 4, 10, 24, 58, and 72-hours after dermal treatment. Fifty microliters of an aqueous suspension containing either the low (0.05 mg/rat), mid (1.0 mg/rat) or high (10.0 mg/rat) dosage level was applied with a 50 µl Hamilton syringe equipped with a Teflon tip coated needle and plunger tip assembly. The tip of the syringe was used to uniformly spread the suspension over the entire treatment area. The amount of ^{14}C-cyromazine applied to the rat was calculated by radioassay of 50 µl of ^{14}C-cyromazine delivered with the same syringe. The amount of ^{14}C-cyromazine remaining on the Teflon coated needle after application to the rats was considered to be minimal and not measured directly. Calculation of a balance (total ^{14}C recovered) would indicate excessive variation or variances from the intended dose. Skin-Bond® cement (Pfizer Corporation) was evenly spread around a one centimeter border surrounding the dose area. The Stomahesive® was placed on top of the glued area to form a "well" enclosing the treated skin area.

After dosing, the treated area was allowed to air dr for five to ten minutes and the entire treated area enclosed by a nonocclusive covering consisting of fi paper and an aluminum bridge. The aluminum foil bri which was slightly curved to elevate the filter pape was glued to each side of the Stomahesive in order t cross directly over the center of the dose area. Th entire area was "covered" by Whatman No. 1 filter pa which was glued to the Stomahesive barrier.

After treatment, the animals were housed in separate metal metabolism cages with free access to food and water. For the 2, 4, 10 and 24-hour time points, at time of sacrifice the animal was anesthetized with e ether in a dessicator. The entire Stomahesive appli was removed by hand pressure and each component (Stomahesive, aluminum bridge, and paper) was separat and placed into individual glass jars with 50 ml of acetone. The treated skin area was then washed with following solutions: first with Dove liquid/water (20 ml:1 liter) and secondly, with deionized water. Each wash was done using a sterile gauze square. Th soap rinse, water rinse and rinses of the Stomahesive bridge and paper were brought to a 100 ml total volu with acetone. After washing, two samples of skin we removed; the dosed area (skin I) and the surrounding skin covered by the appliance (skin II). The skin samples were placed into 30-40 ml of Beckman Solubil and digested at 50°C overnight (16 hours) in a water shaker bath. The dissolved skins were brought to a ml total volume with toluene. Urine and feces were collected at each time interval and a cage wash was using acetone and water. The carcasses were also analyzed to account for all recoverable radioactivity

For the 58-hour animals, ten hours after dermal exposure and for the 72-hour animals, 24 hours after dermal exposure, the Stomahesive covering was removed and the treated area washed as described in the previous paragraph. After washing, the animals remained uncovered and were placed into clean metal metabolism cages. Urine and feces were collected at 10, 34, and 58 hours after treatment for the 58-hour time period and at 24, 48, and 72 hours for the 72-hour time period. At the time of sacrifice, a second soap and water rinse was done of the treated skin area. The following samples were analyzed at the time of sacrifice: skin I (treated area), skin II (remaining area covered by bandage), blood, carcass, cage wash I (first 10 or 24 hours), cage wash II (last 48 hours), soap rinse I (first 10 or 24 hours), water rinse I (first 10 or 24 hours), soap rinse II (at 58 or 72 hours), water rinse II (at 58 or 72 hours), Stomahesive rinse, paper rinse, bridge rinse, paper and gauze squares.

RADIOASSAY PROCEDURES

Cage wash, bandage rinse, soap rinse, water rinse, paper rinse, bridge rinse, solubilized skin and urine were aliquoted directly. Carcasses were ground with a Hobart food cutter and homogenized using a food grinder attachment. Feces samples were homogenized with dry ice and a micromill. Paper, gauze squares, feces, blood, and carcass homogenates were combusted using a Harvey Oxidizer[1].

The scintillation liquid used for directly aliquoted samples was Scintiverse I (Fisher Scientific Co., Fair Lawn, New Jersey). Combusted samples were collected and counted in Carbon-14 cocktail (R. J. Harvey, New Jersey). All counting was carried out by a Mark III, Model 6881, liquid scintillation counter and efficiencies determined by external standardization. Only solubilized skin samples were counted in a Tracor 6895 scintillation counter. Combustion efficiencies were determined using ^{14}C-mannitol.

CALCULATIONS

Calculation of radioactive metabolic data was done using a desk top computer network using the Hewlett-Packard A600 model. The statistical data were based on radioactive counting[2]. Twice background was just about the break point for a quantitative measurement. To calculate percent of total ^{14}C for the blood, a blood volume of 6.39% was used[3].

Calculations were as follows:

$$DPM = \frac{\frac{Cpm_1 + Cpm_2}{2} - \text{Background}}{\text{Scintillation Counter Efficiency}}$$

$$\text{Total DPM} = \text{DPM} \times \frac{\text{Total Volume/Weight}}{\text{Volume/Weight Assayed}}$$

$$PPM = \frac{DPM/g}{2220 \times \text{weight (g)} \times \text{Sp. Act. } (\mu Ci/mg)}$$

$$\text{Percent } ^{14}C \text{ Recovery} = \frac{\text{Total dpm of sample}}{\text{Total dpm applied}} \times 100\%$$

Total Recovery = The sum of recovery percentages from various fractions radioassayed

EPA Reviewers: Waheeda Mani Tehseen, Ph.D.
OPP-HED Registration Action Branch 3 (7509C)

Date 8/14/2002

EPA Secondary Reviewer: Steve Dapson, Ph. D.
OPP-HED Registration Action Branch 3 (7509C)

Date 09/13/2002

DATA EVALUATION RECORD (DER)

This summary is an upgrade to previously written executive summary and DER (MRID no.00150471, Accession no. 256348 and TXR no. 004263).

STUDY TYPE: Prenatal Developmental Toxicity in Rabbit (gavage); OPPTS 870.3700 [§83-3b]

DP BARCODE: D284318

SUBMISSION CODE: S590374

P.C. CODE: 121301

TEST MATERIAL (PURITY): Cyromazine- CGA-72662 (95.2%)

SYNONYMS: Lavardex Technical, N-cyclopropyl-1,3,5-triazine-2-4-6,-triamine

CITATION: Hagen, C. (1985). A Teratology study (Segment II) in albino Rabbits with Cyromazine Technical: Final Report: Project No. WIL-82001. Study prepared by WIL Research laboratories, Inc. Ashland, Ohio 44805. Jan 23, 1985. MRID no. 00150471. TXR no. 004263. Unpublished.

SPONSOR: Ciba-Geigy Corporaion.

EXECUTIVE SUMMARY:

In a prenatal developmental toxicity study (MRID no.00150471, Accession no. 256348 and TXR no. 004263) in New Zealand White rabbits (16/group- artificially inseminated females), 95.2% Cyromazine was administered by gavage at doses of 0, 5, 10, 30, or 60 mg/kg/day on GD 7-19. The test substance was delivered in 0.5% aqueous carboxymethylcellulose at a dose volume of 1 ml/kg.

All animals were observed daily for moribundity and mortality and clinical signs of toxicity. Females which aborted during the experimental period were sacrificed and necropsied on that day. A gross necropsy was performed on the females which died during the course of the study. Maternal body weights were recorded on gestation days 0, 7, 10, 14, 20, 24, and 29. Mean gravid uterine weights were determined for each female at the scheduled cesarean section. Individual

food consumption was recorded daily from days 0 through 29 of gestation. On gestation day 29, all surviving females were sacrificed by an iv injection of T-61 euthanasia solution. Following sacrifice, the uterus was weighed prior to removal of fetuses. The number and location of viable and nonviable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. All fetuses were individually weighed and examined for malformations and variations. Fetuses were then examined for visceral and skeletal examination after fixation.

Three animals died, one each in the vehicle control, 30 mg/kg and 60 mg/kg groups. The two treated animals died due to intubation errors and not due to treatment. A low pregnancy rate noted in the 10 mg/kg group (38.9%) restricted the extent of information that could have been obtained from this group. Only 7 litters with viable fetuses were obtained from this group with a limited number of fetuses (45). At 5 and 10 mg/kg/day doses, cyromazine did not produce toxic symptoms or any significant changes in food consumption, body weight, and reproductive status in pregnant rats. Three dams with only resorprtions were found at the 30 mg/kg level and one each in the vehicle, 0 and 5 mg/kg groups. The number of corpora lutea was similar between the treated and control groups except for the 10 mg/kg group which had a slightly higher number of corpora lutea. However, these maternal toxicities were not considered treatment related. No compound related changes in preimplantation loss were observed. However, the incidences of postimplantation loss was slightly increased in the two high dosage levels. Significant decreases in the number of female fetuses/dam were noted in the lower doses of 5, 10, and 30 mg/kg, but not in the higher dose of 60 mg/kg group. No significant changes were observed in fetal weights at any dose level. Slight decreases in fetal weight were noted only in the mid doses of 10 and 30 mg/kg groups, but apparently resulted from a larger litter size associated with these two dosage levels. The only treatment related effects observed in pregnant rabbits were decreased body weight gain and food consumption at 30 and 60 mg/kg/day.

For developmental toxicity, several malformations were noted in this study, but none of them were clearly related to the treatment. Cyclopia [1(1) fetus (litter) each in the 10 and 30 mg/kg groups] and diaphagrmatic hernia [1(1) and 3(2) fetuses (litters) in the 10 and 30 mg/kg groups] were observed in the 10 and 30 mg/kg groups. These malformations were not dose related and were not observed in the group of 60 mg/kg. Hydrocephaly was noted in the three highest dose groups (10, 30, and 60 mg/kg/day). However, the incidences of hydrocephaly were comparable to the historical controls. According to the developmental toxicity risk assessment screening committee (TXR numbers 009774 and 006877; Sept. 1992 and Oct. 1992, respectively) these developmental effects were likely due to chance and were not considered treatment-related. This was based on the conclusion that the incidence was within historical control range for the strains utilized. Moreover, these developmental effects were not consistently reproducible across various developmental studies, and clearly not dose-related. Hence, no clear evidence of developmental toxicity in study was noted.

For maternal toxicity, the NOAEL was 10 mg/kg/day and the LOAEL was 30 mg/kg/day, based on decreased body weight gain and food consumption. For developmental toxicity, the NOAEL was $\geq$ 60 mg/kg/day (HDT); a LOAEL was not established.

The developmental toxicity study in the rat is classified **acceptable-guideline** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 b) in rabbit.

EPA Reviewers: Waheeda Mani Tehseen, Ph.D.
OPP-HED Registration Action Branch 3 (7509C)

, Date 8/14/2002

EPA Secondary Reviewer: Steve Dapson, Ph. D.
OPP-HED Registration Action Branch 3 (7509C)

, Date 09/13/2002

DATA EVALUATION RECORD (DER)

This DER is an upgrade to previously written executive summary and DER (MRID no.00103197, Accession no. 070920 / 070921 and TXR no. 002664).

STUDY TYPE:
Two-Generation Reproductive and fertility effects (feeding-rat); OPPTS 870.3800, §83-4

DP BARCODE: D284318

SUBMISSION CODE: S590374

P.C. CODE: 1121301

TEST MATERIAL (PURITY): Cyromazine- CGA-72662 (95.3%)

SYNONYMS: Lavardex Technical, N-cyclopropyl-1,3,5-triazine-2-4-6,-triamine

CITATION: Blair, M.; Slezak, S.; Allen, S.; et al. (1981) Two-generation Reproduction Study with CGA-72662 in Albino Rats: IRDC no. 382-086. (Study received under project no. 100-631; prepared by International Research and Development Corp - IRDC., submitted by Ciba-Geigy Corp., Greensboro, NC; CDL:070920-D; 070921). Jun 10, 1982. MRID 00103197. Unpublished.

SPONSOR: Ciba-Geigy Corporation.

EXECUTIVE SUMMARY:

In a two-generation reproduction study (MRID No. 00103197, TXR No. 002664, IRDC, Study No. 382-086) in Sprague-Dawley rats COBS®CD® rats (15 males and 30 females/group), 95.3% cyromazine was administered at dietary levels of 30, 1000, or 3000 ppm (1.5, 50, or 150 mg/kg/day).

The F0 rats were mated once to produce the F1 litters. F1 rats were randomly selected to mate to produce the F2 litters. Animals were observed for toxicity, behavioral changes, mortality, clinical signs, body weight, food consumption, pregnancy rate, litter size and weight, pup weight, gross pathology, organ weights, and histopathology.

No significant treatment-related effects were observed in general appearance, behavioral changes, parturition and length of gestation in the animals.

There was a significant decrease in parental mean body weights in the F_0 and F_1 generation at 1000 and 3000 ppm. The decreased body weights which ranged for high dose (3000 ppm) males from 14.5% for the F_1 to 17.5% for the F_0, and for high dose females from 17.0% for the F_0 to 20.0% for the F_1, these values represent average differences observed during weeks 1 through 55 of the study, excluding the gestation and lactation days. The mid dose groups had approximately half the differences noted for the high dose groups. At 30 ppm there was a very slight weight loss in the females (4%) but not in the males. The effect was not considered treatment related. Food consumption data followed a similar pattern. The corresponding significant decrease in food consumption of F_0 and F_1 generations at 1000 ppm (slightly decreased) and 3000 ppm group (moderately decreased) was a clear indication of a toxic response to the treatment. No effect was noted on pup survival rates in all groups and generations. Fertility rates were unaffected by treatment. No differences in mean litter size were noted. The difference between the group mean pup weights at birth and on postnatal days 7, 14, and 21 for the high dose and control groups in each generation was consistently statistically significant (p,0.05 or p>0.01). The pup weights in the high dose (3000 ppm) group averaged 10 to 20 % less than that of the control group from birth to weaning.

The parental systemic NOAEL was 1000 ppm (50 mg/kg/day), and the parental systemic LOAEL was 3000 ppm (150 mg/kg/day), based on decreased body weights that were associated with decreased food efficiency. The "offspring systemic / developmental" NOAEL was 1000 ppm (50 mg/kg/day) and the "offspring systemic/developmental" LOAEL was 3000 ppm (150 mg/kg/day), based on decreased body weights at birth and through weaning. The "reproductive" NOAEL is ≥ 3000 ppm (150 mg/kg/day - HDT). No reproductive LOAEL was determined.

The reproductive study in the rat is classified **acceptable-guideline** and does satisfy the guideline requirement for a two-generation reproductive study (OPPTS 870.3800, §83-4) in rat.

Note: See the supplementary documents - (TXR numbers 009774, Developmental peer review, July 1992, memo 11/7/191,)

EPA Reviewers: Waheeda Mani Tehseen, Ph.D. Date 8/14/2002
Registration Action Branch 3 (7509C)

EPA Secondary Reviewer: Steve Dapson, Ph. D. Date 09/13/2002
Registration Action Branch 3 (7509C)

DATA EVALUATION RECORD (DER)

This summary is an upgrade to previously written executive summary and DER (MRID no.00103193, Accession no. 070919 and TXR no. 002664).

STUDY TYPE: Chronic (6 months) Oral Toxicity in Dogs (feeding);

OPPTS 870.4100, Guideline: §83-1b

DP BARCODE: D284318 SUBMISSION CODE: S590374

P.C. CODE: 121301

TEST MATERIAL (PURITY): Cyromazine- CGA-72662 (96.3%)

SYNONYMS: Lavardex Technical, N-cyclopropyl-1,3,5-triazine-2-4-6,-triamine

CITATION: Marshall, P.; Voelker, R.; Dawkins, K. (1980) Subchronic Toxicity Study in Dogs: CGA-72662 Technical. Final rept. Project no. 100-631; prepared by Hazleton Laboratories America, Inc., submitted by Ciba-Geigy Corp., Greensboro, NC; CDL:070919-B. Hazelton Lab. No. 483-180. Jun 10, 1982. MRID no. 00103193. TXR no. 002664. Acession no. 070919. Unpublished.

SPONSOR: Ciba-Geigy Corporation.

EXECUTIVE SUMMARY:

In this study (MRID No. 00103193, TXR No. 002664) groups of male and female beagle dogs (3/sex/dose at 30 and 300 ppm and 4/sex/dose at 3000 ppm and control) were fed diets containing cyromazine at 0, 30, 300 or 3000 ppm (0, 0.75, 7.5 or 75 mg/kg/day, respectively) for 6 months.

All dogs were housed individually in controlled environments. All dogs were observed twice daily for mortality and morbundity and once daily for appearance, behavior, appetite, elimination, and signs of toxicity. Food consumption were recorded twice weekly. Hematology, clinical chemistry, and urinalysis were performed on all dogs at various intervals during the treatment period and for recovery animals at week 30. Ophthalmologic examinations were performed on all dogs prior to treatment and during week 12 and 26. Selected surviving animals were sacrificed

for gross and microscopic pathology.

No treatment-related effects were observed in survival, clinical signs, food consumption, body weights, ophthalmologic parameters and gross and microscopic pathology. Mean body weight and body weight gain values were minimally decreased in males at the highest dose, and in females at all dose levels. Mean food consumption values of the high dose animals were also slightly decreased. These changes were slight, hence may not be considered treatment related.

Pronounced treatment-related effects on hematological parameters, were manifested in males and females as decreases in hematocrit and hemoglobin levels at 3000 ppm. Decreased mean cholesterol and serum glutamic oxaloacetic transaminase (SGOT) values were noted at high dose (3000 ppm) during the treatment time period, however, the values reached normal during the recovery period. Slight increases were noted for the relative brain, heart, and liver weights of the high dose (3000 ppm) males and females and the ovary weights of the high dose females.

The systemic toxicity NOAEL was 300 ppm (7.5 mg/kg/day) and the systemic toxicity LOAEL was 3000 ppm (75 mg/kg/day) based on decreases in hematological parameters (hematocrit and hemoglobin), body weights, and slight increases in brain, heart and liver weights .

This chronic toxicity study is classified **acceptable-guideline** and does satisfy the guideline requirement for a chronic oral study (82-1b) in dogs.

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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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.

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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

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Pages 57 through 67 are not included in this copy.

The material not included contains the following type of information:

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_____ Identity of product impurities.

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_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

✓ Information about a pending registration action.

_____ FIFRA registration data.

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_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

EPA Reviewer: Waheeda Mani Tehseen, Ph. D. Date 1/17/2003
OPP-HED Registration Action Branch 3 (7509C)
EPA Secondary Reviewer: Steve Dapson, Ph. D. Date 07/01/2003
Registration Action Branch 3 (7509C)

DATA EVALUATION RECORD (DER)

This DER is an upgrade to previously written executive summary and DER (MRID no.00103202; 00115735, and TXR no. 002617).

STUDY TYPE: Combined Chronic Toxicity/Carcinogenicity Study in Rats
OPPTS Number: 870.4300 OPP Guideline Number: §83-5

DP BARCODE: D284318 SUBMISSION CODE: S590374
P.C. CODE: 121301

TEST MATERIAL (PURITY): Cyromazine, (95.5%)

SYNONYMS: Lavardex Technical, N-cyclopropyl-1,3,5-triazine-2-4-6,-triamine

CITATION: Blair, M.; Kruger, S.; Nair, K. (1982) 2-year Chronic and Oncogenicity Study with CGA-72662 Technical in Albino Rats (12-month Interim): IRDC No. 382-081. Study received under 100-631; prepared by International Research and Development Corp., submitted by Ciba-Geigy Corp., Greensboro, NC; CDL:070923-A. Jun 10, 1982. MRID No. 00103202. Unpublished.

Blair, M.; Vander Meer, D.; Marroquin, F.; et al. (1982) 2-year Chronic and Oncogenicity Study with CGA-72662 Technical in Albino Rats: 382-081. Study received under IRSD No. 100-631; prepared by International Research and Development Corp., submitted by Ciba-Geigy Corp., Greensboro, NC; CDL:071186-A; 071187; 071188; 071189. Oct 20, 1982. MRID No. 00115735. Unpublished.

SPONSOR: Ciba-Geigy Corporation

EXECUTIVE SUMMARY:

A 24-month chronic toxicity/oncogenicity study in Charles River Sprague-Dawley rats was conducted by IRDC, Mattawan, Michigan, and issued June 30, 1982, for Ciba-Geigy Corporation, Greensboro, North Carloina (IRDC Study No. 382-081).

The study design allocated groups of 60 rats per sex to dose levels of 0, 30, 300 and 3000 ppm (0, 1.5, 15, 150 mg/kg/day) of Cyromazine (95.5% pure cyromazine - Larvadex technical). An additional 10 animals per sex in the control and 3000 ppm groups were designated for interim sacrifice at one year.

The statistical evaluation of mortality provided by the registrant indicated no significant incremental charges with increasing doses of Cyromazine in male or female rats (41% survival in HDT vs. 42% survival in controls). Survival in males and females were excellent in all groups after 24 months.

Female rats had decreased body weight gain at Cyromazine doses of 300 and 3000 ppm, as did males at doses of 3000 ppm. Body weight gains were reduced by 22% in the high-dose males and 33% in females, and in the mid-dose females body weight gain was reduced by about 10% from week 49 until termination. Food consumption was also decreased at these dose levels; however food efficiency was unchanged from control.

Cystic hyperplasia of the mammary gland was observed only in 1/9 control female rat at the interim sacrifice. At the 24-month sacrifice, cystic hyperplasia was found in 0/55, 1/59, 5/56, and 4/59 rats in the 0, 30, 300, and 3000 ppm groups, respectively.

Female rats showed a number of different neoplasms of the mammary glands. There was no increase in the incidence of benign tumors of the mammary gland due to cyromazine treatment. Although the number of animals with the malignant tumors in the mammary gland was slightly greater in the highest dose group (150 mg/kg/day) as compared to the control females, this difference was not statistically significant and there was an absence of dose response. Neither the adenomas nor the fibroadenomas nor the combination of adenomas, fibroadenomas and adenocarcinaomas showed a significant trend ($p<0.01$) and pair-wise comparison ($p<0.05$). Moreover the incidence of mammary tumors in treated rats did not exceed the historical control data, and the tumors were not considered treatment related (HED CPRC, Jan. 6, 1995, TXR No. 0011384).

Based on statistical analysis, there was a slight increased incidence of renal interstitial cell tumors in high dose males (6/57 or 11%) compared to controls (1/60 or 2%), however, these lesions were within the historical control range and the lesions were not considered treatment related.

Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing may have been excessive for assessing the carcinogenic potential of Cyromazine in rats, based on depressions in mean body weight gain of 22% in males, and 33% in females at doses of 3000 ppm. This rather large depression in body weight gain was not, however, accompanied by increases in mortality or signs of toxicity. Morever, the body weight gain seemed to be reversible, increasing towards control levels during the four-week post treatment period.

The systemic toxicity LOAEL is 300 ppm (15 mg/kg/day) based on decreased body weight and the systemic toxicity NOAEL is 30 ppm (1.5 mg/kg/day).

This study is **acceptable-guideline** and satisfies the requirement for a combined chronic/carcinogenicity study (OPPTS # 870.4300 / 83-5) in rat.

EPA Reviewers: Waheeda Mani Tehseen, Ph.D. , Date 8/14/2002
OPP-HED Registration Action Branch 3 (7509C)

EPA Secondary Reviewer: Steve Dapson, Ph. D. Stephen C. Dapson, Date 07/01/2003
OPP-HED Registration Action Branch 3 (7509C)

DATA EVALUATION RECORD (DER)

This DER is an upgrade to previously written executive summary and DER (MRID no.00027488 and TXR no. 003873).

STUDY TYPE: Prenatal Developmental Toxicity in Rat (gavage); OPPTS 870.3700 [§83-3]

DP BARCODE: D284318

SUBMISSION CODE: S590374

P.C. CODE: 121301

TEST MATERIAL (PURITY): Cyromazine- CGA-72662 (96.3%)

SYNONYMS: Lavardex Technical, N-cyclopropyl-1,3,5-triazine-2-4-6,-triamine

CITATION: Tasker, E.J. ; Rodwell, D.E. (1979) International Research and Developmental Corporation (IRDC). Mattawan, Michigan 49071. Teratology study in rats. Study Number 382-070. December 21, 1979. MRID no. 00027488. TXR no. 003873. Unpublished.

SPONSOR: Ciba-Geigy Corporaion.

EXECUTIVE SUMMARY:

The developmental toxicity range finding study (MRID no: 00027488; IRDC no: 382-069 & TXR no: 003873 - August 7, 1979) was adequate to establish dosing levels for this primary study. In this range finding rat study, technical Cyromazine (CGA - 72662 technical) was administered to 30 animals by gavage on gestation days 6-20. Dose levels were 0, 300, 600, 1000, 1500 mg/kg/day. Severe toxicity was observed at 2500 mg/kg/day. At 1500 mg/kg/day significant reduction in maternal weight gains and clinical signs were observed. Reported results at 300, 600 and 1000 mg/kg/day supported the use of these dose levels and below for the primary prenatal developmental toxicity study.

In the primary prenatal developmental toxicity study in COBS® CD® rats (25/group) (MRID no: 0002748; TXR no: 003873 - IRDC no: 382-070), technical Cyromazine (CGA - 72662 technical) was administered by gavage in aqueous 1.0% carboxymethylcellulose at a dose volume of 10 ml/kg on gestation days 6-19. Dose levels were 0, 100, 300, or 600 mg/kg/day.

All animals were housed in individual cages. Animals were observed daily for mortality and clinical signs of toxicity. Individual maternal body weights were recorded on gestation days 0, 6, 9, 12, 16 and 20. No data were provided for food consumption. On gestation day 20, all dams were sacrificed . Following sacrifice, the uterus was weighed prior to removal of fetuses. The number and location of viable and nonviable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for morphological changes. All fetuses were individually weighed and examined for malformations and variations. Fetuses were examined for visceral and skeletal examination after fixation.

At 300 and 600 mg/kg/day dose levels, animals showed red nasal discharge and increase in matting and staining of the anogenital haricoat. At the lowest dose only rats showed clear oral discharge on 2 separate days. The mid dose (300 mg/kg/day) group showed a slight reduction in overall maternal body weight gain (0-20 days), whereas the high dose (600 mg/kg/day) group showed a reduction in both overall maternal body weight gain and body weight gain during the dosing periods (days 6-19). All of the dams survived. There were no statistically significant differences in mean numbers of viable fetuses, late or early resorptions, postimplantation loss, total implantations, corpora lutea or fetal sex distribution in any treatment group compared to control. At the highest dose level, a significant increase in skeletal variations (unossified sternebrae) was observed in fetuses.

For maternal toxicity, the NOAEL was 100 mg/kg/day, and the LOAEL was 300 mg/kg/day, based on increased incidences of clinical observations (red or clear nasal discharge) and decreased body weight gain. For developmental toxicity, the NOAEL was 300 mg/kg/day, and the LOAEL was 600 mg/kg/day, based on increased incidence of minor skeletal variations.

The developmental toxicity study in the rat is classified **acceptable-guideline** and does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 a) in rat.

EPA Reviewers: Waheeda Mani Tehseen, Ph.D. , Date 8/14/2002
Registration Action Branch 3 (7509C)

EPA Secondary Reviewer: Steve Dapson, Ph. D. , Date 08/13/2002
Registration Action Branch 3 (7509C)

DATA EVALUATION RECORD (DER)

This summary is an upgrade to previously written executive summary and DER (MRID no.00135433, Accession no. 098385 and TXR no. 002687).

STUDY TYPE: Subchronic (13 Weeks) Oral Toxicity in Rats (feeding);

OPPTS 870.3100, Guideline: §82-1a

DP BARCODE: D284318

SUBMISSION CODE: S590374

P.C. CODE: 121301

TEST MATERIAL (PURITY): Cyromazine- CGA-72662 (96.3%)

SYNONYMS: Lavardex Technical, N-cyclopropyl-1,3,5-triazine-2-4-6,-triamine

CITATION: Goldenthal, E.; Jessup, D.; Geil, R.; et al. (1979) 90-Day Subacute Toxicity Study with CGA-72662 in Albino Rats: IRDC no. 382-052. Under 100-EX-65; prepared by International Research and Development Corp., submitted by Ciba-Geigy Corp., Greensboro, NC; CDL:098385-B. June 19, 1979 MRID no. 00135433. TXR no. 002687. Acession no. 098385. Unpublished.

SPONSOR: Ciba-Geigy Corporation.

EXECUTIVE SUMMARY:

In this study (MRID No. 00135433. TXR No. 002687) groups of male and female Albino rats (20/sex/dose) were fed diets containing cyromazine at 0, 30, 300, 1000 or 3000 ppm (0, 3, 30, 100, or 300 mg/kg/day, respectively) for 13 weeks.

All animals were observed for general signs of toxicity and mortality twice daily. Ophthalmoscopic exams were conducted pre-treatment and at terminal. Food consumption and body weight were recorded weekly. Hematology, clinical chemistry, and urinalysis were performed on days 29, 61 and 89. All animals were sacrificed for gross and microscopic pathology.

No treatment-related effects were observed in survival, clinical signs, ophthalmologic

parameters, hematology, clinical chemistry, urinalysis, and microscopic pathology. Males showed increased relative heart and testes weights at 1000 and 3000 ppm, however, absolute weights of these organs were not changed. Animals showed decreases in body weights at the highest dose, however, this decrease was associated with a decreased food consumption.

Males showed highly significant ($P < 0.01$) decreases in absolute and relative liver weights at 300, 1000 and 3000 ppm. Males also showed slight decreases in liver weights values at 30 ppm.. The females did not demonstrate any clear compound-related effects on liver weight.

The NOAEL was 30 ppm (3 mg/kg/day) and the LOAEL was 300 ppm (30 mg/kg/day) based on alteration in liver weights in males.

This subchronic toxicity study is classified **acceptable-guideline** and does satisfy the guideline requirement for a subchronic oral study (82-1a) in rats.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

002687

DATE: November 9, 1979

SUBJECT: 100-EUP-65 and 66, PP#9G2230, CGA 72662 (N-Cyclopropyl-1,3,5-triazine-triamine) feedtrough larvicide in poultry; topical for poultry, beef c sheep and hog manure (including feedlots).

FROM: Robert B. Jaeger
Toxicology Branch (TS-769)

TO: Franklin Cee
Product Manager#17

Petitioner: Agricultural Division
CIBA-GEIGY Corp.

Petition No.: 9G2230
100-EUP-65 and 66

Temporary Tolerance: 0.2 ppm - meat, fat, and meat by-products of bee cattle, sheep and hogs

0.6 ppm - eggs and meat, fat, and meat by-produc poultry

Recommendation

Do not grant the EUP's and associated temporary tolerances.

Data Required in Support of EUP's

1. 90-Day Subchronic Oral Dosing Study in a Rodent - demonstration of a NEL.

2. Teratology Study - one species.

3. Reproduction Study (Rodent) - evaluation at least up through the first generation submitted.

4. Mutagenicity Screen - (a) Chromosome (cytogenic)

5. Broiler Chicken Feeding Study - 8-week feeding study (preferably i drinking water) at exaggerated dose; 25 broilers.

6. Laying Chicken Feeding Study - reproduction study to include reari laying, and hatching (to cover 4-week growth period after hatching) day one to 4 months; evaluation of egg production; exaggerated dose; 10 hens for each of 2 roosters.

7. Submit copies of the proposed labels.

Body Weight Changes: There was no clear dose related effect on bo weight. Females were affected to a greater extent than males, showing less weight gain the 3000 ppm level than controls. Males wer affected slightly at 3000 ppm but not signif different from 30 or 300 ppm levels (i.e. we gain for 1000 ppm males was the same on cont

Females (wk - 1 to + 13)

0	30	300	1000	3000
+1.7	+2.6	+1.5	+1.3	+0.8

Males (wk - 1 to + 13)

0	30	300	1000	3000
+2.2	+1.9	+1.7	+2.2	+1.4

Histopathology

Histopathology was unremarkable and there were no compound-related effects on any of the tissues sectioned.

Conclusion

The NOEL for this study is 300 ppm based on the increase in relative liver weight for males at the 1000 ppm and 3000 ppm levels. They wer significant at $p < 0.025$ and $p < 0.1$ respectively.

Classification: CORE Minimum

Accession No. 098385

E. 90-Day Subchronic Oral Toxicity Study in Albino Rats (Tech.) 6/19/ (IRDC)

Animal: Charles River CD Albino Rats

No. & Sex: 110M/110F, 20 rats/sex/group (additional 5 rats/sex/gr for control and high dose groups - so called "Recovery Group")

(18)

002687

3 30 100 300

Doses: 0, 30, 300, 1000, 3000 ppm

Route of Administration: in the diet

Observations:

ophthalmoscopic exam

conducted pretest and terminal (also at 16 wks for recovery group)

general signs of toxicity and mortality

observed twice daily

individual body weight and food consumption

determined weekly

laboratory tests (hematology, biochemistry, urinalysis) - day 29, 61 and 86 (urine collected day 83 and 89);

Recovery group at 118 days as well.

Gross Necropsy: all rats

Histopathology: all rats

organs weighed: liver, kidneys, testes, heart, spleen, brain.

tissues micro:

high dose and control - adrenals, aorta, bone marrow, brain, cecum, colon, esophagus, eye, gonads, Harderian gland, heart, kidney, liver, lung, cervical and mesenteric lymph nodes, mammary gland, skeletal muscle, optic nerve, pancreas, parathyroid and thyroid, peripheral nerve (sciatic), pituitary, prostate, salivary gland, skin, small intestine (duodenum jejunum, ileum), spinal cord, spleen, sternum, stomach (cardi fundus, pylorus), trachea, thymus, urinary bladder, uterus and gross lesions.

low and mid dose - liver, kidney, heart and gross lesio

002687

Results:

Opthalmoscopy - performed with binocular indirect ophthalmoscope after 1% tropicamide solution was placed in the eye to dilate the pupil. No differences between control and treated animals were noted.

Laboratory Tests:

Hematology: total platelet count, erythrocyte counts, total and differential leucocyte counts, hematocrit, hemoglobin, prothrombin time. No compound-related effects were noted.

Biochemistry: Calcium, potassium, SLDH, direct and total bilirubin, albumin, globulin, SGOT, SGPT, SAP, BUN, fastedblood glucose, total cholesterol, total protein. No compound-related effects were noted other than a dose-related decrease in calcium (mg/100 ml) for males, significant at 1000 and 3000 ppm. All the determinations were within normal limits.

Urinalysis: description of appearance, measurement of volume, pH, specific gravity, and qualitative tests for protein, glucose, ketones, bilirubin and urobilinogen; and microscopic examination of sediment. No compound-related effects noted.

General Behavior, Appearances, Survival - No compound-related effects noted. Survival was not affected by treatment.

Gross Necropsy: Mode of death - euthanitized by CO_2 asphyxiation and necropsied.

No compound-related lesions noted.

Organ weight changes:

The following relative organ weight change was considered significant compound related effects:

Relative Heart

Male - significant increase at 1000 and 3000 ppm

Relative Testes

Significant increase at 1000 and 3000 ppm.

Relative Liver

Male - significant decrease (Student t-Test)

002687

30	$p < .05$
300	$p < .01$
1000	$p < .01$
3000	$p < .01$

(Absolute liver weights were also significantly decreased at all dose leve $p < .05$)

The females did not demonstrate any clear compound-related effect except a the high dose in some instances.

Body Weight Changes

The following changes in body weight are noted:

Dose	Male	Female
0	430	267
30	431(-1.6)	270(+1.1)
300	458(+4.6)	264(-1.1)
1000	419(-4.3)	254(-4.9)
3000	410(-6.4)	236(-11.6)

The adverse effects noted at 3000 ppm for both sexes can be associated wit a decreased food consumption. However, the organ: body weight effects noted (liver) for males is considered compound-related since food consumpt was not altered, body weight changes did vary between gains and losses, an yet absolute organ weight (liver) was significantly decreased in both circumstances.

Histopathology

There were no compound-related effects on any of the tissues examined.

Conclusion

The NOEL for this study has not been demonstrated. The significant decrease in relative and absolute liver weight for males at all levels fed even though laboratory evaluation (hemat., biochem.) and both gross and histopathology examination failed to substantiate adverse effects, is justification for a thorough evaluation in this species. The rat is apparently the more sensitive species tested (rat vs. dog). "The weights of the livers were depressed more, in proportion to body weight, than woul have been expected by chance: indicative of potential deleterious effect o this organ" (Weil, C.S. "Significance of organ weight changes in food safe evaluations", pp. 445, in: Francis J.C. Roe, ed. Metabolic Aspects of Foo Safety, (1970).

Classification: CORE-Minimum

TOX/HED:th:RD Initial WOODRUM:11-9-79

EPA Reviewers: Waheeda Mani Tehseen, Ph.D. -- , Date 8/14/2002
Registration Action Branch 3 (7509C)

EPA Secondary Reviewer: Steve Dapson, Ph. D. , Date 09/13/2002
Registration Action Branch 3 (7509C)

DATA EVALUATION RECORD (DER)

This summary is an upgrade to previously written executive summary and DER (MRID no.00135432, Accession no. 098385 and TXR no. 002687).

STUDY TYPE: Subchronic (13 Weeks) Oral Toxicity in dogs (feeding);

OPPTS 870.3100, Guideline: §82-1b

DP BARCODE: D284318

SUBMISSION CODE: S590374

P.C. CODE: 121301

TEST MATERIAL (PURITY): Cyromazine- CGA-72662 (96.3%)

SYNONYMS: Lavardex Technical, N-cyclopropyl-1,3,5-triazine-2-4-6,-triamine

CITATION: Jessup, D.; Geil, R.; Mehring, J.; et al. (1979) 90-Day Subacute Oral Toxicity Study with CGA-72662 in Purebred Beagle Dogs. IRDC no. 382-048 under 100-EX-65; prepared by International Research and Development Corp., submitted by Ciba-Geigy Corp., Greensboro, NC; CDL: 098385-A. June 19, 1979 MRID no. 00135432. TXR no. 002687. Acession no. 098385. Unpublished.

SPONSOR: Ciba-Geigy Corporation.

EXECUTIVE SUMMARY:

In this study (MRID no. 00135432. TXR no. 002687) groups of male and female Beagle Dogs (4M/4F in each low and medium dose and 6M/6F in high dose and control) were fed diets containing cyromazine at 0, 30, 300, 1000 or 3000 ppm (0, 0.75, 7.5, 25, and 75, respectively) for 13 weeks.

All animals were observed for general signs of toxicity and mortality daily. Ophthalmoscopic parameters were observed in all animals on 8, 12 and 17 weeks (withdrawal dogs only). Food consumption and body weights were recorded weekly. Hematology, clinical chemistry, and urinalysis were performed on weeks 4, 7, 12 and 17 (withdrawal dogs only). All animals were sacrificed for gross and microscopic pathology.

No treatment-related effects were observed in survival, clinical signs, ophthalmologic parameters hematology, clinical chemistry, urinalysis, gross necroscopy, and microscopic pathology. Males showed significant increases in relative liver weights at 1000 and 3000 ppm.. Females did not show this effect in the liver. Kidney weights were increased significantly at 3000 ppm in both sexes. There was no clear dose related effect on body weights.

The NOAEL was 300 ppm (7.5 mg/kg/day) and the LOAEL was 1000 ppm (25 mg/kg/day) based on alteration in liver weights in males.

This subchronic toxicity study is classified **acceptable-guideline** and does satisfy the guideline requirement for a subchronic oral study (82-1b) in dogs.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

002687

DATE: November 9, 1979

SUBJECT: 100-EUP-65 and 66, PP#9G2230, CGA 72662 (N-Cyclopropyl-1,3,5-triazine-2 triamine) feedthrough larvicide in poultry; topical for poultry, beef ca sheep and hog manure (including feedlots).

FROM: Robert B. Jaeger
Toxicology Branch (TS-769)

TO: Franklin Gee
Product Manager#17

Petitioner: Agricultural Division
CIBA-GEIGY Corp.

Petition No.: 9G2230
100-EUP-65 and 66

Temporary Tolerance: 0.2 ppm - meat, fat, and meat by-products of beef cattle, sheep and hogs

0.6 ppm - eggs and meat, fat, and meat by-products poultry

Recommendation

Do not grant the EUP's and associated temporary tolerances.

Data Required in Support of EUP's

1. 90-Day Subchronic Oral Dosing Study in a Rodent - demonstration of a NEL.

2. Teratology Study - one species.

3. Reproduction Study (Rodent) - evaluation at least up through the first generation submitted.

4. Mutagenicity Screen - (a) Chromosome (cytogenic)

5. Broiler Chicken Feeding Study - 8-week feeding study (preferably in drinking water) at exaggerated dose; 25 broilers.

6. Laying Chicken Feeding Study - reproduction study to include rearing laying, and hatching (to cover 4-week growth period after hatching); day one to 4 months; evaluation of egg production; exaggerated dose; 10 hens for each of 2 roosters.

7. Submit copies of the proposed labels.

002687

Body Weight Changes: There was no clear dose related effect on body weight. Females were affected to a greater extent than males, showing less weight gain at the 3000 ppm level than controls. Males were affected slightly at 3000 ppm but not significan different from 30 or 300 ppm levels (i.e. weight gain for 1000 ppm males was the same on controls

Females (wk - 1 to + 13)

0	30	300	1000	3000
+1.7	+2.6	+1.5	+1.3	+0.8

Males (wk -1 to + 13)

0	30	300	1000	3000
+2.2	+1.9	+1.7	+2.2	+1.4

Histopathology

Histopathology was unremarkable and there were no compound-related effects on any of the tissues sectioned.

Conclusion

The NOEL for this study is 300 ppm based on the increase in relative liver weight for males at the 1000 ppm and 3000 ppm levels. They were significant at $p < 0.025$ and $p < 0.1$ respectively.

Classification: CORE Minimum

Accession No. 098385

E. 90-Day Subchronic Oral Toxicity Study in Albino Rats (Tech.) 6/19/79 (IRDC)

Animal: Charles River CD Albino Rats

No. & Sex: 110M/110F, 20 rats/sex/group (additional 5 rats/sex/group for control and high dose groups - so called "Recovery Group")

(18)

002687

3 30 100 300

Doses: 0, 30, 300, 1000, 3000 ppm

Route of Administration: in the diet

Observations:

ophthalmoscopic exam

conducted pretest and terminal (also at 16 wks for recovery group)

general signs of toxicity and mortality

observed twice daily

individual body weight and food consumption

determined weekly

laboratory tests (hematology, biochemistry, urinalysis) - day 29, 61 and 86 (urine collected day 83 and 89);

Recovery group at 118 days as well.

Gross Necropsy: all rats

Histopathology: all rats

organs weighed: liver, kidneys, testes, heart, spleen, brain.

tissues micro:

high dose and control - adrenals, aorta, bone marrow, brain, cecum, colon, esophagus, eye, gonads, Harderian gland, heart, kidney, liver, lung, cervical and mesenteric lymph nodes, mammary gland, skeletal muscle, optic nerve, pancreas, parathyroid and thyroid, peripheral nerve (sciatic), pituitary, prostate, salivary gland, skin, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, sternum, stomach (cardia fundus, pylorus), trachea, thymus, urinary bladder, uterus, and gross lesions.

low and mid dose - liver, kidney, heart and gross lesion

Results:

Opthalmoscopy - performed with binocular indirect ophthalmoscope after 1% tropicamide solution was placed in the eye to dilate the pupil. No differences between control and treated animals were noted.

Laboratory Tests:

Hematology: total platelet count, erythrocyte counts, total and differential leucocyte counts, hematocrit, hemoglobin, prothrombin time. No compound-related effects were noted.

Biochemistry: Calcium, potassium, SLDH, direct and total bilirubin, albumin, globulin, SGOT, SGPT, SAP, BUN, fastedblood glucose, total cholesterol, total protein. No compound-related effects were noted other than a dose-related decrease in calcium (mg/100 ml) for males, significant at 1000 and 3000 ppm. All the determinations were within normal limits.

Urinalysis: description of appearance, measurement of volume, pH, specific gravity, and qualitative tests for protein, glucose, ketones, bilirubin and urobilinogen; and microscopic examination of sediment. No compound-related effects noted.

General Behavior, Appearances, Survival - No compound-related effects noted. Survival was not affected by treatment.

Gross Necropsy: Mode of death - cuthanitized by CO_2 asphyxiation and necropsied.

No compound-related lesions noted.

Organ weight changes:

The following relative organ weight change was considered significant compound related effects:

Relative Heart

Male - significant increase at 1000 and 3000 ppm

Relative Testes

Significant increase at 1000 and 3000 ppm.

Relative Liver

Male - significant decrease (Student t-Test)

30	$p < .05$
300	$p < .01$
1000	$p < .01$
3000	$p < .01$

(Absolute liver weights were also significantly decreased at all dose levels; $p < .05$)

The females did not demonstrate any clear compound-related effect except at the high dose in some instances.

Body Weight Changes

The following changes in body weight are noted:

Dose	Male	Female
0	438	267
30	431(-1.6)	270(+1.1)
300	458(+4.6)	264(-1.1)
1000	419(-4.3)	254(-4.9)
3000	410(-6.4)	236(-11.6)

The adverse effects noted at 3000 ppm for both sexes can be associated with a decreased food consumption. However, the organ: body weight effects noted (liver) for males is considered compound-related since food consumption was not altered, body weight changes did vary between gains and losses, and yet absolute organ weight (liver) was significantly decreased in both circumstances.

Histopathology

There were no compound-related effects on any of the tissues examined.

Conclusion

The NOEL for this study has not been demonstrated. The significant decrease in relative and absolute liver weight for males at all levels fed, even though laboratory evaluation (hemat., biochem.) and both gross and histopathology examination failed to substantiate adverse effects, is justification for a thorough evaluation in this species. The rat is apparently the more sensitive species tested (rat vs. dog). "The weights of the livers were depressed more, in proportion to body weight, than would have been expected by chance; indicative of potential deleterious effect on this organ" (Weil, C.S. "Significance of organ weight changes in food safety evaluations", pp. 445, in: Francis J.C. Roe, ed. Metabolic Aspects of Food Safety, (1970).

Classification: CORE-Minimum

TOX/HED:th:RD Initial MODIFICATION:11-9-79

20

Supplement to Tox. Document NA, review for Accession No 257488, dermal absorption study in rats. This supplement provides an updated Executive Summary.

EPA Reviewer: Becky Daiss Becky Daiss, Date 6/24/03
Branch (7509C)
EPA Secondary Reviewer: Robert Zendzian, PhD, Date 6/24/03
Branch (7509C)

LDO 07/01/2003

AMENDED DATA EVALUATION RECORD

STUDY TYPE: Dermal Absorption in Rats

OPP Number: 85-2
DP BARCODE: D284318
PC CODE: 121301

OPPTS Number: 870.7600
SUBMISSION CODE: S590374
TOX CHEM NO: 167B

TEST MATERIAL (PURITY): ^{14}C- Cyromazine -75W formulated product in an aqueous suspension. (95% radioactive purity)

CHEMICAL NAME: Cyromazine

CITATION: T.G. Murphy & B.J. Simoneaux. (1985) Percutaneous Absorption of Cyromazine in Rats, Biochemistry Dept., Agricultural Division, Ciba-Geigy, M7-329-5A, 329950. Report No. ABR-85022, April 3, 1985. Accession No. 257488. MRID 00149656

SPONSOR: CIBA GEIGY Corporation, Agricultural Division, P.O. Box 18300, Greensboro, NC, 27419

EXECUTIVE SUMMARY:

In a dermal absorption study (Accession No. 257488), a formulation of "simulated" 75W formulation of ^{14}C-Cyromazine in aqueous solution was administered to 36 male Harlan Sprague-Dawley albino rats (3 rats per dose group per time period). ^{14}C-Cyromazine was dermally applied at doses of 0.1, 1.0, and 100 mg/rat to a 10 cm^2 area on the upper back portion of the rats. Animals were exposed for durations of 1, 2, 4, and 10 hours followed by skin wash and termination. Table 1 summarizes mean percent absorption and the mean percent remaining in/on the skin after skin wash at each time period for all doses.

Cyromazine **Dermal Absorption in Rats**

Table 1 - Mean Percent Absorption

Dose Level	Mean Percentage of Dose Absorbed & In/On Skin							
	1 hour		2 hours		4 hours		10 hours	
	absorbed	skin*	absorbed	skin	absorbed	skin	absorbed	skin
0.01 mg/cm^2 (0.1 mg/rat)	7.72	29.14	4.47	34.69	8.13	30.14	9.99	34.69
0.1 mg/cm^2 (1.0 mg/rat)	3.48	18.50	4.84	25.87	6.57	20.04	11.43	27.37
10 mg/cm^2 (100 mg/rat)	2.23	7.64	11.72	26.29	3.27	6.37	7.08	15.21

* amount of radioactivity in/on skin after skin wash

Mean total recoveries of applied radioactivity from all dose groups ranged from 85 to 101%. Mean absorption based on blood, urinary/fecal excretion, and carcass, ranged from 2% to 11%. Total radioactivity absorbed generally increased with increasing exposure time but decreased with increasing dose indicating saturation of penetration with increasing dose. The majority of the absorbed radioactivity was found in the urine and carcass. Most of the unabsorbed radioactivity was found in the skin washes from each dose/duration (35-90%). However, based on measurements of skin absorption, a significant amount of radioactive dose was also found in the skin itself (9-40%). Mean absorption with inclusion of radioactivity in dissolved skin ranged from 10 to 45%. The ratio of the amount of radioactive dose in the skin wash to the radioactivity in the skin itself decreased with time indicating penetration into the subsurface of the skin with time after treatment.

There were no deficiencies observed in the study.

The study is classified **Acceptable** and satisfies the guideline requirement 870.7600 (85-3) for a dermal absorption study.

Data Evaluation Report

Compound Cyromazine (Larvadex)

Citation
Percutaneous Absorption of Cyromazine in Rats, T.G. Murphy & B.J. Simoneaux, Biochemistry Dept, Agricultural Division, Ciba-Geigy, M7-329-5A, 329950. Report No. ABR-850[illegible], 4/3/85

Reviewed by Robert P. Zendzian PhD 4/22/85
Pharmacologist

Core classification Acceptable

Conclusion
Cyromazine is rapidly absorbed into the skin and more slowly into the body, with total absorption in the range of 30 to 44 percent of doses of 0.1 and 1.0mg/rat over durations of 1 to 10 hours. Data from the 100mg/rat dose was extremely variable and cannot be used.

Materials

Cyromazine (CGA-72662), ^{14}C labeled in the tirazine ring.
75WP formulated product
Specific activity 16.6 uCi/mg, low dose
2.26 uCi/mg, mid dose
0.0176 uCi/mg, high dose

Male Harlan Sprague-Dawley albino rats weighing 225 gms.

Methods

The hair on the back of the rats was shaved 24 hours prior to dosing and the shaven area washed with acetone. "A 10 sq. cm area, 2.5 by 4 cm, was marked before the dose was applied." The hind legs of the rats were restrained with jewelers chain.

Dermally applied doses were 0.1, 1.0 or 100mg/rat.

"The low dose was prepared by throughly mixing 25mg of ^{14}C-cyromazine with 75 mg of blank formulation (WP) and 12.5ml deionized water. The mid-dose was prepared by throughly mixing 200mg of ^{14}C-cyromazine and 66mg of blank formulation (WP) and 10 ml deionized water. The resutant mixtures are representative of a typical 75WP formulation."

"The high dose was prepared by throughly mixing 50gm 75WP which contains 37.5 g of unlabeled cyromazine and 40 mg of ^{14}C-cyromazine and 30ml of deionized water. The resultant mixture would contain one percent of ^{14}C-cyromazine and 99.9% (sic) unlabled cyromazine to give a representative 75WP formulation. This mixture had the consistancy of a smooth paste."

Zero-time sampling

In order to test the effectiveness of the skin washing procedure, samples of skin were taken from live rats ($10cm^2$, three rats per dose) and treated with the low and medium doses of ^{14}C-cyromazine. The skin was allowed to dry and immediately rinsed with water. The rinse water and the solubilized, digested skin were radioassayed.

Dose application

1. Low (0.1 mg/rat) and the mid (1.0 mg/rat) dose.

"Fifty microliters of an aqueous suspension containing the low (0.1 mg/rat) and the mid (1.0 mg/rat) dose levels were applied with a 50 ul Hamilton Syringe equiped with a teflon tip coated needle and plunger assembly." " The tip of the syringe was used to uniformly spread the suspension over the entire treatment area. The amount of ^{14}C-cyromazine applied to the rat was calculated by radioassay of 50 ul of the ^{14}C-cyromazine delivered with the same syringe. The amount of ^{14}C-cyromazine remaining on the teflon coated needle was considered to be minimal and not measured directly."

2. High dose level (100 mg/rat)

"The high dose level (100 mg/rat) was applied with the blunt end of a glass rod used for packing columns that measured 1.75cm in diameter. Two hundred and eighty milligrams of an aqueous paste (100 mg ^{14}C-cyromazine, 37.5 mg of formulant and 142.5 mg of deionized water) was applied by touching the surface of the paste with the blunt end of the glass rod until the exact amount was transferred. The amount of paste on the tared rod was determined by weighing on an analytical balance. Two drops of deionized water was placed on the treatment area with an eye dropper before the dose was applied. The glass rod tip was rubbed over the wet treatment area until a uniform application took place and by visual inspection very little residue paste remained on the glass rod. The transfer of ^{14}C-cyromazine appeared quantitative and was not measured directly."

Preliminary study

A preliminary study was done on two rats at the mid-dose (1.0mg/rat) for an eight hour exposure. Animals were placed individually in metabolism cages and volatiles, urine and feces collected. At eight hours the rats were sacrificed and blood (plasma and RBCs), carcass, skin, skin wash and cage wash collected for analysis. CO_2 was not collected since metabolism studies have shown that cyromazine metabolism does not produce CO_2.

Main Study

In the main study three rats per dose per time point were used. For each dose three rats were sacrificed at 1, 2, 4 or 10 hours after dosing. Animals were placed individually in metabolism cages and urine and feces collected. At sacrifice blood (plasma and RBCs), carcass, skin, skin wash and cage wash collected for analysis. The preliminary study showed that collection of volitiles was not necessary.

Analysis of samples

Liquid samples were radioassayed directly. Carcasses and feces were homogenized and aliquits combusted.

Results

Zero-time sampling

Table 1. Mean (of two rats) recovery from skin of low and intermediate dose as percent of applied dose. Data from Appendix Table XIII of the report.

	Dose	
	0.1mg/rat	1.0mg/rat
Skin Rinse	99.60	92.58
Skin dissolved	7.52	3.99
Total	107.12	96.57

Preliminary study

Table 2. Mean (of two rats) recovery following a dose of 1.0mg/rat and an eight hour exposure as percent of dose. Date from Table I in the report.

Tissues and Collections	% Dose
Plasma	<0.00
RCB	<0.03
Carcass	4.71
Skin Wash	53.40
Skin Dissolved	18.83
Cage Wash	7.86
Volitiles	<0.44
Urine	1.67
Feces	0.01
Total Recovered	86.95
Missing	13.05

% Dose Absorbed = 25.25 (Totals; Plasma, RCB, Carcass, Skin Dissolved, Urine Feces.)

% Dose Not Absorbed = 61.7 (Totals; Skin Wash, cage wash, volitiles)

Main Study

Table 3. Mean quantity absorbed, as percent of dose, three rats per dose. Date from Appendix Tables I through XIII in the report.

Dose (mg/rat)		0.1	1.0	100.0
Duration (hours)				
1	Total*	36.86	21.98	9.87
	Skin**	29.14	18.50	7.64
2	Total	39.16	30.71	37.41
	Skin	34.69	25.87	26.29
4	Total	38.27	26.61	9.64
	Skin	30.14	20.04	6.37
10	Total	44.68	38.80	22.29
	Skin	34.69	27.37	15.21

*Plasma, RBC, Carcass, Urine, Feces & Skin dissolved.
**Skin after washing (skin dissolved).

Table 4. Mean quantity not absorbed (skin wash) as percent of dose, three rats per dose. Date from Appendix Tables I through XIII in the report.

Dose (mg/rat)	0.1	1.0	100.0
Duration (hours)			
1	50.51	63.82	89.79
2	52.13	56.41	44.17
4	59.00	58.55	88.37
10	41.04	47.88	79.05

Discussion

The compound shows a rapid and high absorption into the skin followed by a slower release into the body. The variation with time of the 100mg dose indicates a possible problem in consistancy of dose appplication, perhaps in spreading. Because of this variation and a general inability to determine its cause the 100mg/rat data should be discarded.

EPA Reviewer: Waheeda Mani Tehseen, Ph. D. , Date 1/17/2003
OPP-HED Registration Action Branch 3 (7509C)
EPA Secondary Reviewer: Steve Dapson, Ph. D. , Date 07/01/2003
Registration Action Branch 3 (7509C)

DATA EVALUATION RECORD (DER)

This DER is an upgrade to previously written executive summary and DER (MRID no. 00115736 and TXR no. 002617).

STUDY TYPE: Carcinogenicity Study in Mouse

OPPTS Number: 870.4200

OPP Guideline Number: §83-2

DP BARCODE: D284318

SUBMISSION CODE: S590374

P.C. CODE: 121301

TEST MATERIAL (PURITY): Cyromazine, (95.3 - 95.5%)

SYNONYMS: Lavardex Technical, N-cyclopropyl-1,3,5-triazine-2-4-6,-triamine

CITATION: Blair, M.; VanderMeer, D.; Marroquin, F.; et al. (1982) Oncogenicity Study with CGA-72662 in Albino Mice: IRDC No. 382-082. Study received Oct 20, 1982 under 100-631; prepared by International Research and Development Corp., submitted by Ciba-Geigy Corp., Greensboro, NC; CDL:071189-A; 071190; 071191; 071192). MRID No. 00115736. Unpublished.

SPONSOR: Ciba-Geigy Corporation.

A 24-month chronic toxicity/oncogenicity study in Charles River CD-1 mice was conducted by IRDC, Mattawan, Michigan, and issued June 30, 1982, for Ciba-Geigy Corporation, Greensboro, North Carolina (IRDC Study No. 382-082).

The study design allocated groups of 60 mice per sex to dose levels of 0, 50, 1000 and 3000 ppm (0, 7.5, 150, 450 mg/kg/day) of Cyromazine. An additional 8 animals per sex per dose were designated for interim sacrifice at one year.

Male mice had decreased body weight gains from 8 weeks (high-dose, 450 mg/kg/day) onward and 19 weeks onward (mid-dose, 150 mg/kg/day). The body weights of the males were consistently 5-9% less than controls. Female weights were unaffected. Hematological parameters and behavioral signs were not affected by the test substance. There was no increase in incidence of non-neoplastic lesions as compared to controls. Relative liver weights were

increased at terminal sacrifice in the high-dose (450 mg/kg/day) males and these animals also had a slightly increased incidence of liver masses. Cystic hyperplasia of the mammary gland occurred, however the incidence did not increase with higher cyromazine doses.

Malignant lymphomas (lymphatic lymphomas and histiocytic lymphomas) were found in both control and treated groups in both sexes. However, two independent pathology labs have reviewed these data and both determined that these tumors did not appear to be treatment-related.

Liver neoplasms (hepatocellular carcinoma/adenomas, hemangiosarcomas and hemangiomas) also were seen in both sexes in treated and control animals but there was no apparent dose-response relationship.

Hemangiosarcoma of the spleen is a frequently observed tumor type in mice and was seen in a few male and female treated mice with no strong dose-response relationship.

Female mice showed various mammary tumors (adenocarcinomas and adenocanthomas). However, the incidence of mammary tumors is similar to the control groups and there is no clear dose-response relationship. Mammary gland neoplasms (adenocarcinomas) were slightly increased in high-dose females (6/57 or 4%) as compared to controls (2/56 or 11%), however, the difference was not statistically different than controls. Hence, there was no statistically significant increase in tumors in the treated groups; there were no statistical trends and no dose response (HED CPRC, Jan. 6, 1995, TXR No. 0011384).

The historical control data for female albino mice conducted at a contract laboratory showed that the incidence of adencarcinomas in the mouse study slightly exceed the range of historical controls but not concurrent controls. Reliable historical control data could not be located for mammary gland adenoacanthomas in the female albino mouse.

Adequacy of Dosing for Assessment of Carcinogenic Potential

Body weight gains in female mice were comparable to controls and there were no indications of toxicity reported. The study report stated that "there is suggestion of a possibly slightly increased mortality" in high dose females; based on this the dosing in the female mouse was considered "marginally adequate"(HED CPRC, Jan. 6, 1995, TXR No. 0011384).

The dosing in male mice was considered adequate, based on body weight gain reductions (12% at the mid dose, 23% at the high dose) relative to controls.

The systemic toxicity LOAEL is 1000 ppm (150 mg/kg/day) based on decreased body weight and the systemic toxicity NOAEL is 50 ppm (7.5 mg/kg/day).

This study is **acceptable-guideline** and does satisfy the requirement for a carcinogenicity study (OPPTS # 870.4200 / 83-2) in mouse.