

CYROMAZINE

(LARVADEX)

RISK CHARACTERIZATION DOCUMENT

**MEDICAL TOXICOLOGY AND WORKER HEALTH AND SAFETY BRANCHES
DEPARTMENT OF PESTICIDE REGULATION
CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY**

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CYROMAZINE

EXECUTIVE SUMMARY

Introduction

Cyromazine is a systemic insecticide and an insect growth regulator which is effective against fly larvae and leaf miners. Cyromazine belongs to the s-triazine class of chemicals, but unlike other compounds of the triazine class, it lacks herbicidal activity. While the exact mechanism of action remains unknown, it has been postulated that cyromazine acts through the hormone which controls ecdysis and the deposition of cuticle in insects.

Cyromazine is the active ingredient in the formulation, Larvadex 2SL, the formulation currently being requested for registration in California. The only proposed use for Larvadex 2SL is as a fly larvicide for direct application on chicken manure. Larvadex 2SL and other cyromazine formulations are registered for use in other states through the United States Environmental Protection Agency (U.S. EPA). Trigard 75W is a wettable powder formulation that is used on celery and lettuce. Larvadex Premix formulations consist of coarsely ground cyromazine that is mixed with chicken feed and used as a "pass-through" pesticide to inhibit the development of fly larvae in chicken manure.

The Risk Assessment Process

A basic principal of toxicology is that at a high enough dose, virtually all substances will cause some type of toxic manifestation. Although chemicals are often referred to as "dangerous" or "safe", as though these concepts were absolutes, in reality, these terms describe chemicals that require low or high dosages, respectively, to cause toxic effects. Toxicological activity is determined in a battery of experimental studies which define the kinds of toxic effects which can be caused, and the exposure levels (doses) at which an effect is first seen. State and federal testing requirements, including California's Birth Defect Prevention Act of 1984 (SB 950, Petris), mandate that chemicals be tested at doses high enough to produce toxic effects, even if that testing requires levels many times higher than those to which people might be exposed. The critical parameters in determining the risk of any chemical, including pesticides, are the intrinsic toxicological activity of the chemical, and the level and duration of exposure to the chemical. The purpose of risk assessment is to determine potential human exposures, and to relate toxic effects in laboratory studies at high dosages to the probability of adverse health effects in people who may be exposed to the pesticide through various routes and activities.

EXECUTIVE SUMMARY (continued)

Toxicology

A human health risk assessment has been conducted for cyromazine because of adverse effects reported in animal studies. This risk assessment specifically addresses the potential exposure of workers performing the mixing and application of cyromazine to chicken manure and the field workers who spread the treated manure as a fertilizer. The toxicology endpoints used in the assessment were developmental toxicity for acute exposure, systemic toxicity for repeated chronic exposure and oncogenicity for potential lifetime exposure. Another potential adverse effect addressed in this risk assessment was in the area of reproductive toxicity, based on the results reported in a chronic dog study.

Several rabbit developmental toxicity studies were conducted using cyromazine. The lowest No Observable Effect Level (NOEL) was established at 5 mg/kg/day for fetal malformations. The NOEL for maternal toxicity was 10 mg/kg/day, based on decreased body weight gain. The NOEL of 5 mg/kg/day was used to calculate the margins of safety for potential acute exposure to cyromazine.

Chronic toxicity from repeated exposure to cyromazine was identified in a 6 month dog study. Toxicity to the seminiferous tubules and a concomitant decrease in spermatogenesis was observed in male animals. The NOEL for these endpoints was 9.27 mg/kg/day.

In a two generation rat reproduction study, the lowest NOEL for systemic toxicity was 1.7 mg/kg/day, based on decreased body weight gain in adult males. The corresponding NOEL for reproductive toxicity was 57 mg/kg/day, based on decreased male pup viability and decreased body weight at birth.

The lowest NOEL from repeated oral exposure to cyromazine was 1.7 mg/kg/day reported in the rat reproduction study. This NOEL was used to characterize the margins of safety from potential repeated daily exposure to cyromazine.

In the rat chronic toxicity/oncogenicity study, interstitial cell tumors occurred in male animals, and mammary adenomas/adenocarcinomas were reported in female rats. The incidence of the benign interstitial cell tumors was marginally statistically significant only in the high dose group; however, the review of all of the scientific evidence, including historical control data, concluded that the ISC tumors were not clearly the result of treatment with cyromazine. Mammary adenomas and adenocarcinomas appeared in female rats with the combined tumor incidence achieving statistical significance in the high dose group.

EXECUTIVE SUMMARY (continued)

In the mouse oncogenicity study male animals exhibited both histiocytic and lymphocytic lymphomas. The incidence of these malignant tumors was not statistically significant when evaluated individually or combined and was not considered related to treatment with cyromazine.

The combined incidence of mammary adenomas/adenocarcinomas in the female rat was used to assess the potential oncogenic risk to workers exposed to cyromazine. These data were used primarily because of the general concern that similar tumors have been reported in rats exposed to other triazine pesticides and that a quantitative assessment of the oncogenic potential of cyromazine was a prudent public health approach.

The weight of scientific evidence did not support conducting a quantitative oncogenic risk assessment for melamine, a metabolite of cyromazine. This perspective is currently shared by the U. S. EPA, which recently concluded that the oncogenic risk of melamine is "non-existent, or, at worst, extremely low".

Occupational Exposure

A worker study using Larvadex 2SL was conducted to determine occupational exposure from applying cyromazine directly on chicken manure in poultry houses. The highest potential exposure was for the backpack sprayer, followed by the power sprayer, with the hand-held sprayer having the lowest exposure. Additionally, since the cyromazine-treated manure can be applied to fields as a fertilizer, potential exposure was estimated for the worker performing this job task. The estimated exposure for the field worker was the same as calculated for the hand-held sprayer.

Risk Assessment

The lowest margin of safety for potential acute (daily) exposure was 132 for the backpack sprayer. Chronic exposure was estimated based on the assumption that workers would only be applying cyromazine to chicken manure 10 times annually. However, probable application practices would suggest that workers would not be exposed to repeated, daily amounts of cyromazine over a period of time that would constitute a chronic exposure which would be similar to that used to characterize the toxicity observed in the rat. The lowest margin of safety from this repeated potential exposure was approximately 1,600 for the backpack sprayer. Both the acute and chronic margins of safety were considered adequate.

The additional lifetime oncogenic risk was calculated using the Global 86 Linearized Multistage Model. In extrapolating from the rat to humans, equivalent human dosages were calculated using a default cross-species scaling of animal body weight to the $3/4$ power. This methodology has been recently proposed by the U.S. EPA.

EXECUTIVE SUMMARY (continued)

The highest additional theoretical lifetime oncogenic risk from applying cyromazine was $3.5E-06$ (95% upper bound) for the backpack sprayer. This calculation assumes that the worker would be performing this job 10 times a year, for 40 years of a 70 year lifetime. Because of the shape of the dose-response curve for the combined mammary tumors, the model was constrained from calculating a positive value for the maximum likelihood estimate (MLE) potency term. Therefore, the MLE was assumed to be zero. The discrepancy between the MLE and the upper bound potency estimates indicates that the latter may not closely reflect the dose-response relationship of the experimental data, and, therefore, may not be a realistic estimation of potential human oncogenic risk.

An evaluation of the rat study using a margin of safety paradigm showed that applicators of cyromazine would be theoretically exposed to a lifetime average daily dosage of at least 3,000 times below the NOEL at which there was no biologically relevant decrease in body weight in female rats exposed to daily amounts of cyromazine for 2 years. This biologically conservative NOEL was 10-fold lower than the dosage which did not significantly increase the frequency of combined mammary adenomas/adenocarcinomas. Workers applying cyromazine to chicken manure are unlikely to attain a chronic dosage comparable to the rat because the larvacide is applied periodically throughout the year, rather than on consecutive days.

Based on the following considerations, the overall chronic risk, including oncogenicity, to workers applying cyromazine to chicken manure appears to be minimal:

- 1) the lack of actual chronic (repeated daily) exposure based on the anticipated patterns of use and projected frequency and duration of applications.
- 2) no evidence that cyromazine accumulates in biological systems.
- 3) the lack of clearly defined positive genotoxicity.
- 4) the limitation of the Global 86 program to only calculate the 95% upper bound potency and, therefore, limit the presentation of a realistic range of potential human risk.

The presumable work practices for the application of cyromazine to chicken manure suggest that the acute, daily exposure may be the most scientifically relevant scenario for assessing the potential deleterious effects to these workers.

I SUMMARY

Cyromazine is a systemic insecticide and an insect growth regulator which is effective against fly larvae and leaf miners. Cyromazine belongs to the s-triazine class of chemicals, but unlike other compounds of the triazine class (e.g. atrazine, cyanazine, simazine), it lacks herbicidal activity. While the exact mechanism of action remains unknown, it has been postulated that cyromazine acts, directly or indirectly, on the metabolism of ecdysone, the hormone which controls ecdysis and the deposition of cuticle in the insects.

Cyromazine is the active ingredient in the formulation, Larvadex 2SL, a 2% water soluble concentrate. This formulation is the only one currently being requested for registration in California. The only proposed use is as a fly larvicide for direct application on chicken manure. Larvadex 2SL and other cyromazine formulations are registered for use in other states through the United States Environmental Protection Agency (U.S. EPA). Trigard 75W is a wettable powder formulation that is used on celery and lettuce. Larvadex Premix formulations consist of coarsely ground cyromazine that is mixed with chicken feed and used as a "pass-through" pesticide to inhibit the development of fly larvae in chicken manure.

A human health risk assessment has been conducted for cyromazine because of adverse effects reported in animal studies. This risk assessment specifically addresses the potential exposure of workers performing the mixing and application of cyromazine to chicken manure and the field workers who spread the treated manure as a fertilizer. The toxicology endpoints used in the assessment were developmental toxicity (malformations in the rabbit) for acute exposure, systemic toxicity (decreased body weight gain in the rat) for repeated chronic exposure and oncogenicity (mammary tumors in the female rat) for potential lifetime exposure. Another potential adverse effect addressed in this risk assessment was in the area of reproductive toxicity, based on the results reported in a chronic dog study.

Several rabbit developmental toxicity studies were conducted using cyromazine. The lowest No Observable Effect Level (NOEL) was established at 5 mg/kg/day for malformations, including cyclopia, using New Zealand White rabbits (Buckshire strain). The NOEL for maternal toxicity in this study was 10 mg/kg/day, based on decreased body weight gain. Another developmental toxicity study using New Zealand White rabbits (Hazelton-Dutchland strain) also reported cyclopia and other malformations with a NOEL of 10 mg/kg/day. While the incidence of cyclopia in these two studies was not indicative of a strong dose-response relationship, this malformation was considered toxicologically relevant because it did not appear in the concurrent or historical control animals. The NOEL of 5 mg/kg/day was used to characterize the margins of safety from potential acute exposure to workers using cyromazine.

I SUMMARY (continued)

Chronic toxicity from repeated exposure to cyromazine was identified in a 6 month dog study. Bilateral degeneration of the seminiferous tubules and a concomitant decrease in spermatogenesis was observed in these animals. The NOEL for these endpoints was 9.27 mg/kg/day.

In a two generation rat reproduction study, the lowest NOEL for systemic toxicity was 1.7 mg/kg/day, based on decreased body weight gain in adult males. The corresponding NOEL for reproductive toxicity was 57 mg/kg/day, based on decreased male pup viability and decreased body weight at birth.

The lowest NOEL from repeated oral exposure to cyromazine was 1.7 mg/kg/day reported in the rat reproduction study. This NOEL was used to characterize the margins of safety to workers from potential repeated daily exposure to cyromazine.

In the rat chronic toxicity/oncogenicity study, interstitial cell tumors occurred in male animals, and mammary adenomas/adenocarcinomas were reported in female rats. The incidence of the benign interstitial cell tumors was marginally statistically significant only in the high dose group; however, the review of all of the scientific evidence, including historical control data, concluded that these tumors were not clearly the result of treatment with cyromazine. Mammary adenomas and adenocarcinomas appeared in female rats with the combined tumor incidence achieving statistical significance in the high dose group.

In the mouse oncogenicity study male animals exhibited both histiocytic and lymphocytic lymphomas. The incidence of these malignant tumors was not statistically different from concurrent controls when evaluated individually or combined, and, therefore, not considered to clearly result from treatment with cyromazine.

The combined incidence of mammary adenomas/adenocarcinomas in the female rats was used to assess the potential oncogenic risk to workers exposed to cyromazine. The decision to use these data was based, primarily, by the general concern that similar tumors have been reported in rats exposed to other triazine pesticides and that a quantitative assessment of the oncogenic potential of cyromazine was a prudent public health approach. In extrapolating from the rat to humans, equivalent human dosages were calculated using a default cross-species scaling of animal body weight to the 3/4 power. This methodology has been recently proposed by the U.S. EPA. The additional lifetime oncogenic risk was calculated using the 95% upper bound confidence interval for potency using the Global 86 Linearized Multistage Model.

The weight of scientific evidence did not support conducting an oncogenic risk assessment for melamine, a primary metabolite of cyromazine found in the rat. This perspective is shared by the U. S. EPA, who recently concluded that the oncogenic risk of melamine is "non-existent, or, at worst, extremely low".

I SUMMARY (continued)

A worker study using Larvadex 2SL was conducted to determine occupational exposure from applying cyromazine directly on chicken manure in poultry houses. The highest potential exposure was for the backpack sprayer, followed by the power sprayer, with the hand-held sprayer having the lowest exposure. Additionally, since the cyromazine-treated manure can be applied to fields as a fertilizer, potential exposure was estimated for the worker performing this job task. The estimated exposure for the field worker was the same as calculated for the hand-held sprayer.

The lowest margin of safety for potential acute (daily) exposure was 132 for the backpack sprayer. Chronic exposure was estimated based on the assumption that workers would only be applying cyromazine to chicken manure 10 times annually. The lowest margin of safety from this repeated potential exposure was approximately 1,600 for the backpack sprayer. Both of these margins of safety are considered adequate.

Since it is highly improbable that cyromazine would be applied to chicken manure on 10 successive days during a year, workers would not be subjected to repeated, daily exposures, and, therefore, potential chronic exposures, using the assumptions presented in this document, are unlikely. Additionally, there is no evidence that cyromazine accumulates in humans or test animals; therefore, the single day, acute exposure would most likely characterize the potential deleterious effects to these workers.

The highest additional theoretical lifetime oncogenic risk from applying cyromazine was $3.5E-06$ (95% upper bound) for the backpack sprayer. This calculation was based on an upper bound potency slope (Q_1) of $5.9E-03$ (mg/kg/day)⁻¹ and assumes that a worker would be performing this job task 10 times a year, for 40 years out of a 70 year lifetime. Because of the shape of the dose-response curve for the combined mammary adenomas and adenocarcinomas, the model was constrained from calculating a positive value for the maximum likelihood estimate (MLE) potency term. Therefore, the MLE was assumed to be zero. The discrepancy between the MLE and the upper bound potency estimates indicates that the latter may not closely reflect the dose-response relationship of the experimental data, and, therefore, may not result in a realistic estimation of potential human oncogenic risk.

An evaluation of the rat study using a margin of safety paradigm showed that applicators of cyromazine would be theoretically exposed to a lifetime average daily dosage of at least 3,000 times below the NOEL at which there was no biologically relevant decrease in body weight in female rats exposed to daily amounts of cyromazine for 2 years. This biologically conservative NOEL was 10-fold lower than the dosage which did not significantly increase the frequency of combined mammary adenomas/adenocarcinomas. Workers applying cyromazine to chicken manure are unlikely to attain a chronic dosage comparable to the rat because the larvacide is applied periodically throughout the year, rather than on consecutive days.

I SUMMARY (continued)

Based on the following considerations, the overall chronic risk, including oncogenicity, to workers applying cyromazine to chicken manure appears to be minimal:

- 1) the lack of actual chronic (repeated daily) exposure based on the anticipated patterns of use and projected frequency and duration of applications.
- 2) no evidence that cyromazine accumulates in biological systems
- 3) the lack of clearly defined positive genotoxicity
- 4) the limitation of the Global 86 program to only calculate the 95% upper bound potency and, therefore, constrain the presentation of a realistic range of potential human risk.

The presumable work practices for the application of cyromazine to chicken manure suggest that the acute, daily exposure may be the most scientifically relevant scenario for assessing the potential deleterious effects to these workers.

CONTRIBUTIONS AND ACKNOWLEDGEMENTS

Principal Author: Keith Pfeifer, Ph.D., DABT
Senior Toxicologist
Health Assessment Section
Medical Toxicology Branch

Toxicology Reviews: Medical Toxicology Branch

SB-950 Review Section

Charles Aldous, Ph.D., DABT
Staff Toxicologist

Judith Parker*, Ph.D., DABT
Staff Toxicologist

Fred Martz*, Ph.D., DABT
Staff Toxicologist

Joyce Gee, Ph.D.
Senior Toxicologist

Product Review Section

Ronald Morgan⁺, Ph.D.
Staff Toxicologist

Peter Leung, Ph.D., DABT
Staff Toxicologist

Gary Patterson, Ph.D.
Senior Toxicologist

Exposure Assessment: Worker Health and Safety Branch

Tian Thongsinthusak, Ph.D.
Staff Toxicologist

Michael Dong, Ph.D.
Staff Toxicologist

David Haskell, B.S.
Assoc. Envir. Research Scientist

* Currently with Department of Toxic Substances, Cal-EPA
+ No longer with the State of California

CONTRIBUTIONS AND ACKNOWLEDGEMENTS (continued)

Peer Review:

Earl Meierhenry, D.V.M., Ph.D., ACVP
Staff Toxicologist
Health Assessment Section
Medical Toxicology Branch

Nu-may R. Reed, Ph.D., DABT
Staff Toxicologist
Health Assessment Section
Medical Toxicology Branch

Jay Schreider, Ph.D.
Primary State Toxicologist
Medical Toxicology Branch

James Sanborn, Ph.D.
Staff Toxicologist
Worker Health and Safety Branch

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II INTRODUCTION

A. CHEMICAL IDENTIFICATION

Cyromazine is a systemic insecticide and an insect growth regulator which is effective against fly larvae and leaf miners. Cyromazine belongs to the s-triazine class of chemicals, but unlike other compounds of the triazine class (e.g. atrazine, simazine), it lacks herbicidal activity. While the exact mechanism of action remains unknown, it has been concluded that cyromazine does not directly inhibit chitin synthesis in the sheep blowfly larvae (Friedel, et al., 1988). It was postulated that cyromazine acts, directly or indirectly, on the metabolism of ecdysone, the hormone which controls ecdysis and the deposition of cuticle.

B. TECHNICAL AND PRODUCT FORMULATIONS

Cyromazine is the active ingredient in Trigard 75W, a wettable powder formulation containing 75% cyromazine. It is used on celery and lettuce.

Larvadex (1.0% Premix) is a coarsely ground powder mixed with chicken feed for use as a "pass-through" pesticide to control fly larvae in chicken manure.

Larvadex 2SL contains 2% cyromazine as the active ingredient in a water soluble concentrate. It is used by direct application on chicken manure to control fly larvae. This formulation is the only one currently being requested for registration in California.

C. USAGE

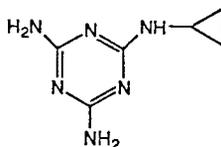
Trigard is registered by the United States Environmental Protection Agency (U.S. EPA) to control leaf miners on celery and lettuce. Larvadex is used to control fly larvae in chicken manure, either by direct application to the manure or indirectly as a "pass-through" feed additive. The Larvadex 2SL formulation can be used in poultry operations that include layers and breeder chickens. Mixing instructions, methods of application and application rates are detailed in the product label (Appendix B).

D. ILLNESS REPORTS

Cyromazine has not been previously registered in California; therefore, no worker illness data from actual work-related activities in California are available. The incidence of worker illnesses from the use of cyromazine in other states is not known.

E. PHYSICAL AND CHEMICAL PROPERTIES (Technical/Formulation)

Common Name: Cyromazine
Chemical Name: N-cyclopropyl-1,3,5-triazine-2,4,6-triamine
Trade Names: Larvadex, Trigard, CGA-72662
CAS Registry No.: 66215-27-8 (1)^a
Molecular Weight: 166.2 (2)
Molecular Structure:



Empirical Formula: C₆H₁₀N₆
Physical State: white crystalline powder (technical) (3)
pale yellow liquid (Larvadex 2SL) (4)
Odor: odorless (technical) (3)
vinegar-like (Larvadex 2SL) (4)
Melting Point: ~223°C (technical) (5)
Boiling Point: 100°C (Larvadex 2SL) (4)
Density: 0.538 g/ml (technical) (6)
Solubility (@ 25°C): water 1.16 g/100ml (technical) (2,3)
methanol 2.2%
isopropanol 0.25%
acetone 0.17%
methyl chloride 0.03%
toluene 0.01%
hexane 0.01%
n-octanol < 0.22%
Vapor Pressure: (@ 25°C) 3.36 x 10⁻⁹ mm Hg (3,5)
K_{ow}: <1 (2)
pKa: 5.3 (2)

^a/ (1) Budavari, S., 1989. (2) Honeycutt, R. G. 1982a. (3) Ciba-Geigy Corp., 1990a. (4) Ciba-Geigy Corp., 1990b. (5) Rordorf, B.F., 1988. (6) Lee, T.C., 1987.

F. ENVIRONMENTAL FATE

Hydrolysis

No significant hydrolysis of radio-labeled cyromazine was measured in 100 ppm solutions at pH 5, 7 or 9 when maintained under laboratory temperatures of 30, 50 or 70 degrees Celsius for 28 days (Burkhard, 1979b). Under strongly acidic conditions (0.1 N HCl), cyromazine hydrolyzed at 50 and 70 degrees Celsius with corresponding half-lives of 106 and 8 days. The primary hydrolysis metabolite was 2-amino-4-cyclopropylamino-6-hydroxy-s-triazine. Under strongly basic conditions (0.1 NaOH), measurable hydrolysis only occurred at 70 degrees Celsius with a half-life of 80 days. An additional degradation metabolite was identified under basic conditions as 2-cyclopropylamino-4-6, dihydroxy-s-triazine.

Photolysis

Photolysis has been proposed as the major pathway of cyromazine degradation in the environment (Honeycutt, 1982b). Cyromazine can be photodegraded via dealkylation to melamine and possibly other unknown metabolites. However, laboratory and greenhouse experiments using various substrates have given conflicting results (see below).

Aqueous

The photodegradation of cyromazine in aqueous solutions was examined at the concentrations of 100 ppm with and without a sensitizer (1% acetone) (Burkhard, 1979a). Solutions were maintained at 25°C under laboratory conditions and exposed to artificial sunlight for 168 hours. No photodegradation occurred in the solutions without acetone. However, in solutions containing acetone, cyromazine degraded to melamine with an estimated half-life of 10 hours.

Soil

The photolysis of cyromazine was determined on soil surfaces using a sandy loam soil fortified with 10 ppm of radio-labeled material (Burkhard, 1980). Moist and dry soils were exposed to artificial sunlight, simulating twice the intensity of natural spring or summer sunlight, and maintained at a temperature of 45°C for a 24-hour period. Under both light and dark conditions, 24% of the ¹⁴C-cyromazine degraded in the moist soils, and 11% degraded in the dry soils. No volatile degradation products were formed. Degradation of cyromazine was attributed to hydrolysis and/or thermal degradation rather than photolysis because similar losses of cyromazine occurred under both light and dark conditions.

F. ENVIRONMENTAL FATE (continued)

Other Surfaces

Cyromazine (~98% purity), Trigard 75W (75% cyromazine), and melamine (99% purity) were dissolved in methanol and applied to the surface of glass petri dishes (Lim et al., 1990). Dishes were exposed to direct, natural sunlight under greenhouse conditions for periods of 2 hours, 1 day, and 1, 2, and 3 weeks. A second set of dishes was prepared with cyromazine and incubated under dark conditions for the specified time periods.

The degradation rate of cyromazine was inversely proportional to concentration and directly related to the length of exposure. At the lowest concentration (1.5 nmol), all the substrate was degraded within 7 days. In contrast, at 30 nmol, only 22% of the cyromazine remained after 3 weeks. Degradation rates were similar for the technical compound and formulated material, indicating that the inert ingredients (25% by weight) in the formulation did not have any influence on the degradation reaction. No degradation of cyromazine was detected in dishes kept under dark conditions; therefore, degradation of cyromazine was attributed to photochemical reactions which yielded melamine from the dealkylation of cyromazine. The degradation of melamine was also inversely proportional to concentration and directly related to the time of exposure. The amount of recovered melamine did not completely account for the degradation of cyromazine. There is the possibility that cyromazine may be converted to other degradation metabolites and/or that melamine may be further degraded. However, specific degradation reactions for melamine were not examined. In addition to photodegradation, volatilization was suggested as a possible pathway of loss. However, based on the relatively low vapor pressure for cyromazine, this pathway would be unlikely.

Microbial Degradation

Soil

Sandy-loam and sand/muck soil samples were fortified with 10.7 ppm ¹⁴C-cyromazine and incubated under sterile, non-sterile, aerobic and anaerobic conditions for up to 12 months (Cargile, 1986). The degradation of cyromazine was more rapid under aerobic than anaerobic conditions. The estimated half-life under aerobic study conditions was 3 to 4 months. Although this study was not considered acceptable to fill the study requirement under AB-2021, the study does provide some useful information regarding relative degradation potential in these media.

Soil/Manure

The metabolism of cyromazine in soil-manure mixtures was examined in the laboratory under aerobic, anaerobic, or sterile aerobic conditions (Caplan, 1981). Samples containing a mixture of equal parts of loam soil and chicken manure were fortified with radio-labeled and non-labeled cyromazine to a concentration of 10 ppm. Samples were maintained under aerobic conditions for 10 months, and anaerobic or

F. ENVIRONMENTAL FATE (continued)

sterile aerobic for 3 months in metabolism chambers. No radio-labeled compounds were detected in the chamber gas trapping media during the study periods. Under aerobic conditions there was an apparent slow breakdown of cyromazine accompanied by an increase in melamine and bound residues. The estimated half-life of cyromazine under aerobic conditions was 439 days. Under anaerobic or sterile aerobic conditions, there was no apparent degradation (less than 10%). However, within a 2 month incubation period, 28% of the radioactivity had leached into the aqueous phase under anaerobic conditions. This study indicates that cyromazine is relatively stable in chicken manure under the simulated environmental conditions in the laboratory.

Manure

The efficacy and dissipation of cyromazine was examined in fresh chicken manure over a 21-day study period (Seim and Brown, 1978). Manure was treated with ^{14}C -cyromazine to the concentration of 27 ppm and incubated for 23 days. Core samples were collected at several intervals during the study period. Cyromazine provided 100% fly control; however, no results were provided regarding cyromazine dissipation or degradation.

The degradation of cyromazine topically applied to chicken manure was examined over a 21-day study period (Simoneaux and Cassidy, 1979). ^{14}C -Cyromazine (1% solution) was applied to manure at the rate of 1 gallon per 100 ft². Fresh, untreated manure was added to the treatments following each sample collection. Concentrations of cyromazine appeared to decline over time; however, this decrease was directly proportional to what would be expected from sample dilution. Losses from $^{14}\text{CO}_2$ evolution or volatility were not evaluated.

Effects on Soil Microorganisms:

The effect of cyromazine on the soil nitrification process was examined in two soils under laboratory conditions (Mumma and Bogus, 1981a). Loam and silt loam soils were blended with equal amounts of fine sand and amended with ammonium sulfate and calcium carbonate. Soil samples were fortified with cyromazine to the concentrations of 0, 1, 10, and 100 ppm. Untreated and treated soils were incubated at 28°C and 80% relative humidity under aerobic conditions for an eight-week period. Soil nitrate levels were determined at several intervals during the incubation period. In both soil types, nitrate levels increased in both treated and untreated soils by the end of the study period. Initially, a decrease in nitrification was observed at 100 ppm in the loam soil. Final nitrate levels were similar in all untreated and treated soils, except in the silt loam soil at 1 and 10 ppm, which contained higher nitrate levels, indicating an increase in nitrification. Data indicate that cyromazine had no detrimental effect on the soil nitrification process.

F. ENVIRONMENTAL FATE (continued)

The effects of cyromazine on soil microorganisms that degrade litter of biological origin was examined in two soil types (Mumma and Bogus, 1981b). Microbial activity was monitored by measuring ^{14}C evolution from soils amended with ^{14}C -cellulose, ^{14}C -protein, and ^{214}C -starch. Silt loam and loam soils were amended with ^{14}C -substrates, fortified with cyromazine to concentrations of 0, 1, 10, and 100 ppm, and incubated under laboratory conditions for a 28-day period. A set of sterile soil samples amended with ^{14}C -substrates were also prepared as positive controls. No degradation of substrates was observed in sterile soils. Similar degradation patterns were observed in treated and untreated soils indicating that cyromazine had no effect on microbial activity.

The effects of cyromazine on soil microorganism respiration was examined in two soil types (Mumma and Bogus, 1981c). Loam and silt loam soils were blended with equal amounts of fine sand and amended with 1% alfalfa meal. Soil samples were fortified with 0, 1, 10, and 100 ppm cyromazine; positive control samples were also prepared from sterilized soils amended with alfalfa meal. Microbial respiration was monitored by measuring carbon dioxide production at several intervals during the 28-day study period. A decrease in carbon dioxide production was observed in untreated loam and silt loam soils, and treated silt loam soils. Carbon dioxide formation increased in relation to time and cyromazine concentration in treated loam soils. Data indicate that cyromazine had no detrimental effect on soil microbial respiration.

The effect of cyromazine on nitrogen fixation was examined in loam and silt loam soils (Mumma and Bogus, 1981d). Soils were blended with equal amounts of fine sand, amended with 2% glucose, and fortified with cyromazine to the concentrations of 0, 1, 10, and 100 ppm. Each sample was inoculated with 10^7 propagules of Azotobacter vinelandii and incubated at 26°C , 80% relative humidity for 28 days under aerobic conditions. Additional control samples were prepared from sterile and non-sterile soils amended with glucose. Soil was collected at several sampling intervals during the incubation period and assayed for nitrogenase activity following incubation in 10% acetylene/argon atmosphere for 48 hours. Nitrogen-fixing activity was determined by measuring ethylene evolution resulting from nitrogenase induced reduction of acetylene to ethylene. In the silt loam soil, increases in ethylene generation were noted at the 3 and 7 day sampling intervals at 1 and 10 ppm cyromazine concentrations, and 1 through 14 day sampling intervals at 100 ppm concentration. In the loam soil, increases in ethylene generation was noted at 1 to 14 day sampling intervals at all cyromazine concentrations, and at 21 days in the 100 ppm samples.

F. ENVIRONMENTAL FATE (continued)

Biodegradation-Melamine

The degradative pathway of melamine, a primary metabolite of cyromazine, was examined using Pseudomonas Sp. strain A.2 (Jutzi, et al., 1982). The pathway involved four successive hydrolytic deamination reactions, which proceeded in the absence of oxygen, and ultimately resulted in the formation of carbon dioxide and ammonia. The three intermediates formed were ammeline, ammelide and cyanuric acid. The rate limiting reaction appeared to be the formation of ammelide from ammeline.

Plant Residues/Metabolism

The potential for uptake of cyromazine into crops from treated manure was studied under greenhouse conditions (Seim and Brown, 1981). Manure containing cyromazine was applied at the rate equivalent to 0.05 lbs ai/acre to the top 3 inches of sandy loam soil in planting containers. The mixture was allowed to "age" for 28-33 days before planting crops. Spring wheat, lettuce, and sugar beets were grown in individual containers and analyzed for cyromazine at 75% and 100% maturity. However, crop residue data were not provided in the study report.

The uptake of cyromazine by crops fertilized with treated manure was examined under greenhouse and field conditions over a 27 week study period (Simoneaux, 1981). A silt loam soil at the field site was amended with 20 tons of treated chicken manure per acre resulting in 0.2 lbs ¹⁴C-cyromazine per acre. Samples of the amended field soil were used for the greenhouse study. Lettuce, carrots, and spring wheat were planted 30 days after the soil was amended with treated manure and were grown to maturity. Lettuce leaves, carrot roots, and wheat stalks and grain were harvested and analyzed for cyromazine and metabolites. At the end of the study period under field conditions, the amended soils contained 37% parent cyromazine, 41% melamine, and 22% non-extractable compounds. The half-life of cyromazine in the manure amended soil was estimated as 12 weeks. Under greenhouse conditions, the initial levels in amended soils were 81% cyromazine and 15% melamine; after 12 weeks some of the melamine degraded to non-extractable compounds. Lettuce, carrot, wheat stalk and grain samples contained 0.03-0.05 ppm, 0.03-0.06, 0.23-0.76, and 0.15-0.17 ppm cyromazine, respectively.

The uptake of cyromazine and metabolites by crops grown in soil amended with treated chicken manure applied to the rate of 0.05 lbs ai/acre was studied under greenhouse conditions (Simoneaux, 1982). Lettuce, sugar beets and spring wheat were grown in soil amended with ¹⁴C-cyromazine treated manure and "aged" for 30 days. The initial

F. ENVIRONMENTAL FATE (continued)

level of cyromazine in the 0-3 inch layer of soil was 0.05 ppm. Thirty days after treatment, the soil contained 72.3% cyromazine and 6.5% melamine; after 121 days the soil contained 65% cyromazine and 18% melamine with the remaining radioactivity identified as non-extractable compounds. Only spring wheat had detectable levels of radioactivity over the limit of quantification (0.05 ppm). Spring wheat hulls contained 0.078 ppm and straw contained 0.112 ppm which was 72% parent compound and 7% melamine.

Mobility

Soil Adsorption/ Desorption

The batch equilibrium technique was used to determine the adsorptive and desorptive qualities of cyromazine in four soils with varying organic matter content and texture (Spare, 1988). Duplicate samples of clay, sandy loam, silt loam, and sand soils were treated with solutions of analytical grade ¹⁴C-cyromazine at the concentrations of 0, 0.25, 0.61, 1.22, 6.02, and 12.08 ug/ml until equilibrium was reached. Adsorption constants (Kd) were determined as follows: 50.4 for clay (highest binding), 5.62 for silt loam, 1.09 for sand, and 0.61 for sandy loam (lowest binding). Desorption constants were 54.6 for sand (highest binding), 51.5 for clay, 17 for sandy loam and 14.6 for silt loam (lowest binding). Soil adsorption (Koc) coefficients ranged from 70 for sandy loam (lowest binding) to 1784 for clay soils (highest binding); desorption coefficients ranged from 2217 for sandy loam (lowest binding) to 10,319 for sandy soil (highest binding). The results indicated that adsorption, desorption constants and coefficients are a function of several soil factors, including the concentration of organic matter.

Field Dissipation

The dissipation of cyromazine and the primary metabolite, melamine, was examined in a sandy loam soil under field conditions for one year (Ballantine, 1985). Trigard 75W was applied at the rate of 5 lbs active ingredient per acre as a broadcast, soil incorporated treatment. Soil samples were collected from 0-6" depth at 0, 15, and 34 days post-application (DPA); 0-6" and 6-12" depths at 60, and 120 DPA; and 0-6", 6-12", and 12-18" depths at 181, 271, and 366 DPA. Initial levels of cyromazine and melamine in the upper layer of soil were 0.39 ppm and <0.05 ppm, respectively. Cyromazine was detected only in the 0-6" soil layer during the study period. Levels ranged from 0.26 ppm to 0.93 ppm from 0 to 181 DPA with no pattern of degradation; at the remaining two sampling intervals levels dropped to 0.07 ppm and <0.05 ppm, respectively. Melamine was first detected in the 0-6" soil layer at 15 DPA and ranged in concentration from 0.15-0.74 ppm during the study. Melamine was detected in the 6-12" depth at all sampling intervals, and levels ranged from 0.09-0.2 ppm with a general increase over time. Melamine was detected in the 12-18' depth at 0.08 ppm at the 181 DPA sampling interval.

F. ENVIRONMENTAL FATE (continued)

Leaching Potential

The leaching potential of cyromazine was examined in silty loam, sandy loam, and two sand (Lakeland and Collombey) soils under laboratory conditions (Guth, 1980). The results were compared with data for a reference compound, monuron, considered a "moderate leacher". A suspension of an 80% wettable powder formulation containing radio-labeled cyromazine was applied to the top layer of soil in a 40 cm column at a rate equivalent to 4.45 lbs ai/acre. Approximately 7.9 inches of simulated rainfall was applied to the soil columns in a two-day period. Cyromazine was found to be considerably more mobile than the reference compound in the sandy loam and sand (Collombey) soils, but similar or less mobile in the silty loam and sand (Lakeland) soils. Cyromazine leached to the depth of 14, 16, 18, and >30 cm in the silty loam, sand (Lakeland), sandy loam, and sand (Collombey) soils, respectively. It appeared that the leaching behavior of cyromazine was primarily related to soil pH rather than soil organic matter content. Cyromazine which is weakly basic in solution was less mobile in the silty loam and sand (Lakeland) soils which are slightly acidic in nature.

III TOXICOLOGY PROFILE

A. PHARMACOKINETICS

Oral-Rat

An initial metabolism study was conducted with Charles River white rats given radiolabeled cyromazine (90.7% purity) as a single oral dose of 0.5 mg/kg (Simoneaux and Cassidy, 1978). This study was not considered acceptable as per U.S. EPA guidelines since only 4 males and 2 females were used, and there was not adequate justification of the dose level. The study results indicated that by 72 hours approximately 95% of the recovered dose was excreted in the urine and 3% in the feces. Ion exchange quantification of urinary components showed ~80% as parent compound and 3 minor metabolites, each approximately 5%.

An acceptable metabolism study was conducted using Cr1:CD(SD)BR rats (5 animals/sex/dose group, except 4 females used for IV study) given ¹⁴C-Cyromazine (97% purity) via oral (single or multiple doses) or IV routes (Capps, 1990). The single oral study had two dose groups, 3 mg/kg or 300 mg/kg; the multiple oral study (14 days of 96% unlabeled and 1 dose of ¹⁴C-Cyromazine) was administered at 3 mg/kg, and the IV was given at 3 mg/kg. There were no differences in metabolic patterns regardless of the route of administration, dose level or sex. Renal excretion was the primary route of elimination for cyromazine with 82-92% of the administered dose excreted in the urine, and 5% recovered in the feces during the 7 day collection period. Excretion appeared to be rapid with 52-78% and 2-5% of the administered radioactivity recovered in the urine and feces, respectively, at 24 hours. Approximately 50% of the radiolabeled material recovered in the feces (i.e. ~2.5% of the administered dose) probably came from biliary excretion, although this was not quantified. Unchanged cyromazine (72%), melamine (7%), hydroxycyromazine (9%) and methylcyromazine (2%) were found in the urine. Similar percentages were established for feces: 71% cyromazine, 7% melamine and 8% methylcyromazine, hydroxycyromazine and other metabolites. There was no evidence of preferential distribution or bioaccumulation of cyromazine in specific tissues or organs.

Dermal-Rat

Two dermal absorption studies have been conducted with ¹⁴C-Cyromazine applied to the shaved backs of male rats (See Appendix B). The initial study was not considered acceptable because the collection periods were too short and the large quantity of cyromazine bound in the skin (Murphy and Simoneaux, 1985).

The second dermal absorption study was conducted using three dose levels: 0.1, 1.0 and 10 mg/rat, which is equivalent to 0.01, 0.1 and 1 mg/cm², respectively (Murphy, 1987). The results indicate that cyromazine was rapidly absorbed into the skin but slowly released into the blood. The percent of dermal absorption ranged from 10% at the high exposure dose (1 mg/cm²) to 17% at the low dose. The low dose was considered more typical of potential human exposure; therefore, 17% was used to estimate dermal absorption for workers using cyromazine under conditions indicated on the product label.

B. ACUTE TOXICITY

Several acute toxicity studies have been conducted using Technical Cyromazine ~95% purity, Larvadex Premix (0.3% a.i.), Larvadex Premix (1% a.i.), Trigard 5% SC-C (5% a.i.) and Trigard 75W (75% a.i.). The results of these studies are summarized in Table 1.

Table 1 Acute toxicity of technical cyromazine and product formulations

Route/Species	Sex	Dosage/Effect	Category	Ref. ^a
<u>TECHNICAL GRADE</u>				
<u>Oral LD₅₀</u>				
Rat	M/F	3387 mg/kg	III	1
Mouse	M/F	2029 mg/kg	Supp. ^b	2
Rabbit	M/F	1467 mg/kg	Supp. ^b	3
<u>Dermal LD₅₀</u>				
Rat	M/F	>3170 mg/kg	III	4
<u>Inhalation LC₅₀</u>				
Rat	M/F	>0.049 mg/l ^c	I	5
<u>Eye Irritation</u>				
Rabbit	M	Non-irritating	IV	6
<u>Dermal Irritation</u>				
Rabbit	M/F	Non-irritating	IV	7
<u>Dermal Sensitization</u>				
Guinea pig		Negative		8
<u>Intraperitoneal LD₅₀^d</u>				
Rat	M/F	7-17 mg/kg	Supp. ^b	9

a/ (1) Sachsse and Bathe (no date). (2) Sachsse and Bathe (no date). (3) Sachsse and Ullmann, 1978a. (4) Sachsse and Bathe (no date). (5) Ulrich and Blair, 1979. (6) Sachsse and Ullmann, 1978b. (7) Sachsse and Ullmann, 1978c. (8) Sachsse and Ullmann, 1978d. (9) Sachsse and Bathe, 1978.

b/ Supplemental study since not required under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

c/ Actual measured concentration

B. ACUTE TOXICITY (continued)

Table 1 Acute toxicity of technical cyromazine and product formulations (continued)

Route/Species	Sex	Dosage/Effect	Category	Ref. ^a
<u>LARVADEX PREMIX (0.3%)</u>				
<u>Oral LD₅₀</u> Rat	M/F	>5033 mg/kg	IV	10
<u>Dermal LD₅₀</u> Rabbit	M/F	>2004 mg/kg	III	11
<u>Inhalation LC₅₀</u> Rat	M/F	>1.87 mg/l ^b	III	12
<u>Eye Irritation</u> Rabbit		Slight	III	13
<u>Dermal Irritation</u> Rabbit	M/F	Non-irritating	IV	14
<u>Dermal Sensitization</u> Guinea pig		Positive		15
<u>LARVADEX PREMIX (1.0%)</u>				
<u>Oral LD₅₀</u> Rat	M/F	>5050 mg/kg	IV	16
<u>Dermal LD₅₀</u> Rabbit	M/F	>2010 mg/kg	III	17
<u>Inhalation LC₅₀</u> Rat	M/F	>3.59 mg/l ^b	III	18

a/ (10) Sabol, R. 1979. (11) Cannelongo, 1979a. (12) Ulrich, 1979. (13) Cannelongo, 1979b. (14) Cannelongo, 1979c. (15) Sabol, E. 1979. (16) Maedgen, 1986a. (17) Maedgen, 1986b. (18) Maedgen, 1986c.

b/ Actual measured concentration

B. ACUTE TOXICITY (continued)

Table 1 Acute toxicity of technical cyromazine and product formulations (continued)

Route/Species	Sex	Dosage/Effect	Category	Ref. ^a
<u>LARVADEX PREMIX (1.0%) (continued)</u>				
<u>Eye Irritation</u>				
Rabbit		Slight	III	19
<u>Dermal Irritation</u>				
Rabbit		Non-irritating	IV	20
<u>Dermal Sensitization</u>				
Guinea pig		Negative		21
<u>TRIGARD 5% SC-C</u>				
<u>Oral LD₅₀</u>				
Rat	M/F	>5010 mg/kg	IV	22
<u>Dermal LD₅₀</u>				
Rabbit	M/F	>2010 mg/kg	III	23
<u>Inhalation LC₅₀</u>				
Rat	M/F	>2.90 mg/l ^b		24
<u>Eye Irritation</u>				
Rabbit		-----	ND ^c	25
<u>Dermal Irritation</u>				
Rabbit		Non-irritating	IV	26
<u>Dermal sensitization</u>				
Guinea pig		Negative		27

a/ (19) Maedgen, 1986d. (20) Maedgen, 1986e. (21) Maedgen, 1986f. (22) Cannelongo, 1983a. (23) Cannelongo, 1983b. (24) Maedgen, 1983a. (25) Cannelongo, 1983c. (26) Cannelongo, 1983d. (27) Cannelongo, 1983e.

b/ Reported value; study unacceptable, only one concentration used

c/ Not determined; study unacceptable

B. ACUTE TOXICITY (continued)

Table 1 Acute toxicity of technical cyromazine and product formulations (continued)

Route/Species	Sex	Dosage/Effect	Category	Ref. ^a
<u>TRIGARD 75W</u>				
<u>Oral LD₅₀</u> Rat	M/F	4460 mg/kg	III	28
<u>Dermal LD₅₀</u> Rabbit	M/F	>2010 mg/kg	III	29
<u>Inhalation LC₅₀</u> Rat	M/F	1.99 mg/l ^b	III	30
<u>Eye Irritation</u> Rabbit		Slight	III	31
<u>Dermal Irritation</u> Rabbit	M/F	Slight	III	32
<u>Dermal Sensitization</u> Guinea pig		Negative		33

a/ (28) Cannelongo, 1982a. (29) Cannelongo, 1982b. (30) Maedgen, 1983b. (31) Cannelongo, 1982c. (32) Cannelongo, 1982d. (33) Cannelongo, 1983f.

b/ Actual measured concentration

Clinical Observations (acute)

The following clinical observations were reported in acute, single dose, oral LD₅₀ toxicity studies using rats, mice or rabbits: sedation, dyspnea, exophthalmos, curved position, ruffled fur, tremor, ataxia, salivation.

C. SUBCHRONIC TOXICITY

Dietary-Rat

In a 90 day study, rats (no number or strain reported) were fed dietary concentrations of 0, 30, 300, 1000 or 3000 ppm of cyromazine (no purity reported) (Ciba-Geigy, 1982a). Recovery animals in the control and high dose groups only received the basal diet for an additional 4 weeks. No signs of overt toxicity or mortality were observed in any animal. There were no compound related effects on food consumption, ophthalmic examination, hematology, biochemical tests or urinalysis. The NOEL was estimated at 1000 ppm based on slightly reduced mean body weights measured in both sexes at 3000 ppm. This study was considered unacceptable by the Medical Toxicology Branch since only a brief summary was submitted by the registrant, and no individual animal data were included.

Dietary-Dog

Beagle dogs (4-6 animals/sex/dose) were given technical cyromazine (96.3% pure) at 0, 30, 300, 1000 or 3000 ppm in their diet for 13 weeks, with a four week recovery period for 2 dogs/sex in the 3000 and 0 ppm groups (Jessup, 1979). No mortality occurred at any dose level. When compared to concurrent controls, males in the 3000 ppm group exhibited significant reduction ($p < 0.01$) in hematocrit (19-21%) and hemoglobin (17-19%) on days 58 and 85. The toxicological significance of these findings is unclear, since the pre-test values for these parameters in these animals were similar to those measured on study days 58 and 85. In addition, the significant differences ($p < 0.05$) in absolute thyroid, kidney, heart and liver weights in the treated groups were not considered toxicologically meaningful since there were no compound-related changes (gross or microscopic) observed at necropsy. The study NOEL was established at ≥ 3000 ppm, the highest dose tested. However, this study was considered unacceptable by the Medical Toxicology Branch because there was no analysis of cyromazine in the feed to determine stability, the homogeneity and content of cyromazine in the feed was not reported, and the dose level selections were inadequate to permit the identification of target organ toxicity.

D. CHRONIC TOXICITY

Dietary-Rat

Technical grade cyromazine (95.5% and 95.3% purity) was administered in the diet to CD Sprague-Dawley albino rats at 0, 30, 300 or 3000 ppm for 2 years (Blair, 1982a). The equivalent dosages for these treatment groups were 0, 1.5 (males)/1.8 (females) mg/kg/day, 14.7 (males)/18.8 (females) mg/kg/day or 156 (males)/210 (females) mg/kg/day, respectively. There were either 60 (low and mid dose) or 70 (control and high dose) males and females per treatment group. Satellite studies included a one year interim sacrifice where five animals per sex in the control and high dose groups were necropsied. An additional five animals per sex in the high dose and control groups were withdrawn from treatment after one year and sacrificed for necropsy four weeks later.

D. CHRONIC TOXICITY (continued)

Antemortem findings were unremarkable, and animal survival was not affected by dietary exposure to cyromazine. However, although this study was considered acceptable as per FIFRA testing guidelines, it was generally concluded by the reviewers in the Medical Toxicology Branch that this study was poorly designed with regard to dose selection. The high dose selection was particularly poor in the case of the females, where animals typically weighed on the order of 30% less than concurrent controls. Previous U.S. EPA position documents on the "maximum tolerated dose" (MTD), suggested that acceptable body weight decrements in the high dose group should be roughly 10-15% less than the concurrent controls and should be based on subchronic testing (Farber, 1986; Harris et al., 1986). Mean food consumption of the high dose females was only slightly reduced (~9.3%) compared to concurrent controls on a g/rat/day basis, suggesting that the decreases in body weight were not just the result of decreased palatability. Body weights in the 300 ppm females were generally about 10% lower than the concurrent controls or than the 30 ppm (low) dose group. This indicates that 300 ppm was at the lower end of an MTD, since there was no apparent increase in mortality in the 3000 ppm females. The results at the interim sacrifice were unremarkable except for a body weight decrement in the high dose group.

Males in the high dose group had body weight decreases of approximately 23% compared to males in the concurrent control group. Body weights of males in the 300 ppm group were not statistically different ($p \leq 0.05$) from concurrent controls, and there were no other apparent treatment effects. Although the high dose elicited body weight decrements that were somewhat greater than the 10-15% target range, the 3000 ppm dose was considered as an acceptable high dose for male rats since it was only slightly above the target range for an MTD and since there was no effect on survival or no pronounced systemic toxicity. The results at the interim sacrifice were likewise unremarkable except for the body weight decrement in the high dose group.

Non-neoplastic effects included renal pelvic epithelial hyperplasia in high dose females and suppurative bronchitis, with an increased incidence of alveolar macrophages and bronchiectasis, in high dose males.

Non-Oncogenic Effects

A No Observed Effect Level (NOEL) for non-oncogenic effects was established for male rats at 300 ppm based on decreased body weight at the next highest dose of 3000 ppm. This NOEL is equivalent to an approximate daily dosage of 14.7 mg/kg/day for the male animals. The NOEL for non-oncogenic effects was established for female rats at 30 ppm based on the decreases in body weight at the next highest dose of 300 ppm. This NOEL is equivalent to an approximate daily dosage of 1.8 mg/kg/day for the female animals.

D. CHRONIC TOXICITY (continued)

Mammary Tumors

The incidence of mammary adenomas or mammary adenocarcinomas in the treatment groups was not significantly different ($p \leq 0.05$) from concurrent controls. The significance levels for adenomas and adenocarcinomas in the high dose females were $p=0.13$ and $p=0.089$, respectively. Combining the incidence of adenomas with adenocarcinomas resulted in a $p=0.019$ for the high dose females when compared to the concurrent controls (Table 2). The incidence in the concurrent control group for these combined mammary tumors was within the historical range and below the historical mean. The incidence (28.8%) for combined adenoma/adenocarcinomas in the high dose group was greater than the historical mean (15.3%) and greater than the historical range (Table 2).

Table 2 Incidence of neoplastic lesions in Sprague-Dawley rats after two years of treatment with cyromazine in feed

Tumor Type	Concentration (ppm)			
	Control	30	300	3000
Interstitial cell ^a (Testes)	1/60 ⁺⁺ (1.67%)	2/59 (3.39%)	1/58 (1.72%)	6/57* (10.5%)
Mammary adenoma ^b	3/53 (5.66%)	8/58 (13.8%)	6/58 (10.3%)	8/59 (13.6%)
Mammary adenocarcinoma ^c	3/53 ⁺⁺ (5.66%)	2/58 (3.45%)	1/58 (1.72%)	9/59 (15.3%)
Combined mammary adenoma/adenocarcinoma ^d	6/53 ⁺⁺ (11.3%)	10/58 (17.2%)	7/58 (12.1%)	17/59* (28.8%)

a/Historical control: Mean (7.7%); Range (0-22.2%) (Blair, 1982a)
b/Historical control: Mean (4.9%); Range (0-21.7%) (Blair, 1982a)
c/Historical control: Mean (9.5%); Range (1.5-21.4%) (Blair, 1982a)
d/Historical control: Mean (15.3%); Range (0-21.7%) (Blair, 1982a)

* Significantly different from concurrent control (Fisher's Exact Test); ($p=0.049$ for interstitial tumor and $p=0.019$ for combined mammary adenoma/adenocarcinomas at 3000 ppm)
 ++Significant for trend at $p < 0.01$ (dose weighted chi-square test (Peto, et al., 1980))

D. CHRONIC TOXICITY (continued)

Combining the benign adenomas with the malignant adenocarcinomas does not infer that there is a continuum for these tumors in the rat, i.e. that the adenomas eventually progress to the malignant form. The protocol for rodent oncogenicity studies is generally limited in its scope to adequately address this important issue. However, from a regulatory perspective, when considering the overall potential impact of a chemical to human health, it is equally important to protect against potential benign tumors, as it is to protect against malignant neoplasia. Therefore, the adenomas and adenocarcinomas observed in this study were combined for a statistical evaluation for overall oncogenic risk. This procedure is consistent with generally acceptable regulatory and scientific perspective use in evaluating animal oncogenicity studies (State of California, 1985; McConnell, et al., 1986; U. S. EPA, 1986; Huff and Haseman, 1991). Paynter (1985) did provide guidelines for combining benign and malignant neoplasms but did not specifically address combining mammary adenomas and adenocarcinomas in his U. S. EPA standard evaluation procedure document, "Oncogenicity Potential: Guidance for Analysis and Evaluation of Long Term Rodent Studies".

The application of the results from the rat study to assess the potential oncogenic risk to workers applying cyromazine is discussed in the Hazard Identification Section.

Interstitial Cell Tumors

There was a marginally statistically significant ($p=0.049$) increase in interstitial cell (ISC) tumors in the high dose males (Table 2). Only one animal in the high dose group exhibited this tumor prior to the terminal sacrifice. Historical control data indicated that in studies conducted at International Research and Development Corporation (IRDC) from 1975 to 1979 the mean incidence rate for interstitial cell adenoma was 7.7% with a range of 0 to 22%.

The incidence of ISC tumors in the concurrent control group was within the historical range, but below the historical mean (Table 2). The incidence in the high dose males (10.5%) was also within the historical range, but higher than the historical mean (7.7%).

The ISC tumors had been initially designated as a "possible adverse effect" by reviewers in the Medical Toxicology Branch (Martz, 1986a; Morgan, 1989a). Subsequent reexamination of these data indicated that there was not sufficient evidence to consider the slight increase in ISC tumors in the 3000 ppm group to be treatment related (Aldous, 1992a). Therefore, the Toxicology Summary was amended to reflect this change in the status of this effect (See Toxicology Summary, Appendix A).

Other than ISC tumors, the only other notable pathology in the testes was "testicular atrophy". This effect was apparently not related to treatment with cyromazine since the total incidence of "atrophy" was somewhat lower in the 300 and 3000 ppm groups than in the 30 ppm and concurrent controls. There were no other indications of treatment-related pathology in the testes.

D. CHRONIC TOXICITY (continued)

Dietary-Mouse

Technical grade cyromazine (95.3% and 95.5% pure) was administered in the diet to CD-1 mice at 0, 50, 1000 or 3000 ppm for 2 years (103 weeks) (Blair, 1982b). The equivalent dosages for these treatment groups were 0, 6.5(males)/9.5(females) mg/kg/day, 126(males)/187 (females) mg/kg/day, or 383(males)/525(females) mg/kg/day, respectively. Each treatment group consisted of 68 males and females. After one year of treatment, 8 animals/sex/dose level were sacrificed and necropsied.

Survival was slightly reduced in the high dose male group and in all female treatment groups (study weeks 91-103). Additionally, the high and intermediate dose male groups gained 24% or 12% less weight than the control animals, respectively, over the 103 week treatment period. Female mice in the high dose group experienced a 13% decrease in weight gain when compared to control animals.

Non-Oncogenic Effects

There were no remarkable non-neoplastic responses in either sex from treatment with cyromazine. The body weights of various treatment groups of male animals were often significantly reduced ($p < 0.05$) compared to concurrent controls; however, the differences were generally small and of questionable biological significance. Generally, high dose males weighed approximately 3 g less than control animals. Mid-dose males also exhibited a slight reduction in body weight, but it was generally less than the reduction found in the high dose group. The NOEL for non-oncogenic effects in this study was 1000 ppm, approximately 126 mg/kg/day, based on the body weight reduction in the high dose males.

Neoplasia

No increase in neoplastic lesions was reported for female mice in this study. In fact, the incidence of histiocytic lymphoma in the females decreased with increasing cyromazine concentration (i.e. from 16% in the concurrent controls to 1.6% in the high dose group). In male mice the incidence of histiocytic lymphomas, lymphocytic lymphomas or combined lymphomas was not statistically significant ($p \leq 0.05$) for any treatment group when compared to the concurrent controls (Table 3). However, the incidence for combined lymphomas in the high dose males was marginally insignificant (one-tailed $p = 0.053$). In addition, the incidence for combined lymphomas was significant for trend at $p < 0.05$. The incidence of combined lymphomas in all treatment groups was greater than the historical mean (9.4%) and greater than the historical range at the high dose. The concurrent control males exhibited tumors at rates that were similar to the mean historical values for each respective tumor type (Table 3).

D. CHRONIC TOXICITY (continued)

The combined incidence of lymphocytic/histiocytic lymphomas in the male mice had been initially characterized as a "possible adverse effect" by reviewers in the Medical Toxicology Branch (Martz, 1986b; Morgan 1989b). Subsequent reexamination of these study data indicated that these neoplasia in the mouse were not clearly the result of treatment with cyromazine and did not warrant a "possible adverse effect" designation (Aldous, 1992b). This conclusion was primarily based on the lack of clear statistical significance (pair-wise) between treatment groups and concurrent controls, the relatively small increase in tumor incidence over concurrent and mean historical controls, and the negative dose-response in the female animals. Therefore, the Toxicology Summary was amended to reflect this change in the status of this effect (See Toxicology Summary, Appendix A).

Table 3 Incidence of neoplastic lesions in male CD-1 mice after two years of treatment with cyromazine in feed

Tumor Type	Concentration (ppm)			
	Control	50	1000	3000
Histiocytic lymphoma^a				
-All dead/morib/sacr.	2/60 ^b (3.3%)	2/60 (3.3%)	2/56 (3.6%)	5/59 (8.5%)
Lymphocytic lymphoma^c				
-All dead/morib/sacr.	3/60 (5.0%)	5/60 (8.3%)	6/56 (10.7%)	7/59 (11.9%)
Histiocytic and lymphocytic lymphomas^d				
-All dead/morib/sacr.	5/60 ⁺ (8.3%)	7/60 (11.7%)	8/56 (14.3%)	12/59 ^e (20.3%)

^a/Historical control incidence for histiocytic lymphoma in CD-1 male mice at IRDC from 16 studies: range = 0-6.1%, mean = 2.5% (Campbell and Stevens, 1991a)

^b/Denominator includes animals considered "at risk"; i.e., animals that died after the first death of a male mouse having either lymphoma

^c/Historical control incidence for lymphocytic lymphoma in CD-1 male mice at IRDC from 16 studies: range = 0-12.5%, mean = 7%

^d/Historical control incidence for combined malignant lymphomas in CD-1 male mice at IRDC from 16 studies: range = 2.9-17.5%, mean = 9.4%

^e/p = 0.053 (Fisher's Exact Test)

+ Significant for trend at p<0.05 (dose weighted chi-square test) (Peto, et al., 1980)

D. CHRONIC TOXICITY (continued)

Dietary-Rat (Melamine)

A National Toxicology Program (NTP) carcinogenesis bioassay evaluated the oncogenic potential of the cyromazine metabolite, melamine, in Fischer 344 rats (Melnick, 1983a; Melnick, et al., 1984). Fifty male animals were fed melamine (100% purity) at 2250 ppm (~134 mg/kg/day) or 4500 ppm (~284 mg/kg/day), while 50 females were given 4500 ppm (~252 mg/kg/day) or 9000 ppm (~499 mg/kg/day) of melamine in their feed for 103 weeks. The control groups consisted of 50 males and 49 females.

Female survival was 68%, 60% and 54% in the control, low dose group and high dose group, respectively. Male rat survival was only 38% at the high dose ($p < 0.05$ compared to controls), 60% at the low dose and 61% in the control group. No compound-related clinical signs of toxicity were reported for males or females. There was no effect on average daily food consumption with increasing dose. Body weight was marginally affected, but mean body weights of treatment groups were still within 10% of control groups throughout the study.

The urinary bladder was the primary organ affected in male rats. The incidence of urinary bladder lesions for the male rats are presented in Table 4. Transitional-cell carcinomas occurred in male animals with a statistically significant positive trend ($p < 0.002$). The incidence of this tumor in the high dose group was 8/49 (16%) and was significantly ($p < 0.016$) greater than control animals. A transitional-cell papilloma was observed in the urinary bladder of an additional high dose male. There were no transitional-cell papillomas or carcinomas diagnosed in the urinary bladder of the low dose male rats. The incidence rate of urinary bladder transitional-cell carcinomas in the control males (0/45) reflected the historical rate for this tumor in untreated male F344 rats at Litton Bionetics, Inc. (0/789), or throughout the NTP bioassay program (0/3888). Transitional-cell carcinomas were not observed in female rats.

There was also an increased incidence of urinary bladder calculi in the high dose male rats. Seven of the 49 urinary bladders from the high dose male group that were examined microscopically had transitional-cell carcinomas with calculi, one had this tumor without calculi, three had calculi without evidence of the carcinoma, and 38 had neither calculi nor transitional-cell carcinomas. Bladder calculi were also observed in the one high dose male with the transitional-cell papilloma. Bladder calculi were observed in one low dose group male. Overall, there was a statistically significant association ($p < 0.001$) between the presence of bladder calculi and bladder tumors (Melnick, et al., 1984). Although bladder calculi were not observed in one male rat with the transitional-cell carcinoma and in other male rats without bladder tumors, it was suggested by the study investigators that bladder calculi could have developed but were passed in the urine before examination.

D. CHRONIC TOXICITY (continued)

Table 4 Incidence of urinary bladder lesions and calculi in F344 male rats fed diets containing melamine for 103 weeks

	Control	Low Dose (2250 ppm) ^a	High Dose (4500 ppm) ^b
Animals examined microscopically	45	50	49
Transitional-cell carcinoma	0	0	8 (16%)
Transitional-cell papilloma	0	0	1 (2%)
Transitional-cell hyperplasia	0	1 (2%)	2 (4%)
Bladder calculi	0	1 (2%)	10 (20%)

a/ Approximately 134 mg/kg/day
b/ Approximately 284 mg/kg/day

Chronic inflammation of the kidney was significantly increased ($p < 0.01$) in the low dose females (34%) and high dose females (82%), relative to the control females (8%). Chronic inflammation of the kidney was not significantly increased in the treated male rats, (i.e. 4% control group, 6% low dose and 12% high dose).

NOELs were estimated for non-oncogenic endpoints in male and female rats at <2250 ppm (134 mg/kg/day) and <4500 ppm (252 mg/kg/day), respectively, based on bladder calculi/transitional-cell hyperplasia (male) or kidney inflammation/transitional-cell papilloma (female), which occurred at the lowest doses tested.

The results of this study indicate that in the male F344 rat there was an apparent biological threshold for the occurrence of transitional-cell carcinomas at >2250 ppm and <4500 ppm. In the female F344 rat there were no transitional-cell tumors up to the highest dose tested, 9000 ppm; therefore, also indicating a plausible biological threshold for tumor formation in these animals. For the purposes of this risk assessment, the NOEL from this study for the occurrence of this tumor has been established at 2250 ppm (134 mg/kg/day). The relationship of this result to the quantification of oncogenic risk is discussed in greater detail in the **Hazard Identification Section**.

D. CHRONIC TOXICITY (continued)

Dietary-Mouse (Melamine)

An NTP carcinogenesis bioassay evaluated the oncogenic potential of the cyromazine metabolite, melamine, in B6C3F₁ mice (Melnick, 1983a; Melnick, et al., 1984). Fifty male and female¹ animals were fed melamine (100% purity) at 2250 ppm (~328 mg/kg/day, males; ~488 mg/kg/day, females) or 4500 ppm (~688 mg/kg/day, males; ~992 mg/kg/day, females), for 103 weeks. The control groups consisted of 49 males and 50 females.

Female survival was 74%, 86% and 82% in the control, low dose group and high dose group, respectively. Male mouse survival was 56% at the high dose compared to controls (p<0.05), 72% at the low dose and 80% in the control group. Average daily feed consumption per male mouse in the low and high dose groups was 93% and 95%, respectively, of the control group. The average daily feed consumption for female mice was 88% in the low dose group and 87% in the high dose group when compared to control animals. There was no consistent effect of melamine ingestion on the body weights of male or female mice. No compound-related clinical signs of toxicity were reported for males or females.

Male mice exhibited a marked increase in the incidence of urinary bladder calculi and in acute and chronic inflammation and epithelial hyperplasia of the urinary bladder (Table 5). Urinary bladder calculi were observed in 4/50 (8%) high dose female mice, and bladder inflammation or hyperplasia were only seen in high dose females at 4-8%.

Table 5 Incidence of urinary bladder lesions and calculi in B6C3F₁ male mice fed diets containing melamine for 103 weeks¹

	Control	Low Dose (2250 ppm) ^a	High Dose ^b (4500 ppm)
Animals examined microscopically	45	47	44
Bladder calculi	2 (4%)	40 (85%)	41 (93%)
Inflammation (acute/chronic)	0	25 (53%)	24 (55%)
Inflammation (chronic)	2 (4%)	10 (21%)	14 (32%)
Hyperplasia -epithelial	1 (2%)	11 (23%)	13 (30%)

a/ Approximately 328 mg/kg/day

b/ Approximately 688 mg/kg/day

D. CHRONIC TOXICITY (continued)

In both the 13 week subchronic and 103 week chronic dietary studies with mice, the urinary bladder was the only organ exhibiting a toxic insult from melamine exposure (Melnick, et al., 1984). In the chronic study, there was an increase in the incidence of urinary bladder calculi, inflammation and mild hyperplastic changes in the urinary bladder of the male mice. The incidence of these lesions in the female mice was not significantly different from control animals ($p \leq 0.05$). There was no evidence of tumor development in either the female or male B6C3F₁ mice. The systemic NOEL for male mice was estimated at <2250 ppm (~328 mg/kg/day) based on bladder calculi, inflammation and hyperplasia of the urinary bladder. The NOEL for female mice was established at 2250 ppm (~488 mg/kg/day), based on the same effects as in the males, at the high dose of 4500 ppm (~992 mg/kg/day).

Dietary-Dog

Beagle dogs (6-8 animals/sex/group) were given technical cyromazine (96.3% pure) in their feed at 0, 30, 300 or 3000 ppm for 26 weeks, with a 4 week recovery period for 2 dogs/sex at 0 and 3000 ppm (Voelker, et.al., 1980; Hardisty, 1990). Generally, a 26 week (6 month) dog study is not acceptable to fulfill the chronic study (1 year) data requirement because 6 months is an insufficient time period for the development of a complete tissue response from repeated, low dose chemical exposure. This study was initially considered unacceptable by the Medical Toxicology Branch because the dose level and/or duration of exposure were not sufficient to identify a target organ (Voelker, et. al., 1980). The initial review indicated that there was a decrease in both absolute and relative testes weights for dogs in the high dose group when compared to concurrent control animals. However, without histopathological information, the toxicological significance of these findings could not be established. A subsequent submission of results from a histological reevaluation of selected tissues from the dogs in this study demonstrated that a target organ (testes) and an adverse effect (seminiferous tubule degeneration) had been identified (Hardisty, 1990). Therefore, the 6 month study was considered acceptable to fill the chronic non-rodent study requirement, and the study was given a "possible adverse effect" designation (See Toxicology Summary, Appendix A).

The histomorphological examination of the testes from the males demonstrated that there was a bilateral degeneration of the seminiferous tubules with decreased spermatogenesis in the testes of two of five males dogs in the 3000 ppm group at 26 weeks. One animal exhibited a decreased number of sperm cells and an increased number of immature sperm types in the epididymides. The other dog had complete aspermia in the tubules of the epididymides. This effect in the testes was not seen in the concurrent control or other treatment groups; therefore, the NOEL for this effect was established at 300 ppm.

D. CHRONIC TOXICITY (continued)

Other effects related to body weight changes, feed consumption and hematology were also reported. These physiological responses were considered equivocal in nature and of minor toxicological importance. The high dose group of both sexes had alternating weight loss and gain with the amount of gain during the period of exposure being approximately 19% of control animals. There was also a reduction in weight gain in the intermediate female group, which gained approximately 50% of the control females. Feed consumption in the high dose males was reduced slightly throughout the treatment period, with the difference from controls achieving significance ($p < 0.05$) at week 8. Feed consumption in the high dose females was slightly reduced during the first 4 weeks, with no dose-related differences recorded during the remainder of the study. It is not known if these changes were related to the palatability of the feed. Because the reduction in body weight gain and feed consumption were parallel, the exposure to cyromazine on a mg/kg basis generally remained proportional to the feed concentration.

At all sampling intervals the high dose males had decreased mean total cholesterol levels, and at 5/6 sampling times there was a significant increase in mean SGOT activity. The values for both parameters returned to control levels by the end of the recovery period at week 30.

Hemoglobin and hematocrit values decreased in the high dose groups, with the difference from controls for both parameters being significant in males ($p < 0.05$) at all sampling intervals and achieving occasional significance in females. The lowest values were measured at week 17 where hemoglobin was 79% of controls and hematocrit was 83% of controls. At various sampling intervals, hematocrit was decreased in the intermediate dose males and high dose females, as was hemoglobin in the intermediate and low dose males. During the 4 week recovery period, both hemoglobin and hematocrit values in the males approached control values, while there was no change in females.

Erythrocyte counts were decreased at week 4 in females and week 8 in males in the high dose group when compared to pretreatment and control values. During the recovery phase, erythrocyte counts in the high dose animals increased to the highest levels during the study.

Platelet counts in the intermediate and high dose females, and high dose males, were significantly greater than controls ($p < 0.05$) at week 8 and weeks 17 and 21, respectively.

The sporadic hematological changes reported in the intermediate (300 ppm) and low (30 ppm) dose male groups were addressed by the registrant in a separate submission (Ciba Geigy Corp, 1982). The registrant disagreed with the testing laboratory, who had concluded that the NOEL for the hematological changes was 30 ppm. The registrant proposed that the hematocrit and hemoglobin values at the start of the study in the concurrent control group were abnormally high, when

D. CHRONIC TOXICITY (continued)

compared to historical controls, and, therefore, made the responses in the low and intermediate groups appear more significant than they actually were during the course of the study. The review of this study by the Medical Toxicology Branch was in agreement with the registrant that the hematocrit and hemoglobin values for the control group were higher than the animals in the low and intermediate dose groups at the initiation of the study. The values in the low and intermediate groups generally paralleled control values during the study and never changed in relationship to each another in a dose-related manner. The NOEL based on the hematological effects was established at 300 ppm, approximately 9.27 mg/kg/day and 8.85 mg/kg/day for males and females, respectively.

In their last revision of the cyromazine data base on Integrated Risk Information System (IRIS), the U.S. EPA concluded that the NOEL for the hematological effects seen in the 6 month dog study was 30 ppm (U.S. EPA, 1991). The U.S. EPA used the default assumption that food consumption was 2.5% of body weight (i.e. 1 ppm = 0.025 mg/kg/day) which gave a NOEL = 0.75 mg/kg/day. (Note: The adjusted NOEL, based on the actual food consumption in the study report, was approximately 0.90 mg/kg/day for males and females). Using an uncertainty factor of 100, the RfD was established by U.S. EPA at 0.0075 mg/kg/day (7.5 ug/kg/day). The 6 month dog study was given the classification of "core grade minimum".

E. GENOTOXICITY

Gene Mutation

Five gene mutation assays were conducted with cyromazine in bacterial or mammalian test systems. Three out of the five assays were considered acceptable based on federal guidelines. The two acceptable in vitro assays were negative for mutation potential in the respective bacterial or mammalian cell culture assay systems. The in vivo assay, the Mammalian (mouse) Spot Test, was considered positive but not indicative of a clear mutagenic response; therefore, the cover page of the Toxicology Summary (Appendix A) reflects this conclusion by not listing gene mutation as a "possible adverse effect". A memorandum from Dr. J. Gee to Dr. K. Pfeifer (4/2/92) further explained the rationale used in coming to this conclusion, and this memorandum is part of Appendix A.

As part of the NTP evaluation on the oncogenic potential of melamine, a mutagenicity study using the TA98, TA100, TA1535 and TA1537 strains of S. typhimurium was conducted with and without metabolic activation with S-9 hamster or rat liver fractions (Melnick, 1983b). This study was acceptable and indicated no mutagenic potential for melamine under the test conditions.

E. GENOTOXICITY (continued)

Structural Chromosomal Aberrations

The potential of cyromazine to induce chromosomal aberrations was evaluated in five different in vitro or in vivo test systems. Although all of the studies were considered negative, most (4/5) of the assays were not acceptable because of technical flaws in study design. The micronucleus test in the mouse was acceptable and considered negative for increasing the frequency of micronuclei in polychromatic erythrocytes (Hool, 1980).

Other Genotoxic Effects

Two studies using either rat or mouse primary hepatocytes were conducted using technical cyromazine. There was no unscheduled DNA synthesis in either in vitro test system; however, the rat study was considered unacceptable because the number of cells scored and the dose level were not reported. A D7/Mammalian-Microsome Mutagenicity Test using Saccharomyces cerevisiae was negative for mitotic cross over, gene conversion and gene reversion with and without metabolic activation.

A summary of the genotoxicity studies with cyromazine is presented in **Table 6**.

F. REPRODUCTIVE TOXICITY

Dietary-Rat

A two-generation reproduction study using Sprague-Dawley COBS CD rats was conducted with cyromazine (95.3% purity) at dietary concentrations of 0, 30, 1000 or 3000 ppm (Note: animals in high dose group received 4000 ppm for weeks 1-4) (Schardein, 1981). Using food consumption and body weight data for the F_0 generation (weeks 1-15) and the F_1 generation (weeks 28-43), the approximate equivalent dosages were 0, 1.8, 58 and 199 mg/kg/day for males, and 0, 2.1, 72 and 224 mg/kg/day for females. Both F_0 and F_1 generations consisted of 15 males and 30 females. Each generation was¹ exposed to cyromazine for approximately 28 weeks. There was no effect on the appearance or behavior of the offspring at any dose level or time period. There was no treatment-related effect on male spermatogenesis or on fertility indices.

F_0 Parents/ F_1 Litters

Males and females in the F_0 generation experienced significant ($p < 0.01$) decreases in mean body weight gain at 1000 ppm (9.3% for males; 9.7% for females) and 3000 ppm (20.9% for males; 18% for females). There was a concomitant reduction in mean food consumption at 1000 ppm (7.8% for males; 6.4% for females) and at 3000 ppm (14.8% for males; 15.5% for females).

E. GENOTOXICITY (continued)

Table 6 Summary of genotoxicity studies conducted with cyromazine

Test Type/ System	Strain/ Species	S-9	Results	Comments/(Ref) ^a
<u>GENE MUTATIONS</u>				
S. typhimurium	TA98,TA100 TA1535,TA1537	±	Neg	Unaccept.FIFRA (1)
Mouse lymphoma	L5178Y/TK ^{+/-}	±	Neg	Unaccept.FIFRA (2,3)
Chinese hamster embryonic lung	V79	±	Neg	No base pair/ frame shift (4)
Mammalian spot	C57B1/6	NA ^b	Pos	See text (5)
S. typhimurium	TA98,TA100 TA1535,TA1537	±	Neg	No increase in revertant colonies (6)
<u>STRUCTURAL CHROMOSOMAL ABERRATIONS</u>				
Dominant lethal	Mouse	NA	Neg	Unaccept.FIFRA (7)
Micronucleus	Chinese hamster	NA	Neg	Unaccept.FIFRA (8)
Peripheral lymph.	Human	±	Neg	Unaccept.FIFRA (9)
Dominant lethal	Mouse	NA	Neg	Unaccept.FIFRA (10,11)
Micronucleus	Mouse	NA	Neg	No increase in micronuclei (12)
<u>OTHER GENOTOXIC EFFECTS</u>				
DNA repair/ hepatocyte	Mouse	NA	Neg	No unsched. DNA synthesis (13)
DNA repair	Rat	NA	Neg	Unaccept.FIFRA (14)
Saccharomyces cervisiae	D-7	±	Neg	Unaccept.FIFRA (15,16)

a/(1) Arni and Muller, 1978. (2) Beilstein and Muller, 1985a. (3) Beilstein and Muller, 1985b. (4) Dollenmeier and Muller, 1986. (5) Strasser, 1986. (6) Deparade, 1988. (7) Hool, 1981. (8) Hool, 1980. (9) Strasser and Arni, 1985. (10) Campbell and Stevens, 1981. (11) Campbell and Stevens, 1988. (12) Strasser, 1987. (13) Tong and Williams, 1983. (14) Tong and Williams, 1982. (15) Hool and Arni, 1984a. (16) Hool and Arni, 1984b.

b/Not applicable to test system

F. REPRODUCTIVE TOXICITY (continued)

In the F₁ litters, pup survival was slightly reduced (90.4% vs. 95.5% in controls) on lactation day 4 in the 3000 ppm group. There was a statistically significant (p<0.01) reduction in mean pup body weight at birth in the 3000 ppm group. This reduction in mean body weight persisted with statistical significance (p<0.01) for both males and females on days 4, 7, 14 and 21 of lactation. In the 1000 ppm group, mean male body weight was reduced on day 21 (p<0.05).

F₁ Parents/F₂ Litters

F₁ females experienced significant (p<0.01) decreases in mean body weight gain at 1000 ppm (11.8%) and 3000 ppm (15.5%). There was a 5.9% reduction in weight gain at 30 ppm. Males had a significant (p<0.01) decrease in mean weight gain only in the high dose group (14.6%). Mean food consumption also decreased in the 1000 ppm group (5.4% for males; 5.6% for females) and in the 3000 ppm group (10.4% for males; 15.8% for females).

In the F₂ litters, the number of viable pups per total pups born was reduced² (94.4% vs. 99.3% in controls) in the 3000 ppm group. This response was statistically significant at p<0.05. Mean pup body weight at birth was reduced in the 3000 ppm group (p<0.01). Mean body weights for both male and female pups were reduced (p<0.05) on lactation days 4, 7 and 21 in the 3000 ppm group. In the 1000 ppm group, mean body weights were statistically different from controls (p<0.05) only on day 7 of lactation.

The reproductive NOEL was 1000 ppm (~57 mg/kg/day for males and 72 mg/kg/day for females), based on the decreased pup weight at birth and the decreased number of viable pups in the 3000 ppm, F₂ litters. The systemic NOEL for the parents was at the low dose of 30 ppm (~1.7 mg/kg/day for males and 2.1 mg/kg/day for females), based on decreased body weight gain at 1000 and 3000 ppm (F₀ males and females, F₁ females) and at 3000 ppm (F₁ males).

G. DEVELOPMENTAL TOXICITY

Gavage-Rat

Technical cyromazine (96.3% pure) was given by gavage to pregnant female Charles River COBS CD rats, 25 animals/dose group, at 0, 100, 300 or 600 mg/kg/day on days 6-19 of gestation (Rodwell, 1979). At 300 and 600 mg/kg/day clinical signs included facial staining, ano-genital staining and red vaginal discharge. There were no treatment-related necropsy findings at any dose. At 300 and 600 mg/kg/day, the maternal body weight gains were 86% and 74%, respectively, of control values. Fetal body weights also decreased in the 300 and 600 mg/kg/day groups. No malformations were seen in the study. In the high dose group, the number of fetuses with variations increased to approximately 55%, compared to 25-26% in the control and other treatment groups. This

G. DEVELOPMENTAL TOXICITY (continued)

study was initially considered unacceptable by the Medical Toxicology Branch, but upgradable pending the submission of: 1) an analysis (initial or retrospective) of the dosing suspensions, 2) individual data for clinical observations, necropsy and fetal variations, 3) an explanation of laboratory classification of fetal variations. A subsequent submission addressed these questions, and the study was considered acceptable (Campbell and Stevens, 1982). Fetal variations were defined by the contract laboratory (IRDC) as "alternations in anatomical structure that are considered to have no significant biological effect on animal health or body conformity and represent slight deviations from normal". Most examples placed in this category are "minor variations in size and form of normally present ossification centers". Also included in this category are "slight misshapening or misalignment of structures, and processes involving continued development (bilateral skeletal centers not yet fused, incomplete maturation of renal papillae, presence of vestigial structures, etc.), and development of extra ossification sites". The NOEL for both maternal and developmental effects was 100 mg/kg/day, based on decreased maternal and fetal body weights.

Gavage-Rabbit

In two separate experiments, technical cyromazine (CGA-72662; 96.3% purity) was administered by gavage on days 6-27 of gestation at dosages of 0, 25, 50 or 75 mg/kg/day (Experiment #1), and 0, 10, 30 or 60 mg/kg/day (Experiment #2), to 16 artificially inseminated Dutch Belted (Langshaw Farms) rabbits per treatment group (Blair, 1981). The maternal NOEL was established at 10 mg/kg/day, based on decreased weight gain at 30 and 60 mg/kg/day (Experiment #2) and dam deaths at 25, 50 and 75 mg/kg/day (Experiment #1). The causes of death were determined as heart failure (2 animals), pneumonitis-pleuritis (1 animal), abortion-related (2 animals) and unknown causes (2 animals). Developmental effects of biological significance noted in this study were:

- 75 mg/kg/day: significant decrease in mean number of live fetuses
- 75 and 60 mg/kg/day: increased number of resorptions
- 60 mg/kg/day: 1 fetus with hydrocephaly; slight decrease in fetal weight
- 30 mg/kg/day: significant decrease in fetal weight; one fused and one malformed sternebrae
- 10 mg/kg/day: one hydrocephaly; slight decrease in fetal weight

The developmental NOEL, based on embryo-feto toxicity occurring to some degree at all dosages, was considered to be <10 mg/kg/day. Since a NOEL was not established for developmental toxicity, this study was considered as unacceptable according to FIFRA testing guidelines, and not considered upgradable by the Medical Toxicology Branch.

G. DEVELOPMENTAL TOXICITY (continued)

Gavage-Rabbit

Technical cyromazine (95.2% purity) was given by oral gavage to 18 inseminated New Zealand White (Buckshire) rabbits per dose group on day 7 through day 19 of gestation (Nemec, 1985). Dose levels were 0 (untreated control), 0 (0.5% carboxymethylcellulose, vehicle control), 5, 10, 30 or 60 mg/kg/day. Animals were necropsied on day 29 of the study.

Maternal Effects

There were three maternal deaths, two of which resulted from intubation errors; one in the 30 mg/kg/day group and one in the 60 mg/kg/day group (Table 7). There were four abortions, one in the untreated control, one at 30 mg/kg/day and two at 60 mg/kg/day. The only clear clinical signs of maternal toxicity were decreased defecation and urination, which were increased in frequency and duration in the 30 and 60 mg/kg/day treatment groups.

Table 7 Maternal effects reported in a developmental study using New Zealand White rabbits after treatment with cyromazine (Nemec, 1985)

Parameter	Dosage (mg/kg/day)					
	Vehicle Control	Untreat. Control	5	10	30	60
Bred	18	18	18	18	18	18
Pregnant	14	14	13	7	13	12
Dams died	1 ^a	0	0	0	1 ^b	1 ^b
Dams aborted	0	1	0	0	1	2
Dams w/ total resorptions	1	1	1	0	3	0

a/ animal sacrificed

b/ intubation death

G. DEVELOPMENTAL TOXICITY (continued)

Mean maternal body weight changes during the treatment period ranged as follows:

Vehicle control:	6.0% increase
Untreated control:	4.5% increase
5 mg/kg/day:	5.6% increase
10 mg/kg/day:	6.7% increase
30 mg/kg/day:	2.5% increase
60 mg/kg/day:	3.7% decrease

The changes measured in the 60 mg/kg/day group were statistically significant ($p < 0.01$) when compared to the vehicle control group. After the treatment period (gestation days 20-29), mean body weight gain in the 60 mg/kg/day group was significantly increased ($p < 0.01$). The body weight gain in the 30 mg/kg/day group was decreased over the entire interval of days 7-29 when compared to the vehicle or untreated control groups.

All values for mean food consumption in the untreated control, 5 and 10 mg/kg/day groups were comparable to the vehicle control. Mean food consumption (g/kg/day) was significantly decreased ($p < 0.01$) during the treatment period (gestation days 7-19) in the 60 mg/kg/day group. The decrease ranged from 36% of the vehicle control during days 7-10 to 73% of the vehicle control during days 14-20. Animals in the high dose group had a significant increase ($p < 0.01$) in mean food consumption after the treatment period (days 20-29). Mean food consumption in the 30 mg/kg/day group ranged from 78% to 80% ($p < 0.05$) of the vehicle control group for the same time intervals.

The NOEL for maternal toxicity was established at 10 mg/kg/day, based on reduced body weight gain at the next two higher dosages.

Fetotoxicity

There were no biologically or statistically significant differences between the vehicle control group and all treatment groups concerning the mean numbers of viable or dead fetuses, implantation sites, corpora lutea or mean fetal weights (Table 8). There was a statistically significant difference ($p < 0.05$) in the mean fetal sex ratios in the 5, 10 and 30 mg/kg/day groups, which was not considered biologically meaningful since the effect was not seen in the high dose group. The mean number of early resorptions in the 30 and 60 mg/kg/day dose group, and the mean number of late resorptions in the 60 mg/kg/day group were increased. These effects were also reflected in the total and mean number of post implantation loss sites in the 30 and 60 mg/kg/day groups (Table 8). These responses were reported to be statistically insignificant. However, there was statistical significance ($p < 0.05$) for the 60 mg/kg/day group when the incidence of post implantation loss was calculated as a percentage of the total number of implantation sites.

G. DEVELOPMENTAL TOXICITY (continued)

The live number of fetuses per litter in all groups was low and not related to treatment with cyromazine. With the exception of the 10 mg/kg/day group, all groups had mean values below the historical range of 6.1 to 9.1 and the historical mean of 7.3.

Table 8 Fetal effects reported in a developmental study using New Zealand White rabbits after treatment with cyromazine^a

Parameter	Vehicle Control	Dosage (mg/kg/day)				
		Untreat. Control	5	10	30	60
Litters w/ viable fetuses	12	13	12	7	10	11
Viable fetuses						
-Male/Female	22/36	28/25	41/21	28/17	46/25	27/27
-Total	58	53	62	45	71	54
-Mean ^b	4.5	3.8	4.8	6.4	5.5	4.9
Dead fetuses	0	0	0	0	0	0
Fetal weight (g)						
-Mean	44.3	47.8	46.7	41.5	42.6	45.7
Corpora lutea						
-Total	111	116	129	77	112	104
-Mean	8.5	8.3	9.9	11.0	8.6	9.5
Total implant. sites	70	65	73	51	87	80
Resorptions						
-Early	11	12	11	6	16	19
-Late	1	0	0	0	0	7
Total resorpt. implant. site (%)	12/70 (17%)	12/65 (18%)	11/73 (15%)	6/51 (12%)	16/87 (18%)	26/80* (33%)

^a/ Adapted from Table 7 (Nemec, 1985)

^b/ Mean values were calculated using number of gravid females at day 29: Veh. control (13), Untreat. control (14), 5 mg/kg/day (13), 10 mg/kg/day (7), 30 mg/kg/day (13) and 60 mg/kg/day (11).

* p<0.05 (Fisher's Exact Test)

G. DEVELOPMENTAL TOXICITY (continued)

Fetal Malformations

Examination of the fetuses revealed a number of external, visceral and skeletal malformations in all groups, including controls (Table 9). Malformations, which were not present in either of the concurrent control groups, consisted of open eyelid, micrognathia, short, bent or absent tail, umbilical hernia, cyclopia with multiple head anomalies, hydrocephaly, diaphragmatic hernia and heart anomaly (Table 10). There did not appear to be a pattern to the malformations observed, and in most cases, they were found in only one fetus. There were some skeletal malformations in all dose groups, with a higher prevalence in the 60 mg/kg/day group. Additionally, in the 60 mg/kg/group, there was a statistically significant increase in the incidence of fetuses with external malformations ($p < 0.001$) and total (external, visceral and skeletal) malformations ($p < 0.05$) (Table 9). Although the number of litters with fetuses having any one malformation was not different from the vehicle control group, there was a significant increase ($p < 0.05$) in the number of litters with fetuses with any external malformation at 60 mg/kg/day (Table 11).

One fetus with cyclopia was observed in the 10 mg/kg/day group and another in the 30 mg/kg/day group. Cyclopia was not observed in the 5, 60 mg/kg/day groups or in either concurrent control group. Additionally, there was no incidence of cyclopia in the 306 fetuses (42 litters) submitted as historical control data from the laboratory which conducted this study. The markedly greater incidence of total resorptions (33%) at 60 mg/kg/day may have limited the ability to detect cyclopia in that dose group (Table 8). The NOEL for developmental toxicity was 5 mg/kg/day, based on the occurrence of malformations (cyclopia) at 10 mg/kg/day.

Table 9 Incidence of malformations observed in the fetuses of New Zealand White rabbits after treatment with cyromazine^a

Malformation	Dosage (mg/kg/day)					
	Vehicle Control	Untreat. Control	5	10	30	60
External	0/58	0/53	1/62	1/45	2/71	10/54 ^{***}
Visceral	1/58	0/53	1/62	2/45	6/71	3/54
Skeletal	6/58	2/53	4/62	2/45	5/71	11/54
Total	7/58	2/53	5/62	3/45	10/71	15/54 [*]

^a/ Adapted from Table 8, (Nemec, 1985)

* Significantly different from vehicle control ($p < 0.05$) (Fisher's Exact Test)

*** Significantly different from vehicle control ($p < 0.001$) (Fisher's Exact Test)

G. DEVELOPMENTAL TOXICITY (continued)

Table 10 Malformations observed in the fetuses of New Zealand White rabbits after treatment with cyromazine^a

	Dosage (mg/kg/day)					
	Vehicle Control	Untreat. Control	5	10	30	60
Number examined	58	53	62	45	71	54
<u>Malformation</u>						
Open eyelid	0	0	0	0	0	4
Micrognathia	0	0	0	0	1	0
Short tail	0	0	0	0	0	3
Umbilical hernia	0	0	0	0	1 ^a	1
Cyclopia	0	0	0	1	1 ^a	0
Spina bifida	0	0	0	0	1 ^a	0
Bent tail	0	0	0	0	0	1
Absent tail	0	0	1	0	0	0
Hydrocephaly	0	0	0	1	2	1
Diphragmatic hernia	0	0	0	1	3	0
Spleen absent	0	0	0	0	0	1
Heart/great vessel anomaly	0	0	0	0	0	1
Lung anomaly	0	0	0	0	0	1
Sternebrae fused	0	0	0	0	0	2

^a/ Adapted from Table 8, (Nemec, 1985)

G. DEVELOPMENTAL TOXICITY (continued)

Table 11 Incidence of malformations observed in the litters of New Zealand White rabbits after treatment with cyromazine^a

Malformation	Vehicle Control	Dosage (mg/kg/day)				
		Untreat. Control	5	10	30	60
External	0/12	0/13	1/12	1/7	2/10	5/11*
Visceral	1/12	0/13	1/12	2/7	4/10	3/11
Skeletal	6/12	2/13	2/12	1/7	5/10	6/11
Total	6/12	2/13	3/12	2/7	6/10	6/11

a/ Adapted from Table 8, (Nemec, 1985)

* Significantly different from vehicle control (p<0.05)
(Fisher's Exact Test)

Gavage-Rabbit

The previous teratology study using New Zealand White rabbits (Buckshire strain) reported a slight increase in the incidence of cyclopia in two dosage groups (Nemec, 1985), and both fetuses exhibiting this rare malformation had been sired by the same male. Therefore, another study was conducted to: 1) determine if untreated females inseminated with semen from the male in the previous teratology study would produce cyclopia and/or related head effects, suggesting a genetic etiology 2) evaluate the rate of spontaneous malformation in New Zealand White rabbits from Buckshire Farms, when compared to Hazleton Dutchland and Lanshaw Farms 3) determine the effect of stress by sham-gavage on the rate of spontaneous malformations (Nemec, 1986a)

This study consisted of three control groups composed of a minimum of 56 females. No animals were given cyromazine. Semen from the male used in the previous teratology study was utilized to impregnate does from Groups 1 and 3, termed control and sham control, respectively. Maternal animals from Group 2 were inseminated with semen from alternate males from the animal colony.

There was no difference in the percentage of fetuses with malformations between the test groups described above. Buckshire rabbits exhibited a greater incidence (5.8%) in total fetal malformations when compared to the Hazleton/Dutchland (3.9%) or the Langshaw rabbits (2.8%). Although cyclopia was not seen in the Buckshire rabbits in this study, there were incidences of cranial defects, including hydrocephaly, acrania and cleft palate.

G. DEVELOPMENTAL TOXICITY (continued)

Other malformations reported were spina bifida, fused sternbrae, diaphragmatic hernia among others. The study investigators concluded that the cyclopia reported in the previous teratology study was not attributable to the male used in the current study and that many of the malformations seen in the previous study may have occurred spontaneously, and may not have been the result of treatment with cyromazine.

Gavage-Rabbit

Technical cyromazine (95.2% pure) was given by oral gavage to 74 inseminated New Zealand White (Hazelton-Dutchland) rabbits per dose group on days 7-19 of gestation (Nemec, 1986b). Dosage levels were 0 (0.5% carboxymethyl cellulose), 5, 10 or 30 mg/kg/day. Thirty-three to 36 dams per dose level were necropsied on day 29 of gestation, and the remaining dams were allowed to litter, with kits necropsied on day 28 of lactation.

During the treatment period, dams in the 30 mg/kg/day group had a marked decrease in mean body weight (115 g loss) compared to a 78 g mean weight increase in control animals; this difference was statistically significant at $p < 0.01$. Mean food consumption in the 30 mg/kg/day group was decreased by approximately 32% when compared to control animals during the treatment period; this difference was statistically significant at $p < 0.01$. There was a compensatory increase in food consumption and weight gain during the period after dosing. The NOEL for maternal toxicity was established at 10 mg/kg/day based on the body weight changes that occurred in the 30 mg/kg/day group.

There were no treatment-related effects on the number of corpora lutea, implants, resorptions or fetal weights. Additionally, there was no treatment-related effect on pup weight or survival during the lactation period. The initial review of this study (6/2/88) concluded that the malformations (cyclopia, cleft palate, omphalocele) seen in one dead kit of the 30 mg/kg/group was not treatment related, and, therefore, did not constitute an adverse (teratogenic) effect since it may have been a spontaneous occurrence or sire-related (See Toxicology Summary in Appendix A for incidence tables). A subsequent review of this study (9/19/89) reported spina bifida, short tail, microphthalmia/anophthalmia and hydrocephaly in fetuses other than the one where cyclopia occurred. The incidence of these malformations is presented in Table 12. Cyclopia and hydrocephaly occurred at the high dose, and these responses were considered treatment-related since they did not appear in the concurrent and historical controls. Spina bifida may be a manifestation of the teratogenic effect that also causes cyclopia (Johnston et al., 1979), even though spina bifida occurred at a very low incidence (0.03%) in the historical controls. The developmental NOEL for these malformations was established at 10 mg/kg/day.

G. DEVELOPMENTAL TOXICITY (continued)

Gavage-Rabbit

Technical grade cyromazine (no purity given) was administered by oral gavage to 18 inseminated female Dutch Belted rabbits (Langshaw Farms) per dose group from day 7 through day 19 of gestation (Blair, 1985). Dose levels of cyromazine were 0 (untreated control), 0 (1% carboxymethyl cellulose), 5, 10, 30 or 60 mg/kg/day. Animals were necropsied on day 28 of the study. This study was considered acceptable under FIFRA testing guidelines.

Dams in the 30 and 60 mg/kg/day groups experienced weight loss during the dosing period, and there was a decrease in food consumption by animals in the high dose group. Maternal deaths in the 10, 30 and 60 mg/kg/day exposure groups were not considered compound related but may have been related to intercurrent disease that appeared in all treatment and control groups. However, the incidence of the disease was not considered of sufficient severity to invalidate the results of the study. The NOEL for maternal toxicity was established at 10 mg/kg/day, based on the decreases in body weight and food consumption at the next higher dosages.

There were no treatment-related effects on any of the fetal parameters measured in this study; therefore, the NOEL for developmental toxicity was \geq 60 mg/kg/day.

Table 12 Incidence of malformations in NZW rabbits after treatment with cyromazine during gestation (Nemec, 1986b)

Effect	Dosage (mg/kg/day)				Historical Control
	Control	5	10	30	
Cyclopia	0/355	0/398	0/325	1/317 ^a	0/3024
Spina bifida	0/355	0/398	0/325	1/317 ^b	1/3024
Short tail	1/355 ^c	0/398	0/325	1/317 ^d	1/3024 ^e
Microphthalmia/ Anophthalmia	0/355	1/398 ^f	0/325	0/317	1/3024
Hydrocephaly	0/355	0/398	0/325	2/317 ^g	0/3024

a/ Dam and fetus numbers not reported

b/ Dam #1267, Fetus #1

c/ Dam #1405, Fetus #7 (tail 0.4 cm long)

d/ Dam # 1267, Fetus #3 (thread-like tail)

e/ Reported as tail anomaly

f/ Dam # 1251, Fetus # 8 (microphthalmia, left)

g/ Dam and fetus numbers not reported

G. DEVELOPMENTAL TOXICITY (continued)

A summary of the rat and rabbit developmental toxicity studies with cyromazine is presented in Table 13. The data indicate that the rabbit is more sensitive than the rat to cyromazine for both maternal and fetal effects. The New Zealand White rabbit (Buckshire) appears to be the most sensitive species treated with cyromazine. The lowest NOEL for developmental toxicity was 5 mg/kg/day, based on malformations (cyclopia) seen in this strain of rabbit. This NOEL was used to calculate the margins of safety from the potential acute exposure from the use of cyromazine on chicken manure.

Table 13 Summary of developmental toxicity studies in rats and rabbits given cyromazine

Species	Strain	Maternal NOEL (mg/kg/d)	Endpoint	Develop. NOEL (mg/kg/d)	Endpoint	Ref.
Rat	Charles River CD BSCD	100	Decreased BW ^a	100	Decreased BW	(1,2)
Rabbit	Dutch Belt (Langshaw)	10	Decreased BW gain/death	<10	Malform./ fetotox.	(3) ^b
Rabbit	NZW (Buckshire)	10	Decreased BW gain	5	Malform. (cyclopia)	(4)
Rabbit	Dutch Belt (Langshaw)	10	Wt. loss	≥60	Fetotox.	(5)
Rabbit	NZW (Hazelton-Dutch.)	10	Decreased BW/ food consumpt.	10	Malform.	(6)

a/ body weight

b/ Not acceptable per FIFRA testing guidelines

References: (1) Rodwell, 1979. (2) Campbell and Stevens, 1982. (3) Blair, 1981. (4) Nemeč, 1985. (5) Blair, 1985. (6) Nemeč, 1986.

H. NEUROTOXICITY

Delayed neurotoxicity studies for cyromazine are not required under current FIFRA study guidelines.

IV RISK ASSESSMENT

A. HAZARD IDENTIFICATION

Potential adverse effects have been identified in the following animal studies using cyromazine: 1) Developmental toxicity (teratology), rabbit. 2) Chronic toxicity, dog. 3) Combined chronic/oncogenicity, rat.

Acute Toxicity

The most sensitive species exhibiting teratogenic effects from treatment with cyromazine was the New Zealand White rabbit (Buckshire) (Nemec, 1985). One fetus with cyclopia was found in the 10 mg/kg/day group and one in the 30 mg/kg/day group. However, the fact that there was a 33% implantation loss (resorptions) at the high dose may have precluded the detection of additional malformations, such as cyclopia. The incidence of total external malformations (other than cyclopia) in the litters of high dose animals was statistically different ($p < 0.05$) from the concurrent controls (Table 9). Cyclopia did not occur in either the vehicle or untreated concurrent control groups. Additionally, there was no incidence of cyclopia in the 306 fetuses (42 litters) submitted as historical control data for Buckshire rabbits from the laboratory which conducted the cyromazine teratology study. The developmental NOEL for this study was established at 5 mg/kg/day, based on the occurrence of cyclopia at 10 mg/kg/day. The NOEL for maternal toxicity was established at 10 mg/kg/day, based on a difference in maternal weight gain.

Another study with New Zealand White rabbits from a different breeding facility (Dutchland), also had a single incidence of cyclopia at 30 mg/kg/day (Nemec, 1986b). There was a small incidence of spina bifida and hydrocephaly in the fetuses of the 30 mg/kg/day dose group (Table 12). Cyclopia did not appear in the concurrent controls or in the historical control animals (i.e. 0/3024 fetuses). Spina bifida and hydrocephaly did not occur in the concurrent controls, and the incidence in the historical control animals was 1/3024 for spina bifida and 0/3024 for hydrocephaly. The developmental NOEL for these malformations was established at 10 mg/kg/day. The NOEL for maternal toxicity was also considered to be 10 mg/kg/day, based on body weight decreases in the high dose dams during the treatment period.

The U. S. EPA initially (1985) set the NOEL for developmental toxicity (fetotoxicity) in the Wil Labs. 82001 study (Nemec, 1985) at 5 mg/kg/day. In 1989, the U. S. EPA reexamined the issues surrounding the interpretation of this study and considered a "weight of evidence" approach (primarily miscellaneous historical control data) proposed by the registrant (Campbell and Stevens, 1991b). The U. S. EPA concluded that although "the Agency was being conservative during the initial (1985) evaluation of the Wil 82001 studydue to the unusual findings of cyclopia in two dose groups....., the developmental effects observed in the submitted studies are not consistently reproducible across studies, are clearly not cyromazine dose-related, and appear to have occurred by chance" (U. S. EPA, 1989a).

A. HAZARD IDENTIFICATION (continued)

Therefore, the U. S. EPA raised the NOEL for developmental toxicity from 5 mg/kg/day to 10 mg/kg/day, based on increases in skeletal anomalies at 30 mg/kg/day, and "cyromazine is not longer considered to be a developmental toxicant".

The Medical Toxicology Branch maintained that the lowest NOEL for developmental toxicity was 5 mg/kg/day, established in the Buckshire New Zealand White rabbit study for cyclopia (Nemec, 1985). The additional historical control data submitted by the registrant (Campbell and Stevens, 1991b) was not sufficiently convincing to change this conclusion. The Mid-Atlantic Regional Teratology Association (MARTA) reference on historical controls indicated that the normalized incidence of cyclopia in rabbits was 1.4 per 10,000 fetuses (MARTA, no date). However, the actual incidence of cyclopia was 1 in 7,312 fetuses (0.014%). The overall concerns with these historical control data are: 1) the names or number of the laboratories were not included, 2) the strain of rabbit or name of the breeding facility was not indicated, 3) the dates for data collection were not given. In another historical control reference, the incidence of "cyclopia-otocephaly sequence" in New Zealand White rabbits was 7.9 per 10,000 (~0.08%) (Palmer, 1972). Apparently, these data had also been normalized per 10,000 fetuses, so the actual incidence of malformations is not known. The concerns expressed above for the MARTA data are also applicable to this historical data set. A third historical control reference presented spontaneous malformations in rodents and rabbits used for chemical testing in Japanese laboratories (Morita, et al., 1987). Cyclopia appeared in one Japanese white rabbit fetus out of a total of 15,556 fetuses. The relevance of this finding to the occurrence of cyclopia in the New Zealand White rabbits used in the cyromazine studies is highly questionable. In conclusion, the miscellaneous historical data submitted by the registrant do not support the contention that cyclopia occurs in clusters and at a relatively high spontaneous rate. Therefore, 5 mg/kg/day was used to assess the potential acute exposure to workers using cyromazine.

Chronic Toxicity

Chronic toxicity from repeated exposure to cyromazine was identified in the 6-month ("chronic") dog study (Voelker, et. al., 1980; Hardisty, 1990). The identified adverse effect was bilateral degeneration of the seminiferous tubules of the testes with a decrease in spermatogenesis. The NOEL for these effects was 300 ppm, approximately 9.27 mg/kg/day.

In the rat reproduction study, cyromazine-related effects to pups in the first and second generation litters were confined to the high dose group, 3000 ppm (Schardein, 1981). The reproductive NOEL was established at 1000 ppm (~57 mg/kg/day for males), based on pup viability and decreased body weight at birth. The NOEL for systemic toxicity in adult animals was established at the lowest dose of 30 ppm (~1.7 mg/kg/day for males), based on decreased body weight gain experienced by animals at 1000 and 3000 ppm in both the first and second generations.

A. HAZARD IDENTIFICATION (continued)

The lowest NOEL from repeated oral exposure to cyromazine was 1.7 mg/kg/day based on reduced weight gain for male rats in a two generation reproduction study. Additionally, the NOEL in the rat chronic study was established at 1.8 mg/kg/day (females) for decreased body weight. The biological significance of these effects does not appear to be as important as the adverse effect to the seminiferous tubules reported in the 6 month dog study. The NOEL for that effect was 9.27 mg/kg/day. However, cyromazine has demonstrated teratogenic potential to developing organisms, as indicated by the results from the rabbit studies, and possible effects in the testes of rats and dogs. Therefore, a prudent public health approach was to select the lowest chronic NOEL for systemic, non-oncogenic toxicity to insure the reproductive success of adults and the normal development of the offspring. Therefore, the NOEL of 1.7 mg/kg/day was used to calculate a margin of safety for chronic toxicity from repeated daily exposures to cyromazine.

Oncogenicity

Cyromazine

In the rat combined chronic toxicity/oncogenicity study, ISC tumors occurred in male animals, and mammary tumors, including adenomas and adenocarcinomas, were reported in the females. This study was considered acceptable by the Medical Toxicology Branch under FIFRA testing guidelines; however, although animal survival was not affected by exposure to cyromazine, relatively large decrements in body weight, particularly in female rats, indicated that the MTD had probably been exceeded. The marginal increase in ISC tumors only in the high dose males was considered of questionable biological significance and not related to treatment with cyromazine.

Mammary adenomas and adenocarcinomas appeared in female rats with the combined incidence of these tumors reaching statistical significance ($p < 0.05$) in the high dose group. The combined incidence was well above the historical mean and greater than the historical range (Table 2). As previously discussed, there was a general concern regarding the interpretation of the mammary tumor incidence in the high dose females, where the MTD had apparently been exceeded, based on substantial reductions in food consumption and body weight. However, previous studies with rats have indicated that the incidence of mammary tumors generally decreases in relation to decreases in food consumption and body weight (Turnbull, et al., 1985; Boorman, et. al., 1990; Ip, 1991). Since the frequency of mammary adenomas/adenocarcinomas was increased in the high dose females, who had concomitant, substantial decreases in body weight, the resultant combined increase in these mammary tumors was considered biologically significant and identified as a "possible adverse effect".

A. HAZARD IDENTIFICATION (continued)

In the mouse oncogenicity study, only male animals exhibited both histiocytic and lymphocytic lymphomas. The conclusion of the reviewers in the Medical Toxicology Branch was that the individual or combined incidence of these lymphomas was not related to treatment with cyromazine.

Cyromazine belongs to the general class of chemicals known as triazines. However, cyromazine is not unique in the ability to produce mammary adenomas and adenocarcinomas. Studies on file at the DPR, using Sprague-Dawley rats, have reported similar findings for other structurally-related triazines, including cyanazine (Bogdanffy, 1990), atrazine (Wingard and Mayhew, 1986; Thakur, 1991) and simazine (Ciba Geigy, 1988). In addition, the rat study with atrazine reported an increased occurrence of testicular interstitial cell tumors. (Wingard and Mayhew, 1986). It is beyond the scope of this risk characterization document to evaluate the oncogenic potential of other triazine pesticides, either individually or as a group. However, based on the results of the studies with cyromazine and the "weight of evidence" for other structurally-related compounds to elicit similar responses in laboratory animals, an assessment of the oncogenic potential was considered a prudent and justifiable, public health approach.

In using a low dose extrapolation model to characterize the potential oncogenic risk to humans, the most appropriate animal data-set should be used as the basis for the estimation of risk. For reasons discussed in the **Toxicology Profile Section** and above in the **Hazard Identification Section**, the chronic rat study had some limitations because of the selection of the dose levels. However, although cyromazine does not appear to be as potent an oncogen as other triazines, and is not genotoxic based on currently available studies, the ability to cause both benign adenomas and malignant adenocarcinomas in rats appears to be a common biological characteristic of these compounds, including cyromazine. Therefore, the Medical Toxicology Branch concluded that the incidence of mammary adenomas plus adenocarcinomas would be used to assess the oncogenic risk of cyromazine to workers.

Melamine

Compound-related lesions were observed in the urinary tract of F344 rats and B6C3F1 mice fed diets containing melamine ranging from approximately 134 mg/kg/day (2250 ppm) for male rats to approximately 992 mg/kg/day (4500 ppm) for female mice. In male rats, the urinary bladder was the primary organ affected. Transitional-cell carcinomas of the urinary bladder occurred in the male rats with a statistically significant positive trend, and the rate in the high dose group was significantly greater than that of the concurrent control group. There was also an increased incidence of urinary bladder calculi in the high dose males. There was a highly significant statistical association between the formation of bladder calculi and the presence of bladder tumors. Bladder calculi or transitional-cell carcinomas were not observed in female rats.

A. HAZARD IDENTIFICATION (continued)

In the mouse study, the urinary bladder was also the primary organ affected by exposure to melamine. Male mice exhibited an increased incidence of urinary bladder calculi and inflammatory and mild hyperplastic changes in the urinary bladder. However, in contrast to male rats, there was no evidence of tumor development in the male mice, even though the incidence of bladder calculi was greater than 85% in both treatment groups. The incidences of these lesions in the female mice were not significantly different from control animals ($p \leq 0.05$)

It has been postulated that anatomical differences, such as the location of the prostate gland in relation to the urethra, may increase the susceptibility of males to the growth of urinary bladder calculi due to obstruction of the urethra (Melnick, et al., 1984).

The more biologically important aspects of the rat study were the occurrence of transitional-cell carcinomas in the bladder of males fed diets containing at least 4500 ppm of melamine and the mechanistic debate about the possible role of bladder calculi in the development of this tumor. This NTP study showed a significant statistical association between the presence of bladder calculi and the development of urinary bladder tumors. This association between calculi and carcinoma was also reported in a more recent study using male F344 rats (Okumura, et al., 1992). In this study the animals were given 0, 0.3% (3000 ppm), 1%, or 3% (30,000 ppm) melamine (>99% purity) in their diet for 36 weeks, followed by a 4 week recovery period. There was a highly statistical correlation ($p = 0.0065$) between calculus formation and tumor incidence. The lack of tumors in the low dose group in the presence of calculi was attributed to the lower number and/or smaller size of the calculi.

Additionally, previous studies have suggested that rodent bladder tumors may result from the stimulation of epithelial cell division due to the irritation from the calculi (Weil, et al., 1965; Clayson, 1974; Anderson, 1980; Cheng, 1980; Chin, et al., 1981). It has been proposed that the bladder calculi consist primarily of insoluble melamine because of the inability of the male rat to metabolize this triazine compound (Worzalla, et al., 1974; Mast et al., 1983). Also in support of this hypothesis was the report that dietary treatment of male F344 rats for 4 weeks with 1.6 or 1.9% melamine resulted in urinary bladder calculi that were composed primarily of unmetabolized melamine (Melnick, et al. 1984). The hypothesis that the urinary calculi are composed of precipitated melamine cannot be solely explained by simple water solubility, since the solubility of melamine in water (37°C) was higher (calculated as 6.4 mg/ml), when compared to a measured melamine urinary concentration in the NTP rat study of 1.8 mg/ml (Heck and Tyl, 1985).

A. HAZARD IDENTIFICATION (continued)

The association between urinary bladder calculi and bladder tumors is suggestive, but not proof, of a "cause and effect" relationship. Since the specific mechanism of tumor induction in rodent urinary bladders by melamine is unknown, it is not possible to conclude that the tumorigenic response resulting from the ingestion of melamine is solely due to urinary bladder calculi or results from only melamine, or a combination of melamine plus urinary bladder calculus formation.

Standard short-term tests have indicated no genotoxic potential for melamine. Mutagenicity tests with melamine were negative in Drosophila melanogaster (Rohrborn, 1962), and for various strains of Salmonella typhimurium with or without S-9 metabolic activation systems (Seiler, 1973; Lusby et al., 1979; Melnick, 1983b). In addition, melamine was negative in tests for the induction of sister chromatid exchanges in Chinese hamster ovary (CHO) cells (NTP unpub. results). Mast et al. (1982a, 1982b) reported in two abstracts that melamine was negative in the following genotoxicity tests:

- 1) mutagenicity tests for various strains of S. typhimurium (Ames).
- 2) the hypoxanthineguanine phosphoribosyl transfer (HGPRT) locus in CHO cells.
- 3) the induction of sister chromatid exchanges in CHO cells.
- 4) the rat-hepatocyte primary culture DNA repair test.
- 5) the in vivo mouse micronucleus test.

Although no genotoxic effects have been reported from melamine exposure in the standard short-term in vitro test systems using S-9 activation fractions from liver homogenates, NTP apparently initiated mutagenicity and sister chromatid exchange studies with homogenates derived from bladder epithelial cells in order to obtain a more complete evaluation of the genotoxic potential of melamine, (Melnick et al., 1984). The results of these proposed studies have not been submitted to the DPR and are unknown at the present time. Nonetheless, the available genotoxicity studies conducted with melamine have indicated no genotoxic potential.

The weight of scientific evidence available to assess the oncogenic potential of melamine in humans did not support the use of a low dose linearized multi-stage model (e.g. Global 86) for the following reasons: 1) there is no definitive evidence of any genotoxicity 2) there were no bladder tumors formed in female rats or in both male and female mice 3) the presence in the male rat of an apparent biological threshold below which bladder tumors are not found 4) a strong indication that urinary bladder calculi are a prerequisite for the formation of bladder calculi and that these urinary calculi are only formed at abnormally high dietary concentrations of melamine, at which the urine may become supersaturated, and melamine precipitates out of solution.

A. HAZARD IDENTIFICATION (continued)

Although initially concerned about the oncogenic potential of melamine (U.S. EPA, 1984, 1985), the U. S. EPA subsequently concluded that "the oncogenic risk posed by melamine, the major metabolite of the insect growth regulator, cyromazine, is non-existent, or, at worst, extremely low" (U.S. EPA, 1989b). This position by the U. S. EPA was supported by a previous evaluation by the International Agency for Research on Cancer (IARC) which concluded that there was inadequate evidence for the carcinogenicity of melamine to experimental animals (U.S. EPA, 1988). In addition, the U.S. Food and Drug Administration (FDA) and U. S. EPA Cancer Assessment Committee (FDA/CAG) evaluated the results of the melamine studies and concluded: 1) there is a direct correlation between the occurrence of bladder neoplasms and the formation of calculi in the same bladders 2) the presence of bladder calculi completely obfuscates any plausible case which might be made for a treatment-related chemical induction of bladder neoplasms 3) melamine is only indirectly responsible for this occurrence in that "stones" occurred in the bladder only at high melamine doses, and it is the "stones", not melamine, that are tumorigenic (U.S. EPA, 1984). The oncogenic classification of melamine and cyromazine by the U.S. EPA is undergoing internal review and is not currently available. (U.S. EPA, 1992a).

B. OCCUPATIONAL EXPOSURE

Applicators

A study using Larvadex 2SL was conducted by the registrant in 1988 to determine occupational exposure from applying cyromazine to chicken manure in poultry houses (Merricks, 1988). A more detailed discussion of this study is located in **Appendix B, Human Exposure Assessment for Cyromazine**. The three application methods used in the study were: 1) hand-held sprayer 2) backpack sprayer and 3) portable power sprayer. The study was designed to estimate the actual exposure from an application of cyromazine with workers wearing a dust mask and rubber gloves, in addition to normal work clothing (e.g. shoes, socks, long pants and long-sleeved shirt or coveralls. A mean exposure estimate from 9 replicate applications was calculated for dermal and inhalation routes.

The mean Absorbed Daily Dosage for the three application methods is presented in **Table 14**. The exposure study used male workers, and the mean Absorbed Daily Dosage was calculated using default values for body weight and body surface area that are applicable to male workers (**See Appendix B**). However, female workers cannot be excluded from performing the job tasks presented in this document. Additionally, the biological endpoint (i.e. fetal malformations in a developmental toxicity study) used to assess the acute exposure is directly applicable to women of child-bearing age. The exposure assessment

B. OCCUPATIONAL EXPOSURE (continued)

indicated that the body surface area to body weight ratios for males and females at the 50th percentile are nearly identical. Therefore, although female workers are not specifically addressed in the exposure assessment and risk characterization sections, the Absorbed Daily Dosage in ug/kg, which was determined from body surface area exposures in the male workers, would be similar for female workers.

The Absorbed Daily Dosage (ADD) was used to calculate the Annual Average Daily Dosage (AADD) and the Lifetime Average Daily Dosage (LADD) using the assumptions presented in Table 15.

Field Workers

The Larvadex 2SL label indicates that chicken manure treated with cyromazine can be used as a soil fertilizer supplement at a rate of 4 tons per acre per year. Using the assumptions presented in Appendix B, a field worker's potential dermal exposure was calculated as 0.62 mg for an 8-hour work day. The absorbed daily dosage for a 76 kg male would be 1.4 ug/kg/day, i.e. similar to the hand-held sprayer, (Table 14).

Table 14 Mean absorbed daily dosages from dermal and inhalation routes after exposure to cyromazine during three application methods^a

Application Method	Daily Dermal Dosage ^b (ug/kg/day)	Daily Inhalation Dosage ^c (ug/kg/day)	Combined Daily Dosage ^d (ug/kg/day)
Hand-held Sprayer	0.74	0.46	1.2
Backpack Sprayer	37.36	0.46	37.8
Power Sprayer			
-Mixer/loader	0.45	0.14	0.59
-Applicator	8.50	0.14	8.64
-Combined	8.95	0.28	9.23
Field Worker	1.4	---- ^e	1.4

^a/Adapted from Appendix B (Table 4 and text).

^b/Based on 17% dermal absorption factor and 76 kg body weight

^c/Based on 50% of MDL values, breathing rate of 29L/min., 50% pulmonary retention and 100% absorption, and 76 kg body weight

^d/Combined daily dosage = Absorbed Daily Dosage (ADD)

^e/Not estimated but considered insignificant relative to dermal exposure

B. OCCUPATIONAL EXPOSURE (continued)

Table 15 Absorbed daily dosages, annual average daily dosages and lifetime average daily dosages for applicators of cyromazine and for field workers spreading manure

Application Method	Absorbed Daily Dosage ^a ug/kg/day	Annual Average Daily Dosage ^b ug/kg/day	Lifetime Average Daily Dosage ^c ug/kg/day
Hand-held Sprayer	1.2	0.03	0.02
Backpack Sprayer	37.8	1.04	0.59
Power Sprayer			
-Mixer/loader	0.59	0.02	0.01
-Applicator	8.64	0.24	0.14
-Combined	9.23	0.26	0.15
Field Worker	1.4	0.04	0.02

a/ From Table 14

b/ Assumes a maximum of 10 applications (work days) per year (See Appendix B)

c/ Assumes 40 years of potential exposure over 70 year lifetime

C. RISK CHARACTERIZATION

Applicators

Margins of safety were calculated for acute and chronic exposures for applicators using the proposed methods of application (Table 16). The additional theoretical lifetime oncogenic risk was calculated only as a 95% upper bound confidence interval (95th UB) using the Global 86 Linearized Multistage Model (Howe et. al., 1986) (Table 16). Generally, a maximum likelihood estimate (MLE) for risk is also presented in order to give a realistic range of potential oncogenic risk. Because of the relatively similar incidence of mammary adenomas/adenocarcinomas in the control, 30 mg/kg/day and 300 mg/kg/day groups, the Global 86 program could not calculate a positive potency slope for the MLE; therefore, the MLE potency was assumed to be zero, with a resulting risk of zero. In extrapolating from the rat to humans, equivalent human dosages were calculated using a default cross-species scaling of animal body weight to the 3/4 power (See Appendix C). This methodology has been recently proposed by the U. S. EPA (U. S. EPA, 1992b). The computer printouts for the risk estimates using the Global 86 program are included in Appendix C.

C. RISK CHARACTERIZATION (continued)

Table 16 Margins of safety for acute and chronic exposures and additional lifetime oncogenic risk for applicators of cyromazine and for field workers applying manure

Application Method	Acute MOS ^a	Chronic MOS ^b	Lifetime Risk ^c MLE	95th UB
Hand-held Sprayer	4,166	56,666	ND ^d	1.2E-07
Backpack Sprayer	132	1,635	ND	3.5E-06
Power Sprayer				
-Mixer/loader	8,475	85,000	ND	5.9E-08
-Applicator	579	7,083	ND	8.3E-07
-Combined	542	6,538	ND	8.9E-07
Field Worker	3,571	42,500	ND	1.2E-07

a/ Based on ratio of the NOEL of 5,000 ug/kg/day/Absorbed Daily Dosages in Table 15. (Cyclopa in rabbit developmental toxicity study, **Nemec, 1985**)

b/ Based on ratio of the NOEL of 1,700 ug/kg/day/Annual Average Daily Dosages in Table 15. (Decreased body weight gain in rat reproduction study, **Schardein, 1981**)

c/ Based on the Lifetime Average Daily Dosages (Table 15) and potency estimates of zero for the MLE and 5.9E-03 (mg/kg/day)⁻¹ (Q₁) for the 95% upper bound confidence interval from rat combined mammary tumors (Table 2) (**Blair, 1982a**)

d/ Not determined since potency slope for MLE was assumed to be zero

The MLE analysis from the Global 86 program indicated that the incidence for combined mammary adenomas/adenocarcinomas was not characteristic of a rigorous dose-response data set, since the MLE potency slope may not be positive at all dose levels. Although the Global 86 model will proceed to calculate a potency slope for the 95% upper bound term, the biological validity of this mathematical approach is open to question. Therefore, an alternative analysis, using a margin of safety paradigm, was considered as an approach to evaluate the potential lifetime exposure to cyromazine.

In the rat chronic study, the lowest dose of 30 ppm (~1.8 mg/kg/day) was established as a non-oncogenic NOEL for the female animals, based on the decrease in body weights at 300 and 3000 ppm. The ratio of the non-oncogenic NOEL of 1.8 mg/kg/day and the potential LADD of 0.59 ug/kg/day results in a MOS of approximately 3,000 for the backpack sprayer, the worker with the greatest potential exposure.

C. RISK CHARACTERIZATION (continued)

This biologically conservative NOEL of 1.8 mg/kg/day was approximately 10-fold lower than the dosage which did not result in a statistically significant increase in the incidence of the combined adenomas/adenocarcinomas.

Field Workers

The margin of safety for potential acute exposure for a 76 kg worker handling cyromazine-treated chicken manure was greater than 3,500, based on the ratio of a NOEL of 5,000 ug/kg/day from the rabbit teratology study and an Absorbed Daily Dosage of 1.4 ug/kg/day. The margin of safety for potential chronic exposure was greater than 42,000, and the additional oncogenic risk would be similar to the worker using the handheld sprayer, provided that the number of annual work days is similar for both workers.

V RISK APPRAISAL

The risk assessment process evaluates the potential for human exposure and the likelihood that adverse effects, identified primarily in animal studies, will occur in humans under specific exposure conditions. Every risk assessment has inherent limitations on the application of the toxicity and exposure data to characterize the potential risk to human health. Consequently, certain assumptions and extrapolations are used in the hazard identification and exposure assessment processes. Subsequently, these assumptions and extrapolations are incorporated into the risk characterization, resulting in varying degrees of uncertainty. Qualitatively, risk assessments generally have similar types of uncertainty. However, the magnitude of the uncertainty depends on the availability and quality of the data, and the types of specific exposure scenarios being assessed. Assumptions and areas of uncertainty associated with the current risk assessment for cyromazine are presented in the following sections which address the adequacy of the margins of safety or excess risk from the use of cyromazine on chicken manure.

A. APPLICATORS

Acute Toxicity

Margins of safety for potential acute exposure from the direct application of cyromazine to chicken manure ranged from 132 for the backpack sprayer to greater than 500 for the power sprayer who also performs the mixing/loading operations. These margins of safety are considered adequate. The NOEL of 5 mg/kg/day used to calculate the acute margins of safety was based on malformations seen in a rabbit teratology study (Nemec, 1985). Cyclopia occurred in one fetus at 10 mg/kg/day and in one fetus at 30 mg/kg/day. This malformation did not appear in the high dose group of 60 mg/kg/day, indicating no clear-cut dose-response in these animals. However, inherent toxicity, expressed as implantation loss in the high dose animals, may have limited the interpretation of the dose-response in this study. Because cyclopia did not appear in the two concurrent control groups and because of the relatively rare incidence of cyclopia in the historical population of New Zealand White rabbits, the NOEL was established at 5 mg/kg/day. Additional support for the use of cyclopia as a biological endpoint to assess the risk from potential acute exposure appeared in the subsequent study (Nemec, 1986b) using Hazelton-Dutchland New Zealand White rabbits. The NOEL for malformations, including cyclopia, was 10 mg/kg/day, with an incidence of 0/355 for cyclopia in the concurrent control and 0/3024 from historical records (Table 12). Additionally, the U. S. EPA reviewers followed what they called a "conservative" approach in their initial evaluation of this study by also listing the NOEL at 5 mg/kg/day (Nemec, 1985). However, subsequent miscellaneous, but irrelevant, historical control data submitted by the registrant and reevaluations by the U. S. EPA lead to an increase in the NOEL from 5 mg/kg/day to 10 mg/kg/day (U. S. EPA, 1989a). The U.S. EPA is currently addressing the biological relevance of the malformations observed in the rabbit that were the basis for setting the NOEL for developmental toxicity (U.S. EPA, 1992).

V RISK APPRAISAL (continued)

When considering the exposure scenario and the biological endpoint of fetal malformations used to establish the NOEL for acute exposure, women of child-bearing age would be the only work group to which this evaluation could be directly applied. A NOEL for acute, systemic toxicity that could be generically applied to all workers was not available for cyromazine. However, the underlying assumption used in this risk assessment was that using the most sensitive acute NOEL to establish a MOS would be health protective for all workers. This evaluation indicated that there are adequate acute margins of safety for all potential application methods allowed by the product label.

Chronic Toxicity

Margins of safety for chronic toxicity were calculated using a biologically conservative effect (i.e. body weight change) established in a reproduction study and an annual average daily dosage. Margins of safety for all worker activities were greater than 1,600 and are considered adequate.

The calculation of an annual average daily dosage to characterize the potential chronic exposure is primarily academic because of the anticipated annual use patterns and label restrictions which limit application to not more frequently than every 21 days. Workers who apply cyromazine to chicken manure would probably not perform this job on 10 consecutive days during a year, but rather periodically throughout the year on an "as need" basis. Since there would not be any daily, repeated exposure and since there is no evidence that cyromazine accumulates in humans or test animals, the most relevant exposure pattern for these applicators would be characterized by the single day, acute scenario. However, any changes in the number or pattern of applications would require a reevaluation of the potential chronic exposure for these workers.

Oncogenicity

The worker using the backpack method of application had the highest potential exposure to cyromazine. The additional theoretical lifetime oncogenic risk associated with this work category was $3.5E-06$ using the 95% upper bound estimate. It was not possible to calculate a MLE for risk since the potency slope for the MLE was zero. The great discrepancy between the MLE and the upper bound potency estimates suggests a limitation of the Global 86 model in this particular analysis, and indicates that the latter term may not closely reflect the dose-response relationship of the experimental data. When evaluating the potential oncogenic risk of a chemical to humans, it is scientifically meaningful and conceptually responsible to present a range of the potential risk. This range can be reported as a maximum likelihood estimate at the lower end, to a 95% upper bound confidence interval at the upper end. Although zero can always theoretically be used as the "lowest bound" on potential risk, this approach is considered scientifically inappropriate and irresponsible from a regulatory perspective.

V RISK APPRAISAL (continued)

It was previously stated in this document that the incidence of mammary tumors in the rat was not indicative of a potent oncogenic chemical where there would be a proportional increase in tumors with an increase in dosage. However, because similar tumors have been reported in rats exposed to structurally similar triazine pesticides, an estimate of oncogenic risk for cyromazine was considered to be a responsible public health approach. On the other hand, by only considering the theoretical 95% upper bound on risk, particularly when this function in the model is influenced primarily by the response at the high dose, a realistic range of overall oncogenic risk cannot be presented.

In addition to the cancer potency/risk analysis using the Global 86 model, an approach was presented which indicated that the potential lifetime average daily dosage (i.e. 0.59 ug/kg/day) for the backpack sprayer would be approximately 3,000 times lower than the NOEL (i.e. 1.8 mg/kg/day or 1800 ug/kg/day) at which there was no biologically significant decrease in body weight gain in female rats exposed to cyromazine for 2 years. It was assumed that the backpack sprayer would perform this task 10 times a year for 40 years. Workers using other methods of applying cyromazine to chicken manure and field workers would have approximately 4 to 30 times lower exposure than the backpack sprayer, i.e., margins of safety ranging from 12,000 (combined power sprayer) to 90,000 (handheld sprayer). The NOEL of 1.8 mg/kg/day is a biologically conservative no-effect-level since it was based on decreased body weight gain in a dietary study where the animals were given a uniform amount of cyromazine on a daily basis for 2 years. This NOEL is 10-fold lower than the dosage of 18.8 mg/kg/day at which there was no statistically significant increase in the incidence of the combined mammary adenomas and adenocarcinomas. As previously stated, workers applying cyromazine to chicken manure on 10 days during a year are unlikely to achieve a chronic dosage comparable to the female rats.

Based on the following considerations, the overall chronic risk, including oncogenicity, to workers applying cyromazine to chicken manure appears to be minimal:

- 1) the lack of actual chronic (repeated daily) exposure based on the anticipated patterns of use and projected frequency and duration of applications.
- 2) no evidence that cyromazine accumulates in biological systems.
- 3) the lack of clearly defined positive genotoxicity.
- 4) the limitation of the Global 86 program to only calculate the 95% upper bound potency and, therefore, constrain the presentation of a realistic range of potential human risk.

V RISK APPRAISAL (continued)

The presumable work practices for the application of cyromazine to chicken manure suggest that the acute, daily exposure may be the most scientifically relevant scenario for assessing the potential deleterious effects to these workers.

B. FIELD WORKERS

Acute and chronic margins of safety, and additional lifetime oncogenic risk for workers applying cyromazine-treated chicken manure to fields as a fertilizer were similar to those values calculated for the handheld sprayer and are considered adequate. Calculations used to characterize potential chronic risk and margins of safety were based on the assumption that the number of annual work days for the field worker is the same as for the applicator using the handheld sprayer.

VI CONCLUSIONS

Margins of safety for potential acute exposure from the direct application of cyromazine to chicken manure ranged from 132 for the backpack sprayer to greater than 4,000 for the hand-held sprayer and at least 500 for the power sprayer. These margins of safety are considered adequate.

Margins of safety for potential repeated exposures to cyromazine were calculated using decreased body weight gain in an animal reproduction study as a conservative indication of chronic toxicity. Margins of safety for all worker activities using an annualized average daily dosage were greater than 1,600 and are considered adequate.

An estimate of additional oncogenic risk was attempted using the Global 86 Linearized Multistage model, based on the incidence of combined benign adenomas and malignant adenocarcinomas in the mammary gland of the female rat. The model only calculated a positive potency for the 95% upper bound confidence interval. The results indicated a range of theoretical additional lifetime oncogenic risk of $3.5E-06$ for the backpack sprayer to $5.9E-08$ for mixer/loader using the power sprayer. It was not possible to calculate a MLE for risk since the potency slope for the MLE was zero. The discrepancy between the MLE and the upper bound potency estimates demonstrates the limitations of the Global 86 model and indicates that the upper bound term may not closely reflect the dose-response relationship of the experimental data. Therefore, a realistic range of potential human risk could not be presented.

Anticipated work practices for the use of cyromazine to control fly larvae in chicken manure suggest that repeated daily exposures are unlikely; therefore, the overall chronic risk, including potential oncogenicity, appears to be minimal. At the present time, the acute, daily exposure appears to be the most scientifically relevant scenario for assessing the potential deleterious effects to these workers.

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VIII APPENDICES

APPENDIX A

TOXICOLOGY SUMMARIES

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

CYROMAZINE

Chemical Code # 2286, Tolerance # 414
SB 950 # Not Assigned

December 23, 1985
Revised December 7, 1992
I. DATA GAP STATUS

Combined, rat:	No Data Gap, Possible Adverse Effects.
Chronic toxicity, rat:	No Data Gap, (See Combined)
Chronic toxicity, dog:	No Data Gap, Possible Adverse Effects.
Oncogenicity, rat:	No Data Gap, (See Combined)
Oncogenicity, mouse:	No Data Gap, No Adverse Effects.
Reproduction, rat:	No Data Gap, No Adverse Effects.
Teratology, rat:	No Data Gap, No Adverse Effects.
Teratology, rabbit:	No Data Gap, Possible Adverse Effects.
Gene mutation:	No Data Gap, No Adverse Effects.
Chromosome effects:	No Data Gap, No Adverse Effects.
DNA damage:	No Data Gap, No Adverse Effects.
Neurotoxicity:	Not required at this time.

Toxicology one-liners are attached.

All record numbers through 902785 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

indicates a study on file but not yet reviewed.

File name: T921207

Peter Henry 12/7/92

*John
12/7/92*

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED, RAT

**** 001-004 902751-4**, "Two Year Chronic and Oncogenicity Study with CGA-72662 Technical in Albino Rats", (IRDC, 6/30/82), Cyromazine technical, 95.5% and 95.3% pure; 3000, 300, 30 or 0 ppm in the feed of CD* rats. Major treatment-related effects included decreased body weight gain at the high dose (20% and 28% less than controls for males and females, respectively), lesser but treatment-related body weight decrement in 300 ppm females, renal pelvic epithelial hyperplasia at the high dose in females (25%) and bronchiectasis in high dose males and females (31% and 18%, respectively). The NOEL is 30 ppm for females, and 300 ppm for males, both based mainly on body weight decrements. **Possible adverse effect:** increased mammary tumors (significant only when adenocarcinomas are combined with adenomas). Prior to the 1992 review, testicular interstitial cell tumors were also considered to be treatment-related. See 1992 DPR review associated with Record No. 092779, below. Report and study **ACCEPTABLE**. (Martz 5/13/86, Updated 7/31/89, Morgan; and 12/4/92, Aldous).

020 902761 Addendum to 902751-4, 12 month interim report.

020 902762 Addendum to 902751-4, 12 month interim histopathology.

414-083 092779 [addendum to document 414-001:902751], "2-Year chronic and oncogenicity study with CGA-72662 Technical in albino rats", IRDC Study 382-081, 6/30/82, (date of additional data submission: 5/22/91). Interstitial cell (ISC) tumors of testes of 3000 ppm males, and mammary adenomas and carcinomas (combined) in females were previously flagged as "possible adverse effects". On re-examination, there is not sufficient evidence to consider the slight increase in ISC tumor incidence in 3000 ppm males to be treatment related (considering especially historical control data). High dose females had body weight decrements exceeding U.S. EPA recommendations for an MTD, making this dose of questionable value for tumor analysis. The rebuttal argued that CDFA reviewers used inappropriate statistical comparisons for evaluating female mammary tumor incidences: the CDFA analysis had isolated mammary adenomas from among the benign tumors, and grouped these with mammary adenocarcinomas for statistical evaluation. DPR notes that NTP considers such groupings for analysis, and does not consider this an invalid procedure. The combined incidence of adenomas plus adenocarcinomas was 6/53 for controls vs. 16/59 at 3000 ppm. This was significant ($p = 0.03$) by Fishers Exact test. Mammary adenoma incidence was 3, 8, 6, and 8 in controls through increasing dosage groups, giving no indication of a dose-response. Combined mammary benign tumor incidence for adenomas plus fibroadenomas was virtually identical between dose groups. Mammary carcinoma incidence was 3, 2, 1, and 9 in controls through increasing dose groups. High dose group mammary carcinoma incidence was well within historical range. Cyromazine data do not establish mammary adenocarcinomas as a clear treatment effect, however the modest increases in mammary tumors in this study, coupled with positive mammary tumor responses in several closely related triazines, do not warrant a change in the "possible adverse effect" status. NOEL is reduced to 30 ppm for females, and remains 300 ppm for males, both due to b.w. decrements. Aldous, 12/4/92.

PZ 12/7/92

94
12/7/92

CHRONIC TOXICITY, RAT

See Combined, Rat

CHRONIC TOXICITY, DOG

008 902743-5 Historical control data and statistical analyses for 902734, 902746.

**** 008, 016, 080; 902734, 902745, 902746, 95971, 95972 902785;** "Subchronic Toxicity Study in Dogs;" (Hazleton, VA, 10/22/80); technical Cyromazine, 96.3%, in the feed at 3000, 300, 30, or 0 ppm to 6-8 beagles/sex/ dose for 26 weeks with 4 week recovery period for 2/sex at 3000 and 0 ppm; decreased testes weights at 3000 ppm, decreased HGB, HCT, cholesterol, increased SGOT (M only), reduced food consumption (M only) and body weight gain; **adverse effect:** histomorphologic lesions in testes with epididymides in 2/5 high-dose males at end of 26 week dosing period consisting of bilateral degeneration of seminiferous tubules with decreased spermatogenesis; NOEL = 300 ppm (based on decreased testes weights and testicular atrophy at the HDT); initially study reviewed as unacceptable (Margolis and Martz, 1/18/88); study rereviewed with results from a pathology examination subsequently submitted; **acceptable;** (upgraded, Leung, 2/7/91).

ONCOGENICITY, RAT

See Combined, Rat

ONCOGENICITY, MOUSE

018 902748 Addendum to 902749, pilot study.

**** 004-007 902749-50, 902755-7,** "Oncogenicity Study with CGA-72662 in Albino Mice", (6/30/82, IRDC). Cyromazine technical, 95.3% & 95.5% pure; 3000, 1000, 50 or 0 ppm in the feed. **No adverse effects** (this is a change of status, since earlier reviews considered malignant lymphomas as a treatment effect in 3000 ppm males). The NOEL is 1000 ppm for males, and 3000 ppm for females, based upon slightly decreased body weights, typically about 3 g, limited to 3000 ppm males (an earlier CDFA review had placed the NOEL at 50 ppm, also based on body weights in males: see Aldous review of 1992). Report and study are **ACCEPTABLE.** (Martz 6/17/86, Updated 8/1/89, Morgan; 12/4/92, Aldous).

019 902759 Addendum to 902749, 12 month interim report.

019 902760 Addendum to 902749, 12 month interim histopathology.

414-083 092780 [addendum to study 414-004:902750, entitled "Oncogenicity study with CGA-72662 in albino mice"], IRDC Study 382-082, 6/30/82 (date of additional data submission: 5/22/91). This submission contains the review of the primary U.S. EPA reviewer, analyses by the original study pathologist and by an outside consultant pathologist, analysis by the pathologist assigned to U.S. EPA Toxicology Branch (in OPTS Health Effects Division) and by the leader of the Biostatistics Team of that branch. More recent historical control data were provided than had been available, and these control data were grouped as to the major tumor subtypes under consideration. Re-examination of the data does not confirm the earlier CDFA determination that malignant lymphomas were elicited by cyromazine treatment. Thus there is no treatment effect on tumors, and **no adverse effect** indicated by this study. This change is

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consistent with the U.S. EPA conclusion, and with the conclusion of the original and reviewing pathologists. The NOEL is 1000 ppm, based upon slightly decreased body weights, typically about 3 g, in males only at 3000 ppm (this is a change from an earlier CDFA review, which had placed the NOEL at 50 ppm based on body weights in males). Aldous, 12/4/92.

REPRODUCTION, RAT

** 017-018 902766-7, "Two Generation Reproduction Study with CGA-72662 in Albino Rats", (12/1/81, IRDC), Cyromazine technical, 95.3% purity, 0, 30, 1000 or 3000 ppm; NOEL for parental (systemic effects) = 30 ppm (decreased bodyweight gain); NOEL for reproductive effects = 1000 ppm (F_1 @ 3000 slight decrease in the number of live pups, F_0 and F_1 pup weight decrease). ACCEPTABLE with NO ADVERSE EFFECTS. (Parker 5/9/86)

TERATOLOGY, RAT

017, 053 36220 Addendum, pilot study to 36220.

** 053 36221, "Teratology Study in Rats", (IRDC, 12/21/79), CGA 72662 technical (96.3% purity); 25/group were given 0, 100, 300 and 600 mg/kg/day on days 6-19 by oral gavage; this rat teratology study shows maternal and fetal toxicity at 300 and 600 mg/kg/day. No treatment-related clinical observations were reported at 100 mg/kg/day. At 300 and 600 mg/kg/day clinical signs included facial staining, ano-genital staining and red vaginal discharge. No treatment-related necropsy findings were observed at any dose level. At 300 and 600 mg/kg/day the maternal body weight gains were 86% and 74%, respectively, of the control value. NOEL (maternal and developmental) is 100 mg/kg/day (decreased maternal and fetal bodyweights, increased clinical signs). NO TERATOGENIC EFFECT observed. ACCEPTABLE. (Originally found unacceptable but Upgradeable with submission of analysis of dosing suspensions or retrospective analysis; individual data for clinical observations, necropsy and fetal variations; and explanation of laboratory classification of fetal variations, Parker 10/28/85; additional data submitted and found acceptable, revised, Morgan, 8/2/89)

TERATOLOGY, RABBIT

017 902764 Addendum, pilot study to 902765.

017 902765, "CGA-72662 (Larvadex®) Technical, Teratology Study in Rabbits", (IRDC, 1981), CGA-72662 technical, 96.3% purity, 0, 10, 25, 30, 50, 60 & 75 mg/kg/day by gavage on days 6-27 of gestation with 16 artificially inseminated Dutch Belted females/group. Maternal NOEL = 10 mg/kg (weight gain); developmental NOEL < 10 mg/kg (decreased fetal weight). ADVERSE EFFECTS: decreased number of live fetuses at 75 mg/kg/day, increased resorptions at 60 and 75 mg/kg/day, decreased fetal weight at all dose levels. Incomplete, UNACCEPTABLE, not upgradeable (no developmental NOEL established). (Parker 6/16/86).

028 22095 Addendum to 902765, Individual clinical observations. (Parker 6/86)

028 22096 Addendum to 902765, Individual fetal variations. (Parker 6/86)

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028 22097 Addendum to 902765, Individual uterine parameters for females that died, aborted or were killed. (Parker 6/86)

028 22098 Addendum to 902765, Historical control values. (Parker 6/86)

028 22099 Addendum to 902765. Statistical analysis of skeletal variants by IRDC. Analyzed 13 ribs and 27 pre-sacral vertebrae, not significant at 10 mg/kg/day, (low dose). Note, EPA statistical analysis disagrees, see Vol.056 #43730. (Parker 6/86)

028 22101 Addendum to 902765. Acclimatization history and health of animals - experiments I and II. (Parker 6/86)

028 22100 Addendum to 902765. Summary by Ciba-Geigy and reviews by Drs. Beaudoin, Holson, Harris and Johnson. Duplicate of IRDC statistical review. Peer review by consultants all agree that a NOEL for maternal and developmental toxicity was established at 10 mg/kg/day. Copy of EPA electronic letter to Ciba-Geigy, 7-3-84, study as inadequate due to poor health, "erratic" weight gain, and "mishandling" of animals. (Note, consultants reviews were previously reviewed by C.S.K. on 9-24-84 as record number 13563). (Parker 6/86)

056 43730 Addendum to 902765. EPA's comments, 12-13-84, on Ciba-Geigy's response to EPA review of IRDC study. EPA used incorrect method to determine pre-implantation loss and as implantation occurs prior to initiation of dosing, this should not be affected by dosing. Parker disagrees with EPA's statement that there was mishandling of animals. One intubation death is not evidence for this. EPA stated concern over the incidence in all treated groups of 13 ribs and 27 presacral vertebrae. As these findings usually occur together (when there are extra ribs, there are extra vertebrae) only 1 should be of concern. EPA concludes that there was no NOEL established for fetal toxicity and that the maternal NOEL was 10 mg/kg/day. (Parker, 5/23/86)

** 034 031090, "CGA-72662 (Larvadex®) Technical, Teratology Study in Rabbits", (1/23/85, WIL Research Labs), Teratology-833-NZW rabbit, vehicle control, untreated control, 5, 10, 30 and 60 mg/kg/day by gavage on days 7-19 of gestation. Possible ADVERSE EFFECT: cyclopia at 10 mg/kg/day and 30 mg/kg/day. NOAEL = 5 mg/kg/day; Maternal NOEL = 10 mg/kg/day (Reduced body weight gain), Developmental NOEL = 5 mg/kg/day (Resorptions). EPA classified as minimum with the above NOELs. ACCEPTABLE. (Parker 10/85, 6/18/86)

063 055963, 055964; "A Study of the Incidence of Fetal Malformations in the Control Population of BUK:(CRL)NZWfBR Rabbits"; (Wil Research Laboratories, Inc., Ashland, OH, Project No. Wil-82005, 2-9-86); No test substance used; Group 1, untreated controls (56 F) and male #2749; Group 2, untreated controls (59 F) and alternate males; Group 3, sham gavage controls (56 F) and male #2749; This study was conducted to compare the incidences of fetal malformations from different sources of NZW rabbits and to investigate the possible relation of malformations seen in study 031090 with male #2749. The report is complete and contains useful supplemental information. (Parker, 4-14-88).

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056 038377 Addendum to 031090. No worksheet. EPA letter, 5-2-85, study remains classified as Core Minimum with maternal NOEL=10 and developmental NOEL=5. EPA letter, 4-14-85, EPA comments on Ciba-Geigy response to EPA review. Peer Review by C.Kimmel, EPA, 4-5-85. Concludes maternal NOEL = 10 mg/kg/day and since some malformations seen at 10 and there were few fetuses to evaluate, fetal NOEL is 5 mg/kg/day. EPA review, 2-5-85, which lists problems with study including low pregnancy rate at 10 mg/kg and low values for implantation sites, both of these events occur prior to dosing. They also mention 1 fetus at 10 and 1 fetus at 30 that had cyclopia. These are considered by EPA to be evidence of a teratogenic effect of the compound even though this was not seen at 60 mg. (Parker, 5/23/86)

057 038378 Addendum to 031090. No worksheet. Ciba-Geigy response to EPA review dated 2/27/85. Letter from WIL, 3/4/85, addressing pregnancy rate, weight range and historical data. Letter from Wil, 1/28/85, with historical control. WIL suggests cyclopia may be of genetic origin in rabbits from Buckshire. Letter from WIL, 2-14-85, which correlated male breeder with fetal malformations observed. This is not the best way to present data as it appears that male #2749 contributes most of the malformed fetuses. However #2749 sired 58% of the fetuses in this study and therefore would be expected to sire most of the malformed fetuses. (Parker, 5/23/86)

** 064 055966; "A Teratology and Postnatal Study in Albino Rabbits with Cyromazine Technical"; (WIL Research Laboratories, Inc., Ashland, OH, Report No. WIL-82008, 2-9-86); Cyromazine Technical 95.2%; Dose levels 0 (0.5% carboxymethylcellulose), 5, 10, and 30 mg/kg/day administered by gavage on days 7-19 of gestation to 74 inseminated NZW female rabbits per dose group; 33-36 dams/dose level necropsied at day 29, remainder allowed to litter and kits necropsied at day 28 of lactation; Maternal NOEL = 10 mg/kg/day (decrease in food consumption and weight gain during dosing); **ADVERSE EFFECTS:** Cyclopia (1/317 at 30 mg/kg) and hydrocephaly (2/317 at 30 mg/kg), these effects were not reported in the historical controls; Developmental NOEL = NOAEL = 10 mg/kg/day; **ACCEPTABLE.** (Parker 8/17/87, 4/15/88, Original review indicated no adverse effects, this study was reviewed again and the adverse effects listed above were indicated, revised 8/3/89, Morgan)

** 065 055967; "Teratology Study in Rabbits"; (International Research and Development Corporation, Mattawan, MI, report no. 382-104, 4-11-85), (technical CGA-72662) Cyromazine technical (no purity stated), administered by gavage at 0 (untreated control), 0 (vehicle control), 5, 10, 30, and 60 mg/kg/day to 18 inseminated Dutch Belted rabbits/group on days 7 through 19 of gestation; Maternal NOEL = 10 (decrease body weight gain during dosing) Developmental NOEL \geq 60 mg/kg/day; **ACCEPTABLE, NO ADVERSE EFFECTS.** (Parker, 8-17-87, 4-18-88).

Cyclopia was seen in # 031090 in 1 fetus each @ 10 and 30 mg/kg/day. In a rigorous study (055966) conducted with a post natal phase, cyclopia and hydrocephaly were present at 30 mg/kg/day. Rabbits used in these two studies were from different suppliers, therefore the developmental effects cannot be supplier-dependent. A repeat study in Dutch Belted Rabbits # 055967, failed to indicate developmental toxicity. The lack of expressed developmental effects when Dutch Belted rabbits were used may be due to insensitivity of the strain or greater resorption of the defective embryos.

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Cyromazine is a unique hazard to the developing organism. The maternal NOEL is 10 mg/kg/day in both NZW and Dutch Belted Rabbits. The developmental NOEL is 5 mg/kg in NZW and > 60 mg/kg/day in Dutch Belted Rabbits. (Parker 6/20/88, Morgan 8/15/89).

GENE MUTATION

NOTE: There is one positive test under this category, as well as two acceptable negative tests. J. Gee evaluated the "Gene Mutation" data base on April 2, 1992, and concluded that the weight of evidence does not indicate that cyromazine is a mutagen.

069 073936 "Salmonella/Mammalian - Microsome Mutagenicity Test" (Ciba-Geigy Limited, Agricultural Division, Basle, Switzerland, Laboratory Study # 78/2572, 12/11/78) CGA-72662 [technical cyromazine] was tested with Salmonella strains TA 98, TA 100, TA 1535, and TA 1537, with and without activation by Aroclor-stimulated rat liver S-9 fraction; 3 plates/strain/dose; 1 trial; 0 (DMSO), 25, 75, 225, 675, and 2025 ug/plate for 48 hours; **no adverse effects noted** (no increase in number of revertant colonies); **not acceptable, upgradable** (no justification for doses selected, inadequate description of the test material, inadequate cytotoxicity evidence, inadequate positive controls, no individual plate counts or standard deviations). (Klein and Gee 9/7/89)

069 072 073937 073949 "L5178Y/TK^{+/-} Mouse Lymphoma Mutagenicity Test, CGA-72662 Technical" (Ciba-Geigy Limited, Agricultural Division, Basle, Switzerland, Laboratory Study # 840942, 12/6/85) CGA-72662 Technical [cyromazine], Batch # EN38440, 96.2% pure, was incubated for 4 hours with mouse lymphoma L5178Y/TK^{+/-} cells, with and without activation by Aroclor-stimulated rat liver S-9 fraction at 0 (medium), 50, 100, 200, 300, 400, 450, and 500 ug/ml; **no adverse effects noted** (no forward mutations); **unacceptable** (no justification for the concentration of S9 and dimethylnitrosamine used). (Klein and Gee 9/6/89)

** 069 073939 "CGA-72662 Technical, V79 Chinese Hamster Point Mutation Test" (Ciba-Geigy Limited, Experimental Pathology, Basle, Switzerland, Laboratory Study # 840798, 8/11/86) CGA-72662 Technical [cyromazine], Batch # P.3, 98.9% pure, was incubated with V79 Chinese hamster embryonic lung cells for 21 hours without activation at 0 (medium), 25, 50, 100, 200, 400, 600, 800, and 1000 ug/ml and for 5 hours with activation by Aroclor-stimulated rat liver S9 fraction at 0 (medium), 100, 200, 400, 800, 1600, 2400, 3200, and 4000 ug/ml; **no adverse effects noted** (no base-pair or frameshift mutations, no deletions); **acceptable**. (Klein and Gee 9/6/89)

** 072 073950 "Mammalian Spot Test, Mouse, 8 Weeks, CGA 72 662 techn." (Ciba-Geigy Limited, Basle, Switzerland, Test # 850616, 2/11/86) CGA 72 662 techn. [cyromazine], Batch # EN 38440, 96.2% pure, was given by intraperitoneal injection to 71 pregnant C57 Bl/6 mice/dose on the 10th day after conception (after mating with male T-stock mice) at 0 (sesame oil), 150, 300, or 600 mg/kg, offspring examined at birth and from 12-14 days to 5 weeks of age; **possible adverse effect** (dose-related increase in frequency of recessive spots); **acceptable**. (Klein and Gee 8/29/89)

** 072 073952 "Salmonella/Mammalian-Microsome Mutagenicity Test, CGA 72662 technical" (Ciba-Geigy Limited, Basle, Switzerland, Test # 871713, 5/31/88) CGA 72662 technical [cyromazine], Batch # FL 870153, 97.5% pure, was tested with Salmonella strains TA98, TA100, TA1535, and TA1537, with and without

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activation by Aroclor-stimulated rat liver S9 fraction, 3 plates/strain/dose, 2 trials/strain; 0(DMSO), 20, 78, 313, 1250, and 5000 µg/plate for 48 hours; **no adverse effects noted** (no increase in number of revertant colonies); **acceptable**. (Klein and Gee 9/6/89)

CHROMOSOME EFFECTS

018 902768, "Dominant Lethal Study - CGA 72662 Mice (Test for Cytotoxic or Mutagenic Effects on Male Germinal Cells)", (Ciba-Geigy Limited, 3/17/81), cyromazine, no purity stated, 20 males/group were given 0, 326 or 678 mg/kg by oral gavage once and mated for 6 consecutive weekly periods with 40 females (34 for high dose - 3 males died). **No evidence of dominant lethal effect** is reported. Incomplete (missing info), **UNACCEPTABLE**, not upgradeable (no positive or historical control). (Gee, 5/5/86)

018 902769, "Nucleus Anomaly Test in Somatic Interphase Nuclei, CGA 72662, Chinese Hamster (Test for Mutagenic Effects on Bone Marrow Cells)", (Ciba-Geigy, 2/6/80), Cyromazine, 98.9% purity; 6/sex/group were given 0, 2000, 4000 or 8000 mg/kg by gavage and sacrificed at 24 hours; 1000 marrow cells of 3/sex/group were analyzed; **no evidence of clinical toxicity or micronuclei formation** is reported. Incomplete (missing data), **UNACCEPTABLE**, not upgradeable (inappropriate protocol: only analyzed marrow of 3/sex, time of sacrifice). (Gee, 3/13/86)

069 073938 "CGA-72662 Technical Chromosome Studies on Human Lymphocytes in Vitro" (Ciba-Geigy Limited, Agricultural Division, Basle, Switzerland, Laboratory study # 850013, 11/12/85) CGA-72662 Technical [cyromazine], Batch # EN38440, 96.2% pure, was incubated with peripheral blood lymphocytes with and without activation by Aroclor-stimulated rat liver S-9 fraction, 2 cultures/dose level at 0 (DMSO), 62.5, 125, 250, 500, and 1000 µg/ml for 3 hours; **no adverse effects noted** (no structural chromosomal aberrations); **unacceptable, possibly upgradable** (cells were harvested 44 hours after exposure to test material, an interval which probably missed the majority of cells in their first mitosis). (Klein and Gee 9/7/89)

018 073 902768 073966 "Dominant Lethal Study, Mouse (Test for cytotoxic or mutagenic effects on male germinal cells) - CGA 72 662" (Ciba-Geigy Limited, Basle Switzerland, Test # 790033, 3/17/81, supplemental report 8/11/88) Cyromazine, Batch P3, 98.9% pure, was given by gavage to 20 male mice/group at 0 (PEG 400), 226, or 678 mg/kg (single dose), the males were mated for 6 consecutive weekly periods with 40 females (34 for the high dose - 3 males died). **No adverse effects noted** (no evidence of a dominant lethal effect). Originally reviewed as unacceptable (no positive control or historical control data). Gee 5/5/86. Reviewed again with the submission of additional data, **unacceptable, possibly upgradable** (inadequate justification of dose levels, inadequate historical positive control data). (Klein and Gee 9/7/89)

** 073 073967 "Micronucleus Test (Mouse), CGA 72 662 tech." (Ciba-Geigy Limited, Basle, Switzerland, Test # 861345, 7/23/87) CGA 72 662 technical [cyromazine], Batch # EN 40025, 96.3% pure, was given by oral gavage to mice at 0 (0.5% carboxymethylcellulose), 360, or 1080 mg/kg, mice sacrificed at 24, 48, and 72 hours, 5/sex/dose/sacrifice time. **No adverse effects noted** (based on frequency of micronuclei in polychromatic erythrocytes); **acceptable**. (Klein and Gee 8/29/89)

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DNA DAMAGE

** 069 073934 "The Hepatocyte Primary Culture/DNA Repair Assay on Compound CGA-72662 using Mouse Hepatocytes in Culture" (Naylor Dana Institute, American Health Foundation, Valhalla NY, Study # 050382, 4/15/83) CGA-72662 [technical cyromazine] was tested with primary mouse hepatocytes in the presence of ³H-thymidine, 3 coverslips/dose, 3 trials, at 0 (DMSO), 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.10, 0.50, and 1.0 mg/ml (Trial 1), at 0 (DMSO), 0.001, 0.005, 0.01, 0.05, 0.10, 0.50, and 1.0 mg/ml (Trial 2), and at 0 (DMSO), 0.005, 0.01, 0.05, 0.10, and 0.50 mg/ml (Trial 3), for 18 hours; **no adverse effects noted** (no unscheduled DNA synthesis); **acceptable**. (Klein and Gee 9/6/89)

069 073935 "The Hepatocyte Primary Culture/DNA Repair Assay on Compound CGA-72662 using Rat Hepatocytes in Culture" (Naylor Dana Institute, American Health Foundation, Valhalla NY, Study # 042782, 7/19/82) CGA-72662 [technical cyromazine] was tested with primary rat hepatocytes in the presence of ³H-thymidine, 3 coverslips/dose, 1 trial at 0 (DMSO), 0.0001, 0.001, 0.005, 0.010, 0.050, 0.10, 0.50, and 1 mg/ml for 18 hours; **no adverse effects noted** (no unscheduled DNA synthesis); **unacceptable, upgradable** (number of cells scored/dose level not reported). (Klein and Gee 9/7/89)

069 072 073940 073948 "CGA-72662 Technical, Saccharomyces cerevisiae D7/Mammalian-Microsome Mutagenicity Test in vitro" (Ciba-Geigy Limited, Agricultural Division, Basle, Switzerland, Laboratory Study # 831167, 10/24/84) CGA-72662 Technical [cyromazine], Batch # P.3, 98.9% pure, was incubated with Saccharomyces cerevisiae, D7 strain, for 16 hours, with and without activation by Aroclor-1254 stimulated rat liver S9 fraction, 2 trials, at 0 (DMSO), 375, 750, 1500, and 3000 µg/ml; **no adverse effects** (no crossing over, no gene conversion, no gene reversion); **unacceptable and upgradable** with reconciliation of the solubility limit statement in Record # 073948 with method description in Record # 073940). (Klein and Gee 9/6/89)

NEUROTOXICITY

Not required at this time.

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TO: Phil Anderson, Registration Specialist
Pesticide Registration Branch

FROM: Medical Toxicology Branch

ORIGINAL: 12/23/85
REVISED: 12/7/92

PRODUCT REGISTRATION RECOMMENDATION SHEET

Formulated Product Name: Larvadex® 2SL

Chemical Code #: 2286

ID #: 125307-N

EPA #: 100-631

SB 950 #: New Active Ingredient

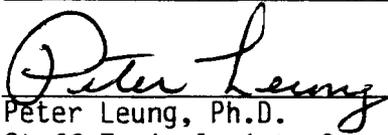
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056, 057, 063-065, 067, 069, 071, 072, 074, 078, 080-083

Company Name: Ciba-Geigy Corp.

RECOMMENDATION:

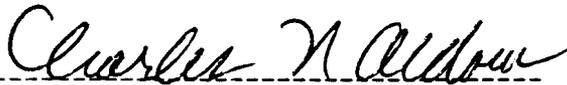
Submitted as a new active ingredient Section 3 Registration request for
terrestrial, non-food use.

Registration is recommended pending completion of health assessment
review.



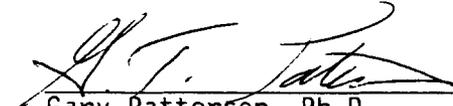
Peter Leung, Ph.D.
Staff Toxicologist, Specialist

12/7/92
Date



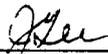
Charles N. Aldous, Ph.D.
Staff Toxicologist, Specialist

12/07/92
Date



Gary Patterson, Ph.D.
Senior Toxicologist

12/7/92
Date



Joyce Gee, Ph.D.
Senior Toxicologist

12/7/92
Date

TO: Phil Anderson, Registration Specialist
Pesticide Registration Branch

FROM: Medical Toxicology Branch

DATE: 12/7/92

DATA PACKAGE SUMMARY AND RECOMMENDATION SHEET

Active ingredient: CYROMAZINE

Formulated product name: Larvadex® 2SL

Formulation (excluding inerts): 2% Cyromazine 98% Inert Ingredients

SB 950#: new active ingredient **ID#:** 125307-N

EPA Reg#: 100-631

Document #'s: 414: 004, 008, 012, 016-020, 028, 029, 031, 033, 034, 053, 056, 057, 063-065, 067, 069, 071, 072, 074, 078, 080-083

Company name: Ciba-Geigy Corp.

SUMMARY: ("DPR One-Liners" from each study worksheet, significant information not mentioned in worksheets, other pertinent information).

Ciba-Geigy Corporation is requesting section 3 registration for the active ingredient cyromazine and for the formulated product Larvadex® 2SL, which is to be used in poultry (chicken) layer operations.

The acute toxicity of the 0.3% Premix 245-18, 1% Premix, and the Trigard 5% SC-C FL-830406 formulations were evaluated.

Cyromazine has also been referred to as CGA 72662.

ACUTE STUDIES - Technical

	<u>Toxicity</u>	<u>Category</u>
Acute Oral Toxicity LD ₅₀	III	
Acute Dermal Toxicity LD ₅₀	III	
Acute Inhalation Toxicity LD ₅₀	I	
Primary Eye Irritation	IV	
Primary Dermal Irritation	IV	

Acute Oral Toxicity

** 067; 58149; "Acute Oral LD50 In The Rat Of Technical CGA 72662"; Ciba-Geigy, Basle, Switzerland, Project No. Siss 6446, no date stated; (Technical Cyromazine CGA 72662); Dose levels 1000, 1670, 3590, 4640, and 6000 mg/kg; Clinical Observations- sedation, dyspnea, exophthalmos, curved position and ruffled fur; Necropsy- no substance related gross organ changes were seen; LD50 (M/F) = 3387 (2524-4547) mg/kg; Toxicity Category III; **Acceptable.** (Berliner, 7-2-87)

Acute Dermal Toxicity

** 067; 58152; "Acute Dermal LD50 In The Rat Of Technical CGA 72662"; Ciba-Geigy, Basle, Switzerland, Project No. Siss 6446, no date stated; (Technical Cyromazine CGA 72662); Dose levels 2150 and 3170 mg/kg; 5/sex/dose; exposure

period 24 hours, occluded patch; Clinical Observations- dyspnea, curved position, ruffled fur, and no local skin irritation seen; No Mortalities; LD50 (M/F) > 3170 mg/kg; Toxicity Category III: **Acceptable**. (Berliner, 7-2-87)

Acute Inhalation Toxicity

** 067; 58157; "Acute Inhalation Toxicity Study In Rats"; International Research and Development Corporation, Mattawan, MI, Study No. 382-074, 7-20-79; (Technical Cyromazine CGA 72662 5SCO-A); Dose level nominal 2.63 mg/l and actual concentration 0.049 mg/l; 5/sex/dose; aerodynamic diameter 1.95 um; Clinical Observations- dyspnea and dried red matter around the nares; LC50 (M/F) > 0.049 mg/l (actual concentration); Toxicity Category I; **Acceptable**. (Berliner, 7-3-87)

Primary Eye Irritation Study

** 067; 58153; "Eye Irritation In The Rabbit Of Technical CGA 72662"; Ciba-Geigy, Basle, Switzerland, Project No. Siss 64446, 3-16-78; (Technical Cyromazine CGA 72662); Dose level 0.1 g; 3M (unwashed) used, guidelines require at least six unwashed eyes; all unwashed eyes had score of 0 at 24 hours, all washed eyes 0 score at 24 hours; Toxicity Category IV; **Acceptable** (see explanation on worksheet). (Berliner, 7-2-87)

Primary Dermal Irritation

** 067; 58154; "Skin Irritation In The Rabbit After Single Application Of Technical CGA 72662"; Ciba-Geigy, Basle, Switzerland, Project No. 6446, 3/16/78; (Technical Cyromazine CGA 72662); Dose level 0.5g; 3M/3F; 24 hour exposure period, occluded patch; 72 hours (intact) all six animals score of 0 for erythema and edema; Toxicity Category IV; **Acceptable**. (Berliner, 7-2-87)

ACUTE STUDIES - CGA 72662 0.3% Premix 245-18

	<u>Toxicity Category</u>
Acute Oral Toxicity LD ₅₀	IV
Acute Dermal Toxicity LD ₅₀	III
Acute Inhalation Toxicity LD ₅₀	III
Primary Eye Irritation	III
Primary Dermal Irritation	IV

Acute Oral Toxicity

** 012; 902736; "Rat Acute Oral Toxicity CGA-72662 0.3% Premix (Male/Female) (Larvadex®)." Rat Acute Oral Toxicity; Stillmeadow Inc., Houston, TX, Project No. 1116-79, 5-10-79; (CGA 72662 0.3% Premix 245-18); administered as a 33.3% (w/v) slurry of the test material in corn oil; Dose level 5033 mg/kg; 5M/5F for the one dose used; Clinical Observations-piloerection in one male animal only; Necropsy-consolidated lungs in one male animal only, all other animals had no observed abnormalities; No mortalities; LD50 (M/F) > 5033 mg/kg; Toxicity Category IV; **Acceptable**. (Berliner, 5-29-87)

Acute Dermal Toxicity

** 012; 902737; "Rabbit Acute Dermal Toxicity For CGA-72662 0.3% Premix (Male/Female) (Larvadex®)." Rabbit Acute Dermal Irritation; Stillmeadow Inc., Houston, TX, Project No. 1117-79, 5-18-79; (CGA 72662 0.3% Premix FL 790372); test material applied as a slurry in saline; Dose level 2004 mg/kg; 5M/5F; exposure period of 24 hours, occluded patch; Clinical Observations-diarrhea, few feces, and little urination; Necropsy-diarrhea, discoloration of the G I contents, red fluid in the abdominal cavity and variations thereof; Death in one male animal before sacrifice on day 14; LD50 (M/F) > 2004 mg/kg; Toxicity Category III; **Acceptable.** (Berliner, 5-29-87)

Acute Inhalation Toxicity

** 012; 902738; "Acute Inhalation Toxicity Study In Rats For CGA-72662 0.3% Premix (Males/Females) (Larvadex®)." Acute Inhalation Toxicity Study In Rats; International Research and Development Corporation, Mattawan, MI, 8-2-79; (CGA 72662 0.3% Premix); Dose level 35.3 mg/l (nominal concentration); 5M/5F; four hour exposure period; No mortalities; Clinical Observations-one animal dried red material around the nares on day 1 postexposure, all other animals appeared normal; Necropsy-all animals appeared normal; Bodyweights-all males and three females had normal weight gains, two females lost weight; LC50 > 1.87 mg/l (actual concn.); Toxicity Category III; **Acceptable.** (Berliner, 6-1-87)

Primary Eye Irritation

** 012; 902739; "Rabbit Eye Irritation Study For CGA-72662 0.3% Premix (Larvadex®)." Rabbit Eye Irritation; Stillmeadow Inc., Houston, TX, Project No. 1119-79, 4-27-79; (CGA 72662 0.3% Premix F1 790372); Dose level 100 mg; 6 (unwashed) and 3 (washed); untreated left eyes served as controls; All eyes had zero scores by day seven; Toxicity Category III; **Acceptable.** (Berliner, 6-1-87)

Primary Dermal Irritation

** 012; 902740; "Rabbit Primary Skin Irritation Study For CGA-72662 0.3% Premix (Larvadex®)." Rabbit Primary Skin Irritation; Stillmeadow Inc., Houston, TX, Project No. 1118-79, 5-10-79; (CGA 72662 0.3% Premix FL 790372); Dose level 0.5 g moistened with 1.0 ml of saline; exposure period of 24 hours, occluded patch; 3M/3F used; 72 Hour Readings (intact 2 sites): Erythema-3/6 animals score of 1 and 3/6 animals score of zero. Edema-1/6 animals score of 1 and 5/6 animals score of zero; yellow discoloration of test site hairs; Toxicity Category IV; **Acceptable.** (Berliner, 6-1-87)

ACUTE STUDIES - CGA 72662 1.0% Premix

	<u>Toxicity Category</u>
Acute Oral Toxicity LD ₅₀	IV
Acute Dermal Toxicity LD ₅₀	III
Acute Inhalation Toxicity LD ₅₀	III
Primary Eye Irritation	III
Primary Dermal Irritation	IV

Acute Oral Toxicity

** 074 73960, "Rat Acute Oral Toxicity", (Stillmeadow, Inc., Houston, TX, Lab. Project No. 3949-86, 2/21/86); Larvadex® 1% Premix, FL-860087, dosed as a 25.0% w/v mixture in corn oil at a volume of 20.2 ml dosing mixture/kg; 5050 mg/kg; 5 animals/sex; No mortality; Clinical Observations- constricted pupils, diarrhea, exophthalmos, piloerection, polyuria, respiratory gurgle; Necropsy- no observable abnormalities; LD50 > 5050 mg/kg (M/F); Toxicity Category IV; **Acceptable.** (Duncan, 7/6/89)

Acute Dermal Toxicity

** 074 73961, "Rabbit Acute Dermal Toxicity", (Stillmeadow, Inc., Houston, TX, Lab. Project No. 3950-86, 2/14/86); Larvadex® 1% Premix, FL-852113; 2010 mg/kg, each dose moistened with 4.0 ml/kg physiological saline; 5 animals/sex; 24-hour exposure, porous wrap; Mortality- male: 1/5, female: 1/5, deaths occurred on Days 7 and 9; Clinical Observations- activity decrease, diarrhea, decreased urination, lacrimation, no defecation; Necropsy- GIT: distended with gas, discoloration of contents, serosal blood vessels pronounced; LD50 > 2010 mg/kg (M/F); Toxicity Category III; **Acceptable.** (Duncan, 7/6/89)

Acute Inhalation Toxicity

** 074 73962, "Rat Acute Inhalation Toxicity", (Stillmeadow, Inc., Houston, TX, Lab. Project No. 3954-86, 4/2/86); Larvadex® 1% Premix, FL-860087, used neat; 3.59 mg/l (gravimetric); 5 animals/sex; dry aerosol, 4-hour whole-body exposure; 45.43% of particles were < 4.7 um at 1.25 h (MMAD = 5.104 um, GSD = 2.807), 50.96% of particles were < 4.7 um at 3.00 h (MMAD = 4.188 um, GSD = 2.878), by cascade impactor analyses; No mortality; Clinical Observations- constricted pupils, diarrhea, dilated pupils, exophthalmos, nasal discharge, piloerection, polyuria, ptosis, salivation; Necropsy- no observable abnormalities; LC50 > 3.59 mg/l (M/F); Toxicity Category III; **Acceptable.** (Duncan, 7/7/89)

Primary Eye Irritation

** 074 73963, "Primary Eye Irritation", (Stillmeadow, Inc., Houston, TX, Lab. Project No. 3951-86, 2/7/86); Larvadex® 1% Premix, FL-852113, dosed neat; 100 mg/eye; 6 animals unwashed, 3 animals washed after 30 seconds; examined 1, 24 (w/fluorescein), 48, and 72 h, and 4, and 7 d; UNWASHED- conjunctivitis only (max. scores = 3/redn., 3/chem., 2/disch.) clear by 7 d; WASHED- conjunctivitis only (max. scores = 2/redn., 3/chem., 3/disch.) clear by 4 d; Toxicity Category III; **Acceptable.** (Duncan, 7/7/89)

Primary Dermal Irritation

** 074 73964, "Rabbit Skin Irritation", (Stillmeadow, Inc., Houston, TX, Lab. Project No. 3952-86, 2/7/86); Larvadex® 1% Premix, FL-852113, dosed neat; 500 mg/site, each dose moistened with 0.5 ml dH2O; 1 site/animal, 6 animals; 4-h exposure, semi-occluded sites; scored 1, 24, 48, 72 h (term.); no irritation; Toxicity Category IV; **Acceptable.** (Duncan, 7/7/89)

ACUTE STUDIES - Trigard 5% SC-C (FL-830406)

	<u>Toxicity Category</u>
Acute Oral Toxicity LD ₅₀	IV
Acute Dermal Toxicity LD ₅₀	III
Acute Inhalation Toxicity LD ₅₀	unacceptable and not upgradeable*
Primary Eye Irritation	unacceptable and not upgradeable*
Primary Dermal Irritation	IV

*See Conclusions

Acute Oral Toxicity

414-078; 91566; Acute Oral Toxicity; 811; Rat; Stillmeadow, Inc., Houston, TX, Project #2936-82, 4/15/83; Trigard 5% SC-C FL-830406; 5/sex/dose; 1 dose of 5010 mg/kg; no mortalities; observations- very slight diarrhea in 1/5 males 6 hrs after exposure, clearing within 1 day; necropsy- no observable abnormalities; LD50 (M/F) > 5010 mg/kg; Category IV; Acceptable. (Corlett, 2/15/91)

Acute Dermal Toxicity

414-078; 91567; Acute Dermal Toxicity; 812; Rabbit; Stillmeadow, Inc., Houston, TX, Project #2937-83, 4/21/83; Trigard 5% SC-C FL-830406; 5/sex/dose; 1 dose of 2010 mg/kg; 24 hr exposure, covered; no mortalities; observations- lacrimation in 1/5 males after exposure, clearing within 2 days; erythema (in 1/5 males and in 2/5 females) and edema (in 4/5 males and in 5/5 females) after exposure, all signs clearing within 2 days; necropsy- a fluid filled cyst against the abdominal wall in 1/5 males; LD50 (M/F) > 2010 mg/kg; Category III; Acceptable. (Corlett, 2/15/91)

Acute Inhalation Toxicity

414-078; 91568; Acute Inhalation Toxicity; 813; Rat; Stillmeadow Inc., Houston, TX, Project #2941-83, 8/4/83; Trigard 5% SC-C FL 830406; 5/sex/dose; 1 dose of 2.90 mg/l (mean analytical concentration); nominal concentration= 6.93 mg/l; MMAD determinations of 1.688 μ m (GSD=2.807) and 2.861 μ m (GSD=2.801); 4 hr exposure; no mortalities; observations- piloerection, constricted or dilated pupils, and respiratory gurgle after exposure, all signs clearing within 4 days; necropsy- dark red or mottled red lungs in all, blue gray or red blue nodules on the lungs of 1/5 males and 2/5 females, and consolidation of portions of the lungs in 3/5 males; Reported LC50 (M/F) > 2.90 mg/l; Category not determined; Unacceptable and not upgradeable because only 1 dose level was used in the study. (Corlett, 2/15/91)

Primary Eye Irritation

414-078; 91569; Primary Eye Irritation; 814; Rabbit; Stillmeadow, Inc., Houston, TX, Project #2938-83, 4/12/83; Trigard 5% SC-C FL-830406; 9 animals (6 with nonwashed treated eyes, 3 with washed treated eyes); 0.1 ml/eye; observations- nonwashed treated eyes: no corneal opacity or iritis; conjunctival irritation (grade 2 in 5/6, grade 1 in 1/6) 24 hrs after exposure, clearing in all within 4 days; Category not determined; Unacceptable and not upgradeable since fluorescein staining done at 1 hr after instillation (Corlett, 2/15/91)

Primary Dermal Irritation

414-078; 91570; Primary Dermal Irritation; 815; Rabbit; Stillmeadow, Inc., Houston, TX, Project #2939-83, 4/12/83; Trigard 5% SC-C FL-830406; 6 animals; 2 abraded and 2 intact sites/animal; 0.5 ml/site; 24 hr exposure, covered; no erythema or edema at any intact test site; Category IV; Acceptable. (Corlett, 2/15/91)

ACUTE STUDIES - Supplemental

033, 23184 "Acute Toxicology Studies," No lab or report date stated; Cyromazine technical, 0.3% Premix, 5% SC; Various LD₅₀ values (rat, mouse, rabbit, quail), skin and eye irritation ratings; Insufficient information present to evaluate - no data; - Summary only. (Hughett 12/9/87, Parker 6/2/88)

067, 58155 "Acute Intraperitoneal LD₅₀ in the Rat of Technical CGA 72662", (Ciba-Geigy Ltd., Basle, Switzerland, project # Siss 6446, 6/12/78), Five, 160 to 180 gram Tif:RAIF (SPF) rats / sex / dose were exposed ip to cyromazine (CGA 72662; 0.1 to 1% suspensions in polyethylene glycol) at 6, 7.75, 10, 14.7, 21.5, or 100 mg / kg body weight and observed for 14 days. Calculated acute intraperitoneal LD₅₀ = 7 - 17 mg/kg. This was supplemental information, not a required study, and therefore not evaluated for acceptability. (Morris and Patterson, 03/07/88.)

067; 58150; "Acute Oral LD50 In The Mouse Of Technical CGA 72662"; Ciba-Geigy, Basle, Switzerland, Project No. Siss 6446, no date; (Technical Cyromazine 72662); Dose level 600, 1000, 2150, 3590, 4640, and 7750 mg/kg; 5 animals/sex/dose; Clinical Observations- sedation, dyspnea, curved position and ruffled fur; Necropsy- no substance-related gross organ changes were seen; LD50 (M/F) = 2029 (1472-2707) mg/kg; **Supplemental**. (Berliner, 7-1-87)

067; 58151; "Acute Oral LD50 In The Rabbit Of Technical CGA 72662"; Ciba-Geigy, Basle, Switzerland, Project No. Siss 6446, 1-11-78; (Technical Cyromazine CGA 72662); Dose level 600, 1000, 2150, and 3590 mg/kg; 3/sex/dose; Clinical Observations- sedation, curved position, ruffled fur, tremor, ataxia, salivation, and ventral position; Necropsy- (survivors) no substance-related gross organ changes were seen, (dead animals) partially congested organs and bloatings along the gut; LD50 (M/F) = 1467 (1012-2127) mg/kg; **Supplemental**. (Berliner, 7-2-87)

SUBCHRONIC STUDIES

008, 902747 "Summary of the Subchronic, Chronic, Reproduction, Teratology and Mutagenicity Studies Made With Respect to the Safety of the Pesticide, Larvadex® (CGA-72662)," Cyromazine (no purity information) in the diet of rats for 90 days at 0, 30, 300, 1000 or 3000 ppm, recovery animals fed basal diet only for four additional weeks; **NO ADVERSE EFFECTS** indicated; NOEL stated to be 1000 ppm based on reduced body weights in both sexes at 3000 ppm; **UNACCEPTABLE** - Very brief summary (No worksheet). (Hughett 12/9/87, Parker 6/2/88)

414-071; 73945; "90-Day Subacute Oral Toxicity Study with CGA-72662 in Purebred Beagle Dogs"; International Research and Development Corp., Mattawan, MI, Report no. 382-048, 6/19/79; cyromazine (CGA-72662 Technical) 96.3% pure; 3000, 1000, 300, 30, or 0 ppm in feed daily; 4-6 beagle dogs/sex/dose for 13 weeks with 4 week recovery period for 2 dogs/sex at 3000 and 0 ppm; no mortality reported in any of the doses tested; general behavior and appearance and findings from physical and ophthalmologic exams were similar for control and treated dogs; hematological and clinical laboratory tests including biochemistry and urinalysis revealed similar findings for control and treated animals; essentially no toxic effects with the highest dose tested; NOEL(M/F) > 3000 ppm; **Study not acceptable and not upgradeable**; analysis of dosing material for stability, homogeneity and content in animal feed not reported; dose level selections were inadequate to permit identification of target organs (Leung, 7/12/89).

METABOLISM STUDIES

031 19242 "Metabolism and Balance Study of ¹⁴C-CGA-72662 in the Rat," (Ciba-Geigy, Greensboro, N.C., report ABR-78072, 9/22/78), ¹⁴C-CGA-72662 (radioactive purity >99% by TLC, chemical purity by LC - 90.7%) dose level was 0.5 mg/kg in a single oral dose (equivalent to 5 ppm by diet route) to 4 male & 2 female rats; overall recovery was 97.8%; 95.0% excreted in the urine and 2.8% excreted in the feces by 72 hours, <0.1% excreted in volatiles & respiratory CO₂ or in cage wash, tissues, intestinal tract & blood; Incomplete, **UNACCEPTABLE** - Not Upgradeable (Too few animals employed per group, inadequate number of dose levels). (Hughett 11/18/87 and Gee 4/7/88)

** 082; 92344; "Characterization and Identification of ¹⁴C-Cyromazine and Metabolites in Rats" (Hazleton Laboratories America, Inc., Madison, WI, Lab. Project ID # ABR-89108, 2/13/90); rat; 851; ¹⁴C-Cyromazine (Lot Nos. CL-XVIII77 and CL-XVIII-78, specific activity: 9.8 and 0.8 uCi/mg, respectively, 97.2% purity for both lots), unlabeled Cyromazine (Batch No. FL 862286, 96.3% purity); single: oral (3 mg/kg, 5M/5F; 300 mg/kg, 5M/5F), IV (3 mg/kg, 5M/4F); multiple (3 mg/kg unlabeled Cyromazine qd @ 14 days followed 24 hrs after final dose by a single dose of ¹⁴C-Cyromazine, 5M/5F); no differences in metabolic pattern regardless of route of administration, dose level or sex; rapid excretion 24 hrs after dosing (52-78% in urine and 2-5% in feces); renal excretion major route of elimination with 82 - 92% of the administered dose excreted in urine and 5% of the total dose recovered in feces; unchanged Cyromazine (72%), melamine (7%), hydroxycyromazine (9%), and methylcyromazine (2%) in urine; 71% of fecal extract radioactivity cochromatographed with Cyromazine, 7% with melamine and 8% with mixture of methylcyromazine, hydroxycyromazine and other metabolites; **Acceptable**; (Leung, 5/7/91).

SB950-MANDATED HEALTH EFFECTS STUDIES

Combined, Rat

** 001-004 902751-4, "Two Year Chronic and Oncogenicity Study with CGA-72662 Technical in Albino Rats", (IRDC, 6/30/82), Cyromazine technical, 95.5% and 95.3% pure; 3000, 300, 30 or 0 ppm in the feed of CD® rats. Major treatment-related effects included decreased body weight gain at the high dose (20% and 28% less than controls for males and females, respectively), lesser but treatment-related body weight decrement in 300 ppm females, renal pelvic epithelial hyperplasia at the high dose in females (25%) and bronchiectasis in high dose males and females (31% and 18%, respectively). The NOEL is 30 ppm

for females, and 300 ppm for males, both based mainly on body weight decrements. **Possible adverse effect:** increased mammary tumors (significant only when adenocarcinomas are combined with adenomas). Prior to the 1992 review, testicular interstitial cell tumors were also considered to be treatment-related. See 1992 DPR review associated with Record No. 092779, below. Report and study **ACCEPTABLE**. (Martz 5/13/86, Updated 7/31/89, Morgan; and 12/4/92, Aldous).

020 902761 Addendum to 902751-4, 12 month interim report.

020 902762 Addendum to 902751-4, 12 month interim histopathology.

414-083 092779 [addendum to document 414-001:902751], "2-Year chronic and oncogenicity study with CGA-72662 Technical in albino rats", IRDC Study 382-081, 6/30/82, (date of additional data submission: 5/22/91). Interstitial cell (ISC) tumors of testes of 3000 ppm males, and mammary adenomas and carcinomas (combined) in females were previously flagged as "possible adverse effects". On re-examination, there is not sufficient evidence to consider the slight increase in ISC tumor incidence in 3000 ppm males to be treatment related (considering especially historical control data). High dose females had body weight decrements exceeding U.S. EPA recommendations for an MTD, making this dose of questionable value for tumor analysis. The rebuttal argued that CDFA reviewers used inappropriate statistical comparisons for evaluating female mammary tumor incidences: the CDFA analysis had isolated mammary adenomas from among the benign tumors, and grouped these with mammary adenocarcinomas for statistical evaluation. DPR notes that NTP considers such groupings for analysis, and does not consider this an invalid procedure. The combined incidence of adenomas plus adenocarcinomas was 6/53 for controls vs. 16/59 at 3000 ppm. This was significant ($p = 0.03$) by Fishers Exact test. Mammary adenoma incidence was 3, 8, 6, and 8 in controls through increasing dosage groups, giving no indication of a dose-response. Combined mammary benign tumor incidence for adenomas plus fibroadenomas was virtually identical between dose groups. Mammary carcinoma incidence was 3, 2, 1, and 9 in controls through increasing dose groups. High dose group mammary carcinoma incidence was well within historical range. Cyromazine data do not establish mammary adenocarcinomas as a clear treatment effect, however the modest increases in mammary tumors in this study, coupled with positive mammary tumor responses in several closely related triazines, do not warrant a change in the "possible adverse effect" status. NOEL is reduced to 30 ppm for females, and remains 300 ppm for males, both due to b.w. decrements. Aldous, 12/4/92.

Chronic Toxicity, Dog

008 902743-5 Historical control data and statistical analyses for 902734, 902746.

**** 008, 016, 080; 902734, 902745, 902746, 95971, 95972 902785;** "Subchronic Toxicity Study in Dogs;" (Hazleton, VA, 10/22/80); technical Cyromazine, 96.3%, in the feed at 3000, 300, 30, or 0 ppm to 6-8 beagles/sex/ dose for 26 weeks with 4 week recovery period for 2/sex at 3000 and 0 ppm; decreased testes weights at 3000 ppm, decreased HGB, HCT, cholesterol, increased SGOT (M only), reduced food consumption (M only) and body weight gain; **adverse effect:** histomorphologic lesions in testes with epididymides in 2/5 high-dose males at end of 26 week dosing period consisting of bilateral degeneration of seminiferous tubules with decreased spermatogenesis; NOEL = 300 ppm (based on decreased testes weights and testicular atrophy at the HDT); initially study reviewed as unacceptable (Margolis and Martz, 1/18/88); study rereviewed with

results from a pathology examination subsequently submitted; **acceptable**;
(upgraded, Leung, 2/7/91).

Oncogenicity, Mouse

018 902748 Addendum to 902749, pilot study.

** 004-007 902749-50, 902755-7, "Oncogenicity Study with CGA-72662 in Albino Mice", (6/30/82, IRDC). Cyromazine technical, 95.3% & 95.5% pure; 3000, 1000, 50 or 0 ppm in the feed. **No adverse effects** (this is a change of status, since earlier reviews considered malignant lymphomas as a treatment effect in 3000 ppm males). The NOEL is 1000 ppm for males, and 3000 ppm for females, based upon slightly decreased body weights, typically about 3 g, limited to 3000 ppm males (an earlier CDFA review had placed the NOEL at 50 ppm, also based on body weights in males: see Aldous review of 1992). Report and study are **ACCEPTABLE**. (Martz 6/17/86, Updated 8/1/89, Morgan; 12/4/92, Aldous).

019 902759 Addendum to 902749, 12 month interim report.

019 902760 Addendum to 902749, 12 month interim histopathology.

414-083 092780 [addendum to study 414-004:902750, entitled "Oncogenicity study with CGA-72662 in albino mice"], IRDC Study 382-082, 6/30/82 (date of additional data submission: 5/22/91). This submission contains the review of the primary U.S. EPA reviewer, analyses by the original study pathologist and by an outside consultant pathologist, analysis by the pathologist assigned to U.S. EPA Toxicology Branch (in OPTS Health Effects Division) and by the leader of the Biostatistics Team of that branch. More recent historical control data were provided than had been available, and these control data were grouped as to the major tumor subtypes under consideration. Re-examination of the data does not confirm the earlier CDFA determination that malignant lymphomas were elicited by cyromazine treatment. Thus there is no treatment effect on tumors, and **no adverse effect** indicated by this study. This change is consistent with the U.S. EPA conclusion, and with the conclusion of the original and reviewing pathologists. The NOEL is 1000 ppm, based upon slightly decreased body weights, typically about 3 g, in males only at 3000 ppm (this is a change from an earlier CDFA review, which had placed the NOEL at 50 ppm based on body weights in males). Aldous, 12/4/92.

Reproduction, Rat

** 017-018 902766-7, "Two Generation Reproduction Study with CGA-72662 in Albino Rats", (12/1/81, IRDC), Cyromazine technical, 95.3% purity, 0, 30, 1000 or 3000 ppm; NOEL for parental (systemic effects) = 30 ppm (decreased bodyweight gain); NOEL for reproductive effects = 1000 ppm (F_1 @ 3000 slight decrease in number of live pups, F_0 and F_1 pup weight decrease). **ACCEPTABLE with NO ADVERSE EFFECTS**. (Parker 5/9/86)

Teratology, Rat

017, 053 36220 Addendum, pilot study to 36220.

** 053 36221, "Teratology Study in Rats", (IRDC, 12/21/79), CGA 72662 technical (96.3% purity); 25/group were given 0, 100, 300 and 600 mg/kg/day on days 6-19 by oral gavage; this rat teratology study shows maternal and fetal toxicity at 300 and 600 mg/kg/day. No treatment-related clinical observations were reported at 100 mg/kg/day. At 300 and 600 mg/kg/day clinical signs

included facial staining, ano-genital staining and red vaginal discharge. No treatment-related necropsy findings were observed at any dose level. At 300 and 600 mg/kg/day the maternal body weight gains were 86% and 74%, respectively, of the control value. NOEL (maternal and developmental) is 100 mg/kg/day (decreased maternal and fetal bodyweights, increased clinical signs). **NO TERATOGENIC EFFECT** observed. **ACCEPTABLE**. (Originally found unacceptable but upgradeable with submission of analysis of dosing suspensions or retrospective analysis; individual data for clinical observations, necropsy and fetal variations; and explanation of laboratory classification of fetal variations, Parker 10/28/85; additional data submitted and found acceptable, revised, Morgan, 8/2/89)

Teratology, Rabbit

017 902764 Addendum, pilot study to 902765.

017 902765, "CGA-72662 (Larvadex®) Technical, Teratology Study in Rabbits", (IRDC, 1981), CGA-72662 technical, 96.3% purity, 0, 10, 25, 30, 50, 60 & 75 mg/kg/day by gavage on days 6-27 of gestation with 16 artificially inseminated Dutch Belted females/group. Maternal NOEL = 10 mg/kg (weight gain); developmental NOEL < 10 mg/kg (decreased fetal weight). **ADVERSE EFFECTS**: decreased number of live fetuses at 75 mg/kg/day, increased resorptions at 60 and 75 mg/kg/day, decreased fetal weight at all dose levels. Incomplete, **UNACCEPTABLE**, not upgradeable (no developmental NOEL established). (Parker 6/16/86).

028 22095 Addendum to 902765, Individual clinical observations. (Parker 6/86)

028 22096 Addendum to 902765, Individual fetal variations. (Parker 6/86)

028 22097 Addendum to 902765, Individual uterine parameters for females that died, aborted or were killed. (Parker 6/86)

028 22098 Addendum to 902765, Historical control values. (Parker 6/86)

028 22099 Addendum to 902765. Statistical analysis of skeletal variants by IRDC. Analyzed 13 ribs and 27 pre-sacral vertebrae, not significant at 10 mg/kg/day, (low dose). Note, EPA statistical analysis disagrees, see Vol.056 #43730. (Parker 6/86)

028 22101 Addendum to 902765. Acclimatization history and health of animals - experiments I and II. (Parker 6/86)

028 22100 Addendum to 902765. Summary by Ciba-Geigy and reviews by Drs. Beaudoin, Holson, Harris and Johnson. Duplicate of IRDC statistical review. Peer review by consultants all agree that a NOEL for maternal and developmental toxicity was established at 10 mg/kg/day. Copy of EPA electronic letter to Ciba-Geigy, 7-3-84, study as inadequate due to poor health, "erratic" weight gain, and "mishandling" of animals. (Note, consultants reviews were previously reviewed by C.S.K. on 9-24-84 as record number 13563). (Parker 6/86)

056 43730 Addendum to 902765. EPA's comments, 12-13-84, on Ciba-Geigy's response to EPA review of IRDC study. EPA used incorrect method to determine pre-implantation loss and as implantation occurs prior to initiation of dosing, this should not be affected by dosing. Parker disagrees with EPA's statement that there was mishandling of animals. One intubation death is not evidence for this. EPA stated concern over the incidence in all treated groups of 13 ribs and 27 presacral vertebrae. As these findings usually occur together (when there are extra ribs, there are extra vertebrae) only 1 should be of concern. EPA concludes that there was no NOEL established for fetal toxicity and that the maternal NOEL was 10 mg/kg/day. (Parker, 5/23/86)

**** 034 031090**, "CGA-72662 (Larvadex®) Technical, Teratology Study in Rabbits", (1/23/85, WIL Research Labs), Teratology-833-NZW rabbit, vehicle control, untreated control, 5, 10, 30 and 60 mg/kg/day by gavage on days 7-19 of gestation. Possible ADVERSE EFFECT: cyclopia at 10 mg/kg/day and 30 mg/kg/day. NOAEL = 5 mg/kg/day; Maternal NOEL = 10 mg/kg/day (Reduced body weight gain), Developmental NOEL = 5 mg/kg/day (Resorptions). EPA classified as minimum with the above NOELs. ACCEPTABLE. (Parker 10/85, 6/18/86)

063 055963, 055964; "A Study of the Incidence of Fetal Malformations in the Control Population of BUK:(CRL)NZWFBR Rabbits"; (Wil Research Laboratories, Inc., Ashland, OH, Project No. Wil-82005, 2-9-86); No test substance used; Group 1, untreated controls (56 F) and male #2749; Group 2, untreated controls (59 F) and alternate males; Group 3, sham gavage controls (56 F) and male #2749; This study was conducted to compare the incidences of fetal malformations from different sources of NZW rabbits and to investigate the possible relation of malformations seen in study 031090 with male #2749. The report is complete and contains useful supplemental information. (Parker, 4-14-88).

056 038377 Addendum to 031090. No worksheet. EPA letter, 5-2-85, study remains classified as Core Minimum with maternal NOEL=10 and developmental NOEL=5. EPA letter, 4-14-85, EPA comments on Ciba-Geigy response to EPA review. Peer Review by C.Kimmel, EPA, 4-5-85. Concludes maternal NOEL = 10 mg/kg/day and since some malformations seen at 10 and there were few fetuses to evaluate, fetal NOEL is 5 mg/kg/day. EPA review, 2-5-85, which lists problems with study including low pregnancy rate at 10 mg/kg and low values for implantation sites, both of these events occur prior to dosing. They also mention 1 fetus at 10 and 1 fetus at 30 that had cyclopia. These are considered by EPA to be evidence of a teratogenic effect of the compound even though this was not seen at 60 mg. (Parker, 5/23/86)

057 038378 Addendum to 031090. No worksheet. Ciba-Geigy response to EPA review dated 2/27/85. Letter from WIL, 3/4/85, addressing pregnancy rate, weight range and historical data. Letter from Wil, 1/28/85, with historical control. WIL suggests cyclopia may be of genetic origin in rabbits from Buckshire. Letter from WIL, 2-14-85, which correlated male breeder with fetal malformations observed. This is not the best way to present data as it appears that male #2749 contributes most of the malformed fetuses. However #2749 sired 58% of the fetuses in this study and therefore would be expected to sire most of the malformed fetuses. (Parker, 5/23/86)

**** 064 055966**; "A Teratology and Postnatal Study in Albino Rabbits with Cyromazine Technical"; (WIL Research Laboratories, Inc., Ashland, OH, Report No. WIL-82008, 2-9-86); Cyromazine Technical 95.2%; Dose levels 0 (0.5% carboxymethylcellulose), 5, 10, and 30 mg/kg/day administered by gavage on days 7-19 of gestation to 74 inseminated NZW female rabbits per dose group;

33-36 dams/dose level necropsied at day 29, remainder allowed to litter and kits necropsied at day 28 of lactation; Maternal NOEL = 10 mg/kg/day (decrease in food consumption and weight gain during dosing); **ADVERSE EFFECTS:** Cyclopia (1/317 at 30 mg/kg) and hydrocephaly (2/317 at 30 mg/kg), these effects were not reported in the historical controls; Developmental NOEL = NOAEL = 10 mg/kg/day; **ACCEPTABLE.** (Parker 8/17/87, 4/15/88, Original review indicated no adverse effects, this study was reviewed again and the adverse effects listed above were indicated, revised 8/3/89, Morgan)

** 065 055967; "Teratology Study in Rabbits"; (International Research and Development Corporation, Mattawan, MI, report no. 382-104, 4-11-85), (technical CGA-72662) Cyromazine technical (no purity stated), administered by gavage at 0 (untreated control), 0 (vehicle control), 5, 10, 30, and 60 mg/kg/day to 18 inseminated Dutch Belted rabbits/group on days 7 through 19 of gestation; Maternal NOEL = 10 (decrease body weight gain during dosing) Developmental NOEL \geq 60 mg/kg/day; **ACCEPTABLE, NO ADVERSE EFFECTS.** (Parker, 8-17-87, 4-18-88).

Cyclopia was seen in # 031090 in 1 fetus each @ 10 and 30 mg/kg/day. In a rigorous study (055966) conducted with a post natal phase, cyclopia and hydrocephaly were present at 30 mg/kg/day. Rabbits used in these two studies were from different suppliers, therefore the developmental effects cannot be supplier-dependent. A repeat study in Dutch Belted Rabbits # 055967, failed to indicate developmental toxicity. The lack of expressed developmental effects when Dutch Belted rabbits were used may be due to insensitivity of the strain or greater resorption of the defective embryos.

Cyromazine is a unique hazard to the developing organism. The maternal NOEL is 10 mg/kg/day in both NZW and Dutch Belted Rabbits. The developmental NOEL is 5 mg/kg in NZW and $>$ 60 mg/kg/day in Dutch Belted Rabbits. (Parker 6/20/88, Morgan 8/15/89).

Mutagenicity, Gene Mutation

NOTE: There is one positive test under this category, as well as two acceptable negative tests. J. Gee evaluated the "Gene Mutation" data base on April 2, 1992, and concluded that the weight of evidence does not indicate that cyromazine is a mutagen.

069 073936 "Salmonella/Mammalian - Microsome Mutagenicity Test" (Ciba-Geigy Limited, Agricultural Division, Basle, Switzerland, Laboratory Study # 78/2572, 12/11/78) CGA-72662 [technical cyromazine] was tested with Salmonella strains TA 98, TA 100, TA 1535, and TA 1537, with and without activation by Aroclor-stimulated rat liver S-9 fraction; 3 plates/strain/dose; 1 trial; 0 (DMSO), 25, 75, 225, 675, and 2025 ug/plate for 48 hours; **no adverse effects noted** (no increase in number of revertant colonies); **not acceptable, upgradable** (no justification for doses selected, inadequate description of the test material, inadequate cytotoxicity evidence, inadequate positive controls, no individual plate counts or standard deviations). (Klein and Gee 9/7/89)

069 072 073937 073949 "L5178Y/TK^{+/-} Mouse Lymphoma Mutagenicity Test, CGA-72662 Technical" (Ciba-Geigy Limited, Agricultural Division, Basle, Switzerland, Laboratory Study # 840942, 12/6/85) CGA-72662 Technical [cyromazine], Batch # EN38440, 96.2% pure, was incubated for 4 hours with mouse lymphoma L5178Y/TK^{+/-} cells, with and without activation by Aroclor-stimulated rat liver S-9 fraction at 0 (medium), 50, 100, 200, 300, 400, 450, and 500 ug/ml; **no adverse effects noted** (no forward mutations); **unacceptable and upgradable** with justification for the concentration of S9 and dimethylnitrosamine used. (Klein and Gee 9/6/89)

** 069 073939 "CGA-72662 Technical, V79 Chinese Hamster Point Mutation Test" (Ciba-Geigy Limited, Experimental Pathology, Basle, Switzerland, Laboratory Study # 840798, 8/11/86) CGA-72662 Technical [cyromazine], Batch # P.3, 98.9% pure, was incubated with V79 Chinese hamster embryonic lung cells for 21 hours without activation at 0 (medium), 25, 50, 100, 200, 400, 600, 800, and 1000 ug/ml and for 5 hours with activation by Aroclor-stimulated rat liver S9 fraction at 0 (medium), 100, 200, 400, 800, 1600, 2400, 3200, and 4000 ug/ml; **no adverse effects noted** (no base-pair or frameshift mutations, no deletions); **acceptable**. (Klein and Gee 9/6/89)

** 072 073950 "Mammalian Spot Test, Mouse, 8 Weeks, CGA 72 662 techn." (Ciba-Geigy Limited, Basle, Switzerland, Test # 850616, 2/11/86) CGA 72 662 techn. [cyromazine], Batch # EN 38440, 96.2% pure, was given by intraperitoneal injection to 71 pregnant C57 B1/6 mice/dose on the 10th day after conception (after mating with male T-stock mice) at 0 (sesame oil), 150, 300, or 600 mg/kg, offspring examined at birth and from 12-14 days to 5 weeks of age; **possible adverse effect** (dose-related increase in frequency of recessive spots); **acceptable**. (Klein and Gee 8/29/89)

** 072 073952 "Salmonella/Mammalian-Microsome Mutagenicity Test, CGA 72662 technical" (Ciba-Geigy Limited, Basle, Switzerland, Test # 871713, 5/31/88) CGA 72662 technical [cyromazine], Batch # FL 870153, 97.5% pure, was tested with Salmonella strains TA98, TA100, TA1535, and TA1537, with and without activation by Aroclor-stimulated rat liver S9 fraction, 3 plates/strain/dose, 2 trials/strain; 0(DMSO), 20, 78, 313, 1250, and 5000 ug/plate for 48 hours; **no adverse effects noted** (no increase in number of revertant colonies); **acceptable**. (Klein and Gee 9/6/89)

Mutagenicity, Chromosome

018 902768, "Dominant Lethal Study - CGA 72662 Mice (Test for Cytotoxic or Mutagenic Effects on Male Germinal Cells)", (Ciba-Geigy Limited, 3/17/81), cyromazine, no purity stated, 20 males/group were given 0, 326 or 678 mg/kg by oral gavage once and mated for 6 consecutive weekly periods with 40 females (34 for high dose - 3 males died). **No evidence of dominant lethal effect** is reported. Incomplete (missing info), **UNACCEPTABLE**, not upgradeable (no positive or historical control). (Gee, 5/5/86)

018 902769, "Nucleus Anomaly Test in Somatic Interphase Nuclei, CGA 72662, Chinese Hamster (Test for Mutagenic Effects on Bone Marrow Cells)", (Ciba-Geigy, 2/6/80), Cyromazine, 98.9% purity; 6/sex/group were given 0, 2000, 4000 or 8000 mg/kg by gavage and sacrificed at 24 hours; 1000 marrow cells of 3/sex/group were analyzed; **no evidence of clinical toxicity or micronuclei formation** is reported. Incomplete (missing data), **UNACCEPTABLE**, not upgradeable (inappropriate protocol: only analyzed marrow of 3/sex, time of sacrifice). (Gee, 3/13/86)

069 073938 "CGA-72662 Technical Chromosome Studies on Human Lymphocytes in Vitro" (Ciba-Geigy Limited, Agricultural Division, Basle, Switzerland, Laboratory study # 850013, 11/12/85) CGA-72662 Technical [cyromazine], Batch # EN38440, 96.2% pure, was incubated with peripheral blood lymphocytes with and without activation by Aroclor-stimulated rat liver S-9 fraction, 2 cultures/dose level at 0 (DMSO), 62.5, 125, 250, 500, and 1000 ug/ml for 3 hours; **no adverse effects noted** (no structural chromosomal aberrations); **unacceptable, possibly upgradable** (cells were harvested 44 hours after exposure to test material, an interval which probably missed the majority of cells in their first mitosis). (Klein and Gee 9/7/89)

018 073 902768 073966 "Dominant Lethal Study, Mouse (Test for cytotoxic or mutagenic effects on male germinal cells) - CGA 72 662" (Ciba-Geigy Limited, Basle Switzerland, Test # 790033, 3/17/81, supplemental report 8/11/88) Cyromazine, Batch P3, 98.9% pure, was given by gavage to 20 male mice/group at 0 (PEG 400), 226, or 678 mg/kg (single dose), the males were mated for 6 consecutive weekly periods with 40 females (34 for the high dose - 3 males died). **No adverse effects noted** (no evidence of a dominant lethal effect). Originally reviewed as unacceptable (no positive control or historical control data). Gee 5/5/86. Reviewed again with the submission of additional data, **unacceptable, possibly upgradable** (inadequate justification of dose levels, inadequate historical positive control data). (Klein and Gee 9/7/89)

** 073 073967 "Micronucleus Test (Mouse), CGA 72 662 tech." (Ciba-Geigy Limited, Basle, Switzerland, Test # 861345, 7/23/87) CGA 72 662 technical [cyromazine], Batch # EN 40025, 96.3% pure, was given by oral gavage to mice at 0 (0.5% carboxymethylcellulose), 360, or 1080 mg/kg, mice sacrificed at 24, 48, and 72 hours, 5/sex/dose/sacrifice time. **No adverse effects noted** (based on frequency of micronuclei in polychromatic erythrocytes); **acceptable**. (Klein and Gee 8/29/89)

Mutagenicity, DNA

** 069 073934 "The Hepatocyte Primary Culture/DNA Repair Assay on Compound CGA-72662 using Mouse Hepatocytes in Culture" (Naylor Dana Institute, American Health Foundation, Valhalla NY, Study # 050382, 4/15/83) CGA-72662 [technical cyromazine] was tested with primary mouse hepatocytes in the presence of ³H-thymidine, 3 coverslips/dose, 3 trials, at 0 (DMSO), 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.10, 0.50, and 1.0 mg/ml (Trial 1), at 0 (DMSO), 0.001, 0.005, 0.01, 0.05, 0.10, 0.50, and 1.0 mg/ml (Trial 2), and at 0 (DMSO), 0.005, 0.01, 0.05, 0.10, and 0.50 mg/ml (Trial 3), for 18 hours; **no adverse effects noted** (no unscheduled DNA synthesis); **acceptable**. (Klein and Gee 9/6/89)

069 073935 "The Hepatocyte Primary Culture/DNA Repair Assay on Compound CGA-72662 using Rat Hepatocytes in Culture" (Naylor Dana Institute, American Health Foundation, Valhalla NY, Study # 042782, 7/19/82) CGA-72662 [technical cyromazine] was tested with primary rat hepatocytes in the presence of ³H-thymidine, 3 coverslips/dose, 1 trial at 0 (DMSO), 0.0001, 0.001, 0.005, 0.010, 0.050, 0.10, 0.50, and 1 mg/ml for 18 hours; **no adverse effects noted** (no unscheduled DNA synthesis); **unacceptable, upgradable** (number of cells scored/dose level not reported). (Klein and Gee 9/7/89)

069 072 073940 073948 "CGA-72662 Technical, Saccharomyces cerevisiae D7/Mammalian-Microsome Mutagenicity Test in vitro" (Ciba-Geigy Limited, Agricultural Division, Basle, Switzerland, Laboratory Study # 831167, 10/24/84) CGA-72662 Technical [cyromazine], Batch # P.3, 98.9% pure, was incubated with Saccharomyces cerevisiae, D7 strain, for 16 hours, with and without activation by Aroclor-1254 stimulated rat liver S9 fraction, 2 trials, at 0 (DMSO), 375, 750, 1500, and 3000 µg/ml; **no adverse effects** (no crossing over, no gene conversion, no gene reversion); **unacceptable and upgradable** with reconciliation of the solubility limit statement in Record # 073948 with method description in Record # 073940). (Klein and Gee 9/6/89)

SUPPLEMENTAL STUDIES

031, 029 19899, "NTP Technical Report on the Carcinogenesis Bioassay of Melamine In F344/N Rats and B6C3F₁ Mice (Feed Study)," (NTP at Litton Bionetics, Kensington, MD); Technical Melamine (100% ±1%) in the diet of 50 F344/N rats/sex/dose level for 103 weeks at 0, 2,250 or 4,500 ppm to males and at 0, 4,500 or 9,000 ppm to females; **Possible Adverse Effects:** Increase in

transitional-cell carcinoma of the urinary bladder in high dose males (8/49), Increase in chronic inflammation of the kidney in females (17/50 at mid-dose and 41/50 at high dose). Supplementary data. (Gee, 4/18/88)

031, 029 19899, "NTP Technical Report on the Carcinogenesis Bioassay of Melamine in F344/N Rats and B6C3F₁ Mice (Feed Study)," (NTP at Litton Bionetics, Kensington, MD, 3/83); Technical Melamine (100% \pm 1%) in the diet of 50 B6C3F₁ mice/sex/group for 103 weeks at 0, 2250, or 4500 ppm; **Possible adverse effects:** Increased incidence of bladder stones, acute and chronic inflammation of the urinary bladder and epithelial hyperplasia in treated male mice; systemic NOEL (males) < 2250 ppm (lowest dose tested), systemic NOEL (females) = 2250 ppm (bladder inflammation, hyperplasia and calculi). No oncogenic effect in either sex. Supplementary data. (Gee 4/18/88)

031, 029 19899, "NTP Technical Report on the Carcinogenesis Bioassay of Melamine in F344/N Rats and B6C3F₁ Mice (Feed Study) - Appendix 1: Mutagenesis Results for Melamine in Salmonella typhimurium", (NTP at Litton Bionetics, 3/83); Melamine, > 95% purity; 3.3 - 3333 ug/plates (3 trials) without activation and 3.3 - 5550 ug/plate (3 trials) with activation with rat and hamster liver, Aroclor 1254 induced; **No adverse effects indicated;** Supplementary data. (Hughett 12/11/87, Gee 4/7/88)

CONCLUSIONS: Do data support registration?

Toxicity data to support Larvadex® 2SL and the active ingredient, cyromazine, were submitted and reviewed for Section 3 Registration for terrestrial, non-food use.

All required acute toxicity studies for the technical grade active ingredient are acceptable. The acute oral and dermal toxicity and primary dermal irritation studies conducted with the Trigard 5% SC-C formulation are acceptable. The primary eye irritation study conducted with Trigard 5% SC-C is unacceptable and not upgradeable because fluorescein staining was done at 1 hour after instillation of the test article. However, data from a primary eye irritation study conducted with the 1% premix formulation is used for bridging to fulfill this data requirement. Although the acute inhalation toxicity study is unacceptable, there are sufficient data to support a toxicity category III.

The metabolism study satisfies all the data requirements necessary for a complete metabolism study.

The subchronic oral toxicity study in dogs is unacceptable and not upgradeable because the dose selections were inadequate to permit identification of target organs and does not provide a good basis for selecting the appropriate doses for a long term chronic toxicity study. Analysis of the dosing material for stability, homogeneity and content in the animal feed was not reported.

In contrast, the status of the chronic oral toxicity study in dogs has been upgraded to acceptable. Histomorphological examinations of selected tissues from male and female dogs treated with 0, 20, 300, and 3000 ppm CGA 72662 technical demonstrated that treatment-related bilateral degeneration of the seminiferous tubules with decreased spermatogenesis were present in the testes of two of five dogs in the 3000 ppm group sacrificed after a 26-week exposure period.

The combined chronic/oncogenicity study in rats and the mouse oncogenicity study are acceptable. An acceptable rat reproductive toxicity has been submitted. The rat teratology and three of the four rabbit teratology studies are acceptable.

Acceptable studies were submitted to fulfill the data requirements for the gene mutation, structural chromosomal aberration, and other genotoxic effects categories.

A summary of the adverse effects observed in the studies reviewed is listed below. This summary includes the incidences of the effects and their statistical significance. (Morgan 9/20/89)

ADVERSE EFFECTS:

- A. Combined, Rat: Mammary adenoma and adenocarcinoma
- B. Chronic, Dog: Bilateral degeneration of the seminiferous tubules associated with decreased spermatogenesis
- C. Teratology, Rabbit: Decreased number of live fetuses
 Increased resorptions
 Decreased fetal weights
 Cyclopia
 Hydrocephaly
- D. NTP Technical Report on the Carcinogenesis Bioassay of Melamine in F344/N Rats and B6C3F₁ Mice (Feed Study):
 Increase in transitional-cell carcinoma of the urinary bladder
 Increase in chronic inflammation of the kidney
 Increased incidence of bladder stones
 Acute and chronic inflammation of the urinary bladder
 Epithelial hyperplasia

STATISTICAL SUMMARY OF ADVERSE EFFECTS

Combined, Rat

Effect	Incidence ¹				Trend ⁴
	0 ppm ³	30 ppm	300 ppm	3000 ppm	
Mammary Adenoma	3/53	8/58 (0.13)	6/58 (0.29)	8/59 (0.13)	0.45
Mammary Adeno- carcinoma	3/53	2/58 (1.0)	1/58 (1.0)	9/59 (0.09)	0.041
Mammary Adenoma and Adenocarcinoma	6/53	10/58 (0.27)	7/58 (0.57)	16 ⁵ /59 (0.030)	0.078

¹ Number affected/number observed

² Values in parentheses are the p values as compared to the control (Fisher's Exact Test-one tailed)

³ Concurrent control values for the study

⁴ Approximate p value for the dose-response relationship

⁵ Female #41282 had adenoma and adenocarcinoma

Effect	HC ³		Incidence ¹			Trend ⁶	
	0 ⁴	5 ⁵	10	30	60		
Record# 31090							
Cyclopia	0/3024	0/58	0/62 (1.0) ²	1/45 (0.014)	1/71 (0.023)	0/54 (1.0)	0.031
Spina Bifida	1/3024	0/58	0/62 (1.0)	0/45 (1.0)	1/71 (0.046)	0/54 (1.0)	0.28
Record# 55966							
Cyclopia	0/3024	0/355	0/398 (1.0)	0/325 (1.0)	1/317 (0.086)	-	0.079
Spina Bifida	1/3024	0/355	0/398 (1.0)	0/325 (1.0)	1/317 (0.16)	-	0.23
Hydrocephaly	0/3024	0/355	0/398 (1.0)	0/325 (1.0)	2/317 (0.007)	-	0.0078
Total Cyclopia and Spina Bifida for record#'s 31090 and 55966							
Cyclopia	0/3024	0/413	0/460 (1.0)	1/370 (0.097)	2/388 (0.010)	0/54 (1.0)	0.027
Spina Bifida	1/3024	0/413	0/460 (1.0)	0/370 (1.0)	1/388 (0.19)	0/54 (1.0)	0.416

¹ Number affected/number observed

² Values in parentheses are the p values as compared to the control values (+ historical control values if available) (Fisher's Exact Test-one tailed)

³ Historical Control values (New Zealand rabbits from various suppliers)

⁴ Concurrent control values for the study

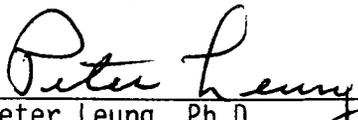
⁵ Doses are in mg/kg/day

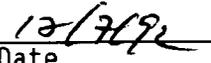
⁶ Approximate p value for the dose-response relationship

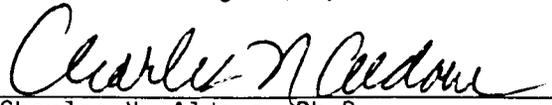
**RECOMMENDATIONS: In case of ongoing registration, register or do not register?
What other specific studies or data requested?**

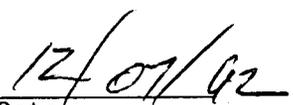
Submitted as a new active ingredient Section 3 Registration request for terrestrial, non-food use.

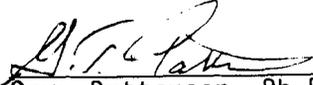
Registration is recommended pending completion of health assessment review.


Peter Leung, Ph.D.
Staff Toxicologist, Specialist


Date

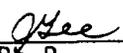

Charles N. Aldous, Ph.D.
Staff Toxicologist, Specialist


Date



Gary Patterson, Ph.D.
Senior Toxicologist

12/7/92
Date



Joyce Gee, Ph.D.
Senior Toxicologist

12/7/92
Date

APPENDIX B

OCCUPATIONAL EXPOSURE ASSESSMENT

**ESTIMATION OF EXPOSURE OF PERSONS IN CALIFORNIA
TO PESTICIDE PRODUCTS THAT CONTAIN CYROMAZINE**

BY

David E. Haskell*, Associate Environmental Research Scientist
Michael H. Dong, Staff Toxicologist
Tian Thongsinthusak, Staff Toxicologist

HS-1645 February 19, 1993

California Environmental Protection Agency
Department of Pesticide Regulation
Worker Health and Safety Branch
1220 N Street, Sacramento, California 95814

ABSTRACT

The Ciba Geigy Corporation has applied for a Section 3 registration to apply cyromazine formulated as Larvadex® 2 SL to chicken manure to control flies. The risk characterization document prepared by the Medical Toxicology Branch of the California Department of Pesticide Regulation for cyromazine indicates the active ingredient has the potential to cause "developmental effects" in laboratory animals. Dermal absorption of a 10 ug/cm² dose is estimated to be 17%. Animal feeding studies have identified the primary metabolites of cyromazine as hydroxycyromazine, melamine, and methylcyromazine with approximately 70% of the radioactivity excreted as unmetabolized cyromazine. Absorbed cyromazine is rapidly excreted, primarily in the urine, with the majority of the dose being eliminated within 24 hours. Dermal and inhalation exposure to cyromazine from applying Larvadex® 2 SL in a chicken house ranged from 0.40-16.77 mg per workday depending on the application method used. Environmental monitoring indicated that unprotected persons entering a chicken house immediately after an application will not be exposed to significant levels of cyromazine.

This report was prepared as an Appendix B to the Department's Risk Characterization Document for cyromazine.

* David Haskell was the lead person for the preparation of this document.

APPENDIX B

California Department of Pesticide Regulation Worker Health and Safety Branch

Human Exposure Assessment

CYROMAZINE

February 19, 1993

PHYSICAL AND CHEMICAL PROPERTIES

Cyromazine [*N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine, CAS Registry Number 66215-27-8, molecular formula $C_6H_{10}N_6$, molecular weight 166.19] is an insecticide of the triazine family. This triazine member has been used primarily as an insect growth regulator to control dipterous (*e.g.*, fly) larvae on animals and to control leafminers in ornamentals and vegetables. The compound has a vapor pressure of $< 1 \times 10^{-6}$ mm Hg (< 0.13 mPa) at 20°C, with a specific gravity of 1.35 g/ml and a melting point of 220 - 222°C. Cyromazine is moderately soluble in water (11 g/L at 20°C and pH 7.5) and only slightly soluble in methanol. The compound, which is commercially available as a colorless crystal and is stable at $< 310^\circ\text{C}$, has been observed to withstand hydrolysis for 28 days at up to 70°C (Royal Society of Chemistry, 1990: Worthing and Hance, 1991).

FORMULATION

Cyromazine was first synthesized by Ciba-Geigy in 1980 and has since been registered to the company under several trade names (Neporex[®], Vetrazine[®], Trigard[®], Armor[®], CGA 72662[®], and Larvadex[®]) for various veterinary and plant protection uses. The cyromazine product currently under review by the Department of Pesticide Regulation (DPR) is Larvadex[®] 2SL. As a soluble concentrate, each gallon of Larvadex[®] 2SL contains 0.17 lb of cyromazine (2% of the product) as the active ingredient (*a.i.*). The maximum label rate is 1 gallon of 0.1% finished spray per 100 sq. ft. of surface area of manure or other sites where maggots are active. The label specifies that Larvadex[®] 2SL shall not be sprayed more frequently than once every 21 days at a particular site.

Some background information is necessary to understand how this product will be used under California conditions. The current Larvadex[®] label limits the use of cyromazine to fly control around chicken layer and breeder operations only. Based on information provided by farm advisors, poultry operators, and staff from the Agricultural Commissioners' offices, cyromazine will probably be used primarily by that segment of the poultry industry involved in egg production. The egg industry utilizes layer chickens that are kept in wire cages and maintained in large houses where their numbers are in the thousands. The manure accumulates on the concrete floor underneath the cages and is removed periodically. How often the removal takes place and the ability to control its moisture content are the principle factors in managing fly problems. Most operations practice some form of integrated pest management centered around sanitary practices to control fly infestations by the systematic removal and drying of the manure. Or the manure can be removed in a slurry and retained in ponds to retain a high level of moisture to control fly propagation.

These practices include the management of fly predators and parasites and the judicious use of pesticides. Only a few products are currently registered for use inside the chicken houses to control adult flies that roost on the inside walls.

Flies are not a year round problem in California. In the San Joaquin Valley, the propagation season lasts from late March into October (Bokhai, 1993). The first frost usually occurs by mid-November and the flies over-winter as pupae until March. In the inland valleys of San Bernardino and Riverside Counties where the egg laying industry is located, the fly season starts in May and continues into November (McKeen, 1993a). Mitch Bernstein (1993) of San Bernardino Vector Control estimated the fly season can start in early spring and go into October. The average fly season was calculated to last for 210 days.

Two species of flies are of major concern for poultry operations in California (McKeen, 1984). The Lessor Housefly, Vania canicularis, is predominately a cool season pest during the early spring and fall months (McKeen, 1993a; Bokhai, 1993). It has the habit of not roosting readily and its constant flying can be very irritating. During the warmer summer months, the Common Housefly, Musca domestica becomes the dominate species (McKeen, 1993; Bokhai, 1993). Unseasonable rains or broken water pipes that prevent the timely drying and removal of manure can cause sudden fly infestations of both species.

Most poultry operations also maintain their own breeding flocks which provide eggs for hatching into laying hens or baby chicks to raise into broilers. As a rule their numbers usually represent about 1% of the broiler or laying population of the ranch (Bokhai, 1993). These flocks are maintained in similar houses as the layers and can experience the same fly problems.

REGISTRATION STATUS

Larvadex[®] is currently registered by the U. S. Environmental Protection Agency for use as a feed supplement or a directed spray to control developing fly larvae. The registrant has applied for a Section 3 registration of the directed spray formulation (Larvadex[®] 2SL) in California.

USAGE

The active ingredient cyromazine currently is not registered for use as a pesticide in California. Consequently, data are not available on the usage of cyromazine in California. However, projected sales by the Ciba Geigy Corporation for the California market are estimated at 6,000 gallons annually or about 1020 lbs of a.i.

LABEL PRECAUTIONS

Larvadex[®] 2SL is labelled as a Toxicity Category III pesticide. Initially, the Larvadex[®] 2SL label did not specify any protective clothing to be worn when handling cyromazine. However, the proposed California label requires applicators to wear a long-sleeved shirt and long pants, boots, rubber gloves and a dust mask. California regulations require some form of eye protection to be worn when applying this pesticide by the methods listed on the label. The label advises users to avoid contact with eyes, skin, or clothing and not to breathe vapors or spray mist. Contact with eyes will cause moderate eye irritation. Should contamination occur, the label directs the user to wash the exposed skin with soap and water, and to flush the eyes with plenty of water. If exposure occurs via inhalation,

handlers shall move or be removed to fresh air. The user is required to seek medical attention if irritation from exposure to cyromazine develops or persists. The label prohibits the direct application of this product to poultry or poultry feed or the feeding of manure treated with Larvadex[®] to animals. The label advises that a lapse of 24 hours be allowed between the last application and slaughter to avoid illegal residues.

WORKER ILLNESSES

Since cyromazine has not been registered for use in California, illnesses have not been reported from its use.

DERMAL HYPERSENSITIVITY

One dermal sensitization study was submitted in support of the registration of cyromazine in California. The test was conducted under the (commonly-accepted) assumption that acute toxicity of the new Larvadex[®] formulation with 2% cyromazine be considered equal to the results from using the old formulation (under the trade name of Trigard[®]) that contains 5% cyromazine. According to the new product label, the only change in the new formulation is a reduction in the active ingredient content by the addition of water.

The study investigated the potential of cyromazine to cause delayed dermal sensitization after repeated exposures (Sabol, 1983). The backs of 10 male Hartley albino guinea pigs were shaved and treated with 0.5 ml of Trigard[®] 5%. The test material was left in contact with their skin for approximately 6 hours. A total of 11 treatments were made in 35 days. Observations for skin reactions were made at approximately 24 hours after each application. In this study, dinitrochlorobenzene (DNCB) at 0.05% (w/v) in ethanol was applied to ten other guinea pigs in a similar manner, as the positive control. As expected, a sensitizing reaction was observed in the animals treated with DNCB. No skin reaction was observed, however, among the test animals following treatment with Trigard[®] 5%. The average skin reaction score for this test group for their virgin site was 0.2, out of a possible 8.0. Consequently, Trigard[®] 5% (as well as Larvadex[®] 2SL) was not considered to be a dermal sensitizer in guinea pigs.

DERMAL AND INHALATION ABSORPTION

Two dermal absorption studies were conducted with ¹⁴C-cyromazine applied to the shaved backs of male rats. The first study observed the rates of absorption using three dose levels: 0.1, 1.0, and 100 mg per rat equivalent to 10, 100, and 10,000 ug/cm², respectively (Murphy and Simoneux, 1985). The animals were exposed for 1, 2, 4, and 10 hours and then sacrificed. The percent of dose recovered in the treated skin was: 29-35% for low dose, 19-27% for mid dose, and 8-15% for high dose. The rates of dermal absorption, calculated as the percent of the dose recovered in urine, feces, carcass, plasma, and RBC were 4.5-11%, 3.5-11.4%, and 2.2-7.1% for low, mid and high doses, respectively. Because of the short sample collection periods and the high amount of bound skin residues, this study was not considered for the determination of dermal absorption.

A second study was conducted using three dose levels: 0.1, 1.0, and 10 mg/rat equivalent to 0.01, 0.1, and 1 mg/cm², respectively (Murphy, 1987). Labelled cyromazine (low and mid doses) and labelled cyromazine plus non-labelled cyromazine (high dose) were mixed with the formulation blank prior to application to the shaved skin sites of the male rats.

Four rats were used for each sacrifice time. The treated skin was covered with a non-occlusive protective appliance. At the conclusion of the exposure, the treated skin was washed with 2% Dove solution in water. There were two distinct observation periods. For the short-term sample collection, the animals were exposed for 2, 4, and 10 hours and then sacrificed. With the long-term study, the exposure times were 10 and 24 hours and the animals were sacrificed 48 hours after the exposure. Samples collected for the analyses included blood, carcass, skin washes, cage washes, treated skin and the skin around the treated area, urine, feces, and rinses of the protective appliance.

The results indicate that cyromazine is rapidly absorbed into the skin and saturated in a short period of time. The percent of the dose absorbed or recovered from the treated skin was similar for all dose levels. Bound skin residues, total dose recovery and dose absorbed are shown in Table 1.

Dermal absorption of cyromazine is apparently time and dose dependent; the absorption rate for the low dose is greater than the higher doses. Also, the percent of dose absorbed is greater with the longer exposure time. At the end of the longest exposure time (72 hours), the amount of bound skin residues as percent of dose for low, mid and high doses were 7.2, 11.5 and 5.9 percent (Table 1), respectively. There is an obvious pattern that bound skin residues are decreasing with increasing sacrifice time. For example, bound skin residues are 22.6 and 7.2 percent for the sacrifice time 2 and 72 hours, respectively (Table 1). A similar pattern is also observed for the excretion of the dose in urine and feces (Table 2). It is unjustifiable to add the percent bound skin residues directly to percent dose absorbed, because it is unlikely that all bound residues will be further absorbed and excreted. Furthermore, there are adequate data to estimate graphically the bioavailability or the amount of bound residues that is available for further absorption and excretion. Cumulative excretion data for any given compound always approaches a maximum upper bound. An asymptotic plot of the percent dose recovered in the excreta for 24, 48 and 72 hour collection times (Table 2) was based upon the exponential saturation equation described by Spain (1982). An example of the plot and output (performed by Steve Saiz, Worker Health and Safety Branch) is shown in Figure 1 and attachment 1. The bioavailability of skin residues is the difference of the percent of dose at asymptote and at the termination of the study. The percent of dose that are bioavailable from bound skin residues are then added to the percent of dose absorbed in 72-hour sacrifice time (Table 3).

This observation is supported by the rate of excretion of the low dose in urine. From 48 to 72 hours, 1.54% of the dose was excreted in urine. Between 24 and 72 hours, the percent of the dose excreted decreases by approximately 50% every 24 hours. If this rate of excretion continues for three more days, an additional 1.35% of the dose will be excreted in urine. This amount is almost equivalent to the percent of the dose (1.24%) estimated to be available by asymptotic extrapolation.

Table 1. Percent of administered dose absorbed or retained in treated skin.

	Exposure time (hours)				Exposure time (hours)	
	2	4	10	24	10	24
Sacrifice time (hours) ^a /	2	4	10	24	58	72
<u>Low dose (0.01 mg/cm²)</u>						
Total recovery	100.8	88.7	95.5	92.2	110.4	101.6
Skin residues	22.6	19.0	18.6	23.9	14.8	7.2
% Dose absorbed ^b	3.3	7.3	7.6	6.9	20.3 ^c	16.1
<u>Mid dose (0.1 mg/cm²)</u>						
Total recovery	100.4	102.3	97.9	99.1	102.0	103.3
Skin residues	9.9	13.6	12.0	21.3	8.4	11.5
% Dose absorbed ^b	6.6	3.5	5.1	2.8	8.8	12.5
<u>High dose (1.0 mg/cm²)</u>						
Total recovery	87.3	77.5	76.8	89.0	94.9	101.1
Skin residues	4.5	6.3	8.6	9.3	3.2	5.9
% Dose absorbed ^b	2.1	0.8	0.8	2.6	11.6	9.1

Thongsinthusak, WH&S, 1991

^a Hours after treatment
^b Percent dose in blood, urine, feces, carcass, and cage washes.
^c Cage washes for rat # 3816 was not included due to abnormally high values compared to other animals.

Table 2. Percent administered dose excreted in urine and feces following 10- and 24-hour exposures.

		sample collection time (hours)		
		10	34	58
<u>Dosages for 10-hour exposure</u>				
0.01 mg/cm ²	Urine (non-cum.)	2.48	4.26	2.27
	Feces (non-cum.)	0.07	0.11	0.24
	Urine + feces (cum.)	2.55	6.92	9.43
0.1 mg/cm ²	Urine (non-cum.)	2.66	1.99	2.39
	Feces (non-cum.)	0.23	0.05	0.08
	Urine + feces (cum.)	2.89	4.93	7.44
1.0 mg/cm ²	Urine (non-cum.)	3.16	2.33	0.66
	Feces (non-cum.)	0.01	0.08	0.05
	Urine + feces (cum.)	3.17	5.58	6.29
		Sample collection time (hours)		
		24	48	72
<u>Dosages for 24-hour exposure</u>				
0.01 mg/cm ²	Urine (non-cum.)	5.64	3.53	1.54
	Feces (non-cum.)	0.16	0.13	0.11
	Urine + feces (cum.)	5.8	9.46	11.11
0.1 mg/cm ²	Urine (non-cum.)	3.31	3.37	1.96
	Feces (non-cum.)	0.09	0.08	0.08
	Urine + feces (cum.)	3.40	6.85	8.90
1.0 mg/cm ²	Urine (non-cum.)	0.45	4.37	1.78
	Feces (non-cum.)	0.01	0.22	0.09
	Urine + feces (cum.)	0.46	5.05	6.92

Thongsinthusak, WH&S, 1991

(cum. = cumulative)

Table 3. Dermal absorption of cyromazine in rats when bound skin residues were taken into consideration.

		Exposure time (hours)	
		10	24
Sacrifice time (hours)/		58	72
0.01 mg/cm ²	% Dose absorbed	20.30	16.10
	% Dose bioavailable ^a	4.42	1.24
	% Dermal absorption	24.72	17.34
	% Dermal absorption ^b	22.39	17.07
0.1 mg/cm ²	% Dose absorbed	8.80	12.50
	% Dose bioavailable ^a	1.14	2.94
	% Dermal absorption	9.94	15.44
	% Dermal absorption ^b	9.75	14.95
1.0 mg/cm ²	% Dose absorbed	11.60	9.10
	% Dose bioavailable ^a	0.30	1.28
	% Dermal absorption	11.90	10.38
	% Dermal absorption ^b	12.54	10.37

Thongsinthusak, WH&S, 1991

^a From asymptotic extrapolation (% dose at asymptote minus % dose excreted in the last sample collected).

^b Corrected percent dermal absorption for total dose recovery.

The low dose in this rat dermal absorption study most closely approximates the estimated human dermal dose. At the exposure rate of 0.01 mg/cm², a 76 kg man with a body surface area of 19,400 cm² (50 percentile man) would experience approximately 194 mg of dermal exposure (U.S. EPA, 1985). The highest exposure rate observed in the exposure studies was 17 mg/day for the backpack sprayer.

Dermal absorption of the three dose groups for the 58-hour sacrifice time (Table 3) appeared atypical because the mid dose showed the lowest absorption. Linear plots of the percent dermal absorption at 58 hours versus dose levels gave a dermal absorption of 16.6% for the low dose with a low coefficient of determination. This percent absorption was similar to the dermal absorption of low dose for the 72-hour sacrifice time (17.3%). The plots of percent dermal absorption and doses for the 72-hour sacrifice time gave a high coefficient of determination ($R^2 = 0.941$). Considering the results obtained from these two sacrifice times, a dermal absorption of 17% was used for worker exposure estimates. This rate is used under the assumption that the dermal dose from occupational exposure to cyromazine is not more than 50 ug/cm². If this is not the case, dermal absorption will be determined from a curve constructed from plotting the percent dermal absorption and dose levels for the 72-hour sacrifice time.

Inhalation absorption data was not available for this chemical in animals. Inhalation absorption was based on the study of several chemicals in humans by Raabe (1988). His study indicates that vapor uptake is proportional to the inverse square root of the molecular weight. The relationship was found for pure vapor in air for several compounds in humans. He observed that chemicals in the form of aerosols and particulates were retained about 50% in the lungs. These residues were held by the alveoli and then 100% absorbed. A 50% inhalation uptake and 100% absorption was used for the worker exposure estimates.

ANIMAL METABOLISM

Two animal metabolism studies were conducted by the registrant to support the registration of Larvadex[®]. Rats were used in both studies to investigate the biologic fate of cyromazine. The first study conducted in 1978, was reviewed by DPR in 1987 and 1988, and found to be incomplete (and not upgradeable) because of too few animals employed per dose group (Simoneaux and Cassidy, 1978).

The second, more recent study was determined to be acceptable, since it was conducted in compliance with the data requirements set for pesticide registration (Capps, 1990). A total of 39 Charles River rats in four dose groups were used in the second study. Three of the four groups, consisting of 5 males and 5 females each, were given single or multiple oral (intragastic) doses of 300 mg/kg or 3 mg/kg (vehicle: Hi Sil 233/1% aqueous carboxymethylcellulose). In the high and the low oral dose groups, the animals were given only a single dose of ring labelled ¹⁴C-cyromazine (with purity > 96%). The third oral dose group was treated *daily* for 14 days with unlabelled cyromazine at 3 mg/kg, and then with a *single* radiolabelled dose of 3 mg/kg on Day 15. In addition, 5 other male and 4 other female rats were given a single intravenous (IV) dose of ¹⁴C-cyromazine (vehicle: deionized water). In all cases, the test animals were monitored over a 7-day period for recovery of radioactivity in the urine, feces, and tissues against time.

The total radioactivity eliminated via urine and feces was > 85% for all four dose groups, with more than 80% of the radioactivity excreted in the urine alone. Regardless of the dose route, the majority of administered radioactivity was eliminated in the urine during the first 24 hours after dosing. The amount of radioactivity eliminated in the feces for the four groups was between 3% and 7% of the total administered radioactivity. Recovery of the radioactivity in the tissues ranged from non-detectable to 0.01 ppm for all test animals except the high oral dose group. The carcass residues in the high dose group averaged 0.67 ppm, with approximately 80% obtained from the liver and 20% from red blood cells. When the radioactivity detected in the carcass was expressed as a percentage of the dose, the values for all dosage groups was < 0.37% of the dose.

The major metabolites present in the urine and fecal extracts were characterized by either high-performance liquid chromatography or thin-layer chromatography. The metabolite distribution in both extracts were shown to be similar, with approximately 70% of the radioactivity associated with unmetabolized cyromazine. The quantifiable secondary metabolites found in the urine and feces were hydroxycyromazine, melamine, and methylcyromazine. None of these three, individually, accounted for more than 9% of the radiolabelled dose in either excretion. The metabolism data presented in the study suggest that cyromazine is metabolized to hydroxycyromazine, methylcyromazine, and melamine. Additional products, which each accounted for < 1% of the administered radioactivity, were believed to be metabolized *directly* from hydroxycyromazine and methylcyromazine.

OCCUPATIONAL EXPOSURE

I. Applicator Exposure

A study of Larvadex[®] 2SL was conducted in 1988 to observe the occupational exposure from applying cyromazine in a poultry house (Merricks, 1988). Since poultry operations employ many different techniques to apply pesticides, the study included a survey of owners and managers of poultry businesses to determine the prevalent methods. Two of the application methods observed in the study; the backpack sprayer and the portable power sprayer were among the most common methods used to apply pesticides. Of the 216 poultry businesses surveyed, 42% and 32%, reported using, respectively, the portable sprayer on wheels and the backpack sprayer to apply insecticides in and around their poultry houses. Also, 40% of the businesses reported using the hand-held fogger. However, this method of application is not permitted by the Larvadex[®] 2SL label. Other reported methods used to apply insecticides including the hand-held sprayer, accounted for less the 9% of the businesses reporting in the survey.

The protocol called for four poultry operations located in Maryland, Pennsylvania and Virginia to participate in the study. The size of their operations ranged from 9,500 birds (45' X 100' house) to 120,000 birds (58' X 500' house). Larvadex[®] 2SL was applied as a 0.1% solution to the piles of manure underneath the cages to control developing fly larvae. Each worker, operating the hand-held or backpack sprayers, mixed and applied a two gallon mixture of cyromazine three times for each replicate. This entailed the handling of 0.024 kg of a.i. per replicate. Two persons, one to mix and load the spray material and the other to spray with a 200' hose, were used to operate the portable power sprayer. Each replicate consisted of mixing one gallon (0.077 kg a.i.) of Larvadex[®] with 19 gallons of water and treating the manure to the point of near run-off. A total of sixteen replications of mixing and applying Larvadex[®] were conducted for each application method at four different sites. However, it was later decided by EPA and Ciba Geigy that analysis of the samples from nine replicates at three different sites was adequate for the study.

The study was designed to estimate the potential and actual exposure from an application of Larvadex[®] with workers wearing a dust mask and rubber gloves in addition to work clothing (shoes, socks, long pants and long sleeved-shirt). Dermal exposure was detected with two sets of patches made of alpha-cellulose attached to a plastic frame with an aluminum foil backing. One set of patches was covered with cloth to represent the protective clothing worn by the applicators. Both types of patches were attached side by side to the outside of the Tyvek[®] coveralls at various body locations. Exposure to the hands was detected with cotton gloves worn underneath the latex gloves.

Respiratory exposure was monitored during the exposure period with a personal air pump equipped with a tee to draw air through two filters. The air pump was calibrated to draw

air through the two filters at a rate of two liters per minute. The polyurethane foam in the filters was covered with dust mask material to simulate the dust mask requirement on the label. Cyromazine has a vapor pressure of $<1 \times 10^{-6}$ mm Hg at 20°C. At this moderately low vapor pressure, a large number of fine particles are not expected from volatilization. Most spray particles were expected to be $>50 \mu$ and will be trapped in the dust mask material.

Quality control procedures were conducted at the application site and in the laboratory. All sample media were spiked in the field at various rates and frozen to serve as positive controls. Additional media samples were spiked prior to the exposure period to determine the stability of cyromazine in samples exposed to the poultry house environment. Samples were taken from each tank mix for all the sprayer types. A freezer storage stability study was initiated in the laboratory for all the sample media. The alpha-cellulose patches, cotton gloves samples and foam filters were extracted with hydrochloric acid, reacted with cation exchange resin, washed, eluted and then subjected to liquid chromatographic analysis.

The results indicate that cyromazine is relatively stable during storage. An average of 85% of the initial spikes were recovered from the field fortified patches, 79% from the gloves and 84% from the foam filters. These rates of recovery were confirmed by results from the laboratory freezer study. All the dermal and inhalation exposure calculations were adjusted for the minor losses that occurred in storage.

The minimum detectable level (MDL) for cyromazine on the patches was 0.001 ug/cm² and 0.2 ug total for the gloves and the foam filters. Cyromazine residues were not detected on any of the foam filters taken from the personal air sampling pumps. In accordance with the exposure guidelines listed in Subdivision U, Applicator Exposure Monitoring, (U.S. EPA, 1987), one-half the limit of detection was used to make the exposure calculations when residues were not detected for a particular sample. The following levels, representing 1/2 the MDL for the foam filter samples, were used to calculate the inhalation exposure for the various work tasks; 0.001 ug/L-backpack sprayer, 0.001 ug/L-handheld sprayer, 0.005 ug/L-power sprayer mixer/loader and 0.002 ug/L-power sprayer applicator (Merricks, 1988). To keep the inhalation exposure equivalent for each of the work tasks, the MDL's vary because the amounts of a.i. handled during the replicates are different for each work task and the exposure times differ for each work task.

Inhalation exposure was derived as the mg of exposure per kg of a.i. handled for each replicate. The inhalation exposures listed in the tables of the study represent the mean values from the nine replications of each work task and were calculated using this formula:

$$\frac{(\text{level of residue in sample}) (29 \text{ L/min, (U.S. EPA, 1987)}) (\text{time of replicate in min})}{(\text{kg of a.i. handled}) (\text{percent recovery of field spikes}) (1,000 \text{ ug/mg})}$$

Example calculation for hand-held sprayer: 0.001 ug/l X 29 L/min X 53 min./ 0.024 kg per replicate X 0.84 X 1,000 ug/mg = 0.076 mg of inhalation exposure per kg of a.i. handled.

Exposure to the body was calculated as the total exposure per 18,980 cm² of surface area excluding the hands. When the surface area of the hands (840 cm²) are included, this value is 102 % of the 19,400 cm² body surface area (50 percentile man) used to estimate the surface area of a 76 kg man (U.S. EPA, 1985). The exposure values from the study are listed in Table 4 without any correction for surface area.

Workers operating the hand-held sprayer had residues below detectable levels with the

exception of unprotected patches located on the ankles and thighs. These patches had residues consistently above the limit of detection. The backpack operators received the highest dermal exposures of all the workers, with the ankles, thighs, shoulders and back patches detecting the greatest residues. All the protected patches for the mixer/loaders loading the portable power sprayers had residues below the detection limits with the exception of one. Most workers applying cyromazine with the portable power sprayer had detectable residues on the thighs, ankles and forearms.

Residues detected on the cotton gloves worn underneath the chemical resistant rubber gloves were considered as actual exposure to the hands. The level of residues present on the cotton gloves were undetectable for eight workers and in the range of 0.20 to 0.42 mg for three other workers (two backpack applicators and one portable sprayer mixer, loader). The 4.25 mg of residue detected on the hands of a portable sprayer applicator was probably the result of an accidental spill.

Occupational exposure to cyromazine was expressed in terms of milligrams of exposure per kilogram of active ingredient handled per replicate in Tables 18-21 of the study. The mg of exposure per kg of a.i. handled per replicate were calculated as the average from the nine replicates for each sprayer type and job function. However, to identify the dermal and inhalation exposures, the values in the following table were listed as the actual exposure per replicate. These were calculated by multiplying the dermal and inhalation exposure values by the number of kg of a.i. handled during the replicate. This value was then multiplied by the number of replicates that can be completed in an eight hour workday in order to estimate the Daily Dermal and Inhalation Exposures. Example for the hand-held sprayer : (1.442 mg of dermal exposure/kg of a.i. handled/replicate) X (0.024 kg of a.i. handled) X (9 replicates per 8 hour workday) = 0.31 mg of Daily Dermal Exposure and (0.072 mg of exposure/kg of a.i. handled/replicate) X (0.024 kg of a.i. handled) X (9 replicates per 8 hour workday) = 0.016 mg of Daily Inhalation Exposure.

Table 4. Mean Daily Exposure to Workers Applying Cyromazine with Various Types of Sprayers^a.

Application Method	Dermal Exposure mg/replicate	Inhalation Exposure mg/replicate	Number of Replicates per/8 hr day	Daily Dermal Exposure mg/person/8 hr day	Daily Inhalation Exposure mg/person/8 hr day
Handheld Sprayer	0.035	0.0017	9.0	0.31	0.016
Backpack Sprayer	1.67	0.0017	10.0	16.7	0.017
Power Sprayer mixer/loader	0.012	0.0018	14.0	0.17	0.025
applicator	0.27	0.0018	14.0	3.8	0.025

Haskell, WH&S, 1991

^a Workers were wearing shoes, socks, long pants, long sleeved-shirt, dust mask and rubber gloves.

Since male workers predominate in agriculture, the exposure assessment was conducted to assess male exposure to cyromazine. Occupational exposure was estimated based on a body weight of 76 kg and a body surface area of 19,400 cm² for the 50th percentile man (U.S. EPA, 1985). The ratio of body surface area to body weight is 255 cm²/kg (19,400 cm²/76 kg). This ratio for the 50th percentile women is 273 cm²/kg based on a body surface area of 16,900 cm² and a body weight of 62 kg. These surface to body weight ratios are fairly constant between males and females. Although female exposure to cyromazine was not quantified in the occupational exposure assessment, the exposure estimates for the male workers are representative for female workers.

Exposure to cyromazine will be limited to the workers and owners of the poultry ranch. Owners of poultry operations in California generally do not engage the services of a pest control operator (PCO) to assist them in their pest control activities (Bokhai, 1993; Bell, 1993). Since chickens are raised in enclosed habitats, they are very susceptible to various avian diseases (University of California, 1977). Operators are concerned with protecting their flocks from Infectious Coryza, New Castle disease, bronchitis, fowl pox and other avian diseases (McKeen, 1993a). An infection in one bird threatens the whole flock. Sanitary measures for people and equipment entering the chicken houses, particularly where the young birds are kept, are important control measures for these infectious diseases (University of California, 1977). Farm advisors make it a practice to visit only one operation per day. Operations may require non company personnel to wear disposable protective clothing upon entering the houses to prevent the spread of infectious diseases (Post, 1993). Because of this desire to isolate each ranch's birds, even from other birds within the same company, each ranch manager is provided with their own spray equipment to make all the necessary pesticide applications (Maust, 1993). Utilizing the services of a

PCO would incur the risk that diseases could be transmitted from one operation to another via infected spray equipment and personnel. An analysis of the pesticide use reports now available under the 100% pesticide use reporting regulations demonstrate this is indeed the case. Riverside County is the leading egg producing county in California (California Agricultural Statistics Service, 1992). A member of the Riverside County Agricultural Commissioner's staff conducted a search for pesticides reported used by PCOs for applications to poultry operations (Riverside Ag. Commissioner, 1993). The search indicated 211 pesticide applications were reported at poultry sites for January-June 1992. All applications were made by the owners or operators of the poultry ranches.

The farm advisor for San Bernardino County (McKeen, 1993b) and the extension poultry specialist at the University of California, Riverside (Bell, 1993) estimate that treatments will be made on problem ranches only on an emergency basis. These ranches are usually located in rural areas that are rapidly urbanizing with residential neighbors that have a low tolerance for flies. The Yucaipa poultry district in San Bernardino County has experienced chronic fly problems even with good integrated pest management. This district has six companies with approximately 1.2 million birds located on 13 ranches (Bernstein, 1993). A typical poultry house (40' by 400') with three hens per cage contains approximately 13,230 birds (McKeen, 1993b). These 1.2 million birds are estimated to be located in 90 houses on 13 ranches with an average of 7.5 houses per ranch. Mr. Maust (1993) operates a 175,000 layer business with 110,000 birds located at the "Home" ranch in 17 houses. Utilizing a motorized spray cart with a 50 gallon tank, he indicated it would take about 20 minutes to spray each house and that the whole ranch could be sprayed in one day. Using this profile as typical for the Yucaipa district, a cyromazine treatment of all the chicken houses on one ranch could be completed in one day. Assuming each ranch manager will make all the applications for their ranch, a maximum treatment program with applications of Larvadex[®] made every 21 days during the fly season will result in ten exposure days per year.

**Table 5. Estimation of Lifetime Average Daily Dosage
for Applicators of Cyromazine.**

Application Method	Absorbed Daily Dosage ^a ug/kg/day	Average Annual Daily Dosage ^b ug/kg/day	Lifetime Average Daily Dosage ^c ug/kg/day
Handheld Sprayer	1.2	0.03	0.02
Backpack Sprayer	37.8	1.04	0.60
Power Sprayer mixer/loader	0.60	0.02	0.01
applicator	8.65	0.24	0.14

Haskell, WH&S, 1991

^a Since the estimated rates of dermal exposure are less than 10 ug/cm², the rate of dermal absorption was 17% (Murphy, 1987). Inhalation absorption is considered as 50% uptake and 100% absorption (Raabe, 1988). Weight of worker was 76 kg.

^b Assumes a maximum of ten applications made per year with the operator completing each application in one day. In California, the large size of most poultry operations dictates that some motorized form of the power sprayer would be the most likely method of application. One person would probably perform both the mixing/loading and application work tasks. Exposure for this worker would be the sum of the exposures for the power sprayer mixer/loader and applicator. The hand-held and backpack sprayers would likely be used for spot treatments only.

^c Assumes 40 years of exposure from application of cyromazine over a 70 year lifespan.

II. Field Worker Exposure

The volatilization of residual cyromazine after the application was monitored with alpha-cellulose cards and personal air sampling pumps fitted with foam filters. The cards were placed on the floors and walls of the treated houses. Additional cards and the air pump samplers were placed in the aisles at a height of five feet to collect residues in the breathing zone. The cards and foam filters from the pumps were collected immediately after the application and at 1, 2 and 4 days post application. Cyromazine residues were not detected (< 0.001 ug/cm² for wall cards and < 0.08 ug/cm² for foam filters) on any of the air pump filters or cards attached to the walls of the poultry houses. Only trace amounts of residues (< 0.001-0.005 ug/cm²) were detected on the cards placed on the

floor of one house. These results indicate that unprotected workers entering a poultry house after an application of Larvadex® 2SL will not be exposed significantly.

According to the label, manure treated with Larvadex® 2SL can be used as a soil fertilizer supplement. For manure treated solely with Larvadex® 2SL, the maximum allowable rate is 4 tons per acre per year. In addition, the label cautions against applying manure treated with Larvadex® 2SL to small grain crops that will be harvested or grazed, as illegal residues may result. Degradation data indicate that the levels of cyromazine in treated chicken manure remain stable 21 days after application (Honeycutt, 1982). Two hundred chickens will excrete approximately 66 lbs of manure per day with a manure surface area of 100 square feet (Bell, 1993). The levels of cyromazine present, 21 days after a treatment of 0.0086 lb. of a.i., are estimated to be 6.2 ppm. Due to the obnoxious nature of chicken manure, workers removing and spreading it are expected to wear a minimum of shoes, socks, long pants and long-sleeved shirt. If they were exposed dermally to 100 grams of treated chicken manure from working an eight hour day, the exposure to cyromazine would be equivalent to 0.62 mg. The absorbed dose for a 76 kg man would be only 1.4 ug/kg. This level of exposure for workers loading and spreading treated manure is not expected to be significant.

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ATTACHMENT 1

Determination of bioavailability of bound skin residues.
 (Cyromazine: 10 ug/cm²; 24 hour exposure with 72 hour sacrifice times)

ITERATION	LOSS	PARAMETER VALUES		
0	.23924100+02	.12000+02	.10000+00	.60000+01
1	.49592260+01	.11150+02	.96170-01	.17110+02
2	.57545500+00	.11070+02	.64580-01	.13670+02
3	.27283560+00	.11140+02	.57730-01	.11640+02
4	.99132260-01	.11520+02	.47680-01	.98410+01
5	.53490850-01	.11720+02	.43580-01	.84740+01
6	.97926970-02	.12170+02	.36850-01	.63420+01
7	.61282110-03	.12310+02	.34930-01	.57870+01
8	.46190810-04	.12340+02	.34780-01	.57520+01
9	.66342400-05	.12340+02	.34740-01	.57390+01
10	.32405680-06	.12350+02	.34670-01	.57080+01
11	.49191590-08	.12350+02	.34670-01	.57090+01
12	.24687370-09	.12350+02	.34670-01	.57090+01
13	.76112290-11	.12350+02	.34670-01	.57090+01

DEPENDENT VARIABLE IS: % Dose excreted = 5.8, 9.5, and 11.1 for 24, 48, and 72 hours after treatment, respectively.

PARAMETER	ESTIMATE
A	12.3502
B	0.0347
C	5.7087

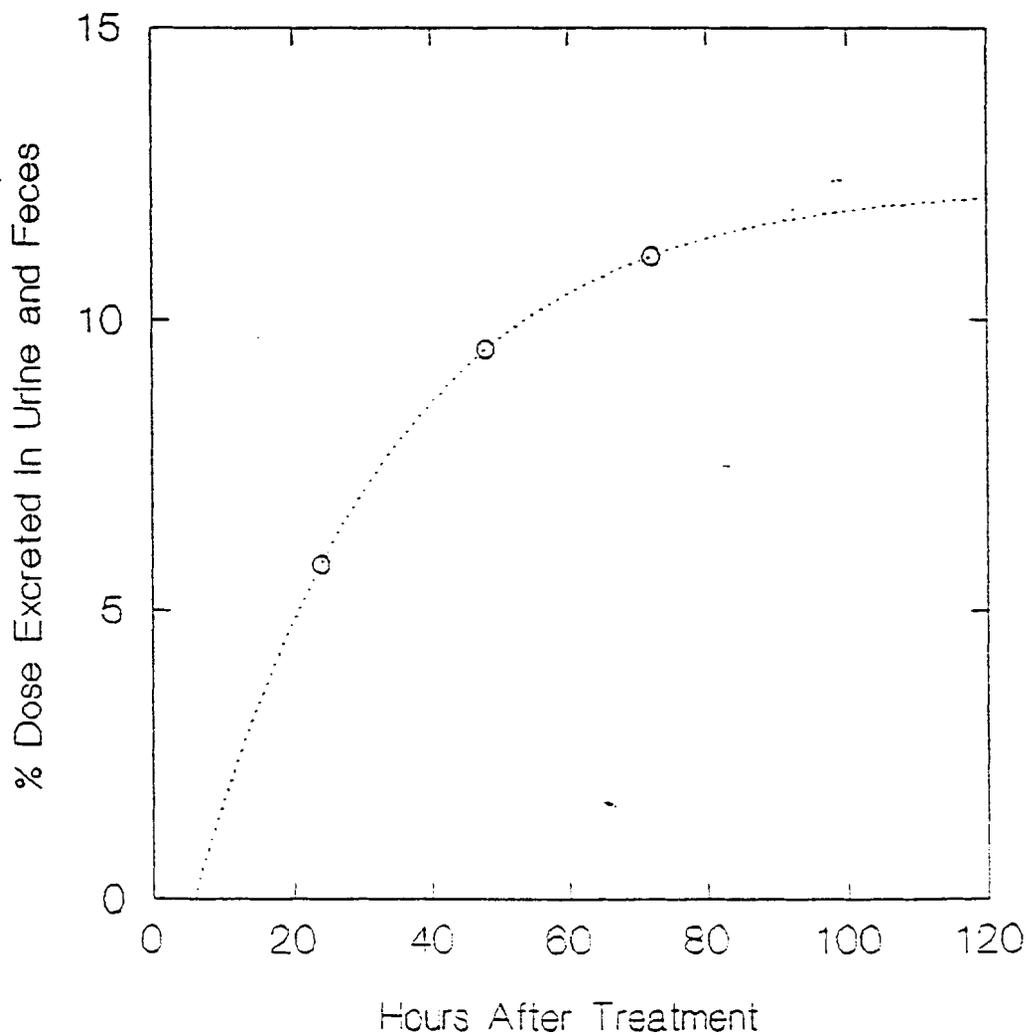
Bioavailability of bound skin residues = 12.35-11.11 = 1.24%

FIGURE 1

Determination of Bioavailability of Bound Skin Residues

(Cyromazine: 10ug/cm²; 24 hour exposure; 72 hour sacrifice time)

$$Y=12.35*(1-EXP(-0.0347*(X-5.709)))$$



APPENDIX C

ONCOGENICITY COMPUTER PRINTOUTS

HUMAN DOSAGE CALCULATION

CYROMAZINE

CALCULATION OF EQUIVALENT HUMAN DOSAGE

Study: Two Year Chronic Toxicity and Oncogenicity

Species: Sprague-Dawley Rat

Sex: Female

Biological Endpoint: Combined benign/malignant mammary adenomas/adenocarcinomas

Group	Concentration in Feed (ppm)	Animal Dosage (mg/kg/day)	Equivalent Human ^a Dosage (mg/kg/day)
1	0	0	0
2	30	1.8	0.50
3	300	18.8	5.26
4	3000	210	58.8

Sample Calculation:

$$\frac{\text{Dosage}_A}{\text{Dosage}_H} \times \frac{\text{BW}_H}{\text{BW}_A} = \frac{\text{BW}_A^{3/4}}{\text{BW}_H^{3/4}}$$

$$\text{Dosage}_H = \text{Dosage}_A \times \left(\frac{\text{BW}_A}{\text{BW}_H} \right)^{1/4}$$

Example (Group 2):

$$\begin{aligned} \text{Dosage}_H &= 1.8 \text{ mg/kg/day} \times \left(\frac{0.338 \text{ kg}}{55\text{kg}} \right)^{1/4} \\ &= 0.50 \text{ mg/kg/day} \end{aligned}$$

a/ Equivalent human dosage based on scaling of body weight to the 3/4 power.

Average body weight for female rat: 0.338 kg

Default body weight for human female: 55 kg

DATE: 03/05/1993

TIME: 08:46:24

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

CLEMENT ASSOCIATES
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

cyromazine, mamm. tumors, human equiv LADD

POLYNOMIAL DEGREE SELECTED BY PROGRAM, (POLY-DEGREE=0)
MONTE CARLO TEST USED IN SELECTION

GROUP	DOSE	#RESPONSES OBSERVED/#ANIMALS	RESPONSES PREDICTED
1	.000000	6/ 53	7.21
2	.500000	10/ 58	7.89
3	5.26000	7/ 58	7.89
4	58.8000	17/ 59	17.00

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 1.0039

P-VALUE FOR THE MONTE CARLO TEST IS .5750000000

FORM OF PROBABILITY FUNCTION:

$P(\text{DOSE}) = 1 - \exp(-Q_0 - Q_1 * D - Q_2 * D^2 - \dots - Q_6 * D^6)$

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q(0) = .146292066159
Q(1) = .000000000000
Q(2) = .000000000000
Q(3) = .000000000000
Q(4) = .000000000000
Q(5) = .000000000000
Q(6) = 4.683677284294E-12

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -102.657908111

CALCULATIONS ARE BASED UPON EXTRA RISK

LINEARIZED MULTISTAGE CONFIDENCE LIMITS

RISK	MLE DOSE	LOWER BOUND ON DOSE	UPPER BOUND ON RISK	CONFIDENCE LIMIT SIZE
----	-----	-----	-----	-----
.10000	53.131	20.188	.24216	90.0
		17.818	.26960	95.0
		16.138	.29310	97.5
		14.499	.32029	99.0
1.00000E-02	35.914	1.9257	.17092	90.0
		1.6997	.19133	95.0
		1.5394	.20901	97.5
		1.3831	.22970	99.0
1.00000E-03	24.450	.19171	.11980	90.0
		.16920	.13461	95.0
		.15325	.14753	97.5
		.13768	.16278	99.0
1.00000E-04	16.656	1.91619E-02	8.32560E-02	90.0
		1.69125E-02	9.37943E-02	95.0
		1.53181E-02	.10304	97.5
		1.37623E-02	.11400	99.0
1.00000E-05	11.348	1.91611E-03	5.75027E-02	90.0
		1.69117E-03	6.48976E-02	95.0
		1.53174E-03	7.14054E-02	97.5
		1.37617E-03	7.91501E-02	99.0
1.00000E-06	7.7310	1.91610E-04	3.95445E-02	90.0
		1.69116E-04	4.46850E-02	95.0
		1.53174E-04	4.92196E-02	97.5
		1.37616E-04	5.46292E-02	99.0
1.00000E-07	5.2671	1.91610E-05	2.71141E-02	90.0
		1.69116E-05	3.06647E-02	95.0
		1.53173E-05	3.38018E-02	97.5
		1.37616E-05	3.75505E-02	99.0
1.00000E-08	3.5884	1.91610E-06	1.85534E-02	90.0
		1.69116E-06	2.09951E-02	95.0
		1.53173E-06	2.31548E-02	97.5
		1.37616E-06	2.57385E-02	99.0

END OF LINEARIZED MULTISTAGE CONFIDENCE LIMITS

GLOBAL 86 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

DOSE ----	MLE RISK -----	UPPER BOUND ON RISK -----	CONFIDENCE LIMIT SIZE -----	COEFFICIENTS FOR CONFIDENCE LIMIT -----
5.90000E-04	-3.21280E-17	3.48872E-06	95.0%	Q(0) = .12492 Q(1) = 5.91310E-03 Q(2) = .00000 Q(3) = .00000 Q(4) = .00000 Q(5) = .00000 Q(6) = .00000

NORMAL COMPLETION!