# CYANAZINE

# (BLADEX®)

## VOLUME I
## RISK CHARACTERIZATION DOCUMENT

(6-26-97)

Medical Toxicology Branch

Department of Pesticide Regulation

California Environmental Protection Agency

## EXECUTIVE SUMMARY

### A. Introduction

Cyanazine is a herbicide which has been registered by U.S. EPA since 1971. Two formulations are currently registered in California, Bladex® 90DF and 4L. Cyanazine is used for the pre- and post emergence control of annual grasses and broadleaf weeds in corn, cotton, grain sorghum, winter wheat and fallow cropland. A Special Review was initiated by U.S. EPA for cyanazine in 1985 because of concerns over fetotoxicity in laboratory animals and evidence of occupational exposure. Subsequently, label amendments and "Restricted Use Classification" overcame these concerns. In 1990, California listed cyanazine as a chemical which was “known to the State to cause reproductive toxicity” under the State Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). Cyanazine is listed under “Developmental Toxicity.” In 1994, another Special Review was initiated by U.S. EPA. In this case, concerns were expressed about possible cancer risks, resulting not just from cyanazine, but also from two other related triazine herbicides, atrazine and simazine, either alone or in combination. Subsequently, the current cyanazine registrants, DuPont and Griffin, have voluntarily agreed to gradually phase out and eventually cancel the use of cyanazine, under certain conditions, by the year 2002.

### B. The Risk Assessment Process

The risk assessment process consists of four aspects: hazard identification, dose response assessment, exposure assessment, and risk characterization.

Hazard identification entails review and evaluation of the toxicological properties of each pesticide. The dose-response assessment then considers the toxicological properties and estimates the amount which could potentially cause an adverse effect. The amount which will not result in an observable or measurable effect is the No-Observed-Effect Level, NOEL. A basic premise of toxicology is that at a high enough dose, virtually all substances will cause some toxic manifestation. Chemicals are often referred to as "dangerous" or "safe", as though these concepts were absolutes. In reality, these terms describe chemicals which require low or high dosages, respectively, to cause toxic effects. Toxicological activity is determined in a battery of experimental studies which define the types of toxic effects which can be caused, and the exposure levels (doses) at which effects may be seen. State and federal testing requirements mandate that substances be tested in laboratory animals at doses high enough to produce toxic effects, even if such testing involves chemical levels many times higher than those to which people might be exposed.

In addition to the intrinsic toxicity of a pesticide, the other parameters which are critical to determining the risk are the magnitude, frequency and duration of exposure. The purpose of the exposure evaluation is to determine the potential exposure pathways and the amount of pesticide likely to be delivered through those routes. This includes occupational exposure on an acute (short-term), a chronic (long-term) or a lifetime basis. Dietary exposure is also estimated on an acute (daily) and chronic (annual) basis. The level of potential exposure is determined by the amount of pesticide residue on specific commodities and processed foods, and the consumption rate.

The risk characterization then integrates the toxic effects observed in the laboratory studies, conducted with high dosages of pesticide, to potential human exposures to low dosages of pesticide residues in the diet. The potential for possible non-oncogenic adverse health effects in human populations is generally expressed as the margin of exposure (MOE), which is the ratio of

the dosage which produced no effects in laboratory studies to the estimated dietary dosage. For oncogenic effects, the probability of risk is calculated as the product of the cancer potency of the pesticide and the estimated dietary dosage.

C. **Toxicology**

Based on the currently available data, the Department of Pesticide Regulation has concluded that the principal toxicological effects of cyanazine consist of fetal eye malformations following short term exposure and cancer and reproductive toxicity following longer term exposure. The mammary tumors which occurred in female rats were malignant, increased in a dose-dependent manner, with high potency and have also been observed with other, related triazine pesticides. There was no oncogenicity in the mouse.

D. **Occupational Exposure**

Estimates of occupational exposure from application(s) to cotton were made from a surrogate study using cyanazine on corn. The exposure data were amortized to the typical use rates for cyanazine on cotton in California, using the Annual Pesticide Use Report by Chemical. Acute, chronic (annual) and lifetime exposure estimates were calculated for cyanazine in order to calculate MOE values for acute and chronic exposure as well as cancer risk from lifetime exposure.

E. **Dietary exposure**

The registrants' crop residue database suggests that residues will not be present at harvest. Calculations conducted by DPR of dietary acute and chronic (annual) exposure used default residue levels or tolerances. The exposure has been calculated for all crops, singly and combined, for which there are U.S. EPA tolerances *i.e.* corn, cotton, grain sorghum and winter wheat. In addition, dietary exposure to cyanazine from the consumption of drinking water containing theoretical residues has been calculated. The dietary exposure for various population subgroups has been calculated for all commodities combined. Non-nursing infants (<1 yr.) had the highest potential acute dietary exposure and children (1-6 yrs.) had the highest potential chronic (annual) dietary exposure to cyanazine.

F. **Conclusions**

A margin of exposure or MOE of at least 100 is generally considered adequate to protect people from the toxic effects of a chemical when the NOEL is based on toxicology data from animal studies. MOE values were calculated using currently available acute exposure and toxicity data. Mean, short-term worker exposure data resulted in MOE values above 100 for both farmers and commercial applicators when calculated using abnormalities in the rabbit fetus as the toxicological endpoint. An estimated 95$^{th.}$ percentile of acute exposure gave MOE values below 100 for these workers. Long-term occupational exposure data resulted in MOE values above 100 for both farmers and commercial applicators when calculated using weight loss in a rat chronic study as the toxicological endpoint. Excess lifetime cancer risk was greater than $10^{-5}$ (1 in 100,000) but less than $10^{-4}$ (1 in 10,000) for commercial applicators and greater than $10^{-6}$ (1 in 1,000,000) but less than $10^{-5}$ (1 in 100,000) for farmers.

Based on the available toxicity and residue data, DPR concluded that the MOE values for potential acute (daily) and chronic (annual) dietary exposure, for all commodities for which U.S. EPA tolerances have been established, were above 100 for all population subgroups studied. The excess lifetime cancer risk for the general population was greater than $10^{-6}$ (1 in 1,000,000) but less than $10^{-5}$ (1 in 100,000).

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## I. SUMMARY

Cyanazine is a pre- or post-emergence herbicide which has been registered since 1971 by U.S. EPA for the control of annual grasses and broadleaf weeds in corn, cotton, grain sorghum, winter wheat and fallow cropland. It is a member of the triazine family of herbicides, which act by the inhibition of the Hill reaction of photosynthesis. Although over 90% of cyanazine use is on corn nationwide, in California, *ca.* 90% of cyanazine use is on cotton.

Field trials conducted by a former registrant indicated that residues of cyanazine in the above crops at harvest were below 0.01 ppm (the Limit of Detection, LOD). Likewise, four corn biotransformation products were not detected at LODs of 0.03 or 0.05 ppm.

Environmental fate studies have indicated that cyanazine has low to moderate persistence in soil ($t_{½}$ values of 6 to 20 days). However soil degradation products containing the triazine ring have much greater persistence. Furthermore, cyanazine has been shown to be mobile in soil while identified degradates were mobile or very mobile. The possibility of soil leaching is relatively high and leaching has been observed in the corn-belt States. In California, there have been no detections of parent cyanazine in groundwater monitoring studies.

A human health risk assessment has been conducted for cyanazine because of reproductive toxicity and because of carcinogenicity in animal studies. The risk assessment specifically addresses the potential exposure of workers mixing-loading-applying cyanazine to cotton. The toxicological endpoints used in the assessment were rabbit maternal body weight loss and developmental toxicity for acute dietary and occupational exposure; systemic toxicity in the rat (reduced body weight) for chronic dietary and occupational exposure; and, cancer (increased malignant mammary tumors in the rat) for lifetime occupational and dietary exposure.

Developmental toxicity was measured in three oral gavage studies in the rat and in an oral gavage and a dermal study in the rabbit. Maternal toxicity expressed itself as reduced body weight, in all studies, and showed similar NOEL values to those measured for developmental toxicity. Severe eye malformations were reported in both species, in the form of microphthalmia and anophthalmia, at dose levels which did not show particularly high maternal toxicity.

Chronic toxicity from repeated exposure to cyanazine was identified as reduced body weight in four studies employing rats, mice and dogs. The lowest NOEL for this effect in an acceptable study was 0.2 mg/kg/day in a rat study.

Oncogenicity was recorded in the form of an increased, dose-dependent incidence of malignant mammary tumors, in the rat. Because there were insufficient data to show that a threshold mechanism was operable, a linear multi-stage model was used as a default for calculating cancer potency. Genotoxicity was evident in four types of assay using mammalian cells, although not in those assays which had the potential for metabolism. There was evidence that plant metabolite(s) of cyanazine may be genotoxic. However, mammalian assays conducted *in vivo* were generally negative.

Reproductive toxicity was determined in a 2-generation rat study. Adult body weight reduction had a NOEL of 150 ppm, while in pups body weight reduction and reduced viability were reported with a NOEL of 75 ppm.

The NOEL of 1 mg/kg/day from a rabbit developmental toxicity study was used to determine MOE values for potential acute occupational and dietary exposure. A chronic NOEL of 0.2 mg/kg/day from the rat chronic toxicity study was used to determine MOE values for potential chronic occupational and dietary exposure. Excess cancer risk was determined using the $Q_1^*$ value of 0.58 $(mg/kg/day)^{-1}$ and the $Q_1$ value of 0.33 $(mg/kg/day)^{-1}$ for humans, obtained using the Global 86 program (Appendix A).

Occupational exposure was derived from a surrogate study in which cyanazine was applied to corn, with the application rates adjusted to typical use-rates in cotton. The absorbed daily dosage (ADD) for the mixer-loader-applicator (M/L/A) for ground applicators, using a closed cab, was 2.6 $\mu$g/kg/day and the 95$^{th.}$ percentile was 24.6 $\mu$g/kg/day. The ADD for the farmer is considered likely to be similar to that for the commercial-applicator. The annual average daily dosage (AADD) was 2.1E-02 (farmer) and 11E-02 $\mu$g/kg/day (commercial-applicator); and the lifetime average daily dosage (LADD) was 1.1E-02 (farmer) and 5.7E-02 $\mu$g/kg/day (commercial applicator).

Dietary exposure was estimated using TAS® software in combination with crop residue studies conducted by the former registrant. No residues were detected in any of these residue studies and so default values were used. Similarly, analysis of groundwater for cyanazine (by CDFA or DPR) has not resulted in any detections. Therefore, the dietary estimates can be considered to be largely theoretical. Acute dietary exposure to cyanazine, at the default residue level (LOD) for each crop was calculated. For all registered commodities combined, at the 95$^{th.}$ percentile, acute dietary exposure ranged from 0.038 to 0.16 $\mu$g/kg/day, for 17 population subgroups examined. Non-nursing infants had the greatest theoretical exposure. For mean chronic dietary exposure to cyanazine, at 50% of the LOD for each crop, the calculated exposures ranged from 0.004 to 0.031 $\mu$g/kg/day (not adjusted for % crop treated). The larger number is for children (1-6 yrs.). For the U.S. population (all seasons), hypothetical residues in drinking water increased chronic dietary exposure from 0.013 to 0.015 $\mu$g/kg/day.

A mean combined occupational and dietary exposure was calculated for the U.S. population (all seasons). The combined acute exposure was 2.7 $\mu$g/kg/day, an increase of 4% above the occupational exposure. Drinking water made no significant difference to the combined acute exposure. The combined chronic exposure was estimated to be 0.037 (farmers) and 0.126 $\mu$g/kg/day (commercial applicators). The inclusion of drinking water (at 50% LOD) increased the chronic exposure to 0.039 $\mu$g/kg/day (8% increase) and to 0.128 $\mu$g/kg/day (2% increase) for the two groups of workers, respectively.

The MOE for acute, occupational exposure, based on the mean exposure at the mean use rate in practice on cotton, was 385. Based on the 95$^{th.}$ percentile of exposure at the mean use rate, it was 41. The MOE values for chronic occupational exposure, based on mean exposure at the mean use rate, were 9,520 (farmer) and 1,820 (commercial applicator). At the mean use rate, the excess lifetime cancer risks, at the 95%UB (upper bound) for cancer potency, were 6.4E-06 and 3.3E-05, respectively. At the MLE (maximum likelihood estimate) for cancer potency estimate, the risks were 3.6E-06 (farmer) and 1.9E-05 (commercial applicator).

The MOE for acute, theoretical, dietary exposure, for all registered commodities combined, was 6,270 for non-nursing infants (<1 yr.) to 26,300 for seniors (55+ yrs.). The equivalent range for chronic exposure was 6,440 for children (1-6 yrs.) to 48,100 for nursing infants. The inclusion of possible theoretical levels of drinking water exposure reduced the MOE values by 5% (acute) to 33% (chronic, adjusted). The excess cancer risk from theoretical dietary

exposure, at the 95%UB, was 7.7E-06 and 8.5E-06, with drinking water.

The MOE values for acute, combined (occupational plus dietary) exposure were reduced from 385 (occupational) to 374, or 373 with drinking water included. Dietary exposure to cyanazine in California is likely to be largely theoretical, as stated. In addition, because it is likely to be considerably less than occupational exposure, it was not considered necessary to calculate safety and risk from combined (dietary and occupational) exposure.

The consumption of commodities with residues of cyanazine at tolerance (0.05 or 0.1 ppm) gave theoretical, acute, dietary exposures (at the $95^{th.}$ percentile) ranging from 0.003 - 0.0367 $\mu$g/kg/day, for sorghum grain, to 0.062 - 0.54 $\mu$g/kg/day, for corn grain. The MOE values for these exposures were 27,000 to 290,000 and 1,900 to 16,000, respectively. The ranges reflect differing dietary exposure patterns for various population sub-groups.

A MOE of at least 100 is generally considered adequate to protect people from the toxic effects of a chemical when the toxicology endpoints are derived from animal studies. Based on toxicology studies indicating maternal and fetal body weight loss combined with reduced pup viability, MOE values were calculated for acute occupational and dietary exposure. The ground application of cyanazine to cotton, the major use crop, at typical use rates and a closed cab resulted in MOE values above 100 for the M/L/A. However, the MOE was below 100 for acute exposure at the upper end ($95^{th.}$ percentile) of exposure. The MOE values were also greater than 100 for combined occupational and theoretical dietary exposures. MOE values were also calculated for chronic occupational and dietary exposure, based on weight loss in chronic, dietary animal studies. Using a closed cab, the MOE values were again above 100 for the M/L/A. The inclusion of theoretical dietary exposure in addition to occupational exposure did not reduce the MOE below 100. The lifetime excess cancer risk from estimated occupational exposure to cyanazine was greater than $10^{-5}$ (1 in 100,000) and below $10^{-4}$ (1 in 10,000) for the commercial applicator and above $10^{-6}$ (1 in 1,000,000) and below $10^{-5}$ (1 in 100,000) for the farmer. For dietary exposure to cyanazine, the excess cancer risk was above $10^{-6}$ (1 in 1,000,000) and below $10^{-5}$ (1 in 100,000). This was the case whether the MLE ($Q_1$) or 95%UB ($Q_1^*$) cancer potency estimate was used in the calculation, for a commercial applicator or farmer.

The dietary consumption of commodities containing theoretical residues of cyanazine at the LOD or tolerance resulted in MOE values greater than 100 for all population subgroups, both for acute and chronic (annual) exposure patterns. The addition of theoretical drinking water exposure did not reduce the MOE values below 100. The calculated excess cancer risk from theoretical dietary exposure to cyanazine, with or without potential drinking water exposure, was above 10,$^{-6}$ regardless of whether the MLE ($Q_1$) or 95%UB ($Q_1^*$) cancer potency estimate was used in the calculation.

The consumption of crops with residues at the U.S. EPA cyanazine tolerance level, on all commodities for which tolerances have been established, gave MOE values, for all population subgroups, which were above 100. This was the case for commodities consumed alone or in combination.

# II. INTRODUCTION

A risk assessment for cyanazine has been conducted based on the possible adverse effects identified in the following studies: chronic toxicity, genotoxicity, reproduction, and oncogenicity. Volume I comprises the toxicology profile, risk assessment, risk appraisal, tolerance assessment and conclusions. Appendix C gives the estimated dietary exposure. Volume II describes the estimates of occupational exposure. These exposure estimates were used for developing the risk characterization section in Volume I.

## A. CHEMICAL IDENTIFICATION

Cyanazine, (2-[[4-chloro-6-(ethylamino)-*s*-triazin-2-yl]amino]-2-methylpropionitrile) is a selective pre- or post-emergence triazine herbicide registered for use to control annual grasses and broadleaf weeds in corn, cotton, grain sorghum, winter wheat, and fallow crop land (U.S. EPA, 1986a). It is a photosynthesis inhibitor, which inhibits the Hill reaction of photosystem II causing chlorosis, necrosis, and plant death (Pauli *et al,* 1991).

## B. REGULATORY HISTORY

Cyanazine was registered in 1971 by U.S. Environmental Protection Agency (U.S. EPA) to be sold by Shell Chemical Company, and subsequently by E.I. DuPont de Nemours & Co., under the trade name, Bladex® (U.S. EPA, 1986b).

On January 3, 1985, U.S. EPA issued a registration standard for cyanazine where data gaps were identified and registrants were required to develop the additional data within a specified time frame (U.S. EPA, 1986a). Besides certain data describing product chemistry, residues and environmental chemistry, toxicological data were required. These included chronic and oncogenicity studies in two species, developmental toxicity studies in two species, a two-generation reproduction study, a dermal absorption study, and a complete set of genotoxicity testing. U.S. EPA also required precautionary statements to be put on the label regarding cyanazine's teratogenic potential (see below).

A special review of cyanazine was initiated by the U.S. EPA in 1985 based on its teratogenic effects in rats, fetotoxicity in rabbits and "sufficient exposure to mixer/loaders and applicators" (U.S. EPA, 1985). The teratogenic effects were reported in Fischer 344 rats where increased incidences of anophthalmia (no eyes) and microphthalmia (small eyes) were observed (Lu *et al*, 1981).

The special review was concluded on December 29, 1987 (U.S. EPA, 1987). Label modifications required: a. the use of protective gloves when mixing or loading cyanazine or when adjusting, repairing, or cleaning equipment; b. precautionary statements concerning the washing of the protective gloves; c. the use of closed systems in connection with aerial use and chemigation; d. the use of a chemical resistant apron when mixing or loading; e. cyanazine products carry a "Warning" sign and " Restricted Use Classification" because "..at doses which caused serious maternal illness in laboratory animals, birth defects were present." and, f. precautionary statement regarding washing of contaminated clothing. In addition, concern was expressed by U.S. EPA about ground and surface water contamination from agricultural uses of cyanazine. Label changes were imposed advising users not to apply cyanazine to highly permeable soils, *i.e.* well drained soils such as loamy sands, or where the water table was close to the surface.

As of September 19, 1991, there were no toxicity data gaps for cyanazine as required under California Senate Bill 950, The Birth Defects Prevention Act of 1984 (SB 950). In 1990, California listed cyanazine as a chemical which was "known to the State to cause reproductive toxicity" under the State Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). Cyanazine is listed under "Developmental Toxicity." A Reference Dose (RfD) of 0.002 mg/kg/day, a Drinking Water Equivalent Level (DWEL) of 0.07 mg/l (both based on a NOEL of 0.2 mg/kg/day for body weight loss in rats in a 2-year feeding study) and a $Q_1^*$ of 1.0 (mg/kg/day),$^{-1}$ based on increased mammary gland tumors in female rats, have been established (U.S. EPA, 1994a). A lifetime U.S. EPA Health Advisory (for non-cancer toxicity) of 1 ppb in drinking water is in effect. The Maximum Contamination Level (MCL) and Allowable Daily Intake (ADI) are pending from U.S. EPA.

## C. TECHNICAL AND PRODUCT FORMULATIONS

There are two products containing cyanazine as the active ingredient registered since August 14, 1987 which are still registered for use in California: Bladex® 90DF herbicide and Bladex® 4L herbicide. The former is a 90% dry flowable granule formulation and the latter, a liquid formulation containing 4 lb. a.i.[1] /gallon.

## D. USAGE

Cyanazine products are registered in California as pre- or post- emergence herbicides for the control of various weed species in cotton, corn, sweet corn, wheat and in conservation tillage and crop fallow land. Cyanazine may be applied alone or in combination with other herbicides and fertilizers. The application rates vary and are dependent on the soil texture and its organic matter content. Higher rates are used on heavier soils and soils with higher organic matter content. Cyanazine is not recommended for pre-emergence use on peat or muck soils. It is not allowed to be used on sandy and loamy sand soils with less than 1% organic matter. The maximum annual application rate for Bladex® 4L and 90DF is 6.5 lb. a.i./acre. On highly erodible land with plant ground cover below 30%, the maximum rate is 3.0 lb. a.i./acre per year.

Cyanazine is currently (1992-93) the fifth most heavily used pesticide in the U.S., with over 30 million lbs. being applied annually, mostly on corn. Its use in California accounts for only about 1% of this total. In contrast to the national use pattern, cyanazine in California is mostly used on cotton: in 1990, 383,163 lbs, with 90% on cotton (DPR, 1991); in 1991, 288,415 lbs. with 84% on cotton (DPR, 1992), in 1992, 348,645 lbs. with 87% on cotton (DPR, 1993) and in 1993, 508,205 lbs. with 87% on cotton. Other California uses included corn, wheat and fallow cropland.

## E. ILLNESS REPORTS

There were no cyanazine-related illnesses in California, from 1980 to 1990 (Mehler, 1991). There is a report in the medical literature describing dermatitis in a farmer following the application of atrazine, Bladex® and propachlor herbicides (Schlicher and Beat, 1972). He developed "painful erythematous eruption with blistering and swelling of both hands and forearms". Although atrazine exposure was to the hands, while mixing, it is unclear where exposure to the other herbicides took place. Healing occurred within a month. It is not clear which of these herbicides was primarily responsible for each symptom.

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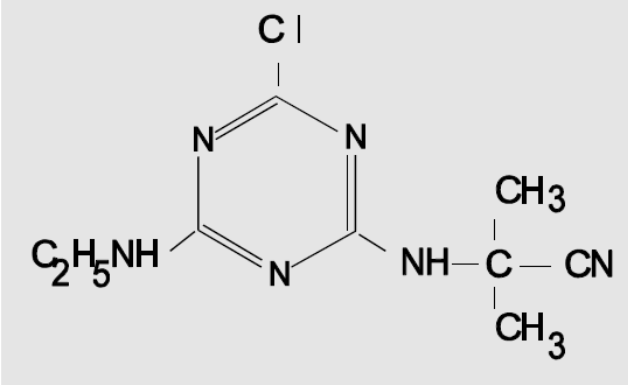
1/ a.i. = active ingredient

**F. PHYSICAL AND CHEMICAL PROPERTIES** (Shell Chemical Company, 1981)

| | |
|---|---|
| 1. Common Name: | Cyanazine |
| 2. Chemical Name: | 2-[[4-chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile |
| 3. Trade Names: | Bladex® 4L Herbicide, Bladex® 90DF Herbicide |
| 4. CAS Registry No.: | 21725-46-2 |
| 5. Molecular Weight: | 240 g/mole |
| 6. Molecular Formula: |  |
| 7. Empirical Formula: | $C_9H_{13}N_6Cl$ |
| 8. Physical State: | Solid, white crystalline |
| 9. Odor: | mild, non-specific chemical |
| 10. Melting Point: | 166.5°C-167°C (purity ≥94%) |
| 11. Solubility: | Water 155 ppm (Hoffman, 1988) and 171 ppm at 25°C (Merck Index, 1989); benzene 15 g/liter, chloroform 210 g/liter, ethanol 45 g/liter, hexane 15 g/liter at 25°C (Merck Index, 1989) |
| 12. Vapor Pressure: | $3.2 \times 10^{-8}$ mm Hg (25°C) (Barefoot, 1989) |
| 13. Henry's Law Constant: | $6.6 \times 10^{-11}$ atmos.-$m^3$/mole at 25°C (Hoffman, 1989) |
| 14. Partition Coefficient ($K_{ow}$): | 127 ± 2 (98.5% purity, 20°C); $\log K_{ow} = 2.1$ (Reinsfelder and Kenney, 1985) |

## G. ENVIRONMENTAL FATE

### Summary

Cyanazine is rapidly degraded in the presence of both soil and sunlight to derivatives which all retain the triazine ring. Hydrolysis of chlorine and nitrile groups occurred under acid conditions (pH 5) or on soil in the dark. An additional reaction, N-dealkylation to the des-ethyl derivatives, occurred through photolysis. Anaerobic soil degradation was similar to aerobic but occurred more slowly (Fig. 1, Section III.A). Cyanazine has medium to high soil mobility, indicating a high leaching potential. There is little tendency for residues to accumulate in crops.

### Hydrolysis

Cyanazine is very stable to hydrolysis. In a study employing $^{14}$C-ring labeled cyanazine, no hydrolysis occurred in pH 7 and 9 buffered solutions at 25°C after 30 days (Woodward *et al*, 1986a). Slight hydrolysis occurred at pH 5 with an extrapolated half-life ($t_{½}$) of 148 days. The major hydrolysis product arose from the hydrolysis of the chlorine atom and the cyano group, yielding the hydroxyacid (N-(4-(ethylamino)-6-hydroxy-2,3,5-triazin-2-yl)-2-methylalanine). A minor hydrolysis product was detected and tentatively identified as the hydroxyamide of cyanazine.

### Photolysis or Photodegradation

Cyanazine was degraded slowly to des-ethyl cyanazine under natural sunlight. A study using $^{14}$C-ring labeled cyanazine (5.1 ppm) in pH buffered solution under natural sun -light showed first order degradation with a $t_{½}$ of 43 days (Woodward & McEuen, 1985a). Des-ethyl cyanazine was the only product and no degradation occurred in the dark.

On soil surfaces exposed to sunlight, cyanazine is extensively degraded. $^{14}$C-ring labeled cyanazine exposed on sandy loam soil plates (0.57 $\mu$g/cm$^2$) during August and September in a California location degraded into three organosoluble and several water soluble products with a $t_{½}$ of 6.5 days (Woodward *et al*, 1985b), later recalculated as 3.5 days (CDFA, 1987). Des-ethyl cyanazine was the major organosoluble product. The principal water soluble product was the hydroxy derivative of cyanazine: N-(4-(ethylamino)-6-hydroxy-2,3,5-triazin-2-yl)-2-methyl-alanine. The des-ethyl product rapidly appeared in the light-exposed samples during the first two days and only increased slightly thereafter, while the hydroxy product increased steadily. Cyanazine degraded also on control soil plates in the dark, with a $t_{½}$ of 41 days, indicating only soil metabolism. The only major degradation product in the dark was the hydroxy material; no des-ethyl was produced in the dark, because it is a photoproduct.

### Soil Metabolism

Cyanazine is extensively metabolized in the soil under aerobic and anaerobic conditions into products retaining the triazine ring (Woodward *et al*, 1986 b,c). Under aerobic conditions, $^{14}$C-ring labeled cyanazine degraded in a Hanford sandy loam soil incubated in the dark at 25°C with a $t_{½}$ of 17 days. The major degradation pathway was hydration of the amide to give the chloro acid followed by the hydrolysis of the chlorine to give the hydroxy acid. No mineralization of the ring was detected and no volatile products were reported. Unextracted radioactivity associated with the humus and humic acid accounted for 16% of the applied dose after 180 days of

incubation. Less than 1% of the parent cyanazine was in the soil at the end of the incubation period. When soil incubated with cyanazine aerobically for 16 days was converted to anaerobic conditions by water-logging, degradation followed a similar degradation path but at a slower rate. The $t_{½}$ of cyanazine was extrapolated to be 108 days (Woodward *et al*, 1986c), compared with 17 days under aerobic conditions (Woodward *et al*, 1986b).

The metabolism of cyanazine in soils with growing field crops was described in a published study (Beynon *et al*, 1972). Cyanazine was applied at the rate of 2 kg/hectare (kg/ha) to three loam soils and one peat soil with maize seeds planted in them. The study was conducted using mainly the $^{14}$C-ring labeled material. $^{14}$C-cyanazine labeled in the isopropyl or ethyl or the nitrile groups was also used. Radioactive residues were determined in the soils after 168 days (28 days following the harvest of the maize plants). Total radioactive residue ranged from 3.08 to 3.62 ppm in the three loam soils. Cyanazine concentration was 0.41 - 0.62 ppm in these soils. The major soil metabolites were the amide and its carboxylic acid derivative arising from the hydrolysis of the nitrile group and its subsequent oxidation along with the hydroxy analog of the latter arising from the hydrolysis of the chlorine atom (Fig. 1). The latter metabolite was associated with the unextractable radioactivity that required acid treatment or hot water for its release. Dealkylated products were present only in trace amounts. The peat soil had higher total residues (5.36 ppm) and higher cyanazine concentration (0.90 ppm) than the other soils.

In a more recent study, the incubation of soil with cyanazine and sodium nitrite resulted in the formation of nitroso-cyanazine (Zwickenpflug & Richter, 1994). Chemical synthesis of (3) possible N-nitroso derivatives showed that the one found in soil had the nitroso attached to the nitrogen bearing the propane-nitrile group. It was found to be relatively stable, compared with other nitroso-triazines. Furthermore, a series of triazines (though not including cyanazine), incubated with human gastric juice and sodium nitrite, was shown to result in nitroso-derivatives being formed in each case (Cova *et al.*, 1996).

## Mobility (soil, air, water, plants)

Laboratory studies employing $^{14}$C-ring labeled cyanazine have shown that it is very slightly adsorbed to soils (Lee, 1982). Adsorption $K_{oc}$ (soil adsorption coefficient, adjusted for % organic carbon content) values ranged from 72.8 to 263 in four soils representing sandy to silty clay loam. These results suggest medium to high potential mobility of cyanazine in soils.

The mobility of cyanazine and its degradation products was evaluated by soil thin layer chromatography (TLC) in the same soils employed for the adsorption studies using the $^{14}$C-ring labeled material (McEuen and Woodward, 1986). However, this study was unacceptable to DPR because adsorption coefficients could not be calculated from the data supplied. The Rf values (movement relative to solvent front) of cyanazine placed it in a soil mobility category of “mobile to intermediate.” The amide and the chloro acid soil degradates of cyanazine were highly mobile; the hydroxy acid was similar in mobility to cyanazine.

## Plant Metabolism/Residues

### Metabolism

Available studies indicate that cyanazine is readily absorbed by plants from treated soils and is extensively metabolized into products retaining the triazine ring. A metabolism study in cotton has not been conducted. In maize plants grown in soil treated with $^{14}$C-ring labeled

cyanazine (2 kg/ha), at harvest (139 days) the total absorbed radioactivity was ≤0.02 ppm in corn cobs. The proportion of this which was the intact parent material was low, below the limit of detection (LOD) *i.e.* <0.01 ppm (Beynon *et al*, 1972). Most of the radioactivity was localized in the leaves (1.41 -2.07 ppm) followed by the stems (0.12 -0.21) and only a trace in the cob (0.02 ppm). Cyanazine was poorly absorbed from a peat soil (0.31 ppm in the leaves). Correspondingly, there was a higher concentration of the radioactivity remaining in peat soil (5.36 ppm) in contrast to the loam soils (3.08 - 3.62 ppm). The radioactive residue of cyanazine in plants was mainly the amide (SD 20196) and its des-ethyl analog (SD 33104), and the 2-hydroxy carboxylic acid (SD 31223) and its des-ethyl derivative (SD 31224), along with conjugates of the latter two. Des-ethyl cyanazine was found in trace amounts. It is apparent from this study that oxidation of the nitrile group, N-dealkylation and hydrolysis of the chlorine atom are the major pathways in the metabolism of cyanazine in plants (Fig. 1).

### Residues

Results of field tests indicate that residues of cyanazine in crops grown in soil treated with cyanazine are non-detectable (limit of detection, 0.01 ppm). Field data (1981 -1984) submitted by the former registrant, Shell Oil Company, are summarized in Table 1.

Table 1 Cyanazine Residues in Crops.

| Formulation | Rate (lb ai/A.) | Crop | Pre-Harvest Interval (days) | Residues[d] (ppm) | Reference |
|---|---|---|---|---|---|
| 90DF, 4L, 80W | 4-12[a] | field/sweet corn | 77-184 | Nd[e] | Shell Oil Co., 1985a |
| 90DF, 4L | 4.0[a] | cotton seed | 92 | ND | Shell Oil Co., 1985b |
| 90DF, 4L, 80W | 1.6-4[b] | wheat grain | 33-253 | ND | Shell Oil Co., 1985c |
| 90DF[c] | 3.9[b] | sorghum | 129 | ND | Shell Oil Co., 1981a |
| 90DF[c] | 2.0-4.0[b] | field corn | 116-138 | ND | Shell Oil Co., 1981b |
| 90DF[c] | 5.6[a] | cottonseed | 99 | ND | Shell Oil Co., 1981c |
| 90DF[c] | 4.0[b] | wheat grain | 343 | ND | Shell Oil Co., 1982 |

a/ multiple application (pre-plant, pre- & post-emergence)
b/ single application (pre-plant or pre-emergence)
c/ tank mix with other herbicides
d/ Label-approved Pre-Harvest Interval (PHI) for cotton is 54 days.
e/ ND is not detected, LOD for cyanazine = 0.01 ppm, for metabolites SD 33104 and SD 20196 = 0.03 ppm , SD 31223 & 31224 = 0.05 ppm (Fig. 1).

## Bioaccumulation

### Field Dissipation

The field dissipation of cyanazine was measured in California, Delaware and Illinois and was found to show a $t_{½}$ of 6 - 20 days (Powley, 1990), which is consistent with the laboratory data. These $t_{½}$ values were recalculated by DPR for cyanazine plus soil degradates, using all of the data, as 14 to 39 days (DPR, 1991), the latter value being for Madera, CA. Cyanazine and its metabolites appeared at depths of 30 to 60 cm in some samples but were discounted and "contamination or sample switching of some sort during collection or processing of soil" was suggested. However, studies discussed above on the soil adsorption and desorption, mobility on soil plates, hydrolysis, and soil metabolism studies point to the potential mobility of cyanazine and its metabolites in the soil. The potential for soil leaching cannot therefore be dismissed.

## III. TOXICOLOGY PROFILE

Acceptability of the studies (except for genotoxicity studies) where noted, is determined according to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines. The acceptability of the genotoxicity studies by the Department of Pesticide Regulation (DPR) is based on the guidelines of the Toxic Substances Control Act (TSCA), published in 1985 (Federal Register, 1985). Wherever appropriate, the term NOEL (no observed effects level) is used to refer to adverse effects and it is therefore synonymous with "NOAEL" (no observed adverse effects level). Developmental toxicity studies were also considered for estimating acute toxicity. A Toxicology Summary is not included with this document but is available from the Registration Branch of DPR.

### A. PHARMACOKINETICS

#### Summary

Urinary excretion in the rat following oral dosing with $^{14}C$ cyanazine was approximately 34%, with 18% in the feces, within the first 24 hours. Assuming that the $^{14}C$ in the feces was not absorbed, 82% of the dose was absorbed from the gut. Elimination of radiolabel was fairly rapid and was nearly complete within 4 days. An experiment in the dog showed 52 to 64% absorption by the oral route. Based on several rat studies, cyanazine rapidly undergoes metabolism via N-deethylation, dechlorination and conjugation with glutathione with subsequent formation of mercapturic acids, and oxidation of the nitrile group (Fig. 1). Dermal absorption of cyanazine in the male rat, from an aqueous solution of Bladex®4L, averaged 0.9% at the end of a 10-hour exposure period, peaked at 2.0% (group mean) and 4.6% (highest individual value) at 24h. Dermal absorption in the female rabbit was similar: maximally, 1 to 3% occurred after a 6 h exposure period.

#### Oral-Rat

The only study reported in detail examined the excretion of cyanazine after oral administration of uniformly $^{14}C$-ring labeled compound to Carworth Strain E rats (Griffiths, 1968). Three rats/sex were dosed by gavage with 0.8 mg cyanazine (3.2-4.0 mg/kg). Urine, feces, skin, gut, carcass and expired air were collected for 4 days and radioactivity measured. The results indicated cyanazine is excreted fairly rapidly. Over 90% of the radioactivity was eliminated within four days, 40.6% in urine, 49.6% in feces with only 3.0% remaining in the carcass. Over 50% of the elimination occurred within the first 24 hours (34% and 18% for urine and feces, respectively). Trace amounts of $^{14}C$ were observed in the feces for four days. There was no indication of cleavage of the triazine ring based on $^{14}CO_2$ in expired air. No blood measurements of the radioactivity were made and only preliminary metabolite identification was attempted. Only 2% of the total urinary radioactivity was parent compound, and at least 7 other labeled compounds were detected, indicating extensive metabolism of cyanazine. Females excreted slightly less radioactivity through the urine and slightly more through the feces than males.

Since 18% fecal elimination of $^{14}C$ occurred within 24 hours of oral administration, it was assumed that $\geq$82% was absorbed from the gut. Approximately 50% of the total radioactivity was eliminated in the feces by 4 days; therefore, some of the fecal radioactivity may have resulted from biliary excretion of absorbed material. The low molecular weight (240) and moderate lipophilicity ($\log K_{ow}$=2.1) of cyanazine argue against biliary excretion. However, Crayford & Hutson (1972) showed that the bile duct cannulated rat excreted 21% of $^{14}C$-cyanazine, as metabolites, in

the bile in 20 h. Thus, biliary excretion could therefore account for the (18%) fecal elimination, above. The four main biliary metabolites of cyanazine were desethyl cyanazine glutathione conjugate > desethyl cyanazine > hydroxyacid cyanazine > glutathione conjugate of cyanazine.

A summary of a study investigating the metabolism of cyanazine in the rat was submitted to DPR (Shell Toxicology Laboratory, 1972). A number of flaws existed in this study. The study design was vague as to how many animals were involved. Either 1/sex, 12 females, or 1 female were dosed orally with cyanazine labeled with $^{14}C$ either in the ring or the ethyl group. No data were presented and it was unclear which results were derived from 1 animal and which were from the group of 12. Given these limitations, the proposed biotransformation of cyanazine occurs without degrading the triazine ring structure and involves metabolism of the substituents via N-deethylation, dechlorination and conjugation with glutathione with subsequent formation of mercapturic acids, and oxidation of the nitrile group. The urinary metabolites reported in this study were the N-deethylated metabolite, the mercapturic acid metabolites with and without N-deethylation, the 2-hydroxy-6-carboxylic acid metabolites, and the amide metabolite formed by oxidation of the nitrile group. The major fecal metabolite reported was the 2-hydroxy-6-carboxylic acid, which was also detected in urine.

Several studies were collectively summarized in a brief document (Shell Chemical Company, 1985). Since no data were submitted to substantiate the information, and the methods used were not presented, the utility of this information is clearly limited. The importance of the N-deethylation reaction in the metabolic fate of cyanazine was reportedly demonstrated in rats treated with $^{14}C$-ethyl-labeled cyanazine from which 50% of the administered dose was recovered in the expired air. The biotransformation of cyanazine labeled with $^{14}C$ in either the ring, the isopropyl-, or the cyano-group was reported as being similar. Only a small amount of the administered dose (less than 5%) was excreted unchanged. The major urinary metabolites were reported to be N-deethylated parent and its N-acetyl-cysteine conjugate. Minor urinary metabolites reported were amides (formed by oxidation of the cyano group) of (1) the parent and (2) the N-deethylated compound. The major fecal metabolite was reportedly identified as the 2-hydroxy-6-carboxylic acid of the parent compound.

A literature report (Crayford & Hutson, 1972) indicated that an alternative route to the biotransformation of cyanazine to its hydroxy acid derivative in the rat was through the formation of hydroxy-cyanazine followed by its amide (Route 2, Fig. 1).

A summary of a study of the mammalian metabolism of the major plant metabolites was submitted (Shell Toxicology Laboratory, 1970a). Rats received oral doses of $^{14}C$-ring labeled 2-hydroxy-6-carboxylic acid and the N-deethylated, 2-hydroxy-6-carboxylic acid derivatives of cyanazine, separately. Radioactivity was excreted mostly in the feces (60% and 85%, respectively) with smaller amounts in urine (25% and 10%, respectively). No further metabolism was detected. Again there are many limitations to the usefulness of this report, notably the lack of information on the number of animals and the methods used.

**Oral-Dog**

A summary of the elimination of a single oral dose of $^{14}C$-ring labeled cyanazine (0.8mg, equivalent to 0.05-0.09 mg/kg) administered by capsules to 2 beagles per sex indicated that 52% of the dose was excreted in the urine and 36% in the feces during the first 96 hours (Shell Toxicology Laboratory, 1968).

**Dermal-Rat**

Two dermal absorption studies have been conducted using male F344 rats and both indicate that cyanazine is poorly absorbed through the skin of experimental animals. In the first study, 3 dosage levels of Bladex® 4L formulation were used (0.5, 5.0 and 50 mg cyanazine/rat) and the absorption was assessed over a 10 hour duration of exposure in 4 rats/dose/time point (Mitschke and Logan, 1985). Interpretation of the data was complicated by poor and variable recovery due to solubility problems. These resulted in non-homogeneous suspensions and consequently an inability to reproducibly aliquot the doses, especially at low concentrations of the dosing preparation.

In the second study, 4 male rats per time point were exposed for up to 10 hours to $^{14}$C-ring labeled Bladex® 4L (44-45% cyanazine) in water. They were dosed with 50 mg cyanazine/rat (167-184 mg/kg) applied over a 12 $cm^2$ shaved area (Logan, 1986a). The application site was washed after 10 hours and again at sacrifice, to mimic occupational exposure. Absorption was monitored as $^{14}$C in urine, feces, expired air, blood, carcass, skin and skin washings, for up to 8 days after the application. Rats were sacrificed and absorption determined at time intervals of 0.5, 2.0, 4.0, 10, 24, 48, 72, 120 and 192 hr. Absorption increased over the 10 hour exposure period. The average percent of the dose absorbed in urine, feces, blood, carcass and skin, after 10 hours exposure, was 1.2%. The maximum percent of the dose absorbed by any one group was 2.0% (mean, 24 hour). The maximum amount absorbed through the skin (into urine, feces, blood, carcass) at any time was 0.3%. Maximum body burden (blood, carcass) at any time was 0.1%. Less than 0.01% of the applied dose was recovered in expired air over 192 hours. The maximum absorbed by any rat was 4.6% (range 0.8 to 4.6%), at 24 hour. The majority of the absorbed dose was still located in the skin at 10 hours, some of which was slowly absorbed over the 8 day monitoring period.

**Dermal-Rabbit**

The dermal penetration of $^{14}$C-ring labeled cyanazine was investigated in female rabbits following exposure to Bladex® 4L formulation at 0.2 and 1.2 ml/kg (approximately 200 and 1,200 mg/kg), for 6 hours/day, followed by washing after each application, for 13 days; only the final dose was radiolabeled with $^{14}$C-Bladex® 4L, at 98.5 to 99.7% radiochemical purity (Logan, 1986b,c). For comparison, two groups of rabbits received 1 or 4 mg/kg of technical $^{14}$C-cyanazine, orally and the blood $^{14}$C levels were monitored for 96 hours (Logan, 1986c). The study demonstrated that absorption by the dermal route is very low compared to the oral route through assessment of peak plasma levels of cyanazine. The peak levels occurred at 10 hr. and formed a plateau until 96 hr. These levels were 56 ng/ml and 274 ng/ml, at 0.2 and 1.2 ml/kg, respectively. After oral dosing, the analogous peak plasma levels were 204 and 662 ng/ml after 1 and 4 mg/kg, respectively, occurring at 2-4 hr. after dosing. The amount of absorption from oral administration was thus several hundred-fold higher than from dermal application. Chromatography revealed that cyanazine comprised 13% (dermal, at 4-18 hr.) and 11% (oral, at 2-6 hr.) of the recovered radioactivity. It was found that 97 to >99% of the $^{14}$C was removed from the skin by washing the application site, indicating that maximally 1 to 3% was absorbed during each dermal exposure period.

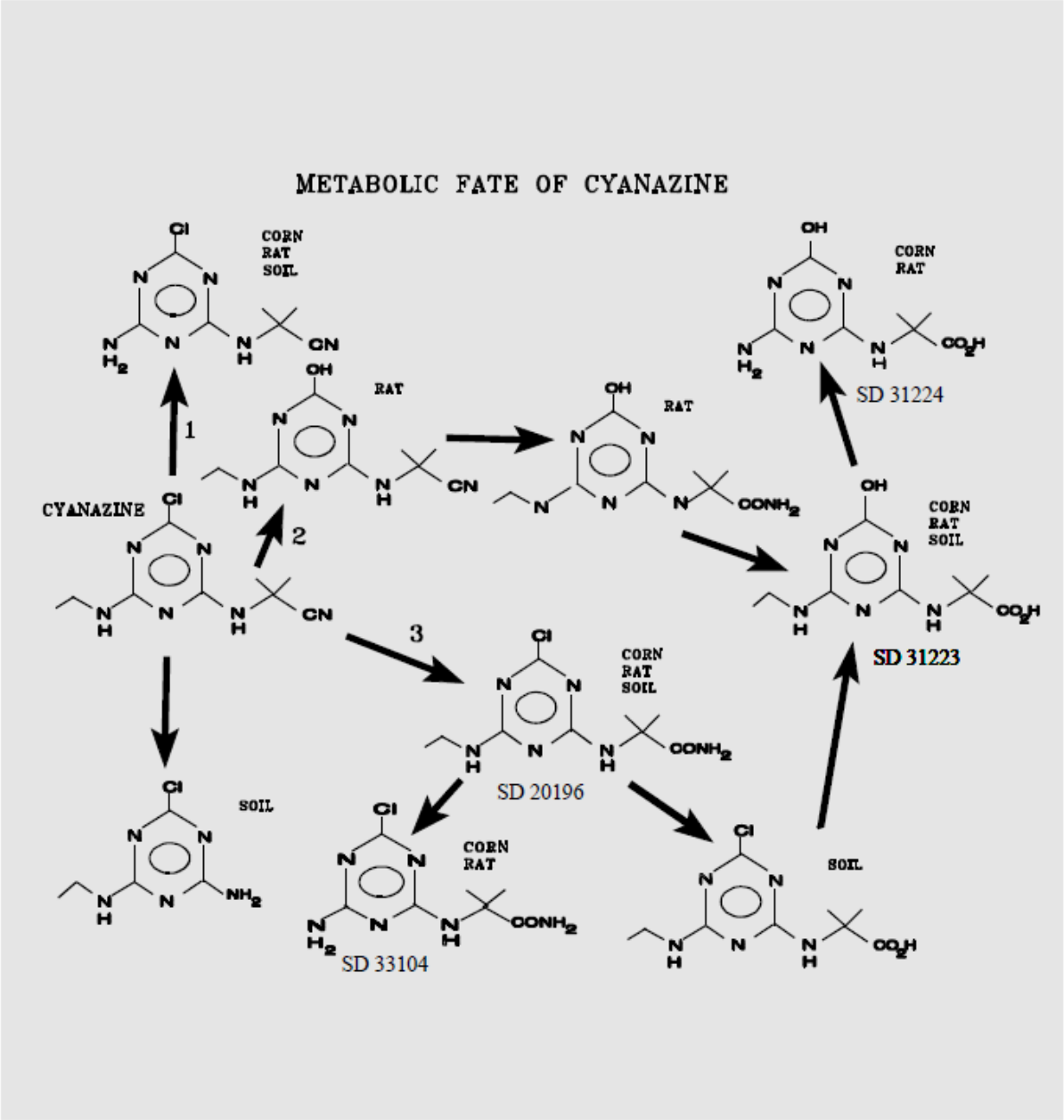



Figure 1. Principal metabolites of cyanazine in rat, soil and corn.[a]
Cyanazine is initially metabolized in the rat in one of three ways:
1. de-ethylation to the amine, 2. dechlorination to the hydroxide, or 3. nitrile oxidation to the amide (SD 20196) This may undergo de-ethylation to the amine (SD 33104) or de-amination to the acid. Subsequent oxidation reactions of the acid result in the hydroxy acid (SD 31223) followed by the hydroxy amine acid of cyanazine (SD 31224), the terminal metabolite. Conjugates of the parent and des-ethyl cyanazine with glutathione are not shown.
a/ References: Beynon *et al*, 1972; Shell Toxicology Laboratory, 1972; Crayford & Hutson, 1972; Shell Chemical Company, 1985.

## B. ACUTE TOXICITY

### Summary:

Cyanazine and its formulations were not acutely toxic to the rabbit by dermal exposure, at ≥2,000 mg/kg. There was only mild dermal and eye irritation resulting from cyanazine dosing in the rabbit and no dermal sensitization in the guinea pig. Acute oral toxicity studies in the rat gave $LD_{50}$ values of 835 (male) and 369 (female) mg/kg. By inhalation, cyanazine dust had a $LC_{50}$>906 mg/m$^3$ ($LD_{50}$>152 mg/kg) after a 4-hour exposure, with an estimated NOEL of 1.6 mg/kg. Data describing the acute toxicity of metabolites are limited; two major metabolites were considerably less toxic to the rat orally, having $LD_{50}$ values ≥4-fold that of the parent. Bladex®4L and 90DF had toxicities which were similar to cyanazine by oral and dermal routes in rat and rabbit, respectively. The acute toxicity of technical and formulated cyanazine is summarized in Table 2.

### Systemic Effects

Rats (Sprague Dawley CD) dosed with technical cyanazine by gavage at ≤1000 mg/kg showed clinical signs only at 1000 mg/kg (WIL Research Laboratories Inc., 1979a). These included depression, ataxia, depressed righting and placement reflexes, within one hour of dosing, followed by pale extremities, labored respiration, epistaxis and death. Necropsy (14 days) revealed reddened lungs and/or mottled organs.

Rats (Sprague Dawley) receiving single oral doses of Bladex® 4L at ≤1143 mg/kg (Stillmeadow Inc., 1979a) showed clinical signs, at ≥366 mg/kg. These included those noted above for technical, plus diarrhea, piloerection, salivation, polyuria, hematuria, constricted pupils, mucoid diarrhea, tremors, lacrimation, chromodacryorrhea, aggression, ptosis, melanuria, dilated pupils. Similar effects as for the technical were observed at necropsy, plus discoloration of the mesenteric lymph nodes, salivary gland, thymus and pancreas. The acute oral NOEL for Bladex® 4L was 206 mg/kg. Rats dosed by gavage with Bladex® 90DF at 200 to 500 mg/kg, displayed similar signs to those dosed with 4L (Haskell Laboratory, 1988a). Necropsy of survivors showed small, soft testes, the number affected increasing with dose.

Dermal dosing of the New Zealand White rabbit with cyanazine technical at 2,000 mg/kg for 24 hr. caused no mortality or remarkable signs (WIL Research Laboratories Inc., 1979b). Likewise, Bladex 4L at 2,300 mg/kg (2.0 ml/kg) and Bladex® 90DF at 2,000 mg/kg caused no remarkable signs (Stillmeadow Inc., 1979b; Haskell Laboratory, 1988b).

Charles River rats exposed to cyanazine (technical) dust by inhalation for 4 hours at 2.46 mg/L, equivalent to 413 mg/kg, exhibited the following signs: ptosis, enophthalmus, clear nasal discharge, salivation, diuresis and rhinitis for up to 18 hours. No mortality occurred during the next 14 days (Industrial Bio-Test Laboratories, 1976). Rats (Fischer 344) exposed (whole body) to cyanazine dust, at 0, 95, 280 and 906 mg/m$^3$ for 4 hours, showed no mortality (Evancheck *et al.*, 1983). Clinical signs included repeated mastication, rubbing of mouth with forepaws, head nodding, lacrimation, red/yellow material around eyes, tiptoe gait, hyperventilation, piloerection and hypoactivity, at all doses, to varying degrees. Recovery was dose-related, occurring after 3, 4 and 6 days at 95, 280 and 906 mg/m,$^3$ respectively. Reduced ($p<0.05$) weight gain (5-10%) was reported for high dose males (at 0-7 days) and for mid- and high-dose females (at 0-14 days). Testicular atrophy was found in mid- and high-dose rats. Since toxic effects were noted at all doses, the LOEL was the lowest dose of 95 mg/m,$^3$ or 15.9 mg/kg (U.S. EPA, 1988). An estimated NOEL of 1.6 mg/kg was obtained by dividing the LOEL by an uncertainty factor of 10.

## Metabolites

Two major cyanazine metabolites were tested for acute oral toxicity in rats, Carworth Farm E strain (Walker *et al*, 1974). One of them (SD 31223, Fig. 1), the hydroxy carboxylic acid (2-(1-carboxyl-1-methylethylamino)-4-ethylamino-6-hydroxy-1,3,5-triazine) had an $LD_{50}$ of 789 mg/kg, with clinical signs similar to those for cyanazine. Cyanazine administered in the same solvent, 3% dimethyl sulfoxide, had a $LD_{50}$ of 182 mg/kg. The other metabolite (SD 31224, Fig. 1) was desethyl hydroxy carboxylate (2-amino-4-(1-carboxy-1-methylethylamino)-6-hydroxy-1,3,5-triazine), with a $LD_{50}$ >2000 mg/kg.

**Table 2 Acute Toxicity of Cyanazine.**

| Route/Species | Sex | Dosage/Effect | Reference[a] |
|---|---|---|---|
| | | **TECHNICAL** | |
| **Oral $LD_{50}$** | | | |
| Rat | M | 835 (481-1143) mg/kg | 1 |
| | F | 369 (274-449) mg/kg | 1 |
| **Dermal $LD_{50}$** | | | |
| Rabbit | M/F | >2000 mg/kg | 2 |
| **Inhalation $LC_{50}$** | | | |
| Rat | M/F | >2.46 mg/L; >413 mg/kg[b] | 3 |
| Rat | | >0.906 mg/L; >152 mg/kg[b] | 4 |
| **Skin Irritation**:rabbit | M/F | none | 5 |
| **Eye Irritation**:rabbit | M/F | mild | 6 |
| **Skin Sensitization**:guinea pig | M/F | none | 7 |
| | | **BLADEX® 90DF (90% cyanazine)** | |
| **Oral $LD_{50}$** | | | |
| Rat | M | 313 (235-390) mg/kg | 8 |
| | F | 238 mg/kg | 8 |
| **Dermal $LD_{50}$** | | | |
| Rabbit | M/F | >2000 mg/kg | 9 |
| | | **BLADEX® 4L (43% cyanazine)** | |
| **Oral $LD_{50}$** | | | |
| Rat | M | 510 (357-729) mg/kg | 10 |
| | F | 473 (346-648) mg/kg | 10 |
| **Dermal $LD_{50}$** | | | |
| Rabbit | M/F | >2300 mg/kg | 11 |
| **Skin Irritation:**rabbit | M/F | mild | 12 |
| **Eye Irritation:**rabbit | --- | mild | 13 |
| **Skin Sensitization:**guinea pig | M/F | none | 14 |

a/ (1) WIL Research Laboratories Inc., 1979a. (2) WIL Research Laboratories Inc., 1979b. (3) Industrial Bio-Test Laboratories, Inc. 1976. (4) Evanchek *et al.* 1983. (5) WIL Research Laboratories Inc., 1979c.
(6) WIL Research Laboratories Inc., 1979d. (7) WIL Research Laboratories Inc., 1979e. (8) Haskell Laboratory, 1988a. (9) Haskell Laboratory, 1988b. (10) Stillmeadow Inc., 1979a (11) Stillmeadow Inc., 1979b. (12) Stillmeadow Inc., 1979c. (13) Stillmeadow Inc., 1979d.(14) Stillmeadow Inc., 1979e.
b/ based on a default inhalation rate of 0.175 L/min. for a 250 g rat (U.S. EPA, 1988).

## C. SUBCHRONIC TOXICITY

### Summary:

All of the subchronic studies which have been submitted to DPR are considered unacceptable. It is therefore difficult to determine the subchronic toxicological consequences of cyanazine administration to the rat, mouse and rabbit. The major, consistent dose-related effect of cyanazine was loss of body weight. In the dietary studies there was a concomitant reduction in food intake in the rat, but not in the mouse. In dermal (rabbit) and inhalation (rat) studies, the body weight reduction did not appear to be consistently accompanied by a loss of appetite. The subchronic studies using cyanazine are summarized in Table 4.

### Dietary-Mouse

A summary of a subchronic dietary study in mice was submitted (Shell Chemical Company, 1980). Technical cyanazine (purity not specified) was administered in the diet to CD mice (12/sex/group, except control group, 24/sex) at 0, 10, 50, 500, 1000, and 1500 ppm (0, 1.5, 7.5, 75, 150, and 225 mg/kg/day[1]) for 13 weeks. No changes in general health or behavior of mice were observed which were compound-related. No quantitative data were provided, so that the severity of the effects reported and possible toxicological significance of the following findings cannot be assessed: significant ($p<0.05$) reductions in body weight gain were reported in both sexes at $\geq$500 ppm, throughout the experiment, without a significant reduction in food intake, along with significant ($p<0.05$) increases in relative liver weight. Alterations in clinical chemistry parameters were inconsistent and not treatment-related. It was concluded that the NOEL was 50 ppm, equivalent to 7.5 mg/kg/day, based on reduced body weight gain at 500 ppm.

### Dietary-Rat

A summary of a subchronic dietary rat study was also submitted (Shell Chemical Company, 1968). Carworth Farm 'E' strain rats (12/sex/group, except control group, 36/sex) were fed technical cyanazine (>97% purity) at 0, 1.5, 3, 6, 12, 25, 50, or 100 ppm (0, 0.15, 0.3, 0.6, 1.2, 2.5, 5, and 10 mg/kg/day[1]) for 13 weeks. No quantitative data were provided, so that the severity and possible toxicological significance of the following cannot be assessed: reduction in final body weights in 100 ppm females and 3, 50, and 100 ppm males; decreased food consumption for 50 and 100 ppm males during the first 3 weeks; decrease in spleen and kidney weights in males at 50 and 100 ppm and of heart in 100 ppm males. Similar changes were present in the females at 100 ppm. Clinical chemistry effects were inconsistent and not dose-related. The NOEL was considered to be 25 ppm (2.5 mg/kg/day), based on reduced body weight and food consumption.

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1/ 1 ppm equivalent to 0.15 mg/kg/day for young mice and 0.10 mg/kg/day for young rats, assuming 5% of body weight per day (Lehman, 1959).

**Dermal-Rabbit**

Cyanazine, as a 4 lb/gallon water dispersible liquid (WDL) formulation, was applied daily (5 days/week) dermally, to groups of rabbits (5/sex/group) with intact or abraded skin for 19 days at 500 and 2000 mg/kg (Newell, 1970). Six hours after each application, the skin was washed with warm tap water. No significant adverse effects were seen in the treated groups. Body weight losses occurred during the first week for both sexes at 2000 mg/kg. For abraded skin, these losses were 15% (male) and 18% (female) and for intact skin, weight losses were 10% (male) and 19% (female). By the study end, males with abraded sites had not regained their original body weights. No other effects were reported on necropsy or histopathological examination. This study was not conducted according to FIFRA guidelines for subchronic toxicity testing. The report lacks details of the area of the application site, the type of covering and necropsy data and additionally, 5 rather than 10 rabbits/sex/ dose were employed.

**Inhalation-Rat**

A summary of a subchronic rat inhalation study was submitted (Industrial Bio-Test Laboratories, Inc., 1976b). Charles River (COBS) rats (12/sex/dose) were subjected to a dust of technical cyanazine (purity not stated) at 0, 3, 10 and 30 mg/m$^3$ (0, 0.88, 2.9 and 8.8 mg/kg/day[1]) for 7 hours/day, 5 days/week for 13 weeks. This study was considered invalid by U.S. EPA and therefore offers no useful conclusions.

**Dietary-Rat, Metabolites**

Summaries of studies conducted with the two major plant metabolites of cyanazine, fed to Carworth Farm E strain rats (12/sex/dose) for 13 weeks at 400, 1000 or 3000 ppm and at 3000/10,000 ppm (10,000 ppm for the last 5 weeks, 6/sex/dose), reported no adverse effects (Walker *et al.*, 1974; Shell Toxicology Laboratory, 1970b,c). These metabolites were hydroxy carboxylic acid (2-hydroxy-4-ethylamino-6-(1-methyl-1-carboxy-ethylamino)-*s*-triazine, SD 31223, Fig. 1), and N-deethylated hydroxy carboxylic acid (2-hydroxy-4-amino-6-(1-methyl-1-carboxy-ethylamino)-*s*-triazine, SD 31224, Fig. 1).

## D. CHRONIC TOXICITY AND ONCOGENICITY

**Summary:**

Chronic toxicity manifested itself as severe weight loss in all species tested *i.e.* rat, mouse and dog, usually in conjunction with reduced food intake. Many of the chronic effects *e.g.* chronic inanition, poor skin and fur condition and anemia, could thus have been a consequence of inadequate nutrition. In an early rat chronic toxicity study, an increased incidence of thyroid adenomas was reported in males at the highest dose, but without showing a clear dose-response relationship. In a later rat study, reduced body weight gain was reported for males. Other effects noted include increased alveolar macrophages, reduced creatinine kinase and atrophy of the seminiferous tubules. Cyanazine resulted in an increase in malignant mammary gland tumors in females. The incidence of adenocarcinomas, considered with carcinosarcomas, was elevated significantly at the 3 highest doses.

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1/ based on default inhalation rate of 0.175 L/min. for a 250g rat (U.S. EPA, 1988b).

**Dietary-Rat**

In two studies, cyanazine (>97% purity) was fed to Carworth Farm E strain rats (24/sex/group, treated; 48/sex/group, controls) for two years. In the first study (Walker and Thorpe, 1970a), doses were 0, 6, 12, 25, and 50 ppm (0, 0.3, 0.6, 1.25, and 2.5 mg/kg/day,[1]). An interim sacrifice at 44 weeks included 9 additional rats/sex/dose with 18/sex, controls. There was an increased incidence of thyroid C-cell tumors, particularly adenomas, in 50 ppm males compared with concurrent controls. This was not considered by investigators, nor by the original CDFA reviewer to represent a treatment effect. A re-evaluation of thyroid C-cell tumor incidence was undertaken, considering this study, plus the related 1973 study of Simpson & Dix (below), and the acceptable "combined" study (Bogdanffy, 1990). The re-evaluation likewise concluded that there was not a treatment effect on the incidence of thyroid tumors. The study of Walker & Thorpe (1970) was conducted well before current study guidelines and is thus unacceptable to DPR, principally because inadequate numbers of animals were employed for meaningful statistical evaluation. The only effect noted was lower body weight gain (usually <10% below controls) in 25 and 50 ppm females and 50 ppm males. The NOEL for this effect was 12 ppm, equivalent to 0.6 mg/kg/day.

The doses of cyanazine (>97% pure) used in the second study (Simpson and Dix, 1973) were 0, 1, 3, and 25 ppm (0, 0.05, 0.15, and 1.25 mg/kg/day,[1] respectively), fed to rats at 24/sex/dose. No adverse effects were reported in this study. The only noticeable effect was lower mean body weight (up to 10%) in the 25 ppm group compared to controls early in the study. Convulsions were reported, in a large proportion (42%) of both treated and untreated rats, three months after dosing and no cause was identified. The NOEL for the reduced body weight was 3 ppm, equivalent to 0.15 mg/kg/day. This was an unacceptable study because of many deficiencies with respect to current FIFRA guidelines. Major problems included the aforementioned clinical signs (indicating an animal health management problem), lack of diet analysis, poorly selected dose levels (in view of the equivocal thyroid effects in the 1970 study), inadequate group sizes for oncogenicity assessment, no clear indication of the extent of the pathology examinations, inadequate clinical chemistry protocol and a lack of individual data, except for histopathology findings in key organs.

1/ 1 ppm equivalent to 0.05 mg/kg/day for adult rats, or 5% body weight (Lehman, 1959).

Potential chronic and oncogenic effects in rats were comprehensively evaluated in a more recent study (Bogdanffy, 1990). Cyanazine (>96% purity) was given in the diet for 24 months at 0, 1, 5, 25, or 50 ppm to rats (Crl:CD®BR, 62/sex/group). An interim sacrifice (10/sex/dose) occurred at 1 year. Measured cyanazine intakes (over 2 years) were 0, 0.04, 0.20, 0.985, and 2.06 mg/kg/day in males and 0, 0.053, 0.259, 1.37, and 2.81 mg/kg/day in females. Chronic toxicity included reduced body weight and body weight gain in both sexes at 25 and 50 ppm, accompanied by slight decreases in mean daily food intake of 4% and 9%, respectively. Mean body weight gain of male and female rats was reduced at 50 ppm by 20% ($p<0.05$, Dunnett's test) and 16% ($p<0.05$), respectively at 1 yr. At 2 yrs., the corresponding reductions in body weight gain were 19% and 17%. At 25 ppm, body weight gain of males and females was reduced by 7% ($p<0.05$) and 13% ($p<0.05$), respectively at 1 yr. By 2 yrs., the corresponding reductions were 7% and 4%. With cyanazine administration, longevity was increased significantly only for males, at the highest dose tested (HDT), as follows: the number surviving to Day 721 was increased ($p<0.02$, Fisher's exact test); for females, the increased survival was not significant ($p=0.08$). The increased incidence of malignant mammary tumors in females (see below) may have compromised the increased lifespan which would have been anticipated from reduced food intake. For example, females at 50 ppm, but not at lower doses, had a shorter mean lifespan when malignant mammary tumors were present (540±106 days, n=6) than when they were not present (617±107 days, n=22), excluding animals killed by study design. Other chronic effects of dosing included an increased incidence of hyperreactivity in males at 25 ppm in 24/280 observations ($p<0.05$, Fisher's exact test) and 50 ppm in 34/329 observations ($p<0.01$), from 280 days onwards, with a positive dose-response ($p<0.01$, Peto's trend test). However, the occurrence of instances of hyperreactivity in untreated rats (12/259) makes this sign of doubtful toxicological relevance. Furthermore, the dose response relationship was discontinuous: although 1 ppm cyanazine caused a significant ($p<0.05$) increase in hyperreactivity (17/174), 5 ppm did not (17/273). In males, increased foamy alveolar macrophages were reported at 2 years ($p<0.05$, Peto's trend test), without being significantly elevated at any particular dose. Significant effects on organ weights were: decreased mean absolute kidney weight (16%, $p<0.05$ Dunnett's test) and increased mean relative testis weight (34%, $p<0.05$ Dunnett's test) at two years, in males at 50 ppm, without histopathological changes. Creatinine kinase, a marker enzyme for energy production in muscle, was significantly reduced ($p<0.05$) at 5, 25 and 50 ppm, in males, at two years, by 57%, 49% and 75%, respectively. However, because of the lack of a clear dose-response and as this enzyme was not affected at 3, 6, 12 or 18 months, the toxicological significance of inhibition is also uncertain.

Three other, chronic effects were reported by U.S. EPA (1994a) to be specific to cyanazine among the triazine herbicides: granulocyte hyperplasia of bone marrow in males, extramedullary hematopoiesis of the spleen in males and demyelination of the sciatic nerve in females. However, although there was an increased level above control at the highest dose tested, none of the effects was statistically significant (Fisher's exact test). Following the inclusion of interim sacrifice (1-year) data, the increase in extramedullary hematopoiesis in the spleen of males was significantly elevated ($p<0.05$) at the highest dose tested. Atrazine, a related triazine herbicide, also caused an increase in extramedullary hematopoiesis in the spleen of the female rat, in a 2-year study.

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1/ 1 ppm equivalent to 0.05 mg/kg/day for adult rats, or 5% body weight (Lehman, 1959).

Oncogenic effects were apparent as increased incidences of malignant mammary gland tumors in females at 5, 25, and 50 ppm (Table 3), with no increase in males. Rats at risk were considered as those animals which were autopsied after the first incidence (335 days) until the end of the study, but excluding the interim sacrifice (Table 3). There was a significantly increased incidence of tumors even at the NOEL for the principal non-oncogenic effects (body weight loss) of 5 ppm. It is therefore seems unlikely that these tumors resulted from a secondary effect of dosing, such as impaired homeostasis or increased cell death, which is often considered to result in tumors in chronic studies with high doses of xenobiotics. The increase in malignant tumors, which were principally adenocarcinomas, showed a dose-related positive trend ($p<0.001$, Peto's trend test). There was a lower rate of adenocarcinoma incidence at the highest dose (29%) compared with the next highest dose (35%). This could be associated with the large relative fall in mean body weight gain at 2 yrs., at the highest dose, of 17% *versus* only 4% at 25 ppm. It is well established that reduced food consumption and decreased body weight lead to reduced incidences of neoplastic lesions in untreated rodents *(e.g.* Tannenbaum, 1948; Gellatly, 1975; Conybeare, 1980). Similarly, in rats treated with specific carcinogens *e.g.* N-methyl-N-nitrosourea (Beth *et al.*, 1987; Chevalier *et al.*, 1993) or 7,12-dimethylbenz[*a]*anthracene (Klurfeld *et al.*, 1989; Kritchevsky *et al.*, 1989), dietary restriction resulted in a reduced incidence of mammary tumors compared with free-feeding rats. The additional cancers resulting from these carcinogens were abolished at 30% and 40% dietary restriction. It is therefore possible that the reduced food intake and fall in body weight compensated, to some extent, for the increased incidence of mammary tumors that would be anticipated at the HDT compared with the next lower dose. The figures showing tumor incidences were considered, by the study authors, to be significant only at 25 and 50 ppm when compared to the laboratory historical controls. These indicated that the concurrent control group level (10%) of malignant mammary tumors was below the Haskell Laboratory mean of 18% (87/476) from 1984-9 and at the low end of the range of 10 to 23%. There was, however, no significant increase in benign mammary tumors resulting from cyanazine administration. For combined (malignant plus benign) mammary tumors, the dose-related increase (Table 3) showed a positive trend ($p<0.01$, Peto's trend test). However, only the incidence in the 25 ppm group was significantly different from the concurrent control. There were no statistically significant, dose-related increases in other tumors or in total tumors (Table 3).

The NOEL for non-oncogenic effects was 5 ppm (0.20 mg/kg/day) based on reduced body weight gain in both sexes, at 25 ppm, of 7% and 13% at 1 yr. This study was accepted by DPR and demonstrated a possible adverse effect of oncogenicity.

**Table 3 Malignant and benign mammary tumors in female rats fed cyanazine for 2 years.[a]**

| Tumor type | Dose, ppm | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 5 | 25 | 50 |
| No. rats at risk[b] | 49 | 43 | 41 | 48 | 51 |
| **Malignant** | | | | | |
| Adenocarcinoma[c] | 5 | 6 | 12* | 17** | 15* |
| Carcinosarcoma | 0 | 0 | 0 | 1 | 0 |
| Combined Malignant | 5[+++] (10%) | 6 (14%) | 12* (29%) | 18** (38%) | 15* (29%) |
| **Benign** | | | | | |
| Adenoma | 4[d] | 4[e] | 3[e] | 5[f] | 2[e] |
| **Combined** | | | | | |
| Malignant + Benign | 9[++] (18%) | 9 (21%) | 14 (34%) | 20[*] (42%) | 16 (31%) |
| **Other Tumors** | | | | | |
| Fibrosarcoma | 0 | 0 | 1 | 0 | 1 |
| Fibroma | 1 | 0 | 1 | 0 | 0 |
| Fibroadenoma | 21[g] (43%) | 19 (44%) | 18[h] (44%) | 17[i] (35%) | 24[j] (47%) |
| Granuloma | 0 | 0 | 0 | 0 | 2 |
| **Total Tumors**[k] | 28 (57%) | 28 (65%) | 31 (76%) | 28 (58%) | 37 (73%) |

a/ data are from Bogdanffy, 1990.
b/ incidences are expressed as the number of animals bearing tumors per animals at risk, defined as rats subjected to necropsy after at least 335 days, excluding interim sacrifice.
c/ rats with multiple tumors account for the following proportions: 2/5 (control), 2/6 (1 ppm), 5/12 (5 ppm), 4/17 (25 ppm) and 7/15 (50 ppm); the others are single tumors.
d/ includes 2 rats which also had fibroadenoma.
e/ includes 1 rat which also had adenocarcinoma.
f/ includes 1 rat which also had adenocarcinoma, 2 rats fibroadenoma and 2 rats had both.
g/ includes 1 rat which also had adenocarcinoma and 2 adenoma.
h/ includes 3 rats which also had adenocarcinoma.
l/ includes 5 rats which also had adenocarcinoma, 2 adenoma and 2 rats had both.
j/ includes 5 rats which also had adenocarcinoma, 1 fibrocarcinoma and 1 sarcoma.
k/ includes all rats bearing 1 or more tumors, listed above.
++ significant trend ($p<0.01$) based on dose-weighted chi-square test (Peto *et al.*, 1980).
+++ significant trend ($p<0.001$) based on dose-weighted chi-square test (Peto *et al.*, 1980).
* significantly different from control ($p < 0.05$) based on Fisher's Exact test.
** significantly different from control ($p < 0.01$) based on Fisher's Exact test

**Dietary-Mouse**

Cyanazine technical (98% purity) was fed to CD mice (50/sex/level; 100/sex, controls) at dietary concentrations of 0, 10, 25, 250, or 1000 ppm (0, 1.5, 3.75, 37.5, 150 mg/kg/day[1]) in a two year feeding study (Gellatly, 1981). Mean body weights were depressed significantly, in both sexes at all treatment levels, and were dose-related. Reductions of 9% (M), 11% (F, $p<0.01$) were reported at 1000 ppm, within a week of study initiation; at termination, body weights were reduced by 25% (M) and 32% (F, $p<0.01$) at 1000 ppm, and by 11% (M) and 15% (F, $p<0.01$) at 10 ppm. A corresponding reduction in mean food intake was reported *e.g.* over weeks 1 to 52. Food intake was reduced by 7% in males at 250 ppm ($p<0.01$) and by 10% at 1000 ppm ($p<0.01$); for females, the reductions were 7% ($p<0.05$) and 5% ($p<0.05$), respectively. Reduced food intake probably contributed to the lower body weights of dosed mice, but there were also significant reductions in food conversion efficiency for both sexes at 250 and 1,000 ppm. This was measured as the mean body weight gain per unit weight of food consumed. These reductions were apparent during the first week ($p<0.01$), week 1 to 13 ($p<0.05$) and also at the conclusion of the study ($p<0.01$). Lower food intake resulted in symptoms of poor skin condition, fur loss, reduced blood glucose, anemia and adrenal cortical lipid depletion, at 250 and 1000 ppm. Also observed at the conclusion of the study were increased cases of cutaneous ulceration, myocarditis in males at 1000 ppm ($p<0.001$), myocardial fibrosis in females, both basal and non-basal, at 250 ppm ($p<0.05$) and 1000 ppm ($p<0.001$), focal renal cortical tubular dilation in females at 250 ppm ($p<0.05$) and 1000 ppm ($p<0.05$) and epithelial vacuolation in females at 250 ppm ($p<0.05$) and 1000 ppm ($p<0.001$). There were no oncogenic effects from treatment. Because of the significant reduction in body weight at all doses tested, 10 ppm (1.5 mg/kg/day) was the LOEL. An estimated NOEL of 0.15 mg/kg/day was established using the default approach of dividing the LOEL by an uncertainty factor of 10. This study was acceptable to DPR.

**Oral-Dog**

Cyanazine (>97% purity) was administered daily by capsule to beagle dogs (4/sex/treatment group and 6/sex/control group) at 0, 0.625, 1.25 or 5 mg/kg/day for two years (Walker and Thorpe, 1970b). Toxic effects related to treatment occurred at the highest dose level. Dogs in this group frequently vomited, within 1 hour of dosing, and showed reduced mean body weight, absolute liver weight and total serum protein, throughout the test. The mean body weight was reduced, at the highest dose, for the duration of the test: even at 4 weeks, males ($p<0.05$) and females ($p<0.01$) had reduced body weights. At 1.25 mg/kg/day females had body weight reductions of 7% (4 weeks) to 17% (104 weeks), but only at 12 weeks was the (14%) decrease significantly different from control ($p<0.01$). Food consumption data were not provided. There were no consistent hematology or clinical chemistry findings. The NOEL was 0.625 mg/kg/day, based on reduced mean body weight at the two higher doses. This study was unacceptable to DPR due to inadequate pathology and lack of individual data.

**Dietary-Dog**

In a 1 year dietary study, cyanazine (98% purity) was administered to beagle dogs (6/sex/level) in the feed at 0, 10, 25, 100, or 200 ppm (males: 0, 0.27, 0.68, 3.20 or 6.11 mg/kg/day;[2] females: 0, 0.28, 0.72, 3.02, 6.39 mg/kg/day,[2] Dickie, 1986). Mean body

1/ 1 ppm equivalent to 0.15 mg/kg/day for adult mice (Lehman, 1959).
2/ reported dosages were calculated from dog body weight and food consumption data.

weight and body weight gain for both sexes were depressed at 100 and 200 ppm. At 13 weeks, mean body weight was reduced, at 100 and at 200 ppm, by 15% (M) and 16% and 20% (F). At termination, the decrements were 12% (M) and 25% (F) for both 100 and 200 ppm. Mean food consumption was also depressed, particularly in the 200 ppm group. For males, a significant reduction in food intake was reported only for the first week, of 28% at 200 ppm ($p<0.05$) and 18% at 100 ppm (n.s.). The food intake of females was reduced significantly for 3 of the first 6 weeks and subsequently, only at weeks 39 and 43. During these weeks, food intake was reduced by 10% to 28% at 100 ppm and 16% to 28% at 200 ppm. Thus, the reduction in body weight may not have been caused entirely by reduced food intake. Absolute organ weights were depressed by 10% to 30% for heart, lung, and spleen, and were increased (20%) for adrenals, in both sexes. Absolute liver weight was reduced only in females. None of the absolute organ weights were statistically different from control but relative organ weights (heart, lung, liver, adrenals, and kidneys) were increased significantly by 19% to 43%, in one or both sexes, largely because of the reduced body weight. All were elevated in the 200 ppm group ($p<0.05$) whereas at 100 ppm, significant increases were limited to lung (19%) and kidney (20%), in females.

**Table 4 Summary of subchronic and chronic effects caused by cyanazine.**

| Species | Route | Effect | LOEL (mg/kg/day) | NOEL (mg/kg/day) | Ref.[a] |
|---|---|---|---|---|---|
| SUBCHRONIC TOXICITY | | | | | |
| Mouse 13-wk. | oral diet | decreased body weight<br>clinical chemistry changes | 75 | 7.5 | 1 |
| Rat 13-wk. | oral diet | decreased body wt., food consumption | 5 | 2.5 | 2 |
| CHRONIC TOXICITY & ONCOGENICITY | | | | | |
| Dog 2-yr. | oral capsule | reductions in body wt. gain, absolute liver wt. and total serum protein | 1.25 | 0.625 | 3 |
| Dog 1-yr. | oral diet | decreased body wt.<br>increased relative organ wts. | 3.0 | 0.70 | 4[b] |
| Mouse 2-yr. | oral diet | decreased body wt.; renal cortex tubular diln.<br>no oncogenicity at $\leq$HDT (150 mg/kg/day) | 1.5 | 0.15[c] | 5[b] |
| Rat 2-yr. | oral diet | decreased body wt. | 1.25 | 0.15 | 6 |
| Rat 2-yr. | oral diet | males: hyperreactivity, decreased body wt.<br>females: malignant mammary gland tumors | 1.0<br>- | 0.2<br>- | 7[b] |

a/ References: 1. Shell Chemical Company, 1980; 2. Shell Chemical Company, 1968; 3. Walker & Thorpe, 1970b; 4. Dickie, 1986; 5. Gellatly, 1981; 6. Simpson & Dix, 1973; 7. Bogdanffy, 1990.

b/ study acceptable to DPR, according to FIFRA guidelines.

c/ estimated NOEL

Sporadic increases in platelet count and inorganic phosphorus with reduced total serum protein, albumin and calcium were dose-related but not always statistically significant. Neither the organ weight changes nor the hematological/clinical chemistry changes were associated with any histopathological changes. The NOEL from this study was 25 ppm (0.7 mg/kg/day) based on decreased body weight and body weight gain along with increased relative lung and kidney weights in both sexes. This study was acceptable to DPR.

## E. GENOTOXICITY

### Summary

Cyanazine caused genotoxic effects in 4 types of assay using mammalian cells, *in vitro*: clastogenic activity in chromosomes of human lymphocytes; gene mutations in mouse lymphoma cells, with and without metabolic activation; unscheduled DNA synthesis in rat primary hepatocytes; transformation in a mouse cell line, although only without metabolic activation. In non-mammalian cells, cyanazine caused an increased response in the *Drosophila* dominant lethal assay, following dosing *in vivo*, as well as a variety of chromosome aberrations in plant cells. However, the *in vivo* evidence suggests that cyanazine may not be genotoxic in mammals. For example, in rat hepatocytes and spermatocytes, cyanazine did not cause UDS after *in vivo* administration. Genotoxicity tests with cyanazine are summarized in Table 5.

### Gene Mutation

Cyanazine (96% purity) was tested at 10 to 5000 $\mu$g/plate on *Salmonella typhimurium* strains TA1535, TA97a, TA98 and TA100 with and without S-9 rat liver homogenate activation and found negative (Arce, 1987). Technical grade cyanazine (purity unstated) was mutagenic (2-3 fold increase above background) to mouse lymphoma cells L5178Y in the presence or absence of S-9 rat liver homogenate activation (Jannasch and Sawin, 1986). Mutagenesis was concentration-dependent, up to the solubility limit of 0.5 mg/ml, and was repeated in both trials. The mutagenic potential of cyanazine (96% purity) was also evaluated in the CHO/HPRT assay in the presence or absence of a S-9 rat liver homogenate activation system and was negative (Rickard, 1987). All of these reports were acceptable to DPR, according to TSCA guidelines.

In a literature report (Venkat *et al.*, 1995), the PQ37 strain of *E. coli* was used to measure the genotoxic activity of 47 pesticides in a SOS microplate assay, both in DMSO (10%) and also in sodium taurocholate solution. The latter was used to simulate conditions in the small intestine. Mutagenicity was assessed by measuring the potency of induction of the gene for β-galactosidase and comparing this with the activity of the standard mutagen, 4-nitroquinoline oxide. Cyanazine was the most potent mutagen in the DMSO solution, having approximately 50% of the activity of the standard, but it ranked only 37$^{th.}$ when assayed in the taurocholate solution.

### Structural Chromosomal Aberrations

Cyanazine (purity unstated) did not induce chromosomal aberrations in bone marrow cells of CF1 mice following two daily oral doses of 50 or 100 mg/kg (8/sex/dose) (Dean and Senner, 1974). This study included a positive control (cyclophosphamide). However, it was considered unacceptable by DPR due to lack of individual animal data, limited doses and lack of dose justification.

Cyanazine (purity unstated) did not cause dominant lethal mutations in male CF1 mice following single oral doses of 0, 80, 160, or 320 mg/kg (Dean, 1974). However the study was unacceptable to DPR due to lack of a positive control and individual data.

In a well documented assay, cyanazine (96% purity) was not clastogenic to human lymphocytes *in vitro*, at 12.5 to 350 $\mu$g/ml, with and without S-9 rat liver homogenate activation (Stahl, 1987). This report was acceptable to DPR, according to TSCA guidelines. In a literature publication however, cyanazine *did* cause clastogenic activity in human lymphocytes *in vitro*, at 1 $\mu$g/ml, but not at two lower concentrations (Roloff *et al.*, 1992). Positive and negative controls responded appropriately in this study.

Cyanazine genotoxicity in a variety of non-mammalian cells has been reported in the literature. In *Drosophila melanogaster*, cyanazine supplied in the diet at 0.01% caused an increased response in the dominant lethal assay and reduced egg hatch (Murnik & Nash, 1977). However, the authors stated that, because cyanazine had not been shown to be a strong mutagen, this dominant lethal effect was due to physiological factors, such as sperm toxicity. In barley shoot tips, cyanazine induced chromosome aberrations, including dicentric bridges ($p<0.01$) and multipolar anaphases, correlated with seedling injury (Kahlon, 1980). The percentage of cells with chromosome aberrations increased 2-3 fold above control at 250 to 1000 ppm. Similarly, in root tips of broad beans and *Tradescantia*, chromosomal aberrations were found following spraying of the plants with cyanazine at 200 to 600 ppm (Ahmed & Grant, 1972). The types of abnormalities were similar to those caused by the standard mutagen, ethyl methane sulfonate, and included those aberrations seen in barley (Kahlon, 1980). Cyanazine was toxic to both plant species at the rates used and it is possible that some of this plant injury resulted directly from genetic toxicity.

**Other Genotoxic Effects**

In the host-mediated assay (male mice) cyanazine had no effect on the frequency of mitotic gene conversion in a double auxotrophic strain of *Saccharomyces cerevisiae* (Dean *et al*, 1974). The doses used were 160 or 320 mg/kg, *in vivo*, and $\leq$4 mg/ml for 4 or 24 hours, *in vitro*. However this report was judged unacceptable by DPR due to incomplete details of individual plate data or cyanazine purity.

Cyanazine (96% purity) induced unscheduled DNA synthesis (UDS) *in vitro* in primary hepatocytes of the rat (Crl:CD® BR, male) in a concentration-dependent manner, starting at the lowest concentration tested 1 µM, up to 1,450 µM (Vincent, 1987). There was a parallel concentration-dependent increase in the activity of lactate dehydrogenase in the medium, indicating cytotoxicity. This report was acceptable to DPR. However, cyanazine did not induce DNA unwinding or strand breaks in hepatocytes when it was administered *in vivo* to rats by intraperitoneal injection, in a published report (Grilli *et al.,* 1991). Similarly, cyanazine (97.3-98.6% purity) did not cause UDS in rat spermatocytes, *in vivo*, following dosing by oral gavage at 125 to 500 mg/kg/day for 5 days (Bentley, 1993). Although the highest dose caused mortality, suggesting that a high enough dose may have been achieved, and two positive control compounds caused UDS, the absence of analytical data for the dosing solutions precluded acceptance by DPR.

The mouse BALB/c 3T3 cell transformation assay was used to study genotoxic and cytotoxic properties of cyanazine (Perocco *et al.,* 1993). In the absence of a rat liver S-9 homogenate, cyanazine was cytotoxic, concentration-dependently, from 10 to 100 $\mu$g/ml. Cyanazine also caused cell transformation at 50 $\mu$g/ml, the only concentration tested ($p<0.01$, Mann-Whitney unpaired test). Cyanazine which had been exposed to the S-9 rat liver homogenate was ineffective in both the cytotoxicity and cell transformation assays.

**Table 5 Summary of genotoxicity tests with cyanazine.**

| Test | Route | Results | Reference |
|---|---|---|---|
| | **Gene Mutation** | | |
| bacteria, *S. typhimurium* | *in vitro* | - | Arce,1987[a] |
| bacteria, *E. Coli* | *in vitro* | + | Venkat *et al.*, 1995[b] |
| mouse lymphoma | *in vitro* | + | Jannasch & Sawin, 1986[a] |
| CHO cells | *in vitro* | - | Rickard, 1987[a] |
| | **Structural Chromosomal Aberration** | | |
| mouse bone marrow | *in vivo* | - | Dean & Senner, 1974 |
| *S. cerevisiae* gene conversion | *in vivo* | - | Dean *et al.,* 1974 |
| mouse dominant lethal | *in vivo* | - | Dean, 1974 |
| *Drosophila* dominant lethal | *in vivo* | + | Murnik & Nash, 1977[b] |
| human lymphocytes | *in vitro* | - | Stahl, 1987[a] |
| human lymphocytes | *in vitro* | + | Roloff *et al.,* 1992[b] |
| barley shoot tips | *in vivo* | + | Kahlon, 1980[b] |
| broad bean roots | *in vivo* | + | Ahmed & Grant, 1972[b] |
| Tradescantia roots | *in vivo* | + | Ahmed & Grant, 1972[b] |
| | **Other Genotoxic Effects** | | |
| rat hepatocytes, UDS | *in vitro* | + | Vincent, 1987[a] |
| rat hepatocytes, UDS | *in vivo* | - | Grilli *et al.,* 1991[b] |
| rat spermatocytes, UDS | *in vivo* | - | Bentley, 1993 |
| BALB/c-3T3 cell, cytotoxicity | *in vitro* w/ S-9 | - | Perocco *et al.,* 1993[b] |
| and transformation | w/o S-9 | + | Perocco *et al.,* 1993[b] |

a/ study acceptable to DPR, according to TSCA guidelines.
b/ literature publication

## F. REPRODUCTIVE TOXICITY

### Summary

The toxicity of cyanazine in a 2-generation rat reproduction study included reduced food intake and body weight in adults. Pup body weight and food intake were also lowered, in a dose-dependent manner, and pup viability (survival) was reduced. In pups the effects on body weight and viability occurred at lower doses than did reduced body weight in adults, indicating a possible adverse effect on reproduction. A summary of the reproductive toxicity studies is presented in Table 11.

### Dietary-Rat

The reproductive effects of cyanazine in rats are reported in two studies. In the first study, cyanazine (>97% purity) was tested in Long Evans rats at 0, 3, 9, 27 and 81 ppm in the diet (10 males and 20 females/dose level) over three generations (Hine, 1969). The report contained very limited data and showed slight reduction in terminal body weights at the 81 ppm level of 5-13% (M) and 5-10% (F), in all generations. The study was unacceptable by DPR due to inadequate study design, lack of diet analysis and food consumption data and limited necropsy and weight data. No NOEL was derived from this study due to limited data.

The second investigation was a 2-generation reproduction study of cyanazine (Nemec, 1987) in Sprague Dawley COBS CD rats (28 rats/sex/dose level). These were fed cyanazine (98% pure) in the diet at 0, 25, 75, 150 or 250 ppm over two generations, commencing 72 days prior to the first pairing. These concentrations are approximately equivalent to 1.9, 5.6, 11.2 and 19.5 mg/kg/day, respectively, using the means of the reported chemical consumption of the $F_0$ during the $F_1$ and $F_2$ gestation periods. Decreases in body weight gain and food intake during the $F_0$, $F_1$ and $F_2$ generations were reported at the 75, 150 and 250 ppm levels (Table 6). The NOEL for decreased body weight in adults ($F_o$) was determined to be 150 ppm, equivalent to 11.2 mg/kg/day, based on a 10% fall in body weight increase in males from week 6 to 30 at 250 ppm ($p<0.01$, Dunnett's test). At 150 ppm, there was a statistically significant fall in body weight of 5% ($p<0.01$), but this was not considered biologically significant. Body weight gain in $F_{1a}$ pups was decreased by 18% ($p<0.01$) from day 4 to day 21 at 150 ppm, but by only 10% at 75 ppm. Subsequent generations were not clearly affected by cyanazine dosing. Because body weight was reduced significantly by over 10%, for most of the dosing period, at both 150 ppm and 250 ppm but not at 75 ppm, the latter value was selected as the NOEL for this effect in pups. Reduced pup viability occurred (Table 7) on days 14 and 21 in $F_{1a}$ pups at 250 ppm ($p<0.01$) and on days 1 and 4 in the $F_{2a}$ pups at 150 ppm ($p<0.01$) and at 250 ppm ($p<0.05$). Five out of 22 dams had total litter loss between day 11 and 19 at 250 ppm in $F_{1a}$. The NOEL for reduced pup viability was 75 ppm, equivalent to 5.6 mg/kg/day. The reproductive parameters (male and female fertility, gestation length and parturition) were not affected by cyanazine. The study was acceptable to DPR.

**Table 6 Weight change in parents ($F_0$) and successive litters (a, b) of 2 generations ($F_1$, $F_2$) of Sprague Dawley rats receiving dietary cyanazine.[a]**

| Generation | Dose, ppm[b] | | | |
|---|---|---|---|---|
| | 25 | 75 | 150 | 250 |
| $F_0$ - Male | | | | |
| start (week 9)[c] | +6%**[d] | 0% | -6%* | -5%*[e] |
| end (week 30) | +3% | -2% | -11%** | -15%** |
| $F_0$ - Female | | | | |
| start (week 10) | +2% | -1% | -6%* | -6%**[f] |
| end (week 30) | -2% | -4% | -10%** | -13%** |
| $F_{1a}$ | | | | |
| start (day 4) | 0% | -3% | -12%** | -12%** |
| end (day 21) | -5% | -13%* | -30%** | -26%*[g] |
| $F_{1b}$ | | | | |
| start (day 4) | -2% | 0% | -9% | -3% |
| end (day 21) | 0% | 0% | -9% (21) | -18%** |
| $F_{2a}$ | | | | |
| start (day 4) | -12%* | -9%* | -13%*[g] | -15%**[g] |
| end (day 21) | -6% | -4% | -10%* | -17%** |
| $F_{2b}$ | | | | |
| start (day 7) | -4% | -13%** | -13%** | -14%**[h] |
| end (day 21) | -4% | -8% | -17%** | -22%** |

a/ data are from Nemec, 1987.
b/ mean dosages, based on the measured $F_0$ cyanazine consumption during the $F_1$ and $F_2$ gestation periods, were 1.9, 5.6, 11.2 and 19.5 mg/kg/day, at 25, 75, 150 and 250 ppm, respectively.
c/ weeks (or days) for the start or end of continuous weight loss period, except where stated below.
d/ mean percentage weight change relative to control.
e/ week 6, onwards, instead of week 9.
f/ week 5, onwards, instead of week 10.
g/ reduced pup viability, $p<0.05$ (Dunnett's test).
h/ day 1, onwards, instead of day 7.
* body weight change significantly different from control, $p<0.05$ (Dunnett's test).
** body weight change significantly different from control, $p<0.01$ (Dunnett's test).

**Table 7 Pup viability in the rat following dietary cyanazine administration.[a]**

| Mean pup viability index, %[b] | Dose, ppm[c] | | | | |
|---|---|---|---|---|---|
| | 0 | 25 | 75 | 150 | 250 |
| $F_{1a}$ | | | | | |
| DAY 0 | 99 | 98 | 98 | 98 | 98 |
| DAY 1 | 100 | 98 | 100 | 98 | 95 |
| DAY 4 | 99 | 98 | 99 | 95 | 93 |
| DAY 14 | 100 | 99 | 98 | 99 | 76** |
| DAY 21 | 100 | 99 | 93 | 90 | 75** |
| $F_{2a}$ | | | | | |
| DAY 0 | 97 | 99 | 98 | 94 | 98 |
| DAY 1 | 99 | 100 | 98 | 83** | 86* |
| DAY 4 | 99 | 99 | 97 | 82** | 84* |
| DAY 14 | 98 | 100 | 100 | 100 | 96 |
| DAY 21 | 98 | 100 | 100 | 100 | 91 |

a/ data are from Nemec, 1987.
b/ pup viability index is the number of viable pups per litter on a specific day divided by the number of viable pups per litter on day 1 or day 4 (after culling) x 100.
c/ mean of the measured $F_0$ consumption during the $F_1$ and $F_2$ gestation periods
* significantly different from control, $p<0.05$ (Dunnett's test).
** significantly different from control, $p<0.01$ (Dunnett's test).

## G. DEVELOPMENTAL TOXICITY

### Summary

Developmental toxicity of cyanazine has been described in 3 oral gavage studies in the rat and in oral gavage and dermal exposure studies in the rabbit. Fetal malformations (microphthalmia and anophthalmia) were noted in two oral rat studies and in a single litter in the rabbit, after oral gavage. Maternal toxicity was noted in all studies in the form of weight loss and reduced food intake. Quantitatively, these effects occurred at similar dose levels as the fetal effects, in the rat and rabbit. A summary of the developmental toxicity studies is presented in Table 11.

### Gavage-Rat

Cyanazine (98.5% purity) was given by oral gavage to Charles River SD-CD rats at 0, 1, 3, or 30 mg/kg/day (30/group) on gestation days 6-15 (Lu, 1983). The only toxic manifestations of this treatment were a significantly lower ($p<0.05$) mean maternal weight gain (80% of control) during the treatment period and lower ($p<0.05$) mean absolute maternal weight gain (weight gain during the gestation period minus gravid uterine weight), 82% of control, for dams in the 30/mg/kg/day group. All other parameters evaluated, including those of the fetus, were not

affected by cyanazine treatment. The NOEL for maternal toxicity (reduced weight gain) was 3 mg/kg/day, and the developmental NOEL was ≥30 mg/kg/day. This study was acceptable to DPR.

In another gavage study, groups of 30 mated female rats (Fischer 344) were given oral doses of cyanazine (98.5% purity) at 0, 1, 2.5, 10 or 25 mg/kg/day during gestation days 6-15 (Lu *et al*, 1981). A positive control group dosed with vitamin A was included. Toxic manifestations in the pregnant rats included lower body weight gains in the 10 and 25 mg/kg/day groups, on day 12. Mild clinical signs included transient incidences of vaginal discharge and irritated swelling of the footpad. Fetotoxic effects were manifested by a dose-related increase in the incidence of the skeletal variation of lumbar spur. This was statistically significant ($p<0.05$) only in the high dose group, where it was reported in 74% of the litters and 30% of the fetuses. Additionally, anophthalmia and microphthalmia were seen in this group (5 cases in 3 litters). It is likely that these were direct developmental effects rather than arising as a result of maternal toxicity. The reason is that, although the mean maternal weight gain was reduced at 25 mg/kg/day, during the 6 to 15 day period, consideration of individual data showed no correlation between the severity of symptoms of maternal toxicity and developmental malformations. For example, the dam giving rise to 3 of the 5 cases of eye malformations (in one litter) showed only slight clinical signs (transient footpad irritation) and a weight gain which was paradoxically much higher than the mean for that dose level. The other 2 dams showed weight gains during the 6-15 day period which were slightly below the group mean (13g and 15g *vs.* 18g). The individual with the lower weight gain demonstrated a transient vaginal discharge and the other dam had no clinical signs. A low incidence of diaphragmatic hernia was seen in all the cyanazine treated groups, in 5 to 15% of the litters, but was not dose-related. In a subsequent study (Lochry, 1985), it was concluded that this hernia is a genetic variation in the Fischer 344 rat and therefore has little toxicological significance. Thus, the maternal NOEL of 2.5 mg/kg/day was based on reduced body weight gain and the developmental NOEL of 10 mg/kg/day was based on eye malformations. The study was acceptable to DPR.

In a third study, groups of 70 mated female rats (Fischer 344) per dose were given technical cyanazine (purity not specified) by oral gavage at 0, 5, 25, or 75 mg/kg/day during gestation days 6-15 (Lochry, 1985). There was a dose-dependent decreased weight gain, totaling 73%, 23% and -45% of control weight gain (33g) for days 6-15 at 5, 25 and 75 mg/kg/day, all significant at $p<0.01$ (Dunnett's test). Food consumption comprised 92%, 78% and 68% of the mean control value, from day 6 to 15, at these three doses. The effects on these parameters persisted throughout the post-natal phase of lactation in the mid and high dose groups. Daily food consumption was significantly lower ($p<0.01$) on days 1 to 9 of lactation at 25 mg/kg/day and on days 1 to 21 (study end) of lactation at 75 mg//kg/day. Body weight changes were not significantly different between dose groups during the post-natal period. Increased incidences ($p<0.01$) of clinical signs (lacrimation and excess salivation in about 90% of animals, soft or liquid feces in about 50%) were observed between gestation days 6 to 25, at 25 and 75 mg/kg/day. At the high dose, during the same time period, the occurrence of more severe signs of ataxia, tip-toe walk, chromodacryorrhea, chromorhinorrhea, a thin dehydrated appearance, hyperpnea, and inflamed perineum, alopecia, arched back, red vaginal discharge and ptosis was also observed ($p<0.01$, for each clinical sign). The high dose was lethal to 13/70 (19%) of dams, usually after 2 or 3 dosages, and was associated with gastrointestinal and liver lesions. The NOEL for maternal toxicity was 5 mg/kg/day, based on decreased body weight gain and increased incidences of clinical signs at 25 and 75 mg/kg/day. Developmental effects included an increased number of fetuses and pups with micro- or anophthalmia, liver and diaphragmatic changes at 25 and 75 mg/kg/day (Table 8). The latter effect was distinct from the "diaphragmatic hernia" which was reported in Lu *et al.,* 1981 and

Lochry concluded that this "hernia" was the result of a genetic variation in the Fischer 344 rat rather than a true developmental malformation. Cyanazine also reduced mean litter weight; high dose litters weighed 75% of controls ($p<0.01$). The number of viable fetuses was also affected, being 2.6-fold higher in control *versus* high-dose litters ($p<0.01$). The number of resorptions increased at the high dose ($p<0.01$). Accordingly, the NOEL for developmental toxicity was 5 mg/kg/day. This study was acceptable to DPR.

The hypothesis that maternal toxicity was the cause of developmental toxicity (Lochry, 1985) was studied by examining the body weight gain and clinical signs of individual animals during day 8-12 of presumed gestation, which is when eye malformations are thought to occur in the Fischer 344 rat (Yoshitomi & Boorman, 1990). The proportions of rats which showed reduced maternal body weight gains or signs were the same, regardless of whether the offspring had malformations. Each of the 3 dams which had fetuses with these eye defects following Cesarian section showed body weight gains (instead of reductions), which were actually greater than the means for both 25 and 75 mg/kg/day. Two of these dams showed severe clinical signs and one had only slight signs, similar to the proportion of dams with signs in the dams which did not produce offspring with eye malformations. Similarly, of the 8 dosed dams undergoing natural delivery of pups with eye malformations, 3 showed severe and 5 had slight clinical signs. This strongly suggests that the production of offspring exhibiting eye malformations was not simply a function of maternal toxicity but was instead due to a direct developmental effect. The proportion of concurrent control offspring showing eye malformations was much higher than for the historical control data, provided by the registrants (Table 8). As a result of this, the number of eye malformations for fetuses and pups ($p<0.001$) and litters ($p<0.05$ or $p<0.001$) was statistically elevated above control only at the highest dose level.

**Table 8 Occurrence of microphthalmia or anophthalmia in litters of the Fischer 344 rat following maternal dosing with cyanazine by oral gavage.[a]**

| Eye Malformation | Dosage (mg/kg/day) 0[b,c] | 5 | 25[c] | 75 |
|---|---|---|---|---|
| **Microphthalmia** | 2/55 (3.6%) | 0/55 | 2/51 (3.9%) | 4/16[*] (25%) |
| **Anophthalmia** | 1/55 (1.8%) | 0/55 | 3/51 (5.9%) | 3/16[*] (19%) |
| **Combined** | 2/55 (3.6%) | 0/55 | 4/51 (7.8%) | 7/16[***] (44%) |

a/ data are from Lochry, 1985.
b/ historical control data showed 1/705 (0.14%) litters and 1/9183 (0.01%) fetuses or pups with microphthalmia. Concurrent controls were 2/55 (3.6%) litters and 2/583 (0.3%) for fetuses/pups.
c/ includes cases of (different) pups with anophthalmia and microphthalmia in the same litter.
* significantly different from control at $p<0.05$ (Fisher's exact test).
*** significantly different from control at $p<0.001$ (Fisher's exact test).

**Gavage-Rabbit**

Cyanazine (98% purity) was given by oral gavage to mated New Zealand rabbits (22/group) at 0, 1, 2, or 4 mg/kg/day on days 6-18 of gestation (Dix, 1982). The 1 mg/kg/day level produced no adverse effects on the dams or fetuses (Table 9). Slight maternal toxicity, manifested by reduced food consumption and weight gain was observed at 2 and 4 mg/kg/day. Decreased live litter size and increased resorptions were reported at the mid- and high dose. At 4 mg/kg/day there were increases in the number of dead fetuses/dam ($p<0.05$), post-implantation losses/dam ($p<0.01$) and an increased number of fetuses with a $13^{th.}$ rib or a $13^{th.}$ pair of ribs along with a concomitant decrease in the number of litters and fetuses with 12 pairs of ribs ($p<0.001$, Peto's trend test). There was also a decrease in the mean weight of live fetuses. All of these effects were also observed at 2 mg/kg/day, and although not statistically different from control, collectively, they indicate that fetal toxicity was probably occurring at this dosage, which is similar to that which causes maternal toxicity. Fetal malformations reported at 2 mg/kg/day included a case of one fetus lacking both a kidney and ureter (Table 9). At 4 mg/kg/day, multiple visceral malformations were noted in several fetuses from two dams: one produced a fetus with acephali and thoracoschisis, 3 fetuses with a domed cranium (two of which also had dilated brain ventricles), plus 3 fetuses which had flexed carpi (one of which also had a dilated renal pelvis). The second dam produced fetuses with microphthalmia, a flexed carpus, dilation of the renal pelvis and a fourth fetus with a domed head, dilation of the brain ventricles and renal pelvis. Most of these malformations were considered by the study authors to be related to fetal immaturity due to loss of appetite and body weight by the dams at the high dose. Quantitatively, these two dams lost 6.8% and 6.4%, respectively, of their body weights between days 6 and 18, compared with the group mean loss of 0.22% during this period. There was no compound-related increase in the number or severity of skeletal malformations. Accordingly the maternal and developmental NOEL in the rabbit was 1 mg/kg/day, based on decreased body weight gain (maternal) and decreases in litters with 12 pairs of ribs, live litter size and weight, increased resorptions, post-implantation losses and instances of $13^{th.}$ ribs (fetus). This study was acceptable to DPR.

The role of maternal toxicity in the developmental toxicity of cyanazine was then addressed by considering individual data along with group mean data. The dam which gave rise to the fetus with microphthalmia had a body weight loss of 6.4% during the 6 to 18 day gestation period. This compared with the mean control group gain of 1.2% and a high-dose group mean loss of 2.5%. However, rabbit eye development occurs mainly during the 8 to 12 day period (Edwards, 1968) and this may be the time during which teratogens affecting eye development are most likely to be effective. In the present study, during the 6 to 12 day period of gestation, for which figures are available from the report, the dam producing the fetus with microphthalmia had a body weight gain of 1.2%, compared with the control group mean body weight gain of 1.1% and the high-dose group mean of -1.0%. Thus the data do not support the conclusion that the microphthalmia was a result of maternal toxicity in the rabbit. Instead, when the data are analyzed in this way, it is suggested that there is evidence for developmental toxicity which is independent of maternal toxicity.

**Table 9 Occurrence of developmental effects in the New Zealand rabbit following maternal dosing with cyanazine by oral gavage.[a]**

| Parameter | Dosage (mg/kg/day) 0 | 1 | 2 | 4 |
|---|---|---|---|---|
| **Maternal body wt.** | | | | |
| Day 9,[b] mean (g) | 4485±50 | 4464 | 4435** | 4410** |
| Day 18, mean (g) | 4491±137 | 4518 | 4436 | 4339** |
| **12 pairs of ribs** litters | 19/19+++ (100%) | 18/20 90%) | 17/20 (85%) | 8/16 (50%) |
| **13th. single extra rib** fetuses | 13.4% | 11.2% | 19.4% | 23.2% |
| **13th. pair of ribs** fetuses | 24.7% | 36.9% | 36.1% | 46.0% |
| **mean number resorptions/dam** | 0.8 | 0.6 | 1.2 | 1.6 |
| **mean number dead fetuses/dam** | 0.2 | 0.3 | 0.4 | 1.5# |
| **mean number post-implant. losses/dam** | 1.0 | 0.9 | 1.5 | 3.2## |
| **mean number live fetuses/litter** | 7.5 | 7.5 | 7.0 | 6.1 |
| **mean live fetal wt./litter, g** | 42.5 | 42.6 | 40.5 | 41.3 |

a/ data are from Dix *et al.*, 1982.
b/ daily dosing on days 6 to 18 of gestation; 20 to 22 dams per dose level.
* significantly different from control at p<0.05 (Fisher's exact test)
** significantly different from control at p<0.01 (Dunnett's test)
# significantly different from control at p<0.05 (Wilcoxon test)
## significantly different from control at p<0.01 (Wilcoxon test)
+++ significant trend (p < 0.001), dose-weighted chi-square test (Peto *et al.*, 1980).

**Dermal-Rabbit**

Artificially inseminated rabbits (New Zealand White, 20/dose) were exposed dermally to Bladex 4L formulation (44.7% cyanazine) at 0, 0.2, 0.6, 1.2 and 2 ml/kg/day (calculated as 0, 89, 268, 536 and 894 mg/kg/day a.i.) during gestation days 6 through 18 once daily for 6 hours (WIL Research Laboratories, 1985; Gardiner *et al.*, 1986). In the preliminary, unaudited study (WIL Research Laboratories, 1985), severe maternal toxicity was reported in the form of mortality, as follows: 0/20 (controls), 1/20, 1/20, 2/20 and 3/20, with increasing dose. It was determined that oral ingestion of cyanazine, presumably from grooming had taken place in this experiment, contributing to the toxicity observed, and therefore, in the subsequent, definitive study (Gardiner *et al.*, 1986), Elizabethan collars were used to overcome this technical problem. The preliminary study has not been considered here, except from the standpoint of weight-of-the-evidence. Sham controls were exposed to formulation blank. Mortality was reported at 894 mg/kg/day (15%) and at 268 mg/kg/day (5%) (Table 10). Other maternal toxic effects of cyanazine exposure were manifested as a dose-related reduction in weight gain and food consumption (Table 10). Significant mean body weight reduction was reported at the first measurement, 3 days after dosing started, at the highest dose ($p<0.01$) and at 6 days at the 3 highest doses ($p<0.01$, Dunnett's test) and was continuous until study termination. Based on these effects, the maternal LOEL was 0.6 ml/kg/day (268 mg/kg/day) and the NOEL was 0.2 ml/kg/day (89 mg/kg/day). Developmental effects included increased numbers of resorptions at two of the doses and the formulation blank, when compared with the historical control (Table 10). Mean fetal weight was reduced at the two highest dose levels, by 9% at 536 mg/kg/day, compared with the formulation blank. No malformations were observed as a result of the test material. The developmental NOEL, based on reduced fetal weight, was 268 mg/kg. This study was acceptable to DPR. The U.S. EPA concluded that the maternal NOEL was <96 mg/kg (0.2 ml/kg) and the fetal NOEL was 573 mg/kg (1.2 ml/kg) and therefore, that cyanazine was not teratogenic in this test (U.S. EPA, 1986). Slightly different dosage calculations were used by DPR and U.S. EPA., based on percentage purity and percentage active ingredient.

**Table 10 Maternal and developmental toxicity of cyanazine to the New Zealand rabbit following dermal dosing with Bladex®4L.[a]**

| Parameter | Dosage (mg/kg/day) control | 89 | 268 | 536 | 894 |
|---|---|---|---|---|---|
| Food eaten (mean, g/day) | 144±34[b] | 140±28 | 111±31* | 94±36** | 69±34** |
| Weight change, g (mean, day 6-18) | +81±170 | +38±174 | -100±145** | -226±151** | -355±179** |
| Maternal mortality | 0/20 | 0/20 | 1/20 | 0/20 | 3/20 |
| # of litters with Resorptions | 1/18 (6%)[b] | 2/19 (11%) | 0/18 | 2/19 (11%) | 0/18 |
| Fetal weight, g (mean±s.d.) | 42.1±4.3 | 45.8±6.3 | 42.5±5.6 | 38.3±7.6 | 40.3±6.2 |

a/ data are from Gardiner *et al.*, 1986.
b/ historical control litter data: mean food eaten, 180 g/day; resorptions, 15/370 (4%).
* significantly different from formulation blank (control) at $p<0.05$ (Dunnett's test)
** significantly different from formulation blank (control) at $p<0.01$ (Dunnett's test)

**Table 11 Summary of reproductive and developmental toxicity studies with cyanazine.**

| Species/Route | Results | LOEL mg/kg/day | NOEL mg/kg/day | Ref.[a,b] |
|---|---|---|---|---|
| | | **Reproductive** | | |
| Rat/diet | adult toxicity, lower body wt. | 19.5 | 11.2 | 1 |
| | lower pup viability, body wt. | 11.2 | 5.6 | |
| | | **Developmental** | | |
| Rat/gavage | maternal toxicity | 30 | 3 | 2 |
| | developmental effects | - | ≥30 | |
| Rat/gavage | maternal toxicity | 10 | 2.5 | 3 |
| | developmental effects micro- and anophthalmia | 25 | 10 | |
| Rat/gavage | maternal toxicity | 25 | 5 | 4 |
| | developmental effects micro- and anophthalmia | 25 | 5 | |
| Rabbit/gavage | maternal & fetotoxicity: resorptions, 13th. ribs decreased litter size | 2 | 1 | 5 |
| Rabbit/dermal | maternal toxicity: | 268 | 89 | 6 |
| | developmental toxicity | 536 | 268 | |

a/ 1. Nemec, 1987; 2. Lu, 1983; 3. Lu *et al*, 1981; 4. Lochry, 1985; 5. Dix, 1982; 6. Gardiner, 1986.
b/ All of these studies were acceptable to DPR.

## H. NEUROTOXICITY

Neurotoxicity studies are not required under current FIFRA study guidelines and under SB 950 because triazines are not chemically related to any of the known classes of neurotoxic agents. None of the signs of dosing appeared to be related to those caused by acetylcholinesterase inhibitors. However, a dose-related increase in hyperreactivity was reported in the rat chronic study (Bogdanffy, 1990). The mechanism for this effect, if compound-related, is unknown.

# IV RISK ASSESSMENT

## A. HAZARD IDENTIFICATION

The Birth Defect Prevention Act of 1984 (SB 950) requires DPR to review the toxicological data for all active ingredients currently registered in California. DPR placed cyanazine in risk assessment based on the possible adverse effects identified in the following studies: chronic toxicity, genotoxicity, reproduction, and oncogenicity. In acute, sub-chronic and chronic studies, cyanazine consistently suppressed appetite in experimental animals, usually with a concomitant fall in body weight. A reduction in body weight was observed after the administration of cyanazine by gavage, inhalation, dermal or dietary exposure. In all but the latter case, loss of appetite could not have resulted directly from reduced palatability. Other triazine pesticides, such as atrazine, simazine and cyromazine, also cause this effect, regardless of the duration of exposure. U.S. EPA has used the endpoint of reduced body weight, along with increased hyperreactivity, to define the RfD for cyanazine (0.002 mg/kg/day). Following maternal dosing, cyanazine caused anophthalmia and microphthalmia in the rat and rabbit fetus/pup, sometimes at dose levels causing little or no maternal toxicity. Evidence for genotoxicity was produced in a variety of *in vitro* tests. A 2-generation rat reproductive toxicity study resulted in reduced pup viability at doses below that which reduced adult body weight. In a chronic study using the Sprague-Dawley rat, evidence of a compound-related increase in malignant mammary tumors in females was produced.

### Acute Toxicity

Cyanazine and its formulations were not acutely toxic to the rabbit dermally, with no mortality at 2000 mg/kg. There was only mild dermal and eye irritation resulting from cyanazine dosing in the rabbit; no dermal sensitization occurred in the guinea pig. Acute oral toxicity studies in the rat indicated $LD_{50}$ values of 835 (M) and 369 (F) mg/kg. By inhalation, in the rat, cyanazine dust had a $LD_{50}$ > 152 mg/kg after a 4-hour exposure, with an estimated NOEL of 1.6 mg/kg (Evancheck *et al.,* 1983). Data describing the acute toxicity of metabolites are limited, but two major metabolites (SD 31223 and 31224, Fig. 1) were considerably less toxic to the rat orally; the more toxic of the metabolites (SD 31223) was only 23% as toxic as the parent (Walker *et al.,* 1974). Bladex®4L and 90DF had toxicities which were quantitatively very similar to technical cyanazine by oral and dermal routes in rat and rabbit, respectively (Stillmeadow Inc., 1979a,b; Haskell Laboratory, 1988a,b).

The most sensitive groups of animals for determining the acute toxicity of cyanazine were dams and/or offspring of rats and rabbits. Cyanazine caused developmental as well as maternal toxicity. For example, in a study using the Fischer 344 rat (Lu *et al.*, 1981), microphthalmia and anophthalmia were reported at 25 mg/kg/day, with a developmental NOEL of 10 mg/kg/day. A maternal NOEL of 2.5 mg/kg/day, in this study, was based on reduced mean body weight gains at higher doses. In a subsequent study, using the same strain of rat, loss of mean maternal body weight gain and clinical signs were reported after the first dosing, at all dose levels tested (Lochry, 1985). However, these signs were no worse in individual dams producing fetuses or pups with anophthalmia and microphthalmia, than in those dams producing normal offspring. It is therefore concluded that the developmental effects were not a direct consequence of maternal toxicity. The NOEL for developmental and maternal toxicity in this study was 5 mg/kg/day. In rabbits treated with cyanazine by gavage, maternal toxicity (depressed body weight within 3 days of first dosing) was observed, with a LOEL of 2 and a NOEL of 1 mg/kg/day. Developmental toxicity (decreased litters with 12 pairs of ribs and decreases in size and weight of

live litters, increased resorptions, increased post-implantation losses and increased litters with fetuses having 13th. ribs) had the same LOEL and NOEL values as for maternal toxicity (Dix, 1982). Microphthalmia was observed in a single litter at 4 mg/kg/day. It is possible that increased post-implantation fetal losses (3-fold increase, $p<0.01$) in dosed animals obscured the occurrence of further cases of microphthalmia and anophthalmia, as seen in the rat. Cyanazine was not teratogenic in rabbits treated dermally; it is poorly absorbed through the skin. The dermal NOEL for maternal toxicity, which was based on decreased body weight gain, continuously from three days until study termination, was 89 mg/kg/day (Gardiner, 1986). This is equivalent to an absorbed dosage of 1.3 mg/kg/day, based on a mean dermal absorption of 1.5% in the rabbit (Logan, 1986).

The NOEL value of 1 mg/kg/day for oral exposure of the rabbit (Dix, 1982) was used as the critical NOEL to assess the acute dietary and occupational exposures. This **(**rabbit**)** oral NOEL is of very similar magnitude to the rabbit dermal NOEL of 1.3 mg/kg, as noted above.

**Subchronic Toxicity**

No subchronic toxicity studies have been submitted which are acceptable under FIFRA guidelines. Summaries of studies suggest that the only, consistent dose-related effect of cyanazine was the loss of body weight. In the dietary studies there was a concomitant reduction in food intake in the rat, but not in the mouse. In dermal (rabbit) and inhalation (rat) studies, the body weight reduction did not appear to be consistently accompanied by a loss of appetite.

Cyanazine feeding to rats during the reproduction cycle reduced pup viability and body weight at doses below those causing body weight loss in adults, indicating a possible adverse reproductive effect (Nemec, 1987).

**Chronic Toxicity**

Chronic dietary ingestion of cyanazine consistently reduced body weights, often the result of reduced food intake. This effect was observed in rats, mice, and dogs. The NOEL values for this effect were:

0.2 mg/kg/day in rats, 2-year, acceptable study (Bogdanffy, 1990)
0.15 mg/kg/day in rats, 2-year, unacceptable study (Simpson & Dix, 1973)
0.15 mg/kg/day (estimated) in mice, 2-year, acceptable study (Gellatly, 1981)
0.7 mg/kg/day in dogs, 1-year, acceptable study (Dickie, 1986)

Cyanazine feeding generally caused food to be poorly palatable, resulting in lower food intake which may partly explain the reduced body weight gain in dietary studies. In addition to reduced body weight gain in mice, cyanazine feeding resulted in toxicological adverse effects of increased renal cortical tubular dilation and epithelial vacuolation and myocarditis (Gellatly, 1981). The lowest measured NOEL from an acceptable study (5 ppm or 0.2 mg/kg/day), for systemic toxicity in the rat (Bogdanffy, 1990), was used as the critical NOEL for evaluating non-oncogenic effects. This is the same chronic NOEL value used by U.S. EPA (1994a). The next highest dose, 25 ppm, resulted in significantly reduced body weight and body weight gain, in both sexes, and hyperreactivity in males. However, because hyperreactivity was not clearly defined in the report and showed a discontinuous dose-response, it is considered by DPR to have doubtful toxicological relevance.

**Oncogenicity**

Cyanazine chronic feeding in rats resulted in a statistically significant increase in combined malignant mammary gland tumors (adenocarcinomas and carcinosarcomas) in female rats administered cyanazine in the diet at 5, 25 and 50 ppm, but not at 1 ppm (Table 3; Bogdanffy, 1990). There was no compound-related increase in benign mammary tumors (adenomas); however, when rats having adenoma(s) were combined with those having malignant tumors, an elevated incidence of tumors was observed, but only at 25 ppm.

The significant increase in mammary tumors at the highest dose tested (HDT) 50 ppm, was accompanied by significantly reduced food intake and body weight. In the untreated rat a *reduction* of mammary tumor incidence accompanies reduced food intake and body weight (Turnbull *et al.*, 1985; Boorman *et al.*, 1990; Ip, 1991). Characteristically, a lower caloric intake is associated with increased lifespan and reduced carcinogenicity in rodents (*e.g.* Kritchevsky & Klurfeld, 1987; Seilkop, 1995). The increase in rat mammary tumors which resulted from cyanazine administration was considered toxicologically significant. However, the Carworth Farm E strain rat did not show an increase in mammary gland tumors in two earlier, unacceptable studies (Walker & Thorpe, 1970a; Simpson & Dix, 1973) nor were there increased tumors in a mouse study (Gellatly, 1981). It should be noted that other triazine pesticides cause elevated levels of mammary tumors (adenocarcinomas and adenomas) in Sprague-Dawley female rats: atrazine (Wingard & Mayhew, 1986; Thakur, 1991) simazine (Ciba-Geigy, 1988), cyromazine (Blair, 1982), propazine (U.S. EPA, 1991 a) and terbutryn (U.S. EPA, 1991 a). A significant body weight reduction also accompanied the increased mammary tumor incidence caused by cyromazine (Pfeifer, 1993) and atrazine (Gammon, in preparation).

There is evidence that cyanazine has genotoxic potential, as shown by results from 4 types of *in vitro* assay using mammalian cells. The same assays using S-9 metabolic systems were inactive. *In vivo* studies suggest that cyanazine may not have genotoxic potential in mammals. For example, in rat hepatocytes (Grilli *et al.*, 1991) and rat spermatocytes (Bentley, 1993), cyanazine did not cause unscheduled DNA synthesis (UDS) after *in vivo* administration.

The assessment of the potential oncogenic risk of cyanazine in humans was evaluated using a quantitative, low-dose extrapolation approach. A non-threshold mechanism was assumed because a biological mechanism has not been convincingly demonstrated (see Section V). This approach is consistent with that used by U.S. EPA. The linearized multi-stage model, Global 86 (Howe *et al.,* 1986), as shown in Appendix A, was used to calculate cancer potency factors in female rats. By extrapolating the dose-response curve (linearly) to low doses, potency values were estimated based on the incidences of rat combined malignant mammary tumors (Table 3). The malignant mammary tumors were considered because of the greater statistical significance of the increased incidence with dose and because they are of greater relevance to human health than benign tumors. Both the maximum likelihood estimate (MLE, $Q_1$) and the 95% upper confidence limit (95% UB, $Q_1^*$) of the linear term of the multi-stage model were calculated as estimates of oncogenic potency.

Equivalent human potency values were estimated using a body-weight conversion factor assuming an interspecies dose equivalence of body weight to the 3/4 power (Appendix B), from the rat values, using the equation: $Q_1$, human/$Q_1$, rat = [body weight, human/body weight, rat].$^{¼}$ Using combined malignant mammary tumors, the human cancer potency values for cyanazine were 0.33 $(mg/kg/day)^{-1}$ for the maximum likelihood estimate (MLE) $Q_1$ and 0.58

$(mg/kg/day)^{-1}$ for the 95% upper bound confidence interval (UB), $Q_1^*$ (Table 12). These values were used to estimate potential oncogenic risk from occupational and dietary exposures. These potency values are greater than values for other structurally-related triazines. In comparing $Q_1^*$ values, cyanazine was 8.3-fold more potent than simazine, 4.5-fold more potent than atrazine (U.S. EPA, 1991b; U.S. EPA, 1994a) and *ca.* 100-fold more potent than cyromazine (Pfeifer, 1993). However, it should be noted that only for cyanazine are the $Q_1$ values based on combined malignant mammary tumors; for the others, the $Q_1$ values are based on combined malignant and non-malignant tumors.

**Table 12 Potency estimates for MLE ($Q_1$) and 95% Upper Bound ($Q_1^*$) for combined malignant mammary tumors[a] in humans.**

| Tumor Type[a] | Human MLE ($Q_1$)[b] $(mg/kg/day)^{-1}$ | Human 95% UB ($Q_1^*$)[c] $(mg/kg/day)^{-1}$ |
|---|---|---|
| **Mammary[d]** | 0.33 | 0.58 |

a/ Data are from Bogdanffy, 1990.
b/ Based on rat $Q_1$ values of 0.097 $(mg/kg/day)^{-1}$ see Appendix B.
c/ Based on rat $Q_1^*$ values of 0.17 $(mg/kg/day)^{-1}$ see Appendix B.
d/ Combined malignant (adenocarcinomas and carcinosarcomas).

U.S. EPA originally estimated a $Q_1^*$ of 0.159 $(mg/kg/day)^{-1}$ for the rat and 0.84 $(mg/kg/day)^{-1}$ for the human, for adenocarcinomas and carcinosarcomas, using the linearized multi-stage model (Global 86). This calculation, which was used in the U.S. EPA risk characterization, included interim sacrifice animals (U.S. EPA, 1991b). Subsequently, U.S. EPA decided that interim animals should not, in general, be included in the calculation of a potency factor for lifetime exposure, regardless of whether some animals already exhibited tumors (U.S. EPA, 1993). U.S. EPA recalculated a $Q_1^*$ value of 1.0 $(mg/kg/day)^{-1}$ for humans, equivalent to 0.2 $(mg/kg/day)^{-1}$ for the rat, based on a body weight scaling factor of the 2/3 power (U.S. EPA, 1993). It has been DPR policy to use a $(body\ weight)^{3/4}$ instead of a $(body\ weight)^{2/3}$ scaling factor in the calculation of animal-to-human dose equivalence. This accounts for most of the difference between the two calculations of the human $Q_1^*$ values. Another difference is the calculation of daily dosage in the rat chronic study (Bogdanffy, 1990). DPR used average, measured chemical intake and U.S. EPA used rat default mean dietary intake (Lehman, 1959).

## B. EXPOSURE ASSESSMENT

### Occupational Exposure

The Absorbed daily dosage (ADD), annual average daily dosage (AADD) and lifetime average daily dosage (LADD) were estimated for workers using a study of the ground application of Bladex® 4L to corn (Sanborn and Mehler, 1996). Although *ca.* 90% of cyanazine use in California is on cotton, the corn study is considered a suitable surrogate, once it had been adjusted for the lower application rate for cotton in California (averaging 2.0 lb./A. *versus* 4.5 lb./A. for corn). The 4L (liquid) formulation is only one of two cyanazine products currently registered in California, the other being the 90DF (granule). They are used in approximately equal amounts on cotton in California (see Section V).

### Mixer/Loader/Applicator (Commercial or Farmer)

The exposure of a mixer-loader-applicator (M/L/A) involved in the ground application of Bladex® to corn resulted in an estimated mean absorbed daily dosage (ADD) of cyanazine of 2.6 $\mu$g/kg/day (95% C.I.= 5.0 $\mu$g/kg/day), a 95$^{th.}$ percentile (high-end) exposure of 24.6 $\mu$g/kg/day and an annual average daily dosage (AADD) of 0.11 $\mu$g/kg/day, based on 15 days' use per year. These dosage estimates are based on 2% dermal absorption, from a rat dermal penetration study (Logan, 1986a). This was the same dermal absorption used by U.S. EPA (U.S. EPA, 1994a,b). Over an occupational lifetime of applying cyanazine *i.e.* 40 of 75 years, the lifetime average daily dosage (LADD) would be 0.057 $\mu$g/kg/day (Table 13). For a farmer applying cyanazine, the ADD is expected to be the same as for the commercial applicator but, because of the reduced number of days of exposure per year (3), the AADD and LADD values would be correspondingly lower than for the commercial applicator.

**Table 13 Occupational exposure to cyanazine.[a]**

| WORKER | ADD[b] ($\mu$g/kg/day)[c] | AADD[b] ($\mu$g/kg/day)[d] | LADD[b] ($\mu$g/kg/day)[e] |
|---|---|---|---|
| Commercial M/L/A[g] | 2.6[f g] | 11E-02 | 5.7E-02 |
| Farmer[g] | 2.6[f g] | 2.1E-02 | 1.1E-02 |

a/ see Volume 2 for calculations of worker exposure, based on a Bladex® 4L study on corn.
b/ ADD = Absorbed daily dosage; AADD = Annual average daily dosage; LADD = Lifetime average daily dosage
c/ Geometric mean ADD
d/ Applications per year = 3 days (farmer), 15 days (commercial applicator).
e/ Assumes 40 years of exposure, over a 75 year lifetime.
f/ 95$^{th.}$ percentile = 24.6 $\mu$g/kg/day
g/ Bladex® study conducted with 12 replicates: 3 workers and 4 loads each.

## Dietary Exposure

DPR evaluates the dietary exposure to an active ingredient in the diet using two processes: (1) use of residue levels detected in RACs (raw agricultural commodities) to estimate the exposure from all label uses, and (2) use of tolerance levels to estimate the exposure to individual commodities (see Section VI). For the evaluation of risk to detected residue levels, the total exposure in the diet is determined for all label-approved raw agricultural commodities, processed forms, and animal products (meat and milk) that have established U.S. EPA tolerances. Tolerances may be established for the parent compound and associated metabolites. DPR considers these metabolites and other degradation products that may be of toxicological concern in the dietary assessment.

The percentage of a commodity (crop) which is treated with a particular pesticide is often considered relevant for dietary exposure. For short-term (acute) dietary exposure, it is assumed that 100 percent of each commodity has been treated and therefore contains a residue. However, for long-term (chronic) dietary exposure, it is reasonable to suppose that only a proportion of any specific commodity has been treated with a particular pesticide. Therefore, a percentage crop-treated adjustment can be made for specific commodities.

### Residue Data

Primary and Secondary Residues

Data for potential pesticide residues associated with U.S. EPA and California label-approved direct food uses with tolerances, and with any secondary residues in animal tissues, are necessary for estimating human dietary exposures. The sources of residue data for dietary exposure assessment include DPR and federal monitoring programs, field trials, and survey studies by registrants. Residue data obtained from the monitoring programs are often preferred because they represent a realistic estimate of potential exposure. When residues are at levels higher than established tolerances, they are not utilized in the dietary exposure assessments since they are illegal. Additionally, DPR evaluates the potential risk from consuming commodities with residues over tolerance levels using an expedited acute risk assessment process. In the absence of data, surrogate data are used from the same crop group as defined by U.S. EPA, or theoretical residues equal to U.S. EPA tolerances are used.

Residue studies in RACs were conducted by the former registrant, Shell Oil Co. (Table 1). The reasons that they were used by DPR to assess dietary exposure are as follows: a very low LOD (Limit of Detection) of 0.01 ppm, a complete range of crops for which registrations were being sought and for which tolerances were obtained, and a range of application rates, including levels above the current maximum label application rates. In addition, residues of four plant metabolites of cyanazine were measured in these studies, with LODs of 0.03 or 0.05 ppm.

DPR has two major sampling programs: priority pesticide and marketplace surveillance. However, the residue analysis used by DPR for cyanazine had a LOD of 0.2 ppm, twenty-fold higher than the LOD used by the former registrant. No cyanazine residue detections were made in DPR's crop residue program from 1988 to 1993. When considered in combination with the registrant’s data (Table 1), it is clear that residues in crops at harvest are hypothetical.

The U. S. Food and Drug Administration (FDA) has three monitoring programs for determining residues in food: (1) regulatory monitoring, (2) total diet study, and (3) incidence/level monitoring. For cyanazine, the LOD for residues in crops was 0.04 ppm, using a multi-residue screen.

The U. S. Department of Agriculture (USDA) is responsible for the Pesticide Data Program (PDP), a nationwide cooperative monitoring program. The PDP is designed to collect objective, comprehensive pesticide residue data for risk assessments. However, USDA has not monitored for cyanazine residues in RACs in California. Similarly, there have been no determinations of residues in secondary animal products such as beef, pork, poultry, sheep and eggs. This is probably because registrant studies have demonstrated that cyanazine does not concentrate in animal tissues (Shell Chemical Co., 1985b).

Drinking Water

Cyanazine has been frequently detected in ground and surface water of the principal corn-growing States of the central USA *i.e.* IL, IN, KS, MO, NE and OH (see Wiles *et al.*, 1994; Cohen *et al.*, 1995). Consequently, since 1990, DPR has monitored for (parent) cyanazine in ground water from regions of California with a high usage of this pesticide. Cyanazine has never been detected at a LOD of $\leq$ 0.1 ppb. A degradation product of cyanazine, des-isopropyl atrazine (DIPA) is also a common degradate of atrazine and simazine. DIPA was the fourth most frequently detected compound in California groundwater in 1995 (DPR, 1996). Simazine and atrazine (parents) ranked first and fifth, respectively, for number of detections in groundwater in 1995. This ranking was similar to previous years.

**Acute Exposure**

Estimates of potential acute dietary exposure use the highest measured residue values at or below the tolerance for each commodity. The following assumptions are used to estimate potential acute dietary exposure from measured residues: (1) the residue does not change over time, (2) the concentration of residue does not decrease when the raw agricultural commodity is washed, (3) processing is assumed to be at a level equivalent to the raw agricultural commodity residue level that may be multiplied by an adjustment factor, and (4) all foods that are consumed will contain the highest reported residue.

None of the field trial or surveillance data showed any detectable residues, at LODs of 0.2, 0.04 or 0.01 ppm, for any of the RACs listed in Table 1. Therefore, the limit of detection (LOD) of 0.01 ppm was used as a default for the estimation of acute dietary exposure (Table 14). For the estimation of drinking water exposure, there have been no detections of cyanazine at a LOD of $\leq$ 0.1 ppb (1,111 wells from 24 counties, see Appendix B). Therefore, 0.1 ppb was used as the default concentration in drinking water. Judging from the use patterns of these triazines, it is possible that some of the DIPA detections could have resulted from the use of cyanazine, although most of them probably resulted from the use of simazine (on citrus).

**Chronic Exposure**

Estimates of potential chronic dietary exposure used the average of measured and "below detection limit" residue values for each commodity. The default procedure assumed that "below detection limit" residues were equal to 50% of the LOD for each commodity. The following assumptions were used to estimate potential chronic dietary exposures from measured residues:

(1) the residue level does not change over time, (2) residues are not reduced by washing the RAC, (3) processing is assumed to be at a level equivalent to the RAC residue level that may be multiplied by an adjustment factor (4) exposures to a commodity at all reported residue levels do occur, *i.e.* a commodity with the average calculated residue is consumed every day at an annual average level (dosage) and (5) except where stated, 100% of each crop was treated with a particular pesticide.

Field residue trials (Table 1) showed that cyanazine (parent) residues were not detected in any crop at harvest at the LOD of 0.01 ppm and that (four) identified transformation products were not detected at LODs of 0.03 or 0.05 ppm. Therefore, default residues of 0.005 ppm (50% of LOD) were used for each commodity for the estimation of potential chronic (annual) dietary exposure (Table 15). The values presented in Table 15 assume that 100 percent of the commodities were treated with cyanazine. Percentage of crop-treated data indicate that approximately 30% of corn and cotton and 10% of sorghum or wheat are treated with cyanazine in California (Appendix B). Therefore, the theoretical residue values, and resultant chronic exposure values, would be reduced accordingly. For the potential exposure to cyanazine residues in drinking water, as mentioned above, there were no detections in ground water at a LOD of 0.1 ppb. Therefore, 0.05 ppb was used as a default residue level to estimate potential chronic exposure through drinking water.

## Dietary Exposure Analysis

### Acute Exposure

Acute dietary exposure analyses were conducted using the Exposure-4™ program of Technical Assessment Systems, Inc. (TAS). This program estimates the distribution of user-day (consumer-day) exposure for the overall U.S. population and specific population subgroups (TAS, 1992a). A user-day is any day in which at least one food from the specific commodity list is consumed. The analysis uses data from the USDA Nationwide Food Consumption Survey (USDA, 1987-88).

Based on the 95$^{th.}$ percentile of user-day exposures for all specific population subgroups, the potential acute dietary exposure to cyanazine from all labeled uses ranged from 0.038 to 0.160 $\mu$g/kg-day (Table 14). Infants (non-nursing, <1 yr.) had the highest and seniors (55+ yrs.) the lowest potential acute dietary exposure to cyanazine. Appendix B gives the complete dietary exposure analysis. Potential exposure through drinking water was also estimated using the TAS Exposure-4™ program. This would increase the potential exposure to non-nursing infants to 0.176 $\mu$g/kg-day, a 10% increase. The potential exposure of the U.S. population would be 0.074 $\mu$g/kg-day, without water and 0.078 $\mu$g/kg-day, with water, a 5% increase. Exposure of nursing infants would be reduced, from 0.102 to 0.066 $\mu$g/kg-day, with the inclusion of drinking water and children (1 - 6 yrs.) would have an increased exposure, from 0.132 to 0.138 $\mu$g/kg-day, a 5% increase, with the inclusion of drinking water.

**Table 14 Potential acute dietary exposure to cyanazine in all commodities with U.S. EPA tolerances and in drinking water.**

| Population subgroup | ACUTE EXPOSURE[a] ( $\mu$g/kg-day) | ACUTE EXPOSURE[a] w/ drinking water @ 0.1 ppb ($\mu$g/kg-day) |
|---|---|---|
| US Pop. all seasons | 0 .074 | 0.078 |
| Western Region | 0 .069 | |
| Hispanics | 0. 070 | |
| Non-Hispanic Whites | 0.073 | |
| Non-Hispanic Blacks | 0. 083 | |
| Non-Hispanic Other | 0. 071 | |
| Infants (nursing) | 0 .102 | 0.066 |
| Infants (non-nursing) | 0. 160 | 0.176 |
| Children (1-6 yrs) | 0 .132 | 0.138 |
| Children (7-12 yrs) | 0 .093 | |
| Females (13-19 yrs, not pregnant or nursing) | 0.056 | |
| Females (13+ yrs, pregnant, not nursing) | 0.042 | |
| Females (13+ yrs, nursing) | 0.045 | |
| Females (20+ yrs, not pregnant or nursing) | 0.040 | |
| Males (13-19 yrs) | 0 .067 | |
| Males (20+ yrs) | 0 .046 | |
| Seniors (55+ yrs) | 0 .038 | |

a/ 95$^{th.}$ percentile of dietary exposure (residues = LOD *i.e.* 0.01 ppm for corn, sorghum, wheat and cottonseed).

**Chronic Exposure**

The potential chronic dietary and drinking water exposure was calculated using the Exposure-1™ software program (TAS, 1992b). The food consumption data for the chronic analysis was also based on the 1987-88 USDA Nationwide Food Consumption Survey (USDA, 1987-8). The program estimates the annual average exposure for specific population subgroups. In addition to calculations of theoretical dietary exposure assuming that 100% of each registered crop was treated with cyanazine, calculations were made adjusting for percentage of crop-treated in California (Appendix B).

All potential dietary exposure was pooled by combining cyanazine residues in all commodities on which cyanazine use is registered (Table 15). The mean potential annual dietary exposure ranged from 0.004 (nursing infants) to 0.031 $\mu$g/kg-day (Children, 1-6 yr.), based on 100% of crop treated. Percentage of crop-treated adjustment factors were 30% for corn and cotton; 10% for sorghum and wheat. The equivalent mean potential chronic dietary exposure levels, adjusted for percentage of crop-treated, were 0.001 and 0.006 $\mu$g/kg-day, for the same sub-populations (not shown). In addition, potential exposure to cyanazine through drinking water was also calculated, at 0.05 ppb (50%LOD). For the U.S. population, all seasons, drinking water increased the potential chronic exposure to cyanazine from 0.013 to 0.015 $\mu$g/kg-day. Potential exposure for nursing infants would be increased from 0.004 to 0.006 $\mu$g/kg-day. At the upper end of the chronic exposure range, children (1 - 6 yrs.) would experience a calculated increase from 0.031 to 0.033 $\mu$g/kg-day (not adjusted) with the inclusion of potential drinking water residues, a

6% increase. Thus, the theoretical chronic dietary exposure to cyanazine using residue data adjusted for percentage of crop-treated was reduced to between 18% and 28% of the exposure calculated for 100% crop-treated. The complete dietary exposure analysis is in Appendix B.

**Table 15 Potential chronic (annual) dietary exposure to cyanazine in all commodities with U.S. EPA tolerances and in drinking water.**

| | CHRONIC EXPOSURE (µg/kg-day)[a] | |
|---|---|---|
| **Population subgroup** | **ALL COMMODITIES** | **ALL COMMODITIES incl. drinking water[b]** |
| US Pop. all seasons | 0.013 | 0.015 |
| Western Region | 0.013 | |
| Hispanics | 0.013 | |
| Non-Hispanic Whites | 0.013 | |
| Non-Hispanic Blacks | 0.014 | |
| Non-Hispanic Other | 0.013 | |
| Infants (nursing) | 0.004 | 0.006 |
| Infants (non-nursing) | 0.018 | |
| Children (1-6 yrs) | 0.031 | 0.033 |
| Children (7-12 yrs) | 0.022 | |
| Females (13-19 yrs) (not pregnant, not nursing) | 0.013 | |
| Females (13+ yrs) (pregnant, not nursing) | 0.010 | |
| Females (13+ yrs) (nursing) | 0.011 | |
| Females (20+ yrs) (not pregnant, not nursing) | 0.009 | |
| Males (13-19 yrs) | 0.016 | |
| Males (20+) | 0.010 | |

a/ annual average dietary exposure (residues = 50% of LOD *i.e.* 0.005 ppm for corn, sorghum, wheat and cottonseed). Based on 100% crop-treated.
b/ includes theoretical drinking water residues of cyanazine, 0.05 ppb (50% of LOD).

## Combined Occupational and Dietary Exposure Assessment

### Acute Exposure

The combined acute exposure was obtained by summing the mean (occupational) ADD of 2.6 $\mu$g/kg/day (Table 13) and the acute dietary exposure for males, 13-19 yrs. (0.067$\mu$g/kg/day) or 20+ yrs. (0.046 $\mu$g/kg/day) , the subgroups most likely to experience occupational exposure (Table 14). This gave a total, acute, combined occupational and dietary exposure of 2.7 $\mu$g/kg/day, with or without drinking water included, for males 13-19 yrs. For males of 20+ yrs., the estimated combined acute exposure was 2.6 $\mu$g/kg/day.

### Chronic Exposure

The combined chronic exposure was obtained by summing the AADD values of 0.021 and 0.11 $\mu$g/kg/day, for farmers and commercial applicators, respectively (Table 13) and the potential chronic dietary exposure for males, 13-19 yrs. or 20+ yrs., of 0.016 or 0.010 $\mu$g/kg/day, respectively (Table 15). The inclusion of theoretical drinking water exposure at 0.05 ppb

(50%LOD) increased the dietary exposure value by approximately 0.002 μg/kg/day. Total, chronic, combined occupational and dietary exposure estimates were 0.037 μg/kg/day for farmers and 0.126 μg/kg/day for commercial applicators, or 0.039 μg/kg/day and 0.128 μg/kg/day, with the inclusion of drinking water exposure.

## C. RISK CHARACTERIZATION

The risk characterization process consists of calculating a margin of exposure (MOE) by dividing the critical acute or chronic NOEL value for a specific toxicological endpoint (Section IV A) by an estimate of human exposure (Section IV B). The probability of excess cancer risk in a lifetime was calculated by multiplying the LADD values (occupational) and/or the chronic annual average dietary exposure, by the cancer potency factors. Additionally, the cancer risk was calculated for combined occupational and dietary exposure, through the consumption of theoretical crop residues, with and without the inclusion of theoretical drinking water residues.

### Occupational Exposure

The estimates of occupational exposure, following Bladex® application to cotton, are given as the ADD, AADD and LADD (Table 13). These estimates were used to calculate the acute and chronic MOE, as well as the probability of excess cancer risk in a lifetime, respectively (Table 16).

The acute MOE, based on the mean ADD, for farmers and commercial applicators was 385. For workers exposed to the 95$^{th.}$ percentile of the ADD, the MOE was 41. The annual MOE, based on the mean AADD, was 1820 for commercial applicators and 9520 for farmers. The probability of excess cancer risk in a lifetime was 1.9E-05 (MLE) and 3.3E-05 (95%UB) for commercial applicators and 3.6E-06 (MLE) and 6.4E-06 (95%UB) for farmers.

**Table 16 Margins of exposure and excess risk from potential occupational exposure to cyanazine.**

| WORKER | ACUTE MOE[a,b] | | CHRONIC | LIFETIME RISK[d] | |
|---|---|---|---|---|---|
| | MEAN | 95$^{th.}$ Percentile | MOE[c] | MLE | 95% UB |
| Commercial M/L/A | 385 | 41 | 1820 | 1.9E-05 | 3.3E-05 |
| Farmer | 385 | 41 | 9520 | 3.6E-06 | 6.4E-06 |

a/ MOE = $\frac{\text{NOEL}}{\text{ADD}}$

NOEL of 1 mg/kg/day from a rabbit oral developmental toxicity study (Dix, 1982).

b/ Mean ADD (2.6 μg/kg/day) and 95$^{th.}$ Percentile (24.6 μg/kg/day), from Table 13.

c/ MOE = $\frac{\text{NOEL}}{\text{AADD}}$

NOEL (chronic) of 0.2 mg/kg/day from a 2-year rat study (Bogdanffy, 1990). AADD values of 11E-02 (commercial applicator) and 2.1E-02 (farmer) μg/kg/day, from Table 13.

d/ Based on the product of LADD values (Table 13) and human cancer potency factor ($Q_1$ , MLE and $Q_1^*$ 95% confidence interval, UB, in Table 12),) derived from malignant mammary tumors in the female rat (Bogdanffy, 1990).

## Dietary Exposure

### Acute Exposure

The margin of exposure (MOE) for each population subgroup for theoretical acute dietary exposure to cyanazine is given in Table 17. These values were derived from the theoretical dietary exposure values (Table 14) in which all registered commodities were assumed to contain residues at the default level of the LOD. The MOE values ranged from 6,270, for non-nursing infants (<1 yr.), to 26,300 for seniors (55+ yrs.). The inclusion of theoretical drinking water residues at 0.1 ppb (LOD) reduced the MOE for the U.S. population, all seasons, from 13,500 to 12,800, a 5% decrease.

**Table 17 Margins of exposure for theoretical acute dietary exposure to cyanazine residues in all commodities with U.S. EPA tolerances.[a]**

| Population subgroup | Margin of Exposure[b] |
|---|---|
| US Pop. all seasons | 13,500[c] |
| Western Region | 14,400 |
| Hispanics | 14,200 |
| Non-Hispanic Whites | 13,700 |
| Non-Hispanic Blacks | 12,100 |
| Non-Hispanic Other | 14,100 |
| Infants (nursing, <1 yr.) | 9,850 |
| Infants (non-nursing, <1 yr.) | 6,270 |
| Children (1-6 yrs) | 7,560 |
| Children (7-12 yrs) | 10,800 |
| Females (13-19 yrs) (not pregnant, not nursing) | 17,700 |
| Females (13+ yrs) (pregnant, not nursing) | 23,800 |
| Females (13+ yrs) (nursing) | 22,400 |
| Females (20+ yrs) (not pregnant, not nursing) | 24,700 |
| Males (13-19 yrs) | 14,900 |
| Males (20+ yrs) | 21,800 |
| Seniors (55+ yrs) | 26,300 |

a/ Residues = LOD *i.e.* 0.01 ppm for corn, sorghum, wheat and cottonseed.
b/ MOE= NOEL / Dietary intake, 95$^{th.}$ percentile
NOEL of 1 mg/kg/day based on maternal and fetal toxicity from a rabbit oral developmental toxicity study (Dix, 1982).
c/ MOE including theoretical drinking water exposure at 0.1 ppb (LOD) = 12,800.

### Chronic Exposure

The margin of exposure for each population subgroup following theoretical chronic (annual) dietary exposure to cyanazine is given (Table 18). These values were derived from the theoretical exposure values (Table 15) in which all registered commodities were assumed to contain residues at the default level of 50% of the LOD. The MOE values ranged from 6,440, for children (1-6 yrs.), to 48,100 for nursing infants. Crop-treated adjustment factors elevated these MOE values to 31,600 and 186,000 for these two groups, respectively. The inclusion of theoretical drinking water residues at 0.05 ppb (50% LOD) reduced the MOE values for the U.S. population, from 15,000 to 13,300 (unadjusted), a 11% fall, and from 75,100 to 50,000 (adjusted for percentage of crop-treated).

**Table 18 Margins of exposure and percentage of U.S. EPA Reference Dose for theoretical chronic (annual) dietary exposure to theoretical cyanazine residues in all commodities with U.S. EPA tolerances.) and in drinking water.[a]**

| Population subgroup | MARGIN OF EXPOSURE[b] | % of RfD[c,d] |
|---|---|---|
| US Pop. all seasons | 15,000[e] | 0.7% |
| Western Region | 15,500 | 0.6% |
| Hispanics | 16,000 | 0.6% |
| Non-Hispanic Whites | 15,100 | 0.7% |
| Non-Hispanic Blacks | 14,300 | 0.7% |
| Non-Hispanic Other | 15,300 | 0.7% |
| Infants (nursing) | 48,100 | 0.2% |
| Infants (non-nursing) | 11,300 | 0.9% |
| Children (1-6 yrs) | 6,440 | 1.6% |
| Children (7-12 yrs) | 8,950 | 1.1% |
| Females (13-19 yrs) (not pregnant, not nursing) | 15,300 | 0.7% |
| Females (13+ yrs) (pregnant, not nursing) | 20,000 | 0.5% |
| Females (13+ yrs) (nursing) | 18,600 | 0.5% |
| Females (20+ yrs) (not pregnant, not nursing) | 22,100 | 0.5% |
| Males (13-19 yrs) | 12,700 | 0.8% |
| Males (20+) | 19,400 | 0.5% |

a/ Residues = 50% of LOD *i.e.* 0.005 ppm for corn, sorghum, wheat and cottonseed.
b/ MOE= $\frac{\text{NOEL ( 0.2 mg/kg-day)}}{\text{AADD}}$
c/ RfD or Reference Dose = 0.002 mg/kg/day, using same NOEL value (U.S. EPA, 1994a).
d/ % of RfD for all commodities with U.S. EPA tolerance.
e/ MOE = 13,300 if theoretical drinking water residue included at 0.05 ppb.

**Lifetime Exposure**

The excess risk of oncogenicity calculated to result from theoretical dietary exposure to cyanazine was estimated for the U.S. population (Table 19). It is assumed that dietary exposure would be the same every year over a lifetime. Using the MLE for cancer potency, $Q_1$ (0.33 per mg/kg/day) and the range of potential chronic dietary exposures (0.003 to 0.013 $\mu$g/kg-day, based on adjustment for percentage of crop treated), the cancer risk was 1.0 to 4.3E-06. For the upper bound cancer potency factor, $Q_1^*$ (0.58 per mg/kg/day), the excess cancer risk from potential dietary exposure was 1.5 to 7.7E-06.

Potential exposure to cyanazine through drinking water would increase the theoretical cancer risk, from dietary exposure, by 10 to 16% (unadjusted for percentage of crop treated) or 30 to 50% (adjusted for percentage of crop treated, Table 19).

**Table 19 Excess cancer risk from theoretical dietary exposure to cyanazine**.

| DIETARY EXPOSURE | LIFETIME RISK[a] | |
|---|---|---|
| | MLE | UB |
| No drinking water | 4.3E-06 | 7.7E-06 |
| With drinking water | 5.0E-06 | 8.5E-06 |

a/ Calculated by multiplying the cancer potency factor $Q_1$ or $Q_1^*$ by the theoretical, annual average dietary exposure (U.S. population), not adjusted for % crop-treated.

U.S. EPA (1994a) calculated a 95% UB cancer risk estimate of 2.9E-05 for potential dietary exposure to all registered RACs. However, the anticipated residues used in this calculation were above tolerances, as follows: corn, 0.12 ppm; cottonseed, 0.09 ppm; sorghum, 0.10 ppm and wheat, 0.16 ppm. The tolerances for these RACs are 0.05 ppm, except for wheat, 0.10 ppm. In addition, U.S. EPA included anticipated secondary residues in milk, poultry, eggs and red meat at 0.28 ppb, 2.3 - 4.2 ppb and 3.5 - 10.3 ppb, respectively. Any residue which is detected above tolerance in a RAC or detected, at all, in a commodity for which tolerances do not exist, would be illegal and the food would not be allowed to be sold for human consumption. It is the current DPR policy not to include such illegal residues in dietary exposure calculations.

U.S. EPA (1994) calculations of 95%UB cancer risk estimates were conducted on an individual crop basis: 1.2E-05 (corn), 9.3E-08 (cotton), 1.2E-07 (sorghum), 2.3E-06 (wheat) plus secondary residues in milk, eggs, chicken and red meat, totaling 2.9E-05 (UB) or 1.6E-05(UB) for just the 4 RACs with tolerances. If U.S. EPA had based their calculations on the residue levels at the tolerances for the RACs, the excess cancer risks would likely be similar to those calculated by DPR (Table 19).

**Combined Occupational and Dietary Exposure**

Because dietary exposure to cyanazine is largely theoretical, and because it is much less than occupational exposure, margins of exposure and excess oncogenic risk were not calculated for combined occupational and dietary exposure. For example, for acute exposure (U.S. population), the MOE decreased by only 3%, from 385, for occupational exposure, to 374, adding dietary exposure. The addition of drinking water exposure to combined gave a MOE of 373, also a decrease of 3% below occupational exposure alone.

# V RISK APPRAISAL

## A. Introduction

Risk assessment is the process which is used to evaluate the potential for exposure and the likelihood that the toxic effects of a substance will occur in humans under specific exposure conditions. Every risk assessment has inherent limitations and uncertainties in the application of existing data to estimate the potential risk to human health. Therefore, certain *a priori* assumptions are incorporated into the hazard identification, dose-response assessment and exposure assessment processes. These, in turn, result in uncertainty in the risk characterization, which integrates all of the information in these three processes. Qualitatively, risk assessment for all chemicals has similar types of uncertainty. However, the degree or magnitude of the uncertainty varies depending on the availability and quality of the data and the exposure scenarios being assessed. Varying degrees of uncertainty are involved in the estimation of these parameters, affecting the accuracy of the risk characterization. Specific areas of uncertainty associated with this risk assessment for cyanazine are delineated in the following discussion.

## B. Hazard Identification

Acute toxicity tests measure the effects of a chemical after a single or brief period of exposure. Developmental toxicity studies are a special case in the battery of such tests. Typically, daily dosages are administered to pregnant animals during the period of organogenesis of the fetus. In the absence of data to the contrary, it is assumed that a reported developmental effect can result from a single dose on a particular day during this time period (U.S. EPA, 1991a). Cyanazine is not removed from the rat body within 24 hours; it requires *ca.* 4 days to remove at least 90% (Griffiths, 1968). It is therefore possible that an effect could occur after repeated dosing and result from an accumulation of chemical above a critical threshold. In such a case, the acute NOEL value would underestimate the "true" NOEL and the “true” MOE. The NOEL value used to determine the acute MOE values for cyanazine was derived from such a study, using New Zealand white rabbits. The maternal NOEL was based on decreased body weight gain, occurring early in the study. The NOEL for developmental toxicity was based on decreases in litters with 12 pairs of ribs, live litter size and weight, increased resorptions, post-implantation losses and cases of $13^{th.}$ ribs.

In the evaluation of chronic toxicity, the most prevalent non-cancer toxicological endpoint in rats, mice and dogs, was loss of body weight and body weight gain. This effect was not solely a result of lower food intake due to poor palatability because reduced food intake was reported following dermal and inhalation as well as dietary exposure. The NOEL for this effect in the rat was used to assess chronic exposure. However, the toxicological significance of body weight loss is difficult to assess, giving rise to another area of uncertainty about this endpoint. Indeed, male rats (though not females) showed significantly increased longevity associated with reduced body weight at the highest dose. Other toxicological effects observed in chronic studies included inanition, poor skin and fur condition and anemia which may have all been secondary to poor nutrition.

Oncogenicity was assessed using a linear multi-stage model which assumes a non-threshold mechanism. It is possible that mammary tumors resulting from cyanazine exposure in the female rat arose from an estrogenic (receptor-mediated) effect (Stevens *et al.*, 1994; Tennant *et al.*, 1994), which might be expected to show a threshold. This has been suggested for atrazine, where malignant mammary tumors have been found in the same strain and sex of rat. However,

DPR believes that the currently available data are insufficient to support this hypothesis. Cyanazine showed some positive responses in genotoxicity assays, depending on the specific assay; therefore, a genotoxic mechanism cannot currently be excluded. Additionally, a potentially genotoxic degradate of cyanazine, nitroso-cyanazine, was identified in soil which had been incubated with cyanazine and sodium nitrite (Zwickenpflug & Richter, 1994). Although cyanazine was not tested, other triazines, which were incubated in human gastric juice containing sodium nitrite, were transformed into nitroso-derivatives (Cova *et al.*, 1996). The relationship between potentially genotoxic degradates of cyanazine and the expression of oncogenicity has not been studied or delineated. Current analytical methods for residues of cyanazine and cyanazine degradates in water and crops do not detect nitroso-triazines; therefore, potential human exposure to these compounds is not known.

## C. Exposure Assessment

### Occupational Exposure

Occupational exposure studies using Bladex® formulations on cotton, the major use crop in California, were not available to DPR. A ground study using Bladex® 4L on corn (pre-emergent) was considered to be a suitable alternative (Sanborn, 1996). However, several possible sources of error may exist. For example in 1993, 223,355 lbs. of a.i. were applied to cotton as 4L (51%) and 216,080 lbs. as 90DF (49%). No calculations were made to estimate possible occupational exposure to the 90DF formulation, although exposure to 90DF could be quite different for two reasons. First, because 90DF is a solid, unlike the 4L formulation, which is a liquid. Second, because unlike for the 4L formulation, chemical-resistant gloves are not required on the label for M/L/As using 90DF; only waterproof gloves are currently required.

Human dermal penetration data were not available and the absorption was assumed to be 2%, the same as for the rat. This value may be an overestimate of dermal penetration since rates in rodents are generally greater than rates in humans (Feldmann & Maibach, 1974; Wester & Maibach, 1985). However, rat laboratory studies involve only a small area of skin, compared with the larger areas which are generally associated with human exposures. Because absorption tends to increase over a larger surface area of exposure (*i.e.* the rate and total amount of absorption are generally inversely proportional to the concentration of chemical) the rat data may under-estimate human dermal absorption.

Another assumption, which would tend to increase the occupational exposure estimates, was the use of a maximum number of loads per day. On the other hand, a factor which would reduce occupational exposure was the use of a mean application rate (2 lb. a.i./A) rather than the maximum label rate (4.5 lb. a.i./A). The information on application rates was obtained from the California pesticide usage database, 1991-1993. Applications of cyanazine to cotton in California are largely made early post-emergence, when application rates are lower than pre-emergent ones. This justifies the use of the lower application rate in the occupational exposure calculations. Since cotton is the major crop on which cyanazine is used in California, accounting for ~90% of the total pounds a.i. applied, the majority of occupational exposure to cyanazine will be from applications to cotton.

The current label for cyanazine in California indicates that an open system can be used by the mixer/loader/applicator and that an open tractor cab can be used during application. However, 8 of the 12 data points pooled to derive the ADD value were obtained using a closed cab, which is currently not a label requirement. The data indicated approximately a 3 to 4-fold

protection factor. Therefore the current ADD value would underestimate the "actual" ADD, based on current label requirements. The label for cyanazine is proposed to require a closed cab, from January 1, 1998 (U.S. EPA, 1995).

**Dietary Exposure**

As discussed in Section II, it is unlikely that residues of cyanazine will be found at harvest in any RACs. Therefore, the default residue values used for calculating possible dietary exposure are considered theoretical values which result in a "worst-case" situation. In practice, the actual MOE values for dietary exposure are thus likely to be considerably higher than those calculated. In addition, the residues in drinking water, which were used for calculating MOE values and excess cancer risk, were also default values at the LOD or 50% of LOD.

It is unlikely that an individual will consume commodities which have been treated with cyanazine for a lifetime. Pesticide usage reports indicate, for example, that only 5 to 8% of California corn is treated with cyanazine and 18 to 20% in the 17 major corn production states, (Appendix B). When the chronic dietary exposure values were adjusted using conservative estimates of percentage of crop-treated, they were reduced to between 18% and 28% of the dietary exposure values calculated in Section IV.B, which used 100% of crop-treated. The chronic MOE values and excess cancer risk were reduced correspondingly.

Drinking water

Cyanazine and other triazine herbicides have a long history of being detected in groundwater and surface water in the mid-western states *e.g.* IL, IN, KS, MO, NE and OH. Triazines, such as simazine and atrazine, along with selected degradates, are also among the most frequently detected pesticides in California groundwater. However, cyanazine has not been found in groundwater in California. The current residue methods used by U.S. EPA and DPR do not identify cyanazine degradates. It has recently been reported that detections of parent-cyanazine in mid-West wells were only 50% as frequent as were detections of the cyanazine amide (Fig. 1, SD 20196), a primary soil degradate (Kolpin *et al.*, 1996). In the most recent DPR report (DPR, 1996) on groundwater testing results for 1994/1995, desisopropyl atrazine (DIPA) was the fourth most frequently detected pesticide (or degradate) in wells. This compound is a common degradate of both cyanazine and simazine, in addition to atrazine. Therefore, it is possible that some detections of DIPA resulted from cyanazine usage. The calculation of MOE and risk from drinkingwater exposure to DIPA would reduce the former and increase the latter.

# VI TOLERANCE ASSESSMENT

## A. INTRODUCTION

A tolerance is the maximum amount of pesticide residue that may remain in or on a food, or animal feed (U.S. EPA, 1991). The U.S. EPA tolerance program was developed as an enforcement mechanism to identify illegal residue concentrations resulting from potential non-compliance with the product label requirements (e.g. improper application rates or methods, inadequate pre-harvest intervals, direct or indirect application to unapproved commodities). Tolerances are enforced by the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), and state enforcement agencies (*e.g.* Pesticide Enforcement Branch of DPR).

The data requirements established by U.S. EPA for tolerances include: (1) residue chemistry which includes measured residue levels from field studies, (2) environmental fate studies, (3) toxicology studies which evaluate the hazards to humans, domestic animals, and non-target organisms, (4) product performance such as efficacy, and (5) product chemistry which includes physical-chemical characteristics and analytical method (Code of Federal Regulations, 1992). The field studies must reflect the proposed use with respect to the rate and mode of application, number and timing of applications, and formulations proposed (U.S. EPA, 1982).

Currently, the tolerances set by U.S. EPA are at levels necessary for the maximum application rate and frequency, and are not expected to produce deleterious health effects in humans from chronic dietary exposure (U.S. EPA, 1991). U.S. EPA uses the Reference Dose for non-cancer risks, and negligible risk level for cancer as guides to determine the appropriate levels for dietary exposure.

Assembly Bill 2161 (Bronzan and Jones, 1989) requires the DPR to "conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides". In the situation where "any pesticide use represents a dietary risk that is deleterious to the health of humans, the DPR shall prohibit or take action to modify that use or modify the tolerance.....". As part of the tolerance assessment, a theoretical dietary exposure for a specific commodity and specific population subgroups can be calculated from the product of the tolerance and the daily consumption rate.

For a pesticide allowed to be used on numerous commodities, tolerance assessments are conducted for selected fruits and vegetables. Generally, commodities are selected from all the uses based on the potential for high levels of dietary exposure. For cyanazine, the tolerances for the following commodities were evaluated: fresh corn, corn grain, cottonseed, sorghum grain and wheat grain. These were selected because they constitute all registered commodities in the United States.

## B. ACUTE EXPOSURE

An acute exposure assessment using the residue level equal to the tolerance was conducted for each individual label-approved commodity. The TAS Exposure-4™ software program and the USDA National Food Consumption Survey (USDA, 1987-1988) were used in this assessment. The acute tolerance assessment does not routinely address multiple commodities at the tolerance levels since the probability of consuming multiple commodities at the tolerance decreases as the number of commodities included in the assessment increases.

The range of potential dietary exposure values at the 95th. percentile for each registered commodity is given in Table 20. The least dietary exposure was from cotton (0.006 to 0.016 μg/kg-day) and the most, from wheat grain (0.22 to 0.67 μg/kg-day). These theoretical acute dietary exposure levels would give MOE values of approximately 63,000-160,000 (cottonseed) to 1,500-4,600 (wheat grain).

**Table 20 Theoretical acute dietary exposure to commodities with residue values of cyanazine at tolerance and corresponding margins of exposure**.

| COMMODITY | %USER-DAYS[a] | TOLERANCE ppm | DIETARY EXPOSURE 95th.% (μg/kg-day) (sub-population range) | MARGIN OF EXPOSURE[b] (sub-population range |
|---|---|---|---|---|
| CORN<br>fresh (sweet)<br>grain | <br>18<br>100 | <br>0.05<br>0.05 | <br>0.11 - 0.41<br>0.062 - 0.54 | <br>2,400[c] - 9,400[d]<br>1,900[e] - 16,000 |
| COTTONSEED | 97 | 0.05 | 0.006 - 0.016 | 63,000[e] - 160,000[g] |
| SORGHUM<br>grain | <br>1 | <br>0.05 | <br>0.003 - 0.037 | <br>27,000[h] - 290,000[g] |
| WHEAT<br>grain | <br>100 | <br>0.1 | <br>0.22 - 0.67 | <br>1,500[c] - 4,600[f] |

a/ a user-day is any day on which at least one food item from the specific commodity is consumed.
b/ MOE = NOEL (1 mg/kg/day) / Exposure (95th. percentile)
c/ Children, 1-6 yrs.
d/ Females, 13+, nursing.
e/ Non-nursing infants.
f/ Seniors, 55+
g/ Females, 20+, non-pregnant/non-nursing.
h/ Males, 20+

## C. CHRONIC EXPOSURE

A chronic exposure assessment using residues equal to the established tolerances for individual or combinations of commodities was not conducted because it is highly improbable that an individual would habitually consume single or multiple commodities with pesticide residues at tolerance levels. This conclusion is supported by data from both federal and DPR pesticide monitoring programs which indicate that less than one percent of all sampled commodities have residue levels at or above the established tolerance (CDFA, 1990-1993).

# VII CONCLUSIONS

## Occupational Exposure

A margin of exposure of at least 100, whenever it is based on animal toxicity data, is conventionally recommended to protect the population from the toxic effects of a pesticide. Using mean, acute occupational exposure estimates, the margins of exposure for the ground application of cyanazine to cotton were above 100 for both farmers and commercial applicators. Using an upper-end ( $95^{th.}$ percentile) exposure estimate, the MOE value was below 100 (41). For mean annual (chronic) exposure, and using chronic toxicity data, margins of exposure were also above 100. For lifetime exposure, however, the risk of excess cancer was calculated to be above $10^{-5}$ (1 in 100,000) for the custom applicator and above $10^{-6}$ (1 in 1,000,000) for the farmer, using either the MLE or 95%UB cancer potency estimate in each case.

Cyanazine is listed as a chemical which is “known to the State to cause reproductive toxicity” under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). Cyanazine is listed under “Developmental Toxicity.” Since the acute exposure scenarios for cyanazine have been evaluated in this document based on developmental toxicity, some of the requirements of the Proposition may be applicable.

## Dietary Exposure

Residue trials have indicated that residues would not be present in crops at harvest. In addition, cyanazine has not been detected in groundwater in California. Using default residues in raw agricultural commodities and/or drinking water, margins of exposure were nonetheless above 100 for all population subgroups. Excess lifetime cancer risk for the U.S. Population as a whole was greater than $10^{-6}$ (1 in 1,000,000), when based on the theoretical residues in RACs and drinking water.

## Combined Exposure

The margins of exposure for combined occupational and dietary exposure were not significantly different from the margins of exposure estimated for occupational exposure alone.

## Tolerances

U.S. EPA tolerances for cyanazine on all commodities for which tolerances have been established, whether consumed alone or in combination, provided acute margins of exposure for all population subgroups which were above 100.

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# IX APPENDICES

# APPENDIX A

DATE: 09/28/1994 TIME: 08:08:43

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

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

Cyanazine Malignant Mammary Gland Tumors In Female Rats

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

| GROUP | DOSE | #RESPONSES OBSERVED/#ANIMALS | #RESPONSES PREDICTED |
|---|---|---|---|
| 1 | .000000 | 5/ 49 | 8.40 |
| 2 | 5.3000E-02 | 6/ 43 | 7.55 |
| 3 | .259000 | 12/ 41 | 7.87 |
| 4 | 1.37000 | 18/ 48 | 13.18 |
| 5 | 2.81000 | 15/ 51 | 18.83 |

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 8.3924

P-VALUE FOR THE MONTE CARLO TEST IS .1500000000E-01

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp( -Q0 - Q1 * D - Q2 * D^2 )

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q( 0) = .188053292804
Q( 1) = 9.705483604692E-02
Q( 2) = .000000000000

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -125.089939328

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

| RISK | MLE DOSE | LOWER BOUND ON DOSE | CONFIDENCE LIMIT SIZE | COEFFICIENTS FOR CONFIDENCE LIMIT |
|---|---|---|---|---|
| 1.00000E-06 | 1.03035E-05 | 5.87816E-06 | 95.0% | Q( 0) = .15158<br>Q( 1) = .17012<br>Q( 2) = .00000 |

NORMAL COMPLETION!

DATE: 09/28/1994 TIME: 08:10:07

GLOBAL 86 (MAY 1986)

BY RICHARD B. HOWE AND CYNTHIA VAN LANDINGHAM

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

Cyanazine Mammary Gland Tumors In Female Rats

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

| GROUP | DOSE | #RESPONSES OBSERVED/#ANIMALS | #RESPONSES PREDICTED |
|---|---|---|---|
| 1 | .000000 | 9/ 49 | 12.13 |
| 2 | 5.3000E-02 | 9/ 43 | 10.76 |
| 3 | .259000 | 14/ 41 | 10.69 |
| 4 | 1.37000 | 20/ 48 | 15.08 |
| 5 | 2.81000 | 16/ 51 | 19.28 |

CHI-SQUARE GOODNESS OF FIT STATISTIC IS 6.0796

P-VALUE FOR THE MONTE CARLO TEST IS .7000000000E-01

FORM OF PROBABILITY FUNCTION:
P(DOSE) = 1 - exp( -Q0 - Q1 * D - Q2 * D^2 )

MAXIMUM LIKELIHOOD ESTIMATES OF DOSE COEFFICIENTS

Q( 0) = .284462877457
Q( 1) = 6.771290462907E-02
Q( 2) = .000000000000

MAXIMUM VALUE OF THE LOG-LIKELIHOOD IS -139.078437160

CALCULATIONS ARE BASED UPON EXTRA RISK

GLOBAL 86 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

| RISK | MLE DOSE | LOWER BOUND ON DOSE | CONFIDENCE LIMIT SIZE | COEFFICIENTS FOR CONFIDENCE LIMIT |
|---|---|---|---|---|
| 1.00000E-06 | 1.47682E-05 | 6.87734E-06 | 95.0% | Q( 0) = .23798<br>Q( 1) = .14541<br>Q( 2) = .00000 |

NORMAL COMPLETION!

# APPENDIX B

B. Calculation of Equivalent Human Dosage

Study: Two Year Chronic Toxicity and Oncogenicity (Bogdanffy, 1990)
**Species:** Charles River CD®BR Rat
**Sex:** Female
**Biological Endpoint:** Combined malignant mammary adenocarcinomas/carcinosarcomas

| GROUP | CONC.[N] (PPM) | ANIMAL DOSAGE (MG/KG/DAY) | EQUIVALENT HUMAN[a] DOSAGE (MG/KG/DAY) |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 1 | 0.053 | 0.0156 |
| 3 | 5 | 0.259 | 0.0764 |
| 4 | 25 | 1.37 | 0.404 |
| 5 | 50 | 2.81 | 0.829 |

a/ Equivalent human dosage based on scaling of body weight to the 3/4 power.
Average body weight for female rat = 0.414 kg.
Average body weight for female human = 55 kg.

**Sample calculation**

$$\frac{Dosage_A}{Dosage_H} \times \frac{BW_H}{BW_A} = \frac{BW_A^{3/4}}{BW_H^{3/4}}$$

$$Dosage_H = Dosage_A \times (BW_A / BW_H)^{1/4}$$

Example (Group 2):

$$Dosage_H = 0.053 \text{ mg/kg/day} \times (0.414 \text{ kg}/55 \text{ kg})^{1/4}$$
$$= 0.0156 \text{ mg/kg/day}$$

Thus,

$$\frac{Q_1 \text{ human}}{Q_1 \text{ rat}} = \frac{(\text{body weight, human})^{1/4}}{(\text{body weight, rat})}$$

$$Q_1 \text{ human} = Q_1 \text{ rat} \times (55/0.414)^{1/4}$$
$$= Q_1 \text{ rat} \times 3.4$$

# **CYANAZINE** (Bladex ®)

# DIETARY EXPOSURE AND ACUTE

# TOLERANCE ASSESSMENTS

Wesley C. Carr, Jr.

HEALTH ASSESSMENT SECTION

MEDICAL TOXICOLOGY BRANCH

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

April 16, 1996

## Cyanazine Dietary Exposure Analysis Summary

Cyanazine (40 CFR #180.307) Acute Tolerance assessment, Acute dietary and chronic non-oncogenic and oncogenic dietary exposure assessments were started and completed in 1994 (33, 34). All available cyanazine raw agricultural commodity (RAC) residue data were evaluated (Table 1). The 40 CFR 180.307 tolerance is characterized as cyanazine parent material alone without a toxicologically significant degradation product (5).

All of the residue monitoring programs do not sample and check for cyanazine. The Food and Drug Administration (FDA) and the California Department of Pesticide Regulation (DPR) monitoring programs analyze for the pesticide while the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) meat program does not monitor for cyanazine.

The FDA multiple residue screen minimum detection level (MDL) for parent material is 0.04 ppm for all RACs (14). There were no detected residues found on any RACs during the 1988 - 1992 FDA monitoring programs.

The DPR cyanazine parent material MDL was 0.2 ppm for the 1988 to 1992 years program's 1, 3, and 4 residue data. The consulted DPR programs were; a. priority pesticide program (program 1), b. preharvest program (program 3), and c. market basket surveillance (program 4). No cyanazine residues were detected on the current label approved RACs in any of the DPR programs in 1988, 1989, 1991 or 1992 (6, 7, 8, 9, and 10).

The USDA has not monitored for cyanazine and there has been no indication in the Program Residue Plan Annual that any change to begin monitoring is likely. The USDA Food Safety Inspection Service (FSIS) meat monitoring program MDL has not been established for cyanazine (37).

Dupont Chemical Company currently owns the herbicide compound cyanazine which it acquired from Shell Chemical. The Shell Chemical Company cyanazine product compound name used in all the submitted field residue studies is: Bladex$^{R}$ (Shell product number: SB15418) - cyanazine, chemical name: (2-[[4-Chloro-6-(ethylamino)-s-triazin-2-yl] Amino]-2-methylpropionitrile) (15...32). The potential residues on commodities from RACs treated with cyanazine at various label rates, including the maximum, were evaluated by Shell and reported in the submitted field studies. The registrant MDL for cyanazine was 0.01 ppm for all the submitted field studies that were cited (15...32). Registrant supplied degradation studies indicate that cyanazine residues in and on plant material (leaf surfaces, etc.) and animal tissues break down and do not concentrate in these fractions or tissues (26, 31, and 32).

All of the RAC residue data used for the cyanazine dietary exposure analysis were obtained from several years of field residue data supplied from the registrant commodity field studies. The FDA residue monitoring program data were used to validate that the registrant field data were representative and consistent with results found from the national channels of trade. EPA commodity tolerance values were not used in the dietary exposure analysis since adequate residue data were available for all of the assessed commodities. Table 2 contains a summary of relevant margin of safety data from conducting acute and chronic dietary exposure assessments.

### Primary RAC Residues (corn, cotton, sorghum, and wheat)

### Corn; fresh and grain

The U.S. EPA section 408 raw food tolerance for both fresh (sweet) and grain corn is 0.05 ppm. The registrant did not require a section 409 food or feed additive tolerance from the U.S.

EPA based on the registrant field residue data (5, 42). The current cyanazine use rates from the three product registrations call for no more than 6.5 pounds (lbs) active ingredient (a.i.) per acre for corn. There were 18 registrant field studies for corn residue data conducted in several states submitted to DPR (15, 17, 18, 23, 28 and 31). The residue values were generated from these registrant supplied data. The registrant supplied data reported residues lower than the level of detection (0.01 ppm). DPR and FDA pesticide monitoring programs data reported all non-detectable residue (6...10 and 14). One of the field trials included processed corn oil and meal data that were also below the limit of detection. The corn food form acute residue value of 0.01 ppm (MDL) was used in the dietary analysis since all data from the regulatory agency were non detectable and the registrant data at the limit of detection or lower had the lowest detection capability. The corn food form residue value of 0.005 ppm (1/2 MDL) was used in the chronic dietary analysis.

The FDA 1988 - 1992 U.S. domestic monitoring programs tested 383 corn or corn product (oil, meal, etc.) composite samples. There were no detected cyanazine residues found on any of the food form types during this period (14). The FDA MDL for cyanazine parent material is 0.04 ppm for corn. The DPR 1988 - 1992 state domestic monitoring programs MDL was 0.2 ppm for the 1988 to 1992 years program's 1, 3, and 4 residue data and no residues were detected (6...10).

**Table 1. Summary of Cyanazine Residues as of April, 1996.**

| RAC | Source (reference) | | Tolerance (ppm) | Residue Used (ppm) Acute | Chronic | N[2] | Additional Information |
|---|---|---|---|---|---|---|---|
| Corn, fresh | Reg-f[1] | (15, 17, 18, 23, | 0.05 | 0.01 | 0.005 | 44 | Registrant MDLs |
| Corn, grain | Reg-f | 28 and 31) | 0.05 | 0.01 | 0.005 | 44 | Registrant MDLs |
| Cottonseed, meal & oil | Reg-f | (16,24,25,27,30) | 0.05 | 0.01 | 0.005 | 37 | Registrant MDLs |
| Sorghum grain | Reg-f | (19) | 0.05 | 0.01 | 0.005 | 2 | Registrant MDLs |
| Water | DPR | (13 and 43) | N.A. | 0.0001 | 0.00005 | 1282 | DPR well monitoring data |
| Wheat grain | Reg-f | (20...22, 29) | 0.1 | 0.01 | 0.005 | 24 | Registrant MDLs |

1/ FDA = U.S. Food and Drug Agency, Reg-f = Registrant supplied field residue data, DPR = 1983-93 well monitoring data
2 N = The number of RAC composite samples analyzed from the selected submitted studies

## Cotton (meal and oil)

The U.S. EPA section 408 raw food tolerance for cottonseed is 0.05 ppm (5, 42). The current maximum cyanazine use rate is for no more than 6.5 pounds (lbs) active ingredient (a.i.) per acre for cotton. This rate maximum includes preplant, preemergence, and post emergence layby applications of cyanazine for any single calendar year. There were 20 registrant submitted cotton field studies available to DPR. The residue values were generated from registrant field studies indicating no detectable residues at 0.01 ppm (MDL) for the acute or 1/2 MDL for the chronic analyses (16, 24, 25, 27 and 30). Additional collected data recording non-detected residues but with higher MDLs from the FDA pesticide monitoring programs were supportive (14). There were no detected cyanazine residues found on or in the cotton products examined in the registrant field studies or FDA programs.

The 14 registrant field studies each examined two (or more) composited samples for residues at 4.0 or more lbs a.i. per acre (16, 24, 25, 27 and 30). The residue value of 0.01 ppm (MDL) was used in the acute dietary analysis. The value of 0.005 ppm (1/2 MDL) was used in the chronic dietary analysis. The FDA 1988 - 1992 U.S. domestic monitoring programs tested 8 cottonseed product samples. There were no detected residues found during this period (14). The FDA MDL for cyanazine is 0.04 ppm for the RAC cottonseed. The DPR 1988 - 1992 state programs did not select and test samples of cottonseed products for cyanazine residues.

**Sorghum**

The U.S. EPA section 408 raw food grain sorghum tolerance is 0.05 ppm. The registrant did not require a section 409 food or feed additive tolerance from the U.S. EPA based on the registrant field residue data (5, 42). The current cyanazine use rates from the three product registrations are for a maximum of 4.6 lbs a.i. per acre but in all cases not to exceed 6.5 lbs a.i. per acre for any calendar year from all sources. The residue values were generated from the registrant supplied data (19). There were no detected cyanazine residues on grain sorghum in the FDA program (14).

The single registrant supplied field study data was examined for cyanazine residues using the two composited samples representing the 1.6 lbs a.i. per acre application rate. There were no detectable residues found in the study. The residue value of 0.01 ppm (MDL) was used in the acute dietary analysis. The value of 0.005 ppm (1/2 MDL) was used in the chronic dietary analysis.

The FDA residue data was used to supplement the information obtained from the registrant field trial data in the dietary analysis. The FDA 1988 - 1992 U.S. domestic monitoring programs tested 5 sorghum product samples. There were no detected cyanazine residues found on the food form products during this period using the FDA MDL for cyanazine of 0.04 ppm for sorghum (14). The DPR 1988 - 1992 state programs did not test samples of sorghum grain for residues (6, 7, 8, 9, and 10).

**Wheat**

The U.S. EPA section 408 raw food wheat grain tolerance is 0.1 ppm. The registrant did not require a section 409 food or feed additive tolerance from the U.S. EPA based on the registrant field residue data (5, 42). The current cyanazine use rate from the single product with a wheat registration is for a maximum of 6.0 lbs a.i. per acre from both wheat preplant and fallow crop land applications in the same growing cycle, but in all cases 6.5 lbs a.i. per acre from all sources should not be exceeded for any calendar year. The residue values were generated from the registrant supplied data (20, 21, 22 and 29). In addition, there were no detected cyanazine residues found on wheat products in the FDA monitoring program (14).

There were 9 registrant supplied field studies submitted. Nine registrant studies that included the labeled application rate of 1.6 lbs a.i. per acre or greater were examined for cyanazine residues. There were not any detectable residues found in the studies (20, 21, 22 and 29). The residue value of 0.01 ppm (MDL) was used in the acute dietary analysis. The value of 0.005 ppm (1/2 MDL) was used in the chronic dietary analysis.

The FDA residue data was used to supplement the information obtained from the registrant field trial data in the dietary analysis. The FDA 1988 - 1992 U.S. domestic monitoring programs tested 42 wheat product samples. There were no detected cyanazine residues found on the food forms during this period using the FDA MDL of 0.04 ppm (14). The DPR 1988 - 1992 domestic state monitoring programs tested wheat for residues. There were no detectable residues of cyanazine on wheat reported. The DPR cyanazine parent material MDL was 0.2 ppm for the 1988 to 1992 years program's 1, 3, and 4 residue data (6, 7, 8, 9, and 10).

**Secondary RAC Residues**

**Milk, Eggs and Meats**

A summary of the secondary residue data for other RACs: Beef, all tissues, eggs, goat, horse, pork, poultry, and sheep is not required and therefore does not appear in Table 1 because there are no current secondary tolerances. Residues of cyanazine do not concentrate in animal

tissues or animal products, such as milk or eggs, at a level to require any separate secondary RAC commodity tolerances (31, 32). Also there were no adjustments required to be made to the sources of the animal feed (41).

**Water**

There is not a currently established U.S. EPA section 408 or 409 food or feed additive tolerance established for water. The U.S. EPA has established a health advisory (HA) for cyanazine of 1 ppb in drinking water (43, 44). Cyanazine products have been detected in the surface and ground water of several mid-western states (43, 44). The DPR has conducted surveys of California's ground water to look for and measure cyanazine residues (13). Based on the concerns of potential cyanazine contaminated water being consumed and used in the preparation of foods (commercial and homeowener reconstitution of frozen juices, etc), DPR has included dietary exposure scenarios which present the potential exposures of diets with and without the addition of cyanazine containing water.

The results of a comprehensive DPR California well water cyanazine residue survey reported that there were no detected residues found between 1983 and 1993. The highest limit of detection (LOD) used during these analyses was 0.1 ppb. There were 1111 wells from 24 counties sampled during this period with a total of 1282 analyses performed without a single detected cyanazine residue (13). The acute (0.1 ppb = LOD) and chronic (0.05 ppb = 1/2 LOD) cyanazine residue values used in one of the DPR presented dietary scenarios is likely an overestimation of the amount of cyanazine found in the California water supply. California drinking water comes from many sources; surface sources (rivers and lakes), agricultural and non agricultural use wells. Many California municipalities blend water from multiple sources, ground and surface water, and also same source (wells) assets. These uncertainties and likely overestimation of cyanazine levels in the state of California's drinking water, DPR did use these values (non-detects) to provide a starting point for dietary estimation of exposure from water.

**Dietary Exposure Summary (Acute and Chronic)**

The acute exposure values resulting from the use of the cyanazine NOEL of 1 mg/kg/day were examined and the results are found in Table 2. The values varied depending on whether the contribution from water exposure were included. The acute exposures without any water contribution ranged from 0.000038 mg/kg/day, Seniors $55^{+}$ Years (cyanazine MOS: 26,312) to 0.000160 mg/kg/day, Non-Nursing Infants, less than 1 Year (cyanazine MOS: 6,265). The acute dietary exposure that included water contribution ranged from 0.000041 mg/kg/day, Seniors $55^{+}$ Years (MOS: 24,220) to 0.000176 mg/kg/day, Non-Nursing Infants, less than 1 Year (MOS: 5,685).

The chronic non-oncogenic dietary exposure values obtained from using a NOEL of 0.2 mg/kg/day were examined (Table 2). There were two chronic exposure scenarios. The first consisted of dietary exposure data without the use of any percent of the crop treated adjustments. The second has the chronic dietary exposure data modified with percent of the crop treated adjustments based on CDFA, DPR, and USDA data. These were further subdivided by the inclusion of dietary exposure from water contribution. The chronic exposures without any water contributions ranged from 0.000004 mg/kg/day, Nursing Infants (MOS: 48,114) to 0.000031 mg/kg/day, Children 1-6 Years (MOS: 6,442). The chronic exposures without water contribution but modified with the percent of the crop treated adjustments ranged from 0.000001 mg/kg/day, Nursing Infants (MOS: 185,892) to 0.000006 mg/kg/day, Children 1-6 Years (MOS: 31,616).

The chronic exposures with the addition of water contributions ranged from 0.000006 mg/kg/day, Nursing Infants (MOS: 35,465) to 0.000033 mg/kg/day, Children 1-6 Years (MOS:

6,011). The chronic exposures with water contribution and also modified with the percent of the crop treated adjustments ranged from 0.000003 mg/kg/day, Nursing Infants (MOS: 78,172) to 0.000011 mg/kg/day, Non-Nursing Infants < 1 Year (MOS: 18,955).

The chronic oncogenic dietary exposure values for the U.S. Population (all) are presented in Table 2. The cancer risk from chronic exposure to cyanazine was determined and the cancer potency Q1* value of 0.58 was used (Table 2). The chronic dietary exposure risk, without including water consumption data, ranged from 1.5E-06 for percent of the crop treated exposures to 7.7E-06 for the U.S. population without any modifications. The chronic dietary exposure risk, using data modified with water consumption values, ranged from 2.3E-06 for percent of the crop treated exposures to 8.5E-06 for the U.S. population without any modifications.

**Table 2. Dietary Exposure, Margin of Safety[a] and Risk from Cyanazine Residues on Raw Agricultural Commodities.**

| Population | Exposure ($\mu$g/kg/day) | Margin of Safety | Q1* Risk[b] | Additional Information |
|---|---|---|---|---|
| **ACUTE** (Using Cyanazine Acute NOEL {1.0 mg/kg body-wt/day} | | | | |
| No Water Codes | | | | |
| Children (1-6 Years) | 0.132 | 7,555 | N.A. | **Q1*** shown for chronic |
| Infants (non-nursing, <1 year) | 0.160 | 6,265 | N.A. | exposure only |
| Infants (nursing, <1 year) | 0.102 | 9,848 | N.A. | |
| U.S. Population, all | 0.074 | 13,464 | N.A. | |
| Water Codes Added | | | | |
| Children (1-6 Years) | 0.138 | 7,239 | N.A. | **Q1*** shown for chronic |
| Infants (non-nursing, <1 year) | 0.176 | 5,685 | N.A. | exposure only |
| Infants (nursing, <1 year) | 0.066 | 15,132 | N.A. | |
| U.S. Population, all | 0.078 | 12,883 | N.A. | |
| **CHRONIC** (Using Cyanazine Chronic NOEL {0.2 mg/kg body-wt/day}) | | | | |
| No Water Codes | | | | |
| No Percent Crop Treated Adjustment | | | | |
| Children (1-6 Years) | 0.031 | 6,442 | | Chronic **Q1*** results shown |
| Infants (non-nursing, <1 year) | 0.018 | 11,303 | | for U.S. Population, all |
| Infants (nursing, <1 year) | 0.004 | 48,114 | | DPR **Q1*** value was 0.58 |
| U.S.Population, all | 0.013 | 15,045 | 7.7E-06 | |
| **CHRONIC** (Using Cyanazine Chronic NOEL {0.2 mg/kg body-wt/day}) | | | | |
| No Water Codes (continued) | | | | |
| Percent Crop Treated Adjustment factor used | | | | |
| Children (1-6 Years) | 0.006 | 31,616 | | Chronic **Q1*** results shown |
| Infants (non-nursing, <1 year) | 0.005 | 43,103 | | for U.S. Population, all |
| Infants (nursing, <1 year) | 0.001 | 185,892 | | DPR **Q1*** value was 0.58 |
| U.S. Population, all | 0.003 | 75,108 | 1.5E-06 | |

(Continued)

**Table 2. Dietary Exposure, Margin of Safety[a] and Risk from Cyanazine Residues on Raw Agricultural Commodities. (Continued)**

| Population | Exposure ($\mu$g/kg/day) | Margin of Safety | Q1* Risk[b] | Additional Information |
|---|---|---|---|---|
| **CHRONIC** (Using Cyanazine Chronic NOEL {0.2 mg/kg body-wt/day}) | | | | |
| Water Codes Added | | | | |
| No Percent Crop Treated Adjustment | | | | |
| Children (1-6 Years) | 0.015 | 13,650 | | Chronic **Q1*** results shown |
| Infants (non-nursing, <1 year) | 0.024 | 8,472 | | for U.S. Population, all |
| Infants (nursing, <1 year) | 0.006 | 35,465 | | DPR **Q1*** value was 0.58 |
| U.S.Population, all | 0.015 | 13,650 | 8.5E-06 | |
| Water Codes Added (continued) | | | | |
| Percent Crop Treated Adjustment factor used | | | | |
| Children (1-6 Years) | 0.009 | 23,381 | | Chronic **Q1*** results shown |
| Infants (non-nursing, <1 year) | 0.011 | 18,955 | | for U.S. Population, all |
| Infants (nursing, <1 year) | 0.003 | 78,172 | | DPR **Q1*** value was 0.58 |
| U.S. Population, all | 0.004 | 49,734 | 2.3E-06 | |

a: Acute margin of safety values taken from the 95th percentile of consumption. Means represent the chronic values.
b: $Q1^*$ risk represents additional tumor potency in $(mg/kg/day)^{-1}$

**Special Crop Adjustment Factors and Usage data**

**Usage data**

There are currently three active registrations of cyanazine approved for use in California. All of the registrations are for agricultural uses. These products are exclusively used as herbicides for general weed control. There are two Dupont products; Bladex 4L and Bladex 90DF and one Ciba Geigy product; Cycle. The cyanazine percent active ingredient ranges from 22% for Cycle, which also contains 22% Metolachlor, to 90% active for Bladex 90DF. There is no crop pre-harvest interval (PHI) required for the three registrations because they are applied primarily as preemergence herbicide products. There were a total of 288,415 pounds of cyanazine applied in California during the 1991 season (11). A total of 348,645 pounds were applied in California during the 1992 season (12).

**Crop Adjustment Factors**

The current DPR chronic dietary exposure analysis default assumption is that 100% of any crop is treated with the pesticide under consideration. When quality data are available that indicate that less than 100% of a commodity is treated with a specific pesticide, then on an individual commodity by pesticide combination basis, exceptions to the default assumptions can be made. The assumption that 100% of the crop is treated with and will contain averaged

residues for up to 70 years is unrealistic. Using the existing percent crop treated data, it is reasonable to revise the 100% treated assumption downward using more realistic pesticide treatment and use patterns. This method has been employed as an additional comparison for 4 commodities that have cyanazine tolerances. These commodities; sweet corn, grain corn, cottonseed, and wheat all have detailed cyanazine use histories at both the state, DPR 100% Pesticide Use Report (11, 12), and the federal, USDA Ag Field Crops Summary annuals (35, 36, 38, 39 and 40), levels. Very conservative assumptions were made when setting the percentage of crop treated adjustment factors for the chronic dietary exposure section for these commodities. Multiple years of cyanazine use and acreage harvested data were evaluated at the state and federal level.

**Corn (grain and sweet)**

The California grain corn acreage harvested during the 1990 season totaled 160,000 acres, for 1991 it was 115,000 and in 1992 it was 145,000 acres (2, 3, and 4). The total harvested California grain corn acreage constitutes less than 1% of the total annual U.S. grain corn production. Cyanazine was applied to 9,582 acres of California grain corn in 1991 (8%) and 7,290 acres in 1992 (5%) (11, 12). The United States grain corn acreage (17 major production states) harvested during 1990 was 74,171,000 acres, 1991 was 68,580,000, 1992 was 71,375,000 acres and for 1993 it was 65,700,000 (35, 36, 38 and 40). Based on USDA Agriculture Marketing Statistics division data, Cyanazine was applied to 18% of the 1990 acreage, 19% of the 1991 acreage, 20% of the 1992 acreage and for 1993 it was applied to 20% of grain corn acreage in the 17 major production states.

Based on the U.S. grain corn use information, a 20% crop adjustment factor could be used for grain corn. The actual DPR selected adjustment factor used in the chronic dietary residue TAS file is 30% of the crop treated. The 30% crop adjustment factor means that the DPR chronic dietary exposure analysis will assume, derived from California and U.S. use data, that at least 70% of the U.S. grain corn crop is not treated with cyanazine in a season and therefore would not be expected to have any residues. The actual use data indicates that at least 80% of the grain corn crop is not treated, however the 30% adjustment value is conservative and reflects the consideration of the less defined use patterns that may exist in the minor grain corn production states.

The production acreage of sweet corn, both fresh and fresh processed combined, in the United States (11 and 7 major states respectively) totaled 640,400 for 1992 (39). Only data for the 1992 U.S. crop production are available. The fresh sweet corn major production states are California, Florida, Georgia, Ilinois, Michigan, New Jersey, New York, North Carolina, Oregon, Texas, and Washington. The processed fresh sweet corn major production states are Ilinois, Michigan, Minnesota, New York, Oregon, Washington, and Wisconsin (1, 3, and 38).

Fresh sweet corn production totaled 154,100 acres for the 11 major sweet corn states in 1992. Cyanazine herbicide was applied to 7% of the total U.S. fresh sweet corn acres harvested from the 11 major states surveyed (CA included). Harvested 1992 California fresh sweet corn totaled 16,500 acres based on the USDA data. Cyanazine was applied to about 44% of the California acreage in 1992 (12, 39). California production of fresh sweet corn represented 11% of the 11 major U.S. states production totals for 1992.

The total 1992 U.S. production of processed sweet corn amounted to 486,300 acres from the 7 major production states. Cyanazine use was reported by the USDA to total 50% of the major production states processed sweet corn acreage. California was not one of the 7 primary processed sweet corn production states (1, 39).

Based on the 1992 USDA data, 26% of fresh sweet corn acreage was treated with cyanazine, a higher value than the percentage for processed sweet corn. The fresh and

processed sweet corn acreage will be combined together and the composite value of 26% crop treated will be used as the representative value. The actual selected crop adjustment factor used in the chronic dietary exposure residue file will be 30%. This means that for the combined fresh and processed sweet corn acreage the DPR chronic dietary exposure analysis will assume that at least 70% of the total annual U.S. crop will not have received any cyanazine herbicide treatments. Actual use indicates that up to 7% of the fresh and 26% of the processed sweet corn crop is treated however, the 30% crop treated adjustment value is conservative and reflects the considerations that cyanazine use patterns may be less defined in the minor sweet corn production states and also the availability of only a single year of U.S. data (1992).

**Cotton**

The total planted California cotton acreage during 1991 was 1,041,000 and for 1992 it was 1,105,000 acres (2, 3, and 4). The California cotton acreage represents approximately 10% of the total annual U.S. cotton production (35, 36, 38 and 40). Cyanazine was applied to 131,374 acres of cotton in 1991 and 179,571 acres in 1992 (11, 12). The DPR agricultural statistics information indicates that cyanazine was applied to 16% or less of the California cotton acreage during the previous two years. The United States cotton acreage is produced primarily in six states; Arizona, Arkansas, California, Louisiana, Mississippi, and Texas. Production during 1990 was from 9,830,000 acres, 10,900,000 acres in 1991, 10,100,000 acres in 1992, and during 1993, 10,130,000 acres (35, 36, 38 and 40). Based on USDA Agriculture Marketing Statistics and DPR data, cyanazine use consistantly ranged from 15% - 21% of the 1990 -1993 acreage in the 6 major production states (11, 12, 35, 36, 38 and 40). Derived from this cotton use data, a 30% crop adjustment factor will be used for cotton in the chronic dietary residue TAS file. The actual use data indicates that on average 80% of the national cotton crop is not treated.

**Sorghum**

There was no reported treatment of California grown sorghum with cyanazine during either the 1991 or 1992 seasons (11, 12). Total California sorghum planted acreage data were not available. Also, there were no statistics for the United States sorghum planted acreage available either. Based on the absence of U.S. use data, no percent crop adjustment factor was used in the chronic dietary residue TAS file.

**Wheat**

The California wheat acreage harvested during 1991 totaled 442,000 acres and for 1992 it was 605,000 acres (2, 3, 4). The harvested California wheat acreage amounts to an average of approximately 1% of the total annual U.S. wheat harvest. Cyanazine was applied to 1,304 acres of California wheat in 1991 and 65 acres in 1992 (11, 12). The United States wheat acreage (12 major production states) harvested during 1990 was 58,950,000 acres, 1991 was 56,720,000, 1992 was 55,890,000 acres, and for 1993 it was 56,120,000 acres. Based on USDA Agriculture Marketing Statistics division information cyanazine was applied to 1% or less of the wheat acreage in the 12 major producing states (35, 36, 38 and 40). Based on this U.S. wheat use information, a 10% crop adjustment factor will be used for wheat in the chronic dietary residue TAS file. The 10% crop adjustment factor means that the DPR chronic dietary exposure analysis will assume, derived from use data, that at least 90% of the U.S. wheat crop is not treated with cyanazine. The actual use data indicates that 99% of the wheat crop is not treated, however the 10% adjustment value is conservative and takes into consideration less defined use patterns that may be found in the minor wheat production states.

**Acute Tolerance Assessment**

An acute tolerance assessment was performed for cyanazine using the current U.S. EPA tolerances. The cyanazine acute NOEL of 1.0 mg/kg-body wt/day was used in the examination of all the grain and seed-meal RACs with cyanazine tolerances. There are only five human consumption RACs having cyanazine tolerances (9). The current U.S. EPA tolerances have not changed from the values listed in the Registration Standard document (9, 42).

All margins of safety were greater than 1500 when using the cyanazine acute NOEL value of 1.0 mg/kg/day. The highest Theoretical Maximum Residue Contribution (TMRC) exposure was 0.000665 mg/kg-bw which occurred in the Children 1-6 Years population from potential wheat (all sources) consumption. The highest MOS was obtained from the sorghum tolerance Females $20^{+}$ Years, not pregnant, not nursing population with a value of 287,000.

**Table 3. Margin of Safety and Acute Tolerance Level Exposures from Cyanazine.**

| **Commodity** | Consumption Estimate % | Tolerance (in ppm) | TMRC $95^{th}$ % (mg/kg-bw) | Theoretical MOS $95^{th}$ % |
|---|---|---|---|---|
| Corn, fresh | 18% | 0.05 | 0.000106 - 0.000414 | 2,400 (B) - 9,400 (D) |
| Corn, grain | 100% | 0.05 | 0.000062 - 0.000537 | 1,900 (A) - 16,000 (C) |
| Cottonseed | 97% | 0.05 | 0.000006 - 0.000016 | 63,000 (A) - 161,000 (E) |
| Sorghum | 1% | 0.05 | 0.000003 - 0.000037 | 27,000 (F) - 287,000 (E) |
| Wheat | 100% | 0.1 | 0.000216 - 0.000665 | 1,500 (B) - 4,600 (C) |

**Population Subgroups Key:** **A**= Non-Nursing Infants, **B**= Children (1-6 Years), **C**= Seniors (55+ Years), **D**= Females (13+ Years/Nursing), **E**= Females (20+ Years/Not Pregnant/Not Nursing), **F**= Males (20+ Years)

April, 1996

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# ASSESSMENT OF HUMAN EXPOSURE TO CYANAZINE

by

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HS-1526

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## SUMMARY

Cyanazine is under review because of teratogenic and reproductive effects in laboratory animals. In addition, it has been demonstrated to have carcinogenic potential in a rat feeding study where mammary tumors were in excess of the controls in female rats. Because of the results of the toxicology studies, cyanazine has been the subject of two U.S. EPA special reviews. After the first review by the U.S. EPA, completed in 1984, a few additional requirements concerning the use of protective equipment while handling cyanazine were added to the label. Currently, cyanazine is in special review at the U.S. EPA because of worker exposure issues, its presence in ground and surface-derived drinking water in the Midwest and some concerns about dietary exposure. Reports of worker illness have been low in California. Dermal absorption of cyanazine has been investigated in rats and determined to be about 2%. Workers handling cyanazine during ground boom, post-emergent applications to control weeds in cotton with hand pour and either open or closed cabs, are estimated to absorb 2.6 ug/kg bw (geometric mean) of cyanazine per day. This report on cyanazine will be included as Volume 2 in the cyanazine risk characterization document.

VOLUME 2

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
WORKER HEALTH AND SAFETY BRANCH

HUMAN EXPOSURE ASSESSMENT

# CYANAZINE

Revised May 21, 1996

## INTRODUCTION

Cyanazine, (2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-yl]amino]-2-methylpropionitrile (EPA Reg. No 21725-46-2), is a pre- and post-emergent herbicide used primarily for the control of weeds in field corn in the Midwest and in cotton in California. The toxicological justifications for development of this exposure assessment are described in Volume 1 of the Risk Characterization Document.

## PHYSICAL PROPERTIES

Some physical properties of cyanazine are listed in below:

| Physical Property[a/] | Value |
|---|---|
| Melting Point (°C) | 167.5-169 |
| Vapor Pressure (nPa, 20 °C) | 200 |
| Water Solubility (mg/L) | 171 |
| Octanol/Water ($K_{ow}$) | 126 |

[a/] Tomlin, 1995

## REGULATORY STATUS

The U.S. EPA indicated that a special review for cyanazine, atrazine and simazine was in progress through publication in the Federal Register Notice of November 23, 1994, Volume 59:60412-60443. This review was initiated due to concerns about adverse toxicology outcomes in animal studies and human exposure (occupational and non-occupational). To date, this review has not been completed. An earlier review by the U.S. EPA, completed in 1984, resulted in some additional statements on the label regarding the toxicology, as well as, a requirement for protective equipment for handlers of products containing cyanazine.

## REGISTERED PRODUCTS

Only two products containing cyanazine are presently registered in California: a ready-to-use liquid/solution containing 43 percent active ingredient (a.i.) and a dry flowable containing 90 percent a.i. The water liquid formulation is the predominantly used formulation in California.

## USAGE

In the United States, the major use of cyanazine is for the control of weeds in field corn. In California, however, application to control weeds in cotton is the most significant use. Since cyanazine is a federally-restricted use pesticide, all applications must be reported and made by licensed applicators. In California, applications of cyanazine are restricted to ground equipment. The use (rounded to the nearest pound) of cyanazine in California in 1990-1993 is compiled in Table 1 (ISB, 1991, 1993, 1994 and 1995).

**Table 1:** Cyanazine Use in California in 1990-1993

| | Pounds Used | | | |
|---|---|---|---|---|
| **Application Site** | **1990** | **1991** | **1992** | **1993** |
| Cotton (General) | 342,757 | 242,735 | 302,384 | 444,878 |
| Corn (Human Consumption) | 29,632 | 22,499 | 12,347 | 6,065 |
| Corn (All other) | 5,723 | 19,514 | 29,512 | 48,776 |
| Uncultivated Agric. Land | 3,764 | 960 | 3,614 | 8,324 |
| Wheat (General) | 1,097 | 2,199 | 77 | 162 |
| Other | 211 | 508 | 731 | 0 |
| TOTAL | 383,184 | 288,415 | 348,645 | 508,205 |

Sanborn, WH&S, 1996

Since data for four years of full-use reporting of pesticides in California are available, it is possible to determine whether there has been a significant change in the use during this time period (Table 1). These data indicate there was an increased use in 1993, above the previous three years. In each year, application to cotton comprised the major use, and in 1993, it constituted nearly 88% of the use of cyanazine.

A useful parameter for exposure assessment in addition to the annual trends discussed above, is the relationship between the number of acres of cotton treated and the number of acres of cotton harvested in California. These data are presented in Table 2.

**Table 2:** Use of Cyanazine in California on Cotton: 1990-1993

| Year | Acres Treated[a/] | Acres Harvested[b/] | Percent Treated |
|---|---|---|---|
| 1990 | 176,435 | 1,224,438 | 14.4 |
| 1991 | 131,373 | 1,230,423[c/] | 10.7 |
| 1992 | 179,571 | 1,204,686 | 14.9 |
| 1993 | 229,876 | 1,262,146 | 18.2 |

[a/] ISB, 1991, 1993, 1994, 1995
[b/] ASB - Reports for 1990, 1992 and 1993
[c/] Data for 1991 not available, estimate based on average of 1990, 1992, and 1993

Sanborn, WH&S, 1996

The mean value for the percentage of the cotton acreage treated was 14.5%. There are two conclusions that can be drawn from these data. The first is not all of the cotton acreage in a given year is treated with cyanazine. This supports the thesis that it is not used as a pre-plant, prophylactic treatment, but rather as a treatment for weeds that have escaped other weed control measures. The observation that about 14.5% of the cotton acreage is treated suggests that the number of workers potentially exposed is less than if all of the acreage were treated. The second conclusion is that the percent acres treated can be quite variable, more than 50% (10.7-18.2%), reflecting the variability in need as a post-emergent application foliar treatment.

Table 3 lists use rates for cyanazine as stipulated by the label, actual use in the rest of the country and as used in cotton in California. The California applications in cotton are post-emergent, directed sprays by ground application. Aerial applications in this crop obviously will not provide the necessary application site selectivity (cotton plants and weeds present in the field) and are not allowed. Given the timing of the application in cotton, other cultural activities, such as insect scouting, which may occur after the cyanazine application, will not result in significant worker exposure to cyanazine.

**Table 3:** Recommended and Typical Rates of Cyanazine Application (Pounds of Active Ingredient per Acre)

| | Label Rates | | Typical Use Rates | |
|---|---|---|---|---|
| Crop | Minimum | Maximum | U.S. | California |
| Corn | 0.62 | 6.0 | 2.5[a/] | |
| Sorghum | 0.8 | 3.2 | 1.5[a/] | |
| Wheat (fallow) | 1.6 | 4.0 | 2.8[a/] | |
| Cotton | 0.75 | 2.0 | | 1.7[b/] |

[a/] United States, Current Label and Reregistration Document, 1984
[b/] ISB, 1992

Sanborn, WH&S, 1996

Since cyanazine is a herbicide, the primary focus for estimation of worker exposure will be mixing/loading and applying of cyanazine to control weeds in cotton.

## LABEL PRECAUTIONS

Signal Word: **WARNING**

**MAY BE FATAL IF SWALLOWED. HARMFUL IF INHALED OR ABSORBED THROUGH THE SKIN. CAUSES TEMPORARY EYE INJURY. THIS PRODUCT MAY BE HAZARDOUS TO YOUR HEALTH. IT IS CLASSIFIED 'RESTRICTED USE' BECAUSE, AT DOSES WHICH CAUSED SERIOUS MATERNAL ILLNESS IN LABORATORY ANIMALS, BIRTH DEFECTS WERE PRESENT. USE OF PROTECTIVE CLOTHING AND EQUIPMENT AND FOLLOWING THE PRECAUTIONS BELOW CAN REDUCE RISK.**

**AVOID BREATHING SPRAY MIST. AVOID CONTACT WITH SKIN, EYES, OR CLOTHING. DO NOT GET IN EYES OR ON CLOTHING. WEAR A FACE SHIELD WHEN MIXING AND LOADING. WASH THOROUGHLY WITH SOAP AND WATER AFTER HANDLING AND BEFORE EATING OR SMOKING.**

**APPLICATORS AND OTHER HANDLERS MUST WEAR:**

- **LONG PANTS AND LONG-SLEEVED SHIRT;**
- **CHEMICAL-RESISTANT GLOVES, SUCH AS BARRIER LAMINATE OR BUTYL RUBBER OR NITRILE RUBBER OR POLYVINYL CHLORIDE OR VITON OR NEOPRENE RUBBER;**
- **CHEMICAL-RESISTANT FOOTWEAR PLUS SOCKS**
- **PROTECTIVE EYEWEAR**
- **CHEMICAL RESISTANT APRON WHEN CLEANING EQUIPMENT, MIXING OR LOADING.**

## ILLNESS REPORTS

Illness or injury attributed to exposure to cyanazine has not been reported in California during the past ten years. (Mehler, 1995).

## DERMAL TOXICITY

No acute systemic toxicity was demonstrated following dermal cyanazine application at rates up to 2,000 mg/kg. Skin irritation was mild to moderate, depending on formulation, and guinea pig sensitization tests were negative (Tomlin, 1995).

## DERMAL PENETRATION

Two sets of dermal absorption experiments have been reported using rats. The first, a 10-hour dermal penetration study, using nominal doses of 0.5, 5 and 50 mg/rat. Interpretation of this work was complicated by poor and variable recovery (Logan, 1986a). Subsequent investigation demonstrated that dilute suspensions in water were not sufficiently homogeneous to deliver low doses reliably (Mueller and Logan, 1986). Subsequent investigation also indicated that more of the dose was removable from the skin by careful washing than had originally been reported. The second study used a single 50 mg dose that was applied to 12 $cm^2$ of shaved skin. This study was continued for eight days, though the skin was washed after 10 hours (Mueller, 1986b). There were 4 animals per sacrifice period and the time periods for the sacrifice after treatment were 0.5, 2, 4, 10, 24, 48, 72, 120, 192 hours.

The dose of 50 mg applied to 12 $cm^2$ (~4200 $ug/cm^2$) is much higher than was measured during the exposure monitoring study, if the dose is assumed to be evenly distributed over the body surface area of a mixer/loader/applicator. However, since 80-90% of the exposure is on the hands (Green, 1985), the dose applied to rats is more in line with the worker's hand exposure during the handling of cyanazine.

Based on the data for urine and fecal excretion in Table 4, a dermal absorption value of 1.80% is estimated from the curve at infinite time where the slope of the excretion curve is zero. This value does not include the amount of residue in the carcass and blood at the time of the final sacrifice. Addition of the skin and carcass data (0.12%) to 1.80% at the time when the slope of the excretion curve is zero, yields a total dermal absorption of 1.92%. The advantage of this kinetic method over a point-estimate at some time interval (*e.g.*, 24 hours), is that all of the data points are used in the calculation of a dermal absorption value. A graphical representation of these data are shown in Figure 1 (placed after the references). The kinetic method of estimation of dermal absorption also takes into account the pesticide still residing in

the skin at the end of the experiment. Thus the pesticide may continue to serve as a reservoir for absorption. Since the value of 1.92% from the kinetic method is similar to the 24-hr point-estimate of 2.0%, the latter value will be used in the calculation of exposure. In the case of cyanazine, the kinetic method for estimating dermal absorption provided almost the same point-estimate value at 24 hours.

**Table 4:** Cumulative Dermal Absorption of Cyanazine in Rats Dosed at 4,200 ug/cm$^2$

| Time Post Application (hr) | Cumul. Absorption(%) Urine + Feces | Tissue Residues[a/] % Applied Dose |
|---|---|---|
| 0.5 | 0 | 0.02 |
| 2 | 0.0015 | 0.05 |
| 4 | 0.010 | 0.13 |
| 10 | 0.046 | 0.13 |
| 24 | 0.097 | 0.06 |
| 48 | 0.017 | 0.03 |
| 72 | 0.29 | 0.05 |
| 120 | 0.49 | 0.06 |
| 192 | 0.69 | 0.12 |

[a/] Blood, carcass only; does not include skin site of application

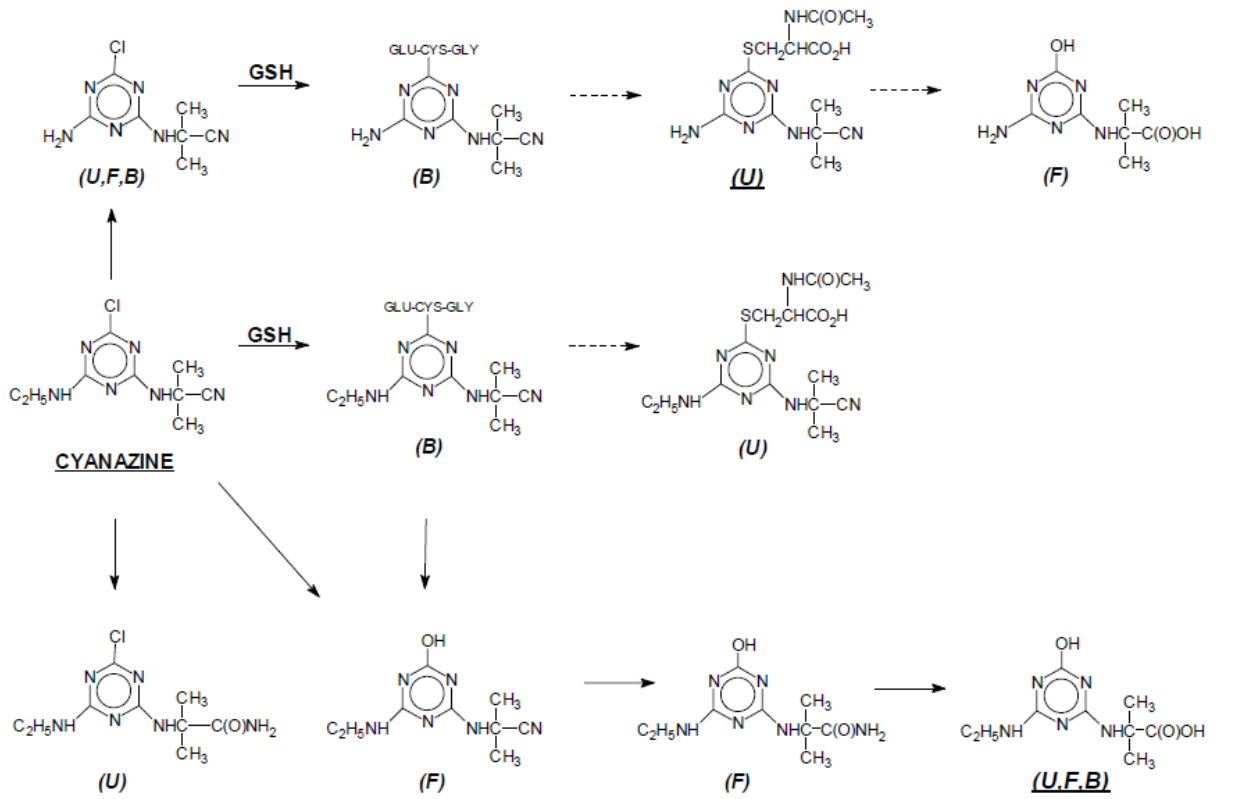
Sanborn, WH&S, 1996, after Logan, 1986b

**METABOLISM**

Cyanazine is metabolized by the following mechanisms: hydrolysis of the chlorine to a hydroxyl; dealkylation (removal of the ethyl leaving an amine); and hydrolysis of the nitrile to an amide and then finally to a carboxylic acid. In addition, cyanazine is conjugated in rats through the glutathione pathway to ultimately yield a mercapturate that is excreted in the urine. All of these transformations have been demonstrated to occur individually and in combination following oral administration of cyanazine to rats (Galley, 1985) and to cows (Beynon *et al*., 1970). The metabolite pattern of cyanazine in rats is shown below (taken directly from the work of Hutson *et al.*, 1970 and Crayford and Hutson, 1972).

Recovery following oral administration of labeled cyanazine to rats varied from 93 to 107 percent after four days. Of that amount, three or four percent remained in the carcass. Distribution among the organs was not specified. About the same amount was recovered from urine as from feces. This held true whether the $^{14}C$ label was in the triazine ring or the isopropyl or nitrile substituents. When the *N*-ethyl was labeled, half the labeled carbon was recovered as expired $CO_2$ within four days. The majority of the urinary metabolites undergo *N*-de-ethylation, but not oxidation or hydrolysis. The fecal metabolites included evidence of *N*-de-ethylation, oxidation and hydrolysis, singly and in combination, and were less likely to be conjugated than the urinary metabolites. In these respects, the metabolites found in feces resembled the plant and soil metabolites more than they resembled the urinary metabolites.

Two metabolites that had undergone all of the degradative mechanisms described, and the one that retained the *N*-ethyl but had undergone hydrolysis and oxidation of the nitrile to a carboxyl, were fed to rats. Recovery in these experiments was excellent (85-95 percent), and excretion was primarily in feces, though appreciable amounts were found in urine. In both cases, the metabolite fed to the rats was the only chemical species recovered.




blood (B), feces (F) and urine (U).

No residues were detectable in the tissues of cows fed cyanazine at 0.2 or 4.6 ppm or in the tissues or eggs of hens fed cyanazine at 0.3 or 1.0 ppm (Beynon, 1972). The highest tolerance for cyanazine, in or on corn fodder or corn forage, is 0.2 ppm. At the 4.6 ppm level in feed, the *N*-de-ethylated metabolite was found in cow's milk at 0.04 ppm. Cows were also fed two *N*-de-ethylated metabolites at 0.3 ppm and one *N*-de-ethylated metabolite at 8.8 ppm. No residues were detected at the low level. At the high level, unchanged metabolite was found in the milk at 0.03-0.07 ppm. The feeding studies in cows and chickens (hens) lasted 21 and 30 days, respectively.

Bioavailability After An Oral Dose

In order to provide an estimate of the bioavailability after oral dosing, a single female rat was cannulated and then given 1 mg radiolabeled cyanazine by oral administration (Crayford and Hutson, 1972). After twenty hours, 21% of the administered radioactivity was eliminated via biliary excretion. If this experiment had been carried out for the same length of time as the rat metabolism study (4 days), a greater proportion (*i.e.,* > 21%) of the administered radioactivity would have been excreted via this route. However, since cannulation experiments with animals is relatively traumatic, it is not possible to extend these studies much beyond 20 hours.

If the cannulation study had been extended, the amount excreted in bile would have been greater which then would increase the estimate of bioavailability after an oral dose.

In addition to the elimination of the radioactivity via biliary excretion over a twenty-hour period, in the 4-day rat (three rats/sex) metabolism study, 40.1 and 42.2% of the administered dose was eliminated in the urine of female and male rats, respectively (Hutson *et al.*, 1970). Therefore, the estimated bioavailability after an oral dose for females is 61.1% (40.1 urinary + 21%, biliary).

Suitability of Urinary Metabolites For Human Exposure Monitoring

The use of urinary metabolites for the assessment of worker exposure may be possible based on the rat metabolism work (Hutson *et al.*, 1970), if it is assumed that humans and rats metabolize cyanazine similarly. The major rat urinary metabolite was *N*-acetyl-*S*-[4-amino-6-(-1-methyl-cyanoethylamino)-*s*-triazinyl-2]-*L*-cysteine. In both males and females, urinary radioactivity constituted 41.1 and 40.1%, respectively, of the administered dose at 0.8 mg/kg. In the female rats, the percentage of this major metabolite in the urine constituted ~60% of the radioactivity. However, when rats were treated with a 62-fold higher dose, this metabolite constituted ~40% of the radioactivity. This reduction of the quantity of the mercapturate metabolite as the dose was increased is not unexpected as saturation of a degradation route, such as the glutathione degradation pathway, is a known phenomenon in metabolism of xenobiotics.

If the assumption is made that rats and humans metabolize cyanazine in a similar fashion, and the glutathione metabolism pathway is not saturated (reasonable assumption as the highest exposure observed in the worker exposure study was ~0.03 mg/kg/day or about 30-fold less than the oral dose level of 0.80 mg in the rat study), it might be expected that workers' urine could contain 24% [~40% administered radioactivity in urine; one metabolite, 60% of this 40% (0.40 x 0.60 = 0.24)] of the major metabolite that was isolated from rat urine. The estimated amount of a major urinary metabolite from the rat study (24%) almost fits the one of the criteria (~30% of the administered dose) suggested by Woolen (1993) as being suitable for biological monitoring. Whether, the major metabolite found in the rat metabolism study would be a major metabolite in humans requires confirmation in a human pharmacokinetic study before it could be considered useful in a worker exposure study.

## WORKER EXPOSURE

Three different workers were monitored during a combined exposure study in which mixing/loading and applying of a water dispersible suspension of cyanazine to fields prior to planting corn was monitored (Green, 1985). Each worker mixed, loaded and applied four loads of cyanazine and the exposure for each load was monitored individually. All mixing/loading was done by hand pouring and applications were made by ground boom equipment. The application rates were 4.3, 4.5, and 4.7 lbs a.i./acre (near maximum allowable label rates and well above the average application rate in California for cotton as shown in Table 3). The mixer/loader/applicators wore work clothing and protective gloves during mixing and loading, as required by the label, but removed the gloves during application, which was permitted by the label at that time. Two of the workers used tractors with enclosed cabs for the application, while one used a tractor that did not have a cab. The total exposure time ranged from 71-129 minutes per replicate. The data for each replicate were then normalized to an 8-

hr day. Dermal exposure was measured by placing gauze patches (arms, legs, torso) in foil covered cardboard holders under the subjects' clothing. Respiratory exposure was obtained by drawing air at the rate of two liters per minute through a 37 mm glass fiber filter from a collector attached to the subject's lapel. Hand exposure was estimated by handwashes in water containing detergent. Since the normal application rate for cotton in California based on use data reports was about 0.75-2.0 lbs/acre (mean ~ 1.7), exposure was normalized for the highest use rate in cotton which is 2.0 lbs/acre. Further, as indicated above, the study conducted in field corn had exposure scenarios that included a tractor with an enclosed cab which currently is not required in California. Table 5 summarizes the exposure data for three workers involved in a total of 4 cycles per day.

**Table 5:** Estimation of an Absorbed Daily Dosage (ADD) of Workers Mixing/Loading and Applying Cyanazine for Treatment of Cotton at 2.0 lbs/acre (from surrogate involving application to field corn)

| | Field-Corn Mixer/Loader/Applicator Study[a/] | | | California-Cotton |
|---|---|---|---|---|
| | | Exposure (µg) | | ADD[b/] |
| Site (Replicate) | Cab Type | Dermal | Inhalation | (µg/kg) |
| 1 (A) | closed | 42,488 | 0.2 | 27.15 |
| 1 (B) | closed | 1,487 | 0.1 | 0.95 |
| 1 (C) | closed | 5,366 | 0.1 | 3.43 |
| 1 (D) | closed | 2,518 | 0.1 | 1.61 |
| 2 (A) | none | 15,898 | 2.4 | 10.57 |
| 2 (B) | none | 12,254 | 2.6 | 7.50 |
| 2 (C) | none | 8,022 | 2.4 | 4.93 |
| 2 (D) | none | 8,891 | 2.4 | 5.69 |
| 3 (A) | closed | 656 | 0.2 | 0.28 |
| 3 (B) | closed | 1,697 | 0.89 | 0.72 |
| 3 (C) | closed | 2,780 | 0.1 | 1.35 |
| 3 (D) | closed | 1,730 | 0.1 | 0.97 |
| | | 8,649[c/] | | 2.6[d/] |
| | | | | 5.0[e/] |

[a/] Corn mixer/loader/applicator exposure study submitted by registrant (Green, 1985)

[b/] Cotton - Absorbed Daily Dosage:
- 2.0 lb ai/A for post-emergence, directed cotton treatment in California
- Normalized to 8 hours
- Dermal absorption - 2.0%, rat study
- Body weight - 76 kg default value assumed, body weights not provided in study
- Respiratory uptake 100%

[c/] Arithmetic mean (calculated for comparison to U.S. EPA values in Federal Register Notice, November 23, 1994)

[d/] Geometric estimate of central tendency
- W: Normal = 0.67, $p<0.05$, distribution not normally distributed
- W: log normal = 0.97, $p = 0.89$, $p>0.05$ distribution could be log normally distributed (Shapiro and Wilk, 1965)

[e/] 95% CI = GM $(GSE)^t = 2.57(1.44)^{1.8} = 4.95$ where GSE = geometric mean standard error

Sanborn, WH&S, 1996, after Green, 1985

95 Percent Confidence Interval of the Mean

The estimation of the 95% confidence interval for the mean provides a measure of the variability of the central tendency. In this case, it is 1.9-fold (5.0/2.6). The calculation of a

confidence interval of the mean value of exposure gives some indication of the spread of the distribution about the central tendency. If these exposure data were used in a stochastic model for estimation of exposure for risk assessment, it would be important to understand the variability about the mean value.

95th Percentile: Population Exposure Estimate for "High-End Exposure"

In addition to estimates of central tendency of exposure for comparison with chronic toxicology endpoints, there is precedence and need to provide estimates of exposure derived from the upper end of an exposure distribution (U.S. EPA, 1990, 1991). The requirement for these latter estimates is justified by concerns for the "highly exposed individual" and/or comparison of the exposure data with acute endpoints derived from animal toxicology studies. It is possible to calculate a "high-end exposure estimate" for the eventual estimation of a population rather than an individual exposure. This "high-end exposure estimate" or 95th percentile is calculated using the equation below:

$$95^{th}\ \text{Percentile} = GM\ (GSD)^{t} = 2.57(3.51)^{1.8} = 24.6$$

GSD, Geometric Standard Deviation

This value indicates that for this exposure scenario, 5% of the population would be expected to experience an exposure above this value. Whenever upper-end exposure estimates are provided, an important caveat must be understood. The uncertainty of the estimates at the upper extremes of the exposure distribution of a log-normal distribution are greater than either the uncertainty at the lower end of this type of distribution or at the central tendency. In contrast, the uncertainty regarding the upper or lower end estimates of a normal distribution are similar and greater than the uncertainty at the central tendency.

Dermal vs. Inhalation Exposure

The ADD data in Table 4 reflect the combined dermal and inhalation exposure. The contribution of inhalation exposure averaged 0.01%. The small contribution of inhalation to the overall exposure may be expected in light of the type of application and the low vapor pressure of this herbicide ($3 \times 10^{-9}$ mm). However, it is possible that applicators may be exposed to aerosolized cyanazine during handling. Since there was a low contribution of the inhalation component, it is likely that exposure to aerosolized cyanazine did not occur in this study.

Effect of Cab Type

Due to several confounding factors, nothing very conclusive can be stated about the degree of protection offered by a closed cab. On the surface, the data from this exposure study suggest that the protection offered by a closed cab (vs. no cab) could be approximately 3.8-fold. Given the variability in the exposure data, the design of the experiment (three workers and four replicates each), the observation that 85-90% of the exposure was to the hands (likely during mixing/loading), one handler moved the spray booms during the monitoring period with no gloves, and the small number of replicates, the protection provided by a closed cab cannot be accurately determined. If the high exposure value for the first replicate at site 1, replicate A (head patch had very high amounts) is removed from the comparison of closed cab and no cab, then there is nearly a seven-fold difference between the scenarios (cab vs. no cab). While it is likely that most of the exposure occurred during mixing/loading (see following PHED estimate for confirmation of this assumption), some could have occurred during the application as it was noted that the conditions were quite windy during some of the replicates and the workers did not wear gloves during this operation.

Cyanazine Dermal Exposure from Federal Register: U.S. EPA Estimate

The dermal exposure data from the Federal Register Notice, November 23, 1994, are summarized in Table 6.

**Table 6:** Exposure Values for Cyanazine Developed by the U.S. EPA, Federal Register Notice November 23, 1994, Table 10. Exposure in mg/kg a.i./day: Corn Application 3.0 lbs/Acre

| Method | Tasks | Dermal Dose (mg/person/day) | Dermal Dose (mg/kg bw/day) | ADD[b/] (µg/kg/day) |
|---|---|---|---|---|
| Grower/ | M/L/A-o/o [a/] | 1180 | 16.85 | 337 |
| Ground Boom | M/L/A-o/c | 345 | 4.94 | 98.8 |
| | M/L/A-c/o | 872 | 12.46 | 249 |
| | M/L/A-c/c | 38.5 | 0.55 | 11 |
| Commercial/ | M/L/A-o/o | 2017 | 28.82 | 576 |
| Ground Boom | M/L/A-o/c | 1151 | 16.44 | 328 |
| | M/L/A-c/o | 919 | 13.14 | 263 |
| | M/L/A-c/c | 53.2 | 0.76 | 15.2 |

[a/] o-open system, c-closed system (first symbol is mix/load; second is for application)
[b/] Absorbed Daily Dosage:
2.0% dermal absorption, rat study
70 kg body weight default used by U.S. EPA

Sanborn, WH&S, 1996, after U.S. EPA, 1994

The estimates of exposure as summarized in Table 6 distinguish between commercial applicator and grower-applicator. While the reason for reporting these two exposure scenarios separately was not explained, it is likely related to the greater number of exposure days per year for a commercial applicator as compared to a grower-applicator and the concern for chronic effects over a lifetime. The range of exposures in the table above are much higher than the estimates from the cyanazine-specific study reported in Table 5 where an ADD of 2.6 µg/kg/day was reported. The basis for the cyanazine exposure estimates of the U.S. EPA study is an atrazine exposure study in grain sorghum. The citation for the atrazine-surrogate study is on page 60430 of the Federal Register Notice of November 23, 1994.

The difference between the ADD values estimated for the cyanazine-specific study and those estimated in the Federal Register document is the result of estimating hand exposure using cotton gloves worn by the workers. In this study, workers handled ~12 lbs atrazine active ingredient and the hand exposures ranged from **25-42 mg (mean 34 mg)**. This is in contrast to the cyanazine-specific study where the workers handled about 120 lbs per replicate and the hand exposure ranged from **0.64-15.2 mg (mean 3.6 mg).** The very large contribution of the hands in the atrazine exposure study where rubber gloves were not worn (now required by the label) leads to overestimating the exposure. This is especially clear when workers handling 10-fold more active ingredient in the cyanazine study have one-tenth the exposure because rubber gloves were worn during mixing/loading and the residues to the hands were monitored with handwashes. **The use of the atrazine-sorghum study as a surrogate for cyanazine is inappropriate as it has some serious flaws in terms of dosimetry techniques for the estimate of hand exposure.**

Effect of Closed Mixing/Loading And Closed Cabs On Exposure

The most significant aspect of the data from the Federal Register Notice written by the U.S. EPA is the reduction in exposure when a closed mixing/loading system is combined with a closed cab during application. For the grower-applicator and commercial applicator, the reduction in exposure was reported to be 30 and 38-fold, respectively, from the open mix/load and application scenarios. Should mitigation be required for occupational exposure during mixing/loading and applying of cyanazine in California, this information on the effect closed mixing/loading and applying systems could be used to reduce exposure. These data indicate more than a 95% reduction in exposure when both mixing/loading and application work tasks are conducted using engineering controls. Since, the source of these closed mixing/loading and cab mitigation data are not specified in the Federal Register Notice it is not possible to assess their validity.

ADD for Application Rates Higher Than 2.0 lbs/acre for Subchronic Toxicology Endpoints

Cyanazine application rates can be as high as 2.8 lbs a.i./A for sorghum. Based on the use data for the other crops grown in California, corn has the next highest use after cotton in terms of total pounds applied. Application rates for pre-plant treatment in California are generally lower than the Midwest because of the significantly lower organic matter in most soils in California (1-2% in sandy loam) as compared to the major field corn growing areas of the United States were cyanazine is applied as a pre-emergent herbicide in soils that can have organic matter content up to 6%. The organic matter in the soil reduces the amount of herbicide available for absorption. While the Federal label for cyanazine allows higher labeled rates (up to 6 lbs) for applications in corn, based on use data for California, these higher application rates do not occur for agronomic reasons that relate to soil type.

Annual Average Daily Dosage (AADD) and Lifetime Average Daily Dosage (LADD)

The data in Table 7 provide values that can be used in the estimate of risk from exposure. The days of exposure per year were taken from a memorandum from Haskell, 1994 who surveyed a county in California where cyanazine is used for weed control in the post-emergent application in cotton. These estimates for days of exposure per year for the commercial applicator (10-15) and a farmer-grower (1-3) for cotton application are similar to the U. S. EPA's estimates for the numbers of days per year for corn applications. The exposure days per year, taken from Table 10 of the Federal Register Notice, November 23, 1994, indicate that a commercial applicator and grower-applicator have 15 and 1-2 days of exposure per year, respectively.

**Table 7:** ADD, AADD and LADD Exposure Estimates for Growers and Custom-Applicators Applying Cyanazine by Ground Equipment

| Applicator | ADD[a/] (µg/kg bw) | AADD[b/] (µg/kg bw) | LADD[c/](µg/kg bw) |
|---|---|---|---|
| Farmer | 2.6 | 0.021 | 0.011 |
| Custom | 2.6 | 0.11 | 0.056 |

[a/] From Table 5

[b/] Annual Average Daily Dosage:
3 days/year for a farmer-grower (Haskell, 1994)
15 days/year for a custom applicator (Haskell, 1994)

[c/] Lifetime Average Daily Dosage: 40 years exposure; 75-year life

Sanborn, WH&S, 1996

Estimation of Exposure To Cyanazine Using the Pesticide Handlers Exposure Database

The Pesticide Handlers Exposure Database (PHED) has been developed to provide generic pesticide worker (*i.e.,* handler) exposure estimates for specific work scenarios. This database was developed by the U.S. EPA, Health Canada and the National Agricultural Chemicals Association. The dermal and inhalation exposure estimates are based on field exposure studies and are reported generically (*i.e.*, not chemical specific). PHED allows exposure assessments to be developed that are based on a larger sample size (more replicates) than is normally found in a single exposure study. The increased sample size offered by PHED is suggested to provide a more representative estimate of exposure than a single study with 10-15 replicates. The theory behind this exposure database is two-fold: (1) the type of equipment used in the pesticide treatment plays a greater role in the exposure outcome than the physical/chemical properties of the active ingredient and (2) exposure is positively related to the amount of active ingredient handled. To provide some idea of the size of the databases, for mixer/loaders, applicators, and combined mixer/loader/applicators there are 556, 715, and 349 replicates, respectively. (The flagger file has 92 replicates.) In general, most of the studies in PHED have utilized the patch dosimetry methodology of Durham and Wolfe (1962) where residues on patches, placed on different regions of the body, are extrapolated to the surface area to estimate exposure to that region. Then all extrapolated residues are summed to provide a total body exposure estimate.

It is the opinion of the U.S. EPA that the increased sample size offered by PHED to estimate occupational pesticide exposure will be more representative of the level of exposure than any single study even though the compound-specific study may have the requisite number of replicates required by Subdivision U. Because PHED estimates are considered to be more representative, comparison of exposure data from a compound-specific study with PHED can provide some useful information and allow the exposure assessor to determine whether or not an individual study should be used as one of the exposure estimates.

PHED estimates cannot provide high end exposure values. This is related to the multiple studies that are used to derive the exposure estimate. Sometimes in the risk assessment process there are needs for upper end exposure values for comparison to acute animal toxicological data to make a judgment of a margin of safety. This requires from the PHED, in addition to the central tendency, a statistically-derived upper value for the exposure parameter. Since the exposure data are often log-normally distributed, one or two standard deviations added to the mean will not provide a statistically relevant estimate of the upper end exposure. A 95% upper confidence interval, which can be obtained from PHED, is virtually meaningless, as it can be up to one-hundred fold greater than the geometric mean. The large confidence interval is the result of combination of multiple studies with different active ingredients that may have different application rates, different formulation types and likely different physical properties.

PHED Applied To Cyanazine Exposure Scenario In California

In order to gain additional perspective of the utility of the cyanazine-specific study to estimate worker exposure during treatment of cotton, PHED (Version 1.1, 1995) was used to develop three dermal exposure scenarios, mixing/loading, applying and combined mixing/loading and applying monitored as one task. These dermal exposure values were used to calculate ADD values. The search parameters listed in Table 8, from PHED, were utilized to develop the three exposure assessments.

Table 8: Parameters Used in PHED Exposure Assessment for Cyanazine: Ground Application

| Parameter | Comments |
|---|---|
| Dermal grade uncovered | A, B Studies |
| Hand grade | A, B Studies |
| Formulation | Emulsifiable concentrate |
| Study location | Outdoors |
| Application method | Ground boom tractor |
| Mixing/Loading | Open pour |
| Exposure units | µg/pound handled |
| Inhalation rate | 25 l/min (PHED default) |
| Exposure | Combined inhalation/dermal |
| Head patches | Observed values, not extrapolated |
| Normal work clothing | Long sleeve pants, shirt and rubber gloves |

Sanborn, WH&S, 1996

Two of the parameters require some justification, the type of formulation and the use of observed residue values on the head patches instead of a combination of observed and extrapolated values. While one of the formulations of cyanazine is a liquid formulation, strictly speaking, it is not an emulsifiable concentrate. The two formulations of cyanazine in commerce are a DF (dry flowable) and a 4L (liquid). Since this exposure database does not have a large data set with either of these two specific formulations, the most appropriate way to use PHED to develop these exposure scenarios, is to use one of the largest data sets which are products formulated as an emulsifiable concentrate. While this formulation type differs from the two for cyanazine, the large number of data entries compensate for this to some extent. It seems more appropriate to use a PHED data set with a sufficient number of replicates than to develop an exposure estimate based on a data set of the exact formulation containing only a few number of replicates.

The other parameter requiring justification is the use of observed residue values on the head patches rather than basing head exposure on extrapolated values from patches located on another portion (chest, back, shoulders) of the body. This is especially important for exposure studies involving mixer/loaders. While handling the undiluted formulation, some may splash on the patch used to estimate the exposure to the head. When extrapolated this could lead to an excessive exposure estimate for the head. Because of the possibility of inadvertent formulation splashing on this patch, it is more appropriate to use observed residue data from the head patch (if it exists) rather than extrapolated values from other patches used for the head exposure estimate.

The observation that the sum of the ADD values for the separately monitored tasks, (5.1 µg/kg bw/day) is two-fold greater than the combined (2.4 µg/kg bw/day) is of minor concern as these estimates were derived from different data sets. Further, the cyanazine-specific study, a combined mix/load/apply work scenario, provided an ADD value of 2.6 µg/kg bw/day. The similarity of this value to the combined PHED mix/loading/application exposure estimate in the table below (2.4 µg/kg bw/day), provides additional support for the use of the cyanazine-specific study to assess exposure while handling this herbicide during treatment of cotton.

Table 9 PHED-Exposure Estimate for Cyanazine for Ground Treatment of Cotton

| Activity (replicates) | Exposure (µg/lb a.i. handled) Dermal | Inhalation | ADD (µg/kg-bw/day) [a/] |
|---|---|---|---|
| Mixing/Loading (77) | 37.8 | 0.58 | 3.5 |
| Application (38) | 7.8 | 0.46 | 1.6 |
| Mixing/Loading/Applying (25)[b/] | 31.4 | 0.28 | 2.4 |

[a/] Absorbed Daily Dosage:
100 acres/day @ 2.0 lbs/acre;
dermal penetration 2% rat study;
body weight 76 kg

[b/] Combined mixing/loading/application exposure scenario

Sanborn, WH&S, 1996

**EXPOSURE APPRAISAL**

Endpoint: Chronic Toxicology

In the development of an exposure assessment for an agricultural chemical, it is uncommon to have data from several sources to arrive at an estimate of an absorbed daily dosage. In the case of cyanazine, there are estimates from: (a) an exposure study specific to the active ingredient (2.6 µg/kg bw); (b) a pesticide exposure database, PHED, (2.4 or 5.1 µg/kg bw, depending how it is calculated, mixing/loading/applying separately monitored or combined); and (c) the U.S. EPA from the Federal Register (225 µg/kg bw). Since the value derived from the U.S. EPA estimate is much higher and is lacking supporting documentation, the ADD value, 2.6 µg/kg bw for a combined mixer/loader/applicator exposure from the cyanazine worker study, should be used for comparison with animal toxicology endpoints for the calculation of risks or margins of safety.

Endpoint: Acute Developmental Toxicology and the Upper End Exposure Estimate

Since the worker exposure data in Table 5 are log-normally distributed, estimation of a high end exposure for acute effects cannot be made using the same statistical methodology used for normally distributed data (*i.e.*, mean + two standard deviations). However, it is possible to estimate an upper end ADD value for this distribution as a 95$^{th}$ percentile which can be used for a population exposure estimate. The calculated 95$^{th}$ percentile was found to be 24.6 µg/kg bw. This is less than the highest measured value of 27.2 µg/kg bw. It is important to remember the caveat previously stated, *i.e.*, the greater degree uncertainty for any estimate derived from the ends of a distribution as compared to the uncertainty associated with the central tendency. This is especially true for the upper end of log-normally distributed data.

Further, in the cyanazine-specific study, a 10-fold difference is observed, when the highest ADD value (27.2 µg/kg bw) is compared to the geometric mean (2.6 µg/kg bw) in Table 5. This is much less than ~160-fold difference observed between the upper 95% confidence interval and the geometric mean derived by the PHED estimate involving multiple studies with different active ingredients. In contrast, the 95% confidence interval of the geometric mean for the cyanazine-specific study is ~2-fold greater (5.0 vs. 2.6 µg/kg-bw/day) than the geometric mean.

With respect to PHED and the possible estimation of an upper end exposure value for comparison to the acute animal endpoint, a statistical problem exists as this program provides a geometric mean for exposure in terms µg exposed/lb handled. In the PHED program, a 95% upper confidence interval for the dermal geometric mean for combined mix/load/apply (Table 9) is 5149 µg/lb handled or ~160-fold greater than the geometric mean (5149/31.4). The extreme variation in the PHED data, as assessed by the 95% upper confidence interval of the geometric mean, is likely the result of combination of exposure data for several different active ingredients that may have different physical properties, application rates and formulation characteristics.

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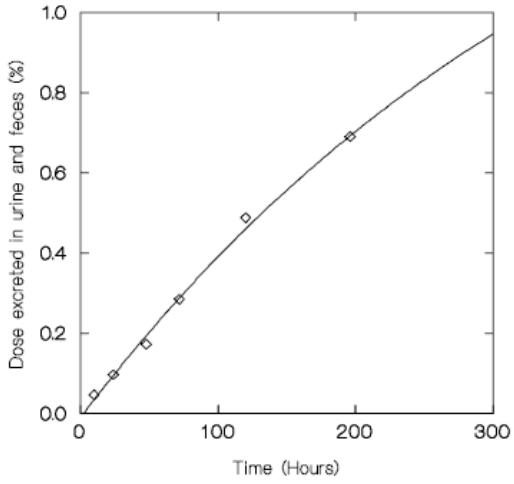
c,h,i: CYANB7.DOC

**FIGURE 1**




THU 10/27/94 2:25:58 PM

| ITERATION | LOSS | PARAMETER VALUES | | |
|---|---|---|---|---|
| 0 | .5398371D+00 | .1000D+00 | .1000D+00 | .1000D+00 |
| 1 | .2655539D+00 | .3421D+00 | .7755D-01 | -.1626D-01 |
| 2 | .9142415D-01 | .6846D+00 | .1379D-01 | -.1149D+00 |
| 3 | .1314859D-01 | .9630D+00 | .5196D-02 | -.1108D+00 |
| 4 | .5962363D-02 | .1193D+01 | .4176D-02 | -.1027D+00 |
| 5 | .3252754D-02 | .1292D+01 | .3767D-02 | -.6565D-01 |
| 6 | .2075769D-02 | .1557D+01 | .2941D-02 | -.1504D-02 |
| 7 | .1760786D-02 | .1717D+01 | .2648D-02 | .2141D-01 |
| 8 | .1601224D-02 | .1895D+01 | .2336D-02 | .3699D-02 |
| 9 | .1505689D-02 | .2113D+01 | .2054D-02 | -.8224D-02 |
| 10 | .1492198D-02 | .2134D+01 | .2028D-02 | -.1598D-01 |
| 11 | .1490511D-02 | .2130D+01 | .2033D-02 | -.5414D-01 |
| 12 | .1468004D-02 | .1978D+01 | .2228D-02 | -.1135D+01 |
| 13 | .1399944D-02 | .1964D+01 | .2263D-02 | -.1660D+01 |
| 14 | .1394772D-02 | .1923D+01 | .2328D-02 | -.1967D+01 |
| 15 | .1383923D-02 | .1871D+01 | .2419D-02 | -.2251D+01 |
| 16 | .1378994D-02 | .1847D+01 | .2454D-02 | -.2246D+01 |
| 17 | .1377941D-02 | .1819D+01 | .2503D-02 | -.2329D+01 |
| 18 | .1377227D-02 | .1802D+01 | .2531D-02 | -.2364D+01 |
| 19 | .1377197D-02 | .1804D+01 | .2528D-02 | -.2348D+01 |
| 20 | .1377196D-02 | .1805D+01 | .2527D-02 | -.2347D+01 |
| 21 | .1377196D-02 | .1805D+01 | .2527D-02 | -.2348D+01 |
| 22 | .1377196D-02 | .1805D+01 | .2527D-02 | -.2348D+01 |

DEPENDENT VARIABLE IS RECOV

| SOURCE | SUM-OF-SQUARES | DF | MEAN-SQUARE |
|---|---|---|---|
| REGRESSION | 0.8370 | 3 | 0.2790 |
| RESIDUAL | 0.0014 | 3 | 0.0005 |
| TOTAL | 0.8384 | 6 | |
| CORRECTED | 0.3097 | 5 | |

RAW R-SQUARED (1-RESIDUAL/TOTAL) = 0.9984
CORRECTED R-SQUARED (1-RESIDUAL/CORRECTED) = 0.9956

| PARAMETER | ESTIMATE | A.S.E. | LOWER <95%> | UPPER |
|---|---|---|---|---|
| MAX | 1.8046 | 0.1169 | 1.4325 | 2.1767 |
| RATE | 0.0025 | 0.0002 | 0.0019 | 0.0031 |
| LAG | -2.3477 | 3.1825 | -12.4758 | 7.7804 |

ASYMPTOTIC CORRELATION MATRIX OF PARAMETERS

| | MAX | RATE | LAG |
|---|---|---|---|
| MAX | 1.0000 | | |
| RATE | -0.8642 | 1.0000 | |
| LAG | -0.2453 | -0.1182 | 1.0000 |