Agriculture
Canada

Food Production
and Inspection Branch

Plant Industry Directorate

Direction générale,
Production et inspection des aliments

Direction de l'industrie des produits végétaux

Canada

E93-01

Decision Document

Myclobutanil

As part of the ongoing effort to provide a summary of the data received and to outline the regulatory action on the active ingredient myclobutanil, a Decision Document has been prepared. This document reflects input from specialists within Agriculture Canada and from key interdepartmental advisors. Based on the review of all available information and in consideration of the agronomic benefits, a regulatory decision has been made to grant temporary registration for myclobutanil and the end-use product NOVA 40W® .

(aussi publié en français)

June 15, 1993

This bulletin is published by the Information Division of the Plant Industry Directorate. For further information, please contact:

Plant Industry Directorate
Agriculture Canada
Ottawa, Ontario
K1A 0C5
(613) 993-4544

Facsimile: (613) 998-1312
Telex: 053-3282
Envoy 100: Pesticide
Information Service: 1-800-267-6315

Table of Contents

Abbreviations Used in this Document

ADI	Acceptable Daily Intake
bw	body weight
^{14}C	Carbon 14
DI	Deionised
DT_{50}	Decline Time 50%
EEC	Expected Environmental Concentration
GC/MS	Gas Chromatography/Mass Spectrometery
ha	hectare
K_{ow}	Octanol/Water partition coefficient
LC_{50}	Lethal Concentration 50%
LD_{50}	Lethal Dose 50%
LOEL	Lowest Observed Effect Level
MFO	Mixed Function Oxidase
MOS	Margin of Safety
MRL	Maximum Residue Limit
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
PCP No.	Pest Control Products Number
PHI	Preharvest interval
ppm	parts per million (also mg/kg, ml/L, etc.)
$t_{1/2}$	half life of 1^{st} order elimination
TA	Triazol-1-yl Alanine
T3A	Triazol-3-yl Alanine
TDI	Theoretical Daily Intake

1.0 Summary

The purpose of this document is to summarize information on the risks and benefits associated with the use of myclobutanil fungicide and to announce the formal regulatory decision on this active ingredient and its end-use product NOVA 40W®.

The benefits of myclobutanil have been assessed by Agriculture Canada while the risks associated with its use have been characterized by Health and Welfare Canada and Environment Canada.

Myclobutanil has been shown to be an effective fungicide for the control of scab and powdery mildew on apples and powdery mildew and black rot on grapes.

When the end-use product NOVA 40W® is applied following label directions (e.g., use of protective clothing and adherence to re-entry directions) there is considered to be an adequate margin of safety (MOS) with respect to occupational exposure. The proposed maximum residue levels for fresh and processed fruit should not pose any health concern to consumers.

Myclobutanil is not expected to pose a direct hazard to wildlife or soil organisms. Avian reproduction studies did not demonstrate any toxicologically significant effects. However, the highest dose included in the study is less than the worst case, maximum residue estimates. Further information on this aspect will be provided by the company.

The potential for persistence and mobility has raised questions about possible off-target effects. To minimize the risk, the label was modified to include a 15-meter buffer zone between last spray swath and aquatic habitats. The appropriateness of the buffer zone will be reassessed once additional data on environmental fate and effect on off-target species is available.

Based on the identified risks associated with the use of myclobutanil and in consideration of the economic benefits to Canadian apple and grape production particularly in Integrated Pest Management (IPM) programs, this fungicide has been granted temporary registration (myclobutanil technical - PCP No. 22398 and NOVA 40W® - PCP No. 22399). The temporary registration will allow for the submission/ review of additional environmental data. The situation will be re-assessed in 1993.

2.0 Pesticide Name and Properties

2.1 Pesticide Name

Common Name:	Myclobutanil
Chemical Name:	a-butyl-a-(4-chlorophenyl) 1H-1,2,4-triazole-1-propanenitrile
Trade Names:	Systhane
CAS Registry No:	88671-89-0

2.2 Physical and Chemical Properties

Empirical Formula:	$C_{15}H_{17}ClN_4$
Molecular Weight:	288.78
Physical Appearance:	Odorless white solid
Melting Point:	70-71.5°C
Vapor Pressure:	1.6×10^{-4} torr/25°C
Solubility:	
Water	142 ppm @ 25°C
Hexanes	< 1g/100g
Most Organics	>50g/100g

2.3 Technical Material

Physical Appearance:	Light yellow solid with moderately intense odor of organosulfur compounds
Density:	1.22 g/cc @ 23°C
Thermal Stability:	Stable at 25°C indefinitely. No major instability below 300°C.

End-Use Product

RH-3866 40 WP (NOVA 40W®)

Physical State:	Fine off-white powder
Odor:	Moderately intense odor of organosulfur compounds
Particle Size:	3-5 microns
Wetting Time:	2 g/150 ml water, less than 30 seconds
% Suspendibility:	1 g in 100 ml water for 30 minutes at about 30°C is greater than 75% in DI water, 342 ppm hardness water and 1000 ppm hardness water
Bulk Density:	Loose 0.24g/cc Packed 0.26 g/cc
Storage Stability:	At room temperature the end-use product is stable for up to 30 months.

3.0 Development and Use History

Myclobutanil is manufactured by Rohm and Haas Company Ltd. Rohm and Haas Canada Inc. is the registrant for both the technical active ingredient myclobutanil and the end-use product, NOVA®. Field tests were initiated in eastern and western Canada in 1984 and the original submission for registration was received by Agriculture Canada in 1988.

4.0 International Considerations

Myclobutanil is registered for many uses in 40 countries worldwide including Australia, New Zealand, France, Germany, Israel, Japan, U.K., and U.S.A. It was first registered in 1986 in Europe and has been used in Europe for the past six years and in the United States for the past three years. Myclobutanil is registered on many crops ranging from fruits, vegetables and cereals to ornamentals and turf. Myclobutanil was reviewed in 1992 at the Joint Meeting on Pesticide Residues* for the purpose of establishing international maximum residue limits.

5.0 Regulatory Position and Rationale

Myclobutanil/NOVA 40W® has low acute oral, dermal and inhalation toxicity in rats and/or rabbits. Subchronic and long term feeding studies as well as reproduction, teratogenicity and mutagenicity studies performed on different mammals were considered favorable to the registration of myclobutanil. The data indicate that when myclobutanil is used on apples and grapes in accordance with label directions, total residues of parent material and major metabolites will be less than 0.5 ppm in apples, 1.0 ppm in grapes, and 10.0 ppm in raisins. Residues of a naturally occurring metabolite, triazolyl alanine, in apples and grapes are not expected to exceed the established MRL of 2.0 ppm. The maximum residue levels for apples, grapes, raisins, whole milk, eggs and livestock products are not considered to pose a health hazard to consumers, even to young children.

A worker exposure study was conducted with Rally 40W (same formulation as NOVA 40W®). The estimated margin of safety (MOS) obtained for mixer/loader/applicator is considered acceptable. An estimate of exposure to re-entry personnel could not be made. However, the use of protective clothing and appropriate label re-entry statements are considered acceptable risk reduction measures.

Studies on aquatic and terrestrial invertebrates suggest that myclobutanil has a low toxicity to these non-target organisms. On the basis of the LD_{50} data, estimated risk factors are low for both birds and mammals. Avian reproduction studies indicate no toxicologically significant effects. However, the highest dose included in the study is less than the worst case, maximum residue estimates. Further information on this aspect will be submitted by the company.

Myclobutanil is not expected to pose a direct hazard to wildlife. However, its potential for persistence and mobility has raised questions about the possible off-target effects. To minimize the risk of any significant off-target effect, the label was modified to include a 15-meter buffer zone between the last spray and aquatic habitats. This 15-meter buffer zone statement will be kept on the label until additional data are

* *JMPR is an international meeting of experts in toxicology and chemistry and is sponsored jointly by the World Health Organization and the Food and Agriculture Organization of the United Nations.*

submitted on the effect of myclobutanil on off-target species. After submission of new studies, the appropriateness of the buffer zone will be reassessed and modified if considered necessary.

6.0 Use Summary and Benefits

6.1 Description of Market

Apples are produced in significant volumes in British Columbia, Ontario, Quebec, Nova Scotia, and New Brunswick. The area involved is 34,000 hectares. In 1989, farm cash receipts were $107,903,000 with exports valued at $31,722,000.

Grapes are produced on 7,300 hectares mainly in Ontario and British Columbia. Value of the crop was $29,960,000 in 1989.

6.2 Pest Problem

a) Apples - The two major diseases affecting quality and yield are apple scab and powdery mildew.

Crop losses from scab development on the fruit can be 70% or greater where cool humid weather occurs during the spring months. These conditions exist annually in eastern Canada (which represents 78% of the total apple growing area) and occasionally in British Columbia.

Severely infected fruit often drop prematurely from the tree and can represent a total loss in yield. Lightly infected fruit is downgraded from fresh pack to processing grade with a corresponding loss of value of 68-70%.

Powdery mildew is a serious problem in British Columbia and can be a problem on susceptible varieties in southern Ontario. The netlike russeting on the fruit can downgrade the produce from fresh to processing grade resulting in a corresponding loss of value. Powdery mildew can reduce tree vigor thereby reducing fruit size in the current year and fruit bud set for the next year. Under severe conditions, growth is not only stunted but new shoots may be killed.

b) Grapes - Two major diseases of grapes are powdery mildew and black rot.

Powdery mildew is a serious problem in all grape-growing areas of Canada. Severely infected shoots desiccated during the winter result in winter-damaged canes. Early berry infection on susceptible cultivars may result in russeted berries that split and allow the infection by other fruit-rot fungi. Severe infections on the rachis may give an off-flavored juice or wine. Grapes infected with fungal pathogens are not acceptable for high quality wine production. Non-treated grapes may result in a complete loss of yield at harvest.

Black rot of grapes infects leaves and berries in Ontario which represent 91% of the total grape area in Canada. Early infection causes unseen losses when the flowers or very young fruit are killed. Infection, at later stages, causes the berries to decay, shrink and mummify.

6.3 Proposed Uses and Alternatives

Myclobutanil is a systemic fungicide with proposed uses for the control of apple scab, powdery mildew, and rust on apples and for the control of powdery mildew and black rot on grapes. Research trials conducted since 1984 have proven this fungicide to be very effective in the control of these diseases on apples and grapes with no phytotoxicity.

Apples - Currently registered fungicides for scab control must be applied within 18-24 hours after an infection period to be effective. Myclobutanil can be applied up to 96 hours after infection providing growers with additional time in case of windy or wet weather, equipment breakdown, etc. With this fungicide, a grower can delay the application of his first spray and extend the interval between sprays, thus reducing the number of pesticide applications. Myclobutanil is also used at low-use rates (136 g ai/ha) thereby reducing the pesticide load in the orchard and minimizing any adverse effects on the beneficial predators and parasites. An additional benefit of myclobutanil is compatibility with oil.

Grapes - Some compounds registered for powdery mildew control are phytotoxic to certain cultivars. There is no observed phytotoxicity with myclobutanil.

6.4 Mode of Action

As with similar triazole compounds such a triadimefon (Bayleton®) and propiconazole (Tilt®), myclobutanil appears to be a specific inhibitor of sterol 14-demethylase. In tests conducted with yeast enzyme extract (Quinn et al; 1986)[1], incorporation of radioactivity into 4-demethyl sterols was inhibited while at the same time an increase in radioactivity was recorded in 4,4-dimethyl sterols. In this way, myclobutanil disrupts the ergosterol biosynthesis pathway which is vital to fungal cell wall formation.

6.5 Other Pesticide Features

Myclobutanil is packaged in water soluble bags offering convenience of use, reduction of mixer/loader exposure, and elimination of container disposal problems.

---

1 *Quinn, J.A. et al. Pesticide Science. 1986. 17, 357-362.*

This fungicide provides Canadian growers with new technology that allows for extended intervals between applications thereby reducing the number of applications and production costs. Because myclobutanil is a systemic fungicide, rainfall does not wash the compound off the foliage. This characteristic eliminates the need for immediate reapplication, as is the case with current fungicides.

Myclobutanil fits into Integrated Pest Management programs because of the low rate of active ingredient, fewer applications required, and its safety to beneficial predators, parasites and honey bees.

7.0 Toxicology and Exposure: Health and Welfare Canada

Triazol-1-Alanine (TA)

Triazolyl alanine (TA) is formed in plants grown in soil previously treated with triazole fungicides. Although soil is not normally treated directly with triazole fungicides, soil residues normally result from crop treatments. TA is also purported to occur naturally in plants not exposed to triazole fungicides. For a detailed discussion of plant metabolic processes of myclobutanil and TA, refer to pp 21-22.

There are a number of triazole fungicides under development in Canada, including triadimefon, triadimenol, tebuconazole, and bitertanol fungicides from Miles Corp. (Bayer), propiconazole from Ciba-Geigy, flutriafol, paclobutrazole, and hexaconazole from Chipman (ICI), flusilazole from DuPont, myclobutanil from Rohm and Haas and azaconazole from Janssen.

Triadimefon fungicide is registered for use in the U.S.A. on food crops and a number of tolerances, ranging from 1.0 to 4.0 ppm, have been established on crops such as wheat, barley, apples, apricots, grapes, nectarines, peaches, pears, pineapples and plums as well as a number of other food crops. Triadimenol has just recently been registered in the U.S.A. for wheat and barley seed treatment. Bitertanol has an interim tolerance in the U.S.A. on apples and grapes of 0.05 ppm. Propiconazole has been registered in the U.S.A. on grains at 0.05 ppm and on other edible commodities at up to 0.2 ppm. Two of these fungicides (triadimefon and propiconazole) have been registered for use in Canada, triadimefon on a temporary basis and propiconazole on a full registration basis.

An association of manufacturers has developed and submitted a package of toxicology, chemistry, and residue data on TA, and has asked that TA be exempted from normal requirements in view of its low toxicity and apparent natural occurrence.

7.1 Toxicology (TA)

a) Acute Toxicity:

Acute oral studies in rats and mice showed LD_{50} values of over 5000 mg/kg bw.

b) Short Term Toxicity:

A 3-month rat feeding study showed a No Observed Effect Level (NOEL) of 1250 ppm (equivalent to 62.5 mg/kg bw/day); decreases in levels of serum triglycerides, urea and creatinine were observed at the next highest dietary level of 5000 ppm (equivalent to 250 mg/kg bw/day) but the magnitude of the decreases were not considered to be of toxicological significance and thus this level of 250 mg/kg bw/day is considered to be the No Observed Adverse Effect Level (NOAEL) for this study; significant decreases in body weights of males were observed at the next highest dietary level of 20,000 ppm (equivalent to 1000 mg/kg bw/day).

A 90-day dog feeding study showed a NOEL of 5000 ppm (125 mg/kg bw/day); decreases in food intake and body weight gains in females were observed at the next highest dose level (20,000 ppm or 500 mg/kg bw/day); the magnitude of these changes was not considered to be toxicologically significant and thus a NOAEL of 500 mg/kg bw/day, the highest dose tested, was established for this study.

c) Reproductive Toxicity:

In a 2-generation rat reproduction study with 2 litters per generation, there were no adverse effects on reproduction at dietary levels of 500, 2000 and 10,000 ppm; the NOEL is considered to be 2000 ppm (equivalent to 100 mg/kg bw/day) based on a slight but toxicologically significant decrease in pup weight observed at the highest dose level.

d) Teratogenicity:

No teratogenic effects were noted in a rat teratology study and an NOEL of 100 mg/kg bw/day was demonstrated based on an increased incidence of fetuses with delayed ossification observed at the next highest level of 300 mg/kg bw/day.

e) Mutagenicity:

The following studies were conducted:

- bacterial point mutation: negative in *Salmonella*
- mammalian point mutation (CHO-V79): negative
- chromosome aberration: *in vivo* micronucleus negative
- DNA repair (rat hepatocytes): negative
- cell transformation: positive in two studies but cell transformation studies have not been demonstrated to be useful as a prediction of carcinogenicity;

Based on all results from mutagenicity studies it is concluded that triazolyl alanine is neither genotoxic nor mutagenic.

f) Metabolism:

The available animal metabolism data indicates triazolyl alanine is **not** an animal metabolite of the triazole fungicides evaluated to date.

In rat metabolism studies, 95-105% of the ^{14}C-labelled TA was excreted within 24 hours mainly in the urine; only trace amounts were detected in tissues; 79% of the dose was excreted as TA parent compound, 14% as its acetyl derivative and only very small amounts as unidentified metabolites.

g) Toxicology Summary:

TA is considered acutely non-toxic and no specific target organ was identified in any of the short term studies.

In the most sensitive study performed, TA was given to F_0 and F_1 parent rats in the reproduction study for 9 months and only a slight decrease in pup weight was observed at the 10,000 ppm dose level with a resulting NOEL of 100 mg/kg bw/day.

In the absence of significant findings in parental animals treated for 9 months at 10,000 ppm (500 mg/kg bw/day), it is considered unlikely that any new chronic toxic effects would develop at lower levels if TA was administered for the lifetime of these animals (i.e. an additional 15 months). Further, TA was not considered mutagenic or genotoxic, is not structurally related to any known carcinogen, and in the rat reproduction study no pre-neoplastic changes were noted in the liver, bladder epithelium, thyroid or stomach mucosa after 9 months of treatment at 10,000 ppm (500 mg/kg bw/day). Thus, chronic carcinogenicity studies are not considered necessary.

A rabbit teratology study was not available but is not considered necessary in view of TA's low toxicity and the fact that no teratogenic effects were observed in the rat.

7.2 Dietary Exposure (TA)

a) Acceptable Daily Intake (ADI) Assessment:

An ADI would be 0.1 mg/kg bw/day, based on a NOEL of 2,000 ppm (100 mg/kg bw/day) in the rat reproduction study and use of 1,000 fold safety factor.

b) Residue Levels:

Extensive data on analyses of **untreated** cereal control samples, some from fields documented as never treated with triazole fungicides, has demonstrated the apparent presence of TA residues (generally <0.05 ppm) with occasional levels up to 0.5 ppm and one barley sample showing residues up to 1.58 ppm.

The high barley control residue values are suspect however due to the lack of field history data and the possibility that the test fields could have been exposed to triazole fungicides either directly, by previous field treatments, or indirectly, by runoff, overspray, and/or wind borne soil.

Residue data from **treated** crops at the recommended treatment rates for the triazole fungicides indicate that for a single season application with flusilazole, TA residues occurred up to 0.07 ppm in grapes and 0.13 ppm in apples. Crops treated at recommended rates for one season with propiconazole indicate that TA residues occurred up to 1.02 ppm in barley and up to 1.07 ppm in wheat. Cereal crops treated annually for 5-6 years with bitertanol, triadimenol, and triadimefon, either singly or in combination, show residues of TA ranging from 0.1 ppm to 1.6 ppm. It appears unlikely however, from the data provided, that residues of TA would exceed 2.0 ppm.

Insofar as the question of the natural occurrence of TA a search of available relevant scientific data bases, covering the last 20 years, did not produce any reference to TA as a natural compound. There was however a reference to a naturally occurring structural isomer, triazol-3-yl alanine (T3A). Due to the similarity in structure and the semi-aromatic nature of the triazole ring, the exact nature of the background analytical response was questioned, i.e. were the background residues due to TA or T3A?

To address the question of identity of the background peaks and the lack of references in the literature to the natural occurrence of TA, the industry established a Task Force to consider this matter. The Task Force has provided data showing that TA and T3A are well separated when analyzed by capillary column GC/MS. The separation times, by this method, are approaching 2 minutes and indicate that the analytical methods used previously would also have separated these two compounds. This separation data coupled with the original background residue data and analytical methodology, indicate that although TA has not previously been substantiated in the literature as a naturally occurring compound, TA is in fact a naturally occurring compound.

c) Risk Assessment:

A maximum residue limit of 2.0 ppm in all food crops would result in a maximum theoretical daily intake (TDI) not in excess of 0.05 mg/kg bw/day. This TDI is only 50% of the ADI of 0.1 mg/kg bw/day.

7.3 Toxicology and Occupational Exposure (Myclobutanil)

a) Health and Welfare Canada:

A toxicology data package was submitted by the registrant, Rohm and Haas Canada Inc. The following data were considered in the assessment of potential human health hazards and the following status report prepared by Health and Welfare Canada was considered in our regulatory decision on myclobutanil. The manufactured technical myclobutanil has a purity of 90-96%. Thus, the NOELs established in the toxicity studies conducted with less pure technical materials (76.4%-79.6%) were corrected accordingly. The company was undecided as to a trade name for the product and therefore the product Rally 40W is referred to occasionally in this document. Rally 40W is identical in every aspect to NOVA 40W®.

b) Acute Toxicity - Technical:

i) Oral:

Technical myclobutanil was slightly toxic to rats and mice. The following oral LD_{50} values were obtained:

Species	Strain	Compound Purity(%)	LD_{50} (mg/kg bw) Male	Female
Rat	CRCD	91.9	1600	2300
Rat	Sprague-Dawley	78.4	1750	1800
Rat	CRCD	78.4	1100-2000	-
Mouse	CD1	91.9		-
Mouse	CD1	79.6	3230	-
Mouse	CD1	91.9	>4420	1360
Mouse	CD1	91.9	1910	1840

Toxic effects were observed on the central nervous system (passiveness, ataxia, tremors, prostration, and convulsions) and autonomic nervous system (lacrimation, salivation, and diarrhoea).

ii) Dermal:

In New Zealand White rabbits, myclobutanil was virtually non-toxic by the dermal route (LD_{50} > 5000 mg/kg bw).

iii) Irritation:

Myclobutanil was a mild dermal irritant and a moderate eye irritant in New Zealand White rabbits.

c) Acute Toxicity - Formulation (Rally 40W, NOVA 40W®):

i) Oral:

Rally 40W was slightly toxic to CRCD rats via oral dosing with LD_{50} values of 1870 and 2090 mg/kg bw respectively for males and females.

ii) Dermal:

The formulation was virtually non-toxic in New Zealand White rabbits with a dermal LD_{50} > 5000 mg/kg bw.

iii) Inhalation:

The inhalation toxicity (LC_{50}) of Rally 40W in rats in a 4-hour study exceeded 5 mg/L.

iv) Irritation:

Rally 40W was a slight dermal irritant and a moderate eye irritant in the New Zealand White rabbit.

v) Sensitization:

Rally 40W did not induce dermal sensitization in guinea pigs.

d) Short-term Toxicity - Technical:

i) Rats:

COBS-CD(SD) BR rats were fed diets containing myclobutanil (79.6% purity) at concentrations of 0, 10, 30, 100, 300, 1000, 3000, 10000 and 30000 ppm for 3 months. The No Observed Effect Level (NOEL) was 100 ppm for males (equal to 4.9 mg ai/kg bw/day) based on an increase in hepatic mixed function oxidase (MFO) activity at 300 ppm. For females, the NOEL was 300 ppm (equal to 18.5 mg ai/kg bw/day) based on gross liver lesions (accentuated lobular architecture which was seen in both sexes), increased MFO activity, and an increase in relative liver weight at 1000 ppm. The No Observed Adverse Effect Level (NOAEL) for both males and females was 1000 ppm (equal to 49 mg ai/kg bw/day); the increased activity of hepatic mixed function oxidase at 300 and 1000 ppm in males and females respectively and the accentuated lobular architecture at 1000 ppm in females were considered a normal hepatic compensatory response to the increased demand for the oxidative metabolism of the test compound.

At 3000 ppm (equivalent to 150 mg ai/kg bw/day) there were histological alterations in the liver (hypertrophy and necrosis), kidneys (increased pigmentation in convoluted tubular epithelium), adrenals (increased cortical vacuolization), and thyroid (increased number of small follicles); increased liver and kidney weight; gross kidney and liver lesions; decreased bodyweight gain; and slight serum chemistry

changes (increased serum cholesterol and globulin; decreased albumin/ globulin ratio). All rats at 30,000 ppm died during the first nine weeks of treatment.

ii) Mice:

Crl:CD-1 (ICR) BR mice were fed diets containing myclobutanil (79.6% purity) at concentrations of 0, 3, 10, 30, 100, 300, 1000, 3000 and 10000 ppm for 3 months. The NOEL was 300 ppm for both males and females (equal to 44 mg ai/kg bw/day) based on dose and treatment-related changes in the liver at 1000 ppm (equal to 147 mg ai/kg bw/day). At this level, the histological changes seen were hepatocytic hypertrophy, vacuoles and necrosis, and necrotic hepatitis. Also at 1000 ppm significant changes consisted of increases in liver weight, accentuated lobular liver architecture, increased liver MFO activity in males, decreased serum cholesterol and cytoplasmic eosinophilia in the adrenal zona fasciculata cells. Additional significant changes seen at the next dose level of 3000 ppm were lower body weights in males, decreased glucose in females, increased alanine aminotransferase, swollen/enlarged livers and increased pigmentation in the spleen and in liver Kupffer cells.

iii) Dogs:

In a range-finding study, two beagle dogs/sex/dose were fed diets containing myclobutanil (78.4% purity) at concentrations of 0, 50, 250, 1000 or 4000 ppm for 4 weeks. The study NOEL was 250 ppm (equal to 7 mg ai/kg bw/day) based on a slight decrease in body weight and food consumption of the female dogs dosed at 1000 ppm. The NOAEL was 1000 ppm (equal to 36 mg ai/kg bw/day). The dogs dosed at 4000 ppm exhibited sharp decreases in food consumption and consequently a decrease in body weight and blood glucose; they could not tolerate the dose and hence were killed after 2 weeks instead of after 4 weeks of dosing.

Beagle dogs were fed diets containing myclobutanil (79.6% purity) at concentrations of 0, 10, 200, 800 or 1600 ppm for 3 months. The primary target organ, as evidenced by dose-related hepatocellular hypertrophy, was the liver with a NOAEL of 200 ppm in males, and 800 ppm in females. NOELs were 10 and 200 ppm in males and females, respectively. At 800 ppm in males, hepatocellular hypertrophy had increased in severity, and liver weights were increased. At 1600 ppm, liver weights were increased in both sexes as was serum alkaline phosphatase (also increased at 800 ppm in females) and platelet counts. Body weight and food consumption were also decreased at 1600 ppm, probably due to palatability problems. The critical NOAEL was 200 ppm (equal to 5.9 mg ai/kg bw/day) in male dogs.

Purebred beagle dogs were fed diets containing myclobutanil (91.4% purity) at concentrations of 0, 10, 100, 400 or 1600 ppm for 12 months. Once again the primary target organ was the liver, with a NOEL of 100 ppm (equal to 3.1 mg ai/kg bw/day for males and 3.8 mg ai/kg bw/day for females) and a NOAEL of 400 ppm (equal to 14 and 16 mg ai/kg bw/day for males and females respectively). Liver effects at the NOAELs were characterized by mild hepatocellular hypertrophy, and in females, slight liver weight increase and increased serum alkaline phosphatase. At 1600 ppm the severity of the effects on the liver increased. Increased incidence of accentuated lobular architecture, hepatocellular hypertrophy (predominately centrilobular, but panlobular in severe cases), increased serum alkaline phosphatase, and significantly increased liver weights were observed. Additionally, alanine aminotransferase was increased in males, and gamma-glutamyl transferase in females.

e) Short Term Toxicity - Formulation (Rally 40W):

i) Rats:

The only dose level tested, 100 mg formulation/kg bw/day was determined to be the NOEL in a 21 day dermal study (dosing was not continuous) in Sprague Dawley rats. The only effect observed at this dose was chronic inflammation and epidermal thickening of the skin at the dose site.

f) Long Term Toxicity/Carcinogenicity - Technical:

i) Rats:

Sprague-Dawley rats were fed diets containing myclobutanil (90.4% and 91.4% purity) at concentrations of 0, 50, 200 and 800 ppm for 24 months. There was no evidence of oncogenicity. The study NOEL was 50 ppm (equal to 2.5 mg ai/kg bw/day for male rats and 3.2 mg ai/kg bw/day for female rats). At 200 ppm there was a decrease in testicular weight and an increase in testicular atrophy at 24 months. At 800 ppm testicular weights were significantly decreased at 12 and 24 months and the incidence of testicular atrophy was increased at 12, 17, and 24 months. These incidences were within the range of historical controls; however, the data did not provide sufficient evidence to negate the possibility of a toxicologically significant treatment-related effect at 200 or 800 ppm in males. Other adverse effects at 800 ppm included slightly but increased liver weight in females at 3 and 6 months, and slightly increased liver MFO activity at 3, 6 and 12 months (and at 3 months for the 200 ppm dose level). These effects, however, were not considered to be toxicologically significant and therefore a NOAEL was set for females at 800 ppm (equal to 52 mg ai/kg bw/day).

The high dose of 800 ppm was insufficient to induce grossly observed toxicity. However, the Health Protection Branch was informed that this study was being repeated with a high dose of 2500 ppm and the study should be available in 1994. Further information on the testicular atrophy in relation to treatment should be forthcoming from this study which may permit a change in the NO(A)EL. This change is not likely to decrease the assessment of the NO(A)EL.

ii) Mice:

COBS CD-1 mice were fed diets containing myclobutanil (90.4% purity) at concentrations of 0, 20, 100, and 500 ppm. There was no evidence of oncogenesis. The study NOEL was 20 ppm (equal to 2.7 mg ai/kg bw/day for male mice and 3.2 mg ai/kg bw/day for female mice). At 100 ppm, increased MFO activity in both sexes at 6 months and in females only at 3 and 12 months was not considered to be toxicologically significant; no measurements were made at 24 months. The NOAEL was therefore set at 100 ppm (13.7 and 16.5 mg ai/kg bw/day for males and females respectively). At 500 ppm, the liver was affected: at 3 months, alanine aminotransferase was increased in females and liver weight was increased in both sexes; at 3, 6, and 12 months there was increased hepatocyte hypertrophy and an associated increase in periportal punctate hepatocyte vacuolation; at 6 months there was increased Kupffer cell pigmentation and at 12 months increased individual cell hepatocellular necrosis. At 24 months in both sexes there was focal hepatocellular alterations and multifocal hepatocellular vacuolation, which were not associated with any hypertrophy. The incidence of these changes was within the historical control range at 24 months.

In this study the high dose of 500 ppm was insufficient to elicit overt signs of toxicity; there was evidence of some toxicity but the high dose was not maximal. The Health Protection Branch was informed that this study was being repeated with a high dose of 2000 ppm and should be available in 1994. This new study is not likely to result in a decrease in the assessment of the NO(A)EL.

g) Teratogenicity - Technical:

i) Rabbits:

In a dose range-finding study, myclobutanil (78.4%) was administered by gavage to groups of six New Zealand White rabbits, artificially inseminated on day 0 of gestation, on gestational days 7 through 19 at dose levels of 0 (vehicle control), 9.3, 29, 93, 200, 431 or 650 mg ai/kg bw/day. Surviving rabbits were killed on Day 29 and necropsied. The study NOEL was 93 mg ai/kg bw/day for maternal and embryo/fetal toxicity. At 200 mg ai/kg bw/day there was embryotoxicity

(increased resorptions) and maternal toxicity (decreased body weight, irregular feces and red urine). Surviving fetuses appeared normal on gross examination. All does at the two highest doses died; treatment-related effects included lung aberrations and ulcerated/reddened gastric mucosa.

Myclobutanil (90.4% purity) was administered by gavage to New Zealand White rabbits, artificially inseminated on day 0, on gestational days 7 through 19 at doses of 0 (distilled water control), 0 (vehicle control), 20, 60 and 200 mg/kg bw/day. Surviving rabbits were killed on Day 29 and the fetuses were examined for developmental alterations. There was no evidence of teratogenicity in this study. The study NOEL was 60 mg/kg bw/day for maternal and embryo/fetal toxicity. At 200 mg/kg bw/day there was a reduced maternal weight gain throughout gestation and increased frequencies of irregular feces and/or bloody urine. Embryo/fetal toxicity was indicated by an increased frequency of resorptions and abortions, and by decreased fetal body weights.

ii) Rats:

In a dose range-finding study, myclobutanil (79.6% purity) was administered by gavage to 8 pregnant Sprague-Dawley rats/group on gestational days 6-15 at concentrations of 0 vehicle control), 31, 67, 98, 211, 455 and 687 mg ai/kg bw/day. Surviving rats were killed on Day 20 and necropsied. The NOEL for maternal toxicity was 211 mg ai/kg bw/day. At 455 mg ai/kg bw/day, there were two deaths, decreased body weights, clinical signs including red exudate around the mouth and scant feces, and irritated gastro-intestinal tract at necropsy. At 687 mg ai/kg bw/day, all the rats died. The NOEL for embryo/fetal toxicity was 31 mg ai/kg bw/day, based on increased resorptions per litter and decreased number of live fetuses per implantation at 67 mg ai/kg bw/day and above. At 455 mg ai/kg bw/day, fetal body weights were significantly depressed.

Myclobutanil (78.4% purity) was administered by gavage to mated Sprague-Dawley rats on gestational day 6-15 at concentrations of 0 (corn oil vehicle control), 29, 87, 290 and 435 mg ai/kg bw/day. All rats were killed on Day 20 and necropsied. The NOEL for maternal toxicity was 87 mg ai/kg bw/day. Myclobutanil was maternally toxic at 290 mg ai/kg bw/day based on clinical signs of rough hair coat, desquamation, and salivation. The NOEL was 29 mg ai/kg bw/day for embryo/fetal toxicity based on significantly increased resorptions per litter and significantly decreased number of live fetuses per implantation at 87 mg ai/kg bw/day and above and increased skeletal developmental variations, mainly in the ribs at 290 mg ai/kg bw/day and above.

h) Reproductive Toxicity - Technical:

i) Rats:

In a two generation two-litter per generation reproduction study, myclobutanil (78.4% purity) was administered in the diet to Sprague-Dawley rats at concentrations of 0, 50, 200 and 1000 ppm. P1 rats were treated for 8 weeks before mating, during mating, gestation, and lactation. F1a (P2) rats were exposed through their lives including at least 8 weeks after weaning at 22 days and before mating. The NOEL for parental effects was 50 ppm (equal to 3.7 mg ai/kg bw/day). General toxic effects were recorded at 200 ppm (15 mg ai/kg bw/day) including increased liver weight in P1 and P2 males and centrilobular hepatocellular hypertrophy in P2 males. At 1000 ppm (equal to 75 mg ai/kg bw/day), the liver of P2 females was affected (centrilobular hepatocyte hypertrophy and increased liver weights) and P2 males showed grossly small flaccid testes, testicular and prostate atrophy, and decreased epididymal spermatozoa or necrotic spermatocytes.

The NOEL for reproductive effects was 200 ppm (15 mg ai/kg bw/day). At 1000 ppm, decreases were observed in the fertility index (F1a, F2a, and F2b), gestation index (F1a and F2b) and mean litter size (F2a) and the incidence of stillborn pups was significantly increased in all matings. Although birth weights of pups in treated and control groups were similar, the weight gain of the 1000 ppm pups was lower than controls by Day 4 or 7 and the difference increased up to Day 21.

i) Mutagenicity - Technical:

Technical myclobutanil was negative in the following mutagenicity tests:

Test System	Test System	Dose Level	Purity
Ames test with and without metabolic activation	*S.typhimurium* TA1535, TA1537, TA98, TA100	0, 75, 250, 750 2500, 7500 mg/plate	90.4%
CHO/HGPRT with and without metabolic activation	Chinese hamster ovary cells	0,25-100, 120-175 mg/mL	79.6%
In vivo Cytogenicity	CD1 male mice bone marrow	0, 65, 260, 650 mg ai/kg bw	79.6%
In vitro Cytogenicity	Chinese hamster ovary cells	50-75 mg/mL	91.9%
Unscheduled DNA synthesis	Rat hepatocytes	0.1-1000 mg/mL	91.9%
Dominant lethal test	Male rats	0, 10, 100, 735 mg ai/kg bw	91.4%

j) Absorption, Distribution, Metabolism and Excretion - Technical

i) Rats:

Male and female Sprague-Dawley rats were given a single 1 ml gavage dose of radioactive myclobutanil (^{14}C incorporated at the 3 and 5 carbons of the triazole ring) of approximately 150 mg/kg bw. Two rats of each sex were killed after 4 days and the remainder at 7 days. Most (99.3%) of the radioactivity was rapidly eliminated in the urine and feces; half-life clearance was 11 hours in females and 15 hours in males. In individual tissues and organs the highest concentrations were in liver, kidney, and intestines. Six more-polar metabolites (RH-9090, RH-9090 sulfate, compound #5, RH-294, RH-9089, and compound #2, Figure 1), all with oxygen substituents on the butyl group, were equally distributed in the feces and urine of males but in females 75% was in the form of the sulfate conjugate of RH-9090.

Young adult male and female Sprague-Dawley (Crl:CD BR) rats were given single doses of ^{14}C-myclobutanil radiolabeled in the chlorophenyl ring. Group A, 4 rats/sex, received a dose of 1 mg/kg bw intravenously (i.v.). Group B, 4 rats/sex, received the same dose orally. Group C, 12 males and 4 females, received a dose of 100 mg/kg bw orally. Group D, 12 males and 4 females, received an oral pulse dose of 100 mg/kg bw following 2 weeks of dietary administration of 1000 ppm unlabelled myclobutanil. Practically the entire dose (89-115%) was absorbed following oral administration with peak plasma concentrations within 1 hour. Clearance from the plasma was biphasic with a rapid phase $t_{1/2}$ of 5.3 hours (Group C) and 2.0 hours (Group D) and a slow phase $t_{1/2}$ of 25.7 hours (Group C) and 31.5 hours (Group D). Most of the dose was eliminated, essentially evenly distributed in the urine and feces, within 24 hours (i.v. dose) or 24-48 hours (oral dose) and 89-98% of the recovered label was eliminated by 96 hours.

The ^{14}C label appeared rapidly in the tissues of male rats and reached peak concentrations within 1 hour (except 6 hours for livers of Group C) with liver concentrations at 1 hour 2.2 (Group C) and 7.7 times (Group D) greater than whole blood. The ^{14}C label was rapidly eliminated from the tissues in a biphasic manner similar to plasma. At 96 hours the amount of ^{14}C label in the tissues of both sexes was < 1% of the dose.

Myclobutanil was extensively metabolized in the rats; only 1.0% to 3.6% of the excreted dose was the parent compound. Although the same metabolites were in the excreta of males and females, there were five major fractions (> 10% ^{14}C label) excreted in males but only one major fraction in females. Pretreatment for 2 weeks with myclobutanil in the diet had little effect on the distribution and metabolism of a pulse oral dose.

ii) Mice:

Young adult male and female Crl:CD-1 (ICR) BR mice were fed myclobutanil in the diet at doses of 10, 100, and 1000 ppm for 2 weeks prior to receiving a single pulse dose of ^{14}C-myclobutanil by gavage of 2, 20, and 200 mg/kg bw. The radiolabel was at all carbons in the chlorophenyl ring. Three mice/sex/dose were sacrificed at 1, 24, and 96 hours after ^{14}C-dose administration. The ^{14}C-myclobutanil was rapidly absorbed with peak concentrations in the blood at 0.25 to 1 hour. The liver had a greater affinity (4 to 11 times greater) for the ^{14}C label than blood although the liver/blood concentration ratio decreased with increasing dose. The blood, plasma and liver concentrations were proportional to the dose as was the area under the curve of the whole blood concentration time curve. Clearance from the blood was biphasic with a rapid phase $t_{1/2}$ of 0.63 to 0.88 hours (absent in high-dose males) and a slow phase $t_{1/2}$ of 6.0 to 30.1 hours. Excretion of the ^{14}C label was rapid and complete. After 96 hours 81-107% of the dose was excreted. Most of the dose was excreted, approximately equal in the urine and feces of both sexes, within 24-48 hours.

^{14}C-myclobutanil was extensively metabolized to more-polar compounds; only 1-7% of the dose was excreted unchanged. Metabolic profiles were similar between males and females. Four of the 15 isolated metabolics each accounted for > 10% of the excreted radioactivity. The disposition and metabolism of ^{14}C-myclobutanil was similar over the dose range studied.

k) Toxicology Summary:

The acute studies indicated that a single oral dose of technical myclobutanil was slightly toxic to male and female rats and mice.

The short-term (3-month) studies showed that the liver was the primary target organ. In rats, the NOAEL was 1000 ppm (equal to 49 mg/kg bw/day) with effects observed at lower dose levels typical of a normal hepatic compensatory response to the increased demand for the oxidative metabolism of the test compound. The NOEL for mice was 300 ppm (equal to 44 mg/kg bw/day). In dogs, NOELs were 10 ppm (equal to 0.3 mg/kg bw/day) in males and 200 ppm (equal to 5.9 mg/kg bw/day) in females. The NOAEL for males was 200 ppm (equal to 5.9 mg/kg bw/day). In the 1-year dog study, adverse effects, mainly with respect to the liver, were found at 1600 ppm resulting in a NOAEL of 400 ppm (equal to 14 mg/kg bw/day).

At the doses employed in the long-term rodent studies, myclobutanil was not oncogenic. However, in the rat study the high dose was only 800 ppm (equal

to 39 mg/kg bw/day) whereas the 3-month rat study had a NOAEL of 1000 ppm. In the mouse study the high dose was 500 ppm (equal to 70 mg/kg bw/day) whereas the 3-month study had a NOEL of 300 ppm (equal to 44 mg/kg bw/day). There were few effects of toxicological significance at the high doses therefore, the high doses in both long term rodent studies appeared to be low. The registrant is currently repeating the two rodent long term studies with high doses of 2500 ppm in the rat and 2000 ppm in the mouse. These studies should be available in 1994. Mutagenicity/ genotoxicity tests were negative.

In the 2-year rat study the NOEL was 50 ppm (equal to 2.5 mg/kg bw/day in males) based on possible treatment-related effects on the testes (atrophy and decreased weights). In females, a NOAEL of 800 ppm (equal to 52 mg/kg bw/day) was based on adaptive hepatic changes observed at this highest dose level tested.

In the 2-year mouse study the NOEL was 20 ppm (equal to 2.7 mg ai/kg bw/day for male mice and 3.2 mg ai/kg bw/day for female mice) and the NOAEL was 100 ppm (equal to 14 mg/kg bw/day in males) based on adaptive hepatic changes at this dose level.

There was no evidence of teratogenicity in the rabbit or rat. In the rabbit, the NOELs were 60 and 93 mg/kg bw/day, respectively, for both maternal and embryo/fetal toxicity. In the rat, the NOELs were 87 and 29 mg/kg bw/day, respectively, for maternal toxicity and embryo/fetal toxicity.

In a multi-generation rat reproduction study, decreases in the fertility and gestation indices, increased incidence of stillborn pups, decreased pup weight gain and in F_{2a} litters, a lower mean litter size resulted in a NOEL of 200 ppm (equal to 15 mg/kg bw/day) for reproductive effects. A NOEL of 50 ppm (equal to 3.7 mg/kg bw/day) was established for parental toxicity.

The pharmacokinetic/metabolism studies in rats and mice showed that myclobutanil was rapidly absorbed from the gastrointestinal tract, extensively metabolized to more polar metabolites, and rapidly excreted in the urine and feces. Most of a ^{14}C label was excreted within 24 hours and by 96 hours was essentially completely eliminated from the body. A proposed metabolism scheme is given in Figure 1.

At the doses tested myclobutanil was not oncogenic to rats or mice and was not teratogenic to rabbits or rats. Mutagenicity tests were also negative. At high doses myclobutanil caused embryo/fetal toxicity (87 mg/kg bw/day in rats; 200 mg/kg bw/day in rabbits) and reproductive effects in rats (75 mg/kg bw/day).

The primary target organs were the liver and in rats, probably also the testes. The lowest doses for which hepatocellular hypertrophy, as an adaptive response, was recorded were in male dogs at 5.9 mg/kg bw/day in a 3-month study and in male rats at 15 mg/kg bw/day in a reproduction study. Other liver changes found at higher dose levels were increases in liver weight and MFO activity, additional liver histology and, in dogs, increases in liver enzymes. At termination of the 2-year studies, however, no treatment-related histopathological liver changes were found in rats. In mice, histopathological changes not associated with any hepatocellular hypertrophy (individual cell necrosis and vacuolation) were observed. In rats, at 9.8 mg/kg bw/day and above, there were dose-related decreases in testes weight and increased incidence of testicular atrophy. In the rat reproduction study, atrophy of the testes and prostate was recorded at
75 mg/kg bw/day.

In rats and mice, myclobutanil was rapidly absorbed from the gastrointestinal tract and rapidly eliminated in a metabolized form in the urine and feces.

The NOEL of 2.5 mg/kg bw/day obtained in the 2-year rat study was considered appropriate for the estimation of an ADI. This is a conservative NOEL based on testicular effects, which may or may not be treatment-related, at the next higher dose of 9.8 mg/kg bw/day. The two-year rat study is being repeated at higher doses and may clarify the relationship between testicular effects and treatment, but is unlikely to result in a decrease in the assessment of the NOEL.

7.4 Food Exposure (Myclobutanil)

a) Acceptable Daily Intake (ADI):

An ADI of 0.025 mg/kg bw/day has been assessed based on a NOEL of 2.5 mg/kg bw/day in a 2-year rat study and use of a 100-fold safety factor.

b) Food Residue Exposure:

i) Label:

The company label states that the present product is effective to control certain fungal diseases on apples and grapes. Label directions suggest multiple applications per season (for grapes, the label states a maximum of 5 applications during the growing season with a 14-day interval between applications and for apples, a maximum of
6 applications are to be made with a 14-day interval between applications) at application rates of 136 grams ai/ha and 80 grams ai/ha on apples and grapes respectively, and a preharvest interval (PHI) of 14 days.

ii) Plant Metabolism:

Apple trees and grape vines were treated under field conditions with multiple applications of either ^{14}C-phenyl labelled or ^{14}C-triazole labelled myclobutanil at rates equivalent to the recommended application rates. Samples of apples and grapes harvested 7-14 days after the last application and samples of juice and pomace processed from these fruit samples were radioassayed.

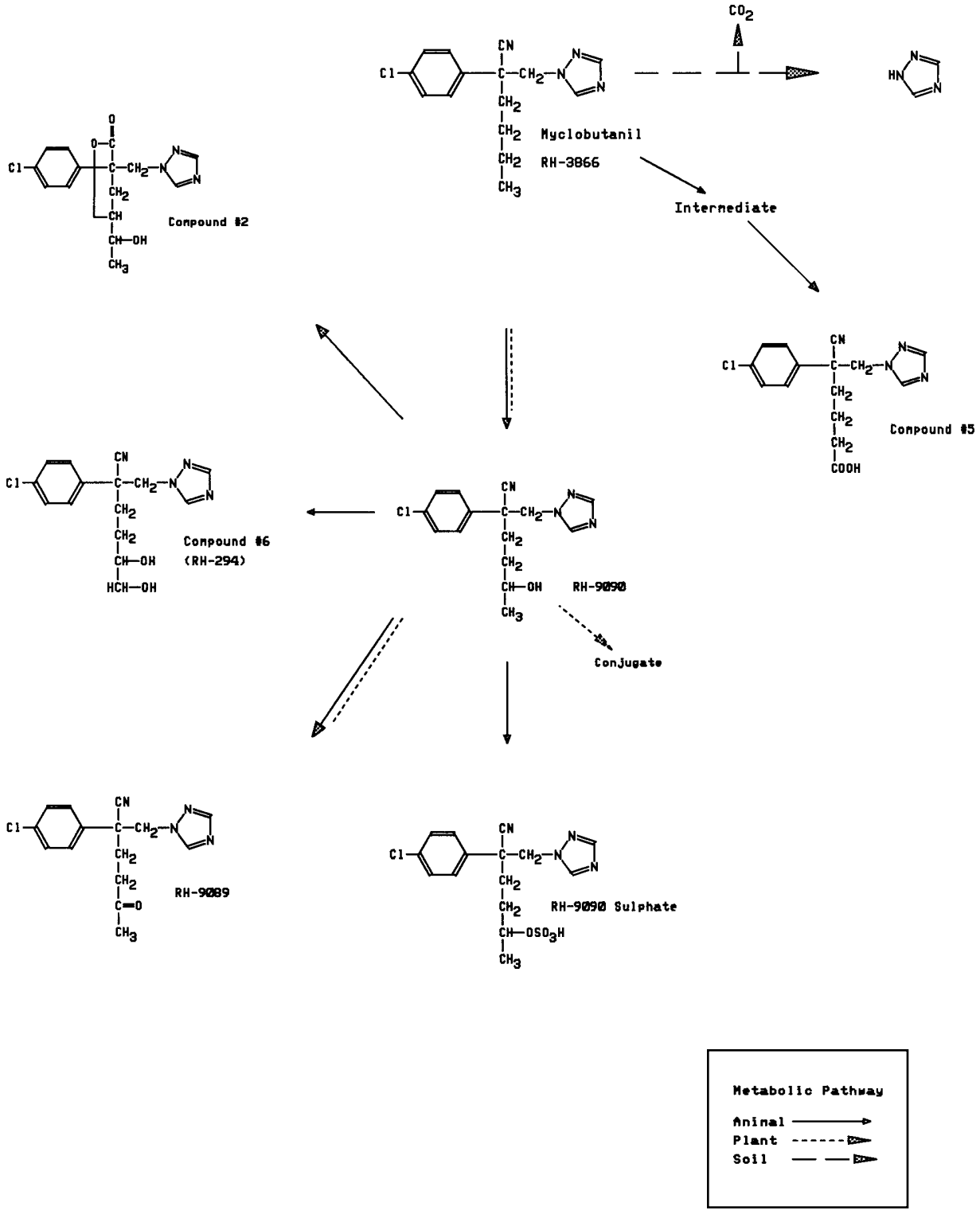
Radioassay analysis indicated that no significant amount of radioactivity was translocated from the treated plant leaves into the untreated leaves and roots. The parent compound was metabolized through oxidation and conjugation in plant tissues. At harvest, the parent compound (myclobutanil) and its metabolites RH-9090, RH-9090 conjugates, and RH-9089 were identified as components in plant terminal residues. These compounds were reported to account for up to 95% of the total radioactive residues found in various apple and grape samples. The distribution of these compounds may differ somewhat in various samples and a summary of typical metabolite distributions is given below. The metabolite RH-9089 was found as a minor component in all cases. A proposed metabolic pathway is given in Figure 1.

The metabolism of myclobutanil in apples and grapes is summarized in Table 1 below.

Compound	% of ^{14}C Label in Commodity			
	Grape (16 day PHI)		Apple (14 day PHI)	
	fruit	juice	fruit	juice
myclobutanil	36 - 55	21.7	48.5 - 66	26 - 33
RH-9089	----	1.3	1 - 1.8	3 - 4
RH-9090	7 - 8	26.5	7 - 11.5	14 - 23
RH-9090 conjugates	11	40.7	5 - 23.7	17 - 24
polar metabolites	1 - 13	----	----	----
bound residues	12 - 15	----	1.8	----

Free 1H-1,2,4-triazole is formed by soil metabolism, is very water soluble, not bound by soil, and is readily absorbed by the plant root systems. Once absorbed the free 1H-1,2,4-triazole reacts in the roots with O-acetyl-serine to form 1H-1,2,4-triazole alanine (TA) which is stable in the plant. This TA is also water soluble and is translocated upward to the aerial portions of the plant. Residues of TA tend to concentrate in the fruiting portions of the plant. This mode of metabolism of free 1H-1,2,4-triazole residues is common to all of the triazole fungicides.

Figure 1: Proposed Major Metabolic Pathways for Myclobutanil



Metabolites Indicated in Figure 1

RH-3866	Myclobutanil; (RS)-2-p-chlorophenyl-2-(1H-1,2,4-triazole-1-ylmethyl)hexanenitrile
RH-9090	(RS)-2-p-chlorophenyl-2-(1H-1,2,4-triazol-1-ylmethyl)-5-hydroxy-hexanenitrile
RH-9090 sulfate	(RS)-2-p-chlorophenyl-2-(1H-1,2,4-triazol-1-ylmethyl)-5-hydroxy-hexanenitrile sulfate
Triazole	1,2,4-H-triazole
Compound #5	(RS)-2-p-chlorophenyl-2-(1H-1,2,4-triazol-1-ylmethyl)-6-carboxy-hexanenitrile
Compound #2	3-lactone-4-hydroxy myclobutanil
RH-294 or Compound #6	(RS)-2-p-chlorophenyl-2-(1H-1,2,4-triazol-1-ylmethyl)-5,6-hydroxy-hexanenitrile
RH-9089	(RS)-2-p-chlorophenyl-2-(1H-1,2,4-triazol-1-ylmethyl)-5-keto-hexanenitrile

c) Animal Metabolism:

Animal metabolism studies were carried out on rodents and livestock. In rats and mice, the ^{14}C labelled myclobutanil was rapidly absorbed from the gastrointestinal tract and rapidly excreted in a metabolized form in the urine and feces. Several metabolites were identified, all had oxygen substituents on the butyl group of the parent compound. RH-9090 the major plant metabolite and RH-9089 the minor plant metabolite were both identified in urine and feces.

Metabolism studies carried out in dairy cattle indicated that 98% of the administered dose was eliminated in the urine and feces. In the urine, four metabolites were identified, RH-294 (31%), RH-9090 (23%) and 2 unidentified polar metabolites (19% and 13%). In milk, 80% of the residues were comprised of 4 unidentified polar metabolites. The nonpolar fraction contained 20% of the milk residues and the majority was identified as RH-294. Milk solids and soluble whey contained over 85% of the radiolabel. The polar metabolites in milk were not identified due to the low concentrations of ^{14}C present.

Metabolism studies carried out in laying hens indicated that myclobutanil and its metabolites have little tendency to accumulate in eggs, organs, or meat. 95% of the dose was eliminated in the excreta. In eggs, organs, or meat, RH-9090 was identified as the major metabolite with smaller quantities of RH-9089, RH-294, compound #2 (see Figure 1), and other more polar metabolites.

The livestock metabolism studies evaluated indicate that if residues of myclobutanil and its metabolites do not exceed 3 ppm in total livestock diet then residues in all livestock tissues and products, including milk and eggs, will not exceed 0.1 ppm.

d) Soil Metabolism:

Soil studies to investigate myclobutanil metabolism indicated that myclobutanil was stable under anaerobic conditions. Under aerobic conditions myclobutanil was metabolized to free 1H-1,2,4-triazole, carbon dioxide, and bound polar compounds.

e) Analytical Methodology:

Analytical methods have been developed which are capable of determining the residues of the parent compound myclobutanil and its metabolites RH-9090, RH-9090 conjugates, and RH-9089 in crops, and the parent compound, RH-9090, and RH-0294 in animal products.

f) Residues:

i) Crop Residues:

Extensive residue data generated using the above methods have shown that when crops are treated in accordance with the label directions (multiple applications at 136 g ai/ha for apples and 80 g ai/ha for grapes and the last application at 14 days before normal harvest), the following maximum residue limits (MRL) may be required to cover the total residues of the parent myclobutanil and its major metabolites:

Apples	0.5 ppm
Grapes	1.0 ppm
Raisins	10.0 ppm

The available processing studies indicate that there is no concentration of residues in apple cider (less than 40% of the whole apple residues), grape juice (less than 20% of the whole grape residues), or wine (less than 40% of the whole grape residues). The proposed MRLs should therefore be adequate to cover possible residues of myclobutanil found in the processed products of apples and grapes.

An accumulation of free 1H-1,2,4-triazole in soil, uptake of free triazole by plants, conversion of the triazole to TA by reaction with O-acetyl-serine in the roots, and subsequent accumulation of TA residues in crops may occur under Canadian conditions. While low levels of TA residues may occur in apples and grapes, it is unlikely that residues of TA greater than 2.0 ppm, the proposed MRL for TA, will occur. The petitioner is developing an analytical method to determine the residues of TA in crop samples. The residue analysis of treated apple and grape samples for TA will be submitted for evaluation upon completion.

ii) Animal Residues:

Total residues of 1.0-3.5 ppm in apple pomace may result from the proposed uses. Apple pomace is currently cleared for animal feed uses by Agriculture Canada but if fed, would only constitute a small portion of the livestock diet. If, however, livestock were fed treated apple pomace containing 3 ppm residues at 100% of the diet, then no significant residues would be expected in cattle meat, fat of meat (<0.02 ppm) or milk (<0.03ppm); in chicken tissues, fat (<0.01 ppm) or eggs (<0.02 ppm); but may result in low levels of residues in cattle liver (maximum 0.108 ppm).

Total residues of 2.0 to 25 ppm may be present in grape pomace processed from treated grapes. Residue data from the feeding of animals with myclobutanil at 30 ppm may result in significant residues in milk (0.258 ppm), cattle tissues such as kidney (0.182 ppm) and liver (0.965 ppm), and chicken eggs (0.122 ppm). Grape pomace is not presently cleared for use as an animal feed in Canada. If, however, this product were to become an approved animal feed, maximum residue limits in livestock liver resulting from this use should not exceed 0.3 ppm, residues in livestock fat, meat, meat byproducts, and milk should not exceed 0.05 ppm, and residues in poultry fat, meat, meat byproducts, and eggs should not exceed 0.02 ppm. Residues at these levels are not considered to pose a hazard to human health.

iii) Soil Residues:

Simulated soil residue studies indicate that very little parent compound or its metabolite RH-9090 would be available in soil under the proposed use conditions. Uptake of residues of these two compounds from soil by crops, if any, would be very minimal. Soil metabolism studies have shown that free 1H-1,2,4-triazole may be formed in soil and may persist at low levels from season to season.

g) Dietary Risk Assessment:

The theoretical daily intake (TDI) of residues by the general population for the proposed uses on apples and grapes, including processed products of apples and grapes, the intake from wine consumed by the total adult population, and potential residues in meat and milk, assuming maximum residues at all times, would not exceed 0.0030 mg/kg bw/day, and represents approximately 12% of the ADI of 0.025 mg/kg bw/day.

For young children consuming milk as 100% of their diet, and assuming residues of myclobutanil at 0.1 ppm (the general Regulation MRL limit) in whole milk, the estimated TDI would utilize approximately 50% of the ADI. The residue and livestock metabolism data indicate, however, that residue levels in whole milk are not expected to exceed 0.05 ppm and this represents an intake of 25% of the ADI.

These proposed maximum residue levels for apples, grapes, raisins, whole milk, eggs, and livestock products are not considered to pose a health hazard to consumers, even to young children consuming large quantities of apple juice, grape juice, or milk.

7.5 Occupational Exposure and Safety Assessment (Myclobutanil)

a) Qualitative Exposure Assessment:

NOVA 40W® is proposed for use on apples at an application rate of 136 g ai/ha and for grapes at an application rate of 80 g ai/ha. For grapes, the label states a maximum of 5 applications during the growing season with a 14-day interval between applications. For apples, a maximum of 6 applications are to be made with a 14-day interval between applications.

Recent Agriculture Canada census documents as well as crop specialists indicated that on average, apple orchards in the major apple producing regions (Ontario, Quebec, British Columbia and Nova Scotia) range from 8-20 ha. For grape production, average vineyard sizes in the major grape producing regions are 2.8 ha in British Columbia and range from 20-24 ha in Ontario.

In addition to persons handling and applying the product, those having a potential for occupational exposure include crop harvesters, and persons engaged in various activities between spray applications and harvest. The latter group includes workers performing pruning and thinning activities. In the case of grapes, the majority of harvesting in Canada (85-95%) is performed by mechanical means.

b) Quantitative Exposure Assessment:

The registrant has submitted a worker exposure study for Rally 40W (identical formulation as NOVA 40W®) and a dislodgeable foliar residues study with Rally 60DF to estimate exposure to harvesters and re-entry personnel.

c) Worker Exposure Study - Mixer/Loader/Applicators (M/L/A):

The worker exposure study was conducted in 3 fresh-market vineyards in California, involving 6 trials in total. Rally 40W, formulated in water soluble pouches, was applied at an application rate of 140 g ai/ha with commercial orchard airblast equipment pulled by open cab-tractors. Similar application equipment would be used in apple orchards. Each trial involved licensed personnel consisting of 1 mixer/loader and 1 applicator monitored for 2 mix/load, application phases at sites either 2.6 or 3.8 ha in size. Workers wore rubber (butyl) gloves, long sleeved shirts, long pants, a baseball-type cap and work boots for the study duration. Mixer/loader tasks for both phases ranged from 11.5 - 20 minutes while those for applicators ranged from 71 - 109 minutes. Exposure was assessed by dermal deposition and inhalation monitoring. Samples of urine collected from the workers were analyzed for myclobutanil residues, but due to incomplete collection, it was not possible to use the biological monitoring results.

Exposure for both the apple and grape use was estimated for a 70 kg worker wearing long pants, long sleeved shirt, butyl gloves (worn in the study), a baseball-type cap and work boots, using the product at the maximum label application rates for both crops (grapes 80 g ai/ha, apples 140 g ai/ha). Although the registrant submitted a dermal absorption study, it was not useful to estimate dermal absorption to NOVA 40W® since it was performed using a different myclobutanil formulation (2EC). In the absence of adequate dermal absorption data for NOVA 40W®, dermal absorption was assumed to be 100%. The estimated mean exposures and ranges were calculated to be:

Crop Use		Exposure (mg/kg bw/day)
Apples	(based on 20 ha)	0.0038 (0.0012 - 0.0136)
Grapes	(based on 2.8 ha)	0.0003 (0.0001 - 0.0011)
	(based on 24 ha)	0.0027 (0.0008 - 0.0096)

The monitored time and areas treated are below that considered typical for grape production in Ontario as well as for apple production across Canada. Because a large extrapolation was required in calculating exposure for these scenarios, the derived exposure values should be viewed with caution.

Other limitations of this exposure data include:

i) Exposure calculations did not include monitoring during cleanup and repair activities.

ii) There were concerns regarding the quality assurance portion of the study, notably the field recovery phase.

iii) The same individual was not monitored through all phases of the pesticide application since mixer/loader tasks were monitored separately from application.

iv) Exposure was monitored for only six cycles of mixing/loading/ application.

Despite these limitations, the study was considered adequate to estimate worker exposure to myclobutanil.

d) Re-entry Exposure - Dislodgeable Foliar Residues:

The study was conducted in 3 vineyards in California reported to have had no previous application of Rally 60DF. Rally 60DF was applied with airblast equipment to grapes over the growing season at similar application rates and for the maximum number of applications proposed for use in Canada. Foliar samples were taken after the final application at ten selected intervals (2 hours to day 35 post application) and analyzed for dislodgeable residues of myclobutanil. The residue data were fit to a first-order rate equation using non-linear regression and half-lives of decline for foliar residues calculated. The registrant used a model developed by Popendorf and Leffingwell[2] to estimate potential dermal exposure of
re-entry personnel from foliar residues measured post-application. The study authors reported that dislodgeable foliar residues were present, with levels declining over the 35-day sampling interval. There were concerns regarding the design of the study and the application and use of the model including:

i) The study was conducted with a 60% dry flowable formulation and the product submitted for registration is a 40% wettable powder formulation. A different formulation may have an effect on the physical parameters associated with deposition, retention and subsequent dislodgeability from the leaf surface.

---

[2] *Popendorf, W.J., and Leffingwell, J.T., Regulating OP pesticide residues for farm worker protection, in Residue Reviews, Volume 82, pages 125-201, Springer-Verlag New York, Inc., 1982.*

ii) A key component of the model is a constant, the crop specific dosing coefficient. Selection of this constant was based on values derived from numerous field studies involving other crops (primarily citrus and strawberries), various other pesticides and formulation types as well as varying degrees of protective clothing/ gloves. It is difficult to predict the impact of these variables on this constant for the proposed use scenarios.

iii) Dislodgeable foliar residue data were available from grape leaves only. The relevance of this data to predict dislodgeable residues from apple leaves is uncertain.

The extent of limiting factors in this particular case preclude the use of the dislodgeable foliar residue data in quantifying exposure to re-entry personnel. At this point in time, approaches to the quantitative assessment of re-entry exposure are under development within the Health Protection Branch.

e) Occupational Risk Assessment:

The range of toxicology studies on myclobutanil revealed the liver to be the target organ with evidence of adaptation at low dose levels. Since occupational exposure is short-term in nature, the most relevant study for risk assessment would be a short term toxicity study. For effects resulting from short term exposure, the lowest NOEL (3.7 mg/kg bw/day based on general liver effects) was demonstrated in the parental treatment period of the rat reproduction study.

Exposure to a typical 70 kg worker (mixer/loader/ applicator) wearing long pants, long sleeved shirt, butyl gloves, a baseball-type cap and work boots, using the product at the maximum label application rate and a typical area for apples, was estimated to be 0.0038 (0.0012 - 0.0136) mg/kg bw/day. For the grape use, the estimate of exposure was greatest for large vineyards such as those found in Ontario with a value of 0.0027(0.0008 - 0.0096) mg/kg bw/ day. The estimated margin of safety (MOS) for the apple use is 970 (range 270 - 3080). For the grape use, the MOS is 1370 (range 390 - 4600). The Health Protection Branch considers these MOS for mixer/loader/applicators to be acceptable.

A quantitative estimate of exposure to re-entry personnel could not be made due to a lack of confidence in the available data. Accordingly, margins of safety could not be estimated. In the absence of significant health concerns, the Health Protection Branch is amenable to considering interim risk reduction measures until such time as re-entry exposure has been formally addressed in the regulatory framework. These measures include the use of protective clothing and the addition of appropriate label re-entry statements.

8.0 Environmental Aspects
Environment Canada

Environmental Impact of Myclobutanil Fungicide (NOVA 40W®)

8.1 Summary

Biotransformation in soil under aerobic conditions was the major route of transformation of myclobutanil. Under anaerobic soil conditions, myclobutanil was found to be stable. Hydrolysis, photolysis, and volatilization from water and moist soil are unlikely to be significant processes in the dissipation of myclobutanil. Data from laboratory studies demonstrated that myclobutanil had a low-to-moderate potential for vertical mobility in soil. The results from two Canadian field dissipation studies indicated that myclobutanil has the potential to persist in soil.

Studies on daphnids indicated a low potential for toxicity to aquatic invertebrates. Investigations employing indicator species for terrestrial invertebrates suggest that myclobutanil would have a low toxicity to these nontarget organisms.

Further studies are required to complete the evaluation of bioaccumulation potential, aquatic biotransformation, and the fate of the active and the major transformation products under field conditions in Canada.

Wildlife may be exposed to myclobutanil through consumption of contaminated food, or through dermal contact with contaminated foliage. On the basis of the LD_{50} and LC_{50} data, estimated risk factors are low for birds or mammals (range 10^{-2} to 10^{-3}). However, on the basis of the no-observed-adverse-effect-levels (NOAELs) for three-month dietary studies in mammals, risk factors (i.e., ratio of expected exposure level to level causing toxic effect) are relatively high (e.g. 0.08 for a rat, 0.53 for a shrew, 0.27 for a mouse). Risk factors based on the lowest-observed-effect-levels (LOELs) for systemic and/or reproductive toxicity, in teratogenicity and reproductive studies, are also high (range 0.08 to 1.96).

Avian reproduction studies indicate no statistically significant effects. Nevertheless, some reproductive performance indicators of the Bobwhite Quail did respond, in a negative fashion, with dose. These apparent responses may be biologically significant. Unfortunately, the highest dose used in the avian reproduction studies is less than expected residue levels.

The estimated risk factors are not considered to be overestimates. The dietary scenarios represent the “worst case”, but the residue data are based on specific studies as well as generic data. As well, the projected use pattern of myclobutanil involves up to 8 applications per season at 7 to 10 day intervals on apples or up to five applications per season at 14 to 21 day intervals on grapes. Since the reported half-life of residues on fruit and foliage ranges from 7 to 28 days, it is reasonable

to conclude that mammals and birds could be exposed to myclobutanil residues over a three to four month period. The exposure estimates do not include dermal exposure from direct spray, spray drift, or contact with contaminated foliage.

The registrant has not submitted an algal study, or any other information on toxicity to aquatic or terrestrial plants. The potential for modification of wildlife habitat is therefore unknown.

8.2 Physicochemical Properties and Environmental Chemistry

Myclobutanil had a high solubility in aqueous solution. A high aqueous solubility is an indicator for potential leaching and surface runoff. The value for vapor pressure indicated a low potential for volatilization. Henry's Law Constant, as calculated by the reviewer, indicated a low potential for volatilization from water and moist soil.

A potential for the accumulation of myclobutanil in nontarget organisms was indicated by the reported values of the following factors (used to predict the potential for bioaccumulation): (the value reported for) the Octanol/Water Partition Coefficient (K_{ow}); molecular weight; solubility; persistence; and degree of ionization. A bioaccumulation/depuration study with earthworms has been requested.

Hydrolysis and phototransformation are not expected to be routes of transformation for myclobutanil. Hydrolysis was not detected in aqueous solutions at pH 5, 7, and 9. Phototransformation studies were conducted in pond water and in deionized water and the DT_{50} was reported to be approximately 3 weeks and 7 months, respectively. No major phototransformation products were detected on soil. Laboratory studies on the adsorption/desorption to soils indicated a low to moderate potential for vertical mobility. In a column leaching study it was found that radiolabelled test material remained in the top portion of the column. The results indicated a low potential for vertical mobility in soil for myclobutanil and the transformation products. Biotransformation studies under aerobic conditions in soil indicated that myclobutanil was persistent (DT_{50} of approximately 4 months). In anaerobic soil studies myclobutanil was stable. The only major (>10%) transformation products in soil, 1,2,4-triazole, had a reported DT_{50} in soil of several weeks in laboratory studies.

Aquatic biotransformation studies (aerobic and anaerobic) have been requested. The persistence of myclobutanil in aquatic environments cannot be assessed until these studies have been submitted.

Field dissipation studies in British Columbia and Ontario have shown that the reported DT_{50} value for myclobutanil is approximately 1 year. Multiple applications were employed in accordance with label instructions. These studies clearly indicated the potential for carry-over into the following growing season. Myclobutanil was only sporadically detected at below 15cm in British Columbia and not below 15cm in Ontario, while 1,2,4-triazole was not detected below 7.5 cm. The assessment of the potential for vertical mobility and dissipation of myclobutanil and 1,2,4-triazole under field conditions in all major areas of intended use in Canada could not be completed because of data gaps in the studies that were submitted. Further field dissipation studies have been requested.

8.3 Environmental Toxicology

Myclobutanil displayed slight toxicity to *Daphnia magna*. Acute toxicity studies with honey bees and earthworms indicated that there was a low potential for toxicity to these species when exposed to a single application of myclobutanil at the maximum application rate. Myclobutanil should not pose a significant hazard to soil microorganisms.

Laboratory data indicated that the major soil transformation product, 1,2,4-triazole, should not pose a significant hazard to earthworms, daphnia, and soil microorganisms.

The acute oral LD_{50} in Bobwhite Quail is 380 mg-ai/kg-bw (male) and 604 mg-ai/kg-bw (female), and the LC_{50} in Bobwhite Quail and Mallard Ducks is greater than 4640 mg-ai/kg-diet.

In avian reproductive studies, with Mallard Ducks and Bobwhite Quail, no statistically significant effects were apparent at doses up to 60 mg-ai/kg-diet. In Bobwhite Quail, however, most reproductive performance indicators, including the percentage of eggs set, the percentage of eggs set that were fertile at 11 days, and the number of embryos hatched per eggs set, were decreased from control levels. Although these effects were not statistically significant, a dose response relationship was evident.

Exposure for wild birds was estimated on the basis of consumption of contaminated fruit or vegetation. Additional exposure could result from dermal contact with contaminated foliage or direct overspray of nesting birds. Worst-case acute risk estimates were calculated for a Bobwhite Quail ingesting 8.9% of its bodyweight daily in contaminated vegetation, a Wren ingesting 34% of its bodyweight daily in contaminated insects, and a robin consuming 15% of its body weight daily in earthworms. On the basis of LD_{50} and LC_{50} data, estimated risk factors are less than $2x10^{-2}$. On the basis of the reproductive NOEL (i.e., the highest dose used in the study), the risk factors for a bobwhite quail consuming contaminated vegetation or fruit would be 1.3 and 0.1 respectively.

In laboratory mammals, oral LD_{50}'s for technical and formulated myclobutanil range from 750 to 3200 mg-ai/kg-bw. In three month dietary toxicity studies with rats and mice, the NOAEL, for effects that include decreased food consumption, decreased bodyweight, and increased liver and kidney weight, ranged from 42 to 66 mg-ai/kg-bw/d. In dogs, the NOAEL was higher. Myclobutanil is not considered to be teratogenic or oncogenic. In rat and rabbit teratogenicity studies, the NOEL for systemic toxicity ranged from 20 to 31 mg-ai/kg-bw/d. In a rat reproductive study, the NOEL for systemic toxicity was 50 mg-ai/kg-diet (equivalent to 3.7 mg-ai/kg-bw/d). The LOEL for systemic toxicity, and the NOEL for reproductive effects, was 200 mg-ai/kg-diet (equivalent to 14.0 mg-ai/kg-bw/d).

The most likely exposure routes for wild mammals are consumption of contaminated vegetation or consumption of contaminated prey. Risk factors were estimated for a rat or mouse ingesting a diet of contaminated vegetation, and for a small shrew ingesting 100% of its bodyweight daily in contaminated arthropods. The exposure estimates do not include dermal exposure from contact with spray or contaminated foliage. On the basis of LD_{50} data, estimated risk factors are less than 4×10^{-2}. Risk factors estimated on the basis of the NOAELs from three month dietary studies, or the NOELs/LOELs for systemic or reproductive toxicity in reproductive and teratogenicity studies, are much higher (range 0.08 to 7.4). For example, on the basis of the rat reproductive LOELs, risk factors are 0.08 and 0.6 for the rat and shrew respectively.

No data were available to evaluate the risk to amphibians and reptiles from the use of myclobutanil. These organisms could be exposed by dermal exposure from spray drift or by ingestion of contaminated invertebrates.

8.4 Fish and Fish Habitat Studies

Myclobutanil has the potential to contaminate aquatic environments through spray drift during application, surface runoff, and erosion of soil with bound fungicide. If a body of water 0.15 m deep were to receive a complete overspray at the maximum label rate, the expected environmental concentration (EEC) for a single application would be 90 µg/L. Multiple application indicated the potential for increasing aquatic concentrations of myclobutanil over the growing season if transformation did not occur. The data on hydrolysis and photolysis in water indicated these processes would not play a major role in the transformation of myclobutanil in aquatic environments. However, an assessment of the fate of myclobutanil in aquatic environments cannot be complete until data from studies on aquatic biotransformation have been submitted.

The 96-h LC_{50} values for rainbow trout and bluegill sunfish were reported to be in the 2-5 mg ai/L range. No effect levels for rainbow trout and bluegill sunfish were between 1 and 2 mg ai/L. The LC_{50} values were based on nominal

concentration and, thus, these values were considered to be approximate. The steep slope of the toxicity curves indicated that small changes in concentration could result in major changes in acute toxicity to fish. The margin of safety for acute toxicity to fish is considered to be low if the LC_{50} values are less than two logs greater than the EEC. Using this criteria, myclobutanil had an unacceptable safety margin. Chronic toxicity studies are required to determine the toxicity of myclobutanil to the most sensitive life cycle stages of fish. This study should be carried out using the most sensitive species that were tested in the acute toxicity studies.

The K_{ow} and the rate of transformation of myclobutanil in aquatic environments are two of the parameters that are used to assess the potential for bioaccumulation in fish. A bioaccumulation study is required to examine the accumulation and depuration of myclobutanil in fish tissue following the maximum number of applications indicated on the label.

Aquatic invertebrates are a major food source for fish. The study of toxicity to *Daphnia magna* indicated that myclobutanil would present a low hazard to aquatic invertebrates.

A complete assessment of hazards to fish and fish habitat cannot be made at this time due to the lack of critical data.

8.5 Wildlife Habitat Conservation

The primary mode of action of triazole fungicides is thought to be competitive inhibition of the cytochrome P-450 enzymes that catalyse the oxidation of the ^{14}C methyl group of fungal sterols. As well, phytosterol and gibberellin biosynthesis may be inhibited, which can lead to stunting of treated plants. This suggests that myclobutanil may have some impact on plants in and around the spray areas. The registrant has not submitted an algal study, or any other information on the toxicity of myclobutanil to non-target aquatic or terrestrial plants. Fungicides that are structurally related to myclobutanil are known to be toxic to algae. The potential for modification of wildlife habitat is therefore unknown.