Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Proposed Regulatory Decision Document PRDD96-01

Tebufenozide

The active ingredient tebufenozide and the formulated product Confirm® 240 F Agricultural Insecticide, for control of larval Lepidoptera, are proposed for registration.

This document provides a summary of data reviewed and the rationale for the proposed regulatory decision concerning these products.

This document has been prepared in keeping with the ongoing efforts of the Pest Management Regulatory Agency (PMRA) to regulate pest control products in an open and transparent manner.

The PMRA will accept written comments on this proposal up to March 16, 1996 and expects to make a final regulatory decision by April 1, 1996. Please forward all comments to:

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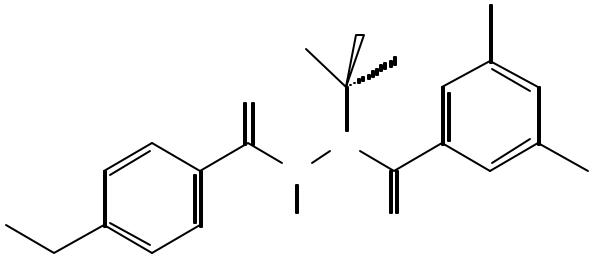
1.0 Introduction

Tebufenozide, a new active ingredient, is an Insect Growth Regulator (IGR) in the chemical family benzoic acid hydrazide. An aqueous flowable formulation, Confirm® 240 F Agricultural Insecticide, for use in apple orchards, is proposed for registration. Tebufenozide has a novel mode of action in that it mimics the action of the insect molting hormone, ecdysone, in larval Lepidoptera (caterpillars), by initiating an unsuccessful (lethal) molt in the larvae. Larvae stop feeding within hours of ingestion of a toxic dose; death occurs in three to seven days.

1.1 Product Identification/Description

Registrant and manufacturer:	Rohm and Haas Company Independence Mall West Philadelphia, PA 19105
Trade names:	Confirm®
Common name:	Tebufenozide
Chemical name:	- IUPAC name: *N*-*tert*-butyl-*NN*-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide - C.A. name: Benzoic acid, 3,5-dimethyl-1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl)hydrazide - Other Name: 3,5-dimethylbenzoic acid 1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl)hydrazide
CAS number:	112410-23-8
Chemical structure:	
Molecular formula:	$C_{22}H_{28}N_2O_2$
Molecular weight:	352.48

Chemical and physical properties of pure substance (99.6% pure tebufenozide):

Colour:	White
Odour:	Faint
Physical State:	Powder
Melting point or range (for solids):	191-191.5°C
UV-visible absorption spectrum:	λ_{max} 233.5 nm in 0.1M NaOH in methanol

Solubility:

Water:	0.83 ppm
Acetone:	6.5 g/100 mL
Acetonitrile:	2.5 g/100 mL
n-butyl acetate:	1.4 g/100 mL
Methanol:	10.8 g/100 mL
Methylene chloride:	24.0 g/100 mL
Toluene:	0.24 g/100 mL

Dissociation constant: Not expected to dissociate in water

Chemical and Physical Properties of Technical Material:

Colour:	White
Odour:	Faint
Physical State:	Lumpy powder
Density:	Bulk density 0.37 g/100 mL
Vapour pressure:	3×10^{-6} Pa (2×10^{-8} Torr) at 25°C
Stability:	No significant change after one year storage at 25°C or after one week at 94°C
Log mean K_{ow}:	4.23

Chemical and Physical Properties of Confirm® 240 F:

Colour:	Cream-coloured liquid suspension
Odour:	Slightly musty
Stability of formulation during storage:	No significant change after one year storage at 25°C
Specific gravity:	1.067 at 20°C

1.2 Chemistry Assessment

Listed specifications of the technical active ingredient included active ingredient and impurities at or above 0.1%. These specifications are considered Confidential Business Information and cannot be disclosed. The specifications submitted for the technical were based on pilot production and will be updated when full-scale production is available.

Active ingredient and impurities were determined by methods which identify the specific compound being analyzed rather than a group of compounds. Some minor impurities were not identified; however, these are expected to be below 0.1% in full-scale production.

The company will confirm the identity of compounds specified in full-scale production.

Technical material was analyzed for nitrosamines. Two nitrosamine compounds were detected at extremely low levels (ppb range) which are not considered significant. Further microcontaminant analyses will be conducted on the technical after full-scale production is initiated.

Quality control data for technical material and formulations will become available when full-scale production has begun.

2.0 Health Assessment

2.1 Toxicology

Toxicokinetics - Technical

Rats:

Three groups of four rats (Crl:CD BR strain)/sex/group, prefasted overnight, were dosed with a single oral dose (by gavage) of 250 mg/kg bw of either [^{14}C-*t*-butyl]-tebufenozide (Group 1), [^{14}C-A-ring]-tebufenozide (Group 2), or [^{14}C-B-ring]-tebufenozide (Group 3). The excretion of radioactivity in male and female rats of all three dose-groups followed similar profiles. Absorption and excretion of ^{14}C-radioactivity were rapid; >70% of the administered dose was eliminated within 48 h of dosing. Mean total excretion over the 7-day period was $82%. Faeces was the major route of excretion, representing at least 98% of total ^{14}C-radioactivity excreted. Only minor amounts (1-2% of dose) were excreted in the urine and trace amounts (#0.05% of dose) were eliminated in the expired air as $^{14}CO_2$ or volatile organic compounds. Retention of ^{14}C-radioactivity in organs/tissues at 7 days post-dosing was very low: <0.1%, <0.03% and <0.01% for Groups 1, 2 and 3, respectively. The

highest tissue concentrations were found in the liver, blood, spleen and fat (0.5-1.3 ：g equivalents ^{14}C-tebufenozide/g) in Group 1 animals and in the fat (<1 ：g equivalents ^{14}C-tebufenozide/g) in Group 2 animals. The concentrations of tissue radioactivity in Group 3 animals were near or below the limit of detection (0.4 ppm).

Four male and four female rats (Crl:CD BR strain), bile duct-cannulated and pre-fasted overnight, were each dosed with a single oral dose (by gavage) of 3 mg [^{14}C-*t*-butyl]-tebufenozide/kg bw. The absorption and elimination of ^{14}C-tebufenozide were followed up to 72 h after dosing. There was no significant difference in the excretion profiles between males and females. Absorption and excretion of ^{14}C-radioactivity were rapid; recovery of the administered dose in excreta was ~100% within 24 h of dosing. The majority of the ^{14}C-radioactive dose (67-70%) was unabsorbed and eliminated directly in the faeces. Systemic absorption was calculated to be 35-39% of the total administered dose; 30-34% was excreted in the bile and ~5% in the urine. Tissue retention of ^{14}C-radioactivity was very low. The mean % of administered dose remaining in the carcass was 0.3-0.4% at 72 h after dosing.

Seventeen groups (3-6 rats/sex/group) of Sprague-Dawley rats of the Crl:CD BR strain were tested. Animals were each administered a single oral dose (by gavage) of either [^{14}C-*t*-butyl]-tebufenozide, [^{14}C-A-ring]-tebufenozide or [^{14}C-B-ring]-tebufenozide at nominal doses of 3 or 250 mg tebufenozide/kg bw. One group of animals was fed a diet containing 30 ppm nonradioactive tebufenozide for 2 weeks before receiving a single oral dose of [^{14}C-*t*-butyl]-tebufenozide at 3 mg/kg bw. Absorption/excretion of the ^{14}C-radioactivity was very rapid. The excretion profiles were similar, regardless of the position of the ^{14}C-label, dose level, sex of the rat, or whether the animals were pre-treated with 30 ppm dietary tebufenozide for 2 weeks. A mean total of 87-104% of the administered dose was excreted within 48 h of dosing, primarily via the faeces which accounted for >90% of the radioactivity excreted. Only minor amounts (<1-8% of the dose) were excreted in the urine. Trace amounts of radioactivity (<0.1-0.4% of the dose) were recovered in the expired air as CO_2 and volatile organic compounds from rats dosed with [^{14}C-*t*-butyl]-tebufenozide, but not from rats dosed with either the [A-ring]- or [B-ring]-label. The results suggest that a small amount of ^{14}C-radioactivity was metabolically cleaved (possibly via N-dealkylation or oxidation) from the *t*-butyl group of the tebufenozide molecule and released in the form of CO_2 and organic volatiles. Maximal levels of ^{14}C-radioactivity in the blood were measured at times ranging from 0.5-12 h after dosing. Clearance of ^{14}C-radioactivity from the circulation was very rapid for the A-ring or B-ring label, with no detectable level of ^{14}C-label in the blood by 24 h after dosing. In contrast, disappearance of ^{14}C-radioactivity from the blood was relatively slow for the *t*-butyl label, with low levels being detected past 10 days post-dosing. In animals dosed with the *t*-butyl label, a differential decline in blood/plasma radioactivity with time (at C_{max}, $1/2C_{max}$ and 168 h successively) was also observed, suggesting that majority of the ^{14}C-*t*-butyl label in blood was probably associated with blood cells at the later

timepoints (representing radioactivity that had been incorporated into endogenous molecules following cleavage and metabolism of the *t*-butyl group). The peak concentration of ^{14}C-radioactivity (C_{max}) in blood was not proportional to the dose level of ^{14}C-tebufenozide administered, suggesting that the pharmacokinetics of ^{14}C-tebufenozide were not linear between the low (3 mg/kg) and high (250 mg/kg) doses. Tissue retention of ^{14}C-radioactivity was very low. At 168 h post-dose, the mean total % of administered dose retained was <1%, <0.2% or <0.01% for the *t*-butyl, A-ring or B-ring label, respectively, at 3 mg/kg bw and #0.01% for any of the three labels at 250 mg/kg bw. The highest concentrations of ^{14}C-radioactivity were consistently found in the liver, fat and kidneys. All other tissues had <0.01 ppm or non-detectable levels of radioactivity, regardless of the position of the ^{14}C-label, dose level or sex of the rat. The tissue distribution results were consistent with the pharmacokinetic data and indicated that the ^{14}C-radio- activity associated with the A-ring or B-ring label was cleared more rapidly from the tissues than that associated with the *t*-butyl label.

The metabolism of tebufenozide in Sprague-Dawley rats (Crl:CD BR strain) which were dosed orally (by gavage) with either 3 or 250 mg tebufenozide/kg bw was determined. ^{14}C-*t*-butyl-, ^{14}C-A-ring- and ^{14}C-B-ring-tebufenozide were used for the low-dose (3 mg/kg bw) study and ^{14}C-*t*-butyl- and ^{14}C-B-ring-tebufenozide were used for the high-dose (250 mg/kg bw) study. Excreta (faeces and urine samples) were collected from five rats/sex/group and analyzed for tebufenozide metabolites. Parent tebufenozide was the major component present in the faeces, accounting for >90% and >35% of the dosed radioactivity at the high dose (250 mg/kg bw) and low dose (3 mg/kg bw), respectively. In addition, 11 metabolites were identified in the high-dose samples and 14 metabolites (10 the same as the high-dose) were found in the low-dose faeces. There were no qualitative differences in the metabolite profiles between the different labelled versions of tebufenozide. No parent tebufenozide was found in the urine. The whole-molecule metabolites identified in the urine samples were the same as many of those observed in the faeces. In addition, three unknown metabolites (A, B, C) were found in the acidified (ethyl acetate-2) fractions of the B-ring-labelled urine, two (similar but not identical to A, B, C) in the A-ring-labelled urine, but none in the *t*-butyl-labelled urine. These unknown metabolites (representing 3-3.5% of total dose) were likely to be partially fragmented metabolites of tebufenozide in which the *t*-butyl group had been metabolically cleaved. Based on the faecal and urine results, a total of 15 metabolites (all except one were present at <1% of the dose) from the high-dose excreta and a total of 14 metabolites (two were present at >10% and nine at >1% of the dose) from the low-dose excreta were identified. The extent of the metabolism of tebufenozide was found to be highly dependent on the amount of test material administered. At the high dose of 250 mg/kg bw, only about 4% of the dose was metabolized, producing about 10 mg/kg bw of metabolites. At the low dose of 3 mg/kg bw, a much greater fraction (~46%) of the dose was metabolized, producing about 1.4 mg/kg bw of metabolites. The major route of metabolism of tebufenozide appeared to be the oxidation of benzylic carbons (A- or B-ring) of the molecule to provide a

number of oxidized metabolites with various combinations of oxidation state at the three carbon centres oxidized. One exception was RH-2703 which was produced by oxidation of a non-benzylic carbon (C), the terminal C on the A-ring ethyl group. Based on the results, a metabolic pathway for tebufenozide in the rat was proposed (Figure 1, page 25).

The metabolism of tebufenozide in Sprague-Dawley rats (Crl:CD BR strain) which were fed 30 ppm of nonradioactive tebufenozide in their diet for 2 weeks prior to receiving a single oral dose (by gavage) of 3 mg/kg bw of [^{14}C-*t*-butyl]-tebufenozide (pulse-dose group) was determined. The excreta (faeces and urine samples) from 5 males and 5 females from the pulse-dose group were analyzed for tebufenozide metabolites. The parent tebufenozide was a major component of faeces, accounting for 26.1-39.3% of the dosed ^{14}C-radioactivity. In addition, a total of 13 metabolites were identified. Significant differences in the levels of several metabolites between the male and female samples were noted. The major faecal metabolites were RH-0282, RH-120898 and RH-0126 (in both sexes) and RH-122777 (in females only). No parent tebufenozide was found in the urine. The metabolites found in the urine samples were the same as many of those observed in the faeces. A total of 13 urinary metabolites were quantitated. A comparison of the overall metabolite profiles of the excreta between rats fed 30 ppm dietary tebufenozide for 2 weeks prior to receiving a single oral dose of 3 mg ^{14}C-tebufenozide/kg bw (pulse dose) and those receiving the single oral ^{14}C-radioactive dose without pre-treatment (single dose) revealed no significant qualitative differences in their metabolism of ^{14}C-tebufenozide. Quantitatively, the amount of unchanged parent tebufenozide was slightly less in the pulse-dose excreta than in the single-dose excreta, suggesting a slightly higher level of metabolism of the compound by the pulse-dose rats which also showed generally higher levels of the more highly oxidized metabolites in the excreta.

The metabolite profile of ^{14}C-tebufenozide in the bile of Sprague-Dawley rats (Crl:CD BR strain) (bile duct-cannulated) administered a single oral dose of 3 mg/kg bw [^{14}C-*t*-butyl]-tebufenozide was determined. The bile samples collected from one male and one female rat during 0-6 h post-dosing (representing ~70% of the total bile excretion over 72 h) were analyzed for tebufenozide metabolites. No parent tebufenozide was found in the bile. A total of 13 biliary metabolites were identified and five unknowns isolated. In general, the biliary metabolites were identical to those from the faeces and urine. Only three new metabolites were observed in the bile: [A-ring]-ketone-[B-ring]-diol, RH-122652 and RH-2652 (the latter two had also been identified by mass spectroscopy in the faeces, but levels were too low for quantitation). In addition, the bile contained five unknowns which appeared to be high-molecular weight amino acid conjugates of some acidic tebufenozide metabolites. It was postulated that in the rat, these conjugates were metabolized prior to excretion (to recover the amino acids), hence their absence in the faeces. The study results support the previously proposed metabolic pathway of tebufenozide in rats (Figure 1, page 25).

Acute Toxicity - Technical

Oral

Tebufenozide technical was practically non-hazardous to mice and rats when given acutely via the oral route (Table 1). No mortalities and no clinical signs of systemic toxicity were observed at dose levels up to 5.0 g/kg bw.

Table 1. Acute Oral LD_{50} in the Mouse and Rat

Species	Sex	LD_{50} (mg/kg bw)
Mouse (Crl:CD-1 ICR BR)	M/F	>5,000
Rat (Crl:CD BR)	M/F	>5,000

Dermal

Tebufenozide technical was practically non-hazardous to rats when given acutely via the dermal route. Signs of transient, mild local irritation (desiccation and pinpoint red foci at the site of application) were noted, but no mortalities or clinical signs of systemic toxicity were evident at a dose level of 5.0 g/kg bw. The LD_{50} for rats (Crl:CD BR strain, both sexes) was >5,000 mg/kg bw.

Inhalation

Tebufenozide technical elicited no mortalities or clinical toxicity in rats when given acutely via the inhalation route at the maximum attainable concentration (with or without particle size constraint) under Limit Test conditions (Table 2). In some animals, accumulation of red or brown material around the nose, mouth or eyes, and/or purulent anogenital discharge (lasting 1-3 days) were noted.

Table 2. Acute Inhalation LC_{50} in the Rat - Tebufenozide Technical

Species	Exposure	MMAD*(μm)	Resp.F.^(%)	Sex	LC_{50}(actual) (mg/L)
Rat (SD:CD)	4-hour, whole-body	2.8	93.3	M/F	>1.7 (± 0.5)[a]
Rat (SD:CD)	4-hour, whole-body	6.0	72.9	F	>4.5 (± 0.5)[b]
Rat (SD:CD)	4-hour, whole-body	5.1	77.5	M	>4.3 (± 0.8)[b]

* : MMAD = Mass mean aerodynamic diameter
^ : Resp.F. = Respirable fraction (particles <9 μm)
a : Maximum attainable concentration at the smallest obtainable particle size.
b : Maximum attainable concentration without particle size constraint.

Irritation Toxicity

Tebufenozide technical (purity 96-97%) was found to be non-irritating to the skin and minimally irritating to the eyes of male New Zealand White rabbits, using the Draize scale of scoring.

Skin Sensitization Potential

Tebufenozide technical (purity 96%) was assessed for skin sensitization potential in a Buehler Test with female Hartley guinea pigs and in a Maximization Test with male Crl:(HA)BR guinea pigs. Tebufenozide technical was not a skin sensitizer in the guinea pig.

Acute Toxicity - Formulation (Tebufenozide 240 F)

Dermal

Tebufenozide 240 F formulation (containing 24% a.i.) was practically non-hazardous to rats when given acutely via the dermal route. Transient desiccation at the site of exposure was noted in some females, but no mortalities or clinical signs of systemic toxicity were evident at a dose level of 5.0 g/kg bw. The LD_{50} for rats (Crl:CD BR strain, both sexes) was >5,000 mg/kg bw.

Inhalation

Tebufenozide 240 F formulation (containing 24% a.i.) was found to elicit no mortality or clinical toxicity in rats when administered acutely via the inhalation route at the maximum attainable concentration (with or without particle size constraint) under Limit Test conditions (Table 3). Wet muzzles and fur, and/or red-stained eyes and yellow-stained anogenital regions were noted upon removal of animals from the exposure chamber.

Table 3. Acute Inhalation LC_{50} in the Rat - Tebufenozide 240 F

Species	Exposure	MMAD*(:m)	Resp.F.^(%)	S e x	LC_{50} (actual) (mg/L)
Rat (Crl:CD BR)	4-hour, nose-only	2.0	65.4	M /F	>0.2[a] (/ >0.05 a.i.)
Rat (Crl:CD BR)	4-hour, nose-only	11.6	14.0	M /F	>2.7[b] (/ >0.65 a.i.)

* : MMAD = Mass mean aerodynamic diameter
^ : Resp.F. = Respirable fraction (based upon ACGIH standards)
[a] : Maximum attainable concentration at the smallest obtainable particle size.
[b] : Maximum attainable concentration without particle size constraint.

Irritation Toxicity

Tebufenozide 240 F formulation (containing 24% a.i.) was minimally irritating to the skin and practically non-irritating to the eyes of New Zealand White rabbits, using the Draize scale of scoring.

Skin Sensitization Potential

Tebufenozide 240 F formulation (containing 24% a.i.) was assessed for skin sensitization potential in a Buehler Test with male and female Hartley guinea pigs. Tebufenozide 240 F formulation was not a skin sensitizer in the guinea pig.

Acute Toxicity - Tebufenozide Metabolites

Oral

Five tebufenozide metabolites were tested for acute toxicity with ICR mice (Crj:CD-1 or Crl:CD-1 BR strain). Tebufenozide metabolites (RH-111788, RH-96595, RH-120970, RH-089886 or RH-112651) were practically non-hazardous to mice when given acutely via the oral route. No mortality or clinical signs of systemic toxicity were

observed at dose levels up to 5.0 g/kg bw. The LD_{50} of these tebufenozide metabolites in the male or female mouse was >5,000 mg/kg bw.

A tebufenozide process intermediate (RH-87051, **not** a tebufenozide metabolite) was also tested for acute toxicity with ICR mice (Crj:CD-1 BR strain). RH-87051 was slightly hazardous to mice when given acutely via the oral route. Clinical signs of intoxication (decreased spontaneous motor activity, abnormal gait, abnormal breathing, sedation and coma) were observed 1-6 h following dosing. The LD_{50} of the process intermediate RH-87051 (**not** a metabolite) was 891 mg/kg bw for the male and 1,000 mg/kg bw for the female mouse.

Short-Term Oral Toxicity - Technical

Mice

Six groups of male albino mice (Crl:CD-1 ICR BR strain), eight mice/group, were fed diets containing tebufenozide technical (purity 94%) at dose levels of 0, 60, 200, 600, 2,000 or 6,000 ppm (equal to 0, 11.6, 38.5, 96.8, 352.5 and 1,093 mg/kg bw/day) for 2 weeks. The study No Observed Effect Level (NOEL) was 600 ppm (equal to 96.8 mg/kg bw/day) based on increased relative liver weights in male mice at the next higher dose of 2,000 ppm. At the highest dose of 6,000 ppm, absolute and relative liver weights were significantly increased. In the absence of available histopathological data, the toxicological significance of a liver weight change could not be fully evaluated.

Groups of 10 male and 10 female mice (Crl:CD-1 ICR BR VAF/Plus strain) each were administered tebufenozide technical (purity 98.6%) orally in their daily diets at dose levels of 0, 20, 200, 2,000 or 20,000 ppm (equal to 0, 3.37, 35.3, 339 and 3,332 mg/kg bw/day) for 13 weeks. At the mid-1-dose of 200 ppm, slightly reduced mean body-weight gain (but not the overall body weight) of males and marginally higher incidence of increased extramedullary haematopoiesis in the spleen and pigment accumulation in kidney tubules (with no concomitant changes in the clinical haematology parameters) of females were observed, which were not considered to be toxicologically significant. The study No Observed Adverse Effect Level (NOAEL) was determined to be 200 ppm (equal to 35.3 mg/kg bw/day). At the next two higher dose levels (2,000 and 20,000 ppm), significant haemolytic changes and reduced mean myeloid/erythroid ratio in bone marrow were evident. There were dose-related increases in the absolute and relative (to-body or to-brain weight) spleen and liver weights. Histopathological findings revealed increased incidence and/or severity of pigment deposition in the liver, spleen and kidney tubules and increased extramedullary haematopoiesis in the spleen of the animals. The pigment was characterized as bile in the liver and iron-positive material (haemosiderin) in the liver, spleen and kidney. The results indicate that the primary target of tebufenozide toxicity was the red blood cells,

leading to an increased erythrocyte turnover and compensatory responses from haematopoietic tissues (a regenerative anemia).

Rats

Groups of six rats/sex/group (Crl:CD BR strain) were fed diets containing tebufenozide technical (purity 94%) at dose levels of 0, 50, 250, 1,000, 2,500 or 10,000 ppm (equal to 0, 3.79, 18.9, 71.1, 181 and 702 mg/kg bw/day) for 2 weeks. The study NOEL was 250 ppm (equal to 18.9 mg tebufenozide/ kg bw/day) based on increased male (relative) and female (absolute and relative) liver weights at the next higher dose of 1,000 ppm. In the absence of available histopathological data, the toxicological significance of a liver weight change could not be fully evaluated. At the highest dose of 10,000 ppm, slightly reduced body weight gain, food consumption and red blood cell parameters, and increased spleen weights (absolute and relative) were observed in males and females. Based on the small magnitude of changes, the reductions in body weight gain, food consumption and red blood cell parameters were considered to be of minimal toxicological significance. The significance of a spleen weight change could not be fully evaluated in the absence of available histopathological data.

Two groups of Sprague-Dawley rats (Crl:CD BR strain), 10 rats/sex/group, were fed daily diets containing tebufenozide technical (purity 96.4%) at dose levels of 0 or 20,000 ppm (equal to 0 and 1516 mg/kg bw/day) for 4 weeks. Decreases in body weight (#9%), body-weight gain and food consumption #12%) were observed in the treated rats, but the mean efficiency of food utilization was not affected suggesting a palatability problem with the test compound-containing diet. There was a slight reduction in the erythrocyte count, haemoglobin and haematocrit. Absolute and relative liver weights (both sexes), absolute and relative spleen weights (males only) and relative kidney weight (females only) were increased in the treated rats. In the absence of histopathological data, the toxicological significance of organ weight changes could not be fully assessed. Based on the results of this range-finding study, a high dietary concentration of 20,000 ppm tebufenozide technical appeared to be a feasible dose level to use in short-term toxicity studies in the rat.

Groups of 10 male and 10 female rats (Crl:CD BR strain) each were administered tebufenozide technical (purity 96.4% or 98.6%) orally in their daily diets at dose levels of 0, 20, 200, 2,000 or 20,000 ppm (equal to 0, 1.30, 13.1, 133 and 1,330 mg/kg bw/day) for 13 weeks. The study NOEL was 200 ppm (equal to 13.1 mg/kg bw/day). At the next higher dose of 2,000 ppm, there were significant decreases in the overall body weight gain and mean food consumption during the first 4 weeks of dosing, and an increase in the relative (organ/brain weight) liver weight (females only). Slight haemolytic anaemia, increased bone marrow erythropoiesis (decrease in the mean myeloid/erythroid ratio) and an increased deposition of pigment in the spleen were observed. At the highest dose level of 20,000 ppm, additional treatment-related effects

included overt haemolytic changes, slightly elevated absolute and relative (organ/brain weight) spleen weights (both sexes), elevated absolute liver weight (females), and tubular nephrosis of the kidney in 4/10 males.

Dogs

Five groups of male Beagle dogs (four dogs/group) were administered tebufenozide technical (purity 96.8%) orally in their daily diet at dose levels of 0, 150, 600, 2,400 or 9,600 ppm (equal to 0, 5.05, 18.8, 77.1 and 289 mg/kg bw/day) for 2 weeks. The study NOEL was 150 ppm (5.05 mg/kg bw/day) based on a significant increase in the mean spleen weight at the next higher dose of 600 ppm. At the highest dose tested (9,600 ppm), mild haemolytic anaemia (significantly reduced erythrocyte count, haemoglobin and haematocrit) was also observed.

Two groups of male beagle dogs (four/group) were administered tebufenozide technical (purity 97.5%) orally in their daily diets at dose levels of 0 or 1,500 ppm (equal to 0 and 41.7 mg/kg bw/day) for 6 weeks. All animals were then maintained on the basal (control diet) for a further 4-week recovery period after which the study was terminated. Haematological examinations were performed on all dogs at Weeks 0 (prior to dosing), 6 (end of dosing), 8 (2 weeks after cessation of dosing) and 10 (study termination, end of 4-week recovery). Administration of tebufenozide at 1,500 ppm for 6 weeks resulted in mild regenerative anaemia. Total recovery from blood toxicity was observed at 4 weeks after cessation of treatment.

Groups of four beagle dogs/sex/group were administered tebufenozide technical (purity 96.1%) in their daily diets at dose levels of 0, 50, 500, or 5,000 ppm (equal to 0, 2.05, 20.1, or 202 mg/kg bw/day) for 90 days. The study NOEL was 50 ppm (equal to 2.05 mg/kg bw/day). At the next higher dose of 500 ppm, there were an increased incidence of Heinz bodies (both sexes), an elevated mean total bilirubin level (females) and increased absolute and relative spleen weights (females). Histopathological findings revealed increased incidence of pigment deposition (haemosiderin in nature) in Kupffer cells of the liver. Increased haemopoiesis and sinusoidal engorgement of the spleen were also noted in these animals. At the highest dose of 5,000 ppm, significant haemolytic changes and increased bone marrow erythropoiesis (reduced mean myeloid/erythroid ratio), were evident at Weeks 6 and 13 of treatment. The mean total plasma bilirubin level was elevated (both sexes) and bilirubin was present in the urine of 3/4 high-dose males. Increased spleen weights (absolute and relative) and slightly higher relative liver weights were observed. There were increased incidences of pigment deposition (haemosiderin) and the presence of erythrocytes in some Kupffer cells of the liver, suggesting active erythrophagocytosis. Increased splenic haemopoiesis and sinusoidal engorgement, and bone marrow hyperplasia were also noted. The results indicated that the primary target of tebufenozide toxicity in the dog was the

erythrocyte, leading to a mild haemolytic anaemia and compensatory responses from the haematopoietic tissues.

Groups of four beagle dogs/sex/group were administered tebufenozide technical (purity 97.5%) in their daily diets at dose levels of 0, 15, 50, 250, or 1,500 ppm (equal to 0, 0.6, 1.8, 8.7, or 52.7 mg/kg bw/day) for 52 weeks. The study NOEL was 50 ppm (equal to 1.8 mg/kg bw/day). At the next higher dose of 250 ppm, there were slight but consistent haemolytic changes and slightly elevated total plasma bilirubin (especially in females) over the 52-week study period. Mean absolute and relative spleen weights (females) and mean relative liver weight (males) were increased. Increased incidence of pigment deposition in the Kupffer cells of the liver, increased splenic haemopoiesis and sinusoidal engorgement, and bone marrow hyperplasia were also observed. At the highest dose of 1,500 ppm, similar treatment-related effects of increased magnitude and severity were observed. The results indicated that the primary target of tebufenozide toxicity in the dog was the erythrocyte, leading to a mild peripheral haemolytic anaemia and compensatory responses from the haemopoietic tissues.

Short-Term Dermal Toxicity - Tebufenozide Technical and Tebufenozide 240 F Formulation

Rats

Groups of Crl:CD BR rats, six/sex/group, were used in a repeated-dose dermal toxicity study with tebufenozide technical (purity 97.2%) or tebufenozide 240 F formulation (containing 24.5% a.i.). Tebufenozide technical at a single dose of 1,000 mg/kg bw/day (moistened with 0.9% saline, 1:6 w/v) or tebufenozide 240 F formulation at dose levels of 0 (solvent control), 62.5, 250, or 1,000 a.i./kg bw/day were applied to the shaved intact back skin under semi-occlusive bandages for 6 h/day, 5 days/week for 4 weeks (a total of 21 applications). No signs of treatment-related systemic toxicity were observed. Minor skin irritation effects were seen in female rats in response to the formulation solvent, but not to the active ingredient. The NOEL for tebufenozide technical or tebufenozide 240 F formulation given via the dermal route was >1,000 mg a.i./kg bw/day, the highest dose level tested in the study.

Long-Term Toxicity/Carcinogenicity - Technical

Mice

Groups of 50 CD-1 mice/sex/group (Crl:CD-1(ICR)BR strain) were fed diets containing tebufenozide technical (purity 96.1%) at dose levels of 0, 5, 50, 500, or 1,000 ppm (equal to 0, 0.8, 7.8, 77.9 or 154.9 mg/kg bw/day) for 78 weeks. Each dose level included an additional satellite group of 10 mice/sex for interim sacrifice after 52 weeks of treatment. The NOEL for general toxicity was 50 ppm (equal to 7.8

mg/kg bw/day). At the next higher dose of 500 ppm, there was a slight reduction in survival rate (males only), and increased pigment deposition in the spleen (males and females) at both interim and terminal sacrifices. At the highest dose of 1,000 ppm, reduced survival rates (both sexes), signs of mild haemolytic anaemia (small but significant increases in blood methaemoglobin, increased incidence of polychromasia and echinocytosis of the red blood cells) and further increases in splenic pigment deposition were observed at interim and/or terminal sacrifice. At terminal sacrifice, increased relative (organ/body weight) spleen weight (males only) and a higher incidence of extramedullary haematopoiesis in the spleen (females) were also noted. There was no evidence of any oncogenic effect of tebufenozide technical on male or female mice at any dose up to and including 1,000 ppm (equal to 155 mg/kg bw/day), the highest dose level tested in the study. Tebufenozide technical was not oncogenic in the mouse under the conditions of the study.

Rats

Groups of 60 male and 60 female rats (Crl:CD BR strain) each received tebufenozide technical (purity 96.1%) orally in their daily diets at dose levels of 0, 10, 100, 1,000 or 2,000 ppm (equal to 0, 0.5, 4.8, 48.4 and 97.3 mg/kg bw/day) for 104 weeks. Each dose level had a satellite group of 10 rats/sex for interim sacrifice during Week 53. Two sentinel groups (five rats/sex/group) were also included in the study for general health screening purposes prior to and at termination of the dosing period. The NOEL for chronic systemic toxicity was 100 ppm (equal to 4.8 mg/kg bw/day). At the next two higher dose levels (1,000 and 2,000 ppm), there were significant decreases in mean body weight and body-weight gain (more pronounced in females) and mean food consumption (females only) throughout the study period. Signs of mild haemolytic anaemia were evident only during the first 52 weeks of treatment, suggesting that the effects on the haemopoietic system were transient and reversible. Slight increases in the incidence and/or severity of pigment deposition (haemosiderin in nature) were noted in the spleen (both sexes) at interim and/or terminal sacrifice, suggesting active splenic erythrophagocytosis. In addition, the 1,000- and 2,000-ppm females also showed increased frequency of swelling of body areas (principally the mammary gland regions) during the first 70 weeks of dosing. However, in the absence of any supporting histopathological findings in the mammary gland tissue/skin, the toxicological significance of these transient swellings in the females could not be ascertained. There were no treatment-related neoplastic lesions noted in any tissue/organ of any treated rat at any dose level up to and including 2,000 ppm (97.3 mg/kg bw/day), the highest dose tested in the study. Tebufenozide technical was not oncogenic in the rat under the conditions of the study.

2.2 **Genotoxicity** - Technical

A battery of mutagenicity studies with tebufenozide technical was conducted to assess the potential for inducing gene mutation, chromosome aberration or unscheduled DNA synthesis. The study results (summarized in Table 4) were clearly **negative**. Tebufenozide technical did not demonstrate any genotoxic potential under the conditions tested.

Table 4. Results of Mutagenicity Assays on Tebufenozide Technical

Test	Test System	Concentration	Results
Reverse Mutation in Bacteria (*in vitro*)	*S.typhimurium* TA98, TA100, TA1535, TA1537	0, 50, 200, 500+, 2,000*, 5,000* ：g/plate	negative[1]
		0, 50, 200, 500, 2,000+, 5,000* ：g/plate	negative[1]
		0, 50, 200, 500, 2,000*, 5,000* ：g/plate 0, 160, 300, 500, 900+, 1,600* ：g/plate	negative[1] negative[1]
		0, 50, 200, 500#, 2,000*, 5,000* ：g/plate 0, 30, 50, 90, 160, 300 ：g/plate	negative[1] negative[1]
	E.coli WP2 *uvr*A	0, 200, 500, 1,000, 2,000*, 5,000* ：g/plate	negative[1]
Forward Mutation (*in vitro*)	Chinese Hamster Ovary (HGPRT)	0, 10, 40, 50, 60 ：g/mL	negative[1]
Chromosome Aberration (*in vitro*)	Chinese Hamster Ovary	0, 5, 10, 20, 30 ：g/mL	negative[1]
Chromosome Aberration (*in vivo*)	Rat (CD, 5-7/sex/dose) bone marrow	0, 0.5, 2.5, 5.0 g/kg bw (single oral gavage dose)	negative
Unscheduled DNA Synthesis (*in vitro*)	Rat (SD) primary hepatocyte cultures	0, 10, 20, 40, 60, 80*, 100* ：g/mL	negative

* precipitation observed in all cultures
+ precipitation observed in some cultures
# minimal precipitation observed in all cultures, did not interfere with colony counting
[1] conducted with and without exogenous metabolic activation

Genotoxicity - Tebufenozide Metabolites

Four tebufenozide metabolites (RH-111788, RH-96595, RH-120970 and RH-089886) and one tebufenozide process intermediate (RH-87051, **not** a metabolite) were tested for mutagenic potential in reverse mutation assays in bacteria (with or without exogenous metabolic activation), using *Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *E. coli* (strain WP2 *uvr*A) and test chemical concentrations up to the limit of solubility (1,250-2,500 ：g/plate, metabolites) or cytotoxicity (1,250-2,500 ：g/plate, process intermediate). The study results (summarized in Table 5) were clearly **negative**. None of the four tebufenozide metabolites nor the tebufenozide process intermediate tested demonstrated any mutagenic potential under the conditions tested.

Table 5. Results of Reverse Mutation Assays in Bacteria on Tebufenozide Metabolites

Compound	Concentration (µg/plate)	Results
RH-87051 (process intermediate)	0, 156, 313, 625, 1,250, 2,500[b], 5,000[a]	negative[1]
RH-89886 (metabolite in rat, rice)	0, 313, 625, 1,250, 2,500*, 5,000*	negative[1]
RH-111788 (metabolite in rat, rice)	0, 313, 625, 1,250, 2,500, 5,000*	negative[1]
RH-96595 (metabolite in rat, rice, soil)	0, 313, 625, 1,250, 2,500*, 5,000*	negative[1]
RH-120970 (metabolite in rat, rice)	0, 313, 625, 1,250, 2,500*, 5,000*	negative[1]

[1] Reverse Mutation Assay in Bacteria (*S.typhimurium* TA98, TA100, TA1535, TA1537; *E.coli* WP2 *uvr*A), with or without exogenous metabolic activation by rat liver S-9 fraction
[a] growth inhibition on all plates
[b] growth inhibition in some strains
* precipitation on all plates

2.3 **Reproductive Toxicity** - Technical

In a two-generation, one-litter/generation reproduction study in Crl:CD BR rats, groups of 25 male and 25 female weanlings/group/generation were fed diets containing tebufenozide technical (purity 97.5%) at dose levels of 0, 10, 150, or 2,000 ppm (equal to 0, 0.7, 9.7 and 142.2 mg/kg bw/day) for 70 days (F_0 rats) or 105 days (F_1 parents) prior to mating. No treatment-related effects were observed in either generation (F_0 or F_1) at the low dose of 10 ppm. At the next higher dose of 150 ppm, increased severity of pigment deposition in the spleen of F_0 and F_1 female rats was noted. At the highest dose of 2,000 ppm, parental (F_0 and F_1 males) body weight and feed consumption were decreased at some time intervals during the pre-mating periods. Increased splenic pigment deposition and extramedullary haematopoiesis were evident in parental animals (both sexes) of either generation. The mean number of implantation sites in F_1 females (parameter was not measured in F_0) was decreased and the length of gestation in F_1 (but not F_0) was increased. There were also small increases in the numbers of pregnant females not delivering (total resorptions) in both generations and in F_1 females dying during delivery. Therefore, the NOEL for general toxicity in this study was 10 ppm (equal to 0.7 mg/kg bw/day) and the NOEL for reproductive parameters was 150 ppm (equal to 9.7 mg/kg bw/day).

2.4 **Teratogenicity** - Technical

Rats

In a range-finding developmental toxicity study in the rat, five groups of mated female rats (Crl:CD BR strain), nine rats/group, were given tebufenozide technical

(purity 96.8%) orally by gavage at dose levels of 0 (vehicle control), 25, 75, 200 or 400 mg/kg bw/day once daily from Days 6 to 15 of gestation. On Day 20 of gestation, all surviving dams were sacrificed, fetuses were delivered by caesarean section and necropsied. No maternal or embryo-fetal toxicity and no fetal external gross malformations/anomalies were noted at dose levels up to and including 400 mg/kg bw/day, the highest dose tested in the study. Based on the results, the study NOEL for maternal and embryo-fetal toxicity and for teratogenicity was 400 mg/kg bw/day in the rat. In a supplementary range-finding study, non-pregnant female rats (Crl:CD BR strain), six rats/group, were administered tebufenozide technical (purity 96.8%) orally by gavage at dose levels of 0, 400 or 1,000 mg/kg bw/day for 10 days. Based on the very limited data provided, the study NOEL was 400 mg/kg bw/day for the female rat. At the next higher dose of 1,000 mg/kg bw/day, a slight increase in the liver weight was noted. In the absence of available histopathological data, the toxicological significance of a liver weight change could not be fully evaluated.

In the main teratology study, groups of 25 mated Sprague-Dawley rats (Crl:CD BR VAF/Plus strain) each were administered tebufenozide technical (purity 96.1%) orally by gavage at dose levels of 0 (vehicle), 50, 250 or 1,000 mg/kg bw/day once daily from Days 6 to 15 of presumed gestation (the day spermatozoa were detected in a vaginal lavage or a copulatory plug was observed *in situ* = Day 0 of gestation). On gestation Day 20, all surviving dams were killed, fetuses were delivered by caesarean section and necropsied. The NOAEL for maternal toxicity was 1,000 mg/kg bw/day. At 1,000 mg/kg bw/day, there was an initial slight, transient reduction in the mean body weight gain and food consumption during dosing, but the mean overall values for the whole dosing/study period were not affected; the noted changes were not considered to be toxicologically significant. All fetuses delivered showed normal development. No signs of fetotoxicity and no treatment-related malformations were observed in any of the fetuses at any dose level tested, up to and including 1,000 mg/kg bw/day. The study results yielded no evidence of any teratogenic potential of tebufenozide technical. Therefore, the NOEL for embryo-fetotoxicity and teratogenicity in the Sprague-Dawley rat was determined to be 1,000 mg/kg bw/day, the highest dose level tested in the study.

Rabbits

In a range-finding developmental toxicity study in rabbits, groups of six mated female Hra:(NZW)SPF rabbits each were administered tebufenozide technical (purity 96.4%) orally by gavage at dose levels of 0 (vehicle), 100, 300, or 1,000 mg/kg bw/day once daily from Days 7-19 of presumed gestation (the day of mating confirmed by the presence of seminal fluids in the vulva of female = Day 0 of gestation). All surviving does were sacrificed, all fetuses were delivered by caesarean section and examined on Day 29 of gestation. The conservative NOEL

for maternal toxicity was 300 mg/kg bw/day, based on the single, unscheduled death (cause not apparent) observed at the next higher dose of 1,000 mg/kg bw/day. No other signs of maternal toxicity or treatment-related disturbance of the intra-uterine development of the conceptuses were observed at any dose level up to and including 1,000 mg/kg bw/day, the highest dose tested in the study. All fetuses delivered showed normal development. No signs of fetal toxicity were evident and no treatment-related malformations were observed in any of the fetuses at any dose level tested, up to and including 1,000 mg/kg bw/day. The study results yielded no evidence of any teratogenic potential of tebufenozide technical in the rabbit. Therefore, the NOEL for embryo-fetotoxicity and teratogenicity in the Hra:(NZW)SPF rabbit was determined to be 1,000 mg/kg bw/day, the highest dose level tested in the study.

In the main teratology study, groups of mated female New Zealand White rabbits (20/group) were administered tebufenozide technical (purity 97.5%) orally by gavage at dose levels of 0 (vehicle), 50, 250, or 1,000 mg/kg bw/day once daily from Days 7 to 19 of presumed gestation (the day of mating confirmed by the presence of seminal fluids in the vulva of female = Day 0 of gestation). All surviving does were sacrificed, fetuses were delivered by caesarean section and examined on Day 29 of presumed gestation. No treatment-related mortality, clinical signs of maternal toxicity nor disturbance of the intra-uterine development of the conceptuses were noted at any dose level up to and including 1,000 mg/kg bw/day (limit dose), the highest dose level tested in the study. All fetuses delivered showed normal development. No signs of fetotoxicity were evident and no treatment-related malformations were observed in any of the fetuses at any dose level tested, up to and including 1,000 mg/kg bw/day. The study results yielded no evidence of any teratogenic potential of tebufenozide technical in the rabbit. Therefore, the NOEL for maternal toxicity, embryo-fetotoxicity and teratogenicity in the New Zealand White rabbits was determined to be 1,000 mg/kg bw/day, the highest dose level tested in the study.

2.5 Toxicological Summary

In the rat, orally administered (by gavage) single doses (3 or 250 mg/kg bw) of ^{14}C-tebufenozide (labelled in either the *t*-butyl, A- or B-ring group) were rapidly absorbed and excreted. The excretion profiles were similar, regardless of the position of the ^{14}C-label, dose level, sex or whether the rats were pre-treated with 30 ppm dietary non-labelled tebufenozide for 2 weeks. A mean total of 87-104% of the administered dose was excreted within 48 h of dosing, primarily via the faeces which accounted for >90% of the ^{14}C-label excreted. Only minor amounts (<1-8% of dose) were excreted in urine and trace amounts (<0.1-0.4% of dose) in expired air (as $^{14}CO_2$ and organic volatiles) from rats dosed with [^{14}C-*t*-butyl]-tebufenozide. At 3 mg/kg bw, systemic absorption was calculated to be 35-39%

of the total dose; 30-34% was excreted in the bile and ~5% in the urine. At 250 mg/kg bw, only about 4% of the administered dose was absorbed and metabolized. Tissue retention of ^{14}C-radioactivity was very low; <1 and #0.01% of the dose were retained at 3 and 250 mg/kg bw, respectively, at 7 days post-dose. The highest concentrations were found in the liver, fat and kidneys. The tissue ^{14}C-distribution profiles were consistent with the pharmacokinetic data and indicated that the ^{14}C-radioactivity associated with the A-/B-ring label was cleared more rapidly from the tissues than that associated with the *t*-butyl label.

^{14}C-tebufenozide was extensively metabolized in rats. The majority of ^{14}C-radioactivity excreted in faeces was in the form of unabsorbed (parent) tebufenozide, accounting for ~60 and >90% of the given dose at 3 and 250 mg/kg bw/day, respectively. No unchanged parent compound was detected in the urine. There were no significant qualitative differences in the metabolite profiles between the different ^{14}C-labelled versions of tebufenozide, the high and low doses, sexes, or rats with or without pre-treatment with dietary tebufenozide (30 ppm) for 2 weeks. In general, the whole-molecule metabolites identified (total of 13-15) in the urine, faeces and bile were identical. The major route of metabolism of tebufenozide technical appeared to be the oxidation of benzylic carbons (A-/B-ring) of the molecule to provide a number of oxidized metabolites with various combinations of oxidation state at the three carbon centres oxidized. One exception was RH-2703 which was produced by oxidation of a non-benzylic carbon, the terminal C on the A-ring ethyl group. Based on the results, a metabolic pathway for tebufenozide technical in the rat was proposed (Figure 1).

In acute studies, tebufenozide technical was practically non-hazardous to mice and rats when given acutely via the oral route. No mortalities and no clinical signs of systemic toxicity were observed at dose levels up to 5.0 g/kg bw. The oral LD_{50} of tebufenozide technical for mice and rats was >5,000 mg/kg bw.

In acute studies, tebufenozide technical was practically non-hazardous to rats when given acutely via the dermal route. No mortalities or clinical symptoms of systemic toxicity were evident at a dose level of 5.0 g/kg bw. The dermal LD_{50} of tebufenozide technical for rats was >5,000 mg/kg bw. Tebufenozide technical elicited no mortalities or clinical toxicity in rats when administered acutely via the inhalation route at maximum attainable concentration (1.7 mg/L at the smallest obtainable particle size or 4.3 mg/L without particle size constraint) under Limit Test conditions. The inhalation LC_{50} of tebufenozide technical for rats was, therefore, >4.3 mg/L. Tebufenozide technical was found to be non-irritating to the skin and minimally irritating to the eyes of male New Zealand White rabbits, and it was not a skin sensitizer in the guinea pig.

In acute studies, tebufenozide 240 F formulation (containing 24% a.i.) was practically non-hazardous to rats when given acutely via the dermal route. No mortalities nor clinical signs of systemic toxicity were evident at a dose level of 5.0 g/kg bw. The dermal LD_{50} of tebufenozide 240 F for rats was >5,000 mg/kg bw. Tebufenozide 240 F formulation elicited no mortalities nor clinical toxicity in rats when given acutely via the inhalation route at the maximum attainable concentration (0.2 mg/L, / 0.05 mg a.i./L at the smallest obtainable particle size; 2.7 mg/L, / 0.65 mg a.i./L without particle size constraint) under Limit Test conditions. The inhalation LC_{50} of tebufenozide 240 F for rats was, therefore, >2.7 mg/L (equivalent to >0.65 mg a.i./L). Tebufenozide 240 F formulation was found to be minimally irritating to the skin and practically non-irritating to the eyes of New Zealand White rabbits, and it was not a skin sensitizer in the guinea pig.

In acute studies, tebufenozide metabolites (RH-111788, RH-96595, RH-120970, RH-089886 or RH-112651) were practically non-hazardous to mice when given acutely via the oral route. No mortality nor clinical signs of systemic toxicity were observed at dose levels up to 5.0 g/kg bw. The oral LD_{50} of these tebufenozide metabolites in mice was >5,000 mg/kg bw. RH-87051 (a process intermediate, **not** a metabolite of tebufenozide) was slightly hazardous to mice when given acutely via the oral route. Clinical signs of intoxication (decreased spontaneous motor activity, abnormal gait, abnormal breathing, sedation and coma) were observed 1-6 h following dosing. The oral LD_{50} of RH-87051 was 891 and 1,000 mg/kg bw for the male and female mice, respectively.

Repeated short-term oral administration of tebufenozide technical to mice (2-week, 13-week), rats (2-week, 4-week and 13-week) and dogs (2-week, 6-week, 13-week and 52-week) resulted primarily in haematotoxic effects - mild regenerative haemolytic anaemia and compensatory responses from the haematopoietic tissues. Based on haematotoxicity, the NOAEL/NOEL was 35.3 mg/kg bw/day for mice (13-week), 13.1 mg/kg bw/day for rats (13-week) and 1.9 mg/kg bw/day for dogs (13-week and 52-week combined). The dog appeared to be the most sensitive species for short-term toxicity.

Repeated short-term (4-week) dermal application of tebufenozide technical or tebufenozide 240 F formulation (containing 24.5% a.i.) to rats resulted in no evidence of treatment-related systemic toxicity at dose levels up to 1,000 mg a.i./kg bw/day. The NOEL was >1,000 mg a.i./kg bw/day for rats.

In long-term rodent dietary studies, the NOEL for chronic systemic toxicity was 7.8 mg/kg bw/day for mice (based on a slightly reduced survival rate and mild regenerative haemolytic anaemia at higher dose levels) and 4.8 mg/kg bw/ day for rats (based on decreased body weight and food consumption and mild

regenerative haemolytic anaemia at higher dose levels). Tebufenozide technical was not oncogenic in the mouse or the rat under the conditions of the studies.

The following mutagenicity studies with tebufenozide technical were **negative**: Ames reverse mutation assays (+/- exogenous metabolic activation) in bacterial systems; a forward gene mutation assay (+/- exogenous metabolic activation) in Chinese hamster ovary (CHO) cells in culture; an *in vitro* cytogenetics test of chromosomal aberration (+/- exogenous metabolic activation) in CHO cells in culture; an *in vivo* bone marrow cytogenetics test of chromosomal aberration in CD rat; and an *in vitro* unscheduled DNA synthesis study in primary hepatocyte cultures of SD rats. In addition, Ames reverse mutation assays (+/- exogenous metabolic activation) in bacterial systems with four tebufenozide metabolites (RH-111788, RH-96595, RH-120970 and RH-089886) and a tebufenozide process intermediate (RH-87051, **not** a metabolite) were also **negative**. Tebufenozide technical and its metabolites did not demonstrate any genotoxic and/or mutagenic potential under the conditions tested.

One rat reproduction study (two-generation, one-litter per generation) was submitted. The study NOEL for parental toxicity was 0.7 mg/kg bw/day based on increased severity of pigment deposition in the spleen (F_0 and F_1 females) at the next higher dose of 9.7 mg/kg bw/day. At the highest dose of 142.2 mg/kg bw/day, additional signs of parental toxicity were reduced mean body weight and food consumption (F_0 and F_1 males only) during pre-mating, and increased splenic extramedullary haematopoiesis (both sexes and generations). Signs of reproductive toxicity were also evident at 142.2 mg/kg bw/day: decreased mean number of implantation sites (F_1 females), prolonged gestation period (F_1 females), a slightly higher number of pregnant females with total resorptions (both generations) and a small increase in the number of dams (F_1 generation) dying during delivery were noted. The NOEL for reproductive toxicity was 9.7 mg/kg bw/day.

In two rat teratogenicity studies, the NOAEL for maternal toxicity was 1,000 mg/kg bw/day, the highest dose level tested. At 1,000 mg/kg bw/day, there was a slight reduction in mean body weight gain and food consumption at initiation of dosing; the decreases were transient and reversible, and therefore were not considered to be toxicologically significant. There were no treatment-related effects on reproductive and litter parameters and no evidence of any teratogenic potential of tebufenozide technical at any dose level. The NOEL for embryo-fetotoxicity and teratogenicity in the rat was determined to be 1,000 mg/kg bw/day, the highest dose level tested in the studies. In two rabbit teratogenicity studies, there were no treatment-related mortalities nor signs of maternal toxicity, no adverse effects on reproductive and litter parameters and no evidence of any teratogenic potential of tebufenozide technical at any dose level. The NOEL for

maternal toxicity, embryo-fetotoxicity and teratogenicity in the rabbit was determined to be 1,000 mg/kg bw/day, the highest dose level tested in the studies.

In summary, the primary target site of tebufenozide toxicity was the peripheral haematopoietic system and the main toxicological end-point, consistent across all species tested, was mild regenerative haemolytic anaemia with compensatory responses from the haematopoietic tissues. Tebufenozide technical was of low acute toxicity to the mouse and rat when given via the oral, dermal or inhalation route. Pharmacokinetics and metabolism studies in the rat revealed that the compound was only partially absorbed, rapidly excreted and there were no signs of bioaccumulation in any tissue/organ examined. Tebufenozide technical was not oncogenic in the mouse or the rat, and it did not demonstrate any mutagenic/genotoxic potential *in vitro* or *in vivo*. There was no evidence of any teratogenic potential of tebufenozide technical in the rat or the rabbit, and no effect on reproduction except at a high dose level that elicited parental toxicity.

2.6 Food Exposure

Acceptable Daily Intake (ADI)

An ADI of 0.019 mg tebufenozide/kg bw has been estimated based on the overall NOEL of 1.9 mg/kg bw/day (50 ppm) for haematotoxicity in the 13- and 52-week feeding studies in the dog and using a 100-fold safety factor.

Residue Levels

Label

Confirm® 240 F Agricultural Insecticide is proposed for use on apples to control larval Lepidoptera. The Confirm® 240 F label contains recommendations for use at 1.0 litre per hectare (240 g a.i./ha). The addition of Companion® spray adjuvant at the rate of 100 mL per 100 L of water is recommended for improved spray coverage. Additional applications may be applied at either 10-14 or 14-21 days after the initial application, depending on prolonged egg hatch or high insect pressure. Apply no more than four applications per year. Do not apply Confirm® 240 F within 14 days of harvest.

Although not present on the current label, a restriction “Do not feed treated apple pomace to livestock” is required on the final version due to the lack of an animal feeding study.

Metabolism

Plant

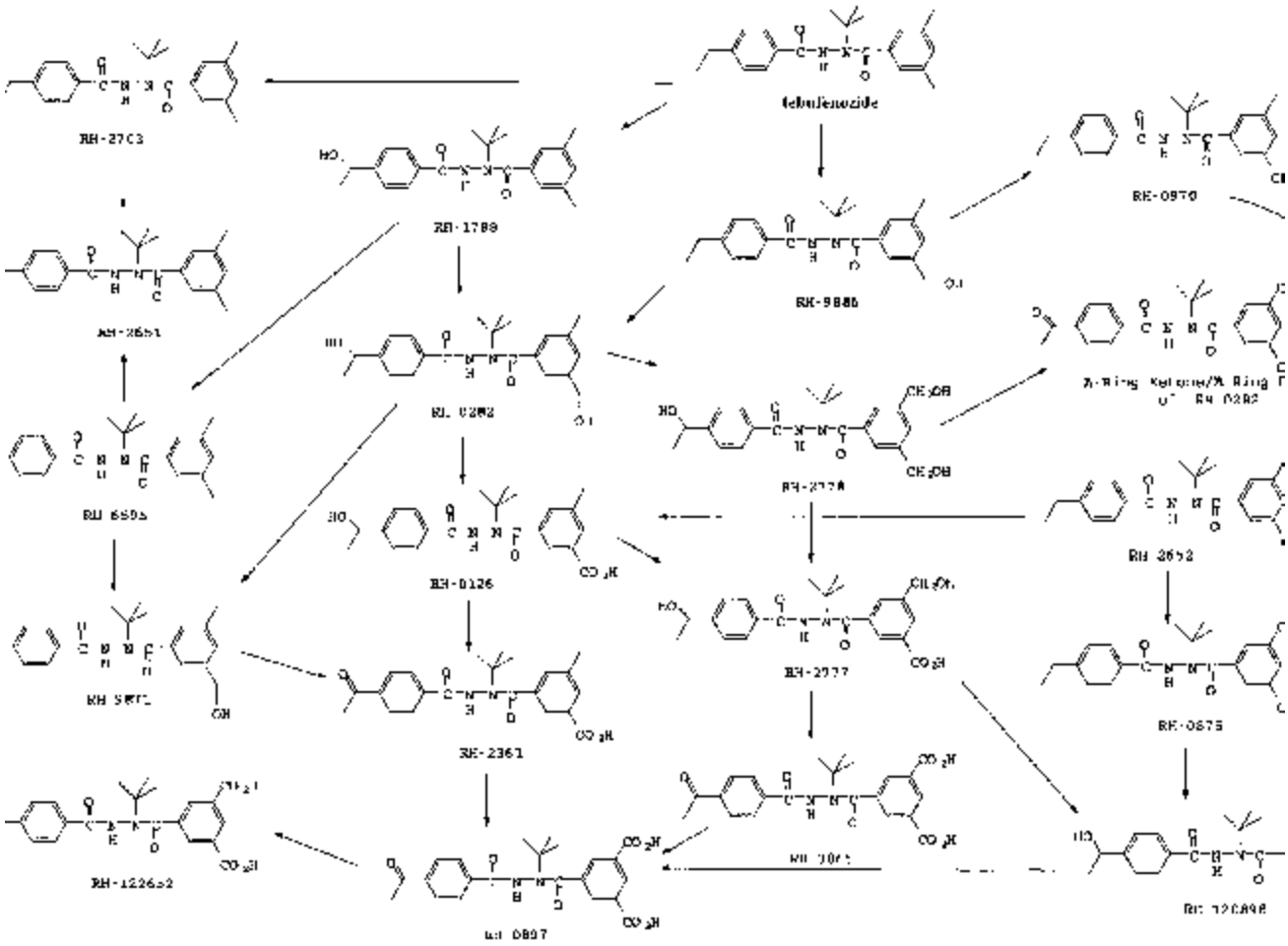
Plant metabolism studies on apples, grapes, rice and sugar beets were conducted with ^{14}C-labelled preparations of tebufenozide. Three radiolabelled versions of tebufenozide, differing in the site of ^{14}C incorporation, were used. One version had the radiolabel uniformly incorporated in the ring carbons of the ethyl substituted phenyl ring (A-ring labelled), another had ^{14}C uniformly incorporated in the ring carbons of the dimethyl substituted phenyl ring (B-ring label), and the third had ^{14}C incorporated in the quaternary carbon of the tertiary butyl group (*t*-butyl label). In all studies, except apple, the three radiolabelled versions of tebufenozide were used (only A-ring labelled material was used in the apple study). Parent tebufenozide was the single major component of the residue in all four studies, comprising 100% of the residue in grapes, 77% in apples, 76% in rice straw, 72% in rice grain, 67% in sugar beetroots and 41% in sugar beet tops. No single metabolite in any study occurred at >10% of the total residue. All metabolites result from the oxidation of the alkyl substituents of the aromatic ring of tebufenozide, primarily at the benzylic positions. On the A-ring side of the molecule, this can result in either an alcohol or a ketone in the benzyl positions. RH-2703 is also formed by oxidation of the terminal carbon of the ethyl group. On the B-ring side, there are two positions which can be oxidized and combinations of one and two oxidations to the alcohol and acid oxidation states (and one case of the oxidation pausing at the aldehyde oxidation state) were observed. This metabolic pathway is found in all of the plants (except grape in which no metabolism was seen), although the extent of metabolism differs among the species. All of the metabolites isolated from plants were observed as metabolites in the rat. In summary, since parent tebufenozide was the single major component from all plant metabolism studies, then this is the residue definition for enforcement purposes. An overall metabolic pathway of tebufenozide is shown as Figure 1.

Animal

The elimination and distribution of ^{14}C-tebufenozide labelled in the A-ring, B-ring and *t*-butyl positions were studied in lactating dairy goats and poultry. In goats, each label was administered to individual animals at approximately 50 ppm for 7 days while leghorn hens were dosed individually (in groups of ten) at 30 ppm for 7 days. Test compound was eliminated primarily via the faeces in the lactating goat (~79-81% for all labels) with urine accounting for ~7-9% of the test material recovered. Milk contributed only 0.08-0.26% (0.065-0.143 ppm based on total over 7-day period) to recovery totals while fat contributed 0.14%-0.26%, liver 0.07-0.40% and muscle 0.02-0.16%. Detectable levels were found in heart and kidney at <0.01%. Results of total radioactive residue analyses in poultry showed

that from 80-103% of the test compound was eliminated primarily via excreta for all labels. Eggs averaged 0.031-0.086% (maximum 0.127 ppm) of the test material recovered and liver retained 0.016-0.523% of the total dose. The individual remaining tissues (gizzard, fat, thigh, breast, and heart) never exceeded 0.049% of the dose. No attempt was made in either study to identify the residues recovered.

Figure 1. Overall Metabolism of Tebufenozide



Analytical Methodology

Analytical methods have been developed which are capable of determining the residues of the parent tebufenozide in apples. Tebufenozide residues are extracted from apples by blending with methanol/0.1 N HCL (9:1) and filtered. A 10% sodium chloride solution is added to the filtrate and partitioned with hexane. The methanol extract is further diluted with sodium chloride and partitioned with methylene chloride. The methylene chloride is concentrated to dryness. A final purification is accomplished by basic alumina column chromatography. Quantitation is accomplished by high performance liquid chromatography with UV detection. The limit of quantitation was demonstrated at 0.02 ppm. Recoveries at fortification levels of 0.01-0.79 ppm ranged from 64-108% (± 11.5) with a mean of 81.3% (n=25).

Residues - Plant

Apple residue data obtained during 1993 and 1994 from Ontario and in 1995 from British Columbia and Nova Scotia indicates that when tebufenozide is used according to the proposed label directions, resulting residues in/on apples are in the range of 0.08-1.10 ppm. As a consequence, when apples are treated in accordance with the Confirm® 240 F label directions, residue data indicates that apples will require a Maximum Residue Limit (MRL) of 1.0 ppm.

An import tolerance of 1.0 ppm was established by the U.S. EPA to cover residues in/on apples exported to the United States from Canada.

A tolerance of 0.05 ppm on walnuts was recently established by the U.S. EPA. Residues of tebufenozide on walnuts imported into Canada will be covered under the *Food and Drugs Regulation* B.15.002 (1).

Results of an apple processing study indicate no concentration of tebufenozide residues in juice and a 2× concentration in wet apple pomace (animal feed).

Residues - Livestock

No livestock feeding study was conducted. A restriction on the label, stating that treated commodities or processed products such as apple pomace should not be fed to livestock, will be required.

Rotational Crops

Results of Canadian accumulation and dissipation studies indicate moderate accumulation of tebufenozide residues and to a lesser extent, soil metabolite RH-6595 one year after application at proposed maximum Canadian rates. Although metabolism studies indicate that tebufenozide exhibits modest translocation, the low levels are of little consequence to the present request for use on apples. However, if a root crop or vegetable crop is requested for future registration, a more

detailed examination of the potential for the accumulation of residues and detailed crop rotation studies would be in order.

Dietary Risk Assessment

An MRL of 1.0 ppm on apples was used to calculate a Potential Daily Intake (PDI). The intake was calculated to be 0.0011 mg/kg bw/day or 5.8% of the ADI (ADI of 0.019 mg/kg bw/day).

2.7 Drinking Water Exposure and Risk Assessment

a) Based on the overall NOEL of 1.9 mg/kg bw/day for haematotoxicity in the 13- and 52-week studies in the dog and using a 100-fold safety factor, an ADI of 0.019 mg/kg bw has been calculated for tebufenozide. Using this ADI, an objective concentration for tebufenozide in drinking water can be calculated as approximately 0.09 mg/L, assuming an adult consumer with a 10% allocation of drinking water.

b) No monitoring data were found on residues of tebufenozide in surface, ground or drinking water.

c) Tebufenozide is sparingly soluble in water with a solubility of 0.83 mg/L at 25°C. Its log octanol/water coefficient (K_{ow}) of 4.23 indicates a high potential for bioaccumulation. Laboratory and field studies (forest pond in Ontario) indicated that tebufenozide would be classified as moderately persistent under aerobic and anaerobic aquatic conditions. Half-lives of tebufenozide in agricultural soils in Ontario, Nova Scotia, New York state and Washington state ranged from 19 to 125 days. These results showed that tebufenozide would be classified as slightly to moderately persistent in soil. Results from a 30-day aged leaching study demonstrated that tebufenozide and RH-96595 would be expected to exhibit low to medium mobility in most soils. The two carboxylic acid transformation products (RH-112651 and RH-112703) would be expected to be mobile in most soils. Results from that leaching study are consistent with the results predicted from adsorption/desorption studies.

Based on the environmental data presented, tebufenozide is not expected to pose a significant health risk through drinking water.

2.8 Occupational Exposure

Qualitative Exposure Assessment

The insecticide Confirm® 240 F is proposed for use on apples using airblast equipment at an application rate of 0.24 kg a.i./ha. It is an aqueous flowable product that is diluted with water prior to application. The area of apple orchards in major apple-producing regions (Ontario, Quebec, British Columbia and Nova Scotia) ranges from 8-20 ha. The use is limited to a maximum of four applications per year with a 14 to 21 day interval between applications. The proposed label

recommends long trousers, long-sleeved shirt, impervious gloves and “splash” goggles during mixing, loading and application. In addition, cartridge respirator is to be worn during application. A reentry interval is not specified, however, the label indicates that treated areas should not be contacted without protective clothing and until residues have dried.

Quantitative Exposure Assessment

Rohm and Haas submitted two surrogate studies on two of their other products (myclobutanil and dinocap) to support the airblast application of Confirm® 240 F in apple orchards. Reentry data were not submitted for the apple orchard use to support a specific reentry interval.

Both the surrogate studies and the registrant's Pesticide Handlers Exposure Database (PHED) estimate have serious limitations for the airblast application (i.e., low number of replicates, using a wettable powder in water soluble bags (WSB) to support an aqueous flowable formulation, large extrapolations of a.i. handled, etc.) to warrant the sole use of this data. Consequently, based on a Health Canada assessment, selected data from surrogate exposure studies were combined with estimates from PHED Version 1.1 (March/95) to yield an estimate for airblast application.

Replicates were subsetted from the mixer/loader (M/L) file and applicator file to estimate total exposure for individuals applying Confirm® 240 F under typical use conditions.

The M/L and applicator files were subsetted for liquid formulations which included emulsifiable concentrates, aqueous suspensions and solutions. As a conservative assumption, data sets were subsetted only for open-cab rather than closed-cab tractors. The number of observations for inhalation and dermal data were acceptable (range from 10-71). Health Canada generally recommends a minimum of 15 replicates for each body location. The sampling time and the amount of active handled fell near the range expected for the proposed use scenarios. All the exposure data were normalized by the kg a.i. handled. Given that the distribution of all the data were lognormal and the sufficient number of observations retrieved, the geometric mean was selected as the best measure of central tendency.

The estimates from the PHED 1.1 assessment and the estimates from the surrogate studies (applicator only) were weighted and combined. The registrant's M/L surrogate data were not used because both studies were based on wettable powders packaged in WSB rather than aqueous flowable formulations.

Results

Table 6 lists the average worker's exposure of tebufenozide from an airblast application. Exposure estimates were based on workers wearing gloves, long pants, and long-sleeved shirt. The estimates are considered representative of the proposed use of Confirm® 240 F.

The inhalation component represented the smallest portion of exposure in the airblast application. The majority of the exposure came from the dermal component (- 77%) which was the result of open cabs in all the replicates.

Table 6. Worker Exposure Estimates of Tebufenozide Use in Apple Orchards

Application Type	Exposure (mg/kg bw/day)[1]
Airblast[2] Mixer/Loader/Applicator	0.013

1 Values based on workers wearing long pants, long-sleeved shirt and gloves; 100% dermal absorption

2 Estimate based on combination of PHED 1.1 and surrogate studies (applicator data only); application rate=0.23 kg a.i./ha; typical area sprayed=20 ha/day

Risk Assessment

The use patterns of tebufenozide indicate a short-term exposure of several days per year. Dermal exposure is regarded as the most relevant route of exposure for airblast application. Tebufenozide primarily acts on the peripheral haematopoietic system resulting in mild regenerative haemolytic anemia with compensatory responses from the haematopoietic tissues. Given the likely route and duration of exposure, the 4-week rat dermal toxicity study was appropriate for risk assessment purposes.

Using the dermal NOEL of 1,000 mg/kg bw/day, the MOS for airblast application was 76,900. Although it is not possible to quantitatively assess risk during reentry, given the low application rate (0.24 kg a.i./ha), low vapour pressure (3×10^{-6} Pa or 2×10^{-8} mm Hg at 25°C) and the low toxicity, the existing reentry statement is adequate. The PMRA considers this MOS acceptable for the proposed use of tebufenozide.

3.0 Environmental Assessment

3.1 Summary of Environmental Chemistry and Fate

Physicochemical Properties

With a solubility of 0.83 mg/L at 25°C, tebufenozide is sparingly soluble in water. The vapour pressure of tebufenozide at 25°C was 3×10^{-6} Pa (= 2×10^{-8} Torr), indicating that tebufenozide is relatively non-volatile. The reciprocal of the Henry's Law Constant, 1/H, calculated by the reviewer

as 2.19×10^6, indicates that tebufenozide is expected to be non-volatile from moist soil and water surfaces. The log octanol/water partition coefficient (K_{ow}) was 4.23, indicating a high potential for bioaccumulation. As tebufenozide has no pronounced acid/base properties, this compound is not expected to dissociate in water.

Laboratory Studies of Transformation and Transport Processes

The hydrolysis half-life of tebufenozide was 568, 1,034 and 517 d at pH 5, pH 7 and pH 9, respectively, indicating that tebufenozide does not undergo appreciable hydrolytic degradation under these conditions. The ultraviolet spectrum of tebufenozide in methanol, which was tested at a wavelength range of 190-350 nm, showed maximum absorbance at 233-234 nm. This demonstrated that absorbance of light did not occur in the environmentally-relevant range (i.e., in the range of 290-350 nm), since wavelengths less than 290 nm are not present in sunlight reaching the earth's surface. Tebufenozide was phototransformed with pseudo first-order half-lives of 98, 66.8 and 1,593 d in soil, pond water and deionized water, respectively. These results suggest that phototransformation is not likely to be an important route of transformation of tebufenozide in the environment.

The half-lives of tebufenozide in aerobic soil under laboratory incubation conditions were 105 d (California loam) and 704 d (Pasquotank sandy loam) indicating that tebufenozide is classified as moderately persistent to persistent in aerobic soils. In a study of the kinetics of tebufenozide in aerobic soil, the half-lives of three minor transformation products were predicted by computer simulation to be 115.3 d (RH-6595; classified as moderately persistent), 3.0 d (RH-2703; classified as non-persistent) and 7.4 d (RH-2651; classified as non-persistent). In an examination of the ready biodegradability of tebufenozide in aerated nutrient medium, 8% biodegradation occurred at 28 days after treatment (DAT), indicating that even under artificial “best-case” conditions, tebufenozide is not readily biodegraded in water. Under aerobic aquatic conditions, the half-lives of tebufenozide were 99 d (Arkansas silty clay hydrosoil) and 101 d (California clay loam hydrosoil) and the three major transformation products were RH-96595, RH-112703 and RH-112651. Under anaerobic aquatic conditions, the half-life of tebufenozide in a Lawrenceville silt loam hydrosoil was 179 d, and there was one major transformation product, RH-112651. According to these results, tebufenozide is classified as moderately persistent under aerobic and anaerobic aquatic conditions. The PMRA environmental evaluators prefer the use of sediments to hydrosoils in aquatic biotransformation studies. Hydrosoils generally have a higher population of microbes and are richer in nutrients than aquatic sediments taken from forestry environments which are generally nutrient poor. Thus studies conducted with hydrosoils would be expected to generate shorter half-lives than studies with sediments, producing a “best case” scenario for dissipation in aquatic systems. A study examining tebufenozide dissipation in a forest pond was also submitted, however, negating the need for a replacement biotransformation study using sediments.

In a study of the bioconcentration and elimination of ^{14}C-tebufenozide by the bluegill sunfish (*Lepomis macrochirus*), the concentration of ^{14}C-residues in edible and nonedible tissues reached a steady state after 1 d of exposure to tebufenozide. Bio-concentration factors (BCFs) ranged from

5.9-8.7 times in edible and 81-150 times in non-edible tissues. Half-lives during depuration ranged from 1 to <3 d in both types of tissue and % elimination after 15 d ranged from 90-98% in edible and 98-100% in non-edible tissues, indicating that tebufenozide did not bioaccumulate in fish. Eight metabolites (RH-0126, RH-2777, RH-2778, RH-2652, RH-2631, RH-0282, RH-9886 and the A-ring ketone/B-ring diol of tebufenozide) were isolated and identified. Four unknown radioactive bands were also characterized, most of which contained multiple components. No single metabolite accounted for >8% of total radioactivity.

For tebufenozide, the adsorption K_{oc}s ranged from 351-894, the first desorption K_{oc}s from 568-1,227 and the second desorption K_{oc}s from 431-1,960. For the transformation product, RH-112651, adsorption K_{oc}s ranged from 76-156, first desorption K_{oc}s ranged from 97-1,135 and second desorption K_{oc}s ranged from 161-415. Based on the adsorption K_{oc}s, tebufenozide is predicted to exhibit low to medium mobility and RH-112651 is predicted to exhibit medium to high mobility in soils. The applicant commented that the increase in K_{oc}s observed from the adsorption to the desorptions may have been due to adsorption being irreversible to some degree, and that the mobility of both compounds might therefore be even lower than predicted based on the adsorption K_{oc}s alone. In an aged leaching study, recoveries of tebufenozide and three of its transformation products in leachates from four soils were as follows: tebufenozide **S** non-detectable (ND) to 19.14%; RH-96595 **S** ND to 1.23%; RH-112651 **S** 0.07 to 15.16%; RH-112703 **S** 0.24 to 2.97%. Tebufenozide and RH-96595 exhibited some mobility on sandy soils with low organic carbon content, indicating that the mobility of these compounds is dependent upon soil texture. By contrast, the two carboxylic acid transformation products (RH-112651 and RH-112703) were mobile in three of four soils (i.e., the soils which had lower organic carbon content). Based on these results, tebufenozide and RH-96595 are predicted to exhibit low to medium mobility in most soils, whereas RH-112651 and RH-112703 are predicted to be mobile in most soils.

Field Studies of Dissipation and Accumulation

In two forestry studies conducted in Ontario, the ranges of the half-lives for tebufenozide in soil were 31-68 d (soil) and 50-181 d (litter). The half-lives from agricultural soils in Ontario and Nova Scotia, and in New York and Washington states, U.S.A., ranged from 19-125 d. Based on these values, tebufenozide is classified as ranging from slightly persistent to moderately persistent in soil. Residues of tebufenozide, however, were still detected at the end of the second season in soil and forest litter, indicating the potential for residue build-up, especially in forest litter. In these dissipation studies, residues of tebufenozide were occasionally found in the soil core segment immediately below the segment which was closest to the soil surface. These observations were consistent with the low to medium mobility for tebufenozide, predicted from adsorption/desorption studies. Small quantities of the transformation products, RH-2651, RH-2703, RH-1788 and RH-6595 were found in the surface segment in the soil compartment and in the litter compartment at some sites. RH-6595 and RH-2651 were also found at depths below the surface segment at New York and Washington.

Based on DT_{50}s in a forest pond in Ontario of 75.8-81.2 d, tebufenozide is classified as moderately persistent in water. The percentage concentration of tebufenozide found in suspended solids was

22-37% at 1 DAT, versus 5-10% at 21-393 DAT. Tebufenozide was shown to partition into and accumulate in bottom sediments. Concentrations of tebufenozide in bottom sediments, which reached maximums at 28-35 DAT, persisted at 393 DAT at concentrations ranging from 13-50% of maximum concentrations. The potential for tebufenozide residues to continue to accumulate in aquatic sediments following annual applications is unknown.

Two foliar dissipation studies conducted in Ontario generated half-lives for tebufenozide ranging from 30-59 d (needles) and 15-31 d (shoots). Small amounts of three transformation products, RH-9886, RH-1788 and RH-6595 were found in the needles. Incidental observations from efficacy studies indicate control of early instar larvae of the spruce budworm (*Choristoneura fumiferana*) in the year following application, thereby demonstrating residue carryover to the next year. No carryover data were provided for residues in apple foliage, however.

3.2 Summary of Environmental Toxicology

Non-target Insects

Based on contact, respiratory and dermal toxicity tests conducted with the adult honey bee (*Apis mellifera*) presented with tebufenozide at concentrations of 0.105% a.i. in water or sugar solutions, tebufenozide does not pose a hazard to honey bees. A valid study examining the toxicity of tebufenozide to honey bee brood would have been desirable, as this would have taken better account of the mode of action of this compound. Tebufenozide was non-toxic to beneficial insects such as the coccinellid mite predator, *Stethorus punctum*, and the predatory mite, *Zetzellia mali* (both at up to nine applications at 347 g a.i./ha), ectoparasitic *Hyssopus* sp. (Eulophidae) which parasitized larvae of the codling moth (*Cydia pomonella*), the bee (*Osmia cornifrons*) (at 133 mg a.i./L), the wasp (*Paracentrobia andoi*) (at 375 g a.i./ha), the mirid (*Cytropeltis lividipennis*) (at 300 g a.i./ha) and spiders (Linyphiidae, Lycosidae and Tetragnathidae) (at 300 g a.i./ha). Based on these results, as well as summaries of other screening data provided by the applicant, the likelihood of deleterious effects on beneficial non-target arthropods following the use of tebufenozide is predicted to be minimal.

Soil Microorganisms and Earthworms

Two studies showed that the effects of tebufenozide (RH-5992 2F) on the nitrogen cycle and respiration were negligible. In a 14-d study of the toxicity of technical tebufenozide to the earthworm (*Eisenia foetida*), the LC_{50} and LOEC were >1,000 mg a.i./kg soil and the NOEC was 1,000 mg a.i./kg soil. Assuming a worst-case scenario in which the concentration of tebufenozide in soil reached an Expected Environmental Concentration (EEC) of 0.359 mg a.i./kg following four applications to an orchard, the safety factor is 2,786, indicating that tebufenozide is unlikely to pose a risk to earthworms.

Birds

The nominal 21-d dietary LC_{50}s and LOECs of technical tebufenozide to both 13-d-old bobwhite quail (*Colinus virginianus)* and 8-d-old mallard duck (*Anas platyrhynchos*) were >5,000 mg a.i./kg diet and the NOEC was 5,000 mg a.i./kg diet. The nominal 21-d acute oral LD_{50} and LOEL of technical tebufenozide to 29-wk-old bobwhite quail were >2,150 mg a.i./kg bw and the NOEL was 2,150 mg a.i./kg bw. Based on these acute and short-term studies, tebufenozide is classified as practically non-toxic to birds. One-generation reproduction studies in which technical tebufenozide was fed for 20 wk to 25-wk-old mallard ducks and 18-wk-old bobwhite quail, resulted in NOECs of 1,000 and 100 mg a.i./kg diet (nominal), respectively. The orchard EEC for food of the bobwhite quail of 56 mg a.i./kg dw, is only 1.8 times less than the lowest NOEC of 100 mg a.i./kg diet. By the time this maximum EEC would be reached in the field, however, the breeding season would be past for most upland birds. Thus, an adequate margin of safety exists for bobwhite quail, indicating that tebufenozide is unlikely to pose a reproductive risk to this species when used according to label directions. No conclusions regarding the potential risk of tebufenozide to passerines can be made, due to the absence of data.

Mammals

Results of mammalian toxicology studies with tebufenozide were summarized from reviews conducted by the PMRA health evaluators. The most likely route of exposure of wild mammals would be through consumption of contaminated prey or vegetation. The dosages consumed by wild mammals were estimated to be less than the NOELs from any of the acute toxicity studies, but exceeded the NOELs from some of the short-term, long-term and reproductive studies. Concentrations of residues in food sources would be expected to decline over time due to processes such as depuration in insects, and transformation and dilution as a result of growth in plants. Tebufenozide is also rapidly metabolized in the rat, suggesting that concentrations in wild mammals would decline in step with reductions in concentrations in their food sources. Because of these considerations, tebufenozide is unlikely to pose a risk to wild mammals when used in accordance with label directions.

Terrestrial Plants

No studies addressing the effects of tebufenozide on terrestrial plants were submitted by the applicant. In the absence of such studies, an assessment of the potential risk could not be made. The applicant has been asked to submit plant-screening data for review.

Aquatic Invertebrates

In two 48-h static acute toxicity studies, the LC_{50}, NOEC and LOEC of technical tebufenozide to *Daphnia magna* were >0.83 mg a.i./L, the limit of solubility in fresh water. However, in a 21-d flow-through toxicity study, the median effective concentration (EC_{50}) to *D. magna* exposed to technical tebufenozide was 0.240 mg a.i./L, and the NOEC and LOEC were 0.029 and 0.059 mg

a.i./L, respectively. These latter values are 0.06 and 0.12 times the EEC for tebufenozide in water of 0.491 mg a.i./L (estimated immediately following four orchard applications), suggesting that the potential exists for lethal and sublethal effects on *Daphnia* and other Cladocera in shallow bodies of water, such as spawning streams. In a 96-h acute toxicity study conducted with the American crayfish (*Procambarus clarkii*) exposed to technical tebufenozide, the LC_{50}, NOEC and LOEC were >0.83 mg a.i./L, suggesting that tebufenozide is unlikely to pose a risk to this species.

A 2-year study was conducted to elucidate the effects of tebufenozide formulation, RH-5992 2F ULV, on zooplankton and phytoplankton in lake enclosures at rates ranging from 0.14-1.34 times as much as the orchard EEC in water of 0.491 mg a.i./L. No measurable differences in abundance of adult and juvenile copepods were observed; however, cladocerans demonstrated distinct concentration-dependant reductions in abundance and some species at the higher rates did not recover until the second season. By contrast, numbers of rotifers increased at higher rates of tebufenozide, but returned to normal by the end of the first season. There were no measurable changes in phytoplankton biomass. The recovery in numbers of cladocerans indicates no long-term effects on ecosystem function.

The stonefly *Pteronarcys* sp. was unaffected when exposed to tebufenozide (RH-5992 2F) in aquatic microcosms for 28 d at up to 0.30 times the orchard EEC in water of 0.491 mg a.i./L. Five other species of aquatic insects were also tested; however, due to high mortality in both the controls and the treatments, results were not accepted.

Tebufenozide did not cause mortality in laboratory flow-through chambers to 11 species of aquatic insects exposed for 1 h at the nominal rate of 3.5 mg a.i./L (or 7.1 times the orchard EEC of 0.491 mg a.i./L). Some mortality was observed with one species of amphipod, *Gammarus* sp.; however, in a subsequent study at rates ranging up to 7.0 mg a.i./L (or 14 times the orchard EEC), *Gammarus* experienced no mortality. Nine species of aquatic insects were unaffected when exposed in outdoor streams to tebufenozide for 1 h at 3.5 mg a.i./L, or for 5 h at rates exponentially decreasing from 3.5 mg a.i./L. Two species of aquatic insects in outdoor streams were unaffected when fed yellow birch leaves for 12 h that had been treated previously with tebufenozide at 50 g a.i./ha. These results indicate that tebufenozide is unlikely to pose a risk to aquatic insects.

In a 96-h flow-through toxicity study with the Eastern oyster (*Crassostrea virginica*), the EC_{50} (95% confidence limit (CL)) was 0.64 (0.33-0.97) mg a.i./L (for 50% reduction in shell deposition), the NOEC was <0.15 mg a.i./L and the LOEC was 0.15 mg a.i./L (both for significant reduction in shell deposition). Compared with the orchard EEC of 0.491 mg a.i./L, these results suggest that tebufenozide has the potential to pose a risk to molluscs, which can be found in freshwater, marine and terrestrial environments. The applicant has been asked to provide data on the toxicity of tebufenozide to freshwater and terrestrial molluscs.

Exposing the mysid shrimp (*Mysidopsis bahia*) to technical tebufenozide in a 28-d flow-through toxicity study, resulted in the LC_{50} (mortality) and LOEC (survival, growth and

reproductive success) of >270 ：g a.i./L and the NOEC of 270 ：g a.i./L. A 96-h static acute toxicity study furnished an LC_{50} (95% CL) of 1.4 (0.82-2.2) mg a.i./L to *M. bahia*, while the NOEC and LOEC were 0.57 and 0.82 mg a.i./L, respectively (all for mortality). Compared with the orchard EEC of 0.491 mg a.i./L, these results for the mysid shrimp indicate a safety factor of 1.2 times when compared with the NOEC of 0.57 mg a.i./L. Tebufenozide is therefore unlikely to pose a risk to the mysid shrimp.

Fish

Two 96-h static acute toxicity studies with technical tebufenozide were conducted with the rainbow trout (*Oncorhynchus mykiss*); in both studies, the LC_{50}, NOEC and LOEC were >0.83 mg a.i./L. In another study, rainbow trout were exposed for 12 h in laboratory flow-through chambers to the tebufenozide formulation RH-5992 2F at the nominal rate of 3.5 mg a.i./L (or 7.1 times the orchard EEC of 0.491 mg a.i./L); no mortality was observed. In two 96-h static acute toxicity studies with bluegill sunfish, exposed to technical tebufenozide, the resulting LC_{50}, NOEC and LOEC were >0.83 mg a.i./L in one study, versus >0.83, 0.39 (considered an artifact) and >0.83 mg a.i./L, respectively, in the other study. In a 96-h flow-through toxicity study with the sheepshead minnow (*Cyprinodon variegatus*) exposed to technical tebufenozide, the LC_{50} and LOEC were >0.72 mg a.i./L and the NOEC was 0.72 mg a.i./L. A 35-d study of the toxicity of technical tebufenozide to embryos and larvae of the fathead minnow (*Pimephales promelas*) resulted in an LC_{50} and LOEC of >0.71 mg a.i./L and a NOEC of 0.71 mg a.i./L (all based on survival at hatch, larval survival and larval growth). Based on the above results, the NOEC for fish of 0.83 mg a.i./L exceeds the maximum estimated EECs in water for orchard use of 0.491 mg a.i./L by a safety factor of 1.7. Because of the low water solubility of tebufenozide (0.83 mg/L), these values underestimate actual safety factors, thus tebufenozide is unlikely to pose a direct risk to fish.

Amphibians

In a 96-h acute toxicity study of the effects of technical tebufenozide on tadpoles of the king frog (*Rana nigromaculata*), the LC_{50}, NOEC and LOEC were >0.83 mg a.i./L. The NOEC exceeds the maximum estimated EEC in water for orchard use of 0.491 mg a.i./L, by a safety factor of 1.7, indicating that tebufenozide is unlikely to pose a direct risk to amphibians.

Aquatic Plants

In a study of the 96-h static toxicity of technical tebufenozide to the freshwater green alga (*Scenedesmus subspicatus*), the EC_{50} was 0.16 mg a.i./L, the NOEC was 0.077 mg a.i./L and the LOEC was 0.15 mg a.i./L (all based on reduction in cell density). The NOEC is less than the maximum estimated EEC in water for orchard use of 0.491 mg a.i./L, suggesting toxicity to algae could occur following exposure to tebufenozide. However, in another study, tebufenozide did not reduce phytoplankton biomass in lake enclosures treated with up to 0.66 mg a.i./L,

suggesting that under actual use conditions, tebufenozide is unlikely to reduce the amount of food available to phytoplankton feeders.

Additional Studies

Additional studies will be submitted to PMRA environmental evaluators over the next year. Two forestry dissipation studies from large-scale research trials (one each from Canada and northeast U.S.A.), as well as studies on the effects of tebufenozide on birds and non-target insects (from the Forest Pest Management Institute) and amphibians (from Canadian Wildlife Service) will be reviewed on a supplementary basis.

3.3 Environmental Concerns

An assessment of the environmental risk associated with the use of tebufenozide has been completed and has identified the following concerns:

- Tebufenozide has the potential for residue carryover into the next season in forest and agricultural soils, forest litter and conifer needles, following application at the proposed maximum label rates.
- Tebufenozide is classified as moderately persistent in pond water in Ontario. Tebufenozide partitioned into and accumulated in bottom sediments in a forest pond, and persisted at 393 DAT. The potential for tebufenozide residues to continue to accumulate in aquatic sediments following annual applications is unknown.
- Tebufenozide was toxic to cladocerans. With a NOEC of 0.029 mg a.i./L to the cladoceran, *D. magna*, versus an orchard EEC of 0.491 mg a.i./L, tebufenozide could present a risk to cladocerans following application at the proposed maximum label rates.
- Tebufenozide was toxic to bivalve molluscs. With a NOEC of <0.15 mg a.i./L to the Eastern oyster, *C. virginica*, tebufenozide could present a risk to molluscs following application at the proposed maximum label rates.

No studies on the effects of tebufenozide on terrestrial plants were submitted. In the absence of such studies, an assessment of potential effects could not be made. The applicant should submit plant-screening data for review.

A study was conducted with a marine mollusc; however, no freshwater or terrestrial species were evaluated. The applicant should provide these data.

Several studies evaluating the effects of tebufenozide on adult honey bees were submitted; however, no valid studies addressing the effects on honey bee larvae were provided. If available, these studies should be submitted.

4.0 Value Assessment: Confirm® 240 F Agricultural Insecticide

4.1 Proposed Uses

The manufacturer, Rohm and Haas, initially proposed that Confirm® 240 F be registered for use on apples to control codling moth, leafrollers (oblique-banded leafroller, red-banded leafroller, three-lined leafroller, European leafroller, fruittree leafroller), winter moth, green pug moth, green fruitworm, spring feeding caterpillars and eye-spotted budmoth. There was also a claim for suppression of spotted tentiform leafminer.

Rohm and Haas has proposed application rates of between 120 to 240 g a.i./ha with the addition of Companion® spray adjuvants (@ 0.1% v/v). They proposed applications during egg laying or first egg hatches of various pests with repeat applications 14 to 21 days if egg laying is prolonged or there are multiple hatches. For insects with multiple generations, re-applications were proposed at egg hatch of each generation. The manufacturer has recommended a maximum of four applications per year with a proposed pre-harvest interval of 14 days.

4.2 Description of Pest Problem

Codling Moth

The codling moth (*Cydia pomonella* (L.)) is one of the most destructive apple pests across Canada (predominantly British Columbia, Ontario, Quebec and Nova Scotia). Direct apple fruit damage is caused by larvae that tunnel into the fruit and feed to the core, sometimes to the seed. Feeding injury also provides infection courts for diseases that further damage the fruit. Injury caused by larvae lowers the quality of the fruit and therefore lowers commercial value.

Mature codling larvae overwinter in silken cocoons on or under loose bark, in the soil or in trash at the base of the trees. Pupation occurs in early spring, and adult moths emerge when apple trees are in bloom (late May to mid June). The adults, living 14 to 21 days, mate and females lay eggs on leaves, twigs and developing fruit. The eggs hatch in 5 to 15 days, and larvae bore into fruits, feed for three to five weeks, then burrow out of the apples and pupate, with emergence of adults approximately one month later in late July or August. The second generation can cause considerable damage to apple fruit, often close to harvest. If weather is warm in late August or early September, a partial third generation may occur in southern parts of Canada.

Fruittree and European Leafroller

Fruittree and European leafrollers (*Archips argyrospila* (Wlk.), *A. rosana* (L.)) are an economic problem in British Columbia, with some impact in Ontario, Quebec and Nova Scotia. The larvae directly damage apple buds and blossoms, older larvae can directly damage fruit, and larvae can indirectly damage the tree by feeding on leaves. Overwintering eggs hatch at the green bud stage, larvae enter buds and feed on flower parts and then move on to feed on leaves and

nearby fruit. Mature larvae pupate within leaf rolls, with adults emerging from June to August. There is one generation per year.

Oblique-banded and Three-lined Leafroller

Oblique-banded and three-lined leafrollers (*Choristoneura rosaceana* (Harr.), *Pandemis limitata* (Rob.)) are a problem in British Columbia, with some impact in Ontario, Quebec and Nova Scotia. The larvae directly damage apple buds and blossoms, older larvae can directly damage fruit, and larvae can indirectly damage the tree by feeding on leaves. Young larvae overwinter in silken cocoons in bark crevices or under bark scales, with some emerging at the green bud stage boring into fruit buds, and others emerging at petal-fall, feeding on flower parts, leaves and young fruit. Mature larvae pupate in rolled leaves and adult moths emerge in early June to late July. The second generation larvae feed on terminal growth and other leaves and then pupate. Adults emerge in late August to October to lay eggs which hatch into larvae for overwintering.

Spotted Tentiform Leafminer

Spotted tentiform leafminer (*Phyllonorycter blancardella* (F.)) have an impact in central Canada (Ontario and Quebec), and are currently managed by biological parasite control in British Columbia and Nova Scotia. Larvae do not attack the fruit but can affect apple quality and size through their damage to the leaves. Leafminer pupae overwinter in leaves on the orchard floor, adults emerge and lay eggs before apple bloom and larvae enter the leaves and feed within leaf mines until pupation. There are two to three generations per year.

Green Fruitworm, Winter Moth, Eyespotted Bud Moth, and Green Pug Moth

Winter moth (*Operophtera brumata* (L.)) are a pest predominantly in Nova Scotia, while green fruitworm (*Lithophane antennata* (Wlk.)), eyespotted bud moth (*Spilonota ocellana* (D. & S.)) and green pug moth (*Choroclystis rectangula* (L.)) are periodic pests across Canada. Generally, the caterpillars of these pests feed on buds, flower parts, new leaves and the young fruit of apples. The life cycles vary among the various species, although they all have one generation per year.

4.3 Efficacy Data Review

Data were submitted from trials which evaluated the efficacy of tebufenozide for control of codling moth, spotted tentiform leafminer, leafrollers (oblique-banded, fruittree, European), winter moth and pug moth on various varieties of apples. All of the trials were conducted in Canada.

Codling Moth

For control of the first generation, Rohm and Haas recommended an application of Confirm® 240 F at 240 g a.i./ha at 200 degree days after bio-fix as determined by pheromone traps (or 3 to 6 days before normal times for application of organophosphorus pesticides). For high insect pressures, a second application was recommended 14 to 21 days later.

Nine studies were submitted to support the claims for control of codling moth: six in Ontario between 1991 and 1994, one in British Columbia in 1993, and two in Nova Scotia in 1993 and 1994. All of the trials used percent fruit damage as control parameters. Four trials used different rates from 120 to 375 g a.i./ha. Various application methods were used, from handguns at 2000 kPa pressure to orchard sprayers at various pressures. Three of the Ontario studies and the Nova Scotia studies contained data analyzed by Duncan's multiple range tests or Tukey's pairwise comparisons of means.

The 1991 and 1992 Ontario studies examined fruit both on the tree and on the ground and found significant reduction in shallow and deep fruit damage on the trees and reductions in deep fruit damage on the ground only. The 1993 and 1994 Nova Scotia studies and a 1994 Ontario study found control of codling moth equivalent to positive controls using azinphos-methyl, phosmet, cypermethrin and *Bacillus thuringiensis* ssp. *kurstaki* (Btk) formulations.

The 1993 British Columbia study provided data that indicated control of moth damage to apples under high population pressures that was equivalent to azinphos-methyl, although a second application was recommended. A 1994 Ontario study conducted a trial on an orchard with high pretreatment populations (>4 moths/trap) and with two applications at 240 g a.i./ha, found no damage to fruit in two post treatment counts. Phytotoxic effects were not observed in any trials.

Fruittree and European Leafroller

The proposed use instructions recommended one application of Confirm® 240 F at 240 g a.i./ha at first egg hatch of each generation for control of leafrollers. For high insect pressures, a second application was recommended 14 to 21 days later.

Two studies conducted in British Columbia in 1993 and 1994 were reviewed in support of the claims for control of these two leafroller species. The 1993 study examined percent mortality of introduced larvae after proposed label rate applications by a handgun. The timing of application was late and no conclusions could be made.

The 1994 study used percent mortality of introduced larvae and percent fruit damage as control parameters. Single applications of two rates of 240 and 360 g a.i./ha were applied with a Turbo mist sprayer at 140 psi. The researchers could not find dead larvae, therefore live larvae were counted. These assumed mortality counts supported a suppression claim for larval populations at

the proposed label rate of 240 g a.i./ha. Data indicated a fifty percent reduction in percent fruit injury at the label rate.

Oblique-banded Leafroller

The proposed use instructions recommended an application of Confirm® 240 F at 240 g a.i./ha at first egg hatch of each generation for control of leafrollers. For high insect pressures, a second application was recommended 14 to 21 days later. For early season control of overwintering populations, the label indicated an application of either 240 g a.i./ha at tight cluster to pink stage or a split application each of 120 g a.i./ha at this stage with the repeat application of 120 g a.i./ha 10 to 14 days later.

Four field studies (one study in Quebec in 1993 and three in Ontario in 1994) and one laboratory study in Quebec in 1993 were reviewed in support of the claims for control of oblique-banded leafroller. The Quebec trials were rejected because of incomplete methods and poor data. The three Ontario trials used percent mortality of larvae and percent blossom clusters without larvae as control criteria. The trials used rates ranging from 120 g a.i./ha applied once and twice, to 240 and 360 g a.i./ha applied once with applications by handgun at 1,500 kPa till runoff. Evaluation of data indicated that the double applications of Confirm® 240 F at the 120 g a.i./ha rates showed the best control. Single applications of 240 g a.i./ha only helped suppress populations and were equivalent to positive controls using deltamethrin.

Spotted Tentiform Leafminer

The proposed use instructions indicated an application of Confirm® 240 F at the first egg hatch of the first generation (pink stage) at a rate of 240 g a.i./ha or as a split application of 120 g a.i./ha with a repeat application 10 to 14 days later for suppression of spotted tentiform leafminer.

Six studies were submitted in support of the claims for suppression of spotted tentiform leafminer; all were conducted in Ontario from 1989 to 1994. The trials used percent control and/or number of mines as control parameters. Rates ranging from 120 g a.i./ha applied once and twice to 240 and 350 g a.i./ha were used and the product was applied by handgun at 1,500 to 2,000 kPa till runoff. Two of the studies contained data analyzed by Duncan's multiple range tests.

The 1991 and 1992 studies applied Confirm® 240 F at 240 g a.i./ha and examined numbers of spotted tentiform leafminers and mines. They found suppression of spotted tentiform leafminers but no reduction of mines.

Other trials (1989, 1993, 1994) indicated a 70.4 to 79.7 percent reduction of spotted tentiform leafminer with double applications of Confirm® 240 F at 120 g a.i./ha and a 62.9 and 71.4 percent reduction at 240 g a.i./ha. A positive control deltamethrin indicated 87.1 percent control of spotted tentiform leafminer. There were no observed effects on parasitism by *Pholetesor ornigis* (Weed) and chalcid abundance in remaining mines.

Green Fruitworm, Winter Moth, Eyespotted Bud Moth, Green Pug Moth, Spring Feeding Caterpillars

The proposed use instructions recommended an application of Confirm® 240 F at 240 g a.i./ha at the tight cluster to full pink stage of the crop for control of these pests.

There was one 1994 British Columbia study submitted in support for control of green fruitworm. The trial examined assumed mortality and fruit injury after applications of 240 and 360 g a.i./ha. While the data did seem to indicate some control and reduction of fruit injury, the absence of corrected mortality values and statistical analysis of the data submitted precludes any definite conclusions and this study was rejected.

Two studies conducted in Nova Scotia in 1993 and 1994 were reviewed in support of the claims for control of winter moth. The trials examined percent fruit injury or damage as control criteria and tested application rates of 180, 240, 300 and 360 g a.i./ha, applied by orchard sprayers. All rates significantly (Tukey's pairwise comparison of means) reduced apple damage to levels equivalent to Btk WP/cypermethrin positive controls.

There was one study submitted in support of control of eyespotted bud moth in British Columbia (1994). The trial examined assumed mortality and fruit injury after applications of 240 and 360 g a.i./ha. The data indicated contamination of the trials, with more budmoth found in the controls that were introduced and this study was rejected.

For control of green pug moth, a 1993 Nova Scotia study indicated suppression of green pug moth with an application of 240 g a.i./ha. Applications with a Btk WP/cypermethrin tank mix achieved greater levels of control.

There were no data submitted to support control of spring feeding caterpillars in apple orchards and these claims have been removed.

4.4 Contributions to Sustainable Agriculture

Integrated Pest Management (IPM)

Increasingly, attention is being focused on IPM programs to reduce reliance on pesticide use in agricultural systems. IPM is an approach to crop protection involving the combined use of chemical, cultural, biological or other methods (population sampling and scouting, action/economic thresholds, population or infestation forecasts) to prevent economic losses due to pests. For an example of reduced pesticide use, a typical Ontario apple orchard following an IPM strategy could use less than half the total pesticide sprays of a traditional chemical strategy.

Confirm® 240 F is being recommended as an IPM compatible pesticide. Data have shown that Confirm® 240 F does not harm predatory mites, wasps, spiders, and beetles that naturally control other insect pests in Canadian apple orchards (see Section 3.0 Environmental Assessment). For example, Confirm® 240 F has low toxicity to predatory mite species which are important predators of European red mites (ERM). Conservation of these predators through use of Confirm® 240 F could provide biological control of ERM which in turn would reduce the need for miticide applications later in the growing season. Another example was the observation during efficacy trials that indicated parasites of spotted tentiform leafminer were present in mines after treatment with Confirm® 240 F. Confirm® 240 F is also not toxic to adult honey bees, which are responsible for 85% of pollination in apple orchards in Canada.

Resistance Management

Resistance of apple pests to currently registered pesticides is becoming a major concern of apple growers. Oblique-banded leafroller resistance to organophosphates has been documented in Quebec and Ontario. European leafrollers in British Columbia are also exhibiting resistance. Spotted tentiform leafminer has become resistant to all synthetic pyrethroids in many orchards in Ontario.

Cross resistance between Confirm® 240 F and other classes of insecticides (organophosphates, synthetic pyrethroids, carbamates) has not been documented. The novel mode of action of Confirm® 240 F, i.e., ecdysone agonist, is unique as compared to other classes, cholinesterase inhibition. With the establishment of a good resistance management strategy using alternate pesticides and the judicious use of Confirm® 240 F in an IPM program, it is hoped that development of resistance by pests to Confirm® 240 F can be managed.

Alternatives

There are numerous insecticides registered in Canada for control of codling moth:

- chlorinated hydrocarbons such as endosulfan and methoxychlor;
- organophosphates (OP) such as azinphos-methyl, diazinon, dimethoate, dichlorvos, malathion, parathion, phosalone and phosmet;
- carbamates such as carbaryl and methomyl;
- synthetic pyrethroids such as cypermethrin, deltamethrin, and permethrin; and
- sulfur.

A number of these pesticides are no longer effective against codling moth.

Broad spectrum pesticides, in general, can be counteractive to IPM strategies, as they all exhibit varying degrees of toxicity to beneficial insects and mites. Current IPM programs will use some of these products with strict conditions and timing to reduce unwanted side effects. For example, British Columbia apple growers are currently engaged in a codling moth eradication program to

supplement IPM programs. Last summer, the province attempted a sterile moth release program in the Okanagan region with limited success. Availability of tebufenozide would obviate the need for up to six applications of azinphos-methyl, which could negatively impact upon present IPM programs.

Apple growers in Ontario are currently using azinphos-methyl, parathion, diazinon, and Btk to control leafrollers. Oblique-banded leafrollers are developing resistance to azinphos-methyl and OPs are not compatible with IPM. Btk is a good alternative; however, timing of application and short availability to insect larvae can affect efficacy. Confirm® 240 F can be another alternative for control of overwintering leafrollers and could suppress subsequent generations of oblique-banded leafrollers.

In Ontario, synthetic pyrethroids are being used by growers to control spotted tentiform leafminers. Unfortunately for IPM programs, synthetic pyrethroids are toxic to invertebrate species which include beneficial predators and parasites. In British Columbia, where synthetic pyrethroids are not used for spotted tentiform leafminers, biological control of spotted tentiform leafminers with parasites reduces numbers of this species to below economic threshold levels. Confirm® 240 F would suppress spotted tentiform leafminers early in the growing season so that beneficial insects, such as *P. ornigis* and chalcids, would be better able to reduce spotted tentiform leafminers later in the season.

4.5 Economics

The apple industry is significant in Canadian agriculture, with a total annual raw commodity value of approximately $140,000,000. British Columbia, Ontario, Nova Scotia and Quebec are the main apple-growing provinces in Canada.

It is difficult to assess the economic value from the use of Confirm® 240 F without any precedents for this kind of product. If various pest management strategies are compared, spray costs of a grower using an IPM strategy are half ($636 per year) compared to that of a grower using a conventional program ($1,451 per year). An IPM program using Confirm® 240 F, instead of a conventional chemical, could possibly reduce costs further, because beneficial insect predators and parasites would not be affected, thereby reducing the need for additional sprays to control secondary pests.

4.6 Conclusions and Label Recommendations

In completing the value assessment, it has been determined that Confirm® 240 F is an effective insecticide that will enhance the use of IPM programs in Canadian apple orchards. With specific activity against lepidopteran insect pests and minimal impact on beneficial arthropods, the product is ideally suited for use in IPM as part of a sustainable agriculture philosophy.

Satisfactory Assessment

The information establishes that the product has merit and value for the following uses with appropriate labelling[1]:

- Control of codling moth with an application of 240 g a.i./ha applied at first egg hatch with a second application of 240 g a.i./ha 14 to 21 days later, only if required after population monitoring;
- Control of oblique-banded leafroller with split applications of 120 g a.i./ha applied at first activity and 10 to 14 days after the initial application. Suppression only of oblique-banded leafroller with an application of 240 g a.i./ha at first activity;
- Control of winter moth with an application of 240 g a.i./ha applied at tight cluster to full pink stage of the crop;
- Suppression of fruittree and European leafroller with an application of 240 g a.i./ha applied at first egg hatch with a second application of 240 g a.i./ha 14 to 21 days later, only if required after population monitoring;
- Suppression of spotted tentiform leafminer with either split applications of 120 g a.i./ha applied at first activity and 10 to 14 days after the initial application or with an application of 240 g a.i./ha at first egg hatch;
- Suppression of green pug moth with an application of 240 g a.i./ha applied at tight cluster to full pink stage of the crop.

More Data Required

Further data are required for fruittree and European leafroller and green pug moth to change claims of suppression to claims of control. Suppression of some of the listed insect pests may be adequate as part of an IPM program.

There were insufficient and/or incomplete data to support any claims for green fruitworm, eyespotted bud moth, red-banded leafroller and three-lined leafroller.

5.0 Regulatory Proposal

5.1 Assessment Conclusions

Chemistry: Specifications, methods of analysis, microcontaminant analysis, and quality control data of the pilot plant production were reviewed and found acceptable. Once full-scale production of tebufenozide is initiated, supplementary chemistry data will be submitted and reviewed.

[1] Control claims may be used on the label only if the product causes greater than 90% mortality of the target insect. Suppression claims must be used for less than 90% mortality of the target insect.

Health Assessment

Toxicology: The primary target site of tebufenozide toxicity was the peripheral haematopoietic system and the main toxicological end-point, consistent across all species tested, was mild regenerative haemolytic anaemia with compensatory responses from the haematopoietic tissues. Tebufenozide technical was of low acute toxicity to the mouse and rat via the oral, dermal or inhalation route. Pharmacokinetics and metabolism studies in the rat revealed that the compound was only partially absorbed, rapidly excreted and there were no signs of bioaccumulation in any tissue/organ examined. Tebufenozide technical was not oncogenic in the mouse or in the rat, and it did not demonstrate any mutagenic/genotoxic potential *in vitro* or *in vivo*. There was no evidence of any teratogenic potential in the rat or the rabbit and no effect on reproduction except at a high dose level that elicited parental toxicity.

Food Exposure: An ADI of 0.019 mg tebufenozide/kg bw has been estimated, based on the overall NOEL of 1.9 mg/kg bw/day (50 ppm) for haematotoxicity in the 13- and 52-week feeding studies in the dog using a 100-fold safety factor. The metabolism of tebufenozide was investigated in apples, grapes, rice, and sugar beets using ^{14}C-tebufenozide. The extent of metabolism and the degree of oxidation appear to be functions of time and differ among the species tested. The parent tebufenozide comprised the majority of the total radioactive residue in all studies and no single metabolite in any study occurred at >10% of the total radioactive residue. All metabolites isolated from plants were observed as metabolites in the rat. The metabolism of tebufenozide was investigated in lactating goats using ^{14}C-tebufenozide. Although no characterization/ identification was reported, results indicate the majority of the administered dose was eliminated in the faeces, with the second largest quantity of test material found in the urine. Only small amounts of the dose were excreted in the milk or retained in the body tissues.

The PMRA is prepared to support the use of tebufenozide on apples. The following Maximum Residue Limits will be required in Table II, Division 15 of the *Food and Drug Regulations* to cover residues on domestic and imported apples and processed products.

Common or (Trade Name)	Chemical Name of Substance	Maximum Residue Limit (ppm)	Foods
tebufenozide (Confirm®)	*N*-*tert*-butyl-*N*'-(4-ethylbenzoyl)-3,5-dimethylbenzo-hydrazide	1.0	Apples

Drinking Water Exposure and Risk Assessment: An objective concentration for tebufenozide in drinking water, using the estimated ADI of 0.019 mg/kg bw, can be calculated as approximately 0.09 mg/L, assuming an adult consumer with a 10% allocation of drinking water. No monitoring data were found on residues of tebufenozide in surface, ground or drinking water. Based on the environmental data presented, tebufenozide is not expected to pose a significant health risk through drinking water.

Occupational Exposure: Based on a Health Canada assessment, selected data from surrogate exposure studies were combined with estimates from the PHED to yield exposure estimates for airblast and aerial application. Exposure estimates were based on workers wearing gloves, long pants, and long-sleeved shirt. The risk assessment indicated that when Confirm® 240 F is used according to label directions, the MOS for occupational and bystander exposure is considered to be acceptable.

Environment Assessment

In laboratory experiments, tebufenozide was relatively non-volatile from moist soil and water surfaces and did not bioaccumulate in fish and mammals tested. Based on laboratory and field studies, the likelihood of deleterious effects on beneficial non-target arthropods, following the use of tebufenozide, is predicted to be minimal. Tebufenozide is unlikely to pose a risk to soil microorganisms, earthworms, birds, wild mammals, fish, amphibians, aquatic plants and most aquatic invertebrates including crayfish, copepods, rotifers, insects and the mysid shrimp.

Tebufenozide was shown to have the potential for residue carryover into the next season in forest soil, forest litter and conifer needles, following application at the proposed maximum label rates. Tebufenozide would be classified as moderately persistent in forest pond water in Ontario and was shown to partition into and accumulate in bottom sediments in a forest pond, and to persist at 393 days after treatment. The potential for tebufenozide residues to continue to accumulate in aquatic sediments following annual applications is unknown. Tebufenozide could present a risk to some aquatic invertebrates, i.e., cladocerans and molluscs, following application at the proposed maximum label rates.

To address the above concerns, and identified data gaps, the applicant has agreed to provide data on the effects of tebufenozide on freshwater and terrestrial species of molluscs, terrestrial plants and honey bee larvae, and additional large-scale forestry dissipation data. Additional studies on the effects of tebufenozide on birds, amphibians and non-target terrestrial insects will be submitted and reviewed on a supplementary basis.

Based on the environmental assessment of tebufenozide, a full registration of the orchard use is acceptable with the required “Environmental Precautions” added to the Confirm® 240 F label.

Value Assessment

Confirm® 240 F agricultural insecticide is an effective insecticide that will enhance the use of IPM programs in Canadian apple orchards. With specific activity against lepidopteran insect pests and minimal impact on beneficial arthropods, it is suited for use in IPM as part of a sustainable agriculture philosophy. A review of efficacy data established that the product has merit and value for the following uses with appropriate labelling:

- Control of codling moth, oblique-banded leafroller, and winter moth at rates and application times listed previously under Section 4.
- Suppression of fruittree and European leafroller, spotted tentiform leafminer, and green pug moth at rates and application times listed previously under Section 4.

Control claims may be used only if the product causes greater than 90% mortality of the target insect; suppression claims must be used for less than 90% mortality of the target insect.

5.2 Proposed Regulatory Decision

Confirm® 240 F Agricultural Insecticide

The PMRA is recommending a full "Commercial" registration for Confirm® 240 F Agricultural Insecticide.

Residues in livestock will be addressed upon completion of the identification and characterization portion of the submitted ruminant metabolism study and submission of an acceptable feeding study. Until that time, the label restriction "Do not feed treated apple pomace to livestock" is required.

Occupational risk assessments indicated that when Confirm® 240 F is used according to label directions, the MOS for occupational and bystander exposure is considered to be acceptable.

Based on the Environmental Assessment of the submitted data and information, the following mitigative labelling statements/precautions are recommended to provide reasonable margins of safety for the identified hazards:

> "Do not apply directly to aquatic systems. Aquatic systems include all permanent lentic (standing) and lotic (flowing) water bodies."
>
> "This product is toxic to certain aquatic invertebrates. To mitigate impact on these organisms, the minimum buffer zone width from aquatic systems should be 15 m for air-blast sprayers, at a maximum wind speed of 11.2 kilometres per hour."

The approved use pattern for Confirm® 240 F will be for use on apples to control and suppress pests as listed under Section 4.1. Optimum rates and timing of applications have been determined from efficacy data submitted with the application for registration in a manner consistent with current IPM practices.

Draft Label

CONFIRM® 240 F

AGRICULTURAL INSECTICIDE

READ LABEL BEFORE USING

GUARANTEE- Tebufenozide..... 240 g/L

REGISTRATION NO. PEST CONTROL PRODUCTS ACT

KEEP OUT OF REACH OF CHILDREN

NET CONTENTS
4 L

ROHM AND HAAS CANADA INC.
2 MANSE ROAD
WEST HILL, ONTARIO
M1E 3T9
1-800-268-4201

Confirm® 240 F agricultural insecticide has a novel mode of action in that it mimics the action of the insect molting hormone, ecdysone, in larval Lepidoptera (caterpillars). Larvae stop feeding within hours of ingestion of a toxic dose of Confirm® 240 F agricultural insecticide and soon thereafter begin to undergo an unsuccessful (lethal) molt. Actual mean time to mortality is somewhat dependent on the physiology of the target species and on local environmental conditions, but is generally 3 to 7 days.

Confirm® 240 F agricultural insecticide is effective against larval Lepidoptera. However, it is essentially non toxic to adult bees. Confirm® 240 F does not adversely affect beneficial insects such as predatory mites, beetles, wasps, and spiders. These characteristics make this compound ideal for integrated pest management systems.

COMPANION® SPRAY ADJUVANT, CONFIRM® 240 F INSECTICIDE, AND THE FLASK SYMBOL ARE TRADEMARKS OF ROHM AND HAAS COMPANY, PHILADELPHIA, PA REGISTERED IN CANADA UNDER WHICH ROHM AND HAAS CANADA INC. HAS BEEN REGISTERED AS A USER.

DIRECTIONS FOR USE:

Confirm® 240 F agricultural insecticide is an aqueous flowable formulation that mixes readily with water (shake container well before using). The addition of Companion® spray adjuvant at the rate of 1 litre per 1000 litres of water (0.1% v/v) is recommended for improved spray coverage. Thorough uniform coverage is essential for good insect control.

FRUIT

Proper timing of application is critical with this product. If application is delayed beyond the recommended time, switch to another insecticide. Monitor other insect pests and apply additional insecticides as required.

Apply no more than 4 applications per year. Do not use this product exclusively to control any one pest. Rotate the application of Confirm® 240 F with other recommended insecticides. Do not apply Confirm® 240 F within 14 days of harvest. Do not feed apple pomace to livestock.

Apples

Pest	Rates (L/ha)	Timing of Application
Codling Moth	1.0	For control of the first generation, apply Confirm® 240 F at 200 degree-days (F) after biofix as determined by the first consistent moth catch in pheromone traps (the lower and upper thresholds for codling moth are 10°C (50°F) and 31°C (88°F)). If degree day model information is not available in your area, apply Confirm® 240 F at first egg hatch which is generally 3 to 6 days before the recommended application timing of organophosphate insecticides. If there is a prolonged egg hatch and/or high insect pressure as determined by population monitoring, a second application for this generation could be required at 500 degree-day (F) heat units or 14 to 21 days after the initial application. Timings for the second generation of codling moth is based on first egg hatch (approximately 1,100 degree days F) plus another application 14 to 21 days later if there is a prolonged egg hatch and/or high insect pressure as determined by population monitoring.
Oblique-banded Leafrollers Overwintering populations	0.5 + 0.5	In the case of early season control of overwintering populations of oblique-banded leafrollers, Confirm® 240 F may be applied to protect blossoms from feeding damage. Apply Confirm® 240 F at tight cluster to pink stage at the first sign of insect activity. Confirm® 240 F is applied as a split application of 0.5 litre at this stage plus 0.5 litre 10 to 14 days after the initial application.
Oblique-banded, Fruittree and European Leafrollers	1.0	Suppression of oblique-banded, fruittree and European leafroller may be achieved by a application of Confirm® 240 F at egg hatch and a second application 14 to 21 days later, if required after population monitoring.
Winter Moth	1.0	For the control of winter moth apply Confirm® 240 F at the tight cluster to full pink stage of the crop.
Green Pug Moth	1.0	Suppression of green pug moth may be achieved by an application of Confirm® 240 F applied at tight cluster to full pink stage.

Spotted Tentiform Leafminer	0.5 + 0.5 or 1.0	For the suppression of this pest, apply Confirm® 240 F at first egg hatch of the first generation (apples are usually at the pink stage). Confirm® 240 F is applied at the rate of 1.0 litre at this stage or used as a split application of 0.5 litre at first egg hatch plus 0.5 litre 10 to 14 days after the initial application.

PRECAUTIONS

KEEP OUT OF REACH OF CHILDREN

IRRITATING TO SKIN UPON REPEATED AND PROLONGED CONTACT. MINIMALLY IRRITATING TO EYES. WEAR PROTECTIVE CLOTHING (LONG TROUSERS, LONG-SLEEVED SHIRTS), IMPERVIOUS GLOVES AND SPLASH GOGGLES DURING ALL MIXING, LOADING AND APPLICATION. WEAR A CARTRIDGE RESPIRATOR DURING APPLICATION. PROTECTIVE CLOTHING SHOULD BE WASHED BEFORE RE-USE.

ENVIRONMENTAL PRECAUTIONS:

DO NOT CONTAMINATE WATER BY CLEANING OF EQUIPMENT OR DISPOSAL OF WASTES. DO NOT APPLY WHEN WEATHER CONDITIONS FAVOUR DRIFT OR RUN-OFF FROM AREAS TREATED.

DO NOT APPLY DIRECTLY TO AQUATIC SYSTEMS. AQUATIC SYSTEMS INCLUDE ALL PERMANENT LENTIC (STANDING) AND LOTIC (FLOWING) WATER BODIES.

THIS PRODUCT IS TOXIC TO CERTAIN AQUATIC INVERTEBRATES. TO MITIGATE IMPACT ON THESE ORGANISMS, THE MINIMUM BUFFER ZONE WIDTH FROM AQUATIC SYSTEMS SHOULD BE 15 M FOR AIR-BLAST SPRAYERS, AT A MAXIMUM WIND SPEED OF 11.2 KILOMETRES PER HOUR.

FIRST AID:

IF IN EYES: FLUSH EYES WITH LARGE AMOUNTS OF WATER FOR AT LEAST 15 MINUTES. CONSULT A PHYSICIAN IF IRRITATION PERSISTS.

IF INHALED: MOVE SUBJECT TO FRESH AIR.

IF ON SKIN: WASH AFFECTED SKIN AREAS WITH SOAP AND WATER AND CONSULT PHYSICIAN IF IRRITATION OCCURS. REMOVE CONTAMINATED CLOTHING PROMPTLY AND WASH BEFORE RE-USE.

IF SWALLOWED: DILUTE BY GIVING TWO GLASSES OF WATER TO DRINK AND CALL A PHYSICIAN OR POISON CONTROL CENTRE. NEVER GIVE ANYTHING BY MOUTH TO AN UNCONSCIOUS PERSON.

TOXICOLOGICAL INFORMATION:

IF SWALLOWED EMESIS IS RECOMMENDED.

STORAGE:

STORE IN A COOL, DRY AREA. DO NOT CONTAMINATE WATER, FOOD OR FEED BY STORAGE OR DISPOSAL. AVOID CONTAMINATION OF STREAMS, LAKES AND PONDS. PESTICIDE WASTES ARE TOXIC. IMPROPER DISPOSAL OF EXCESS PESTICIDE, SPRAY MIXTURE OR RINSATE IS PROHIBITED.

PESTICIDE DISPOSAL:

1. RINSE THE EMPTIED CONTAINER THOROUGHLY AND ADD THE RINSINGS TO SPRAY MIXTURE IN THE TANK.
2. FOLLOW PROVINCIAL INSTRUCTIONS FOR ANY REQUIRED ADDITIONAL CLEANING OF CONTAINER PRIOR TO ITS DISPOSAL.
3. MAKE EMPTY CONTAINER UNSUITABLE FOR FURTHER USE.
4. DISPOSE OF CONTAINER IN ACCORDANCE WITH PROVINCIAL REQUIREMENTS.
5. FOR INFORMATION ON THE DISPOSAL OF UNUSED, UNWANTED PRODUCT AND THE CLEANUP OF SPILLS CONTACT THE PROVINCIAL REGULATORY AGENCY OR THE MANUFACTURER.

RE-ENTRY AND WORKER PROTECTION STATEMENTS:

DO NOT ENTER TREATED AREAS WITHOUT PROTECTIVE CLOTHING UNTIL SPRAYS HAVE DRIED. DO NOT APPLY THIS PRODUCT IN SUCH A MANNER AS TO DIRECTLY OR THROUGH DRIFT EXPOSE WORKERS OR OTHER PERSONS. THE AREA BEING TREATED MUST BE VACATED BY UNPROTECTED PERSONS.

SPILL AND LEAK PROCEDURES:

DIKE AND CONTAIN SPILL WITH INERT MATERIAL (E.G. SAND, EARTH). TRANSFER LIQUID TO CONTAINERS FOR RECOVERY OR DISPOSAL AND SOIL DIKING MATERIAL TO SEPARATE CONTAINERS FOR DISPOSAL. KEEP SPILLS AND RUNOFF OUT OF MUNICIPAL SEWERS AND OPEN BODIES OF WATER. DO NOT TAKE CONTAMINATED CLOTHING HOME TO BE LAUNDERED.

NOTICE TO USER:

THIS CONTROL PRODUCT IS TO BE USED ONLY IN ACCORDANCE WITH THE DIRECTIONS ON THIS LABEL. IT IS AN OFFENSE UNDER THE PEST CONTROL PRODUCTS ACT TO USE A CONTROL PRODUCT UNDER UNSAFE CONDITIONS.

LIMITATION OF WARRANTY:

SELLER'S GUARANTEE SHALL BE LIMITED TO THE TERMS SET OUT ON THE LABEL AND, SUBJECT THERETO, THE BUYER ASSUMES THE RISK TO PERSONS OR PROPERTY ARISING FROM THE USE OR HANDLING OF THIS PRODUCT AND ACCEPTS THE PRODUCT ON THAT CONDITION.