Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Proposed Regulatory Decision Document PRDD2003-04

Fenhexamid

The active ingredient fenhexamid and the formulated products Elevate 50 WDG and Decree 50 WDG, for control of *Botrytis cinerea* are proposed for full registration under Section 13 of the Pest Control Products (PCP) Regulations.

This proposed regulatory decision document (PRDD) provides a summary of data received and the rationale for the proposed full registration of these products. The Pest Management Regulatory Agency (PMRA) will accept written comment on this proposal up to 45 days from the date of publication of this document. Please forward all comments to the Publications Coordinator at the address below.

(publié aussi en français) **March 26, 2003**

This document is published by the Alternative Strategies and Regulatory Affairs Division, Pest Management Regulatory Agency. For further information, please contact:

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ISBN: 0-662-33809-X
Catalogue number: H113-9/2003-4E-IN

Foreword

This proposed regulatory decision document (PRDD) announces the full registration of the active ingredient fenhexamid and the formulated products Elevate 50 WDG fungicide, for the control of *Botrytis cinerea* on grapes and strawberries, and Decree 50 WDG for the control of *Botrytis cinerea* on ornamentals.

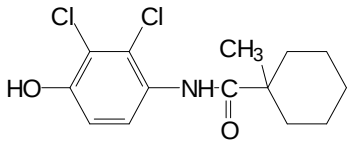
The PMRA had previously issued a temporary registration for these products for grapes, strawberries, and ornamentals with the requirement that Arvesta Corporation (formerly Tomen Agro, Inc.) carry out additional studies (including batch data for the technical active ingredient, bioaccumulation in fish, aerobic soil biotransformation, and terrestrial field dissipation studies). These data gaps were addressed satisfactorily and this document details the scientific rationale used to support these products. Elevate 50 WDG and Decree 50 WDG are identical formulations of fenhexamid. Therefore, studies cited in this document that used the Elevate formulation are also applicable to Decree 50 WDG.

Fenhexamid was jointly reviewed in Canada by the Pest Management Regulatory Agency (PMRA) and the United States Environmental Protection Agency (U.S. EPA).

Table of Contents

1.0 The active substance, its properties, uses, proposed classification and labelling

1.1 Identity of the active substance and preparations containing it

Active substance:	Fenhexamid
Function:	Fungicide
Chemical name (IUPAC):	1-methylcyclohexanecarboxylic acid (2,3-dichloro-4-hydroxyphenyl)amide
Chemical name (CAS):	N-(2,3-dichloro-4-hydroxyphenyl)-1-methylcyclohexanecarboxamide
CAS number:	126833-17-8
Nominal purity of active:	98.6%
Identity of relevant impurities of toxicological, environmental and(or) other significance:	Fenhexamid technical does not contain any byproducts or microcontaminants that meet the Toxic Substances Management Policy (TSMP) Track-1 criteria. Impurities of toxicological concern are not expected to be present in the raw materials nor are they expected to be generated during the manufacturing process.
Molecular formula:	$C_{14}H_{17}Cl_2NO_2$
Molecular mass:	302.2
Structural formula:	

1.2 Physical and chemical properties of active substance

Pure Material Fenhexamid, (KBR 2738)

Property	Result	Comments
Colour and physical state	White powder	N/A
Odour	None	N/A
Melting point/range	153°C	N/A
Boiling point/range	N/A	N/A
Density	1.34 g/cm^3 at 20°C	N/A
Vapour pressure	°C — Vapour pressure (Pa) 20 — 4×10^{-7} 25 — 9×10^{-7}	Low potential for residues on fruits and foliage to decrease as a result of volatilization Not volatile from water
UV/visible spectrum	λ_{max} (nm) — ϵ 203 — 41 340 245 — 10 050 291 — 2810	Photodegradable in the UV range
Solubility (mg/L) in water at 20°C	pH — mg/L 5–7 — 20 8.5 — 200 9.3 — 1000	Soluble at neutral and acidic pHs but very soluble at alkaline pHs
Solubility (g/L) in organic solvents at 20°C	Solvent — Solubility g/L n-hexane — <0.1 toluene — 5–10 dichloromethane — 20–50 2-propanol — 50–100 octanol — 50–100 PEG — 100–200 PEG+C_2H_5OH — >200 acetone — 100–200 DMF — >200 acetonitrile — 1–20 DMSO — >200 PEG = polyethyleneglycol DMF = dimethylformamide DMSO = dimethylsulfoxide	In general, solubility appears to increase with increasing organic solvent polarity
n-Octanol/water partition coefficient (K_{ow})	pH — log K_{ow} — K_{ow} 4 — 3.62 — 4200 7 — 3.51 — 3200 9 — 2.23 — 170	Potential for bioaccumulation under acid to neutral pH conditions
Dissociation constant	pK_a 7.3	

Property	Result	Comments
Oxidizing properties	The product is stable at room temperature under air	Fenhexamid is unlikely to produce any oxidative or reductive processes on plants which may result in a change of the nature and magnitude of residues
Corrosion	The product is compatible with: 1. Aluminum 2. Copper 3. Brass 4. Plain steel (standard) 5. Stainless steel 1.1451 6. Tinplate 7. HDPE 8. PET/AL/PA/PE-laminate 9. Cardboard	N/A
Storage stability	Stable at room temperature for 52 weeks.	Freezer storage stability studies indicated that residues of fenhexamid were stable at ≤-18°C to -20°C for up to 17.5 months (525 days)

End-use product: Elevate 50 WDG Fungicide and Decree 50 WDG Fungicide

Property	Result	Comments
Colour	Brown	N/A
Odour	Weak characteristic odour	N/A
Physical state	Solid	N/A
Formulation type	Water dispersible granule	N/A
Guarantee	50% fenhexamid (nominal)	N/A
Container material and description	Composite foil of paper/LDPE/aluminum/LDPE for 1.0 kg to 10 kg size and plastic drums for 10 and 100 kg sizes	N/A
Specific Gravity	Pour: 0.52 Tap: 0.58	N/A
pH of a 1% solution	8.3, under nitrogen at room temperature	N/A
Oxidizing or reducing action	Not oxidizing or reducing	The plant metabolism study indicated that the metabolic profile did not change following prolonged periods of storage at -20°C
Storage stability	No change after one year under ambient conditions.	N/A
Explodability	Not explosive	

1.3 Details of uses and further information

Elevate 50 WDG and Decree 50 WDG fungicide products are identical wettable granular formulations containing the active ingredient, fenhexamid, for the control of *Botrytis cinerea* (grey mould) on strawberries, grapes and ornamentals. Fenhexamid is a protectant fungicide which inhibits germ tube and hyphal growth.

Elevate 50 WDG may be applied to strawberries at a rate of 1700 g/ha (850 g active ingredient [a.i.]/ha) and a total of four applications per season. Elevate 50 WDG may be applied to grapes at a rate of 1120 g/ha (560 g a.i./ha) and a total of four applications per season. A non-ionic surfactant (0.02% v/v) is tank-mixed with Elevate 50 WDG for application to grapes only. Decree 50 WDG may be applied to ornamentals at a rate of 1120 g/ha (560 g a.i./ha) and no more than six applications per season. Applications may only be made with ground equipment.

2.0 Methods of analysis

2.1 Methods for analysis of the active substance as manufactured

High-performance liquid chromatography (HPLC) and gas chromatography (GC) methods were used for the determination of the active substance and significant impurities (content $\geq$ 0.1%) in the technical product. Based on the validation data and the provided chromatograms, the methods were assessed to be precise, accurate and specific for the determinations.

2.2 Method for formulation analysis

An HPLC method was used for the determination of active substance in the formulation. The method has been shown to have satisfactory specificity, linearity, precision and accuracy.

2.3 Methods for residue analysis

2.3.1 Multiresidue methods for residue analysis

The petitioner submitted data concerning the recovery of residues of fenhexamid using Food and Drugs Administration (FDA) multiresidue method protocols (PAM Vol. I). Recovery of fenhexamid was <30% using Protocols D (extraction with acetone, removal of water with Hydromatrix, gas-liquid chromatography (GLC) determination with various columns and detectors) and E (acetonitrile or water/acetonitrile extraction, partitioning into petroleum ether, Florisil column cleanup, GLC determination with various columns and detectors). The average recovery of fenhexamid from grapes was 82.4% when spiked samples were analysed using Protocol B (extraction from acidified mixture, gel permeation chromatography (GPC), methylation, and Florisil cleanup, determination by GLC).

2.3.2 Methods for residue analysis of plants and plant products

The residue of concern (ROC) was defined from the apple, tomato and grape metabolism studies as the parent, fenhexamid.

An HPLC method with electrochemical detection (ECD) was used for the determination of residues of fenhexamid in/on plant commodity samples collected from the storage stability, crop field, and processing studies. Method validation recoveries indicated that this method adequately recovered residues of fenhexamid from grapes (74–128%), grape juice (77–104%), wine (83–129%), raisins (75–107%) and strawberries (72–113%). The limits of detection (LOD) were not reported, however, the limits of quantitation (LOQs) were reported to be 0.02 ppm for grapes and grape juice, must, and wine, and 0.05 ppm for raisins, raisin waste, wet and dry pomace, and strawberries. The standard deviations measured with respect to recoveries following spiking at the LOQ and 10× LOQ indicated the method had satisfactory repeatability. According to the representative chromatograms, the area of analytical interest was free of interferences from crop matrix components. Furthermore, the retention time appeared to be consistent among the various matrix spiking levels. These factors are indicative of the method having acceptable specificity.

The submitted confirmatory method validation data was marginally adequate to fulfill requirements for a confirmatory method. The data indicated that similar recoveries of fenhexamid were obtained from spiked samples when a UV detector was used instead of an ECD detector as proposed for the enforcement method. In general, confirmatory methods should be significantly different from the enforcement method. When the only difference is in the detector used, it is preferred that the confirmatory detector be more specific, such as a mass selective detector. However, as indicated above, fenhexamid was adequately recovered (82%) from grapes using Protocol B of the PAM I multiresidue methods. Since this method relies on a different chromatographic method, gas chromatography (GC), it should serve as an adequate confirmatory method.

This HPLC/ECD method was proposed for the enforcement of tolerances for residues of fenhexamid in/on various raw agricultural commodities (RAC) and processed plant commodities.

An independent method validation trial was conducted to verify the reliability and reproducibility of this HPLC/ECD method. There were no significant difficulties/problems encountered during the course of the independent laboratory validation (ILV). Recoveries obtained by the ILV were comparable with those of the analytical method.

Although no radiovalidation data were submitted, the plant metabolism data indicated that fenhexamid residues in/on plant commodities, resulting from the requested uses, primarily consisted of surface residues, with the majority of the residues identified as parent fenhexamid; therefore, no radiovalidation data were required for the purposes of this petition.

2.3.3 Methods for residue analysis of food of animal origin

Tolerances for animal commodities were not required to support the proposed uses of fenhexamid. Therefore, no residue analytical methods for animal commodities were required.

3.0 Impact on human and animal health

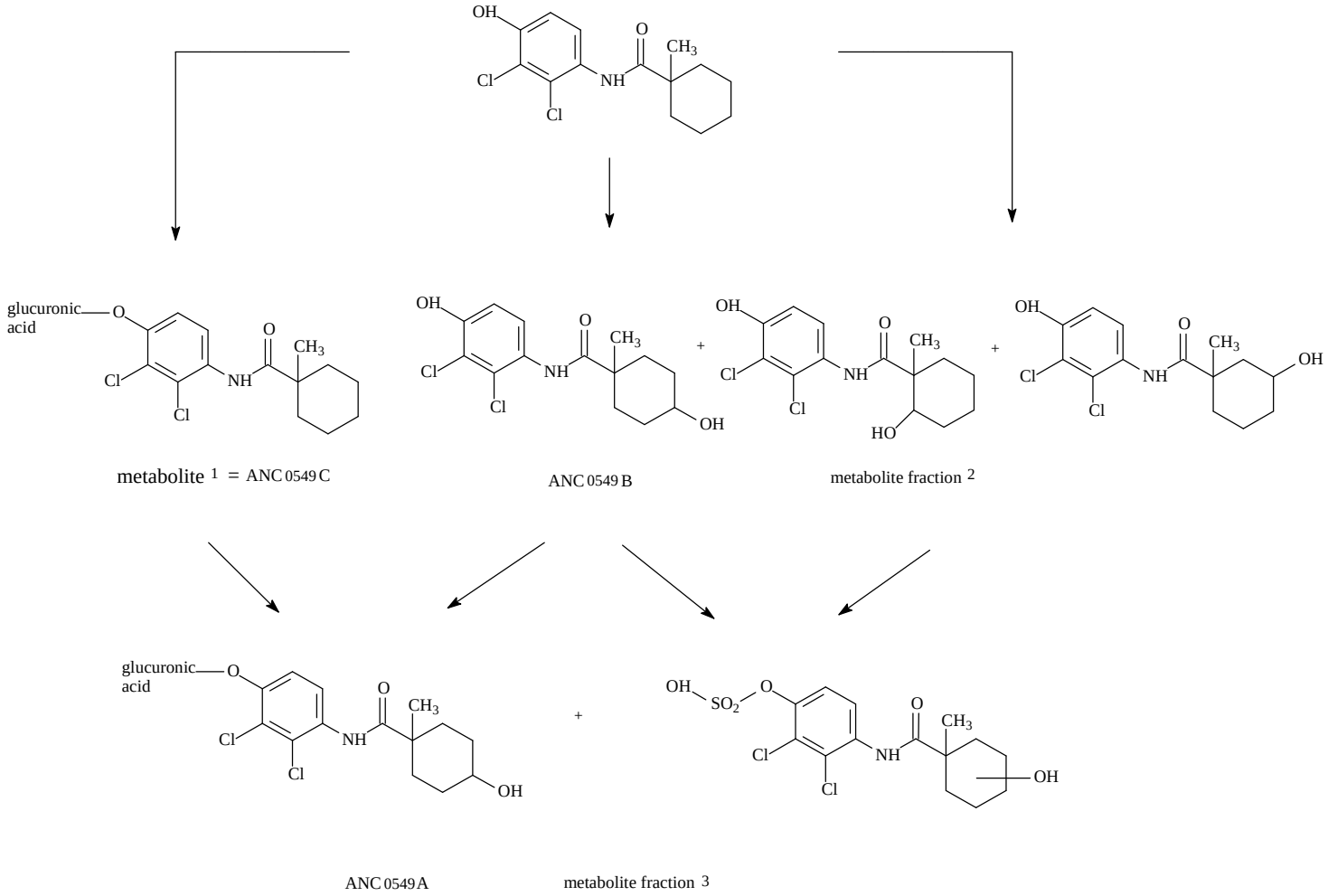
3.1 Effects having relevance to human and animal health arising from exposure to the active substance or to impurities in the active substance or to their transformation products

3.1.1 Absorption, distribution, metabolism and excretion

The absorption, distribution, metabolism and excretion of [Phenyl-UL-^{14}C] KBR 2738 in male and female Wistar rats was determined after a single oral low dose of 1 mg/kg, a single oral high dose of 100 mg/kg and 15 repeated low doses of 1 mg/kg/day. ^{14}C-KBR 2738 was rapidly absorbed from the gastrointestinal (GI) tract in all dose groups. After single and repeated administration of the low dose, the plasma concentration peaked within 5 to 10 minutes. After administration of the high dose, the maximum was detected 40 to 90 minutes post-dosing. The absorption of the test compound was shown to be almost complete in a bile-cannulation experiment, as more than 97% of the administered dose was absorbed from the GI tract 48 hours after intra-duodenal administration. These results are indicative of a pronounced first pass effect and enterohepatic circulation. Tissue residues declined rapidly and after 48 hours the total radioactivity residue in the body, excluding the GI tract, was less than 0.3% of the administered dose in all dose groups. Liver and kidney were the organs with the highest concentrations of radioactivity in all dose groups. There was no evidence of bioaccumulation. Excretion was rapid and almost complete with feces as the major route of excretion. Approximately 62–81% of the recovered radioactivity was found in feces, and 15–36% in urine within 48 hours post-dosing. More than 90% of the recovered radioactivity was eliminated with bile in the bile cannulation experiment. Only 0.02% of the administered radioactivity was recovered in exhaled air. Radioactive residues in rat bodies (excluding GI tract) were significantly lower in females after a single high dose. There was significantly higher renal excretion for females in comparison with males after 15 repeated low doses. In both sexes renal excretion was significantly higher after a single low dose when compared with a single high dose.

Metabolite characterization studies showed that the main component detected in excreta was the unchanged parent compound which accounted for 62–75% of the dose independent of the dosing regime and sex. Metabolite 1, the glucuronic acid conjugate of the parent compound, ranged from 4 to 23% of the dose. Metabolite fractions 2 and 3 accounted for up to 3 and 7% of the dose, respectively. The proposed major pathway for biotransformation is via conjugation of the aromatic hydroxyl group with glucuronic acid. Prior to fecal excretion, hydrolysis in the intestine converts the conjugate back to the parent compound giving rise to enterohepatic circulation. This demonstrates that although the main residues in the feces are due to unchanged parent compound, the absorption rate is close to 100% of the given dose. Furthermore, hydroxylation took place in the positions 2, 3 and 4 of the cyclohexyl ring followed by formation of glucuronic acid and sulfate conjugates of these hydroxylated metabolites. Identification of radioactive residues ranged from 88 to 99% and was independent of dose and sex. Refer to Figure 1.

FIGURE 1 METABOLISM OF TM-402 (KBR 2738) IN RATS



3.1.2 Acute toxicity—technical and formulation

Technical fenhexamid, purity 95.4%, was considered to be of low acute toxicity by the oral, dermal and inhalation routes in Sprague Dawley (SD) rats (oral and dermal LD_{50}s >5.0 g/kg bw; LC_{50} >5.0 mg/L). It was non-irritating when applied to the skin or instilled into the eyes of New Zealand White (NZW) rabbits. Results of skin sensitization testing using guinea pigs, employing the Buehler method were negative.

Based on the results of acute toxicity testing, no signal words are required to be displayed on the primary display panel of the technical material.

Elevate 50 WDG Fungicide formulation (identical to Decree 50 WDG), containing 49.6% technical fenheximid, was considered to be of low acute toxicity by the oral, dermal and inhalation routes in Sprague Dawley rats (oral and dermal LD_{50}s >2.0 g/kg bw; LC_{50} >5.0 mg/L). It was slightly irritating when applied to the skin of NZW rabbits, and was minimally irritating when instilled into the eyes of the same species. Results of skin sensitization testing in guinea pigs, employing the Buehler method, were negative.

3.1.3 Genotoxicity

No evidence of mutagenic potential of technical fenhexamid was observed in vitro with the Ames Bacterial Mutation Test or in an unscheduled DNA synthesis assay with rat hepatocytes. Under the conditions of an in vitro mammalian cell gene mutation assay (cultures of normal ($HGPRT^+$) Chinese hamster ovary (CHO) cells), fenhexamid was considered non-mutagenic for point mutations, frame-shift mutations and deletions. Fenhexamid was not clastogenic in the presence of metabolic activator at any dose level tested in an in vitro chromosomal assay using Chinese hamster lung fibroblasts. In an in vivo study, technical fenhexamid did not induce micronuclei in a mouse micronucleus assay. Based on the data presented, technical fenhexamid was not considered to be genotoxic under the conditions of the tests performed.

3.1.4 Subchronic and chronic toxicity

The subchronic and chronic toxicity of fenhexamid were investigated in mice, rats and dogs. A series of range-finding 28-day and 90-day studies were conducted initially. These studies were used to establish appropriate dose levels to be used in the long-term studies. A 21-day dermal study was also carried out in rabbits.

3.1.4.1 Subchronic and chronic toxicity in the mouse

In a 90-day subchronic toxicity range-finding study, KBR 2738 (97.8% purity) was administered to 10 B6C3F1 mice/sex/dose in the diet at dose levels of 0, 100, 1000 and 10 000 ppm (0, 26.5, 266.5 or 3283.5 mg/kg/day in males and 0, 51.6, 453.9 or 5151.1 mg/kg/day in females) for 14 weeks.

There were no compound-related effects in mortality, clinical signs, body weight, food consumption (females), hematology or gross pathology. The lowest observed adverse effect level (LOAEL) is 10 000 ppm (3283.5/5151.1 mg/kg/day in males/females) based on the observation in both sexes of: increased serum cholesterol, bilirubin and creatinine, decreased kidney weights; increased water consumption, increased food consumption (males) decreased food efficiency (males); renal cortical tubular basophilia (both sexes), renal protein casts and cellular detritus (males); and marginal alterations of liver function (increased serum cholesterol, bilirubin, decreased ASAT, ALAT), marginal increase in liver weights and reduced glycogen content of hepatocytes (males). The no observed adverse effect level (NOAEL) is 1000 ppm (266.6/453.9 mg/kg/day in males/females.

In a carcinogenicity study, KBR 2738 (95.4% purity) was administered to 50 B6C3F1 mice/sex/dose in the diet at dose levels of 0, 800, 2400 or 7000 ppm (0, 247.4, 807.4 or 2354.8 mg/kg/day for males, and 0, 364.8, 1054.5 or 3178.2 mg/kg/day for females) for two years. An additional 10 mice/sex/dose were assigned for the interim sacrifice at 52 weeks.

Survival was not affected by treatment with KBR 2738. There were no compound-related effects on clinical signs, food consumption, hematology or gross pathology. A marginal decrease in body weights (up to 8%) and body weight gain (17%) was observed in males at 7000 ppm. The LOAEL for males is 2400 ppm (807.4 mg/kg/day) based on the observation of decreased kidney weights and decreases in sex-specific vacuolation of the proximal tubules in the kidneys in males. Additional toxicologically significant effects at the highest dose of 7000 ppm (LOAEL for females) included decreased body weights and weight gain in males, significantly increased water consumption (both sexes), increased levels of serum creatinine, bilirubin and albumin (males), decreased kidney weights (females), renal histopathology (increased incidence of basophilic cortical tubules in females; chronic renal disease in males). The LOAEL for females is 7000 ppm (3178.2 mg/kg/day) based on the observations noted above. The NOAEL for males/females is 800/2400 ppm (247.4/1054.5 mg/kg/day, respectively). KBR 2738 is non-oncogenic in mice at doses up to and including 7000 ppm (2354.8 mg/kg/day in males and 3178.2 mg/kg/day in females). There was no treatment-related increase in tumour incidence, tumour spectrum or latency when compared with controls. In this study, KBR 2738 was tested at adequate dose levels for carcinogenicity testing since it was tested at the limit dose of 7000 ppm (2354.8 mg/kg/day in males and 3178.2 mg/kg/day in females) for mice.

3.1.4.2 Subchronic and chronic toxicity in the rat

In a 5-day range-finding inhalation study in rats, technical grade fenhexamid (95.5% purity) was administered to 10 SPF-bred Wistar rats/sex/dose by head-nose only exposure at concentrations of 0, 11.8, 97.7 or 1092.6 mg/m^3 in air for 6 hours per day for a total of 5 days. One-half of the rats were sacrificed 7 days after the first exposure and the other one-half were sacrificed 21 days after the first exposure.

There were no compound-related effects in mortality, clinical signs, body weights, hematology or clinical chemistry parameters. Histopathological examination of tissues was not performed. The LOAEL is 1092.6 mg/m^3 based on the observations of macroscopic grey colouration of the lungs and marginally increased lung weights. The NOAEL is 97.7 mg/m^3.

In a 28-day subchronic range-finding toxicity study, KBR 2738 (97.8% purity) was administered to 10 Wistar rats/sex/dose by gavage at dose levels of 0, 100, 300 or 1000 mg/kg/day for 28 days.

There were no compound-related effects in mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights, or gross and histologic pathology. The NOAEL is 1000 mg/kg/day, the highest dose tested.

In a 56-day bioavailability study, KBR 2738 (95.4% purity) was administered to 10/sex/dose SPF-bred Wistar rats in their diet (1% peanut oil excipient) at dose levels of 0, 1000, 5000, 10 000, 15 000 or 20 000 ppm (57.5, 284.7, 575.7, 943.8, and 1217.1 mg/kg/day for males and 78.0, 407.1, 896.5, 1492.5 and 1896.7 mg/kg for females) for 56 days. The purpose of this study was to determine whether or not there was saturation of intestinal absorption of KBR 2738 when given in the diet at concentrations of 10 000 to 20 000 ppm. Therefore, KBR 2738 levels were determined in plasma and urine samples after a treatment period of 3 or 4 weeks, when steady state conditions were expected. Results showed that plasma samples taken from 20 000 ppm rats had KBR 2738 levels below the limit of detection. Urine samples showed measurable excretion of conjugated KBR 2738 indicating intestinal absorption in the dose range examined. Males had a maximum excretion rate at 15 000 ppm indicating a saturation of intestinal absorption between 15 000 and 20 000 ppm. Urine excretion in females was somewhat lower than in males, at concentrations of 10 000 ppm and above. The highest value was determined at 20 000 ppm suggesting that saturation in intestinal absorption was not achieved with this dose level in females.

In a 90-day oral toxicity study, KBR 2738 (95.4% purity) was administered to 10 Wistar rats/sex/dose in the daily diet, at dose levels of 0, 2500, 5000, 10 000 or 20 000 ppm (0, 202, 415, 904 and 1904 mg/kg/day for males and 0, 270, 549, 1132 and 2824 mg/kg/day for females) mixed with 1% peanut oil (excipient). An additional 2 groups dosed at 0 and 20 000 ppm for 13 weeks were observed for an additional 4 weeks and served as a recovery group.

No treatment-related changes were seen in clinical signs, mortality, ophthalmoscopic examinations, hematology, urinalyses or gross pathology. The LOAEL in males is 10 000 ppm (904 mg/kg) based on decreased terminal body weights and body weight gains, increased food consumption, decreased food efficiency and increased ALAT levels. The LOAEL in females is 20 000 ppm (2824 mg/kg/day) based on increased food consumption, decreased food efficiency, decreased liver weights and liver histopathology (Kupffer cell proliferation and altered hepatocyte morphology). The NOAEL is 5000 ppm (415 mg/kg/day) in males and 10 000 ppm (1132 mg/kg/day) in females.

In a combined chronic toxicity/carcinogenicity study, KBR 2738 (95.4% purity) was administered to 50 Wistar rats/sex/dose in the diet (containing 1% peanut oil excipient), at dose levels of 0, 500, 5000 or 20 000 ppm (0, 28, 292 or 1280 mg/kg/day for males, and 0, 40, 415, or 2067 mg/kg/day for females) for 24 months. An additional 10 rats/sex/dose were sacrificed after 52 weeks.

Survival was not affected by treatment with KBR 2738. The LOAEL for chronic toxicity is 5000 ppm (292/415 mg/kg/day in males/females) based on observations of decreased body weight gain (-6.8%) and food efficiency (-11.8%) in females, increased incidence of cecal mucosal hyperplasia in males, increased cellularity (hyperplasia) of the bone marrow in females and the presence of splenic extramedullary hematopoiesis in males. Additional toxicologically significant findings at the highest dose of 20 000 ppm were observations of increased food consumption, increased numbers of circulating reticulocytes, enlarged spleens observed macroscopically, increased splenic weights and thyroid colloid alterations (both sexes). The NOAEL is 500 ppm (28/40 mg/kg/day in males/females). KBR 2738 is non-oncogenic at doses up to and including 20 000 ppm in the diet. At the doses tested, there was no treatment-related increase in tumour incidence, tumour spectrum or latency when compared with controls. In this study, KBR 2738 was tested at adequate dose levels for carcinogenicity testing since it was tested at the limit dose of 20 000 ppm (1280 mg/kg/day in males and 2067 mg/kg/day in females) for rats.

3.1.4.3 Subchronic toxicity in the dog

In a 90-day subchronic toxicity study KBR 2738 (95.4% purity) was administered to 4 Beagle dogs/sex in their daily diet (mixed with water) at dose levels of 0, 1000, 7000 or 50 000 ppm (33.9, 239.1 or 1747.7 mg/kg/day for males and 37.0, 261.0 or 1866.2 mg/kg/day for females).

There were no compound-related effects in mortality, clinical signs, clinical tests (electrocardiogram (ECG), heart rate, blood pressure, pulse, reflexes, body temperature), ophthalmoscopic examinations, body weight, food consumption, urinalysis, or gross and histologic pathology including liver tissue enzyme analysis. Increased absolute and relative liver weights in high-dose males and in mid and high dose females were considered to be adaptive in nature and, in the absence of any associated histopathological findings, not adverse. The variable nature of alterations in some clinical chemistry parameters (ALAT and ASAT) and the increases in alkaline phosphatase were judged

to be non-adverse, particularly in the absence of any associated gross or histopathological findings in the liver. The LOAEL is 7000 ppm (239/261 mg/kg/day for males and females, respectively) based on significant increases in Heinz bodies in males and females. In addition, at the highest dose of 50 000 ppm, treatment-related effects were seen in other hematology parameters in both sexes (decreased RBC, hemoglobin, hematocrit) and may indicate the potential of KBR 2738 to induce Heinz body anemia in Beagle dogs. The NOAEL is 1000 ppm (33.9/37.0 mg/kg for males and females, respectively).

In a one-year chronic oral toxicity study, KBR 2738 (94.6–95.8% purity) was administered to 4/sex/dose Beagle dogs in the diet at dose levels of 0, 500, 3500 or 25 000 ppm (0, 17.4, 124.3 or 917.8 mg/kg/day for males and 0, 19.2, 132.7 or 947.1 mg/kg/day for females) for 52 weeks.

There were no compound-related effects on mortality, clinical signs, clinical tests (ECG, heart rate, blood pressure, pulse, reflexes, body temperature), ophthalmoscopic examinations, clinical chemistry, urinalysis, or gross pathology. Decreases in RBC, Hb and Hct and increases in Heinz bodies in both sexes were noted in mid- and high-dose dogs. Decreased body weight gain was observed in both sexes of the 25 000 ppm treatment group. The decreased body weight gain by high-dose females may be attributed, in part, to the decreases in food consumption observed sporadically during the latter half of the study period. Treatment-related increases in absolute and relative adrenal weights in mid- and high-dose females were corroborated by the histopathological observations of increases in incidence and severity of intracytoplasmic vacuoles in the adrenal cortex of these animals. No neoplastic changes were observed in any animals of any dose group. The LOAEL is 3500 ppm (124.3/132.7 mg/kg/day in males and females, respectively) based on decreases in RBC, Hb and Hct and on significant increases in Heinz bodies in both sexes; increased adrenal weight parameters in females and the presence of intracytoplasmic vacuoles in the adrenal cortex of 3/4 female dogs. As well as decreased body weight gains (both sexes) and decreased food consumption (females) at the highest dose of 25 000 ppm, more pronounced treatment-related effects were seen in hematology parameters in both sexes (decreased RBC, Hb, Hct, increased Heinz bodies) and may indicate the potential of KBR 2738 to induce Heinz body anemia in Beagle dogs. The hematoxic effect of KBR 2738 was also noted in the 90-day dog study. The NOAEL is 500 ppm (17.4/19.2 mg/kg/day for males and females, respectively). Dosing was considered adequate based on the observation at the high dose of 25 000 ppm of decreased body weight gains, food consumption and hematotoxic effects.

3.1.4.4 Subchronic toxicity in the rabbit

In a 21-day repeated dose dermal toxicity study in NZW rabbits, KBR 2738 (95.4% purity) was applied to the shaved skin of 5 rabbits/sex/dose at a dose level of 1000 mg/kg/day (limit dose), 6 hours/day, for a total of 17 days over a 3-week period.

No rabbits died during this study. No skin irritation was observed in any treated animals. There were no compound-related effects on clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights, or gross and histologic pathology. Dermal administration of KBR 2738 was well tolerated by both sexes for 21 days at the limit dose of 1000 mg/kg/day. The NOAEL is 1000 mg/kg/day (limit dose) and the LOAEL is greater than 1000 mg/kg/day for both systemic and local effects on the skin.

3.1.5 Reproductive and developmental toxicity

In a two-generation reproduction study (1 litter/generation), KBR 2738 (93.8–95.2% purity) was administered to 30 Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 100, 500, 5000 or 20 000 ppm (0, 7.6, 38.2, 406 or 1814 mg/kg/day for males and 0, 9.0, 44.8, 477 or 2043 mg/kg/day for females determined for the 10-week pre-mating period).

There were no compound-related effects on mortality, clinical signs, behaviour or reproductive parameters for adult animals. The NOAEL for reproductive toxicity was 20 000 ppm (1814/2043 mg/kg/day), the highest dose tested.

The neonatal NOAEL was 500 ppm (38.2/44.8 mg/kg/day); the neonatal LOAEL was 5000 ppm (406/477 mg/kg/day) based on significantly decreased pup body weights on lactation days 14 and 21 for F_1 pups (6–11% less than controls) and on lactation days 7, 14 and 21 for F_2 pups (9–11% less than controls). At 20 000 ppm (1814/2043 mg/kg/day), significantly decreased pup body weights were observed on lactation days 7, 14 and 21 for F_1 pups (15–30% less than controls) and for F_2 pups (11–19% less than controls). Treatment-related decreased pup body weights were not observed at birth or on lactation day 4. An additional effect observed at 20 000 ppm was an increase in the number of pups among the post-weaning F_1 pups selected to be F_1 parents which died viz. 0/66, 2/68, 0/68, 0/68 and 10/78 for the control, 100, 500, 5000 and 20 000 ppm dose groups respectively. This effect was attributed to the small size of the pups at weaning (30% less than controls).

The parental NOAEL was 500 ppm (38.2/44.8 mg/kg/day); the parental LOAEL was 5000 ppm (406/477 mg/kg/day) based, in males, on increased creatinine levels in P-generation (but not F_1 generation) males at pre-mating (20%, $p<0.05$) and at termination (20%, not significant); slightly increased alkaline phosphatase levels in P-generation and F_1 generation males at pre-mating and at termination (20–34%, not significant); decreased absolute liver weight in P-generation and F_1 generation males (11–12%, $p<0.05$) and decreased liver/body weight ratios in P-generation and F_1 generation males (8–9%, $p<0.05$ for P-generation and not significant for F_1 generation); decreased absolute kidney weights in F_1 generation (but not P-generation) males (12%, $p<0.05$); and decreased kidney/body weight ratios in F_1 generation (but not P-generation) males (8%, $p<0.05$). The parental LOAEL was based, in females, on increased alkaline phosphatase levels in F_1 generation (but not P-generation) females at pre-mating

(43%, $p<0.05$) and at termination (63%, $p<0.05$); and on very small increases in gammaglutamyl transferase (GGT) (not considered to be biologically relevant). In males at 5000 ppm, the increased creatinine levels and decreased absolute and relative kidney weights suggested a possible treatment-related effect on the kidney and the increased alkaline phosphatase levels and decreased absolute and relative liver weights suggested a possible treatment-related effect on the liver. Histopathological examination of kidney and liver in males, however, did not indicate any treatment-related morphological changes in these organs (i.e., was negative). In females at 5000 ppm, the increased alkaline phosphatase levels and GGT levels suggested a possible treatment-related effect on the liver. Histopathological examination of liver in females, however, was negative.

At 20 000 ppm (1814/2043 mg/kg/day), in males, treatment-related effects on parental parameters were the following: increased creatinine levels in P-generation males (20%, $p<0.05$); increased alkaline phosphatase levels in P-generation and F_1 generation males (16–44%, not significant); slightly increased GGT levels in P-generation males ($p<0.05$, but not considered to be biologically relevant); decreased absolute liver weights in P-generation and F_1 generation males (9–19%, $p<0.05$ for P-generation, not significant for F_1 generation); decreased liver/body weight ratios in P-generation and F_1 generation males (3–11%, $p<0.05$ for P-generation, not significant for F_1 generation); decreased absolute kidney weights in F_1 generation males (13%, $p<0.05$); decreased kidney/body weight ratios in F_1 generation males (8%, $p<0.05$); decreased body weights (6–16%, $p<0.01$); and increased food consumption (12–26%, $p<0.01$). Histopathological examination of kidney and liver was negative. At 20 000 ppm, in females, treatment-related effects on parental parameters were the following: increased urea nitrogen levels in P-generation females (43%, not significant) and F_1 generation females (55%, $p<0.05$); increased creatinine levels in F_1 generation females (17%, $p<0.05$); increased alkaline phosphatase levels in P-generation females (23–25%, not significant) and F_1 generation females (56–87%, $p<0.05$); slightly increased GGT in P-generation females ($p<0.05$, but not considered to be biologically relevant); decreased absolute kidney weights in P-generation and F_1 generation females (15-19%, $p<0.05$); decreased kidney/body weight ratios in P-generation and F_1 generation females (5–6%, $p<0.05$); decreased pre-mating body weights (6–16%, $p<0.01$); decreased gestation body weights (7–9%. $p<0.01$); decreased lactation body weights (7–12%, $p<0.01$); and increased food consumption (4–11%, $p<0.05$). Histopathological examination of kidney and liver was negative. Overall, treatment-related effects observed at 5000 ppm in males and females were also observed at 20 000 ppm, but were slightly increased in severity. Toxicologically relevant additional toxicological effects observed at 20 000 ppm were decreased body weights and increased food consumption in males and increased urea nitrogen and creatinine levels, decreased kidney weights, decreased body weights and increased food consumption in females.

In a developmental toxicity study, KBR 2738 (95.4% purity) was administered to 30 Sprague-Dawley rats/dose by gavage at dose levels of 0 and 1000 (1044 determined analytically) mg/kg/day from days 6 through 15 of gestation.

When tested at the limit dose of 1000 (1044) mg/kg/day, there were no treatment-related effects on maternal mortality, clinical signs, Cesarean parameters or gross pathology. The LOAEL for maternal toxicity is set at 1044 mg/kg/day based on the observed decrease in body weight gain (-12% of controls) during gestation days 6–16 and a decrease in food consumption (10% of controls) during gestation days 6–11. The NOAEL for maternal toxicity is < 1044 mg/kg/day. The NOAEL for developmental toxicity is set at 1044 mg/kg/day (limit dose). No treatment-related effects were noted in any embryo/fetal parameters. Where noted, statistically significant differences from concurrent control values fell within the range of values of the historical control data supplied by the laboratory for those parameters. Under the conditions of this study, therefore, KBR 2738 was not embryotoxic, fetotoxic or teratogenic at a dose of 1044 mg/kg/day (the highest dose tested).

In a developmental toxicity study, KBR 2738 (95.4% purity) was administered to 16 female Russian rabbits (CHBB:HM)/dose by gavage at dose levels of 0, 100, 300 or 1000 mg/kg/day from days 6 through 18 of gestation. Does were naturally inseminated and were sacrificed on gestation day 29.

No treatment-related effects were seen on mortality, general appearance or behaviour. The LOAEL for maternal toxicity is set at 300 mg/kg/day based on observations at this dose and above of alterations of excretory products (discoloured urine, small scybala), decreased body weight gain and feed consumption (mainly during the first week of the treatment period) and decreased placental weights. One abortion at 300 mg/kg/day and one abortion and two total litter resorptions at 1000 mg/kg/day were not considered to be treatment-related because the incidences fell within the ranges of historical control data submitted with the study. Reduced and(or) light feces were also noted at 1000 mg/kg/day. Pale livers were noted in the 2 dams that aborted. The NOAEL for maternal toxicity is set at 100 mg/kg/day. The LOAEL for developmental toxicity is 1000 mg/kg/day based on marginally decreased male fetal body weights and evidence of delayed ossification. Administration of the test compound did not induce any treatment-related fetal malformations or deviations at any of the doses tested under the conditions of this study. All effects on intrauterine development were correlated with maternal toxicity and, therefore, no primary developmental effect was evident. The NOAEL for developmental toxicity is 300 mg/kg/day. KBR 2738 was not teratogenic up to and including 1000 mg/kg/day, the limit dose.

3.1.6 Neurotoxicity (acute, delayed and subchronic)

In an acute neurotoxicity study, a single oral dose of KBR 2738 (95.4% purity) was administered to 12 Wistar rats/sex/dose by gavage at dose levels of 0, 200, 630 or 2000 mg/kg in 2% aqueous Cremophor EL (10 mL/kg). The rats were observed for 14 days. Functional observational battery (FOB) and motor activity testing were performed 7 days prior to dosing, approximately 20 minutes to 3 hours post-dosing, and on days 7 and 14.

There were no compound-related effects on mortality, clinical signs, body weights, brain weights, or gross and histologic pathology or neuropathology. FOB testing revealed no treatment-related effects in any females. High-dose males had a marginally lower ($p<0.05$) mean body temperature (colonic) on the day of treatment (day 0), but which reverted to normal by day 7. No treatment-related effects on measures of motor/locomotor activity or habituation were evident in either sex at doses up to and including 2000 mg/kg. The LOAEL in males is 2000 mg/kg based on marginal acute toxicity as evidenced by the lower body temperatures. The NOAEL in males is 630 mg/kg. The NOAEL in females is 2000 mg/kg, the highest dose tested.

Decreased body temperature may be a sign of acute general systemic toxicity or may possibly be due to a CNS-mediated (neurotoxic) effect of the test material on the brain since the brain controls temperature regulation in the body. There is insufficient data in this study to distinguish between these two possibilities. While this observation could be considered to be a possible neurotoxic effect of the test material, it is only marginal and occurs only at a very high dose level (2000 mg/kg). Therefore, it is not considered to be a toxicologically significant neurotoxic effect.

3.1.7 Integrated toxicological summary

A detailed review of the toxicology database available for the new fungicide, fenhexamid, has been completed. Data submitted were complete and comprehensive, and included the full battery of studies currently required for registration purposes. Studies were well conducted and conform to currently acceptable international testing protocols. The scientific and regulatory quality of the toxicology database is high and is considered sufficient to clearly define the toxicity of this chemical.

Acute dosing revealed that technical fenhexamid and Elevate 50 WDG formulation was of low toxicity by the oral, inhalation and dermal exposure routes to laboratory animals. Technical fenhexamid was non-irritating when applied to rabbit skin and eyes, whereas Elevate 50 WDG induced slight skin irritation and minimal eye irritation. Neither possessed skin sensitizing properties when tested on guinea pigs according to the Buehler method. Fenhexamid did not demonstrate any neurotoxic effects.

In subchronic and chronic oral studies, toxicologically significant effects were hematotoxicity in dogs, and decreased body weights, increased food consumption and mild liver and(or) kidney effects in rats and mice.

In the 90-day and one-year feeding studies in dogs, the most significant treatment-related effects were decreased erythrocyte counts, hemoglobin and hematocrit, indicative of anemia, and increased numbers of erythrocytes containing Heinz bodies. In the 90-day study, increased Heinz bodies were observed at study termination at the LOAEL of 239/261 mg/kg bw/day in males and females, respectively. At the next higher dose of 1749/1866 mg/kg bw/day, which was the highest dose tested, increased numbers of Heinz bodies were first observed at 6 weeks and marginal signs of anemia at 2 weeks in males

and at 13 weeks in females. In the one-year study in dogs, increased numbers of Heinz bodies and signs of anemia were first observed at 13 weeks in both sexes at the LOAEL of 124/133 mg/kg bw/day. At the same dose level, in females only, effects were noted in the adrenal gland (increased adrenal weights, and increased incidence and severity of intracytoplasmic vacuoles in the adrenal cortex). At the highest dose of 918/947 mg/kg bw/day, the severity of effects noted at the LOAEL was increased, and decreased body weight gain and decreased food consumption were also observed. The NOAEL of 17.4/19 mg/kg bw/day in this study was used to establish the acceptable daily intake (ADI) for fenhexamid since it was the lowest NOAEL observed in any of the short- or long-term feeding studies with fenhexamid. The particular effects on which the ADI is based (anemia and increased numbers of Heinz bodies in males and females; adrenal effects in females) are toxicologically significant and were clearly observed in dogs only and not, to any appreciable extent, in any other species. There was, however, an indication of anemia in the two-year chronic feeding study in rats in which enlarged spleens, splenic extramedullary hematopoiesis, bone marrow hyperplasia and increased numbers of circulating reticulocytes were observed in males and(or) females. Although decreases in erythrocyte parameters were not observed in this study, it is possible that these signs of anemia may have occurred early in the study, been transient, and fully compensated for later in the study by the time blood samples were first taken (at 6 months). It appears that treatment-induced anemia occurs in both dogs and rats, but that dogs are more sensitive to the effects of fenhexamid. The LOAEL for anemia in dogs (altered erythrocyte parameters) was 124/133 mg/kg bw/day and for anemia in rats (splenic extramedullary hematopoiesis and bone marrow hyperplasia) was 292/415 mg/kg bw/day in males and females, respectively.

In subchronic and chronic feeding studies in rats and mice, non-specific signs of toxicity such as reduced body weight gain, increased feed and water intake were seen.

In the 90-day feeding study in rats, the primary toxicological effects in males were reduced body weight/body weight gain, increased food consumption, decreased food efficiency and increased serum levels of ALAT (suggestive of slight liver toxicity). The LOAEL/NOAEL for these effects in males is 904/415 mg/kg bw/day. In females, the primary toxicological effects were noted to be increased food consumption, decreased food efficiency, decreased liver weights with associated mild histopathology. The LOAEL/NOAEL for these effects in females is 2824/1132 mg/kg bw/day. Although in this study males appeared to be more sensitive, a biologically meaningful sex difference in sensitivity to fenhexamid was not observed in any other studies on this compound.

In the two-year chronic feeding study in rats, mild treatment-related effects in males at the LOAEL of 292 mg/kg bw/day included increased splenic extramedullary hematopoiesis and increased cecal mucosal hyperplasia. In females at the LOAEL of 415 mg/kg bw/day, treatment-related effects included decreased body weight/body weight gain, decreased food efficiency and bone marrow hyperplasia. At the highest dose tested (1280 mg/kg bw/day in males; 2067 mg/kg bw/day in females), additional treatment-related effects included increased food consumption, decreased food efficiency, enlarged spleens, increased numbers of circulating reticulocytes and slight histopathological changes in the thyroid gland (decreased follicular volume and blue-grey clumps of colloid). No carcinogenic effects were noted.

Of particular interest in the 90-day and two-year feeding study in rats is the regular and consistent observation of decreased body weight, decreased body weight gain coupled with increased food consumption and decreased food efficiency at relatively high dose levels (greater than 415 mg/kg bw/day) in both sexes. At this time there is no available biological explanation for this observation.

In the studies conducted with mice, the main toxicological findings were indicative of kidney pathology. In the 90-day study at the LOAEL of 3284/5151 mg/kg bw/day (M/F), treatment-related effects which were indicative of kidney damage included increased water consumption, increased serum creatinine levels, decreased kidney weights and histopathological changes in the kidneys (increased basophilic cortical tubules and(or) increased protein casts and cellular detritus). The NOAEL in this study was 267/454 mg/kg bw/day (M/F).

In the 18-month study in mice, at the LOAEL of 807 mg/kg bw/day, males had decreased kidney weights and histopathological changes in the kidney (decreased sex-specific vacuolation of the proximal tubules). At the highest dose tested (HDT) of 2355 mg/kg bw/day, additional toxic effects included increased water consumption, increased serum creatinine levels and increased incidence of chronic renal disease, all of which are indicative of kidney damage, as well as increased bilirubin and albumin levels, decreased body weight and body weight gain. In females at the LOAEL of 3178 mg/kg bw/day (HDT), similar observations of increased water consumption, decreased kidney weights and increased numbers of basophilic cortical tubules also suggested kidney damage. No carcinogenic effects were noted.

In a battery of five mutagenicity studies, technical grade fenhexamid was negative for genotoxicity. Taken together with the lack of evidence of carcinogenicity in both sexes of rats and mice, fenhexamid is not likely to pose a carcinogenic risk to humans.

In neither the developmental toxicity studies in rats nor rabbits was there any evidence for increased susceptibility of fetuses to in utero exposure to fenhexamid. Therefore, fenhexamid is not considered to be a developmental toxicant. Fenhexamid did not impair the reproductive capacity of adult rats. On the basis of NOAELs/LOAELs in the reproduction study, no increased susceptibility of pups was demonstrated in this study. However, the severity of the effects observed in the pups (significantly decreased pup body weight) may have been greater than those observed in the adults (minimal alterations of clinical chemistry parameters) at the same dose levels. In addition, several other toxicological considerations, including possibly increased intake of test material in pups resulting from exposure via the milk and feed during the lactation period, as well as a decreased capacity in pups to metabolize (and detoxify) the test material due to decreased levels of UDP-glucuronyltransferase enzyme which is a normally occurring phenomenon in rat pups. This concern for increased neonatal susceptibility to fenhexamid in the diet warrants the use of an additional safety factor (×3) in the determination of the ADI during the regulatory risk assessment for this compound.

Fenhexamid is not considered to be an endocrine distruptor. Although treatment-related effects were observed in the adrenal gland in dogs in the one-year feeding study (slightly increased adrenal weights and increased incidence and severity of intracytoplasmic vacuoles in the cortex of females only), these effects were minimal and not associated with any other significant signs of adrenal dysfunction in this study or in any other study or species. In the two-year chronic rat study, treatment-related histopathological effects were noted in the thyroid glands of male and female rats, but only at the highest dose tested (1280/2067 mg/kg bw/day, respectively). These effects were not accompanied by any other toxicologically significant signs of thyroid dysfunction in this study or in any other species in any other study. Thyroid hormone levels, however, were not measured in the two-year rat study.

In a metabolism study in rats, fenhexamid was rapidly absorbed, distributed, metabolized and almost completely excreted within 48 hours. The major route of excretion was feces (62–81%) with lesser amounts in the urine (15–36%). A pronounced first pass effect and enterohepatic circulation was observed. Bile contained mostly the glucuronide conjugate of fenhexamid, which was subsequently hydrolyzed in the intestine back to the parent compound and re-absorbed. The feces contained almost exclusively parent compound while the urine contained mostly parent compound and its glucuronide conjugate. Considerably smaller amounts of additional metabolites (formed by hydroxylation on the cyclohexyl ring) and glucuronide and sulfate conjugates of these same metabolites were also identified in the urine. All glucuronide and sulfate conjugates of the parent compound and of the hydroxylated metabolites of the cyclohexyl ring are considered to be less toxic than the parent compound. Glucuronide and sulfate conjugation are commonly occurring detoxification mechanisms in mammalian species which result in the formation of polar, more water-soluble metabolites which are readily excreted from the body. Based on similarities of chemical structure, the non-conjugated hydroxylated metabolites of the cyclohexyl ring would be expected to be no more toxic than the parent compound.

3.2 Determination of ADI

The lowest NOAEL was in the one-year dog study at a level of 17.4 mg/kg bw/d, based on treatment-related effects seen in hematology parameters in both sexes (decreased RBC, Hb, Hct, increased numbers of Heinz bodies) and may indicate the potential of KBR 2738 to induce Heinz body anemia in Beagle dogs. The hematotoxic effect of KBR 2738 was also noted in the 90-day dog study at higher doses. For the calculation of the ADI, a safety factor (SF) of 300 is proposed. The additional 3× safety factor is recommended for the protection of infants and children based on the observed increased susceptibility of the neonates to fenhexamid following repeated oral exposure in the two-generation reproduction study and provides a margin of exposure (MOE) of 660 to this endpoint (neonatal toxicity).

The acceptable daily intake proposed is calculated according to the following formula:

$$\text{ADI} = \frac{\text{NOAEL}}{\text{SF}} = \frac{17.4 \text{ mg/kg bw/d}}{300} = 0.058 \text{ mg/kg/day of fenhexamid}$$

The maximum acceptable intake for a 60 kg person, calculated according to the formula ADI × 60 kg is 3.48 mg/day.

3.3 Acute reference dose (ARfD)

In the context of the low order of acute toxicity of fenhexamid, following exposure by oral, dermal and inhalation routes, it is not necessary to propose an acute reference dose.

3.4 Toxicology endpoint selection for occupational and bystander risk assessment

Technical fenhexamid is of low toxicity following acute dosing, non-irritating to eyes or skin and is not a dermal sensitizer. The formulation, Elevate 50 WDG, is not acutely toxic via the oral, dermal or inhalation routes of exposure, is minimally irritating to eyes, slightly irritating to skin and is not a skin sensitizer.

In subchronic and chronic oral studies, noted toxicologically significant effects were anemia in dogs and decreased body weights, increased food consumption and minimal liver and(or) kidney effects in rats and mice. Fenhexamid is not acutely neurotoxic, carcinogenic or mutagenic and is extensively and rapidly metabolised and almost completely excreted within 48 hours. No significant tissue accumulation was evident. There is no evidence of a significant increase in toxicity with increased duration of exposure. Fenhexamid is not a developmental or reproductive toxicant. No increased susceptibility of fetuses to in utero exposure to fenhexamid was demonstrated in the developmental toxicity studies in rats and rabbits. Equivocal evidence from the two-generation reproduction study may suggest that neonates may be more sensitive than adults to the toxic effects of fenhexamid. However, on the basis of the NOAELs/LOAELs in the reproduction study, no increased susceptibility of rat pups was demonstrated.

Based on the above-noted observations, the short- to intermediate-term nature of the occupational exposure and the predominantly dermal route of exposure for workers, it was considered appropriate to base the occupational risk assessment on the 21-day rabbit dermal study. This study was well conducted and did not demonstrate any local or systemic toxic effects at 1000 mg/kg bw/day, the highest dose tested.

A MOE of 100 is considered to be protective of all workers.

3.5 Drinking water limit

Addressed in section 4.2.

3.6 Impact on human and animal health arising from exposure to the active substance or to impurities contained in it

3.6.1 Operator exposure assessment

Elevate 50 WDG would be applied to grapes and strawberries, and Decree 50 WDG would be applied to field and greenhouse ornamentals, using ground equipment. Using air-blast equipment, Elevate 50 WDG would typically be applied to 16 ha of grapes per day, and could be applied to each site up to three times per season. Using groundboom equipment, Decree 50 WDG and Elevate 50 WDG would typically be applied to 32 ha of field ornamentals or strawberries, respectively, per day, and could be applied to each site up to 4–6 times per year. Using hand-held hoses, Decree 50 WDG would be applied to approximately 2 ha of field or greenhouse ornamentals per day, and could be applied to each site up to six times per year for field ornamentals and up to six times per crop cycle for greenhouse ornamentals. Exposure to the mixer/loader/applicator would therefore be short-term (i.e., one to several days per year).

Dermal absorption of technical fenhexamid was characterized in an in vivo rat dermal absorption study. Elevate 50 WP formulation was applied to dorsal skin of rats at doses of 0.00138, 0.0147 or 0.148 mg/cm^2. Rats were sacrificed at 0.5, 1, 2, 4, 10, 24 and 120 hours post-dose. Skin at the application site was washed just prior to sacrifice or, for the 24 and 120 hour post-dose groups, at 10 hours. Dermal absorption was determined to be 20%.

Mixer/loader/applicator exposure was estimated using the Pesticide Handlers' Exposure Database (PHED Version 1.1). The PHED is a compilation of generic mixer/loader/applicator passive dosimetry data with associated software which facilitates the generation of scenario-specific exposure estimates. The following PHED estimates meet criteria for data quality, specificity and quantity outlined under the North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides. As exposure via the inhalation route was a minor component of overall exposure, exposure estimates were based on dermal deposition potential.

To estimate exposure for each use scenario, appropriate subsets of A- and B-grade data were created from the 1) mixer/loader and 2) applicator (i.e., groundboom, air-blast, hand-held hose) database files of PHED. All data were normalized for kg of active ingredient handled. Exposure estimates are presented on the basis of the best-fit measure of central tendency, i.e., summing the measure of central tendency for each body part this is most appropriate to the distribution of data for that body part. Estimates were derived for individuals wearing one layer of clothing during mixing, loading and application, as well as gloves during application. For groundboom application, exposure would be 0.0765 mg/kg bw/day. For air-blast application, exposure would be 0.127 mg/kg bw/day. For application using hand-held equipment, exposure would be 0.0681 mg/kg bw/day. Based on these exposure estimates, the following margins of exposure were derived:

Occupational scenario	Exposure [1] (mg/kg bw/day)	Margin of exposure NOAEL 1000 mg/kg bw/day[2]
Mixer/loader + applicator exposure[3]		
Groundboom	0.0765	13 100
Air-blast	0.127	7900
Hand-held	0.0681	14 700

[1] Based on a 70 kg operator, typical North American use patterns of 32 ha/day for groundboom application; 16 ha/day for air-blast and 2 ha/day for hand-held equipment. Based on an application rate of 0.56 kg a.i./ha for grapes and ornamentals; and 0.85 kg a.i./ha for strawberries.

[2] Based on 21-day rat dermal study; highest dose tested.

[3] Individuals wearing one layer of clothing during all activities, and gloves during application.

These margins of exposure are considered adequate.

3.6.2 Bystanders

Given that application is restricted to agricultural areas, and that the product would be applied using ground equipment only, exposure and risk to bystanders is expected to be negligible.

3.6.3 Workers

Individuals would re-enter treated agricultural (i.e., grapes, strawberries) and horticultural sites to carry out cultivation-related tasks which involve contact with treated foliage. Re-entering treated vineyards was considered to represent the highest exposure potential scenario for the proposed uses of Elevate 50 WDG. Individuals re-enter treated vineyards to conduct various cultivation-related tasks including thinning, pruning, tying, caning, girdling and hand-harvesting.

Chemical-specific dislodgeable foliar residue (DFR) data were submitted. This data provided information on foliar residues on grape foliage available for transfer following an application regime comparable to that proposed for Canada. Together with an appropriate generic transfer co-efficient, these data were used to derive exposure estimates for workers conducting vineyard tasks for an 8-hour workday at various intervals following application. For workers re-entering treated vineyards immediately after residues have dried, exposure was estimated to be 2.2 mg/kg bw/day.

Based on the no observed effect level (NOEL) of 1000 mg/kg bw/day in the 21-day dermal toxicity study in rabbits, a margin of exposure of 450 is obtained for workers re-entering vineyards on the day of application for tasks involving foliar contact. As this was considered to represent the highest potential exposure scenario for re-entry workers, margins of exposure for all post-application activities for proposed uses are considered adequate.

4.0 Residues

4.1 Definition of the residues relevant to maximum residue limits (MRLs)

4.1.1 Definition of the residues relevant to MRLs

Grape metabolism study

Grape vines (variety Muller-Thurgau) were grown in a confined area, in 25 L plant pots filled with loamy sand soil. Total radioactive residues (TRR) were 5.11 ppm in/on grapes harvested 14 days following two applications of [phenyl-UL-^{14}C]fenhexamid, made directly to the grapes using a microsprayer, at rates of 0.73 g a.i./100 L and 0.88 g a.i./100 L. The use pattern recommends treating grapes at a rate of 0.56 kg a.i./ha at early bloom, bunch pre-closure, veraison to 2 weeks after veraison and up to 7 days prior to harvest, without exceeding a maximum seasonal application rate of 1.70 kg a.i./ha. Approximately 96% TRR was identified in/on grapes. The parent fenhexamid was the major residue, accounting for 87.9% TRR (4.49 ppm). Minor amounts of 2-hydroxy-fenhexamid, 4-hydroxy-fenhexamid, and other cyclohexyl-hydroxy metabolites were identified (total of 1.4% TRR, 0.07 ppm). Glucose conjugates of fenhexamid and 2-hydroxy-fenhexamid were identified at low levels (2.7% TRR, 0.14 ppm and 3.2% TRR, 0.17 ppm, respectively); other conjugates of fenhexamid and cyclohexyl-hydroxy metabolites were identified at very low levels (<1% TRR). Data were also presented indicating that there was very little translocation of residues from leaves to fruit.

Tomato metabolism study
Total radioactive residues (TRR) were 1.67 ppm in/on greenhouse-grown tomatoes harvested 10 days following the last of three applications of [phenyl-UL-^{14}C]fenhexamid made directly to the tomatoes, using a pipette with a brush tip, at 3.60, 3.33, and 3.89 mg a.i./application. Approximately 96% TRR was identified in/on tomatoes. The parent fenhexamid was the major residue, accounting for 89.3% TRR (1.49 ppm). Minor amounts of 2-hydroxy-fenhexamid and 4-hydroxy-fenhexamid were identified (total of 2.2% TRR, 0.04 ppm). Glucose conjugates of fenhexamid, 2-hydroxy-fenhexamid, and 4-hydroxy-fenhexamid were identified at low levels (0.2–1.0% TRR, <0.01–0.02 ppm), as well as higher glucose conjugates of fenhexamid and 4-hydroxy-fenhexamid (1.4% TRR, 0.02 ppm each). Data was also presented indicating that there was very little translocation of residues from leaves to tomatoes.

Apple metabolism study
Total radioactive residues (TRR) were 2.10 ppm in/on apples harvested 0 days and 1.34 ppm in/on apples harvested 7 days following two applications of [phenyl-UL-^{14}C]fenhexamid made directly to the apples as solutions containing 0.663 mg a.i./mL. Residues were 5.48 ppm in/on apples harvested 7 days following two applications at an exaggerated rate (using the same solution but applying five times as much). Approximately 82–92% TRR was identified in/on apples. The parent fenhexamid was the major residue, accounting for 79.4–89.4% TRR (1.29–4.35 ppm). Minor amounts of 2-hydroxy-fenhexamid and 4-hydroxy-fenhexamid were identified (0.4–1.1% TRR, 0.01–0.05 ppm). Glucose conjugates of fenhexamid, 2-hydroxy-fenhexamid, and 4-hydroxy-fenhexamid were identified at low levels (<0.1–0.4% TRR, <0.01–0.01 ppm). Data were also presented indicating that there was very little translocation of residues from leaves to apples.

There were minimal differences between the plant (grapes, tomatoes and apples) and rat metabolism profiles. Fenhexamid accounted for the majority of the total radioactive residues (TRRs) in plants, with its glucoside conjugates and the glucoside conjugates of the hydroxylated (cyclohexyl ring) metabolites representing a considerably lower percentage of the TRRs. The parent and its glucuronide conjugates were the predominant residues in the rat with lower levels of metabolites (formed by hydroxylation on the cyclohexyl ring) and glucuronide and sulfate conjugates of these metabolites. Consequently, the definition of the residue of concern (ROC) as the parent fenhexamid only is equivalent in both plants and animals.

Confined Accumulation in Rotational Crops

Total radioactive residues accumulated at levels at or above 0.01 ppm in/on Swiss chard, turnips (root, tops) and wheat (forage, hay, straw, grain) planted in sandy loam soil 30, 134, and 314 days following a single application of [phenyl-UL-^{14}C]fenhexamid at 3.46 kg a.i./ha (~1× the maximum proposed seasonal application rate). Radioactive residues declined significantly at subsequent plant-back intervals of 134 days and 314 days. Fenhexamid was only detected at levels greater than 0.01 ppm in Swiss chard from the 30-day rotation; levels of fenhexamid in all other rotational crop commodities from the 30-day rotation were < 0.01 ppm. No other fenhexamid-related metabolite was detected in any of the rotational crop commodities at > 0.01 ppm; 4-hydroxy-fenhexamid was detected at trace levels in Swiss chard subjected to total hydrolysis. With the exception of Swiss chard and wheat straw, chromatographic analysis of extracts showed that the extractable radioactivity in each commodity consisted of numerous polar and non-polar components, with no single component present at > 0.01 ppm; for Swiss chard and wheat straw, no single component was > 0.04 and > 0.03 ppm, respectively. Further extraction of Swiss chard and wheat straw demonstrated that the majority of the TRR was associated with the lignin and hemicellulose fractions of the plant matrix.

Field accumulation in rotational crops

The confined rotational crop study demonstrated that parent fenhexamid residues may accumulate in Swiss chard at > 0.01 ppm (maximum of 0.03 ppm found). However, the confined rotational crop study was essentially conducted at an exaggerated rate. The study was conducted using one single application at 3.46 kg a.i./ha rather than the maximum proposed rate of 0.85 kg a.i./ha/application, 4 applications/season, with a minimum of 7 days between applications. In addition, the material was applied to bare soil in the confined study versus actual applications that would be directed onto foliage. The LOQ of the validated enforcement method was 0.05 ppm for strawberries (grapes is 0.02 ppm, but this crop is not rotated). Since maximum residues in Swiss chard were only 0.03 ppm from a worst-case/exaggerated rotational crop study, and this is in line with or greater than the validated LOQ of the method, it appears unlikely that residues of fenhexamid will be quantifiable in rotated crops. For the purposes of this registration on strawberries and grapes, a 30-day plant back restriction should be recommended on the label.

Freezer storage stability

Samples of untreated grapes, grape juice, raisins, strawberries and tomatoes were spiked with fenhexamid at levels ranging from 0.02 to 0.5 ppm, stored frozen at ≤-18°C to -20°C, and analysed at various intervals during storage for up to 17.5 months. These studies demonstrated that spiked residues of fenhexamid were stable during frozen storage for up to 12 months in/on grape juice, raisins and tomatoes, up to 17 months in/on grapes, and up to 17.5 months in/on strawberries. These data validate the storage conditions and intervals of samples collected from the grape and strawberry field trials and the grape processing studies.

On the basis of all these considerations, the residue of concern (ROC) can be defined as the parent fenhexamid only.

4.1.2 Definition of the residues in food of animal origin relevant to MRLs

There were no animal feed commodities associated with this petition. Therefore, data pertaining to the metabolism and freezer storage stability of fenhexamid in livestock were not required.

4.2 Residues relevant to consumer safety

Residues of fenhexamid ranged from 0.60 to 2.9 ppm (uncorrected for method recovery) in/on grapes harvested on the day of the last of three sequential foliar applications, at 11- to 56-day retreatment intervals, of the 50% WDG formulation, at 0.56–0.65 kg a.i./ha/application (1.70 kg a.i./ha/season; 1× the proposed maximum seasonal application rate). Although the efficacy data supports the current seasonal maximum application rate (1.70 kg a.i./ha/season), common to both the U.S. and Canada, the data supports a 7-day pre-harvest interval (PHI). Although none of the U.S. and Canadian crop field trials reflected this proposed PHI, the residue decline studies appeared to indicate that residues of fenhexamid will not decrease considerably in/on grapes as a function of time post-treatment (from 0-day to 7-day PHI). In addition, the efficacy data indicated that the control of Botrytis on grapes is more effective when the fenhexamid end-use product (Elevate 50 WDG) is applied with a nonionic surfactant (at a concentration of 0.02% v/v).

Notwithstanding this label recommendation, an MRL of 4.0 ppm in/on grapes is recommended for promulgation.

The submitted grape processing data indicated that fenhexamid residues did not concentrate in juice and wine processed from grapes (red and white) bearing detectable residues. Residues of fenhexamid concentrated 1.8× and 1.9× (average concentration factor = 1.9×) in raisins processed from red grapes and white grapes, respectively, bearing detectable residues. The maximum residues of fenhexamid expected in raisins, based on the highest average field trial (HAFT) (2.8 ppm) and the average concentration factor (1.9×) would be 5.3 ppm. **Based on the highest expected residues, an MRL of 6.0 ppm in/on raisins is recommended for promulgation.**

The submitted strawberry field trial data indicated that residues of fenhexamid ranged from 0.31 to 2.3 ppm when strawberries were harvested on the day of the last of four sequential applications of the 50% WDG formulation at 0.82–1.03 kg a.i./ha/application (3.40 kg a.i./ha/season; 1× the proposed maximum seasonal application rate). Although the efficacy data supports the current seasonal maximum application rate, common to both the U.S. and Canada, these data support a 1-day PHI. Although none of the U.S. and Canadian crop field trials reflected this proposed PHI, the residue decline studies appeared to indicate that residues of fenhexamid will not decrease considerably in/on strawberries as a function of time post-treatment (from 0-day to 1-day PHI). **Therefore, an MRL of 3.0 ppm in/on strawberries is recommended for promulgation.**

There were no animal feed commodities associated with this petition. Therefore, data pertaining to the magnitude of fenhexamid residues in animal commodities were not required

When using the Level I DEEM analysis, the 1994–1996 Continuing Survey of Food Intakes by Individuals (CSFII), the ADI (0.058 mg/kg bw/day) and a 10% water allocation, exposures to the various population subgroups were the following: 12.1% of the ADI for the general population, 10.3% for nursing infants, 11.8% for infants < 1 year, for females 20 years of age and older and adults aged 55 years and above, 16.6% for children 1–6 years, 12.4% for children 7–12 years, 11.2% for nonpregnant and non-nursing females within the ages of 13–19, 10.9% for males aged 13–19 years and 11.5% for men aged 20 years of age and older.

Consequently, the proposed use of fenhexamid on grapes and strawberries does not appear to pose an unacceptable dietary (food and water) risk to any segment of the population including adults, infants and children.

In the context of the low order of acute toxicity of fenhexamid, following exposure by oral, dermal and inhalation routes, it is not necessary to propose an acute ADI.

4.3 Residues relevant to worker safety

This section has been addressed in Section 3.6.3.

4.4 Proposed MRLs and compliance with existing MRLs

4.4.1 Compliance with existing MRLs

Since the active substance is new, there are no existing MRLs. The question of compliance with existing MRLs is therefore not applicable.

4.4.2 Proposed MRLs

The supervised crop field trials carried out on grapes and strawberries indicated that when treated according to the proposed label rate (grapes: 0.56 kg a.i./ha, 3 applications/season, timing of application: at early bloom, bunch pre-closure, veraison to 2 weeks after veraison, maximum seasonal application rate of 1.70 kg a.i./ha/season, PHI of 7 days; strawberries: 0.85 kg a.i./ha/application, 4 applications/season, interval of 7–14 days, maximum seasonal application rate of 3.40 kg a.i./ha, 1-day PHI) and harvested immediately following application, residues ranged from 0.6 to 2.9 ppm (uncorrected for method recovery) and 0.31–2.3 ppm (uncorrected for method recovery), respectively. The efficacy data supports the proposed label rates, common to both the U.S. and Canada, however, the data does not support the 0-day PHI for these crops. Rather the data supports a 7-day PHI for grapes and a 1-day PHI for strawberries. Based on the residue decline studies, residues of fenhexamid do not appear to decrease considerably as a

function of time post-treatment. Furthermore, the grape processing study has indicated that the maximum residues of fenhexamid expected in raisins, based on the highest average field trial (HAFT) of 2.8 ppm and the average concentration factor of 1.9×, would be 5.3 ppm.

MRLs for animal commodities were not required to support the proposed uses of fenhexamid as there were no animal feed commodities associated with this petition.

In light of these considerations and the good agricultural practice proposed for fenhexamid use on grapes (0.56 kg a.i./ha/application, 3 applications/season, maximum rate of 1.70 kg a.i./ha/season, 7-day PHI) and strawberries (0.85 kg a.i./ha/application, 4 applications/season, maximum rate of 3.4 kg a.i./ha, 1-day PHI), MRLs for fenhexamid are proposed as follows:

grapes	4.0 ppm
raisins	6.0 ppm
strawberries	3.0 ppm

4.5 Proposed import tolerances

Domestic uses on grapes and strawberries have been proposed. These are harmonized with the U.S. EPA tolerances and will therefore cover residues of fenhexamid in strawberries and grapes imported from the U.S.

5.0 Fate and behaviour in the environment

5.1 Fate and behaviour in soil

The fate and behaviour of fenhexamid in soil was assessed by studying its transformation (through photolysis and biotransformation) and mobility. Various studies indicated that biotransformation of extractable fenhexamid and formation of bound residues in soil were the principal mechanisms of fenhexamid transformation in soil. A terrestrial field dissipation study, conducted in British Columbia, indicated that fenhexamid will be non-persistent under field conditions, with a DT_{50} of 1.7–1.9 days. The DT_{90} was estimated to be approximately 18 days. The concentration of fenhexamid below 7.5 cm was negligible at all sampling times indicating that the mobility of fenhexamid is low and should not pose a risk to ground water. Concentrations of WAK 7004 (a transformation product of fenhexamid) were negligible after 15 days and below 7.5 cm soil depths.

5.1.1 Phototransformation in soil

Phototransformation of fenhexamid in soil was studied in a biologically active sandy loam soil (Howe, Indiana) by continuous exposure to a xenon lamp for 18 days at 25 ± 1°C. From this study report, the primary transformation process is considered to be biotransformation rather than photolysis (even though the aqueous photolysis showed that

the fenhexamid is photolabile) because similar transformation products were formed in the irradiated and dark samples and the concentration of transformation products was higher in the dark samples than in the irradiated samples. Therefore, the reported DT_{50} of 0.2 days was not that of phototransformation alone. The biotransformation of the extractable fenhexamid and the formation of bound residues were high. None of the 13 identified transformation products were above 10% of the applied ^{14}C. The major transformation route was dimeric and trimeric oxidative coupling involving the polymerization of parent molecules.

5.1.2 Aerobic soil biotransformation

Biotransformation of fenhexamid in aerobic soil was studied (for 365 d) in a sandy loam soil (Howe, Indiana) and the DT_{50} of extractable fenhexamid was less than one day. This DT_{50} value indicated that the extractable fenhexamid in aerobic soil is not persistent. It should be noted, however, that after the first three days, the non-extractable ^{14}C increased to 71% of the applied ^{14}C from 21% at day zero. The evolved CO_2 amounted to 30% of the applied ^{14}C at the end of the study. There were no major transformation products identified. The major routes of aerobic biotransformation in soil are: 1) oxidative C-C or C-O coupling reactions involving the polymerization of two or more fenhexamid molecules; 2) methylation of the hydroxyl group of the aromatic ring; 3) dechlorination and 4) total mineralization of the aromatic nucleus to CO_2 via aerobic ring cleavage. The continuous increase in the evolved CO_2 and the simultaneous decrease in the concentration of transformation products indicated that these transformation products were also biotransformed. Supplementary studies conducted in three German soils indicated similar results.

5.1.3 Anaerobic soil biotransformation

The registrant submitted studies on aquatic anaerobic biotransformation of fenhexamid. See Section 5.2.4.

5.1.4 Field dissipation studies

The DT_{50} of fenhexamid in cropped and non-cropped strawberry plots was 1.7–1.9 days under the conditions of a study conducted in British Columbia. The DT_{90} under these conditions was approximately 18 days. Extractable fenhexamid is non-persistent under field conditions, and residues should not carry-over to subsequent seasons. Fenhexamid was not quantifiable below 7.5 cm depth in soil, indicating that the mobility of fenhexamid in soil is low, and should not pose a threat to ground water. Data from a supplemental study conducted in Branchton, Ontario, supported these results.
WAK 7004, a transformation product of fenhexamid, was below the level of quantitation (<0.005 µg a.i./g soil) by 15 days after the last treatment in samples from the 7.5 cm soil depth, and was not detected below 7.5 cm depth.

5.1.5 Mobility: soil adsorption/desorption

The adsorption/desorption of fenhexamid was studied in six soils (two loamy sand soils from Germany and silty clay, sandy loam and sandy soils from the U.S.) in a batch equilibrium experiment. The amount of adsorbed fenhexamid varied from 24 to 80% of the applied. Desorption ranged from 8 to 20% of the applied ^{14}C. The K_d value ranged from 2.5 to 10.8 mg/L and the K_{oc} values ranged from 446 to 1226. These values indicate that the mobility of fenhexamid is medium to low in the soils studied.

The adsorption/desorption of WAK 7004 (one of the major transient transformation products identified during the phototransformation of fenhexamid in water formed by the dechlorination of fenhexamid) was studied in four soils from the U.S. (used for the adsorption study of fenhexamid). Desorption ranged from 26 to 45% of the adsorbed WAK 7004. The adsorption K_d ranged from 10.1 to 32.1 and the K_{oc} ranged from 2327 to 5037, and these values indicate that the mobility of WAK 7004 is slight to immobile in the soils studied.

The adsorption/desorption studies indicated that fenhexamid has low to medium potential for leaching in the soils studied and WAK 7004 has only a slight potential.

5.1.6 Mobility: soil column leaching

The submitted adsorption/desorption studies (Section 5.1.5) were sufficient to determine the mobility of fenhexamid in soil. Therefore, additional soil column leaching studies are not required.

5.1.7 Mobility: soil thin-layer chromatography

The submitted adsorption/desorption studies (Section 5.1.5) were sufficient to determine the mobility of fenhexamid in soil. Therefore additional soil thin-layer chromatography studies are not required.

5.1.8 Mobility: field leaching data

No acceptable studies were submitted.

5.1.9 Expected environmental concentrations (EECs) in soil

For strawberries, fenhexamid is applied at 0.85 kg a.i/ha four times at 7-d intervals. For ornamentals, fenhexamid is applied at 0.56 kg a.i/ha a maximum of six times at 7-d intervals. For grapes, it is applied at 0.56 kg a.i/ha three times at 14-d intervals. The DT_{50} of extractable fenhexamid in soil is 1.7–1.9 days (terrestrial field study, B.C.). When dissipation in soil and the intervals between applications are taken into account, the maximum cumulative application rates in soil for strawberry, ornamentals and grapes are estimated to be 921.7, 607.3, and 563.4 g a.i/ha, respectively. EECs in soil using these cumulative application rates and assuming a soil bulk density of 1.5 g/cm^3 and a soil depth of 15 cm are 0.410, 0.270, and 0.250 mg a.i/kg soil, respectively.

5.2 Fate and behaviour in aquatic systems

5.2.1 Hydrolysis

The hydrolysis of fenhexamid at pH 5, pH 7 and pH 9 was studied for 30 days under sterile dark conditions at 25 ± 1°C. At the end of the study, all of the ^{14}C was associated with the parent compound and no hydrolytic transformation products were formed in detectable amounts during the study. These results indicate that fenhexamid is stable to hydrolysis, and therefore hydrolysis is not considered to be a major route of transformation in the environment.

5.2.2 Phototransformation in water

The photodegradation of ^{14}C-fenhexamid in sterilized phosphate buffer at pH 7 was studied by continuous irradiation (using a Xenon lamp) for 15 days at 25 ± 1°C. There was no transformation of fenhexamid in the dark samples. In the irradiated samples, however, fenhexamid was transformed and the nature of the transformation products varied with the duration of exposure. The evolved CO_2 increased with exposure time and reached 40% of the applied ^{14}C in 15 days. WAK 7004 (formed by dechlorination of fenhexamid) was identified as a major transient transformation product. Its concentration was 24% of applied after 1 hour of irradiation and decreased to 13% at 5 hours, after which time, it disappeared. At the end of the 15-d study period, dechlorinated/hydroxylated fenhexamid and succinic acid were identified as the major transformation products. Fenhexamid was completely phototransformed after 5 hours of exposure. Aqueous photolysis of fenhexamid proceeded via dechlorination, hydroxylation and cleavage of the phenyl ring. The first-order half-life of photodegradation of fenhexamid in aqueous solution was 1 hour.

In another study, the photodegradation of fenhexamid in natural water (collected from Rhine river, Germany) was studied (for 24 hours) and the half-life was found to be 0.45 hours. In this study, fenhexamid was completely phototransformed after 2 hours of exposure (by Xenon lamp), and WAK 7004 was the major transient transformation product during the first 0.5–1 hour. At the end of the study, no major transformation products were identified except CO_2 (28% of the applied ^{14}C). Based on these results, photolysis is considered to be a major route of transformation of fenhexamid in aqueous solutions.

5.2.3 Aquatic aerobic biotransformation

The aerobic aquatic transformation of phenyl-labelled fenhexamid (1.18 kg a.i/ha) was studied in a water/sediment system (collected from Lake Honniger, Germany and Lake Stanley, Kansas, U.S.) for 100 days in darkness at 20 ± 1°C. Formation of CO_2 increased with time and reached 13% of applied ^{14}C in Stanley and 5% in Honniger in 100 days. Initially, a major portion of the applied ^{14}C was in the surface water. With increasing incubation time, however, applied ^{14}C partitioned more into the sediment (and formed bound residues). After 100 days of incubation, the fenhexamid concentration in the surface water decreased from 74 to 3% of applied ^{14}C in Honniger and from 81 to 7% in Stanley. At test termination, the concentration of bound residues in the sediment increased from 1 to 70–75% of applied. None of the 15 identified transformation products were above 10% of the applied ^{14}C. The main processes occurring in the aerobic water/sediment system were partitioning of the added fenhexamid to sediment and subsequent formation of non-extractable residues.

Methylation and sulfonylation of the hydroxyl group of the aromatic ring are the major routes of fenhexamid transformation in aerobic water/sediment systems. In addition, mineralization of the aromatic nucleus to CO_2 occurs. It should be noted that none of the dimeric and trimeric coupling products of fenhexamid, which were identified in the aerobic soil biotransformation study, were detected in the present study.

The first-order half-lives of fenhexamid in Lake Honniger were 13, 37 and 24 days for the surface water, sediment and the entire system, respectively. The corresponding values for Lake Stanley were 10.2, 19.1 and 14 days. These values indicate that extractable fenhexamid is non-persistent in surface water and slightly persistent in the sediment under aerobic conditions. The half-life of fenhexamid is longer in aerobic water/sediment systems than in aerobic soil.

5.2.4 Aquatic anaerobic biotransformation

Anaerobic transformation of ^{14}C-fenhexamid (at 1.33 µg a.i/g dry weight of soil) in a sediment/water system was studied for 360 days. Air-dried soil (Howe, Indiana) was mixed with saccharose, flooded with deionized water and purged with N_2 to obtain an anaerobic condition before the addition of fenhexamid. The total ^{14}C in the water layer decreased from 86% of applied (day 0) to 3% (day 360), and that in the sediment increased from 17% (at day 0) to 92% (at day 360). At the end of incubation, only 4% and less than 0.1% of the applied ^{14}C remained as the extractable parent compound in sediment and water, respectively. At day 360, 73% of the ^{14}C found in sediment was in bound form. The amount of $^{14}CO_2$ and organic volatiles was negligible (2% of the applied ^{14}C). None of the identified transformation products exceeded 10% of the applied ^{14}C at any time during the incubation.

Major routes of anaerobic transformation of fenhexamid in water/sediment systems are: 1) dechlorination resulting in the formation of the monochloro compound (WAK 6920); 2) hydroxylation of the cyclohexyl part of fenhexamid leading to the formation of hydroxy-cyclohexyl isomers (KBR 6720, KBR 7133 and KBR 7115); 3) oxidation of the OH group of KBR 6720 to form the keto-cyclohexyl compounds; 4) further degradation of the keto-isomers to N-acetyl-2,3-dichloro-p-aminophenol; 5) direct transformation of KBR2738 to N-acetyl-2,3-dichloro-p-aminophenol. These transformation products are gradually (after 2 months) transformed to CO_2 by the oxidation of the phenyl ring.

The fenhexamid first-order half-life was 40 days in the surface water and 87 days in the water/sediment system. It should be pointed out that the half-life in surface water was that of the fenhexamid dissipation mainly through partitioning into the sediment (rather than through transformation). A transformation rate for fenhexamid in the sediment alone, however, could not be clearly quantified owing to the continued partitioning of fenhexamid into sediment. Based on the half-life values, however, fenhexamid is classified as slightly persistent in surface water and moderately persistent in water/sediment systems under anaerobic conditions.

5.2.5 Expected environmental concentration in water

EEC in water from direct overspray: For strawberries, fenhexamid is applied (as Elevate 50 WDG) at 0.85 kg a.i./ha, four times at 7-day intervals. For grapes, it is applied at 0.56 kg a.i./ha, three times at 14 days intervals. For ornamenals, fenhexamid (as Decree 50 WDG) is applied up to six times at 0.56 kg a.i./ha at 7-day intervals. The half-life of extractable fenhexamid in aerobic water is 12 days. When this half-life and the intervals between applications are taken into account, the maximum annual cumulative application rates in water for strawberry, ornamentals, and grapes (calculated from the Excel spreadsheet for multiple application) are estimated to be 2048.8, 1535.2, and 920.7 g a.i./ha, respectively. EECs in water using these cumulative application rates are 0.683, 0.512, and 0.307 mg a.i./L, respectively.

6.0 Effects on non-target species

6.1 Effects on terrestrial non-target species

6.1.1 Wild birds

Fenhexamid is practically non-toxic to bobwhite quail and mallard duck on an acute dietary basis. The acute oral LD_{50} and dietary LC_{50} to bobwhite quail were more than 2000 mg a.i./kg bw and 5000 mg a.i./kg diet, respectively. The acute dietary LC_{50} to mallard duck was more than 5000 mg a.i./kg diet. No fenhexamid treatment-related effects were observed in any of the reproductive parameters (egg production, eggshell thickness, hatchling health and survival) of bobwhite quail. In the reproductive toxicity study on bobwhite quail, the no observed effect concentration (NOEC) was 2000 mg a.i./kg body weight (based on the effect on reproductive parameters).

6.1.2 Wild mammals

The effects of fenhexamid on wild mammals were extrapolated from the review of laboratory mammalian studies by the Health Evaluation Division. There were no clinical signs of toxicity of fenhexamid (technical) to rats observed in acute oral, dermal and inhalation studies. The acute oral and dermal LD_{50} to rats were greater than 5000 mg a.i./kg bw. Fenhexamid (technical) is non-irritating to skin and eyes of rabbits. Acute studies (oral, dermal and inhalation) with Elevate 50 WDG also showed low acute toxicity.

6.1.3 Bees

Fenhexamid is not toxic to bees. The acute oral and contact LD_{50} of fenhexamid to honeybees is more than 200 µg a.i./bee. The acute oral NOEC based on mortality was 100 µg a.i./bee.

6.1.4 Arthropod predators and parasites

Studies on the toxicity of fenhexamid to beneficial insects were done with formulated fenhexamid (50% a.i.). The NOEC based on mortality of predacious mite and rove beetle was 2 kg formulated fenhexamid/ha. The NOEC for parasitic wasp was 4 kg formulated fenhexamid/ha.

6.1.5 Earthworms

The 14-day LC_{50} for the toxicity of fenhexamid to earthworms was higher than 1000 mg a.i./kg dry weight of substrate. The NOEC based on the reduction of body weight of earthworms was 100 mg a.i./kg dry weight of substrate.

6.1.6 Effects on soil microorganisms

No data are required

6.1.7 Terrestrial non-target plants

There were no phytotoxic symptoms observed in strawberry, grapes, geraniums and petunias (as shown in the efficacy studies). Therefore, no phytotoxic effects are expected on non-target terrestrial plants.

6.2 Effects on aquatic non-target species

6.2.1 Bioconcentration in fish

The log octanol–water partition coefficient (log K_{ow}) for fenhexamid was > 3. This value indicates that fenhexamid has a potential for bioaccumulation. The bioconcentration factors for fenhexamid in bluegill sunfish were 185 and 132 µg a.i./L, respectively at 20 and 200 µg a.i./L. The depuration rate constants were 0.71 and 0.94 L/day at 20 and 200 µg a.i./L, respectively. Because of the rapid depuration, bioaccumulation in fish is not expected.

6.2.2 Fish

Fenhexamid is moderately toxic to rainbow trout and bluegill sunfish, and slightly toxic to sheepshead minnow. The 96-h acute LC_{50}s of fenhexamid technical and formulated fenhexamid to rainbow trout were 1.34 and 1.30 mg a.i./L, respectively, and the NOEC based on the behavioural changes was 1 mg a.i./L. The sublethal effects observed were laboured respiration, lying on the bottom of the aquarium, hyperactivity and loss of equilibrium (turned laterally more or less from the normal body position). The 96-h acute LC_{50} of fenhexamid to bluegill sunfish was 3.42 mg a.i./L, and the NOEC based on the transient hyperactive swimming was 1.50 mg a.i./L. The 96-h acute LC_{50} of fenhexamid to sheepshead minnow was 11 mg a.i./L and the NOEC based on mortality was 3.7 mg a.i./L. In the early life cycle toxicity test with rainbow trout, the mean adverse toxic concentration was 144 µg a.i./L and the NOEC (based on swim up—the developmental stage at which the newly hatched fry begin swimming up from the bottom of the test chamber) was 101 µg a.i./L.

6.2.3 Aquatic invertebrates

Fenhexamid is slightly toxic to *Daphnia,* when applied as the technical active ingredient, and practically non-toxic, when applied as the formulated product. The 48-h acute LC_{50} to *Daphnia* is >18.8 mg a.i./L when fenhexamid is applied as the technical active ingredient. When the formulated fenhexamid (49.6% a.i.) was tested, the LC_{50} was 105 mg a.i./L, and the 48-h NOEC (based on immobilization) was 19.5 mg a.i./L. In chronic toxicity tests, the mean adverse toxic effect concentration was 1.4 mg a.i./L, and the NOEC (for survival) was 1.00 mg a.i./L.

6.2.4 Algae

The EC_{50} of fenhexamid to green algae (*Selenastrum capricornatum)* based on the growth inhibition was 4.31 mg a.i./L, and the NOEC was 3.2 mg a.i./L. The 72-h EC_{50} of formulated fenhexamid (49.6% a.i.) to *Selenastrum capricornatum* was 1.33 mg a.i./L and the NOEC was 0.56 mg a.i./L.

6.2.5 Aquatic plants

The 14-day acute LC_{50} of fenhexamid to duckweed (*Lemna gibba*) was greater than 2.3 mg a.i./L (based on the frond number and plant dry weight), and the NOEC based on the inhibition of frond numbers was 0.28 mg a.i./L.

6.3 Effects on biological methods of sewage treatment

Data were not required.

6.4 Environmental risk assessment

6.4.1 Terrestrial organisms

Wild birds

EECs in vegetation and food sources were calculated based on the maximum annual label rate of application (3.4 kg a.i./ha). This did not account for any transformation of fenhexamid on the foliage and represents the worst case scenario (Table 1).

Table 1 EECs on vegetation and other food sources at the maximum label application rate (3.4 kg a.i./ha)

Environmental matrices	Concentration (mg a.i./kg fresh weight)	Fresh/dry weight ratios	Concentration (mg a.i./kg dry weight)
Short range grass	727.6	1:3.3	2401.1
Leaves and leafy crops	380.8	1:11	4188.9
Long grass	333.2	1:4.4	1466.1
Forage crops	176.8	1:5.4	954.7
Small insects	176.8	1:3.8	671.8
Pods with seeds	36.4	1:3.9	141.9
Large insects	30.3	1:3.8	114.9
Grains and seeds	30.3	1:3.8	114.9
Fruit	21.1	1:7.6	160.2

Wild birds, such as bobwhite quail and mallard duck, could be exposed to fenhexamid residues as a result of consumption of sprayed vegetation, contaminated prey or spray drift.

The bobwhite diet consists of approximately 30% small insects, 15% forage crops and 55% grain and seeds. The EECs of fenhexamid on these items were calculated to be 671.8, 954.7 and 114.9 mg a.i./kg dw of diet, respectively, based on the maximum application rate of 3.4 kg a.i./ha (Table 1). Therefore the EEC in bobwhite diet is 408.0 mg a.i./kg dw of diet. The mallard diet consists of approximately 30% large insects and 70% grain and seeds. The EECs of fenhexamid on these items were calculated to be 114.9 and 114.9 mg a.i./kg dw of diet, respectively, based on the maximum application rate of 3.4 kg a.i./ha (Table 1). Therefore the EEC in bobwhite diet is 114.9 mg a.i./kg dw of diet.

In the acute oral toxicity study, the mean body weight of an individual (BWI) of bobwhite quail in the control treatment was 0.193 kg bw/individual, while the mean food consumption (FC) was 0.018 kg dw of diet/individual/day. The daily intake (DI=FC*EEC) was, therefore, 7.34 mg a.i./individual/d. The reported LD_{50} and NOEL values were 2000 and 1050 mg a.i./kg bw, respectively. When expressed on a per individual basis, the $LD_{50(individual)}$ (=LD_{50}*BWI) was 386 mg a.i./individual, and the $NOEL_{(individual)}$ (=NOEL*BWI) was 203 mg a.i./individual.

Based on the DI and the $LD_{50(individual)}$, it would take a bobwhite quail 53 continuous days of feeding to attain the dose equivalent to that administered in the laboratory by gavage that killed 50% of the laboratory population. Similarly, based on the DI and the $NOEL_{(individual)}$, the maximum number of days of continuous intake by a bobwhite equivalent to the dose administered by gavage that had no observable effect on the laboratory population was 28 days. Fenhexamid does not present an acute risk to the bobwhite quail.

Dietary studies with both the bobwhite quail and the mallard duck indicated that the NOECs (5000 and 1250 mg a.i./kg dw of diet, respectively) were greater than the EECs for each species. Safety factors were 12.3 and 10.9, respectively. The bobwhite quail reproductive study resulted in a NOEC of 2000 mg a.i./kg dw of diet. The safety factor (NOEC/EEC) based on reproductive parameters was 4.9. Fenhexamid would not pose a dietary or reproductive risk to birds.

Wild mammals
Wild mammals such as rats, mice, dogs and rabbits could be exposed to fenhexamid residues as a result of consumption of sprayed vegetation and(or) contaminated prey.

The rat diet consists of approximately 70% short grass, 20% grain/seed and 10% large insects. The EECs of fenhexamid on short grass, grain/seed and large insects were calculated to be 1681, 23 and 12 mg a.i./kg diet, respectively, based on the maximum application rate of 3.4 kg a.i./ha (Table 1). Therefore, the EEC in the rat diet is 1715.3 mg a.i./kg diet. The 90-day dietary toxicity studies indicate that the NOAEL (5000 and 10 000 mg a.i./kg diet/day for males and females, respectively) is higher than the EEC in the diet (1715.3 mg a.i./kg diet). Therefore, there is no potential dietary risk to the rat from the application of fenhexamid at the proposed application rate.

The mouse diet consists of approximately 25% short grass, 50% grain/seed and 25% large insects. The EECs of fenhexamid on short grass, grain/seed and large insects were calculated to be 600.3, 57.5 and 1047.2 mg a.i./kg diet, respectively, based on the maximum application rate of 3.4 kg a.i./ha (Table 1). Therefore, the EEC in the mouse diet is 1704.9 mg a.i./kg diet. The 90-day dietary toxicity studies indicate that the NOAEL (1000 mg a.i./kg diet for males and females) is lower than the EEC in the diet (1704.9 mg a.i./kg diet). Therefore, there is a potential dietary risk to mice from the application of fenhexamid at the proposed application rate, only if mice consume contaminated food continuously for 90 days.

Honeybees: Fenhexamid is not toxic to the bees on an acute contact and oral basis (LD_{50} of > 200 µg a.i./bee). Therefore, fenhexamid will not pose a potential risk to bees on an acute contact and oral basis when applied at the proposed label rates.

Earthworms: As the maximum EEC of fenhexamid in soil is 0.410 mg/kg soil, and the NOEC for earthworms is 100 mg/kg substrate, fenhexamid will not pose any potential risk to earthworms at the proposed use rates. The safety factor (NOEC/EEC) is 244.

Parasites and predators: The NOEC for the predaceous mite (based on mortality) and rove beetle (mortality and rate of emergence) is 1 kg a.i./ha. The maximum label application rate of Elevate 50 WDG is 0.85 kg a.i./ha (four times at 7 day intervals for strawberry). Therefore, there is no potential risk to the parasites, predators and beneficial insects when applied at the proposed label rate. The safety factor is 1.12.

Aquatic organisms

Non-target freshwater invertebrates: As the most sensitive endpoint for *Daphnia* (NOEC for survival) is 1.00 mg a.i./L, and the highest EEC in water is 0.683 mg a.i./L, there is no potential risk to the non-target freshwater invertebrates at the proposed Elevate 50 WDG or Decree 50 WDG use rates. The safety factor is 1.5.

Non-target marine invertebrates: As the 96-h acute NOEC (1.7 mg a.i./L) is found to be greater than the highest EEC in water (0.683 mg a.i./L), there is no potential risk to the marine invertebrates at the proposed label application rates for Elevate 50 WDG or Decree 50 WDG. The safety factor is 2.5.

Fish: Fenhexamid is moderately toxic to cold-water and warm-water fish. The early life stages of fish are more sensitive to fenhexamid than adults. The most sensitive endpoint for adults is the NOEC for the behavioural changes in rainbow trout (1 mg a.i./L). As the highest EEC of fenhexamid in water is lower than the NOEC, application of Elevate 50 WDG or Decree 50 WDG at the proposed application rate will not pose a risk to adult fish (the safety factor is 1.5). The most sensitive endpoint in the early life cycle study is NOEC (0.1 mg a.i./L) for swim-up (the developmental stage at which the newly hatched fry begin swimming up from the bottom of the test chamber), which is lower than all of the EECs in water for fenhexamid (0.307–0.683 mg a.i./L). The safety factor for the juvenile fish is 0.15–0.33. Therefore, application of Elevate 50 WDG or Decree 50 WDG at the proposed rates will pose a potential risk to the early life stages of the fish. Consequently, mitigative measures are needed to protect juvenile fish.

Algae: The formulated product is more toxic than technical fenhexamid to freshwater algae. The most sensitive fenhexamid endpoint (NOEC for swelling of cells) is 0.56 mg a.i./L (when formulated fenhexamid is applied). As the highest fenhexamid EEC in water (0.683 mg a.i./L) is higher than the most sensitive endpoint, Elevate 50 WDG at the proposed application rates will pose a potential risk to the fresh water algae. The safety factor is 0.82. Cumulative application rates for Decree 50 WDG are lower than for Elevate 50 WDG, with an EEC in water of 0.512 mg a.i./L. The safety factor is 1.1 and indicates that Decree 50 WDG at the proposed application rates will not pose a potential risk to freshwater algae.

Aquatic vascular plants: As the NOEC (0.28 mg a.i./L based on the inhibition of frond number) was lower than all of the EECs in water for fenhexamid (0.307–0.683 mg a.i./L), application of Elevate 50 WDG or Decree 50 WDG at the proposed application rate will pose a potential risk to aquatic vascular plants. The safety factor is 0.41–0.91.

6.5 Microcontaminant

The technical active ingredient does not contain any impurities that are known to meet the criteria for Track-1 classification under the TSMP.

6.6 Environmental risk mitigation

Based on the data submitted, an assessment of the environmental safety associated with the use of Elevate 50 WDG and Decree 50 WDG has identified the following concerns:

- The application of Elevate 50 WDG or Decree 50 WDG at the proposed label rate will pose a potential risk to juvenile fish.

- The application of Elevate 50 WDG or Decree 50 WDG at the proposed label rate will pose a potential risk to other aquatic organisms, i.e., freshwater algae and aquatic vascular plants.

In order to protect sensitive non-target aquatic organisms, a buffer zone of 5 m is required for ornamentals (Decree 50 WDG) and 5 m is required for grapes (Elevate 50 WDG) when air-blast spray is used. Therefore, mitigative label statements are required in the "Environmental Precautions" section for each product (see Label amendments).

7.0 Efficacy data and information

7.1 Effectiveness

7.1.1 Intended uses

For control of Botrytis bunch rot (grey mould) on grapes.

- Apply 1.0 kg product/ha (500 g a.i./ha) prior to disease establishment when conditions favour disease development at early bloom, bunch pre-closure, veraison to 2 weeks after veraison, up to and including the day of harvest. DO NOT apply more than 3.4 kg of product per hectare per year.

For control of grey mould (*Botrytis cinerea*) on strawberries.

- Apply 1.7 kg product/ha (850 g a.i./ha) beginning prior to disease establishment and no later than 10% bloom, and continue application every 7–14 days or when conditions favour disease development. The final application may be made up to and including the day of harvest. DO NOT apply more than 6.8 kg product per ha per year.

For control of grey mould (*Botrytis cinerea*) on ornamentals.

- Apply 1.0–1.7 kg product/ha (500–850 g a.i./ha) beginning when conditions favour disease development but prior to the establishment of the disease. Use the high rate when conditions favour severe disease development. Applications should be made at 7–14 day intervals.

  Outdoor-grown ornamental: DO NOT apply more than 6.8 kg product/ha/year.

  Greenhouse grown ornamentals: DO NOT apply more than 6.8 kg product/ha/crop.

7.1.2 Mode of action

Fenhexamid is a protectant fungicide which inhibits germ tube and hyphal growth. Fenhexamid seems to be a single site inhibitor with a very specific biochemical mode of action which until now has not been identified. However, studies undertaken to reveal the mode of action of fenhexamid have shown:

- no inhibition of fungal respiration;
- no uncoupling of mitochondrial electron transport;
- no significant inhibitory activity on basic biochemical pathways;
- no effect on pectinase production by the fungus, and
- no cross resistance to benzimidazoles, dicarboximides and anilinopyrimidines.

Work is ongoing to elucidate what appears to be a novel mechanism of action different from all other known botryticides.

7.1.3 Crops

Grapes, strawberries and ornamentals are the crops on which data are presented and for which label claims are made.

7.1.4 Effectiveness against Botrytis bunch rot and blight caused by *Botrytis cinerea* on grapes

Twenty-three trials conducted over three years in the United States were submitted in support of the claim on grapes. The location and number of these trials were: CA 18; NY 1; OR 3; WA 1.

Application rate

No efficacy data were provided using the proposed rate of 0.5 kg a.i./ha, however 18 trials with an average disease incidence of 54% in the untreated check presented data on 0.56 kg a.i./ha. This rate provided a % disease control similar to the 1.12 rate (52% vs. 56% respectively in 7 trials) and typically higher than the 0.42 kg a.i./ha rate (46% vs. 36% in 16 trials). When compared with the untreated check, the 0.56 kg a.i./ha rate provided significantly different (SD) lower disease incidence in 10 out of 18 trials with a % disease control of 47%. The 0.56 kg a.i./ha rate typically had higher % disease control than the commercial standard (46% vs. 35% in 17 trials). These data support an application rate of 0.56 kg a.i./ha.

Number of applications

Eight trials included data on Elevate applied at the proposed 3 applications per season at 0.56 kg a.i./ha as well as a commercial standard. In those trials, Elevate provided 38% disease control compared to 42% for the commercial standard. Four of these trials compared Elevate applied alone and tank-mixed with 0.02% of nonionic surfactant. The addition of 0.02% nonionic surfactant provided a consistent increase in % disease control compared with both Elevate alone and the commercial standard, 61% vs. 31% and 41% respectively. Under high disease pressure the addition of 0.02 % v/v of nonionic surfactant provided SD disease control up to 20 days after the last application (DALA) while Elevate alone did not provide SD disease control at 16 DALA. This indicates that the addition of a nonionic surfactant increases the duration and level of disease control.

Disease resistance management suggests the need to avoid making more than two consecutive applications of Elevate. Supplementary data indicate that 4 applications of Elevate per season would provide increased disease control. This suggests that an additional spray with a different fungicide may be beneficial. The data support the use of three applications per season of Elevate at 0.56 kg a.i./ha + 0.02% v/v of nonionic surfactant on grapes at the proposed application times.

Pre-harvest interval (PHI)

The data do not support a 0-day PHI given that fenhexamid is a protectant fungicide and has no demonstrated curative effect, prevention of infection before harvest minimizes the potential for disease development in storage, the European "standard" protection program (See Ref. 1) recommends the last application 3 weeks pre-harvest, and there is no post-harvest efficacy data available to support a 0-day PHI. The available data support a 7-day PHI. These data support the claim for control of Botrytis bunch rot on grapes with three applications of Elevate (0.56 kg a.i./ha) + 0.02% v/v of nonionic surfactant prior to disease establishment when conditions favour disease development at early bloom, bunch pre-closure, veraison to 2 weeks after veraison, and up to 7 days pre-harvest. DO NOT apply more than 1.7 kg a.i. per hectare per year.

7.1.5 Effectiveness against grey mould caused by *Botrytis cinerea* on strawberries

Twenty trials conducted over three years in the United States were submitted in support of the control claim on strawberries. The location and number of these trials were: CA 15, AL 1, FL 1, NC 1, OR 2.

Thirteen trials with an average disease incidence of 28% in the untreated check provided information supporting the proposed use of 4 applications. Five trials provided data based on more than 4 applications with disease assessment taken after the last application or at harvest and were used as supplemental data. The remaining trials were only used for crop tolerance assessment.

Application rate

Fifteen trials provided information on the proposed rate of 0.85 kg a.i./ha. Nine out of ten demonstrated a significant difference (SD) between Elevate and the untreated check with 75% disease control. The remaining 5 trials showed no significant difference (NSD) between any of the treatments and the untreated check due to a late first application.

The 0.85 kg a.i./ha rate provided higher % disease control than the 0.56 kg a.i./ha rate (75% vs. 55% in 8 trials). Although no trial compares higher application rates with 0.85 kg a.i./ha, rates above 0.85 kg a.i./ha were tested in a limited number of trials and did not provide significantly higher disease control than 0.56 kg a.i./ha rate. When comparing the 0.85 kg a.i./ha rate to 2 commercial standards, Elevate provided 72% disease control compared to 39% and 43% over 7 trials. Elevate + 0.5% v/v nonionic surfactant was tested in 2 trials and did not show improved disease control over Elevate alone. These data support the 0.85 kg a.i./ha application rate of Elevate applied alone.

Number of applications

Out of 11 trials using 4 applications at 0.85 kg a.i./ha or providing disease ratings after the 4th application, 6 trials showed significant difference (SD) between 4 applications of Elevate and the untreated check with 81% disease control. The remaining 5 trials showed NSD between any of the treatments and the untreated check. In each of those 5 trials, the first application was done at the end or after bloom thus allowing infection to occur before the first protective application. These 5 trials support the necessity of bloom protection for successful grey mould control on strawberries. These data support the use of 4 applications of Elevate at 0.85 kg a.i./ha beginning prior to disease establishment and no later than 10% bloom and subsequent applications every 7–14 days or when conditions favour disease development. DO NOT apply more than 3.4 kg a.i./ha per year.

Preharvest interval

A 1-day PHI, compatible with picking schedule and similar to currently registered products is recommended for strawberries. The data support the use of four applications of Elevate at 0.85 kg a.i./ha beginning prior to disease establishment and no later than 10% bloom and subsequent applications every 7–14 days or when conditions favour disease development with a 1-day PHI. DO NOT apply more than 3.4 kg a.i./ha per year.

7.1.6 Effectiveness against grey mould caused by *Botrytis cinerea* on ornamentals

Eighteen trials conducted over three years in the United States were submitted in support of the control claim on ornamentals. The location and number of these trials were: 5 CA; 3 PA; 2 MI; 4 OH; 1 NY; 2 NC. Thirteen trials provided information that could be used to assess disease control, the remaining trials provided phytotoxicity information.

Application rate

No efficacy data were provided using the proposed rate of 0.5 kg a.i./ha. However, 13 greenhouse trials with an average disease incidence of 70% in the untreated check provided information on a range of application rates, i.e., 0.14, 0.28, 0.42, 0.56 and 1.12 kg a.i./ha. The 0.56 kg a.i./ha rate demonstrated consistent disease control equal to or higher than the commercial standards in the 11 greenhouse trials where a commercial standard was included. Although all trials were done in greenhouses, the proposed use also includes outdoor ornamentals. The 0.56 kg a.i./ha application rate is the lowest rate whose efficacy has been demonstrated in outdoor trials on other crops (see 7.1.4 and 7.1.5). For these reasons, 0.56 kg a.i./ha is the minimum application rate which can be considered for use on ornamentals for greenhouse and outdoor uses.

In ten greenhouse trials testing both 0.56 and 1.12 kg a.i./ha application rates, there was NSD between both rates with 72% and 62% disease control respectively. Thus the use of the higher rate is not acceptable since 1.12 kg a.i./ha did not demonstrate a consistent increase in the level of disease control compared to 0.56 kg a.i./ha. These data support the 0.56 kg a.i./ha application rate of Decree for greenhouse and outdoor use on ornamentals.

Number of applications and timing

Elevate (identical to Decree formulation) applied at 0.56 kg a.i./ha was significantly different (SD) from the untreated check in two out of five trials using two applications per season. In 5 trials, using 4 to 6 applications, Elevate was SD from the untreated check in all cases.

Under low disease pressure, Elevate provided 88% disease control with 14 days between applications (DBA) in 3 trials. In one trial under high disease pressure with 15 DBA, Elevate provided negligible disease control thus demonstrating that 15 DBA was too long to maintain disease control under high disease pressure. In two other trials with high disease pressure, Elevate applied at 7 DBA provided consistent disease control equal to the commercial standard. These data support the claim for control of *Botrytis cinerea* on ornamentals at 0.56 kg a.i./ha applied every 14 days under normal disease pressure and every 7 days under high disease pressure, with a maximum of six applications per season or crop. Outdoor-grown ornamental: DO NOT apply more than 3.4 kg a.i./ha/year. Greenhouse-grown ornamentals: DO NOT apply more than 3.4 kg a.i./ha/crop.

Tank-mixing

Seven trials tank-mixed Elevate, 0.56 kg a.i./ha rate, with a nonionic surfactant. Only one of these trials compared Elevate applied alone with Elevate + nonionic surfactant and showed NSD in disease control between treatments. Given that 4 out of the 5 trials supporting the proposed number of applications (4 to 6 per season) and most of the phytotoxicity tests were done using Elevate alone, tank mixing of Elevate with a nonionic surfactant is not supported.

7.2 Information on the occurrence or possible occurrence of the development of resistance

To address the issue of development of fungicide resistance, the following information will be presented on the Elevate and Decree labels:

Elevate 50 WDG Fungicide (and Decree 50 WDG Fungicide) is a cyclohexanecarboxamide protectant fungicide used for the control of grey mould.

This product should be used in a program with other products to provide season-long protection.

There is no indication of cross-resistance between the active ingredient in this product and other fungicides. To preserve the usefulness of botryticides, avoid making more than two consecutive applications of the same fungicide.

Resistance management strategies such as alternation with other botryticides of different chemical classes or broad spectrum fungicides currently recommended for Botrytis control are highly recommended in spray programs.

Avoid making more than two consecutive applications of Elevate (or Decree). After the second application, use an alternative botryticide for two consecutive applications before reapplying the active ingredient in this product. Consult your local advisor for appropriate alternative products.

7.3 Effects on the yield of treated plants or plant products in terms of quantity and(or) quality

1) Grapes
A total of six trials presented yield data. Elevate at 0.56 kg a.i./ha provided significantly higher yield than the untreated control in three trials and a higher yield in the other three. Five trials showed an increase in marketable yield while the last trial showed a significant decrease in the % of total yield rotted. These results indicate that use of Elevate provides a consistent increase in quality and yield at harvest.

2) Strawberries
A total of 13 trials presented yield data. Two trials showed a statistically significant increase in yield. The remaining trials showed NSD between Elevate and the untreated check. Seven trials provided information on the number of rotten berries at harvest, six of which showed a statistically significant decrease in the number of rotten berries at harvest between Elevate and the check. These results indicate that use of Elevate provides an increase in quality but not in yield.

3) Ornamentals
Botrytis cinerea is favoured by cool and wet conditions. This disease can cause heavy losses due to decrease in storability and plant quality. Consistent disease control as demonstrated in the trials reviewed result in an increase in quality.

7.4 Phytotoxicity to target plants (including different varieties), or target plant products

1) Grapes
No phytotoxicity noted in the 23 trials reviewed.

2) Strawberries
No phytotoxicity noted in the 20 trials reviewed.

3) Ornamentals
No phytotoxicity noted in the 17 trials reviewed. One trial tested 98 genera of plants, many in the flowering stage, at two applications with a 1.5× rate.

7.5 Observation on undesirable or unintended side effects

None observed.

7.5.1 Impact on succeeding crops

Impact on succeeding crop not expected from proposed use pattern.

7.6 Conclusion

The data provided indicate that, when used according to amended label directions, Elevate and Decree can be applied to grapes and strawberries, and ornamentals, respectively, for the control of *Botrytis cinerea*.

7.6.1 Summary

Crop:	Grapes	Strawberries	Ornamentals
Varieties:	All	All	All
Application timing:	Early bloom Bunch pre-closure Veraison Up to 7-day PHI*	Early bloom <10% 7–14 days between applications Up to 1-day PHI	Applications should be made on a 7–14 day interval. Use the 7-day interval when conditions favour severe disease development
Product: Rate of application: No. of application:	Elevate 50 WDG 1.12 kg/ha 3 maximum	Elevate 50 WDG 1.7 kg/ha 4 maximum	Decree 50 WDG (formerly Elevate 50 WDG) 1.12 kg/ha 6 maximum
Disease controlled:	*Botrytis cinerea*	*Botrytis cinerea*	*Botrytis cinerea*
Tankmix option:	Nonionic surfactant 0.02% v/v	none	none

* PHI = pre-harvest interval

7.7 References

1) Compendium of Grape Diseases, 1988. American Phytopathological Society.

8.0 Toxic Substance Management Policy

Active ingredient
During the review of fenhexamid, the persistence and bioaccumulation potential of this active ingredient were considered. The PMRA determined that fenhexamid is not persistent in the environment and that bioaccumulation is limited. On this basis, the PMRA concluded that fenhexamid does not meet the criteria for Track-1 classification under the TSMP.

Refer to Regulatory Directive DIR99-03, *The PMRA's Strategy for Implementing the Toxic Substances Management Policy*, March 12, 1999.

Microcontaminants
The technical active ingredient does not contain any impurities that are known to meet the criteria for Track-1 classification under the TSMP.

9.0 Overall conclusions

Elevate and Decree provide commercially acceptable control of *Botrytis cinerea* in grapes, strawberries and ornamentals when applied respectively at 0.56, 0.85 and 0.56 kg a.i./ha.

The product chemistry data for fenhexamid, Elevate 50 WDG, and Decree 50 WDG are complete. The technical material was fully characterized and the specifications were supported by analysis of batches for active, impurities and microcontaminants using specific methods of analysis. The required physical and chemical properties of technical material and of the end-use product were determined using acceptable methods. A fully validated HPLC method for the determination of active in the formulation was submitted.

Acute dosing revealed that technical fenhexamid and Elevate 50 WDG formulation were of low toxicity by the oral, inhalation and dermal exposure routes to laboratory animals. Technical fenhexamid was non-irritating when applied to rabbit skin and eyes, whereas Elevate 50 WDG induced slight skin irritation and minimal eye irritation. Neither possessed skin sensitizing properties when tested on guinea pigs. Fenhexamid did not demonstrate any neurotoxic effects.

In subchronic and chronic oral studies, treatment-related toxicologically significant effects were hematotoxicity in dogs and mild liver and(or) kidney effects in rats and mice. Fenhexamid is not considered to be a developmental toxicant nor did it impair the reproductive capacity of adult rats. Fenhexamid was negative for genotoxicity. Taken together with the lack of evidence of carcinogenicity in both sexes of rats and mice, fenhexamid is not likely to pose a carcinogenic risk to humans.

The recommended ADI was calculated to be 0.58 mg/kg bw, based on the lowest NOEL of 17.4 mg/kg bw/d, established in the one-year dog study (on the basis of hematotoxic effects which may indicate the potential of KBR 2738 to induce Heinz body anemia), and using a safety factor of 300.

A short-term dermal toxicology study was determined to be the most relevant for the occupational risk assessment for both individuals mixing, loading and applying Elevate 50 WDG and Decree 50 WDG and for workers re-entering treated areas. Margins of exposure, calculated on the basis of typical North American use patterns, are acceptable for Elevate 50 WDG.

The fenhexamid plant metabolism studies were performed on grapes, tomatoes and apples. The data indicated that the residues were primarily surface residues with very little translocation (residues of fenhexamid are non-systemic). The studies also demonstrated that the parent fenhexamid represented the predominant residue. There were no animal feedstuffs associated with the uses on grapes and strawberries proposed for this petition, therefore no data were submitted or required. However, metabolite characterization in the rat metabolism study showed that the main component detected in excreta was the unchanged parent compound. Therefore, the residue of concern (ROC) is equivalent in both plants and animals and can be defined as the parent fenhexamid.

A high-performance liquid chromatography method with electrochemical detection (HPLC/ECD) was developed for the quantitation of fenhexamid residues in plant commodities. The limits of quantitation (LOQs) were 0.02 ppm (grapes) and 0.05 ppm (strawberries and raisins). Overall this method showed acceptable method validation, linearity, specificity, repeatability and reproducibility.

An adequate number of geographically representative field trials conducted throughout the U.S. and in limited regions of Canada (Ontario (grapes) and Nova Scotia (strawberries)) were submitted to support the proposed uses on grapes and strawberries. These studies were conducted according to the proposed use patterns. Efficacy data did not support the proposed PHI. However, according to the residue decline studies, residues of fenhexamid on grapes and strawberries did not decrease significantly as a function of time post-treatment, therefore it appears unlikely that amending the label PHIs from 0-day to 7-day for grapes and 1-day for strawberries will have a considerable impact on the magnitude of fenhexamid residues. Based on the grape processing study, there was no apparent concentration of residues in juice and wine, however, residues concentrated an average of 1.9× in raisins. This concentration factor was applied to the highest quantifiable grape residues. On the basis of these results, the following MRLs should be established:

grapes	4.0 ppm
raisins	6.0 ppm
strawberries	3.0 ppm

The chronic dietary risk assessment has indicated that the proposed use of fenhexamid on grapes and strawberries will not pose an unacceptable dietary (food and water) risk to any segment of the population including adults, infants and children.

In the context of the low order of acute toxicity of fenhexamid, following exposure by oral, dermal and inhalation routes, it is not necessary to propose an acute ADI.

Fenhexamid is stable to hydrolysis, but rapidly phototransforms in aqueous solution. Although phototransformation on soil surfaces occurs, it is not considered a major transformation process in the environment. Rapid biotransformation of extractable fenhexamid (DT_{50} < 1 d) and formation of bound residues are the principal mechanisms of fenhexamid dissipation in aerobic soil. No major transformation products are formed. The DT_{50} of extractable fenhexamid in aerobic soil is less than one day. After one year of incubation in aerobic soil, there was 30% mineralization of fenhexamid to CO_2. The transformation pathway includes di- and trimerization of fenhexamid, methylation of the hydroxyl group of the aromatic ring, dechlorination and total mineralization of the aromatic nucleus to CO_2 via aerobic ring cleavage. Terrestrial field studies indicated that fenhexamid is non-persistent in soil (DT_{50} 1.7–1.9 d) and should not pose a risk to groundwater. In the aquatic environment, fenhexamid gradually partitions into sediment (and forms bound residues). Under aerobic aquatic conditions, fenhexamid is non- to slightly persistent (DT_{50} ranged from 12 to 37 d). Under anaerobic aquatic conditions, fenhexamid is moderately persistent (DT_{50} = 87 d). Adsorption/desorption data indicated that fenhexamid has low to medium potential for mobility in soil. Considering the rapid biotransformation of fenhexamid in soil, potential leaching is not a concern.

At the proposed label application rate, fenhexamid is practically non-toxic to bees, beneficial insects and birds. It is toxic to juvenile fish, freshwater algae and aquatic vascular plants.

10.0 Proposed regulatory decision

The Pest Management Regulatory Agency (PMRA) has carried out an assessment of available information in accordance with Section 9 of the Pest Control Products (PCP) Regulations and has found it sufficient, pursuant to Section 18(*b*), to allow a determination of the safety, merit, and value of fenhexamid technical and its end-use products, Elevate 50 WDG and Decree 50 WDG fungicides, manufactured by Arvesta Corporation. The PMRA has concluded that the use of fenhexamid technical, Elevate 50 WDG fungicide, and Decree 50 WDG fungicide in accordance with the label has merit and value consistent with Section 18(*c*) of the PCP Regulations and does not entail an unacceptable risk of harm pursuant to Section 18(*d*). Therefore, based on the considerations outlined above, the use of fenhexamid technical, Elevate 50 WDG fungicide and Decree 50 WDG fungicide for the control of *Botrytis cinerea* on grapes, strawberries, and ornamentals is proposed for full registration, pursuant to Section 13 of the PCP Regulations.

The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document to allow interested parties an opportunity to provide input into the proposed registration decision for this product.

List of abbreviations

a.i.	active ingredient
ADI	acceptable daily intake
ALAT	alanine aminotransferase
ArfD	acute reference dose
ASAT	aspartate aminotransferase
bw	body weight
bwg	body weight gain
BWI	mean body weight
CHO	Chinese hamster ovary
CNS	central nervous system
d	day
DALA	days after last application
DBA	days between applications
DFR	dislodgeable foliar residue
DNA	deoxyribonucleic acid
dw	dry weight
DT_{50}	time required for non first-order dissipation (50%)
EC_{50}	median effective concentration
ECD	electrochemical detection
ECG	electrocardiogram
EEC	expected environmental concentration
EPA	Environmental Protection Agency (U.S.)
F_1	first generation offspring
FC	food consumption
FDA	Food and Drugs Administration (U.S.)
FOB	functional observational battery
GC	gas chromatography
GGT	gammaglutamyl transferase
GI	gastrointestinal
GLC	gas-liquid chromatography
GPC	gel permeation chromatography
h	hour
ha	hectare
HAFT	highest average field trial
Hb	hemoglobin
Hct	hematocrit
HDPE	high-density polyethylene
HPLC	high-performance liquid chromatography
HDT	highest dose tested
ILV	independent laboratory validation
K_d	adsorption quotient
K_{oc}	organic carbon adsorption coefficient
K_{ow}	*n*-octanol–water partition coefficient
LC_{50}	median lethal concentration

LD_{50}	median lethal dose
LDPE	low-density polyethylene
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantitation
MAS	maximum average score
MOE	margin of exposure
MRL	maximum residue level
NAFTA	North American Free Trade Agreement
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NSD	no significant difference
NZW	New Zealand White
PCP	pest control product
PIS	primary irritation score
PHED	Pesticide Handlers' Exposure Database
PHI	pre-harvest interval
PMRA	Pest Management Regulatory Agency
PRDD	Proposed Regulatory Decision Document
RAC	raw agricultural commodity
RBC	red blood cell
ROC	residue of concern
SD	Sprague Dawley
SD	significantly different
SF	safety factor
SPF	specific pathogen free
TRR	total radioactive residue
TSMP	Toxic Substances Management Policy
U.S.	United States of America
v/v	volume per volume

Appendix I

Table 1 Summary of the toxicity studies with fenhexamid

METABOLISM			
Very rapidly and completely absorbed and excreted (<48 hours). Pronounced first pass effect and enterohepatic circulation. Liver and kidney were the organs with the highest concentrations of radioactivity in all dose groups. There was no evidence of bioaccumulation. Excretion was rapid and almost complete with feces as the major route of excretion. Metabolite characterization studies showed that the main component detected in excreta was the unchanged parent compound which accounted for 62–75% of the dose independent of the dosing regime and sex. Metabolite 1, the glucuronic acid conjugate of the parent compound, ranged from 4 to 23% of the dose. Metabolite fractions 2 and 3 accounted for up to 3 and 7% of the dose, respectively. The proposed major pathway for biotransformation is via conjugation of the aromatic hydroxyl group with glucuronic acid. Prior to fecal excretion, hydrolysis in the intestine converts the conjugate back to the parent compound giving rise to enterohepatic circulation.			
STUDY	**SPECIES/STRAIN AND DOSES**	**LD_{50}/LC_{50} NOAEL** (mg/kg bw/day)	**LOAEL** (mg/kg bw/day)**/TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS**
ACUTE STUDIES—TECHNICAL			
Oral	Rat—Wistar, 5/sex, 5000 mg/kg bw	LD_{50}> 5000 mg/kg bw	**LOW ACUTE TOXICITY**
Dermal	Rat—Wistar, 5/sex, 5000 mg/kg bw	LD_{50}> 5000 mg/kg bw	**LOW ACUTE TOXICITY**
Inhalation	Rat—Wistar, 5/sex, 5.057 mg/L (dust)	LC_{50}> 5.057 mg/L	**LOW ACUTE TOXICITY**
Skin Irritation	Rabbit—NZW, 3 females, 0.5 g dose	PIS = 0	**NON IRRITATING**
Eye Irritation	Rabbit—NZW, 3 females, 0.1 mL dose	MAS =0	**NON IRRITATING**
Skin Sensitization (Buehler method)	Guinea pig (Bor:DHPW) 500 mg test material administered for induction and challenge		**NOT A SENSITIZER**
ACUTE STUDIES—FORMULATION ELEVATE 50 WDG Fungicide			
Oral	Rat—Wistar, 5/sex, 2000 mg/kg bw	LD_{50}> 2000 mg/kg bw	Clinical observations of soft feces in 3/rats/sex at 5 hours post-treatment with recovery by day 1. **LOW ACUTE TOXICITY**
Dermal	Rat—Wistar, 5/sex, 2000 mg/kg bw	LD_{50}> 2000 mg/kg bw	No clinical signs of toxicity. **LOW ACUTE TOXICITY**

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}/LC_{50} **NOAEL** (mg/kg bw/day)	**LOAEL** (mg/kg bw/day)/**TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS**
Inhalation			Waiver of the requirement of the submission of an acute inhalation study with Elevate 50 WDG Fungicide. **LOW ACUTE TOXICITY**
Skin Irritation	Rabbit—NZW, 3 females, 0.5 g dose	PIS = 1.3	Observation of slight-to-moderate erythema and slight edema in 3/3 rabbits with clearing by days 7 (2/3) and 14 (all animals). **SLIGHTLY IRRITATING**
Eye Irritation	Rabbit—NZW, 0.1 mL dose; 3 females	MAS = 1.0	Minimal conjunctival irritation (erythema, chemosis) which was fully reversible within 7 days. **MINIMALLY IRRITATING**
Skin Sensitization (Modified Buehler method)	Guinea pig—Hsd Win: DH (previously termed Bor:DHPW) Administered as paste for induction and 50% for challenge.		**NOT A SENSITIZER**
SUBCHRONIC TOXICITY—ORAL STUDIES			
90-day dietary	Mouse—B6C3F1 10/sex/group, 0, 100, 1000 and 10 000 ppm (0, 26.5, 266.5 or 3283.5 mg/kg/day in males and 0, 51.6, 453.9 or 5151.1 mg/kg/day in females)	NOAEL = 1000 ppm (266.6/453.9 mg/kg/day in males/females.	**LOAEL = 10 000 ppm**; ↑ serum cholesterol, bilirubin and creatinine, ↓ kidney weights; ↑ water and food consumption (males) ↓ food efficiency (males); renal effects and marginal alterations of liver function (↑ serum cholesterol, bilirubin, ↓ ASAT, ALAT), marginal ↑ in liver weights and ↓ glycogen content of hepatocytes (males).
4-week dietary	Rat—Wistar, 10/sex/group, 0, 100, 300 or 1000 mg/kg/day	NOAEL = 1000 mg/kg/day	No treatment-related effects at any dose level tested.
56-day oral bioavailability study	Rat—Wistar,10/sex/group 0, 1000, 5000, 10 000, 15 000 or 20 000 ppm (57.5, 284.7, 575.7, 943.8, and 1217.1 mg/kg/day for males and 78.0, 407.1, 896.5, 1492.5 and 1896.7 mg/kg/day for females)		Saturation in intestinal absorption achieved in males at 944 to 1217 mg/kg/day. Not achieved in females at 1897 mg/kg/day.

STUDY	**SPECIES/STRAIN AND DOSES**	**LD_{50}/LC_{50} NOAEL** (mg/kg bw/day)	**LOAEL** (mg/kg bw/day)**/TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS**
90-day dietary	Rat—Wistar,10/sex/group 0, 2500, 5000, 10 000 or 20 000 ppm (0, 202, 415, 904, and 1904 mg/kg/day for males and 0, 270, 549, 1132 and 2824 mg/kg/day for females)	NOAEL = 5000 ppm (415 mg/kg/day) in males and 10 000 ppm (1132 mg/kg/day) in females.	No clinical signs/mortalities. **LOAEL (males) = 10 000 ppm**; ↓bw and bwg, ↑ food consumption, ↓ food efficiency, ↑ ALAT. **LOAEL (females) = 20 000 ppm**; ↑ food consumption, ↓ food efficiency, ↓ liver weights with liver histopathology (Kupffer cell proliferation and altered hepatocyte morphology).
90-day dietary	Dog—Beagle; 4/sex/group; 0, 1000, 7000 or 50 000 ppm (33.9, 239.1 or 1747.7 mg/kg/day for males and 37.0, 261.0 or 1866.2 mg/kg/day for females).	NOAEL = 1000 ppm (33.9/37.0 mg/kg/day for males and females)	No compound-related effects on mortality, clinical signs, clinical tests (ECG, heart rate, blood pressure, pulse, reflexes, body temperature), ophthalmoscopic examinations, body weight, food consumption, urinalysis, or gross and histologic pathology including liver tissue enzyme analysis. **LOAEL = 7000 ppm**; significant ↑ Heinz bodies ♂/♀. At 50 000 ppm, effects seen in other hematology parameters (decreased RBC, Hb and Hct) and may indicate the potential of KBR 2738 to induce Heinz body anemia in Beagle dogs.
1-year dietary	Dog—Beagle; 4/sex/group; 0, 500, 3500 or 25 000 ppm (0, 17.4, 124.3 or 917.8 mg/kg/day for males ; 0, 19.2, 132.7 or 947.1 mg/kg/day for females)	NOAEL = 500 ppm (17.4/19.2 mg/kg/day for males/females).	No compound-related effects on mortality, clinical signs, clinical tests (ECG, heart rate, blood pressure, pulse, reflexes, body temperature), ophthalmoscopic examinations, clinical chemistry, urinalysis, or gross pathology. **LOAEL = 3500 ppm**; ↓ in RBC, Hb and Hct and on significant ↑ in Heinz bodies in both sexes; ↑ adrenal weight in females with intracytoplasmic vacuoles in the adrenal cortex of 3/4 females; At 25 000 ppm: ↓ bwg (both sexes), ↓ food consumption (females) more pronounced treatment-related effects seen in hematology parameters in both sexes (decreased RBC, Hb, Hct, increased Heinz bodies) and may indicate the potential of KBR 2738 to induce Heinz body anemia in Beagle dogs.

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}/LC_{50} **NOAEL** (mg/kg bw/day)	**LOAEL** (mg/kg bw/day)/**TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS**
SUBCHRONIC TOXICITY—INHALATION AND DERMAL STUDIES			
5-day inhalation	Rat—Wistar,10/sex/group 0, 11.8, 97.7 or 1092.6 mg/m^3 in air for 6 hours per day for a total of 5 days	NOAEL = 97.7 mg/m^3 (0.098 mg/L)	No compound-related effects in mortality, clinical signs, body weights, hematology or clinical chemistry parameters. Histopathological examination of tissues was not performed. **LOAEL = 1092.6 mg/m^3**; observations of macroscopic grey colouration of the lungs and marginally increased lung weights.
21-day dermal	Rabbit—NZW, 5/sex, 1000 mg/kg bw/day (limit dose)	NOAEL = 1000 mg/kg/day	No skin irritation was observed in any treated animals. No effects on clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights, gross or histologic pathology.
CHRONIC TOXICITY/ONCOGENICITY			
18-month feeding	Mouse—B6C3F1, 50/sex/group, 0, 800, 2400 or 7000 ppm (0, 247.4, 807.4 or 2354.8 mg/kg/day for males, and 0, 364.8, 1054.5 or 3178.2 mg/kg/day for females) for two years. 10 mice/sex/dose were assigned for the interim sacrifice at 52 weeks.	**Chronic effects** Males, NOAEL = 800 ppm (247.4 mg/kg/day) Females, NOAEL = 2400 pm (1054.5 mg/kg/day) **Oncogenicity** Males, NOAEL = 7000 ppm (2354 mg/kg/day) Females, NOAEL = 7000 ppm (3178 mg/kg/day)	No compound-related effects on survival, clinical signs, body weight, food consumption, hematology or gross pathology. **LOAEL for males = 2400 ppm**; ↓ kidney weights and ↓ in sex-specific vacuolation of the proximal tubules in the kidneys (♂). At 7000 ppm (LOAEL for females) ↓ bw/bwg (♂), significantly ↑ water consumption (♂/♀), ↑ serum creatinine, bilirubin and albumin (♂), ↓ kidney weights (♀), renal histopathology (↑ incidence of basophilic cortical tubules in ♀; chronic renal disease in ♂). KBR 2738 is non-oncogenic in mice at doses up to and including 7000 ppm. (No treatment-related increase in tumour incidence, tumour spectrum or latency when compared with controls.)

STUDY	**SPECIES/STRAIN AND DOSES**	**LD_{50}/LC_{50} NOAEL** (mg/kg bw/day)	**LOAEL** (mg/kg bw/day)**/TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS**
2-year feeding	Rat—Wistar, 0, 500, 5000 or 20 000 ppm (0, 28, 292 or 1280 mg/kg/day for males, and 0, 40, 415, or 2067 mg/kg/day for females)	**Chronic effects** NOAEL = 500 ppm (28/40 mg/kg/day for males/females). **Oncogenicity** NOAEL = 20000 ppm (1280/2067 mg/kg/day for males/females)	Survival was not affected. **LOAEL (chronic toxicity = 5000 ppm)** ↓ bwg and food efficiency in ♀, ↑ food consumption in ♀;↑ incidence of cecal mucosal hyperplasia in ♂, ↑ cellularity (hyperplasia) of the bone marrow in ♀; splenic extramedullary hematopoiesis in ♂. At 20000 ppm: ↑ food consumption, ↑ numbers of circulating reticulocytes, enlarged spleens observed macroscopically, ↑ splenic weights (♂/♀) and thyroid colloid alterations (♂/♀). KBR 2738 is non-oncogenic at doses up to and including 20 000 ppm. At the doses tested, there was no treatment-related increase in tumour incidence, tumour spectrum or latency when compared with controls.

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}/LC_{50} **NOAEL** (mg/kg bw/day)	**LOAEL** (mg/kg bw/day)/**TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS**
REPRODUCTION/DEVELOPMENTAL TOXICITY			
Two-generation, (1 litter/gen.)	Rat—SD, 30/sex/group, 0, 100, 500, 5000 or 20 000 ppm (0, 7.6, 38.2, 406 or 1814 mg/kg/day for males and 0, 9.0, 44.8, 477 or 2043 mg/kg/day for females)	**Systemic effects** Parental NOAEL = 500 ppm (38.2/44.8 mg/kg/day) Neonatal NOAEL was 500 ppm (38.2/44.8 mg/kg/day) **Reproductive effects** NOAEL = 20000 ppm (1814 mg/kg bw/d ♂ 2043 mg/kg bw/d ♀)	**Parental LOAEL = 5000 ppm**; minimal ↑ clinical chemistry parameters (ALP in ♂/♀ and GGT in ♀) ; organ weight changes (↓ liver and kidney weights in ♂) in the absence of associated gross/histopathology. Additionally at 20 000 ppm in P/F_1 parents: ↓ pre-mating body weights (♂/♀), ↓ bw during gestation and lactation, significantly ↑ food consumption in P♂ and F_1 parents) during pre-mating; ↑ clinical chemistry parameters (urea nitrogen and creatinine in ♂/♀), ↑ GGT in ♂; ↓ kidney weights in P females. **Neonatal LOAEL = 5000 ppm**; ↓ bw of F_1 and F_2 pups on lactation days 7 to 21. At 20 000 ppm: ↑ # deaths pups (post-weaning F_1 pups selected to be F_1 parents attributed to small size at weaning.) No reproductive effects were noted at any dose level tested.
Teratogenicity (gavage)	Rat—SD, 30/group, 0 and 1000 (1044) mg/kg bw/day	**Maternal toxicity** NOAEL < 1044 mg/kg/day **Developmental toxicity** NOAEL = 1044 mg/kg/day (limit dose)	**LOAEL (maternal toxicity) = 1044 mg/kg/day**; ↓ bwg during gestation days 6–16; ↓ food consumption during gestation days 6–11. KBR 2738 was not embryotoxic, fetotoxic or teratogenic at a dose of 1044 mg/kg/day (the highest dose tested).

STUDY	SPECIES/STRAIN AND DOSES	LD_{50}/LC_{50} **NOAEL** (mg/kg bw/day)	**LOAEL** (mg/kg bw/day)/**TARGET ORGAN/SIGNIFICANT EFFECTS/COMMENTS**
Teratogenicity (gavage)	Rabbit—Russian rabbits (CHBB:HM), 16/group, 0, 100, 300 or 1000 mg/kg/day	**Maternal toxicity** NOAEL = 100 mg/kg/day **Developmental toxicity** NOAEL = 300 mg/kg/day KBR 2738 was not teratogenic up to and including 1000 mg/kg/day, the limit dose.	**LOAEL (maternal toxicity) = 300 mg/kg/day** based on alterations of excretory products (discolored urine, small scybala), ↓ bwg and feed consumption (during the first week of the treatment period), ↓ placental weights. **LOAEL for developmental toxicity = 1000 mg/kg/day**; marginally ↓ male fetal body weights, delayed ossification (5^{th} sternal and 15^{th} caudal vertebrae). All effects on intrauterine development were correlated with maternal toxicity.
NEUROTOXICITY			
Oral (gavage)	Rat—Wistar, 12/sex/dose 0, 200, 630 or 2000 mg/kg via single oral dose	NOAEL in males = 630 mg/kg. NOAEL in females = 2000 mg/kg	**LOAEL in males = 2000 mg/kg** based on marginal acute toxicity as evidenced by the lower body temperatures(colonic) in one male on the day of treatment (day 0), but which reverted to normal by day 7. FOB not affected in females.
MUTAGENICITY			
STUDY	**SPECIES/STRAIN or CELL TYPE**	**DOSES EMPLOYED**	**SIGNIFICANT EFFECTS/COMMENTS**
Salmonella Ames Assay, in vitro	*S. typhimurium*— TA 1535, TA 1537, TA 98 and TA 100	0, 62.5, 125, 250, 500, 1000, 2000 or 5000 µg/plate (+/- S9)	Negative
Mammalian cell gene mutation assay, in vitro	CHO cells	6, 30 and 150 µg/mL in the absence of and 2, 20 and 120 µg/mL in the presence of S9 metabolic activation	Negative
Chromosomal assay, in vitro	Chinese hamster lung fibroblasts (HGPRT locus)	0, 25, 50, 75, 100, 125, and 150 µg/mL.	Negative
UDS, in vitro	Rat hepatocytes	2.5, 5.0, 10.0, 15.0, 30.0 and 40.0 µg/mL	Negative
Mammalian cytogenetics (micronucleus) assay, in vivo	Mouse—NMRI	750 mg/kg bw with sacrifice at 16, 24 and 48 hours after dosing	Negative