Proposed Regulatory Decision Document PRDD99-01

Difenoconazole

The active ingredient difenoconazole, manufacturing concentrate Dividend® MG, and seed treatments Dividend® 360FS and Dividend® 36FS, for control of certain seed-borne, soil-borne and foliar diseases of spring and winter wheat, are proposed for registration under Section 13 of the Pest Control Products Regulations.

The Pest Management Regulatory Agency (PMRA) has completed its assessment of the active ingredient difenoconazole and Dividend® seed treatment products, based on this active ingredient for control of various diseases in spring and winter wheat. These products have been previously registered in the United States (U.S.) and Europe, and have been given interim status in Canada.

This document provides a summary of data reviewed and the rationale for the proposed Section 13 registration of these products.

The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document.

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Foreword

This regulatory document proposes ongoing registration of Dividend® (difenoconazole) fungicides, which are Novartis products, for control of various diseases of wheat, and details the supporting scientific rationale.

Features of difenoconazole

- The active ingredient is a triazole compound. The proposed products are applied as a seed treatment at low use rates (6–24 g/active ingredient [a.i.]/ 100 kg seed) to control a wide range of pathogens on spring and winter wheat.

- Difenoconazole offers a high level of control against soil-borne dwarf bunt for which chemical control was not previously available. Dwarf bunt was a significant problem for Canadian winter wheat in 1997. The active ingredient and Dividend® 360FS seed treatment were reviewed with respect to this specific use, to allow an expedited registration for wheat seed to be sown in fall 1998. The results of that review are included in this document.

- Difenoconazole has been registered for wheat in the U.S. since 1994; and the current proposal allows harmonization of tolerances.

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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: difenoconazole

Function: fungicide

Chemical name
(International Union of Pure and Applied Chemistry):
cis-trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazole-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether

Chemical name
(Chemical Abstracts Service [CAS]):
1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl}-1H-1,2,4-triazole

CAS Registry Number: 119446-68-3

Nominal purity of active: 95%

Identity of relevant impurities of toxicological, environmental and/or other significance:

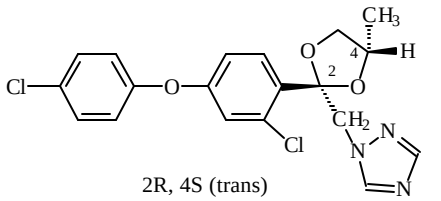
Chloro-p-dibenzodioxins and furans microcontaminants were not detected. The limit of detection (LOD) for 2,3,7,8-TCDD was below the 0.1 parts per billion LOD required by the U.S. Environmental Protection Agency (EPA) for dioxin analysis.

Nitrosamines were not detected at an LOD of 0.5 parts per million (ppm).

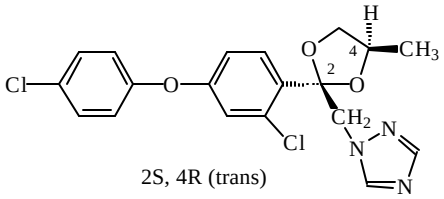
Molecular Formula: $C_{19}H_{17}Cl_2N_3O_3$

Molecular Mass: 406.3

Structural Formula:



2R, 4S (trans)



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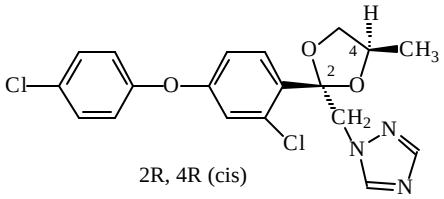
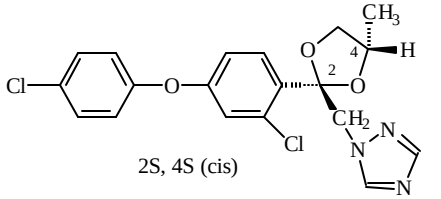


2R, 4R (cis)



2S, 4S (cis)

1.2 Physical and chemical properties of active substance

Table 1.1 Technical product: CGA 169374

Property	Result	Comments
Colour and physical state	Off-white powder	
Odour	Slightly sweet	
Melting point/range	78.6/C	
Boiling point/range	N/A	
Density	1.37 g/cm^3 at 20/C	
Vapour pressure	/C Vapour pressure 20 6.6×10^{-8} Pa 25 3.3×10^{-8} Pa	Non-volatile

Property	Result	Comments
UV/visible spectrum at 26/C	λ_{max} (in methanol) about 200 and 238 nanometres (nm)	
Solubility in water at 20/C	3.3 mg/L	Sparingly soluble
Solubility in organic solvents at 20/C	Solvent — Solubility (g/L) acetone 610 ethanol 330 n-hexane 3.4 n-octanol 95 toluene 490	In general, solubility appears to increase with increasing organic solvent polarity.
Octanol/water partition coefficient (K_{ow})	log K_{ow} = 4.20 at 25/C	Potential for bioaccumulation in fatty tissues
Dissociation constant	None in physiological range	
Oxidizing properties	Stable up to melting point. Stable over 26 weeks (wk) at room temperature and 38/C when exposed to four different metals (carbon and stainless steel, aluminum and tin-plate). Loss on storage is expected to be <0.5%/year (yr) in moderate or tropical climates.	Difenoconazole is unlikely to produce any oxidative or reductive processes on plants that may result in a change of the nature and magnitude of residues.
Storage stability	Not applicable to the technical product.	

Table 1.2 End-use product: Dividend® 360FS and Dividend® MG Manufacturing Concentrate

Property	Result
Colour	Red
Odour	Sweet latex paint odour
Physical state	Liquid suspension

Property	Result
Formulation type	Suspension
Guarantee	32.8% (nominal)
Container material and description	Plastic
Density	1.099 g/mL
pH of 1% dispersion in water at 25/C	5–7
Storage stability	Stable for 52 wk in commercial containers. No significant decomposition observed after 26 wk at 38/C and at 50/C.
Explodability	Not explosive

Table 1.3 End-use product: Dividend® 36FS

Property	Result
Colour	Bright red
Odour	Faint paint odour
Physical state	Liquid suspension
Formulation type	Suspension
Guarantee	3.15% (nominal)
Container material and description	Plastic
Density	1.179 g/mL
pH of 1% dispersion in water at 25/C	5–7
Storage stability	Stable for one year in commercial containers
Explodability	Not explosive

1.3 Details of uses and further information

Difenoconazole is a systemic fungicide for use as a seed treatment on wheat. This active ingredient belongs to the triazole group of fungicides that act by inhibiting sterol demethylation. The proposed products include end-use formulations Dividend® 360FS (32.8% difenoconazole) for application in commercial seed treatment plants, and Dividend® 36FS

(3.15% a.i.) for on-farm seed treatment, as well as technical difenoconazole (95% a.i.) and a manufacturing concentrate (32.8% a.i.). The two seed treatment products are flowable formulations, Dividend® 360FS requires dilution to a slurry and Dividend® 36FS is ready to use.

Dividend® provides control of common bunt, dwarf bunt, loose smut, seed-borne *Septoria* and *Fusarium*, general seed rots, early season control of Septoria leaf blotch and suppression of common root rot and take-all. The application rates for both spring and winter wheat are 6, 12 and 24 g a.i./100 kg seed, depending on disease. The Dividend® 360FS and 36FS labels recommend mid-season application of a foliar-applied fungicide for season-long control of Septoria leaf blotch.

2.0 Methods of analysis

2.1 Methods for analysis of the active substance as manufactured

High pressure liquid chromatography (HPLC) methods and gas chromatography (GC) methods were used for the determination of the active substance and significant impurities (content $ 0.1%) in the technical product. The methods have been shown to have satisfactory specificity, linearity, precision and accuracy.

2.2 Method for formulation analysis

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

2.3 Methods for residue analysis

2.3.1 Multi-residue methods for residue analysis

Method AG-575A was submitted as an analytical and enforcement method for the detection of residues in wheat raw agricultural commodity (RAC). Method AG-544 was presented as the trial and enforcement method for the analysis of residues in meat, milk and eggs. Both these studies were performed according to good laboratory practice standards. Both methods were validated by the analytical labs of the EPA.

2.3.2 Methods for residue analysis of plants and plant products

The residue of concern (ROC) was defined from the wheat metabolism study as ". . . parent (difenoconazole) alone expressed as [(2S,4R)/(2R,4S)]/[(2R,4R)/(2S,4S)] 1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl}-1H-1,2,4-triazole."

A total of two analytical methods were submitted by Novartis to support the analysis of treated crops. Method AG-575 was proposed as the trial and enforcement method to determine residues in plants. These methods analyzed for the parent compound only. Method CER 05303/94 was developed by Envirotest and was used to quantify the residues in Canadian field trials. This method analyzed for the parent compound as well as the three main metabolite observed in the plant metabolism studies, CGA 205375, triazole alanine (TA), and 1,2,4-triazole.

The analytical method used to generate the residue data from crops grown in the U.S. was Method AG-575A. This method was also proposed for enforcement. The method involved the following steps. Frozen wheat samples were homogenized, and residues extracted by boiling the samples in methanol:concentrated ammonium hydroxide solution. The extract was diluted in water and partitioned twice with hexane. The organic layer was then partitioned twice with acetonitrile (ACN). The residues were now transferred to the ACN phase. The ACN was evaporated and re-dissolved in toluene for clean up on a silica Sep-Pak column. The toluene was evaporated, the residue dissolved in hexane, and a second clean up was performed on a phenyl Bond-elute column. A third clean up was then performed with a charcoal column, with toluene as the solvent. Detection was achieved by GC with a nitrogen/phosphorus detector (N/P). The applicant notes that it may be necessary to increase the N/P element power in order to obtain sufficient peak height of the lowest calibration standard. A set of four to six samples can be extracted, cleaned up, and analyzed in a 24-hour (h) period .

Recovery data was generated for the parent compound (CGA 169374). Recovery of a 0.01 ppm spike from wheat grain averaged 79% for four samples (range 70–85%). Wheat straw was spiked at 0.05 and 0.75 ppm (one sample each), with recoveries of 122% and 85%, respectively. Wheat forage was spiked at 0.05 ppm (one sample) with a recovery of 76%. Analysis of control samples (two for grain, one each for straw and forage) showed no residues above the limit of quantitation (LOQ) (0.01 ppm for grain and 0.05 ppm for straw and forage).

In method CER 05303/94, samples were homogenized and extracted with ACN/water and dichloromethane. The aliquot was vortexed with borate buffer solution and FMOC reagent was added. The sample was allowed to stand for about one minute and the reaction terminated by adding 20 mL of ethyl acetate. The ethyl acetate was discarded and the wash repeated twice more with 20 mL of ethyl acetate. The pH was then adjusted to 5–6 with concentrated acetic acid. The TA metabolites were extracted three times with 20 mL of ethyl acetate. The ethyl acetate was removed on a rotovap. The extract was then esterified with distilled diazomethane. Residues were quantified by liquid chromatography/mass spectrometry (MS).

Method CER 05303/94 was validated by spiking wheat samples with either the parent compound or the three metabolites outlined above. Recoveries of the parent compound in

grain straw and green forage were 106 ± 19%, 93 ± 20% and 97 ± 12%, respectively. The average recovery of CGA 205375 in wheat grain and straw was 96 ± 11% and 88 ± 15%, respectively. The average recovery of TA in wheat grain and straw was 97 ± 16% and 73 ± 6.2%, respectively. The average recovery of 1,2,4-triazole in wheat grain and straw was 104 ± 14% and 81 ± 10%, respectively. The LOQs achieved were 0.05 ppm for all metabolites.

2.3.3 Methods for residue analysis of food of animal origin

The ROC was defined from the wheat metabolism study as ". . . parent compound (difenoconazole) alone expressed as [(2S,4R)/(2R,4S)]/[(2R,4R)/(2S,4S)] 1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl}-1H-1,2,4-triazole."

Method AG-544 was used for the analysis of animal samples and was also proposed as the enforcement method. Sample were extracted by homogenization in ACN: concentrated ammonium hydroxide. After filtration, the extract was diluted with water and saturated NaCl and partitioned with hexane. The hexane fraction was partitioned with ACN and the ACN fraction cleaned up on a silica gel SepPak. The final extract was analysed by packed column GC using N/P detection. Samples were fortified with difenoconazole and analysed with the proposed analytical enforcement method. Acceptable recoveries were obtained for each RAC at the level of the proposed maximum residue limits (MRLs). The average recovery was 99 ± 12% (number of samples = 52).

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

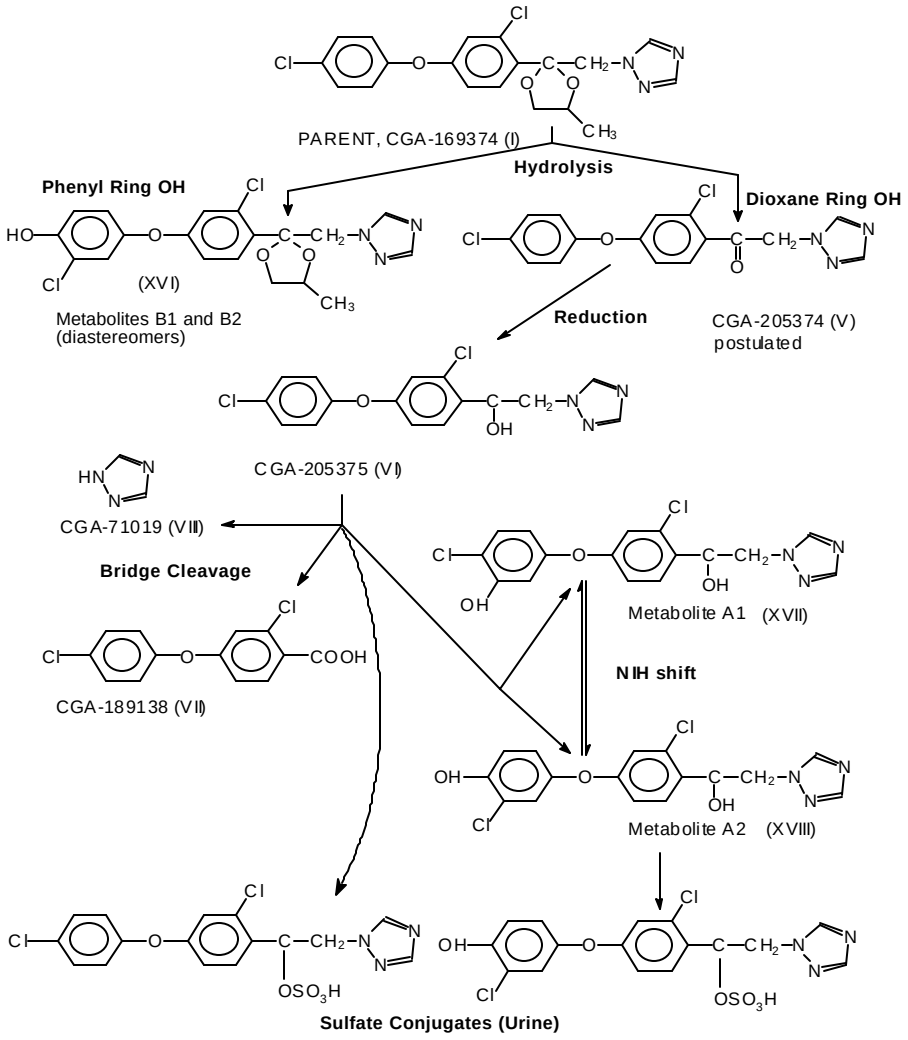
Metabolic and kinetic studies were conducted with ^{14}C-difenoconazole technical (CGA 169374) radiolabelled at the triazole or the phenyl ring of the molecule. In both studies, technical difenoconazole, purity 98.1–98.6%, was administered as either single low oral dose (0.5 mg/kg body weight [bw]), single high oral dose (300 mg/kg bw) or 14 daily low oral doses of unlabelled material followed by radiolabelled test material (0.5 mg/kg bw) to five Sprague Dawley (SD) rats/sex/group. Radiolabelled test material was absorbed and excreted relatively fast with recovery of radioactivity greater than 90%. Irrespective of the label, the sex or the administered dose (AD), the majority of the radioactivity (over 78% in all groups) was found in the feces predominantly through secretion into the bile (~75% in both sexes at the low dose; 56% for males and 39% for females at the high dose). In all groups, the compound was eliminated practically to entirety within 960 h. The half-life of elimination ($t_{½}$) values were approximately 20 h for the low-dose groups and 33–48 h for the high-dose group. The

residual radioactivity was generally low. Seven days (d) after the treatment, residues in tissues represented less than 1% of the AD. Total radioactive residue (TRR) levels ranged from 0.4% to 1.8% in tissues and carcass after seven days with no differences observed with sex or dose. With the triazole label, the highest residual radioactivity was found in the liver while the rats treated with the phenyl-labelled material showed the highest levels of radioactivity in plasma and fat. This apparent difference indicated that the bridge cleavage between the phenyl and the triazole moiety of the molecule occurred to some extent. Pre-treatment with unlabelled difenoconazole had no substantial impact on the residual radioactivity.

The identification of the metabolites was accomplished by co-chromatography with synthetic standards (two-dimensional thin layer chromatography, HPLC) or MS. Isomers were identified with nuclear magnetic resonance spectra. All significant metabolites (i.e., >10% of the AD), were eliminated with the feces. The major metabolites were identified as two isomers of hydroxy-CGA 205375 (Metabolite A; XVII and XVIII) and hydroxy-CGA 169374 (Metabolite B; XVI), representing an range of 40.5–78.52% and 1.5–20.32% of the administered radioactivity, respectively. In the high-dose group animals, CGA 205375 (VI) accounted for an additional 6.7–24.19%.

The urinary metabolite profile was more complex with all individual metabolites below 10% of the AD. Minor amounts of free triazole (CGA 71019) were identified in the urine of rats treated with the triazole label. CGA 189138 (VII) was the major metabolite in the liver of the phenyl-labelled groups. The high organo-solubility of this carboxylic acid may explain the higher residual radioactivity, which was detected in the phenyl- labelled groups. The metabolism of the compound included hydrolysis of the ketal, followed by reduction of the ketone to the corresponding alcohol (CGA 205375); hydroxylation of the (outer) phenyl ring (three metabolites); and some cleavage between the phenyl and triazole ring to produce free triazole (CGA 71019) and the carboxylic acid derivative of the diphenyl ether (CGA 189138).

Figure 1 - Proposed pathway for metabolism of difenoconazole in rats



3.1.2 Acute toxicity - technical and formulation

The acute toxicity data for technical difenoconazole (purity 94.5%), indicated that it was slightly acutely toxic via the oral route (Lethal Dose 50% [LD_{50}] = 1453 mg/kg bw) in the rat and of low acute toxicity via the dermal route (LD_{50} > 2010 mg/kg bw) in the rabbit. Difenoconazole technical was found to be of low acute toxicity to rats by the inhalation route (Lethal Concentration 50% [LC_{50}] > 3.285 mg/L). Technical difenoconazole was found to be mildly irritating to the eyes and minimally irritating to the skin of New Zealand White (NZW) rabbits, and was not a dermal contact sensitizer in Hartley albino guinea pigs.

Based on the data provided, the following label statements are required: "CAUTION - POISON" and "CAUTION - EYE IRRITANT" on the primary display panel; "Harmful if swallowed", "May irritate eyes" and "Avoid contact with eyes" should appear in the precautions on the secondary display panel.

Dividend® 360FS and Dividend® MG, containing 32.7% technical difenoconazole, were considered to be of low acute toxicity by the oral route in the rat (LD_{50} >5050 mg/kg bw), and of low acute toxicity via the dermal route in the rabbit (LD_{50} > 2020 mg/kg bw). Dividend® 360FS/MG were of slight acute toxicity to rats by the inhalation route (LC_{50} >0.985 mg/L). They were slightly irritating when applied to the skin of rabbits, and were minimally irritating when instilled into the eyes of the same species. Results of skin sensitization testing in guinea pigs, employing the modified Buehler test, were negative.

Based on the results of acute toxicity testing, the signal words "CAUTION-POISON" are required to be displayed on the primary display panel of the formulations; "Harmful if inhaled" should appear in the precautions on the secondary display panel.

Dividend® 36FS, containing 3.27% technical difenoconazole, was considered to be of low acute toxicity by the oral route and inhalation in the rat (LD_{50} > 5050 mg/kg bw; LC_{50} > 2.87 mg/L) and of low acute toxicity via the dermal route in the rabbit (LD_{50} >2020 mg/kg bw). It was non-irritating when applied to the skin of 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 test, 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 formulation.

3.1.3 Genotoxicity

No evidence of mutagenic potential of technical difenoconazole was observed in vitro with the Ames Bacterial Mutation Test or in an unscheduled deoxyribonucleic acid (DNA) synthesis assay with rat hepatocytes. A cytogenetics test with human lymphocytes appeared to be negative; however, study protocol and report deficiencies precluded a definitive determination of clastogenic potential in vitro. In an in vivo study, technical difenoconazole did not induce micronuclei in a mouse micronucleus assay. Based on the data presented, technical difenoconazole was not considered to be genotoxic under the conditions of the tests performed.

3.1.4 Sub-chronic and chronic toxicity

The sub-chronic and chronic toxicity of difenoconazole were investigated in mice, rats and dogs. A series of oral sub-chronic (90-d to 6 mos.) feeding studies were conducted initially.

These were used to establish appropriate dose levels to be used in the chronic studies. A 21-d dermal study was also carried out in rabbits.

3.1.4.1 Sub-chronic and chronic toxicity in the mouse

In the 90-d subchronic study in mice, CD-1 mice (15/sex/dose group) were fed diets containing 20, 200, 2500, 7500 or 15,000 ppm (equal to 2.9, 30.8 and 383.6 mg/kg bw/d in males and 4.4, 41.5 and 558.9 mg/kg bw/d in females) difenoconazole technical (purity 94.5%) for 13 wk. A group of 20 mice/sex served as the control group. Necropsy was limited to 10 animals/sex/dose group at the terminal sacrifice; no clinical chemistry analyses were performed.

Significant mortality was observed in the 7500- and 15,000-ppm dose groups as the majority of the animals died on test during the first week. All but two males treated at 7500 ppm were dead by the third week; the remaining two males in the 7500-ppm group were sacrificed *in extremis* during the third week. Signs of toxicity noted in animals dying on test included thinness, hunched posture, languidness and tremors. Pathological examination of the animals dying on test found dark areas in the stomach, mucosal erosion/ulceration of the glandular stomach and hyperkeratosis of the non-glandular stomach, particularly in the 15,000-ppm males. Hepatocellular enlargement was noted in many of the 7500- and 15,000-ppm animals dying on test while hepatocellular necrosis was observed in 3/15 males at 7500 and 15,000 ppm and 2/15 females at 7500 ppm.

At 2500 ppm, body-weight gain was significantly reduced in treated females; liver weights were significantly increased in both sexes with concomitant liver enlargement, hepatocellular hypertrophy and vacuolization. The only effects noted in 200-ppm treated animals were marginally increased liver weights (absolute and relative) in both sexes and an increased incidence of centrilobular hepatocellular hypertrophy (9/10) in males. No treatment-related effects were observed in haematological or ophthalmological parameters. The no observed effect level (NOEL) was 20 ppm (equal to 2.9 mg/kg bw/d in males and 4.4 mg/kg bw/d in females) based on increased liver weights in both sexes and an increased incidence of centrilobular hepatocellular hypertrophy in males at 200 ppm. Since the liver effects noted in the 200-ppm animals may represent an adaptive response to the test material, a no observed adverse effect level (NOAEL) at 200 ppm (equal to 30.8 mg/kg bw/d in males and 41.5 mg/kg bw/d in females) was considered appropriate.

In an 18-month (mo) chronic study, difenoconazole technical (purity 95%) was administered in the diet to CD-1 mice (60/sex/dose; 70/sex/dose for the control, 2500- and 4500-ppm groups) for 78 wk at concentrations of 0, 10, 30, 300, 2500 or 4500 ppm (equal to 0, 1.5, 4.7, 46.3, 423.2 or 818.9 mg/kg bw/d in males and 0, 1.9, 5.6, 57.8, or 512.6 mg/kg bw/d in females). A four-week recovery group at interim sacrifice of 10 mice/sex/dose was included for the control 2500/3000- and 4500-ppm dose groups. However, because of the early mortality in females of the 2500/3000-ppm group, the 10 control females were moved to the 2500-ppm recovery group. Therefore, no control females were available for the recovery portion of the study. All

females receiving 4500 ppm died or were sacrificed due to moribundity during the first two weeks of the study. Animals in the 2500-ppm dose group originally received 3000 ppm of the test material during the first week; dosage was subsequently reduced to 2500 ppm following the unscheduled deaths of 16/70 females at 3000 ppm.

Cumulative body-weight gain was reduced in both sexes treated at 300 and 2500 ppm and males only at 4500 ppm. Mean absolute and relative liver weight was increased at week 53 in the 300-ppm (females only), 2500-ppm (both sexes), and 4500-ppm (males only) dose groups and at termination in the 2500-ppm (both sexes) and 4500-ppm (males only) groups (but not in the recovery group at week 57). Histopathological findings observed in the liver included significant increases in the incidence of necrosis of individual hepatocytes and hepatocellular hypertrophy in the 300-, 2500- and 4500-ppm males and 2500-ppm females; focal/multi-focal necrosis, bile stasis, and fatty changes were observed in males and females treated at 2500 ppm and in males at 4500 ppm. Dose-dependent, statistically and biologically significant increases in the incidence of hepatocellular adenomas were reported in males at 300, 2500 and 4500 ppm and females at 2500 ppm with a significantly increased incidence of combined hepatocellular adenoma/carcinoma in both sexes at 2500 ppm and in males only at 4500 ppm. Clinical pathology investigation revealed increased levels of alanine aminotransferase, alkaline phosphatase (males only), and/or sorbitol dehydrogenase levels in 2500- and 4500-ppm males and 2500-ppm females, which indicated the liver as the target organ for toxicity.

The NOEL for chronic effects was 30 ppm (equal to 4.7 and 5.6 mg/kg bw/d for males and females, respectively) based on reductions in cumulative body-weight gains, increased liver enzymes, and liver pathology consistent with treatment-related hepatotoxicity in the 300-, 2500- and 4500-ppm groups.

The NOEL for tumorigenicity was 30 ppm (equal to 4.7 and 5.6 mg/kg bw/d for males and females, respectively) based on an increased incidence of hepatocellular adenoma in males at 300 ppm. On the basis of the reduced body-weight gain and frank liver toxicity observed in the study, it was concluded that the maximum tolerated dose (MTD) was probably exceeded at 2500- and 4500-ppm dose levels.

3.1.4.2 Sub-chronic and chronic toxicity in the rat

In a 13-wk rat sub-chronic toxicity study, technical difenoconazole (purity 94.5% a.i.) was administered to Wistar rats (20 rats/sex in control- and top-dose groups; 10 rats/sex in low- and mid-dose groups) in the diet at dose levels of 0, 40, 250 or 1500 ppm (equal to 0, 3.3, 19.9 or 120.9 mg/kg bw/d for males and 0, 3.5, 21.4 or 128.5 mg/kg bw/d for females) for 13 wk.

No treatment-related deaths or signs of toxicity were noted during the study. Food consumption was reduced in treated males at 250 ppm and in both sexes at 1500 ppm. Water consumption was reduced in both sexes treated at 1500 ppm. Reduced body-weight gains

were observed in 250 ppm males during weeks 8–13 (-19%) and in both sexes at 1500 ppm during the entire treatment period; mean body weight in the 1500-ppm treatment groups remained below control values after the four-week recovery phase. Hearing and ophthalmological tests did not present any treatment-related findings up to the highest dose tested (HDT). At 13 wk, absolute and relative liver weights were significantly increased in both sexes at 1500 ppm, while the relative liver to body weight ratio was slightly increased in both sexes at 250 ppm. There were no treatment-related macroscopic or histopathological findings in the study. The lowest observable effect level (LOEL) is 250 ppm (equal to 19.9 and 21.4 mg/kg bw/d for males and females, respectively), based on reduced body-weight gain during weeks 8–13 and food consumption in males and slightly increased relative liver weights in both sexes. The NOEL is 40 ppm (equal to 3.3 and 3.5 mg/kg bw/d for males and females, respectively).

In another 13-wk rat study, difenoconazole technical (purity 94.5%) was administered orally in the diet to SD rats (15/sex/dose) at concentrations of 20, 200, 750, 1500, or 3000 ppm (equal to 1.3, 12.3, 48.2, 99.9 or 203 mg/kg bw/d in males and 1.6, 15.8, 62.4, 124.4 or 261.2 mg/kg bw/d in females) for 13 wk. A group of 20 rats/sex served as the control group.

There were no treatment-related signs of toxicity or mortality. There was a significant decrease in body-weight gain in the 3000-ppm males and a dose-related trend for decreased body-weight gains in females treated at 200–3000 ppm concomitant with a negative trend for food consumption. The 200-ppm female rats showed an approximate 10% decrease in body weight gain relative to their controls. Clinical pathology investigations were unremarkable. Pathological examination revealed a significant, dose-related increase in the incidence of modest diffuse hepatocellular enlargement in both sexes at 1500 and 3000 ppm and increased liver weights in both sexes at 750, 1500 and 3000 ppm. Relative liver weight was also slightly increased in females treated at 200 ppm. Additionally, although not statistically significant, compared to the other groups, there was an increase in the frequency and quantity of ketones in the urine of 3000-ppm males. Based on the approximate 10% decrease in body weight (concomitant with a negative trend for food consumption) and increased relative liver weight in the 200-ppm females, the NOEL may be set at 20 ppm (equal to 1.3 mg/kg bw/d in males and 1.6 mg/kg bw/d in females).

In a 104-wk chronic rat study, difenoconazole technical was administered in the diet to SD rats (80/sex/dose) for 104 wk at concentrations of 0, 10, 20, 500, and 2500 ppm (equal to 0, 0.5, 1.0, 24.1, or 123.8 mg/kg bw/d in males and 0, 0.6, 1.3, 32.8, or 169.7 mg/kg bw/d in females). An additional 10 animals/sex were included in the control- and high-dose group and served as recovery animals after 52 wk on test. Ten animals/sex/dose group were utilized as an interim sacrifice group at week 53.

Mean cumulative body-weight gain was reduced for females at 500 ppm and in both sexes at 2500 ppm. Mean food consumption was significantly decreased throughout the study in both sexes at 2500 ppm. Mean absolute and relative liver weight was increased at week 53 and at

termination in the 2500 ppm group (but not in the recovery group at week 57). An increase in the incidence and severity of hepatocellular hypertrophy was observed in male and female animals at 500 and 2500 ppm at termination. Additional findings indicating that the liver is the target organ were observed in the clinical chemistry data. No treatment-related increased incidences of neoplastic findings were observed in this study. The NOEL was 20 ppm (equal to 1.0 and 1.3 mg/kg bw/d for males and females, respectively) based on reductions in cumulative body-weight gains in females and an increased incidence of hepatocellular hypertrophy in both sexes in the 500-ppm dose group.

3.1.4.3 Sub-chronic toxicity in the dog

In a 6-mo dog study, technical difenoconazole (purity 94.5%) was administered in the diet to beagle dogs (three/sex/group) at concentrations of 0, 100, 1,000, 3000 or 6000 ppm (equal to 0, 3.6, 31.3, 96.6 or 157.8 mg/kg bw/d for males and 0, 3.4, 34.8, 110.6 or 203.7 mg/kg bw/d for females) for a minimum of 28 wk.

Compound related bilateral lenticular cataracts were observed ophthalmoscopically in all dogs at 6000 ppm and in one female beagle at 3000 ppm. Additionally, iridic changes (irregular pupillary margins, miosis), secondary to lens-induced uveitis were present in the affected animals. Mean body-weight gain was reduced in both sexes of the 3000- and 6000-ppm treatment groups (% change over baseline from weeks 0 to 29 in males -12.6% and -11.7% at 3000 and 6000 ppm, respectively, versus 28.4% in control; % change over baseline from week 0 to 29 in females -4.2% and -15.1% at 3000 and 6000 ppm, respectively, versus 20.7% in control); weight loss was observed in the 6000-ppm animals during the first three weeks on study. Body weight in the 6000-ppm group remained below baseline levels for the duration of the study. Body-weight loss was accompanied by moderate to severe reductions in mean food consumption in females and males at 6000 ppm during the study. Clinical pathology investigations revealed slight reductions in red blood cell count, hemoglobin and haematocrit in females and males at 6000 ppm; however, these appeared to be secondary to the compound-related reductions in body-weight gain. Decrements in some serum clinical chemistry parameters (calcium and total protein in females at 6000 ppm) and moderate increases in serum alkaline phosphatase in one or both sexes at >3000 ppm were observed but were not correlated with supporting microscopic pathology. Several organ weight measurements were altered at the HDT but were considered secondary to the body-weight gain decrements observed in the 6000-ppm animals. Nevertheless, liver weight measurements were increased in 3,000- and 6000-ppm females. There were no other test article-related changes in any other parameter examined.

On the strength of the available data, the NOEL for male and female beagle dogs was 1000 ppm (equal to 31.3 and 34.8 mg/kg bw/d for males and females, respectively) based primarily on reduced body-weight gain and food consumption, increased absolute and relative liver weights and macroscopic and microscopic evidence of difenoconazole-related development of lenticular cataracts at $3000 ppm.

In a one-year dog study, technical difenoconazole (purity 94.5%) was administered in the diet to beagle dogs (four/sex/dose) at concentrations of 0, 20, 100, 500, or 1500 ppm (equal to 0, 0.7, 3.4, 16.4 or 51.2 mg/kg bw/d for males and 0, 0.6, 3.7, 19.4 or 44.3 mg/kg bw/d for females) for 52 wk.

Females in the 500- and 1500-ppm groups had reduced body-weight gain coupled with significant reductions in food consumption throughout the study (achieving statistical significance on days 7, 35, 70, and 357). Significant increases were observed in the levels of alkaline phosphatase in males receiving 1500 ppm. The NOEL was 100 ppm based on the decreased body-weight gain in females at $500 ppm. Lenticular cataracts were *not* observed in this study at doses of up to 1500 ppm.

Note: The observation of lenticular cataracts in the six-month dog study was not corroborated in the one-year dog study at doses up to 1500 ppm (44.3–51.2 mg/kg bw/d). It was considered most appropriate to combine the results of these two studies to determine the overall NOEL for cataract formation in dogs. Hence, the NOEL for cataracts in male and female dogs was determined to be 1500 ppm (44.3 mg/kg bw/d in males and 51.2 mg/kg bw/d in females).

3.1.4.4 Sub-chronic toxicity in the rabbit

In a 21-d rabbit dermal study, technical difenoconazole (purity 95.5%) was dissolved in absolute ethanol and administered topically to intact skin under six-hour occlusive conditions to three groups of female and male rabbits (five/sex/group) at daily doses of 10, 100, or 1000 mg/kg bw/d for at least 22 consecutive days. An additional group of rabbits (five/sex) served as vehicle controls (100% ethanol) exposed to volumes of ethanol comparable to the rabbits treated with the test material dissolved in the ethanol while another group of rabbits (five/sex) served as untreated controls.

None of the rabbits died on study. Administration of the test article to females at doses $100 mg/kg bw/d resulted in statistically significant decrements in body weight, body-weight gain and food consumption. Macroscopic and microscopic observations ranged from mild to moderate skin irritation localized to the site of application of vehicle or test article-treated rabbits. The 1000-mg/kg bw/d females had increased mean absolute and relative adrenal weights and vacuolation of hepatocytes. Based on decrements in body weight, body-weight gain, and food consumption in females at 100 mg/kg bw/d, the NOEL of technical difenoconazole in this study was determined to be 10 mg/kg/d.

3.1.5 Reproductive and developmental toxicity

A two-generation reproduction study was conducted using SD rats, fed test diets containing technical difenoconazole (purity 94.5%) at 0, 25, 250, or 2500 ppm (equal to 0, 1.8, 17.7 or 172.4 mg/kg bw/d for males and 0, 2.0, 19.6 or 191.6 mg/kg bw/d for females [pre-mating]).

Significant reductions in F_0 and F_1 male body-weight gain were observed at 2500 ppm during days 0–77 and overall (terminal body weight minus day 0 body weight). Significant reductions in F_0 and F_1 body-weight gain of females in the 2500-ppm group were detected during the pre-mating, gestation and lactation periods.

Significant reductions in pup body weight were detected days 0, 4 (pre- and post-culling) 7, 14, and 21 for males and females (day 0 female F_1 were not significant) in the 2500-ppm group of both generations. The percentage of male pups in the F_1 generation surviving post-natal days 0–4 was statistically significantly reduced in the 2500-ppm group. However, this reduction in F_1 pup survival was not considered to be biologically significant (95.2% versus 98.7% in control). There were no treatment-related effects on mortality, clinical signs of toxicity or reproductive parameters in either generation.

The NOEL for maternal toxicity and developmental toxicity was set at 250 ppm (equal to 17.7 and 19.6 mg/kg bw/d for males and females, respectively).

In a rat teratogenicity study, technical difenoconazole (purity 94.5%) was administered by gavage on days 6–15 of gestation to presumed pregnant Crl:COBS CD(SD)BR rats at 0, 2, 20, 100 or 200 mg/kg bw/d.

Significant decreases in maternal body-weight gain and feed consumption were observed during the dosing period for the 100- and 200-mg/kg bw/d groups. Clinical signs of toxicity at 100 and 200 mg/kg bw/d presented as a significant increase in the incidence of excess salivation. There was a biologically significant, treatment-related decrease in the mean number of fetuses per dam, and an increase in the mean number of resorptions per dam and percent post-implantation loss in the 200-mg/kg group. There was a slight decrease in mean fetal body weight at the 200-mg/kg group that was considered treatment-related. The following represents the significant treatment-related alterations in fetal development in the 200-mg/kg group. A significant increase in the incidence of bifid or unilateral ossification of the thoracic vertebrae and the average number of ossified hyoid with decreases in sternal ossification (per fetus per litter). A significant increase in the average number of ribs (with accompanying increases in the number of thoracic vertebrae), and decreases in the number of lumbar vertebrae in this group. These findings may be related to maternal toxicity. The NOEL for maternal toxicity was established at 20 mg/kg and the NOEL for developmental toxicity was 100 mg/kg. No evidence of teratogenicity was observed in the study.

Technical difenoconazole (purity 94.5%) was administered by gavage on days 7–19 of gestation inclusive to presumed pregnant NZW rabbits at 0, 1, 25 or 75 mg/kg.

Maternal toxicity was observed in this study as the death of one doe and abortions observed in two other high-dose does. In addition, significant reductions in body-weight gain of high-dose does were present on days 7–10, 10–14, 7–20 and 0–29. These reductions correspond with reduced feed consumption during these intervals (significant reductions in feed consumption in

the HDT were observed only during the treatment period, not after treatment). Slight biologically significant increases in post-implantation loss and resorptions/doe were observed in the HDT. The significant decrease in fetal weight at the HDT was considered related to treatment. The significant differences in fetal weight observed at the low- and mid-dose were not attributable to treatment since feed consumption was significantly decreased during the post-dosing period days 20–29 with no effect on food consumption observed during the dosing period (7–19). The maternal NOEL was set at 25 mg/kg bw/d and the developmental toxicity NOEL was 25 mg/kg. No evidence of teratogenicity was evident in the study.

3.1.6 Neurotoxicity

No studies on neurotoxicity were performed for difenoconazole.

3.1.6.1 Cataractogenicity

In a sub-chronic toxicity study, technical difenoconazole (purity 95%) was administered in diet to two beagle dogs (one/sex) at dose levels ranging from 3000 to 6000 ppm for 127 d to assess the cataractogenic potential of technical difenoconazole. A second group of two dogs/sex received 6000 ppm technical difenoconazole in week 1 and 3000 ppm in weeks 2–3, then became the recovery group for the remainder of the test period (~15 wk). Administration of 6000 ppm technical difenoconazole to test animals resulted in severe body-weight loss and decreased food consumption during week 1. The dose level was adjusted to 3000 ppm from days 9 to 63 and increased to 4000 ppm at day 64 in an attempt to maintain an MTD. Significant reductions in body weight and decreased food consumption were observed that were reversible after treatment was terminated. No significant effects were noted on haematology or clinical chemistry parameters. Liver weights were significantly increased in male dogs under continuous difenoconazole treatment. No lenticular changes were noted at any observation period. Under the conditions of the test, technical difenoconazole did not display any cataractogenic potential. Given the limitations of this study, no conclusive evidence was presented to refute the findings of the six-month dog-feeding study performed at the same dose levels.

In a sub-chronic toxicity study, technical difenoconazole (purity 95%) was administered to five Hisex chickens/sex in the diet for 56 d at a dose level of 5000 ppm (equivalent to 625 mg/kg bw/d) to assess the cataractogenic potential of technical difenoconazole in chickens. Negative control (Arachis oil) and positive control (2,4-dinitrophenol) groups of three chickens/sex were also included in the study design. Three of five difenoconazole treated males and one of five treated females showed evidence of irreversible lenticular changes consistent with cataract formation at dietary levels of 5000 ppm technical difenoconazole. Concomitant with the lenticular changes was a marked decrease in food consumption and body weight as compared to the negative control. The positive control, 2,4-dinitrophenol, yielded appropriate results. The LOEL is 5000 ppm (equivalent to 625 mg/kg bw/d), based on decreased body weight, food consumption, clinical signs of toxicity and irreversible lenticular changes consistent with the

development of cataracts. There is no NOEL for this study. Under the conditions of the test, technical difenoconazole was cataractogenic in young chickens.

3.1.7 Overall toxicological summary

Absorption and excretion of single or repeat low oral doses (0.5 mg/kg bw) was extensive and rapid in both sexes of SD rats. High dose administration (300 mg/kg bw) resulted in saturation of gastro-intestinal (GI) absorption. Greater than 90% of the AD was eliminated in the excreta within 48 h, with elimination essentially completed by 96 h. The fecal route was the predominant route of excretion (78–88%) primarily via the biliary route (75% low dose; 39–58% high dose); urinary excretion accounted for 19–22%. The half-life of elimination was 20 h for low dose and 33–48 h for the high dose with enterohepatic circulation involved in re-absorption of biliary metabolites. Total terminal residues 7 d post-administration accounted for <1.0% of the AD with the highest radiolabel found in the liver, plasma and carcass. Single or repeat dosing did not alter elimination.

Eleven metabolites were isolated from urine and feces, including two sulfonated metabolites identified in urine. Two metabolites, A and B, contained diastereomers. The proposed metabolic scheme involved hydrolysis of the dioxane ring, followed by reduction of the ketone to the alcohol; hydroxylation of the outer phenyl ring; or bridge cleavage to yield free triazole and the carboxylic acid derivative of the diphenyl ether.

Acute single dosing revealed that technical difenoconazole and the Dividend® formulations were of low to slight acute toxicity by the oral, inhalation and dermal routes to laboratory animals, and were minimally irritating when instilled into the eyes of rabbits. Technical difenoconazole was minimally irritating when applied to rabbit skin, whereas the Dividend® formulations were non-irritating to minimally irritating. Neither technical difenoconazole nor the Dividend® formulations possessed skin-sensitizing properties when tested on guinea pigs according to the modified Buehler methods.

Short-term feeding studies in mice, rats and dogs with difenoconazole technical revealed the liver to be the principle target organ of toxicity with mice exhibiting an exquisite sensitivity to the test material. Mice treated with technical difenoconazole at doses >3 mg/kg bw/d displayed a dose-dependent pattern of increasing liver toxicity ranging from hepatocellular enlargement and vacuolation to focal/multi-focal single cell hepatocellular necrosis. Liver effects in treated rats at doses $12 mg/kg bw/d were considerably less severe consisting of increased liver weights and hepatocellular enlargement. Treatment of dogs with technical difenoconazole revealed a treatment-related reduction in body-weight gain, increased liver weights and lenticular cataracts at doses $97 mg/kg bw/d. A NOEL for cataract formation was established at 51 mg/kg bw/d. Special studies carried out in dogs and chickens to investigate the development of cataracts were inconclusive but did not rule out the potential for difenoconazole-induced cataract formation at doses >51 mg/kg bw/d.

Short-term dermal administration of technical difenoconazole to rabbits produced dermal irritation and reduced body-weight gain at 100 mg/kg bw/d; administration of 1000 mg/kg bw/d resulted in hepatocellular vacuolation in treated females.

Technical difenoconazole was administered chronically to mice and rats. In the mouse dietary study, levels of technical difenoconazole $46mg/kg bw/d (mid- to low dose) were associated with a dose-dependent increase in frank hepatotoxicity and premature mortality and reductions in body-weight gain; the MTD was exceeded at the 423-mg/kg bw/d dose level (mid- to high dose). A dose-related increase in the incidence of liver tumours concurrent with liver toxicity was observed in male mice at levels $46 mg/kg bw/d and in female mice at the 423 mg/kg bw/d dose level. In the rat dietary study, administration of technical difenoconazole produced reduced body-weight gains in females and hepatocellular enlargement in both sexes at levels $24 mg/kg bw/d. There was no evidence of treatment-related neoplasia in rats.

No evidence of mutagenic or clastogenic potential of technical difenoconazole was observed in vitro with the Ames Bacterial Mutation Test, while an in vitro cytogenetics test with human lymphocytes yielded equivocal results. Technical difenoconazole did not induce unscheduled DNA synthesis in vitro. In in vivo studies, difenoconazole did not induce a positive result (i.e., the induction of micronuclei) in a mouse micronucleus assay. On the weight of evidence, difenoconazole was not considered to be genotoxic under the conditions of the tests performed.

In a multi-generation rat reproduction study, parental toxicity was noted at 2500 ppm, HDT, resulting in decreased body weight and body-weight gain. Developmental toxicity was confined to reduced body-weight gain in pups at the 2500-ppm level. The NOEL for reproductive and developmental toxicity in the multi-generation reproduction study was established at 250 ppm, equal to 23.1 mg/kg bw/d in males and 27.4 mg/kg bw/d in females. Difenoconazole was not teratogenic in either rats or rabbits up to doses that produced maternal toxicity.

In most toxicity studies, difenoconazole was shown to induce liver toxicity, with the mouse being the most sensitive species. Mice treated with technical difenoconazole at doses >3 mg/kg bw/d displayed a dose-related pattern of increasing liver toxicity ranging from hepatocellular enlargement and vacuolation to focal/multi-focal single cell hepatocellular necrosis, bile stasis, fatty change and hepatocellular adenoma with progression to hepatocellular carcinoma at 423 mg/kg bw/d, a dose that exceeded the MTD in the mouse. In contrast, liver effects in rats at doses from 12 mg/kg bw/d up to 123 mg/kg bw/d consisted of increased liver weight, altered clinical chemistry and hepatocellular enlargement, with no evidence of oncogenicity demonstrated. Studies in the dog revealed only an increase in liver weights at doses $97 mg/kg bw/d. The tumorigenic response in the mouse was considered to be a threshold phenomenon with a NOEL at 4.7 mg/kg bw/d. The LOEL for tumorigenicity of 46 mg/kg bw/d in the mouse was considered conservative given the lack of genotoxic potential, limited evidence of hepatotoxicity and equivocal tumor data at that dose level.

Based on the weight of the evidence, it is concluded that technical difenoconazole induced liver tumors in the mouse through a non-genotoxic, mitogenic mechanism at doses causing frank liver toxicity. However, the use of technical difenoconazole should not pose a carcinogenic hazard to humans provided that intake is below the threshold concentration required for hepatocellular injury. The current database has established an association between frank liver toxicity and the development of liver neoplasia in the absence of genotoxicity in the mouse only, a species with high sensitivity for epigenetic induction of liver neoplasia. Since a threshold effect for the hepatotoxic effects was demonstrated in mice, application of a margin of exposure (MOE) should provide assurance that technical difenoconazole will not pose a carcinogenic hazard at the levels anticipated in the human dietary intake.

Table 3.1 Summary of the toxicity studies with difenoconazole

METABOLISM

Studies were conducted with triazole- and phenyl-labelled difenoconazole.

Absorption and excretion of single or repeat low oral doses (0.5 mg/kg bw) was extensive and rapid in both sexes of SD rats. High dose administration (300 mg/kg bw) resulted in saturation of GI absorption. Greater than 90% of the AD was eliminated in the excreta within 48 h, with elimination essentially completed by 96 h. The fecal route was the predominant route of excretion (78–88%) primarily via the biliary route (75% low dose; 39–58% high dose); urinary excretion accounted for 19–22%. The half-life of elimination was 20 h for low dose and 33–48 h for the high dose with enterohepatic circulation involved in reabsorption of biliary metabolites. Total terminal residues 7 d post-administration accounted for <1.0% of the AD with the highest radiolabel found in the liver, plasma and carcass. Single or repeat dosing did not alter elimination.

Eleven metabolites were isolated from urine and feces, including two sulfonated metabolites identified in urine. Two metabolites, A and B, contained diastereomers. The proposed metabolic scheme involved hydrolysis of the dioxane ring, followed by reduction of the ketone to the alcohol; hydroxylation of the outer phenyl ring; or bridge cleavage to yield free triazole and the carboxylic acid derivative of the diphenyl ether.

Study	Species/strain and doses	NOEL/NOAEL mg/kg bw/d	Target organ/significant effects/ comments
Acute studies - technical			
Oral	Rat, SD, 5/sex, 1000, 2000 or 3000 mg/kg	LD_{50} = 1453 (both sexes) 95% confidence interval = 933–2263	All animals died prior to day 8 at 3000; 40% mortality at 1000 and 2000. Slight acute toxicity. "CAUTION - POISON"
Dermal	Rabbit, NZW, 5/sex at 2010 mg/kg	LD_{50} > 2010	Dermal irritation noted up to day 14 post-administration. Low toxicity.
Inhalation	Rat, Tif RAI f(SPF), 5/sex, 3.285 mg/L	LC_{50} > 3.285 mg/L	Low acute toxicity.

Study	Species/strain and doses	NOEL/NOAEL mg/kg bw/d	Target organ/significant effects/ comments
Eye irritation	Rabbit, NZW, 0.05g dose Unwashed: 3/sex; Washed: 2 males, 1 female	Maximum average score (MAS) = 19.6 (unwashed) 10.0 (washed)	Mildly irritating. All effects cleared by 96 h. "CAUTION - EYE IRRITANT"
Skin irritation	Rabbit, NZW, 3/sex, 0.5 g dose	Primary irritation index (24 and 48 h) = 0.0	Non-irritating.
Skin sensitization	Guinea pig, Hartley, test material 0.5g induction and challenge; positive control (P.C.) 0.5 mL of 0.05% dinotrochlorobenzene (DNCB) induction and challenge	Test material not irritating but P.C. was sensitizing, demonstrating responsiveness of assay.	Not a sensitizer.
Acute studies - formulation (360FS/MG)			
Oral	Rat, SD, 5/sex, 5050 mg/kg bw	LD_{50} > 5050 mg/kg bw	In both sexes, clinical observations consisted of pilo-erection, diarrhea, decreased activity and ptosis, recovery by day 5; males only polyuria, salivation, epitaxsis and lacrimation; recovery by day 5. One female died on day 3. Low acute toxicity.
Dermal	Rabbit, NZW, 5/sex, 2020 mg/kg bw	LD_{50} > 2020 mg/kg bw	No signs of toxicity, irritation or mortality occurred. Low acute toxicity.
Inhalation	Rat, SD, 5/sex, 2.8 mg/L	LC_{50} > 0.985 mg/L	Mass median aerodynamic diameter (MMAD) = 3.0 ∶m, geometrical standard deviation (GSD) = 2.0 70% < 6 ∶m; 6% < 1.0 ∶m Clinical signs consisted of pilo-erection, decreased activity, and ptosis; recovery by day 1 post-exposure. Slight acute toxicity. "CAUTION - POISON"
Skin irritation	Rabbit, NZW, 2 males and 4 females, 0.5 mL dose	Primary irritation score (PIS) = 0.5	Minimally irritating.

Study	Species/strain and doses	NOEL/NOAEL mg/kg bw/d	Target organ/significant effects/ comments
Eye irritation	Rabbit, NZW, 0.1 mL dose 5 males and 1 female	MAS (1 h) = 6.7	Minimally irritating.
Skin sensitization (modified Buehler method)	Guinea pig, Hartley, test material administered undiluted, 0.5 mL induction and challenge. Positive control reference data with DNCB.	Test material non-irritating. No evidence of sensitization. Positive control was sensitizing, demonstrating responsiveness of assay.	Not a sensitizer.
Acute studies - formulation (36FS)			
Oral	Rat, SD, 5/sex, 5050 mg/kg bw	LD_{50} > 5050 mg/kg bw	Clinical observations consisted of pilo-erection, discoloured crust around snout and diarrhea; recovery by day 4. Low acute toxicity.
Dermal	Rabbit, NZW, 5/sex, 2020 mg/kg bw	LD_{50} > 2020 mg/kg bw	Clinical observations consisted of decreased defecation and diarrhea; recovery by day 14. Low acute toxicity.
Inhalation	Rat, SD, 5/sex, 2.8 mg/L	LC_{50} > 2.87 mg/L	MMAD = 2.5 ∶m, GSD = 2.6 84% < 7 ∶m; 50% < 2.5 ∶m Clinical signs consisted of pilo-erection, decreased activity, and ptosis, recovery by day 3 post-exposure. Low acute toxicity.
Skin irritation	Rabbit, NZW, 2 males and 4 females, 0.5 g dose	PIS = 0.0	Non-irritating.
Eye irritation	Rabbit, NZW, 0.1mL dose, 5 males and 1 female	MAS = 5.3	Minimally irritating.
Skin sensitization (modified Buehler method)	Guinea pig, Hartley, 5/sex, test material administered undiluted, 0.5 mL induction and challenge. Positive control reference data with DNCB.	Test material non-irritating. No evidence of sensitization. Positive control was sensitizing, demonstrating responsiveness of assay.	Not a sensitizer.

Study	Species/strain and doses	NOEL/NOAEL mg/kg bw/d	Target organ/significant effects/ comments
Short term			
21-d dermal	Rabbit, NZW, 5/group/sex, 0 (EtOH), 0 (naive), 10, 100 or 1,000 mg/kg bw/d	NOEL = 10 LOEL = 100	$100 mg/kg: Reduced body-weight gain (BWG) and food consumption (FC), mild to moderate skin irritation at test site. 1000 mg/kg: Females exhibited increased adrenal weights and vacuolation of hepatocytes.
90-d feeding	Mouse, CD-1, 15/sex/group, 0, 20, 200, 2500, 7500 or 15, 000 ppm	NOEL = 20 ppm (~3 mg/kg for males and females) NOAEL = 200 ppm (~30 mg/kg for males and females based on increased liver weight in males and females, and hepatocellular hypertrophy in males). LOEL = 2500 ppm (383 mg/kg in males and 559 mg/kg in females).	2500 ppm: Increased liver weight, hepatocellular hypertrophy and vacuolization in both sexes. Most of mice at 7500 and 15,000 ppm died during first week.
90-d feeding	Rat, CRL:CD(SD), 15/sex/ group, 0, 20, 200, 750, 1500 or 3000 ppm	NOEL = 20 ppm (~1.23 and 1.43 mg/kg for males and females) LOEL = 200 ppm (~11.3 and 15.5 mg/kg for males and females)	200 ppm : Decreased BWG in females reduced; as well as increased absolute and relative liver to body weights. 1500 and 3000 ppm: Decreased BWG in females and males (3000 ppm only); significant. Dose-related increase in hepatocellular hypertrophy in both sexes.
90-d feeding	Rat, Wistar, 20/sex/ group in 0, and 1500 ppm and 10/sex/group in 40 and 250 ppm dose groups 4-wk recovery for control and high dose	NOEL = 40 ppm (~3.3–3.5 mg/kg/d) LOEL = 250 ppm (~11.3 and 15.5 mg/kg for males and females)	250 ppm: Decreased BWG in males , decreased FC in both sexes, increased relative liver to body weight in both sexes at week 13. 1500 ppm : Decreased BWG, FC, and water consumption, increased liver weights (absolute and relative to body and to brain).
6-mo feeding	Dog, beagle, 3/sex/dose, 0, 100, 1000, 3000 or 6000 ppm	NOEL = 1000 ppm = 31.3 and 34.8 mg/kg bw/d for males and females, respectively.	3000 and 6000 ppm: Lenticular cataracts and ocular effects and reduced BWG and FC in both sexes.

Study	Species/strain and doses	NOEL/NOAEL mg/kg bw/d	Target organ/significant effects/ comments
12-mo feeding	Dog, beagle, 4/sex/dose, 0, 20, 100, 500 or 1500 ppm	NOEL = 100 ppm (3.4 and 3.7 mg/kg for males and females) LOEL = 500 ppm (16.4 and 19.4 mg/kg for males and females)	Decreased BWG in females at 500 and 1500 ppm. NOEL for cataracts = 1500 ppm (~37.5 mg/kg bw/d).
Chronic toxicity/oncogenicity			
80-wk feeding	Mouse, CD-1, 60–70/sex/dose, 0, 10, 30, 300, 2500 or 4500 ppm (equal to 0, 1.5, 4.7, 46.3, 423.2 or 818.9 mg/kg bw/d in males and 0, 1.9, 5.6, 57.8, or 512.6 mg/kg bw/d in females)	Chronic Effects: NOEL = 30 ppm (4.7 and 5.6 mg/kg for males and females) LOEL = 300 ppm (46.3 and 57.8 mg/kg for males and females) Oncogenicity: NOEL = 30 ppm (4.65 and 5.63 mg/kg for males and females) LOEL = 300 ppm (46.3 and 57.8 mg/kg for males and females)	Early deaths in females at 2500; all female animals died at 4500 ppm. 300, 2500 and 4500 ppm: Reduced cumulative BWG (both sexes). Males: Increased liver weight hepatocellular lesions (hypertrophy, necrosis, bile stasis and fatty change noted in males. Increased severity with increased dose. Females at 2500: Necrosis, hypertrophy, bile stasis, fatty change Oncogenicity: Hepatocellular tumours, adenoma Males: 300, 2500, 4500 ppm Females : 2500 ppm Adenoma/carcinoma Males: 2500, 4500 ppm Females : 2500 ppm
2-yr feeding	Rat, SD, 80–90/sex/group, 0, 10, 20, 500 or 2500 ppm (equal to 0, 0.5, 1.0, 24.1, or 123.8 mg/kg bw/d in males and 0, 0.6, 1.3, 32.8, or 169.7 mg/kg bw/d in females)	NOEL = 20 ppm (0.96 and 1.27 mg/kg for males and females) LOEL = 500 ppm (24.1 and 32.8 mg/kg for males and females)	500 and 2500 ppm: Decreased BWG, hepatocellular hypertrophy, and clinical chemistry in both sexes. No carcinogenic effect up to HDT.

Study	Species/strain and doses	NOEL/NOAEL mg/kg bw/d	Target organ/significant effects/ comments
Reproduction / developmental toxicity			
Multi-generation	Rat, SD, 30/sex/group/gen. 0, 25, 250 or 2500 ppm (equal to 0, 1.8, 17.7 or 172.4 mg/kg bw/d for males and 0, 2.0, 19.6 or 191.6 mg/kg bw/d for females [pre-mating])	Parental NOEL = 250 ppm (17.7 and 19.6 mg/kg for males and females) Developmental NOEL = 250 ppm (17.7 and 19.6 mg/kg for males and females)	Decreased BW and BWG at 2500 ppm in F_0 and F_1 parental animals; decreased pup BWG and male survival $F_1$and F_2 at 2500 ppm. No effect on reproductive parameters and clinical signs or mortality.
Teratogenicity	Rat, CRL:CD(SD), 25/dose 0, 2, 20, 100 or 200 mg/kg bw/d	Maternal NOEL = 20 mg/kg bw/d Developmental NOEL = 100 mg/kg bw/d	Decreased BWG and FC during dosing at 100 and 200 mg/kg; 9 in mean number of fetuses, increased in resorptions/dam and percent post implantation loss at 200 mg/kg. Delayed ossification and increased number of ribs in 200 mg/kg pups. Not teratogenic.
Teratogenicity	Rabbit, NZW, 19/dose 0, 1, 25 or 75 mg/kg bw/d	Maternal NOEL 25 mg/kg bw/d Developmental NOEL = 25 mg/kg bw/d	Increased abortions, death and significant decreased BWG and FC at 75 mg/kg, decreased fetal weight in pups at 75 mg/kg bw/d. Not teratogenic.
Mutagenicity			
Study	**Species/strain or cell type**	**Doses employed**	**Significant effects/comments**
Bacterial mutation assay (Ames Test)	*S. typhimurium* TA98, 100, 1535, 1537; *E. Coli* CM 881 (WP2 trp UV resistant pKM 101) and CM 891 (WP2 trp uvrA pKM 101)	1st assay: 340 to 5447 ：g/plate 2nd assay: 85 to 1362 ：g/plate	Negative (NEG) (+/- S9)
In vitro mammalian, unscheduled DNA synthesis	Rat-cultured hepatocytes	0, 0.46, 1.39, 4.17, 12.5, 25 or 25 ：g/mL	NEG
In vitro mammalian cytogenetics	Human-cultured lymphocytes	2.5, 5.0, 10.0, 20.0 or 40.0 ：g/mL ± S9	Not able to assess, deficient inconclusive.

Study	Species/strain and doses	NOEL/NOAEL mg/kg bw/d	Target organ/significant effects/ comments
In vivo mouse micronucleus assay	Bone marrow, mouse micronucleus, CD-1 mice (5/sex)	1st part: 1600 mg/kg bw with sacrifice at 16, 24 and 48 h after dosing. 2nd part: 400, 800 or 1600 mg/kg bw with sacrifice at 24 h after dosing.	NEG
Special studies - cataractogenicity			
Cataractogenicity study in dogs	Dog, beagle, 1/sex in Group 1 2/sex in Group 2 (recovery)	Group 1: 6000 ppm (days 1–8) 3000 ppm (days 9–63) 4000 ppm (days 64–127) Group 2: 6000 ppm (days 1–8) 3000 ppm (days 9–21) recovery (days 21–127)	No treatment-related ocular effects were noted at any dose level tested. FC and BWG were reduced during week 1 (6000 ppm dose). Conclusion: Sample size too small for definitive decision on cataractogenic potential in dogs.
Cataractogenicity study in chickens	Chickens, Hisex 5/sex, P.C. 2,4-dinitrophenol 3/sex	5000 ppm (equivalent to 625 mg/kg bw/d P.C. 2,4-dinitrophenol 2500 ppm (equivalent to 312 mg/kg bw/d)	Treatment-related ocular effects were noted in both sexes. Conclusion: Cataractogenic in chickens.

3.2 Determination of acceptable daily intake

The lowest NOEL was in the chronic rat study at a level of 1.0 mg/kg bw/d, based on decreased body-weight gains and increased incidence of hepatocellular hypertrophy in both sexes at the next highest dose of 24 mg/kg bw/d. For the calculation of the acceptable daily intake (ADI), a safety factor (SF) of 100 is proposed.

The ADI proposed is calculated according to the following formula:

$$\text{ADI} = \frac{\text{NOEL}}{\text{SF}} = \frac{1.0 \text{ mg/kg bw/d}}{100} = 0.01 \text{ mg/kg/d of difenoconazole}$$

The maximum acceptable intake for a 60-kg person, calculated according to the formula ADI × 60 kg is 0.6 mg/d.

3.3 Acute reference dose

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

3.4 Toxicology endpoint selection for occupational and bystander risk assessment

The formulation is minimally irritating to eyes, slightly irritating to skin and is not a skin sensitizer. The formulation is not acutely toxic via the oral or dermal routes, but is considered slightly toxic via the inhalation route.

The expected duration of exposure for on-farm seed treaters is two to three days per year, while commercial seed treaters may be exposed over a period of several months. In both cases, the most appropriate toxicity endpoint for occupational exposure assessment is the 21-d rabbit dermal study conducted with technical difenoconazole in which the NOEL was 10 mg/kg-bw/d and the LOEL was 100 mg/kg-bw/d based on reduced body weight and food consumption. The main points considered in this decision are that the predominant route of exposure is dermal and a toxicology study by the dermal route is most appropriate, the dermal study was well conducted and effects observed at higher doses are consistent with the oral toxicity studies, difenoconazole is extensively and rapidly metabolized, and there is no evidence for a major increase in toxicity with increased duration of exposure.

No teratogenic or mutagenic effects were evident. Tumorigenicity was noted in the livers of male and female CD-1 mice at doses $46 mg/kg bw/d with a NOEL at 4.6 mg/kg bw/d. A threshold response was demonstrated. The mechanism of tumorigenicity was consistent with a non-genotoxic, mitogenic process whereby pronounced hepatotoxicity (hepatic fatty change, hepatocellular hypertrophy, biliary stasis, necrosis of individual hepatocytes and focal/multi-focal necrosis) may be the critical determinant in the formation of hepatic tumours. Because this was considered a threshold response observed in a highly sensitive species, a 100-fold MOE to this endpoint is considered adequate.

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

Dividend® 360FS:

Dividend® 360FS is proposed for commercial seed treatment only. It is packaged in 208-L drums and must be diluted 10-fold in water before application as a water-based slurry through standard slurry or mist type commercial seed treatment equipment. The maximum application rate is 23.4 g a.i./100 kg seed. Commercial wheat seed treatment is done from late fall to early spring. Workers may, therefore, be exposed over a period of several months. The draft label specifies that coveralls should be worn over normal work clothes, and chemical-resistant gloves should be worn when handling Dividend® 360FS, treated seed or contaminated equipment.

Work patterns and numbers of people involved in the treatment process vary depending on the size of the operation. Typically, a mixer/operator would make one mix per 8-h shift to treat about 60,000 kg seeds. Wheat seed is typically bagged in large (500 to 1000 kg) bags, or transferred directly from the treatment machine to a large storage bin from which it is augered into the growers' truck or wagon. Smaller bags (25 kg) may be prepared as special orders and would represent maximum handling by, and worst case exposure for baggers and bag sewers. The available information indicates a bagger could handle about 600 25-kg seed bags/h, which equates to handling 120,000 kg seed in an 8-h shift. Treatment machinery may run 24 h/d to allow the treatment operation to "keep up" with the bagging/sewing operation. At an application rate of 23.4 g a.i./100 kg seed, a mixer/operator could handle 14 kg a.i./d, and a bagger or bag sewer could handle seeds treated with a total of 28 kg a.i./d.

Exposure was estimated on the basis of a surrogate worker exposure study that measured potential dermal and inhalation exposure to metalaxyl (Apron® FL) during soybean seed treatment. In each of three trials, conducted at commercial seed treatment plants in Indiana (IN) and Iowa, Apron® FL was applied to soybean seed at the rate of 30 g a.i./100 kg seed using equipment similar to Canadian commercial equipment. Three tasks were monitored: mixer/operator, bagger and bag sewer. In each trial, one worker performed each work function five times for a total of 15 replicates per work function. Most replicates were 3.5 h long. Standard passive dosimetry techniques were used to measure exposure: whole body dosimeters worn under clothing; face and neck wipes with cotton pads; hand rinses; and personal air-sampling devices. All workers wore long clothes and mixer/operators wore goggles, chemical-resistant gloves and chemical-resistant aprons. Because the weather was cold, several workers wore extra layers of clothing, and baggers wore thick cotton gloves for warmth. Some workers also wore dust masks.

Because of irregularities in field recovery data, and the variability in clothing worn by study participants, 90th percentile exposure estimates are considered appropriate for risk assessment. The 90th percentile exposure estimates are 25 ：g/kg bw/d for mixer/operators, 13 ：g/kg bw/d

for baggers and 47 ∶ g/kg bw/d for bag sewers. The bagger and bag sewer estimates are based on seed being bagged into 25-kg bags, which represents the highest potential exposure scenario. In all cases, exposure was predominantly dermal, with about half of the total being to the hands. Inhalation exposure was about 15% of the total for the bag sewers, but many of the inhalation residues were non-detectable or below the LOQ .

Table 3.2 Estimated commercial operator exposure and resulting margins of exposure

Operator exposure scenario	Daily exposure (dermal+ inhalation) 70-kg operator (mg/kg bw/d)	MOE (based on a NOEL of 10 mg/kg bw from a 21-d dermal rabbit study)[a]
Mixer/operator (treating 60,000 kg seed with Dividend® 360FS at 23 g a.i./100 kg)	0.025	400
Bagger (handling 120,000 kg seed treated with Dividend® 360FS at 23 g a.i./100 kg)	0.013	770
Bag Sewer (handling 120,000 kg seed treated with Dividend® 360FS at 23 g a.i./100 kg)	0.047	210

[a] Although long-term exposure is not relevant to the proposed use scenario, it is noted that the MOEs for the tumour endpoint (NOEL = 4.6 mg/kg bw/d) are adequate (i.e., >100) for all exposure scenarios.

The MOEs, calculated on the basis of typical Canadian use patterns, are acceptable for all operators.

Dividend® 36FS

Dividend® 36FS is packaged in 10-L plastic jugs as a pre-diluted formulation. It is proposed for the on-farm treatment of seed using standard gravity flow or mist-type seed treatment equipment or using a "treat-on-the-go" air seeder at a maximum application rate of 23.4 g a.i./100 kg seed. The draft label does not specify any personal protective equipment.

On-farm seed treatment operations generally involve only one person and take place once per year at the time of planting. The maximum quantity of seed treated per day would be about 10,000 kg and this could occur on two to three workdays. At the maximum application rate of 23.4 g a.i./100 kg seed, this equates to handling 2.3 kg a.i./d.

On-farm wheat seed may be treated and planted by a variety of methods. Exposure may occur while loading the product, while handling treated seed (e.g., levelling the seed in small hoppers) or during equipment cleaning and maintenance. The categories of on-farm seed-treatment equipment with the highest potential for occupational exposure are those involving open-pouring of pesticide.

Exposure while pouring Dividend® 36FS was estimated using the Pesticide Handler Exposure Database (PHED) Version 1.1. The PHED is a compilation of generic mixer/loader/applicator

and flagger passive dosimetry data with associated software that facilitates the generation of scenario-specific exposure estimates.

To estimate total dermal and inhalation exposure, appropriate subsets of A and B grade data were created from the mixer/loader PHED database files. The mixer/loader file was subset for open mixing, liquid formulations, and to exclude replicates for packaging in water soluble packets. Exposure was estimated for mixer/loaders wearing long pants, long-sleeved shirts and gloves. PHED Version 1.1 data provide an adequate basis for estimating occupational exposure while pouring Dividend® 36FS into the auger tank. The subsets meet North American Free Trade Agreement criteria for data quality, specificity and quantity.

All data were normalized for kilogram of active ingredient handled and exposure estimated on the basis of the "best-fit" measure of central tendency (i.e., on summing the measure of central tendency for each body part that is most appropriate to the distribution of data for that body part [arithmetic mean if normal distribution, geometric mean if lognormal distribution, median if any other distribution]). The exposure estimate and MOE calculation were based on a farmer pouring 2.3 kg a.i./d. This could occur on two to three days per season.

The PHED estimate does not include other potential sources of exposure. For example, there may be additional exposure associated with handling the treated seed (e.g., smoothing treated seed by hand in an auger), during the seeding operation, or while cleaning and maintaining equipment. Only a qualitative assessment of these exposures is possible. Based on the types of seed treatment equipment described above, such exposures are likely to be small relative to the exposure derived from handling and pouring Dividend® 36FS.

Table 3.3 Estimated farm operator exposure and resulting margins of exposure

Operator exposure scenario	Daily exposure (dermal + inhalation) 70-kg operator (mg/kg bw/d)	MOE (based on a NOEL of 10 mg/kg bw from a 21-d dermal rabbit study)[a]
Farmer, pouring only (treating 10,000 kg seed with Dividend® 36FS at 23 g a.i./100 kg seeds)	0.0037	2700

[a] Although long-term exposure is not relevant to the proposed use scenario, it is noted that MOEs for the tumour endpoint (NOEL = 4.6 mg/kg bw/d) are adequate.

The MOE, calculated on the basis of typical Canadian use patterns, is acceptable.

3.6.2 Bystanders

Given the proposed commercial and agricultural use scenarios, exposure and risk to bystanders should be minimal.

3.6.3 Workers planting treated seeds

Workers in seed treatment plants are considered to have greater exposure potential than farmers planting commercially treated seed due to the much greater volume of product and treated seed handled and the longer duration of exposure. Margins of exposure for workers planting commercially treated seed should exceed those calculated for both commercial seed treaters applying Dividend® 360FS and farmers applying Dividend® 36 FS.

4.0 Residues

4.1 Definition of the residues relevant to maximum residue limits

4.1.1 Definition of the residues in plants relevant to maximum residue limits

The metabolism of difenoconazole in plants was studied in wheat, tomatoes, potatoes and grapes. Based on the qualitative similarities observed in the metabolic profiles of these four crops, the PMRA concluded that the nature of the residue in plants was understood. The metabolism of difenoconazole proceeds by hydroxylation of the phenyl ring and/or oxidative cleavage of the dioxolane ring, followed by cleavage of the carbon-carbon bridge between the phenyl and triazole rings.

The major terminal residues in wheat grain are the metabolites triazole and triazole acetic acid; in wheat straw and forage, the major metabolites are TA, triazole acetic acid and CGA 205375. The parent compound was not detected in grain and comprised 7–8% of the TRRs in forage and 0.3–0.4% of the TRRs in straw.

The applicant has adequately determined the nature of the residue in tomatoes following foliar application. The major terminal residues are the parent compound and its metabolite TA and CGA 131013.

The applicant has established the primary metabolic fate of difenoconazole in potatoes following foliar application. The parent is cleaved at the phenyl-triazole bridge. Triazole-labelling studies indicate that side of the molecule becomes TA, while phenyl-labelling studies indicate the side group becomes conjugated with a number of naturally occurring substrata.

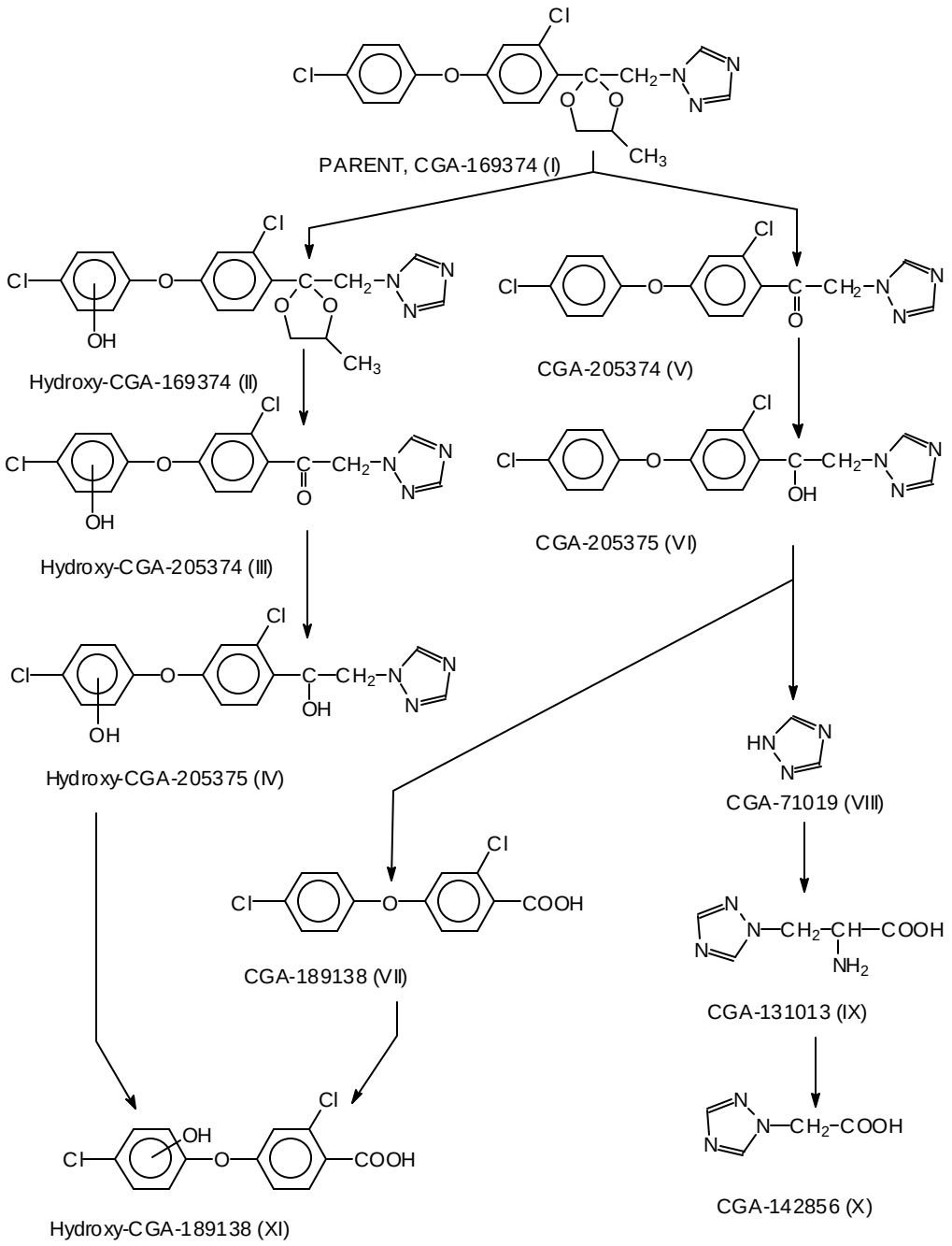
In grapes, difenoconazole *per se* was the major component of the residue, accounting for 32–51% of the TRR. CGA 205375, CGA 205374, CGA 189138 and triazole were also identified, all accounting for <10% of the TRR.

The PMRA concluded that none of the difenoconazole metabolites warrant inclusion in the expression of the ROC. No unique plant metabolite(s) was observed in the plant metabolism studies. No specific toxicological concerns have been raised about the metabolites of difenoconazole; therefore, the PMRA concluded that these metabolites did not pose a dietary

risk. It should be noted that TA and its metabolites are covered by a 2-ppm MRL. The levels of TA observed in these experiments indicate that they are unlikely to exceed the established MRL when difenoconazole is used as a seed dressing. If, however, Novartis wishes to expand the use pattern to include a foliar application, the PMRA will reconsider whether CGA 205375 needs to be included in the difenoconazole tolerance expression.

The metabolic profile of difenoconazole in plants is presented in Figure 4.1.

Figure 4.1. Metabolic profile of difenoconazole observed in plants



Confined crop rotation study

The applicant has provided evidence concerning the fate and disposition of phenyl- and triazole-labelled difenoconazole in lettuce, wheat, sugar beets, mustard, turnips and corn grown as rotational crops in soil treated with difenoconazole. The studies were carried out at exaggerated rates (1.5–5× U.S. rate, based on U.S. planting densities). The recoveries, obtained were high and the level of identification achieved ranged from 67% to 82% of the TRR. The metabolites identified in rotational crops were previously identified in the plant and animal metabolism studies. The major metabolites identified in these crops was TA and further metabolic products of TA. No parent was detected. The confined residue trial did not highlight the presence of novel metabolites that may affect the definition of the ROC.

Environmental chemistry and fate

The fate and disposition of difenoconazole was determined in soil. The results of these experiments show that the biotransformation of the molecule occurred in a similar manner in the soil as was observed in plants and animals.

Abiotic transformation of difenoconazole was observed during aqueous photolysis experiments. These experiments have led to the identification (one identified and three postulated) of four transformation products not observed in either animal, plant or soil metabolism. The potential toxicity of these metabolic intermediates was unknown.

The potential use pattern involved a seed treatment. As this molecule was not highly mobile in the soil, it was unlikely that difenoconazole migrated from the treated seed to the open water where aqueous photolysis could occur. Under these conditions, it was highly unlikely that the transformation product observed in the photolysis experiments would be seen in plants. If Novartis wished to expand the use pattern to a scenario where aqueous photolysis is possible, the identification of these metabolites would have to be confirmed, and their potential toxicity determined.

Storage stability

Novartis has submitted freezer storage stability data illustrating the freezer storage stability of the parent compound in potatoes (Group 1) and tomatoes (Group 8) under frozen storage conditions for at least 2 yr, and in lettuce (Group 4), soybeans (Group 6) and wheat forage for a period of 1 yr. The data indicated that the parent compound is stable in wheat (Crop Group 15) and in cottonseed (not included in a crop group) for up to 2 yr under storage conditions. Therefore, the applicant has demonstrated the freezer storage stability of the parent compound in five crop groupings and cotton seed. The crop groupings were representative of fruiting vegetables (Tomato Group 8), root crops (Potato Group 1), leafy vegetables (Lettuce Group 4), non-oily grains (Wheat Group 15) and oil seeds (Soybean Group 6 and cottonseed). Under these conditions, the PMRA has concluded that residues of difenoconazole in crops were stable for up to 1 yr under freezer conditions.

4.1.2 Definition of the residue in food of animal origin relevant to maximum residue limits

Animal metabolism

The livestock metabolism studies submitted in support of this application were inadequate. Both the laying hen and lactating goat metabolism studies had problems in the accountability of the TRR. This has limited the interpretation of the qualitative information contained in these studies. Using the results of the rat metabolism as well as the potential exposure of animals to residues of difenoconazole in treated feed commodities, the PMRA has assembled enough information to support this application with several restrictions on any further expansion of use.

Qualitatively, the metabolism and excretion data from goats, hens and rats showed a similar pattern with the majority of the dose (87–104% of the dose) excreted in the urine, faeces and bile. The metabolites observed in the three species were similar with TA and CGA 205375 predominating as the terminal residues. In the rat, a minor metabolite of CGA 205375 was also observed (metabolite A1 and A2). The majority of the radioactivity observed in edible tissues was contained in liver and kidney and was identified as CGA 205375. The residues in goats' milk plateaued after 7–8 d. Fractionation of the whole milk into fat, whey and casein showed that the TRR was distributed in the following proportion: 18.7%, 47%, and 21.6% for the triazole label (from day 7 sample) and 32.1%, 12.2%, and 12.4% for the phenyl label (from day 8 sample), respectively. Only triazole activity was characterized; the identified metabolites were CGA 71079, 45.8% and CGA 205375, 3.3%. Also found were the polar "Metabolite D", 2.9%, and other unidentified polar metabolites ~18.2% (- 5 compounds). No parent compound was found. Residues in eggs were the second largest fraction observed in poultry. Residues appeared to be higher in egg yolk. Residue levels ranged from 0.101 to 0.155 and 0.107 to 0.184 ppm for the triazole label, and from 0.011 to 0.016 and 0.006 to 0.010 ppm for the phenyl label. Residues were only characterized with respect to their partitioning into organic or aqueous phases and were not further identified. The majority of the TRRs from the triazole-labelled experiment were aqueous soluble while those from the phenyl experiment were predominantly organo-soluble.

Due to the problems encountered with accountability in the livestock metabolism studies, the PMRA recommended that any further expansion of uses into any major commodities that can be fed to livestock be restricted until an adequate lactating goat and laying hen metabolism studies were submitted and evaluated.

Storage Stability

No freezer storage information was submitted to illustrate the stability of residues in animal matrices.

4.2 Residues relevant to consumer safety

The magnitude of the residue in spring and winter wheat as well as durum wheat was determined in the U.S. and in Canada. Good agricultural practice (g.a.p.) was defined on the proposed Canadian labels as an application of 24 g a.i./100 kg seed. Based on all of the data presented, the PMRA concluded that the applicant has submitted adequate residue data to support a domestic registration of difenoconazole as a seed treatment on wheat. The result obtained from field trials carried out at g.a.p. and at rates up to 2.4× g.a.p., demonstrated that the residues of parent compound in progeny grain, straw and green forage were below the 0.05-ppm LOQ in all cases.

Residue trials carried out in Europe were also considered. These trials were carried out using foliar applications and, therefore, were not representative of the proposed use pattern in Canada. Though these trials had no impact on the regulatory recommendation, they were important as they provided the basis for a scientific rationale requesting a waiver of the livestock-feeding studies.

Based on the results, and in consideration of the tolerances set in the U.S. and in other countries, the PMRA would recommend an MRL of 0.1 ppm to cover the residues of difenoconazole in wheat progeny grain. This MRL is consistent with the legal limits set in other countries.

The potential exposure of consumers to difenoconazole residues through dietary intake was determined to be very low. At the proposed recommended application rate of 24 g a.i./100 kg seed, residues of difenoconazole are not expected to occur in wheat grain at levels greater than the 0.05-ppm LOQ, and residues greater than 0.01 mg/kg are not expected to occur in animal products such as milk, eggs and meat intended for human consumption. Using the Canadian diet and considering consumption of wheat and meat, meat by-products, milk and eggs obtained from animals fed treated commodities, the theoretical maximum daily intake is below 3.8% of the ADI. In children, the potential daily intake (PDI) consumes from 11–19.9% of the ADI (using U.S. Food and Drug Administration consumption figures).

When allocating 10% of the ADI to drinking water and recalculating the dietary risk assessment, taking into account the potential intake from direct wheat consumption and consumption of food of animal origin, the PDIs for all age groups are still below 20% of the ADI.

Accordingly, there is a large safety margin for consumers.

4.3 Residue relevant to worker safety

Addressed in section 3.6.3.

4.4 Proposed maximum residue limits and compliance with existing maximum residue limits

4.4.1 Compliance with existing maximum residue limits in Canada

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

4.4.2 Proposed maximum residue limits

On the basis of the results of the extensive range of supervised trials carried out with respect to the single use proposed for difenoconazole, it is clear that residues in wheat grain and succeeding crops will not exceed the 0.1 mg/kg for difenoconazole residues. The LOQ for the analytical method is 0.05 mg/kg for Canadian residue trials.

Accordingly, it is proposed that the MRL for wheat grain be set at 0.1 mg/kg.

Residues in wheat grain, treated at exaggerated rates, were not detectable (<0.01 mg/kg LOQ of analytical method used in the U.S.). Residues in processed commodities (bran, millings shorts and grems) were also below the LOQ of the method and, therefore, the residues in the processed fractions will be covered by the MRL on the RAC.

When crops are treated in accordance with the proposed label directions, residues of difenoconazole in meat, fat and egg are expected to be less than 0.05 mg/kg. In milk, the residues are expected to be below 0.001 mg/kg.

In light of these considerations, and the good agricultural practice proposed for difenoconazole (use on wheat seed at 24 g a.i./100 kg seed), the following MRLs for difenoconazole have been promulgated.

Table 4.1 Maximum residue limits for difenoconazole

Common name	Chemical name	Maximum residue limit (ppm)	Food(s)
difenoconazole	[(2S,4R)/(2R,4S)]/[(2R,4R)/(2S,4S)]1-{2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-yl-methyl}-1H-1,2,4-triazole	0.1	wheat
		0.05	meat and meat by-products, poultry meat and meat by-products and eggs
		0.01	milk

4.5 Proposed import tolerances

Since only a domestic use on wheat has been proposed, and since MRLs have been proposed for the commodities in which residues are likely to occur, the establishment of import MRLs is not applicable.

4.6 Basis for differences, if any, in conclusions reached having regard to established or proposed maximum residue limits

There are no differences in MRLs.

5.0 Fate and behaviour in the environment

5.1 Physicochemical properties

Difenoconazole is sparingly soluble in water (3.3 mg/L at 20°C). It has a low potential to volatilize under field conditions (vapour pressure 3.3×10^{-8} Pa), and from water and moist soil surfaces (Henry's Law Constant, 8.13×10^{-6} Pa m^3/mol). The log K_{ow} of 4.2 indicates that difenoconazole has a potential of bioaccumulation, however, a bioconcentration/bioaccumulation study with fish showed that the chemical depurates quickly (95% by day 3 of depuration period) and, therefore, that the risk of bioaccumulation should be minimal.

5.2 Fate and behavior in soil

The transformation and dissipation of difenoconazole were investigated in detail under both laboratory and field conditions. Studies were performed using radiolabelled difenoconazole with ^{14}C in the triazole and/or chlorophenyl ring. It was demonstrated that microbial degradation was the principal mechanism of transformation in the terrestrial environment.

5.2.1 Phototransformation in soil

No data required for the proposed use category (seed treatment).

5.2.2 Aerobic soil biotransformation

Difenoconazole transformed in U.S. loam and sandy loam soils under aerobic conditions with Decline Time 50% (DT_{50}) values ranging from 1059 to 1600 days. These values indicate that difenoconazole is persistent in soils under aerobic conditions. A European study designed to determine the effects of varying concentrations (0.1 and 1.0 mg/kg soil) at different temperatures (10, 20 and 30°C) and soil moisture contents (30% or 60% field capacity) on the rate of biotransformation of difenoconazole in a silt loam soil indicated that difenoconazole was

moderately persistent and persistent in aerobic soil at the initial concentration of 0.1 and 1.0 mg a.i./kg soil, respectively. At the lower rate, the Decline Time 90% (DT_{90}) of 739 d indicated a potential for carryover. The biotransformation rate of difenoconazole appeared to increase with decreasing application rate, indicating a possible inhibition of soil microbes at the higher rate.

5.2.3 Anaerobic soil biotransformation

Difenoconazole transformed in U.S. loam and sandy loam soils under anaerobic conditions with DT_{50} values ranging from 679 to 947 days. These values indicate that difenoconazole is persistent in soils under an anaerobic conditions.

5.2.4 Field soil dissipation studies

The field dissipation of difenoconazole was studied at four sites in the wheat-growing areas of the Prairie region (Manitoba, Saskatchewan and Alberta). The design of the study was based on application of the test substance as viable treated seed (dissipation from treated seeds at a nominal concentration of 240 ：g a.i./g of seed not from the bare ground treatment), so that the effects of the seed on the initial deposition of residues and the dispersion of residues in the soil profile would be included in the results. As a result, the test application area was taken to be the area within the seed rows in each plot rather than the total plot area. The transformation and transport of difenoconazole and the transformation product CGA 205375 (1-[2-chloro-4-(4-chlorophenoxy)-phenyl]-2-[1,2,4]triazole-1-yl-ethanol) were monitored until the fall of the year after treatment －500 d). Vegetation samples were collected at each site when biomass was sufficient to provide a sample of adequate size for analysis (approximately 25 d after treatment). CGA 205375 appeared after 1–3 wk and reached a maximum of 0.02 ：g/g soil (<10% of initial deposit). The calculated difenoconazole DT_{50} ranged from 35 to 63 d for the first growing season. These values indicate that difenoconazole is moderately persistent under field conditions. The transformation rate levelled off at the end of the first year, however, and residues persisted until the fall of the second year. The carryover was approximately 20% (48 ：g/g soil). There were no detectable levels of difenoconazole in grain, straw and foliage.

In the Summary Binder submitted by the applicant, some data from the other field dissipation studies were reported. The studies were not submitted for review, as they are not relevant to Canadian ecozones. They were conducted with difenoconazole applied to the bare ground plots in Switzerland, Germany, Spain, South Africa and the United Kingdom at rates between 125 and 800 g a.i./ha. The calculated DT_{50} of difenoconazole under the varying conditions at these sites ranged from 12 to 197 d. There was no apparent correlation between dissipation rate and application rate.

5.2.5 Mobility: Soil adsorption/desorption

An adsorption/desorption study of difenoconazole was conducted with four U.S. soils for 24/24 h, respectively. Adsorption/desorption of difenoconazole (mean of two replicates) ranged between 48.0% and 64.3% (adsorption) and between 24.0% and 40.7% (desorption). The adsorption coefficient values for difenoconazole were 3866.67, 3517.88, 3470.89 and 7734.43 in sand, sandy loam, silt loam and silty clay, respectively. The results of the study indicate that difenoconazole has strong affinity to the soils studied. Difenoconazole would be classified as immobile (silty clay) to slightly mobile (sand, sandy loam and silt loam) in soil and, therefore, has low potential to leach.

5.2.6 Expected environmental concentration

According to the proposed label, the maximum application rate is 23.4 g a.i./100 kg seed; therefore, the expected environmental concentration (EEC) is 234 mg a.i./kg seed.

5.3 Fate and behavior in aquatic systems

5.3.1 Hydrolysis

Difenoconazole was stable to hydrolysis at pH 5, 7 and 9 ($t_{1/2}$ of 1155, 1733 and 6930, respectively.

5.3.2 Phototransformation in water

No data from these sections are required for the proposed use category (seed treatment).

5.3.3 Aquatic aerobic biotransformation

No data from these sections are required for the proposed use category (seed treatment).

5.3.4 Aquatic anaerobic biotransformation

No data from these sections are required for the proposed use category (seed treatment).

5.4 Fate and behaviour in air

No data from these sections are required for the proposed use category (seed treatment).

6.0 Effects on non-target species

6.1 Effects on terrestrial non-target species

6.1.1 Wild birds

Difenoconazole was practically non-toxic to the mallard duck on an acute oral basis with an LD_{50} value of 2150 mg a.i./kg bw, HDT. The NOEL value was 2150 mg/kg bw.

On a dietary basis, difenoconazole was also practically non-toxic to the bobwhite quail (LC_{50} = 4760 mg/kg dry weight [dw], no observable effect concentration [NOEC] = 2500 mg/kg dw) and mallard duck (LC_{50} > 5000 mg/kg dw, NOEC = 625 mg/kg dw).

Table 6.1 Summary of toxicity of difenoconazole to wild birds

Organism	Test type	NOEC (mg a.i./kg)	LC_{50}/LD_{50} (mg a.i./kg)	Classification
mallard duck *(Anas platyrhynchos)*	acute oral	2150	>2150	non-toxic
bobwhite quail *(Colinus virginanus)*	dietary	2500	4760	non-toxic
mallard duck *(Anas platyrhynchos)*	dietary	625	>5000	non-toxic

6.2 Effects on aquatic non-target species

No data from these sections required for the proposed use category (seed treatment).

6.3 Effects on biological methods of sewage treatment

No data from these sections required for the proposed use category (seed treatment).

6.4 Environmental risk assessment

6.4.1 Terrestrial organisms

Wild birds

The major route of exposure to difenoconazole for wild birds under the proposed use pattern is through dietary sources. In the field, birds pick up treated seed from the soil surface; some species may even scratch for incorporated seeds; and some may also pull up and ingest small seedlings with the seed still attached.

Acute risk assessment: Assuming mallard duck will consume 30% small insects and 70% grain (U.S. EPA 1993), the EEC in the diet would be 234 mg a.i./kg dw and the daily intake (DI) would be 18.35 mg a.i./individual [ind]/d. A NOEL value based on an individual

($NOEL_{ind}$) rather than on kg bw was calculated to be 2440 mg a.i./ind. The minimum number of days of intake of difenoconazole required for effects to be observable ($NOEL_{ind}$/DI) would be 133. This indicates that birds are not at risk from exposure to difenoconazole on an acute basis (Table 6.2).

Dietary risk assessment: The NOEC for the bobwhite quail and mallard duck is 2500 and 625 mg a.i./kg dw, respectively, and the EEC in the diet is 234 mg a.i./kg dw. The values of risk factor for bobwhite quail and mallard duck (0.09 and 0.37, respectively) and margin of safety (10.7 and 2.7, respectively) for the dietary risk indicate that the environmental concentration is much lower than the NOEC and that ingestion of this compound at the indicated levels will not pose a dietary risk to birds (Table 6.2).

Table 6.2 Summary of risk assessment to birds

Organism	Test type	NOEC /NOEL mg a.i./kg	EEC mg a.i./kg	DI mg a.i./ind/d	Toxicity	Risk factor (EEC/NOEC)	Safety factor (NOEC/EEC)
Mallard duck	acute oral	2440*	234	18.35	non-toxic	133 d to reach NOEL	
	dietary	625	234	Not Applicable (N/A)	non-toxic	0.37	2.7
Bobwhite quail	dietary	2500	234	N/A	non-toxic	0.09	10.7

* expressed as $NOEL_{ind}$ (mg a.i./ind) = NOEL (mg a.i./kg bw) × BWI (kg bw/ind)

6.5 Environmental risk mitigation

An assessment of environmental safety with the proposed (seed treatment) use of difenoconazole has not identified any concerns requiring mitigation.

7.0 Efficacy data and information

7.1 Effectiveness

7.1.1 Intended uses

Difenoconazole is a systemic fungicide for use as seed treatment on spring and winter wheat to control or suppress a range of diseases. It is effective in controlling common bunt (seed-borne and soil-borne), dwarf bunt (including soil-borne), loose smut, seed-borne *Fusarium* and

Septoria, and general seed rots (e.g., *Aspergillus, Penicillium*), and in providing early season control of Septoria leaf blotch and in suppressing common root rot and take-all.

The proposed formulations are Dividend® 360FS (32.8% difenoconazole) for application in commercial seed treatment plants, and Dividend® 36FS (3.15% difenoconazole) for on-farm seed treatment. Rates of use are 6, 12 and 24 g a.i./100 kg seed, depending on disease. As part of the directions for use, the Dividend® labels recommend a later application of foliar fungicide for season-long control of Septoria leaf blotch.

7.1.2 Mode of action

Difenoconazole is a locally systemic fungicide. This active belongs to the triazole group of fungicides that act by inhibition of demethylation. This results in impaired fungal membrane function and death of hyphae.

7.1.3 Crops

Label claims are for spring and winter wheat, except for fall control of foliar pathogens and dwarf bunt that are specific to winter wheat.

7.1.4 Effectiveness Trials

7.1.4.1 Effectiveness against common bunt (stinking smut, covered smut) *(Tilletia caries)*

Proposed label claim:

> For control of common bunt in spring wheat and winter wheat, apply 6, 12 or 24 g a.i./100 kg seed. Controls both seed-borne and soil-borne common bunt.

Common bunt is a soil-borne and seed-borne disease of wheat that affects grain quality by external contamination of harvested grain. A number of protectant and systemic seed treatments are registered for this disease. The difenoconazole data submitted consisted of seven Canadian trials (spring wheat, Prairies) and eight U.S. trials in appropriate regions (winter wheat, Washington [WA], Kansas, IN). Difenoconazole was applied at 5, 6, 10, 12, 20 or 24 g a.i./100 kg seed in the various trials. Seedling emergence, disease level and yield were assessed.

Spring wheat trials used natural inoculum at Canadian field sites. Disease levels in the check were adequate in seven trials (11–264 smutted heads/plot). Disease incidence in difenoconazole treated wheat was typically nil (100% control in five trials), both in wheat treated at 6 g and at 12 g. The lowest rate (6 g) is, therefore, adequate. There were no data on the 24-g rate. Emergence and yield of treated plants were slightly greater or not significantly different from that of the check.

In winter wheat trials, inoculum was added to soil or seed. Disease levels in the check were adequate in eight trials (10–92% diseased heads). Difenoconazole provided 100% control in six of the trials at rates of 5, 10, 12 and 24 g. The lowest rate (6 g) is, therefore, adequate. In the remaining trials, both of which were furrow irrigated, treated wheat appeared to have up to 30% disease in individual plots, but treatment effects were not clear due to variability between plots and a coarse sampling method (<20 plants assessed). Yield was consistently increased by seed treatment, sometimes by up to100%.

The proposed claim of control of seed-borne and soil-borne common bunt on spring and winter wheat with difenoconazole at the 6-, 12- and 24-g rate is supported. Data showed that the 6-g rate provided adequate control.

7.1.4.2 Effectiveness against Dwarf bunt *(Tilletia contraversa)*

Proposed label claim:

For control of dwarf bunt in winter wheat, apply 12 or 24 g a.i./100 kg seed.

Dwarf bunt is a sporadic but significant disease of winter wheat in Canada, currently limited in distribution to certain counties of Ontario and the Okanagan region of British Columbia (B.C.). In 1996-97, prolonged snow cover resulted in a high incidence of dwarf bunt in Southwestern Ontario and reduced crop value due to downgrading. At that time, there was no effective fungicide for the soil-borne phase of this disease. The review of difenoconazole for this use was expedited to allow treated seed to be available for September 1998 planting, thus, preventing losses should conditions favour dwarf bunt again in 1998-99.

The data that were submitted on dwarf bunt included reports of four trials in B.C. and nine in the U.S. (WA, Utah, Montana, Oregon [OR]), all with winter wheat. The trials were located in fields known to have *T. contraversa*-infested soil. Difenoconazole provided at least 85% control of dwarf bunt and, more typically, close to 100% control. Four other fungicides were also tested; however, none had consistent efficacy. The 12-g rate of difenoconazole provided 100% control in five to eight trials and the slight amount of bunt in the remaining trials was likely within the range of experimental error. The 12-g rate, therefore, was considered adequate.

The proposed claim of control of dwarf bunt in winter wheat with difenoconazole at the 12- and 24-g rate is supported. Data showed that the 12-g rate provided adequate control.

7.1.4.3 Effectiveness against loose smut *(Ustilago tritici)*

Proposed label claim:

For control of loose smut in spring wheat and winter wheat, apply 6, 12 or 24 g a.i./100 kg seed.

Loose smut is a seed-borne disease affecting spring and winter wheat. A number of protectant and systemic seed treatments are registered for this disease. Three Canadian trials, and one U.S. trial on spring wheat were submitted. Difenoconazole was applied at 12 and 24 g a.i./100 kg to seed that was naturally infected (up to 10%) and the number of smutted heads per plot was estimated during ripening. Disease levels in the check were adequate (248–315 heads/plot) in the Canadian trials, and difenoconazole was very effective, providing 92–99% control. There was a slight but consistent benefit from the higher rate in all trials, which may have significance in reducing contamination of grain used for seed. There were no data on the lowest proposed rate (6 g).

The proposed claim of control of loose smut on spring and winter wheat with difenoconazole at the 12- and 24-g rate is supported.

7.1.4.4 Effectiveness against seedling diseases and seed rots (various species of *Septoria, Fusarium, Aspergillus, Penicillium*)

Seedling diseases and poor emergence can result from using seed that is contaminated, either as a result of internal infection prior to harvest (*Fusarium*, *Septoria*), or from surface infestation during storage (*Aspergillus, Penicillium*). A variety of seed treatments are able to improve emergence in fields where several pathogens may be present, but suppression of specific pathogens is more difficult to demonstrate.

Proposed claim:

> For control of seed-borne *Septoria* and seed-borne *Fusarium* on spring and winter wheat, apply 12 or 24 g a.i./100 kg seed.

Six U.S. studies were submitted using wheat seed naturally infected with *Fusarium graminearum* and often having distinguishing tombstone appearance. Seed was treated with difenoconazole at 12 or 24 g a.i./100 kg. In laboratory tests, seed germination after seven days was consistently increased by treatment, typically by 40%. In two plate tests the treated seeds colonized by *Fusarium* sp. were reduced (43–85%) compared with the untreated seeds. Treated seed showed significantly increased emergence in pot trials. The effect was less apparent in parallel field trials, possibly because of microbial buffering of soil. The claim of controlling seed-borne *Fusarium* is, therefore, acceptable. Both the 12-g and 24-g rates were effective.

Two U.S. studies with winter wheat were submitted using *Septoria*-infected seedlots (22% and 44% infected). In one study, there were no data on Septoria disease symptoms at seedling stage and emergence data did not show any increase due to difenoconazole. In the second study, however, assessment of Septoria severity (method not specified) at 27 d after planting showed 22% in the untreated check and 0% in difenoconazole treatment (20-g rate). Excellent control in germination tests was also observed (data not provided). Difenoconazole has also

been shown to control Septoria leaf blotch in spring, resulting from inoculum on leaves (see 7.1.4.5). Although this claim for control of symptoms from seed-borne *Septoria* presents a different infection scenario from the foliar disease, there is sufficient combined evidence to suggest that difenoconazole at 24-g rate would be effective in reducing disease at this stage.

The proposed claim of control of seed-borne *Fusarium* on spring and winter wheat with difenoconazole at the 12- and 24-g rate is supported. Data showed that the 12-g rate provided adequate control.

The proposed claim of control of seed-borne *Septoria* on spring and winter wheat with difenoconazole at the 24-g rate is supported. There were no data on the proposed 12-g rate.

Proposed claim:

> For control of general seed rots, which include those caused by saprophytic organisms such as *Penicillium* and *Aspergillus*, on spring and winter wheat apply 12 or 24 g a.i./100 kg seed.

Two germination studies were provided that showed complete control of these two species with difenoconazole at 6, 12 and 24 g a.i./100 kg seed. In practice, Dividend® would be applied to control a range of other pathogens; therefore, any of the rates specified for seed diseases (above) will suffice.

The proposed claim of control of seed rots (*Aspergillus*, *Penicillium*) on spring and winter wheat with difenoconazole at the 12- and 24-g rate is supported

7.1.4.5 Effectiveness against foliar diseases: powdery mildew, leaf rust and Septoria leaf blotch *(Erysiphe graminis, Puccinia recondita, Septoria tritici* and *S. nodorum)*

Proposed label claim:

> For early season control of powdery mildew, leaf rust and Septoria leaf blotch on winter wheat, apply 24 g a.i./100 kg seed. Dividend® provides control of these diseases for the first six weeks after planting. For full season control, use Tilt® 250E fungicide according to label directions.

The three claimed diseases are most evident in early to mid-summer when foliar fungicides may be applied as needed (e.g., propiconazole, mancozeb, chlorothalonil). The pathogens can infect both spring and winter wheat; however, powdery mildew and Septoria diseases are initiated from infested debris and are more prevalent on winter wheat in Eastern Canada, while rust is initiated from airborne spores and is more prevalent on spring wheat in the Prairies. The original label claim was for fall season control of these diseases, which is appropriate for a seed treatment on winter cereals. However, in the submitted trials, disease was assessed at flag-leaf stage or later, not within six weeks of planting as indicated on the label. Yield data were

collected in some trials; however, the yield impact of these diseases is often difficult to demonstrate and a consistent effect from difenoconazole treatment was not evident.

Powdery mildew: Data were provided; however, difenoconazole did not show any consistent effect on this disease, presumably due to the late assessment dates. There were no data on fall disease levels.

The proposed claim of fall- or early-season control of powdery mildew is not supported.

Leaf rust: Data were provided on winter wheat; however, difenoconazole contributed little to disease control as measured by rust incidence at the late assessment date, either alone or as an addition to systemic foliar fungicides. Studies showing delay of a rust epidemic in spring wheat treated with difenoconazole would be appropriate to support a revised claim in future.

The proposed claim of fall or early season control of leaf rust is not supported.

Septoria leaf blotch: Eight trials were submitted, mainly from U.S. regions (i.e., Georgia, Mississippi, Arkansas) where conditions are not typical of Canadian winter wheat growing areas. Difenoconazole was applied at 12, 20 or 24 g a.i./100 kg seed in the fall, and disease incidence was assessed in early spring (March to June). Percent diseased area was estimated in the leaves of the lower and mid-canopy, or in glumes on the head, on one or more dates. Disease level in the check was adequate in all trials (15–46%). Disease control with difenoconazole in spring was nil in two trials; however, it was typically high in the remaining six trials (mean 64%). These studies showing that difenoconazole is effective in reducing Septoria lesions in early spring suggest, therefore, that it would also be effective in the first six weeks after planting. The 12-g rate was assessed in two trials and provided equal control to the 24-g rate; however, further data would be needed to support this lower rate. Confirmatory data with the proposed use pattern in cooler growing conditions would be valuable, but is not essential to support this claim.

The proposed claim of fall or early control of Septoria leaf blotch in winter wheat with difenoconazole at the 24-g rate is supported.

7.1.4.6 Effectiveness against common root rot *(Bipolaris sorokiniana)*

Proposed label claim:

> For partial control of common root rot in spring and winter wheat, apply 12 or 24 g a.i./100 kg seed. Partial control can mean either erratic control, ranging from good to poor, or consistent control at a level below that generally considered acceptable for commercial disease control.

Common root rot is a soil-borne disease of spring and winter wheat, most significant on the Prairies. Although it can result in whiteheads, the most distinctive symptom is dark brown lesions on the sub-crown internode. It is commonly assessed by determining incidence of plants with lesions, or by a severity index (0 = no lesions, 4 = most of sub-crown internode affected) or a combination of these (incidence in each severity class and the value of the class). The Dividend® label suggests two definitions of partial control (as above). The PMRA considers that widely varying performance is of limited value to growers, and not acceptable from an efficacy standpoint. The term *suppression* is preferred to describe consistent control at a level that is not optimal but is still of commercial benefit.

Ten Canadian trials on spring wheat in the Prairies were submitted, all of which were assessed at or near harvest using the combined index. Difenoconazole was applied at 6, 12 or 24 g a.i./100 kg seed. Both the 12- and 24-g rates were assessed in all trials and were equally effective in reducing common root rot by around 50%, compared with moderate disease indices of 10–56 in the check. A claim of suppression is appropriate for this level of disease reduction and the 12-g rate is sufficient.

Six U.S. studies were submitted from North Dakota (spring wheat) and Oregon (winter wheat). In spring wheat, difenoconazole was applied at 20–24 g a.i./100 kg seed and only symptom severity was measured (2.3–3.5 in the check). The product had a minimal effect on this variable. However, where difenoconazole was applied to winter wheat at 12 g and disease incidence was assessed, this was reduced by 79% compared with 12–40% disease in the check. Therefore, the 12-g rate provides adequate suppression on both spring and winter wheat.

The proposed claim for common root rot in spring and winter wheat with difenoconazole at the 12- and 24-g rate is supported; however, the claim should be described on the product label as "suppression (i.e., consistent control at a level that is not optimal but is still of commercial benefit)". Data showed that the 12-g rate provided adequate suppression.

7.1.4.7 Effectiveness against root rot and crown rot *(Fusarium* spp.*)*

Proposed claim:

> For partial control of Fusarium root rot and crown rot in winter wheat, apply 24 g a.i./100 kg seed. Partial control can mean either erratic control, ranging from good to poor, or consistent control at a level below that generally considered acceptable for commercial disease control.

Root rot and crown rot are typically caused by *Fusarium graminearum* or *F. culmorum* and result in discoloration of plant parts below the soil line and occasionally poor grain fill in mid-season (whiteheads), similar to symptoms of common root rot. Although data have been provided to show activity of difenoconazole against *Fusarium graminearum* on seed (see

7.1.4.4), there is not adequate data to support control or suppression of root rot and crown rot symptoms.

The proposed claim of partial control or suppression of Fusarium root rot and crown rot is not supported.

7.1.4.8 Effectiveness against take-all *(Gaeumannomyces graminis* var. *tritici)*

Proposed label claim:

> For partial control of take-all in spring and winter wheat, apply 24 g a.i./100 kg seed. Partial control can mean either erratic control, ranging from good to poor, or consistent control at a level below that generally considered acceptable for commercial disease control.

Take-all is a sporadic problem in winter wheat and irrigated spring wheat, resulting in root symptoms and whiteheads. Triadimenol seed treatment is currently registered for this disease. The Dividend® label suggests two definitions of partial control for take-all. The term *suppression* is preferred (see 7.1.4.6).

Difenoconazole data consisted of studies from Canadian sites (Prairies, mainly irrigated) and the U.S. Midwest (non-irrigated). Typically, artificial inoculum of *G. graminis* was added to the soil with seed. Seedling emergence, incidence and severity of crown/root symptoms, incidence of whiteheads and yield were assessed.

In eight Canadian spring wheat trials on the Prairies, difenoconazole was applied at 12, 18 and 24 g a.i./100 kg seed in all trials. In four trials, incidence of root symptoms were assessed, with 13–50% of check plants showing disease and 2–16% of these considered severe. These variables were reduced by difenoconazole treatment in all four trials (44–85% control), and this difference was significant at higher disease pressure. In the remaining four trials, incidence of whiteheads and root symptom severity were assessed. The effect of difenoconazole was less prominent in these tests, likely due to the low figures for the variables assessed (less than 5% whitehead and less than three on a 0–5 severity scale in checks). Overall, the 24-g rate did not always provide greatest control of root symptoms compared with 12 or 18 g; however, it typically resulted in fewer whiteheads and in the highest yield figures. Therefore, both 12- and 24-g rates are acceptable.

At seven U.S. sites in the Midwest with winter wheat, inoculum was added to soil or seed, in greenhouse or field studies and difenoconazole was applied at 12, 24, 40 or 60 g a.i./100 kg seed. A more consistent rate effect was seen in these trials, with rates above 24 g providing better take-all control in four of five trials where these were assessed. The 12-g rate was assessed in only one trial, where it gave equivalent control to 24 g, and both rates provided appropriate disease reduction for a suppression claim (51% control). There was no consistent

trend in emergence data; however, yield was consistently and significantly increased with difenoconazole treatment at the 12- and 24-g rates.

The proposed claim for common root rot in spring and winter wheat with difenoconazole at 24-g rate is supported; however, the claim should be described on the product label as "suppression (i.e., consistent control at a level that is not optimal but is still of commercial benefit)". In addition, a 12-g rate should be added.

7.1.4.9 Effectiveness of tank-mix against damping-off *(Pythium* sp.*)*

Proposed label claim:

> For control of damping-off of wheat caused by *Pythium* sp., the recommended rate of Dividend® 360FS should be tankmixed with Apron® flowable seed treatment in accordance with the directions on the Apron® label. Use the Apron® product at the rate of 2 g a.i./100 kg seed. Adhere to all precautions and restrictions on the Apron® label.

Pythium species cause damping-off in a wide range of crops where soils are cool and wet during germination and emergence. In cereals a more common symptom is browning root rot, which occurs in patches of wet soil or phosphorus deficiency. The disease is not easy to quantify; seedlings may show browning root rot at tips, chlorosis and stunting, generally reduced vigour or reduced yield.

The proposed claim was not supported by any data on the tankmix itself. However, Apron® products (either metalaxyl or metalaxyl-M, which contains only the active isomer) are known to control *Pythium* on a variety of crops and have no known activity against the pathogens controlled by difenoconazole. Difenoconazole has no known activity against *Pythium*. Therefore, the two actives, applied at registered rates, are expected to be exclusive and complementary in activity. The tankmix was proposed for the Dividend® 360FS and Dividend® 36FS labels; however, the latter product is for on-farm use and Apron® is not currently registered as an on-farm seed treatment. From a Canadian regulatory aspect, the value of this tankmix is limited as a co-formulation containing both of these actives is under review and expected to be the predominant product in the marketplace.

The efficacy of the proposed tankmix is supported based on weight of evidence, but this use is not accepted for either Dividend® 360FS or Dividend® 36FS due to availability of a co-formulation.

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

Resistance to difenoconazole has not yet been reported. Propiconazole (Tilt® 250E) belongs to the same group of fungicides (triazoles); however, it is recommended on the Dividend® label for

mid-season control of foliar pathogens (i.e., Septoria leaf blotch). A resistance-management statement indicating that both products should not be applied to the same crop would reduce pressure on the pathogen to develop resistance to one or both of these triazoles. However, standard resistance labelling is still under development in Canada and, at present, there are few other chemical control options for wheat growers. Therefore, it is more practical to remove the specific reference to Tilt® and indicate that "for full season control of Septoria leaf blotch, apply a foliar fungicide according to label directions". In future, a resistance-management statement may be required.

7.3 Effects on yield of treated plants in terms of quantity and/or quality

7.3.1 Effects on quality of plant products

Difenoconazole provides control or suppression of plant diseases that affect quality of grain (i.e., smuts, bunts, common root rot and take-all). If not controlled, these diseases can result in downgrading due to reduced kernel size or fungal contamination. In addition, the quality of grain for seed use will be improved by treatment due to reduction in soil-borne and storage contaminants such as *Fusarium*, *Septoria*, *Aspergillus* and *Penicillium*.

7.3.2 Effects on transformation products

Effect on processed grain (e.g., flour) was not assessed. Generally improved quality may be expected (see 7.3.1).

7.3.3 Effects on yield of treated plants

Yield variables (plant count, tiller number, thousand kernel weights, bushel weights, or yield per hectare) were assessed in the majority of field trials. Positive yield effects with difenoconazole can be expected where a yield-limiting disease is present and other determinants such as water, temperature and soil fertility are favourable.

7.4 Phytotoxicity to target plants

Observations on phytotoxicity were recorded in a large number of trials, and no negative effects due to difenoconazole were noted.

7.5 Observations on undesirable or unintended side effects (non-target effects)

7.5.1 Impact on seed viability

Germination and emergence are indicators of seed viability that were measured in the majority of difenoconazole trials. No trends were noted that would indicate reduced viability due to the

product (see 7.4). Tests on stored treated seed were not available. The label includes a disclaimer for reduced germination of seed of poor condition or low viability, and recommends a limited test run before treating entire seed lot.

7.5.2 Impact on beneficial and other non-target organisms

Currently, no biological seed treatments for wheat are registered in Canada. Impact on beneficial or other non-target organisms has not been evaluated; however, no adverse effect is expected from difenoconazole seed treatment due to low application rates (see 6.1).

7.6 Conclusions

Difenoconazole has value as a seed treatment for wheat, providing unique control of dwarf bunt. It also provides another option for growers by controlling common bunt, loose smut, seed-borne *Fusarium* and *Septoria* and general seed rots; in providing early season control of Septoria leaf blotch; and in suppressing common root rot and take-all.

The data showed that a number of claimed diseases were controlled adequately with lowest proposed rates. However, it is recognized that the seed treater cannot normally anticipate presence of individual pathogens; therefore, all effective rates are recommended for the label.

Difenoconazole complements the activity of metalaxyl-M in providing protection of wheat seed and promoting emergence and growth in the presence of a wide range of pathogens. At this time, however, tankmix directions for these two actives are not necessary as they will be available to growers in a co-formulated product.

Table 7.1 Summary

Crop	Winter wheat	Spring wheat
Variety	all	all
Application	seed treatment	seed treatment
Products	Dividend® 360FS Dividend® 36FS	Dividend® 360FS Dividend® 36FS
Diseases	Rates a.i./100 kg seed	
Control Common bunt	6 g 12 g 24 g	6 g 12 g 24 g
Dwarf bunt	12 g 24 g	N/A
Loose smut	12 g 24 g	12 g 24 g

Seedborne Fusarium	12 g 24 g	12 g 24 g
Seedborne Septoria	24 g	24 g
General seed rots	12 g 24 g	12 g 24 g
Early season control Septoria leaf blotch	24 g	N/A
Suppression Common root rot	12 g 24 g	12 g 24 g
Take-all	12 g 24 g	12 g 24 g

8.0 Overall Conclusions

Difenoconazole is formulated as a seed treatment for commercial plant and on-farm application to wheat at rates of 6, 12 or 24 g a.i./100 kg seed. It provides control of common bunt, dwarf bunt, loose smut, seed-borne *Fusarium* and *Septoria*, general seed rots, early control of Septoria leaf blotch and suppression of common root rot and take-all.

The recommended ADI for difenoconazole was calculated to be 0.01 mg/kg/d, based on the lowest NOEL in the chronic rat study at a level of 1.0 mg/kg bw/d (decreased body-weight gains and increased incidence of hepatocellular hypertrophy in both sexes at the next highest dose of 24 mg/kg bw/d) and using a SF of 100.

Metabolism studies submitted demonstrated the fate and disposition of difenoconazole in wheat (grain, straw and forage), potatoes, tomatoes, grapes, ruminants, poultry and rats. In addition, environmental biotic and abiotic transformations were also considered. Based on the results of these studies, the ROC was defined as parent compound only, expressed as difenoconazole equivalents.

The proposed methods of analysis for difenoconazole residues involves sequential extractions and derivitization. The parent compound is quantified by means of GC analysis with N/P detection. Residues unaccounted for by the method are not considered to be of toxicological significance. The LOQs for the method were set at 0.01–0.05 ppm for plant matrices, meat, meat by-products, kidney, liver and fat, and 0.001 ppm for milk.

The program of supervised field trials conducted in Canada and the U.S. involved the application of difenoconazole to wheat seed as a seed dressing. It demonstrated that residues in progeny grain and straw collected at normal harvest were below 0.01–0.05 mg/kg and 0.01 mg/kg, respectively. Considering these results, the proposed MRL for difenoconazole in wheat grain is 0.1 mg/kg.

It is considered unlikely that residue levels greater than 0.01 mg/kg will occur in succeeding crops following use of difenoconazole as proposed (24 g a.i./100 kg seed).

Potential exposure to difenoconazole in the diet is low. At the proposed application rate of 24 g a.i./100 kg seed, residues are not expected to exceed 0.05 mg/kg in animal products such as milk, eggs and meat. On the basis of the Canadian diet, it was estimated that theoretical maximum daily intakes are no more than 20% of the proposed ADI (0.01 mg/kg/d), providing a large safety margin for consumers.

A short-term dermal toxicology study was deemed most relevant for risk assessment for both farmers and commercial seed treaters. Margins of exposure, calculated on the basis of typical North American use patterns, are acceptable for Dividend® 360FS and Dividend® 36FS.

The precautionary label statement for Dividend® 360FS should be modified to include:

> "Wash hands and face after handling and before eating or smoking. When handling Dividend® 360FS, contaminated equipment or seed treated with Dividend® 360FS, wear long-sleeved coveralls over normal work clothing and chemical-resistant gloves. In addition, wear a dust mask when bagging or sewing bags of treated seed or when transferring treated seed to a storage bin."

Dividend® 360FS is for commercial seed treatment only and it is not intended for on-farm use. The label should include a statement clearly indicating this, i.e.:

> "For commercial seed treatment only. Not for on-farm use. Do not use in hopper box, planter box, slurry box or other seed treatment equipment applications at, or immediately before planting."

For consistency with the PHED exposure estimate, and for good hygiene practice, the precautionary label statement for Dividend® 36FS should be modified to include:

> "Wash hands and face after handling and before eating or smoking. When handling Dividend® 36FS, contaminated equipment or seed treated with Dividend® 36FS, wear long pants, a long-sleeved shirt and chemical-resistant gloves."

Difenoconazole is sparingly soluble in water and is not expected to volatilize from moist soil and water surfaces. It is stable to hydrolysis. Laboratory data indicate that difenoconazole is persistent in flooded anaerobic soils. It is also persistent in aerobic soils under various conditions (temperature, moisture content, application rate) when the initial concentration is 1.0 mg a.i./kg soil. The difenoconazole biotransformation rate increased with decreasing application rate, which indicates a possible inhibition of soil microbes at the higher rates. Further laboratory data indicated that difenoconazole has strong affinity to soil and is not mobile; therefore, it has a low potential to leach.

Results from a Canadian terrestrial field dissipation study (dissipation from treated seeds, not from a bare-ground treatment) with the end-use product indicated that the active ingredient difenoconazole was moderately persistent during the first growing season (half-life of 35–63 d). These results were in good agreement with results from laboratory studies with aerobic soil at a low rate of application (DT_{50} of 75 d for an initial concentration of 0.1 mg a.i./kg soil). Carryover of difenoconazole, however, was in the order of 20% with an effective post-application concentration in the fifth year of 300 ：g a.i./g soil. This was in agreement with the potential for carryover indicated by a DT_{90} of 739 d at a low application rate of 0.1 mg a.i./kg soil in the laboratory study. Difenoconazole did not leach in soil under field conditions, confirming laboratory results.

Difenoconazole is not acutely toxic on an oral basis to birds; the LD_{50} for mallard duck is greater than 21,500 mg/kg bw and the NOEL is 2150 mg/kg bw. It is also not toxic to birds on a dietary basis. LC_{50} values for bobwhite quail and mallard duck are 4760 and >5000 mg/kg feed, respectively. The NOEC values are 2500 and 625 mg/kg feed for bobwhite quail and mallard duck, respectively. Comparison of toxicity endpoints with the EEC indicated that difenoconazole, used as a seed treatment, poses low risk to wild birds on an acute and dietary basis.

Proposed decision

It is proposed that full (five-year) registration be granted for Dividend® MG manufacturing concentrate, Dividend® 360FS and Dividend® 36FS.

List of Abbreviations

ACN	acetonitrile
AD	administered dose
ADI	acceptable daily intake
B.C.	British Columbia
bw	body weight
BWG	body-weight gain
CAS	Chemical Abstracts Service
d	day
DI	daily intake
DNA	deoxyribonucleic acid
DNCB	dinotrochlorobenzene
DT_{50}	Decline Time 50%
dw	dry weight
EEC	expected environmental concentration
EPA	Environmental Protection Agency
FC	food consumption
g.a.p.	good agricultural practice (proposed product)
GC	gas chromatography
GI	gastro intestinal
GSD	geometrical standard deviation
h	hour
HDT	highest dose tested
HPLC	high pressure liquid chromatography
IN	Indiana
ind	individual
K_{ow}	octanol/water partition coefficient
LC_{50}	Lethal Concentration 50%
LD_{50}	Lethal Dose 50%
LOD	limit of detection
LOEL	lowest observable effect level
LOQ	limit of quantitation
MAS	maximum average score
MMAD	mass median aerodynamic diameter
mo	month
MOE	margin of exposure
MRL	maximum residue limit
MS	mass spectrometry
MTD	maximum tolerated dose
N/P	nitrogen/phosphorus

List of Abbreviations (cont'd)

NEG	negative
NOEAL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
NZW	New Zealand White
OR	Oregon
P.C.	positive control
PDI	potential daily intake
PHED	Pesticide Handler Exposure Database
PIS	primary irritation score
PMRA	Pest Management Regulatory Agency
ppm	parts per million
RAC	raw agricultural commodity
ROC	residue of concern
SD	Sprague Dawley
SF	safety factor
TA	triazole alanine
TRR	total radioactive residue
$t_{½}$	half-life of elimination
U.S.	United States
WA	Washington
wk	week
yr	year