Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

Regulatory Note

REG2001-10

Spinosad
Success™ 480SC Naturalyte Insect Control Product
Conserve™ 480SC Naturalyte Insect Control Product

The active ingredient spinosad and associated end-use products Success™ 480SC Naturalyte Insect Control Product and Conserve™ 480SC Naturalyte Insect Control Product for the control of insect pests on apples, outdoor ornamentals, and turf have been granted temporary registration under Section 17 of the Pest Control Products Regulations.

This Regulatory Note provides a summary of data reviewed and the rationale for the regulatory decision regarding these products.

(publié aussi en français)

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Foreword

Health Canada's Pest Management Regulatory Agency (PMRA) has issued temporary registration for spinosad technical insecticide, an insecticide developed by Dow AgroSciences LLC, and the associated end-use products Conserve™ 480SC Naturalyte Insect Control Product, for the control of insect pests of outdoor ornamentals and turf, and Success™ 480SC Naturalyte Insect Control Product, for control of obliquebanded leafroller in apple. These products are potential organophosphate replacement products.

Methods for analyzing spinosad in environmental media are available to research and monitoring agencies upon request to the PMRA.

Dow AgroSciences Canada Inc. will be carrying out additional residue, exposure, and value studies as a condition of this temporary registration. Following the review of this additional information, the PMRA will publish a proposed registration decision document and request comments from interested parties before proceeding with a final regulatory decision.

Table of Contents

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

1.1 Identity of the active substance and preparations containing it

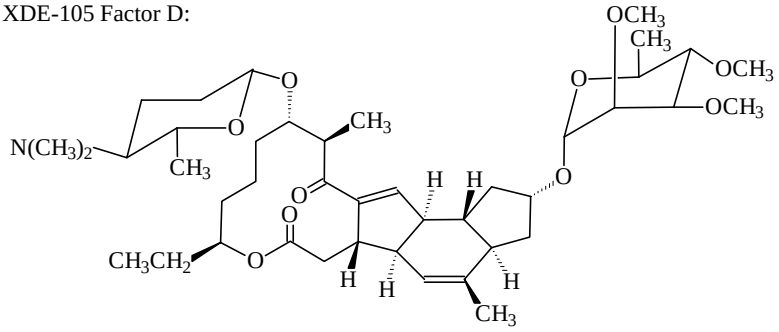
Technical grade active ingredient (TGAI) identification

Active substance:	spinosad containing a combination of XDE-105 factor A and XDE-105 factor D
Function:	Insecticide
Chemical names:	
Proposed International Union of Pure and Applied Chemistry (IUPAC):	**XDE-105 factor A:** (2R,3aS,5aR,5bS,9S,13S,14R,16aS,16bR) -2-(6-deoxy-2,3,4-tri-O-methyl-"-L-mannopyranosyloxy) -13-(4-dimethylamino-2,3,4,6-tetradeoxy-$-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a, 16b-hexadecahydro-14-methyl-1H-8-oxacyclododeca[b]as-indacene-7,15-dione **XDE-105 factor D:** (2R,3aR,5aS,5bS,9S,13S,14R,16aS,16bR)-2-(6-deoxy-2,3,4-tri-O-methyl-"-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetradeoxy-$-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-4,14-dimethyl-1H-8-oxacyclododeca[b]as-indacene-7,15-dione
Chemical Abstract Services (CAS):	**XDE-105 factor A:** 2-[(6-deoxy-2,3,4-tri-O-methyl-"-L-manno-pyranosyl)oxy]-13-[[5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b tetradecahydro-14-methyl-1H-as-indaceno[3,2-d]oxacyclododecin-7,15-dione **XDE-105 factor D:** 2-[(6-deoxy-2,3,4-tri-O-methyl-"-L-manno-pyranosyl)oxy]-13-[[5-(dimethylamino)tetrahydro-6-methyl-2H-pyran-2-yl]oxy]-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b tetradecahydro-4,14-dimethyl-1H-as-indaceno[3,2-d] oxacyclododecin-7,15-dione
CAS numbers:	XDE-105 factor A: 131929-60-7 XDE-105 factor D: 131929-63-0
Molecular formulae:	XDE-105 factor A: $C_{41}H_{65}NO_{10}$ XDE-105 factor D: $C_{42}H_{67}NO_{10}$

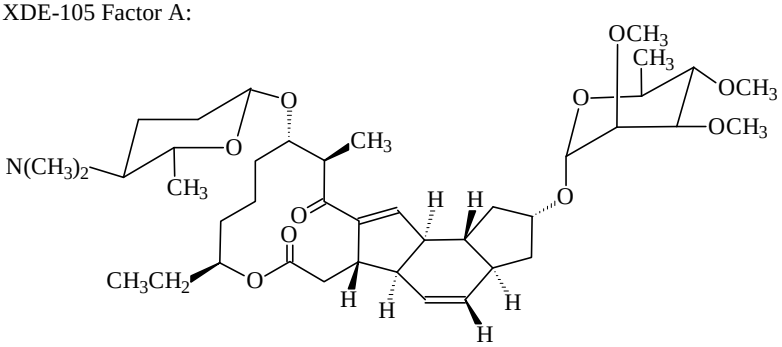
Molecular weights: XDE-105 factor A: 731.45
XDE-105 factor D: 745.45

Structural formulae:

XDE-105 Factor D:



XDE-105 Factor A:



Nominal purity of active ingredient: 90.4%

Identity of relevant impurities of toxicological, environmental, and other significance: The technical grade spinosad does not contain any impurities or microcontaminants known to be Toxic Substances Management Policy (TSMP) Track-1 substances.

1.2 Physical and chemical properties of active substance

Table 1.1 Technical product: spinosad technical insect control product

Property	Result			Comment
	XDE-105	Factor A	Factor D	
Colour and physical state	Light grey – white solid			
Odour	Stale water			

Property	Result			Comment
	XDE-105	**Factor A**	**Factor D**	
Melting point range		84.0–99.5°C	161.5–170.0°C	
Boiling point range	Not applicable			
Density	0.512 g/mL at 20°C			
Vapour pressure at 25°C		3.0×10^{-11} kPa	2.0×10^{-11} kPa	
Henry's law constant at 20°C	1/H	2.65×10^{10}	5.52×10^{7}	Nonvolatile from moist soil and water surfaces
	K	9.22×10^{-13} atm m^3/mol (1 atm = 101.325 kPa)	4.43×10^{-10} atm m^3/mol	
Ultraviolet (UV) – visible spectrum		λ_{max}, (mol/cm) Methanol 243.2 1.10×10^5 201.0 6.77×10^4 Basic 244.0 1.09×10^5 Acidic 244.2 1.08×10^5 200.2 5.73×10^4	λ_{max}, (mol/cm) Methanol 242.6 1.10×10^5 203.0 1.08×10^5 Basic 243.6 1.10×10^5 Acidic 243.8 1.10×10^5 202.8 9.88×10^4	
Solubility in water at 20°C		pH Solubility (ppm) 5 290 7 235 9 16	pH Solubility (ppm) 5 28.7 7 0.332 9 0.053	Factor A: soluble (pH 9, H_2O) to very soluble (pH 5 and pH 7); factor D: practically insoluble (pH 9) to sparingly soluble (pH 7, H_2O) to soluble (pH 5)
Solubility (g/100 mL) in organic solvents		Solvent Solubility Acetone 16.8 Acetonitrile 13.4 CH_2Cl_2 52.5 Amyl acetate 3.69 Hexane 0.448 Methanol 19.0 Isopropanol 3.98 1-Octanol 0.926 Toluene 45.7	Solvent Solubility Acetone 1.01 Acetonitrile 0.255 CH_2Cl_2 44.8 Amyl acetate 2.30 Hexane 743 ppm Methanol 0.252 Isopropanol 0.129 1-Octanol 0.127 Toluene 15.2	
n-Octanol–water partition coefficient (K_{ow})		pH Log K_{ow} 5 2.8 7 4.0 9 5.2	pH Log K_{ow} 5 3.2 7 4.5 9 5.2	Log K_{ow} $ 3; raises concerns about potential bioaccumulation

Property	Result			Comment
	XDE-105	Factor A	Factor D	
Dissociation constant		pK_a = 8.10	pK_a = 7.87	Both compounds will predominate in their neutral forms at most environmentally relevant pH values; adsorption will not be significantly affected by differing soil–sediment pH values
Stability (temperature, metal)	Stable to metals and heat			

Table 1.2 End-use products: Success™ 480SC (NAF-85) and Conserve™ 480SC (Tracer)

Property	Result
Colour	Tan–grey
Odour	Similar to latex paint
Physical state	Liquid
Formulation type	Suspension concentrate
Guarantee	480 g/L (nominal)
Formulants	The product does not contain any U.S. EPA list 1 formulants or formulants known to be TSMP Track-1 substances
Container material and description	Fluorinated, high-density polyethylene plastic containers
Density	1.08 g/mL at 19.7°C
pH of 10% dispersion in water	7.5
Oxidizing or reducing action	Product does not contain oxidizing or reducing agents
Storage stability	Product is stable in fluorinated, high-density polyethylene containers after storage for 1 year at ambient temperature
Explodability	No explosive potential

2.0 Methods of analysis

2.1 Method for analysis of the active substance as manufactured

An isocratic, reverse-phase, high-performance liquid chromatographic (HPLC) method was used for determination of the active components and the significant structurally related impurities (content $ 0.1%) in the technical product. The method has been shown to have satisfactory specificity, linearity, precision, and accuracy.

2.2 Method for formulation analysis

An isocratic, reverse-phase, HPLC method was used for the determination of the active components in the formulations. The method has been shown to have satisfactory specificity, linearity, precision, and accuracy and is suitable for use as an enforcement method.

2.3 Methods for residue analysis

2.3.1 Multiresidue methods for residue analysis

Spinosyns A and D were not recovered using existing multiresidue methods of analysis.

2.3.2 Methods for residue analysis of plants and plant products

The residue of concern (ROC) for spinosad was defined from the plant metabolism studies as two active ingredient compounds, namely spinosyns A and D.

Data gathering method
An HPLC analytical method was submitted for the determination of spinosyns A and D in apples and apple-processed fractions. Recoveries were acceptable and a limit of quantification (LOQ) of 0.01 ppm was reported for both analytes. The analysis was shown to be specific for the two analytes and was free of interferences from coextractives, including a wide range of other potential pesticide residues.

The submitted confirmatory method validation data were adequate to fulfill requirements for a confirmatory method. The data indicated that similar recoveries of spinosad were obtained from spiked apple samples when these samples were analyzed using liquid chromatography – mass spectrometry (LC–MS) operating in the selected ion monitoring mode. Method validation was conducted using spiked apple samples and was found to be adequate for confirmation purposes.

Enforcement method
The proposed analytical enforcement method was equivalent to the data gathering method. The LOQ was reported to be 0.01 ppm. Accuracy and precision of the methods were acceptable as confirmed by validation of recoveries from apples and processed apple products spiked with the two active ingredients of spinosad, namely spinosyns A and D.

Independent laboratory validation (ILV)
An independent method validation trial was conducted to verify the reliability and reproducibility of both methods. There were no significant problems or difficulties encountered during the course of the ILV. Recoveries obtained by the ILV were comparable with those of the data gathering method.

2.3.3 Methods for residue analysis of food of animal origin

The ROC for spinosad was defined from the goat metabolism studies as two active ingredient compounds, namely spinosyns A and D.

The HPLC analytical methods used for plant commodities and processed fractions of apples were shown to be adequate for the analysis of the same analytes determined in livestock meat, meat by-products, fat, and milk. An immunochemical analytical method was submitted for the determination of spinosyns A and D in milk and tissues. Recoveries were acceptable, and an LOQ of 0.01 ppm was reported for both analytes using either analytical method. The analysis using either method was shown to be specific for the two analytes and was free of interferences from coextractives, including a wide range of other potential pesticide residues.

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, exposure to impurities in the active substance, or exposure to transformation products in the active substance

3.1.1 Absorption, distribution, metabolism, and excretion

The absorption, distribution, metabolism, and excretion were determined for ^{14}C-XDE-105 (factors A and D) in male and female Fischer 344 strain rats. Factor A was administered as a single oral low dose of 10 mg/kg, a single oral high dose of 100 mg/kg, and 15 repeated low doses of 10 mg/kg/d, and factor D was given as a single oral high dose of 100 mg/kg.

Factor A
^{14}C-XDE-105 was rapidly absorbed from the gastrointestinal tract (GIT) in all dose groups. Approximately 70–80% of factor A was absorbed, with ~20–30% of the dose eliminated unabsorbed in the feces. Excretion from all sources over a 24-h period was reported to range from ~68 to 80% in single low dose animals and from 67 to 68% for

high-dose animals. The feces were the major route of excretion (82–87% of the doses at 168 h after dosing). Approximately 7–10% of the dose was excreted in the urine, regardless of dose and duration. A sex difference was noted in fecal excretion during the first 24 h after dosing in the high-dose group (52% for males and 28% for females), which indicated a difference in " excretion half-life ($t_{½}$") of fecal excretion between males and females ($t_{½}$" = 13.64 h for males and 28.74 h for females). The multiple low dose group fecal excretion showed some sex differences (76% and 59% for males and females, respectively); however, the $t_{½}$" of fecal excretion was similar between males and females ($t_{½}$" = 9.39 h for males and 10.06 h for females). Blood levels of ^{14}C after the single and multiple 10 mg/kg bw doses were highest at 1 h in both sexes and were reduced by half 6 h (males) and 12 h (females) after dosing, indicating that blood levels remain elevated for longer periods of time in female rats than in male rats. Blood levels of ^{14}C after 100 mg/kg dose were highest at 6 and 2 h in males and females, respectively. At 12 h (males) and 24 h (females) after dosing, the blood levels were reduced by half. Generally, blood levels were observed to remain high for longer periods of time in female rats than in male rats. An increase in activity was noted in some tissues between maximum concentration in the blood phase (C_{max}) C_{max} and ½C_{max}, namely perirenal fat and lymph nodes, which suggested that the test material was still undergoing distribution.

At 168 h after administration of the low dose, the kidneys, liver, and fat of males and females had higher levels than other tissues. In the high-dose group, however, the adrenals (females only), kidneys, lymph nodes, fat, and thyroids had higher levels than other tissues. The total radioactivity remaining in the tissues and carcass of the low- and high-dose animals was <0.6% and <3% of the administered dose (AD), respectively. Also, at 7 d after the 100 mg/kg dose of XDE-105, the radioactivity observed in fat was threefold higher in female rats (40.978 Fg equivalents/g tissue) than in male rats (13.227 Fg equivalents/g tissue). The primary metabolites found in excreta (feces, bile, and urine) were identified as parent (6% of the AD), glutathione conjugates of the parent, and O-demethylated XDE-105. Metabolites in the tissues were characterized as the untransformed XDE-105 and O-demethylated XDE-105. In addition, a cysteine conjugate of XDE-105 and a cysteine conjugated O-demethylated XDE-105 were reported in the feces and were possibly attributed to metabolic action by intestinal microflora, since cysteine conjugates were not detected in any urine and bile. The absorption, disposition, elimination, and metabolism of ^{14}C-XDE-105 demonstrated no appreciable differences based on dose or repeated dosing.

Factor D

At the 100 mg/kg dose, approximately 60% of the AD was absorbed in males and females, with about 35% of the AD recovered from the feces as the unchanged parent. For both sexes, the feces was the major route of elimination (84–96% of the AD at 168 h after dosing); 3–5% of the AD was excreted in the urine. Greater than 68% of the AD was recovered in the feces within the first 24 h following dosing; fecal radioactivity accounted for 34% of the dose (range 23–55%), and the bile contained an average of -36% (range 28–40%). Approximately 21% of the dose was found in the tissues and carcass (range 12–26%) after 24 h. The urine and CO_2 accounted for 3.3 and <0.1% of the dose,

respectively. The excretion kinetics were biphasic, with " and $ excretion half-lives ($t_{½}$) of approximately 6 and 30 h, respectively.

At 168 h after dosing, <1% of the AD was found in the tissues and carcass. The kidneys, liver, and fat of males and females had higher levels of radioactivity than blood or other tissues. The tissue to blood ratio for radioactivity was -100 for fat and 10–30 for the liver, kidneys, and mesenteric lymph nodes. The primary metabolites in excreta were identified as the glutathione conjugates of the parent and O-demethylated factor D; the cysteine conjugate of factor D was tentatively identified as a major metabolite in the feces (-12% of the dose). Metabolites identified in the bile included the glutathione conjugates of the unchanged form and – and O-demethylated forms of factor D. Since cysteine conjugates were not detected in any other specimens, nor were they detected in the bile excretion study, it is possible that these conjugates were formed by gut microflora metabolism of the glutathione conjugates. Metabolites in the tissues were characterized as the – and O-demethylated factor D. The disposition, metabolism, and elimination of ^{14}C-factor D demonstrated no appreciable sex differences.

Marginally lower absorption of factor D was observed (60% of factor D versus 70% of factor A). Based on the results of the toxicokinetics studies for factors A and D, no appreciable differences in absorption, distribution, metabolism, and excretion of spinosad are anticipated.

3.1.2 Acute toxicity: technical and formulation

Technical spinosad, purity 88%, was considered to be of low acute toxicity by the oral route in CD-1 mice and Fischer 344 rats, by the dermal route in New Zealand White (NZW) rabbits, and inhalation routes in Fischer 344 rats [oral and dermal median lethal doses (LD_{50}) > 2.0 g/kg bw; median lethal concentration (LC_{50}) > 5.2 mg/L]. It was nonirritating to the skin and minimally irritating to the eyes of the NZW rabbits. Results of skin sensitization testing of guinea pigs using the Buehler method were negative.

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

Success™ 480SC and Conserve™ 480SC Insect Control Product formulations, containing 48.0% technical spinosad, were considered to be of low acute toxicity by the oral and inhalation routes in Fischer 344 rats and by the dermal route in NZW rabbits (oral and dermal LD_{50} > 2.0 g/kg bw; LC_{50} > 5.0 mg/L). It was minimally irritating when applied to the skin of NZW rabbits and when instilled into the eyes of the same species. Results of skin sensitization testing of guinea pigs using the Buehler method were negative.

3.1.3 Genotoxicity

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

3.1.4 Subchronic and chronic toxicity

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

3.1.4.1 Subchronic and chronic toxicity in the mouse

In a 3-month subchronic toxicity study, XDE-105 (77.6% purity) was administered to 10 CD-1 mice per sex per dose in the diet at dose levels of 0, 0.005, 0.015, 0.045, or 0.12% (equivalent to 0, 7.5, 22.5, 67.5, or 180 mg/kg bw/d) for 13 weeks. The highest dose level was discontinued on day 44 because of mortality (associated with cachexia and hepatic necrosis) of five animals. The lowest observed adverse effect level (LOAEL) was determined to be 0.015% (equivalent to 22.5 mg/kg/d) in mice based on decreased body weight gain in males; clinical signs of toxicity (perineal–ventral soiling and rough, oily hair, **%** only); increased relative and absolute spleen weights in females; treatment-related increases in the incidence of cellular vacuolization in the spleen (**%**) and lymph nodes (**&**); increased incidence of slight multifocal gastric glandular dilation in both sexes; histocytosis in the lymph nodes, hepatocellular cytomegaly in the liver, and cortical tubular regeneration in the kidneys (**%**); and bone marrow necrosis (**&**). At the 0.045% (67.5 mg/kg bw/d) dose level, clinical signs of toxicity (perineal–ventral soiling; rough, oily hair; and decreased body weight gain) were reported in both sexes; hematological [9 HGB (**%**), HCT (**%**), MCV (**&**/**%**), and MCH (**&**/**%**); 8 neutrophils (**&**)] and clinical pathology parameters [8 ALP (**%**), AST (**&**/**%**), and ALT (**&**/**%**); 9 albumin (**%**)] were altered; increased liver and kidney weights were reported in both sexes; and increased spleen weights were reported in females. Treatment-related increases in the incidence and severity of vacuolization were reported in the spleen, lymph nodes, and pancreas in both sexes; the liver (**&**); and the kidneys (**%**), genital tract (ovaries, uterus, cervix, vagina), and adrenal glands (**%**). Additional treatment-related findings were reported at 0.045% (67.5 mg/kg bw/d): necrosis of the bone marrow, lymph nodes (multifocal, both sexes),

and spleen (%); extramedullary hematopoiesis in the spleen (%); skeletal muscle myopathy, multifocal gastric glandular dilation in the stomach, intraalveolar macrophages in the lungs, and histiocytosis in the lymph nodes, stomach, and uterus in both sexes; hepatocellular cytomegaly in the liver (%); and cortical tubular vacuolation–regeneration in the kidneys (%). At 0.12% (180 mg/kg bw/d XDE-105), significant toxicological effects were observed in both sexes which manifested as clinical signs of toxicity (hypoactivity; rough, oily hair coat; rapid respiration and thinness; perineal–ventral soiling); decreased body weight gain; and hematological (9 RBC, HGB, HCT, MCV, MCH, and MCHC; 8 leukocytes), clinical pathology [8 ALP, AST, ALT, and globulin; 9 albumin, glucose, bilirubin, cholesterol (%), and TG (%)], and morphological changes consistent with anemia and renal and liver dysfunction. Treatment-related histopathological changes were similar to those observed at 0.045% (i.e., vacuolation), with increasing severity in both sexes including extramedullary hematopoiesis in the spleen and severe multifocal necrosis in the spleen, lymph nodes, and liver. The no observed adverse effect level (NOAEL) was established at 0.005% (equivalent to 7.5 mg/kg bw/d).

In a carcinogenicity study (MRID 437001505), XDE-105 (88.0% a.i.; 76.14% a.i. factor A and 11% a.i. factor D) was administered to 70 CD-1 mice per sex per dose in the diet, at dose levels of 0, 0.0025, 0.008, or 0.036% (equal to 0, 3.4, 11.4, or 50.9 mg/kg bw/d for males and 0, 4.2, 13.8, or 67.0 mg/kg bw/d for females) for 18 months. Two satellite groups of 10 mice per sex per group were sacrificed at 3 and 12 months. The high-dose females (0.036%) were terminated at day 455 (approximately 15 months) of the study because the maximum tolerated dose (MTD) in this group had been exceeded, as evidenced by decreased feed consumption, marked body weight loss, clinical signs of toxicity, and excessive mortality. The LOAEL is 0.036% (equal to 50.9 and 66.0 mg/kg bw/d in males and females, respectively), based on the decrease in body weight and body weight gain and corresponding decrease in amount of body fat observed at necropsy in both sexes, increased mortality (both sexes), and the effect on hematological parameters [hemoglobin and hematocrit values were significantly decreased at 3 and 12 months in males and at 3 months in females, the white blood cell (WBC) counts were increased in both sexes at 12 months, and the incidence of hypochromasia of erythrocytes was increased in males at 18 months]. The effect on clinical chemistry was manifested as increased activity of aspartate aminotransferase in males. Increase in absolute and relative spleen weights in both sexes was correlated with the increased incidence of extramedullary hematopoiesis. A significant increase in relative liver weights in males was not associated with the histopathological lesions in this organ. The stomach was the most sensitive target organ in mice. Increased incidence of thickened glandular mucosa of the stomach was seen at necropsy in both sexes. In males this correlated histopathologically with an increased incidence and severity of mucosal inflammation and hyperplasia of the glandular gastric mucosa. In addition, the high-dose animals had increased incidence of slight vacuolation and degeneration–inflammation of multiple organs (kidneys, lungs, lymph nodes, pancreas, parathyroid glands, skeletal muscle, tongue, epididymides, ovaries, and uterus). The NOAEL is 0.008% (equal to 11.4 and

13.8 mg/kg bw/d for males and females, respectively). Under the conditions of this study, there was no evidence of carcinogenic potential of XDE-105.

In a supplementary mouse carcinogenicity study (MRID 44123601), XDE-105 (88.0% a.i.) was administered to 50 CD-1 mice per sex per dose in diet at dose levels of 0, 0.0008, or 0.024% (equal to 0, 1.1, or 32.7 mg/kg bw/d for males and 0, 1.3, or 41.5 mg/kg bw/d for females) for 18 months. The satellite group of 10 mice per sex per group was sacrificed at 12 months. The present report was submitted to upgrade a previous study (MRID 43701505) in which the high dose administered to females (67 mg/kg bw/day) caused excessive toxicity and resulted in early termination. This second study was started to comply with test guidelines which require at least three dose levels for a full evaluation of XDE-105. The study was begun during the time of the previous study, and its design included both males and females, but most of the data included in the report are related to the high-dose females. There were no treatment-related effects on mortality, food consumption, food efficiencies, ophthalmological observations, and clinical chemistry. Treatment-related effects occurred in the high-dose males and females. Body weight and body weight gain were reduced in males. Elevated mean WBC counts in both sexes at 12 and 18 months were associated with a chronic inflammation of the glandular mucosa of the stomach. Increased incidence of thickening of the glandular portion of the stomach was observed at necropsy in both sexes. Histopathological evaluations were only conducted on the control and high-dose female mice. Histopathological effects consisted of aggregates of alveolar macrophages in the lungs; sinus histiocytosis of lymph nodes; vacuolation of parathyroid glands; myopathy of skeletal muscle (of the face and tongue); and hyperkeratosis, hyperplasia, and inflammation of the stomach. The incidence of treatment-related histopathological alterations increased with time. There was no increase relative to controls in the incidence of any type of tumor in female mice administered 0.024% XDE-105 up to 18 months.

3.1.4.2 Subchronic and chronic toxicity in the rat

In a 3-month subchronic toxicity study (MRID 43566601), XDE-105 (77.6% purity) was administered to 10 Fischer 344 rats per sex per dose in the diet at dose levels of 0, 0.05, 0.1, 0.2, or 0.4% (equal to 0, 33.9, 68.5, 133.5, or 273.1 mg/kg bw/d in males and 0, 38.8, 78.1, 151.6, or 308.2 mg/kg bw/d in females) for 13 weeks. The high-dose group was discontinued after 44 d due to excessive mortality. The LOAEL for this study was established at 0.05% XDE-105 (equal to 33.9 and 38.8 mg/kg bw/d for males and females, respectively) based on vacuolation in thyroid follicle epithelial cells and lymph node histiocytosis. At 0.1% XDE-105, clinical pathology consisting of increased blood urea nitrogen, increased inorganic phosphorus, and decreased urinary pH in females was observed. Absolute and relative spleen weights were increased in females; histopathological lesions reported were multifocal granulomatous vacuolation of Kupffer cells, mild cardiomyopathy in males, and lymph node enlargement and skeletal muscle multifocal degeneration–regeneration in females. Splenic and lymph node histiocytosis and thyroid cell vacuolation were reported in both sexes. At 0.2% (equal to 133.5 and 151.6 mg/kg bw/day for males and females, respectively), significantly decreased body

weight, body weight gain, and feeding efficiency were reported in both sexes; food consumption was significantly decreased in females only. Hematological [9 HGB (%/&), HCT (%), MCV (&/%), and MCH (&/%); 8 reticulocytes (&/%) and leukocytes (&/%)] and clinical pathology parameters [8 AST (%/&), BUN (&), and inorganic phosphorus (&); 9 triglycerides (%) and urinary pH (%/&)] were altered, and absolute and relative heart, kidneys, spleen, adrenals, and thyroid–parathyroid weights were increased in both sexes; liver and uterus weights were increased in females only, and prostate weight was increased in males. Treatment-related gross pathology was reported as enlarged spleen, thyroid, kidneys, and lymph nodes. Increased incidence and severity of vacuolization were reported in both sexes in liver (Kupffer cells), kidneys, and thyroid; vacuolar change was reported in the lymph nodes, oviduct, uterus, and adrenals in females. Moderate to marked histiocytosis was reported in the spleen and lymph nodes of both sexes at 0.2%. Other histopathological lesions reported were multifocal granuloma in the liver in both sexes, hyperkeratosis in the stomach in both sexes, and alveolar macrophages in females. Persistent clinical signs of intoxication were noted only at 0.4% XDE-105 and consisted of deep, rapid, or labored breathing, hypothermia, thinness, chromorhinorrhea, piloerection, and distended penis. Markedly reduced body weight gain, food consumption, and feeding efficiency were reported in both sexes at 0.4%. There was considerable evidence of disruption of hematopoiesis at doses of 0.4%, with both sexes exhibiting microcytic, hypochromic anemia (9RBC, HGB, HCT, MCV, MCH, and MCHC; 8 nucleated RBCs and leukocytes, polychromasia, and anisocytosis). Clinical pathology was altered significantly in both sexes [8BUN, total bilirubin, ALP, AST, ALT, GGT, inorganic phosphorus, sodium, potassium, and cholesterol (%); 9creatinine and triglycerides (%), albumin, globulin, and total protein]. Histopathological effects observed in both sexes at 0.4% were multifocal granuloma, chronic focal inflammatory necrosis, and multifocal hepatocellular vacuolation in the liver; multifocal tubular vacuolation and tubular necrosis in the kidneys; multifocal degeneration–regeneration in skeletal muscle; hypocellularity in bone marrow; marked diffuse follicular epithelial cell vacuolation in the thyroid; alveolar macrophages and acute multifocal inflammation in the lungs; vacuolar change in the lymph nodes, spleen, and thymus; histiocytosis and necrosis in the lymph nodes; atrophy in the spleen and thymus; diffuse acinar cell vacuolation in the pancreas; and glandular dilation in the stomach. Vacuolation of the reproductive organs (vagina, oviduct, uterus, cervix, epididymis, and prostate) was also evident. Other lesions of note were atrophy of the uterus and adrenal cell vacuolation in females and bilateral hypospermatogenesis in the testes of males. No NOAEL was established for this study.

In a second study, groups of Fischer 344 rats (10–20 per sex per group) were given diets containing 0, 0.003, 0.006, 0.012, and 0.06% XDE-105 for 13 weeks. These doses were equal to 0, 2.2, 4.3, 8.6, and 42.7 mg/kg bw/d for males and 0, 2.6, 5.2, 10.4, and 52.1 mg/kg bw/d for females. There were two “recovery” groups (10 per sex per group) assigned to the control and high dose which received control feed for another 4 weeks.

No deaths occurred in the study. No treatment-related effects were evident on clinical signs, body weights, food consumption, feeding efficiency, ophthalmology, organ weights, clinical pathology [including thyroxine (T_4) levels], or gross pathology. Very slight to slight thyroid follicle epithelial cell vacuolation was seen in most of the high-dose (0.06%) group (10 of 10 males and 8 of 10 females). A similar incidence was seen in the recovery group, but the severity was slightly reduced in the high-dose recovery group with the limited evidence of reversibility. A NOAEL of 0.012% (8.6 mg/kg bw/d for males and 10.4 mg/kg bw/d for females) could be set for this study. The LOAEL was 0.06% (42.7 mg/kg bw/d for males and 52.1 mg/kg bw/d for females) based on very slight to slight thyroid follicle epithelial cell vacuolation noted in both sexes after 13 weeks of treatment followed by a 4-week recovery period.

In a rat chronic–oncogenicity–neurotoxicity study, XDE-105 (88% a.i) was administered in the diet to Fisher 344 rats (65 per sex per group) for 2 years at dose levels of 0, 0.005, 0.02, 0.05, or 0.1% w/w (equal to 0, 2.4, 9.5, 24.1, or 49.4 mg/kg bw/d in males and 0, 3.0, 12.0, 30.3, or 62.8 mg/kg bw/d in females). A group of 15 rats per sex per group was designated as a satellite group and sacrificed at 12 months. Ten satellite rats per sex per group underwent neurobehavioural testing at pre-test and 3, 6, 9, and 12 months, and a subset of 5 rats per sex in the control and high-dose group were assessed for neuropathology. The remaining satellite rats were evaluated for chronic toxicity at 12 months. The dose level in the satellite groups was as follows: 0, 4.6, 9.2, 23, or 46 mg/kg bw/d in males and 0, 5.7, 11.4, 28.5, or 57.0 mg/kg bw/d in females.

The high-dose group (0.1%) was terminated on test days 714 and 611, respectively, for males and females, due to excessive mortality, indicative of exceeding a maximum tolerated dose (MTD). Therefore, 0.05% groups were evaluated as the high-dose group for histopathological findings and organ weights. The LOAEL of 0.02% (equal to 9.5 mg/kg bw/d in males and 12.0 mg/kg bw/d in females) was set based on the increase in the incidence of slight vacuolation of the follicular epithelial cells of the thyroid in both sexes. In the 0.05% group, there was an increase in the incidence of vacuolation of epithelial cells of the thyroid (mild to moderate) in both sexes, mild to moderate necrosis and inflammation of the thyroid gland, and an increase in the absolute and relative thyroid weights in females. The severity of treatment-related alterations in the thyroid progressed with time. The incidence of slight inflammation of the lungs was increased in both sexes at 0.05%. In the 0.1% group, there was decreased body weight and body weight gain and effects on hematology (increased WBC count in females) and clinical chemistry (increase in serum alkaline phosphatase, aspartic aminotransferase, and BUN levels). Histologic examination of tissues of rats receiving 0.1% were not conducted at 24 months because the MTD was exceeded. At 12 months, the following histopathological changes were observed in both sexes: perineal soiling; decreased body fat reserves; degenerative and inflammatory lesions in the heart; hydrothorax; inflammatory changes in the lungs; vacuolation of the tubular epithelial cells of the kidneys; degenerative and inflammatory changes of the skeletal muscles; aggregates of reticuloendothelial cells in the liver, spleen, and mesenteric lymph nodes; degenerative and inflammatory changes in the glandular mucosa of the stomach; and follicular epithelial vacuolation and inflammation

of the thyroid gland. The NOAEL for chronic toxicity is 0.005% (equal to 2.4 and 3.0 mg/kg bw/d for males and females, respectively). Based on the conclusion reached following an independent third-party histopathology peer review, treatment-related histopathological lesions in the thyroid gland from rats given 0.05% support the assessment that this dose level fairly represents the MTD for the purposes of assessing carcinogenic potential in rats. Therefore, it was concluded that technical spinosad has no carcinogenic potential at the dose level up to and including 0.05% (24.1 and 33.3 mg/kg bw/d, respectively, for males and females).

3.1.4.3 Subchronic toxicity in the dog

In a 13-week subchronic toxicity study (MRID 434441-02), XDE-105 (spinosad, 88% purity) was administered to four beagle dogs/sex/dose in diet at dose levels of 0, 150, 300, or 900 ppm (females only), or 1350 ppm (males only) (equal to 0, 4.89, 9.73, or 33.4 mg/kg bw/d in males and 0, 5.38, 10.47, or 29.9 mg/kg bw/d in females). The concentration of 1350 ppm was reduced on day 38 to 900 ppm because one male dog died due to test substance induced weakness. In the high-dose group, clinical signs of toxicity included periocular sebum, decreased spontaneous motor activity, unsteady standing posture, watery, red–black stools, and loose stools. One male was killed in extremis. Decreases in mean body weights (19% in males and 12% in females) and food consumption were reported in both sexes at 1350/900 ppm, particularly in males. Evidence of anemia was found in the hematological examinations (decreases in hematocrit, hemoglobin, and erythrocytes) and in increases in WBC counts, lymphocytes, and reticulocytes. Decreases in albumin and A/G ratio and increases in globulin, total cholesterol, ALT, AST, and ALP were also observed. The increases in the latter three were slight, in only one sex and in one case due to only one dog. There were treatment-related increases in spleen, thyroid, pancreas, and liver weights in both sexes at 1350/900 ppm which were supportive of the microscopic and clinical chemistry results. Cytoplasmic vacuolation or vacuolated cell aggregation was observed in the spleen, lymph nodes (mesenteric, cervical, faucial tonsil, and lymph follicles in the ileum, colon, cecum, and rectum), pancreas, parathyroid, nerve tissue (brain, cervical, thoracic and lumbar spinal cord), and liver in both sexes; foamy cell aggregation in the lungs and atrophic gastric mucosa were also reported in both sexes. Kupffer cell proliferation in the liver and vacuolation in the adrenal cortical cells and thyroid C-cells were reported in females only, and vacuolation in the testes was noted in males. At 1350/900 ppm, arteritis was observed in a variety of tissues in both sexes; atrophic white pulp in the spleen and thymic atrophy (males only) and focal necrosis – cellular depletion in the bone marrow (females only) were also observed. At 1350/900 ppm in males, decreased spermatogenesis and an increase in spermatid giant cells were reported in the testes. At 300 ppm (equal to 9.73 and 10.47 mg/kg bw/d in males and females, respectively), treatment-related increases were reported in the incidence of cellular vacuolation in the spleen, lymph nodes [mesenteric (**%**/**&**), cervical (**&**), faucial tonsil (**%**/**&**); and lymph follicles in the ileum (**%**/**&**), colon (**%**/**&**), cecum (**&**), and rectum (**%**)], and acinar cells in the pancreas (**%**/**&**); foamy cell aggregation in the lungs and atrophic gastric mucosa were noted in females only. The NOAEL is 150 ppm (equal to 4.89 and 5.38 mg/kg bw/d in males and females, respectively). The LOAEL was

300 ppm (equal to 9.73 and 10.47 mg/kg bw/d in males and females, respectively) based on increased incidence of cellular vacuolation in lymphoid tissues and pancreas in both sexes and foamy cell aggregation in the lungs and atrophic gastric mucosa in females only.

In a 1-year toxicity study (MRID 43701504), XDE-105 (87.2% a.i) was administered in the diet to four beagle dogs per sex per dose for 52 weeks. The dose level was initially designated at 0, 50, 100, and 300 ppm but changed to 0, 60, 120, and 360 ppm at 14 weeks when the feeding amount was reduced from 300 to 250 g/dog/d to prevent obesity. Average test compound intakes in the 50/60, 100/120, or 300/360 ppm groups were 1.44, 2.68, or 8.46 mg/kg bw/d for males and 1.33, 2.72, or 8.22 mg/kg bw/d for females. There was no mortality during the study. No treatment-related effects were observed on clinical signs, body weights, food consumption, ophthalmology, hematology, urine analysis, and gross pathological findings. The LOAEL of 300/360 ppm (equal to 8.22 and 8.44 mg/kg bw/d for males and females, respectively) was set based on the changes in the clinical chemistry parameters indicative of hepatotoxicity [increase in serum alkaline aminostransferase (GPT), aspartate aminotransferase (GOT), and triglycerides levels in males]. In addition, histopathological examination revealed vacuolated cell aggregates in the various lymphoid tissues including the spleen, faucial tonsil, lymph nodes, and intestine in both sexes. Arteritis was recorded in the epididymis of one male and in the cerebral meninges of one female. Two males also showed glandular cell vacuolation of the parathyroid. Statistically significantly increased absolute and relative thyroid gland weights observed in three of the four females at the 300/360 dose level were not associated with microscopic changes in this organ. The NOAEL is 100/120 ppm (equal to 2.68 and 2.72 mg/kg bw/d for males and females, respectively).

3.1.4.4 Subchronic toxicity in the rabbit

In a repeat-dose dermal toxicity study (MRID 435575-03), XDE-105 (spinosad, 88.0% a.i.) was applied to the shaved skin of five NZW rabbits per sex per dose at dose levels of 0, 100, 500, or 1000 mg/kg bw/d for 6 h/d, 5 d/week during a 21-d period. Test animals were observed daily for clinical signs and mortality, and weighed weekly. Prior to sacrifice at 21 d, blood samples were collected for hematological and biochemical observations. After sacrifice, animals were subjected to macroscopic and limited microscopic examination and selected organs were weighed. Under the conditions of the test, dermal application of XDE-105 at doses up to 1000 mg/kg bw/d (a limit dose) resulted in no treatment-related toxicity. The NOAEL for dermal and systemic toxicity in this study was 1000 mg/kg bw/d; no LOAEL was established. Although the study was performed according to Organisation for Economic Co-operation and Development (OECD) guidelines, histopathological examination of the target organs and tissues identified in the database was not performed, which limited the utility of the study.

3.1.5 Reproductive and developmental toxicity

In a two-generation (two litters per generation) reproduction study (MRID 43701506), XDE-105 (spinosad, 88.0% a.i.) was administered to 30 Sprague-Dawley rats per sex per dose in diet at target dose levels of 0, 0.005, 0.02, and 0.2% w/w (equivalent to 0, 3, 10, and 100 mg/kg bw /d). First parental generation (P_1) was mated twice to produce first generation offspring F_{1a} and F_{1b} litters. F_{1a} were selected to become the second generation offspring F_2 generation and produce F_2 litters. Exposure to the P_1 animals began at 6 weeks of age and lasted for 10 weeks prior to the first mating to produce the F_{1a} pups. Exposure to the F_{1a} pups (30 per sex) began at weaning and lasted for at least 12 weeks prior to mating to produce the F_2 pups. One week after weaning the F_{1a} pups, the P_1 animals were mated again to produce an F_{1b} generation. All animals were mated on a 1:1 ratio. Parental toxicity was characterized in the high-dose animals by treatment-related increases in dystocia (8 3 and 17%) and vaginal bleeding after parturition (8 24 and 23%) and associated increases in mortality (8 7 and 10%, P_1 and F_{1a} dams). P_1 dams had decreased body-weight gains (about –21%) significantly different by day 21 of gestation for both the F_{1a} and F_{1b} (litters), probably due to smaller litter size. Decreased body-weight gain was associated with a slight (about –9%) decrease in food consumption. Body-weight gains decreased about –6% by day 4 of lactation and recovered by the end of lactation. No significant effects on body weight and food consumption were noted in F_{1a} dams. In high-dose animals, the following treatment-related effects were observed: increased absolute and relative weights of the heart, kidneys, liver, spleen, and thyroid (P_1 and F_{1a}, both sexes); and histopathology in the lungs, mesenteric lymph nodes, spleen, and thyroid (P_1 and F_{1a}, both sexes), heart (P_1 males only), kidneys and prostate (P_1 and F_{1a}, males only), and stomach (P_1 and F_{1a}, females only). Histopathology in the lungs was characterized by an increased incidence of multifocal subacute to chronic inflammation of the interalveolar septae along with multifocal aggregates of alveolar macrophages. For the spleen and mesenteric lymph nodes, the histopathology was described as sinus histiocytosis. The primary lesion in the thyroid was diffuse cytoplasmic vacuolation of the follicular epithelial cells with associated chronic active inflammation and necrosis. Treatment-related histopathologic lesions found exclusively in the high-dose males were degeneration of the myocardium with or without inflammation, tubular degeneration in the kidneys, and chronic active inflammation of the prostate. The treatment-related histopathologic lesions found exclusively in the high-dose females were characterized as dilation of the glandular crypts with cellular debris in the pyloric region of the stomach. There were no treatment-related effects noted in the reproductive function or performance of the high-dose P_1 or F_{1a} adults. For the low- or mid-dose P_1 or F_{1a} adults, no treatment-related effects were noted in the clinical signs, mortality, food consumption, body weights, reproductive function and performance, organ weights, gross pathology, or histopathology. The LOAEL for systemic toxicity is 100 mg/kg bw/d based on decreased body weight and body-weight gain in P_1 dams during F_{1a} and F_{1b} gestation; increases in heart, kidneys, liver, spleen, and thyroid weights (both sexes); corroborative histopathology in the spleen and thyroid (both sexes) and heart and kidneys (males only); and histopathologic lesions in the lungs and mesenteric lymph nodes (both sexes), stomach (females only), and prostate. The NOAEL for systemic toxicity is

10 mg/kg bw/d. Reproductive toxicity, which appears to be related to the systemic toxicity in the dams, was characterized in the high-dose offspring by decreases in the numbers of pups born alive (9 22–35%) and the mean litter sizes (9 23–38%) on days 1 and 4 (F_{1a}, F_{1b}), decreased gestation survival indices (F_{1b} and F_2), and statistically significant decreased pup body weights on lactation days 14 and 21 (up to 11%) for F_{1a} and F_{1b} and on lactation day 21 for F_2 (about –6%). There were no treatment-related gross pathologic changes noted in the offspring of any generation or treatment group. For the low- and mid-dose treatment groups, no treatment-related effects on the body weights, clinical signs, litter size, or survival indices were noted. The LOAEL for reproductive–offspring toxicity is 100 mg/kg bw/d based on decreases in litter size (number of pups born alive), survival (F_2 litters only), and body weights in the offspring and increased incidence of dystocia and vaginal bleeding after parturition. The NOAEL for reproductive–offspring toxicity is 10 mg/kg bw/d.

In a rat developmental toxicity study (MRID 43557505), XDE-105 was administered in 0.5% aqueous Methocel A4M to groups of 30 mated Sprague-Dawley strain rats by gavage at dose levels of 0, 10, 50, or 200 mg/kg bw/d from gestation days 6 through 16 (gestation day 0 was the day sperm were found in vaginal lavage or a vaginal plug was found). Maternal toxicity was reported at the highest dose tested, manifesting as decreased body-weight gain during the gestational dosing period (46% less than that for the control group during gestation days 6–9, 11% less for the days 9–12 interval, and 10% less for the days 6–16 interval). No other treatment-related effects were observed in absolute and relative organ weight changes, and no animals were described in the report as having dose-related clinical signs. Treatment-related increases in delayed ossification of the sternebrae were reported at $50 mg/kg bw/d. The NOAEL for maternal toxicity is 50 mg/kg bw/d. The LOAEL was set at 200 mg/kg bw/d based on treatment-related decrease in maternal body-weight gain during the dosing period (gestation days 6–16). The NOAEL for developmental toxicity was 10 mg/kg bw/d. The LOAEL was set at 50 mg/kg bw/d based on treatment-related increases in delayed ossification of the sternebrae reported at 50 mg/kg bw/d and above. It was important to note that the incidence of delayed ossification in sternebrae in the concurrent control was higher than that in the historical control (both fetuses and litters), and no statistically significant differences between control and treated groups were identified. No other ossification sites were affected, and variations of this type are considered transient effects. The NOAEL for teratogenicity was 200 mg/kg bw/d, the highest dose tested.

In a rabbit developmental toxicity study (MRID 43414521), XDE-105 was administered in 0.5% aqueous Methocel A4M to groups of 20 mated NZW strain rabbits by gavage at dose levels of 0, 2.5, 10, or 50 mg/kg bw/d from gestation days 7 through 19 (gestation day 0 was the day mating occurred). Maternal toxicity was observed at the highest dose tested (50 mg/kg bw/d), indicated by decreased defecation (in 6 of 20 animals compared with 2 of 10 in the control group), decreased body-weight gain (31% less than that for the control group during gestation days 7–20), and reduced food consumption (the high-dose group consumed an average amount that was 74% of the control group value). A marginal increase in abortions (2 of 17) was observed in the high-dose group which was above the

historical control value for abortions (1 of 17). The NOAEL for maternal toxicity is 10 mg/kg bw/d. The LOAEL is 50 mg/kg bw/d based upon decreased defecation, decreased body-weight gain during the dosing period, and decreased food consumption and a marginal increase in abortions in does at 50 mg/kg bw/d which may be treatment related. There were no developmental effects that could be attributed to administration of XDE-105. The NOAEL for developmental toxicity–teratogenicity is $50 mg/kg bw/d.

3.1.6 Neurotoxicity (acute, subchronic, and chronic)

In an acute neurotoxicity study (MRID 435575-01), groups of fasted, 8-week-old Fischer 344 rats (10 per sex per dose) were given a single oral dose of XDE-105 (spinosad, 88% a.i.) in aqueous methyl cellulose at doses of 0, 200, 630, or 2000 mg/kg bw/d and observed for 15 d. The highest dose was considered to be a limit dose. Functional observational battery (FOB) and motor activity testing were performed once prior to test material administration (dosing day –1), approximately 5–6 h postdosing (day 1), and then on days 8 and 15 of the study. Body weights were determined on days –1, 1, 2, 8, and 15. Neuropathology of central and peripheral nervous tissues was conducted in 5 rats per sex per dose at study termination. The only significant finding was a transient decrease in body weight after dosing in the 630 and 2000 mg/kg bw/d males and females. No evidence of neurotoxicological effects was observed at any of the dose levels. There were no effects on FOB, motor activity, and histopathology of the nervous system. The NOAEL for acute neurotoxicity is 2000 mg/kg bw/d. The LOAEL is >2000 mg/kg bw/d. The positive control data provided appropriate positive response (the data were adopted from the chronic–toxicity–carcinogenicity–neurotoxicity study in rats).

In a subchronic neurotoxicity study (MRID 435575-04), XDE-105 (spinosad, 87.9% a.i.) was administered to 10 Fischer 344 rats per sex per dose in diet at dose levels of 0, 0.003, 0.006, 0.012, or 0.06% (equal to 0, 2.2, 4.3, 8.6, or 42.7 mg/kg bw/d for males and 0, 2.6, 5.2, 10.4, or 52.1 mg/kg bw/d for females) for 7 d/week for 13 weeks. All animals underwent neurobehavioural testing at pre-test and at 4, 8, and 13 weeks. At termination, 5 rats per sex per dose were assessed for gross neuropathology, and 5 rats per sex from the control and high-dose groups were examined for histopathological observations of the nervous tissues.

There were no compound related effects on the FOB, motor activity, or histopathological observations of the nervous system. The NOAEL is 0.06% (42.7 and 51.2 mg/kg bw/d for males and females, respectively). The LOAEL is >0.06%.The positive control data provided appropriate positive response (the data were adopted from the chronic–toxicity–carcinogenicity–neurotoxicity study in rats).

In the chronic neurotoxicity portion of this study (MRID 43710503), no effects on the FOB or motor activity were observed after 3, 6, 9, or 12 months of dosing at a dietary level of 0.1% (equal to 46.0 mg/kg bw/d in males and 57.0 mg/kg bw/d in females). Histopathological observations of the central and peripheral nervous systems of the control and high-dose animals revealed no treatment-related lesions. The positive control

data provided appropriate positive response. The LOAEL for neurobehavioural and neuropathic effects was not established. The NOAEL for chronic neurotoxicity is 0.1%, the highest dose tested (equal to 46.0 mg/kg bw/d in males and 57.0 mg/kg bw/d in females).

3.1.7 Integrated toxicological summary

A detailed review of the toxicology database available for the insecticide, spinosad, has been completed. Data submitted were, for the most part, complete and comprehensive and included the full battery of studies currently required for registration purposes. Studies were conducted in conformance with currently acceptable international testing protocols. The scientific and regulatory quality of the toxicology data base is considered sufficient to generally define the toxicity of this chemical. However, mechanistic data to elucidate the etiology of the toxicological effects in multiple tissues were not provided, however information concerning the likely mode of action will be available to the PMRA from an indepepdent expert in the field of compound-induced phospholippidosis.

In metabolism studies in rats, spinosad (factors A and D, XDE-105) was rapidly absorbed, distributed, and metabolized and almost completely excreted within 168 h. The major route of excretion was feces (82–87%), with lesser amounts in the urine (7–10%). Bile accounted for about 36% of the radioactivity and contained mostly the glutathione conjugates of the unchanged form as well as – and O-demethylated forms of factors A and D. At 168 h after administration of the low dose, the kidneys, liver, and fat of males and females had higher levels than other tissues. In the high-dose group, however, the adrenals (females only), kidneys, lymph nodes, fat, and thyroids had higher levels than other tissues. A slight sex difference in elimination was observed in high-dose animals, with females exhibiting a lower rate of excretion of radioactivity compared with males. However, the total radioactivity remaining in the tissues and carcass of the low- and high-dose animals was <0.6% and <3% of the AD, respectively, for both sexes. The primary metabolites in excreta were identified as parent (XDE-105), the glutathione conjugates of the parent, and O-demethylated factor A/D; a cysteine conjugate of XDE-105 and O-demethylated XDE-105 were reported in the feces and were possibly attributed to metabolic action by intestinal microflora, since cysteine conjugates were not detected in any urine and bile.

Acute dosing revealed that technical spinosad and Success™ 480SC and Conserve™ 480SC formulations were of low toxicity by the oral, inhalation, and dermal exposure routes to laboratory animals. Technical spinosad was non-irritating to rabbit skin and minimally irritating to rabbit eyes, whereas Success™ 480SC and Conserve™ 480SC induced minimal skin and eye irritation. Neither possessed skin-sensitizing properties when tested on guinea pigs according to the Buehler method. Spinosad did not demonstrate any acute neurotoxic effects.

Repeated subchronic dietary exposure of spinosad in mice, rats, and dogs resulted in death, marked reductions in body weight, clinical signs of toxicity, and anemia.

Pathological examinations revealed that spinosad induced similar lesions in a wide range of tissues in all species studied [the most prominent lesions consisted of cellular vacuolation, inflammatory changes (including necrosis), and regenerative–degenerative changes, in selected tissues. The most sensitive species to spinosad toxicity was the dog, followed by the mouse, rat and rabbit. Target organ sensitivity was not entirely consistent across species, with the following organs identified as the major targets: thyroid (rat - follicular epithelium and dog - parafollicular cells), kidneys, lymphoid organs (spleen and lymph nodes), liver (rat and dog), bone marrow, and reproductive organs (ovaries - mice, testes - dogs and rats, uterus - rats and mice, vagina - rats and mice, epididymides, and prostate - rats). Changes in clinical pathologic parameters were generally consistent with the type and extent of cellular injury or organ dysfunction reported. The cellular vacuolation in mice, rats and dogs were suggested by study authors to be consistent with phospholipidosis, which was claimed to be a condition resulting from the accumulation of polar lipid lysosomes. Vacuolatory changes at the light microscopic level were associated with the accumulation of cytoplasmic lamellar inclusion within lysosomes.

In chronic–oncogenicity dietary studies with rats and mice, there was no evidence that spinosad was carcinogenic, which was further supported by the negative findings in the genotoxicity studies.

Spinosad is not teratogenic. It did not produce irreversible or deleterious developmental changes in rats or rabbits. Evidence for increased susceptibility of fetuses for delayed ossification following in utero exposure to spinosad was considered equivocal, since concurrent control values exceeded the historical control, only one ossification site was affected, statistical significance was not identified, and the effect is considered transitory in nature.

Administration of spinosad in a multigeneration reproduction study resulted in no clinical signs or body-weight effects in either sex following high-dose spinosad administration during mating and gestation periods. However, following parturition, high dose females had an increased incidence of dystocia and postpartum vaginal bleeding, increased mortality, and decreased litter size (number of pups born alive), pup survival, and pup weights at 100 mg/kg bw/d. The NOAEL for maternal and reproductive–developmental–offspring effects was 10 mg/kg bw/d. On the basis of NOAELs and LOAELs in the reproduction study, no increased susceptibility of pups was demonstrated.

Overall, female animals were slightly more affected by treatment with spinosad than males. A number of end points of concern were identified in endocrine, reproductive, lymphoid, and hematopoietic organs of multiple test species. The apparent steepness of the dose–response curve resulted in the premature termination of high-dose animals (short term: -100–200 mg/kg bw/d; chronic: -50 mg/kg bw/d) insubchronic and chronic studies with mice and rats. Increasing dose and duration of exposure produced an increase in the severity of lesions and a broadening of toxicological effects to include major organ systems in all test species. Toxicity in sentinel organs, such as thyroid, ranged from vacuolar change at a relatively low dose levels and/or short study duration to

inflammation and necrosis at higher doses(maximum tolerated dose) in longer duration studies. Anemia was observed in subchronic studies in rats, mice, and dogs, given high doses of spinosad. Extramedullary hematopoesis indicated that the anemia was regenerative and potentially reversible, however, bone marrow necrosis occurred in a few rats at a dose that exceeded the maximum tolerated dose.

3.2 Determination of acceptable daily intake (ADI)

The recommended ADI for spinosad is 0.009 mg/kg bw/d. The most appropriate study for selection of toxicity end points for chronic dietary exposure was the 1-year study with a NOAEL of 2.7 mg/kg bw/d in dogs where vacuolation occurred in the parathyroid cell vacuolation and lymphoid tissues, increased thyroid weights (females), and increases in serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and triglycerides levels occurred in dogs given 8.2 mg/kg bw/d. An additional safety factor (SF) of 3× (in addition to the usual SF of 100 for inter- and intra-species variation) was deemed necessary based on the concern for the severity of effects noted in the rat multigeneration reproduction study and effects on thyroid, lymphoid, and hematopoietic tissue in several species (mice, rats, and dogs). In the absence of clear evidence of age-related sensitivity, an SF of 300 was applied to the NOAEL of 2.7 mg/kg bw/d as follows:

$$\textbf{ADI for Spinosad} = \frac{\textbf{NOAEL}}{\textbf{SF}} = \frac{\textbf{2.7}}{\textbf{300}} = \textbf{0.009 mg / kg bw / d}$$

The ADI of 0.009 mg/kg bw/d provides a margin of exposure(MOE) of 1111 for reproductive–offspring toxicity (NOAEL = 10 mg/kg bw/d). The maximum acceptable intake for a 60-kg person, calculated according to the formula ADI × 60 kg, is 0.54 mg/d.

3.3 Acute reference dose (ARfD)

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

3.4 Toxicology end-point selection for occupational and bystander risk assessment

Repeated subchronic and chronic dietary exposure to spinosad in mice, rats, and dogs induced a similar spectrum of lesions in a wide range of tissues and in all species studied. Target organ sensitivity was consistent across species, with the following organs identified as the major targets: thyroid (rat and dog), kidneys, lymphoid organs (spleen and lymph nodes), liver, bone marrow, and reproductive organs (ovaries, testes, uterus, vagina, epididymides, and prostate). The most prominent lesions reported in all tissues were cellular vacuolation, inflammatory changes (including necrosis), histiocytosis, regenerative–degenerative changes, increased hematopoiesis, and skeletal muscle myopathy. The most sensitive species to spinosad toxicity was the dog, followed by the rat, rabbit, and mouse. Changes in hematological and clinical pathology were generally

consistent with the type and extent of cellular injury or organ dysfunction reported. The vacuolatory changes in mice and rats were suggested by study authors to be consistent with phospholipidosis, resulting from the accumulation of polar lipid lysosomes. No mechanistic data were available to support these claims.

Chronic administration of spinosad resulted in an increase in severity of adverse effects in all species tested. Chronic dietary exposure to rats resulted in significant mortality to animals in the high-dose group (0.1%) which resulted in termination of the dose group. Based on the conclusion reached following an independent third-party histopathology peer review, treatment-related histopathological lesions in thyroid gland from rats given the 0.05% dose support the assessment that this dose level fairly represents the MTD for the purposes of assessing carcinogenic potential in rats. Therefore, it was concluded that technical spinosad has no carcinogenic potential.

For mixers, loaders, and applicators treating apples, ornamentals, and turf and workers and residential bystanders reentering treated areas, the expected duration of exposure is intermittent, short to intermediate term, predominantly via the dermal route. For this exposure duration, a NOAEL of 5 mg/kg bw/d from a 90-d dog study is considered the most appropriate for use in the occupational and bystander risk assessment to determine the MOEs.

An additional SF of 3× applied to the ususal SF of 100 for inter- and intra-species variation was deemed necessary based on the concern for the steepness of the dose–response curve; severity of effects on thyroid, lymphoid, and hematopoeitic tissue in several species(mice, rats, and dogs); and severity of effects in the rat multigeneration reproduction study.

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

3.5.1 Operator exposure assessment

Application to apples, turf and outdoor ornamentals

On apples, Success™ 480SC would be applied at a rate of 87 g a.i./ha by air-blast equipment, three times per season from May to July. A mixer, loader, and applicator (custom or farmer) would typically treat 20 ha of apples per day and handle 1.7 kg a.i. There is potential for short-term exposure to farmers and intermittent, intermediate-term exposure to custom applicators during mixing, loading, and applying of Success™ 480SC in apple orchards.

On turf, Conserve™ 480SC would be applied at a maximum rate of 49 g a.i./ha with ground or hand-held application equipment between May and September. On ornamentals, Conserve™ 480SC would be applied at a maximum rate of 26 g a.i./ha with hand-held equipment between May and September. Applicators would typically treat 20 ha of commercial turf, 2 ha of residential turf and ornamentals. Applications may be

repeated as required but are unlikely to exceed three per year. Applicators may handle 0.05 kg a.i./d when treating residential turf and ornamentals with hand-held equipment and 1 kg a.i./d when treating commercial turf with ground-boom equipment. Applicators treating turf and ornamentals areas have potential for intermittent, short- to intermediate-term exposure.

Dermal absorption

The applicant submitted a dermal absorption study which was determined to be unacceptable by the PMRA because of a number of study limitations including, but not limited to, a single high dosing level (1 mg/cm^2 of spinosyn A). Although the high dosing level may have underestimated dermal absorption at lower loading levels, the dosing vehicle, total occlusion of the test site, and a 24-h skin wash all may have contributed to a potential overestimation of dermal absorption. After 120 h, male rats absorbed 22% of the AD, of which 90% was trapped in the skin at the dosing site.

Based on the physical–chemical properties of spinosad (physical state, molecular weight, high K_{ow}, low water solubility) and the limited useable data from the dermal absorption study, a 25% default dermal absorption value is deemed appropriate for all assessments of dermal exposure.

Mixer, loader, and applicator exposure

Mixer, loader, and applicator exposure was estimated using the Pesticide Handlers Exposure Database (PHED) version 1.1. PHED is a compilation of generic mixer–loader–applicator passive dosimetry data with associated software which facilitates the generation of scenario-specific exposure estimates. With a few exceptions as noted, the PHED estimates meet criteria for data quality, specificity, and quantity outlined under the North American Free Trade Agreement Technical Working Group on Pesticides. Exposure via the inhalation route was a minor component of overall exposure and was added to the dermal deposition estimates coupled with a default dermal absorption value of 25%.

To estimate exposure for each use scenario, appropriate subsets of A and B (C grade data for hand wand) were created from the mixer–loader and applicator database files of PHED. All data were normalized for kilograms of active ingredient handled. Exposure estimates are presented on the basis of the best-fit measure of central tendency, i.e., summing the measure of central tendency for each body part which is most appropriate to the distribution of data for that body part. Estimates were derived for individuals wearing one layer of clothing and gloves during mixing, loading, and application.

The following exposure estimates and margins of exposure were derived for mixers–loaders–applicators:

Table 3.5.1 Mixer, loader, and applicator exposure

Occupational scenario	Exposure (mg/kg bw/d)[1]	Margin of exposure (based on NOAEL of 5 mg/kg bw/d)[2]
Mixer–loader + applicator exposure[3]		
Apples: air blast	0.0043	1 163
Outdoor ornamentals: hand wand	0.00093	5 376
Commercial turf: ground boom	0.0005	10 000
Residential turf: hand wand	0.0018	2 777
Indoor structural: hand wand	0.0034	1 470

1 Based on a 70-kg operator, typical North American use patterns of 20 ha/d for apples, 20 ha/d for commercial turf, 2 ha/d for residential turf and ornamentals, and 1344 m^2/d of indoor areas, and 25% default dermal absorption.
2 Based on a 90-d dog study.
3 Individuals wearing one layer of clothing and gloves (with the exception of ground-boom applicator not wearing gloves).

The margins of exposure given in Table 3.5.1 are acceptable.

3.5.2 Bystanders

Homeowners and children have potential for post-application exposure after treatment of lawns, and gardens with Conserve™ 480SC. Applications on lawns and gardens are not likely to exceed three per year, thus homeowners and children have potential for short- to intermediate-term exposure intermittently throughout the year. The label includes precautionary statements to keep children and pets off treated areas until dry.

Post-application exposure estimates were generated following the U.S. EPA Draft Standard Operating Procedures (SOPs) for Residential Exposure Assessments. Assumptions included the following: transfer coefficients of 10 000 cm^2/h for pruning of ornamentals; 14 500 and 5200 cm^2/h for activities involving contact with turf for adults and children, respectively; 20% for transferable residues from turf for hand-to-mouth exposure and 5% for transferable residues from turf for dermal exposure; a daily rate of residue dissipation of 10%; a saliva extraction factor of 50%; 20 hand-to-mouth events per hour; a surface area of 20 cm^2 for two or three fingers; and body weight of 70 kg for adults and 15 kg for children aged 1–6 years. Dermal exposure was considered for adults and dermal and oral exposure via hand-to-mouth contact for children aged 3–5 years. Post-application exposure for people reentering golf course turf is considered to be less than that associated with residential turf. All residential assessments are based on a 25% dermal absorption value.

The following exposure estimates and margins of exposure were derived for post-application residential exposure:

Table 3.5.2 Post-application residential exposure

Post-application residential scenario		Exposure (mg/kg bw/d)[1]	Margin of exposure (based on NOAEL of 5 mg/kg bw/d)[2]
Ornamentals	Adult: dermal	0.0063	793
Turf	Adult: dermal	0.0043	1163
	Child: dermal + oral	0.0117	427
Indoor	Adult: dermal	0.668	7.4
	Child: dermal + oral	1.27	4

[1] Values represent exposure on the day of the third application for ornamental and turf uses are based on a 70-kg adult, 15-kg child, exposure duration of 2 h on turf and ornamentals, and 25% default dermal absorption value.
[2] Based on a 90-d dog study.

The margins of exposure given in Table 3.5.2 for ornamental and turf uses are acceptable.

3.5.3 Workers

As application times overlap with summer pruning and hand thinning in apple orchards, rolling and harvesting of treated turf, and handling of treated ornamentals, workers reentering treated areas will have potential for post-application exposure. Exposure is likely to be of short- to intermediate-term duration.

Based on a default residue dissipation of 10% per day, an acceptable MOE ($300) is obtained 14 d post-application for hand thinning, summer pruning, or harvesting of apples and 2 d post-application for turf hand weeding, transplanting, or harvesting.

Table 3.5.3 Post-application worker exposure

Post-application worker scenario	Exposure (mg/kg bw/d)[1]	Margin of exposure (based on NOAEL of 5 mg/kg bw/d)[2]
Apples: thinning, pruning	0.068	73
Outdoor ornamentals: pruning, watering	0.0127	394
Commercial turf: rolling, harvesting	0.0197	254

[1] For the day of the third application, and based on the following: 70-kg worker, 10 000 cm^2/h transfer coefficient for turf and ornamentals, 8000 cm^2/h transfer coefficient for apples, 20% default dislodgeable residues, 25% default dermal absorption value, 8-h exposure duration for thinning–pruning apples and rolling–harvesting turf, and 4-h exposure duration for pruning ornamentals.
[2] Based on a 90-d dog study.

The margin of exposure given in Table 3.5.3 for pruning outdoor ornamentals is considered acceptable.

4.0 Residues

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

4.1.1 Definition of the residues in apples relevant to MRLs

Plant metabolism

Apple

Single applications of either [^{14}C] spinosyn A or spinosyn D containing 885 ppm of spinosyn A and 349 ppm of spinosyn D, respectively, were made to separate dwarf Red Delicious apple trees approximately 1 month prior to fruit maturity. The objectives of this study were to examine the nature of the residues on apple leaves (NOR), determine the influence of photolysis on the formation of terminal residues (PHOTO), and evaluate the translocation of radioactive residues (TRANS). Samples for the PHOTO evaluations were obtained by covering a portion of the tree with a light-blocking material immediately after application of spinosyns A and D. Leaves on a branch that was covered during the application of spinosyn A were used for TRANS samples. Leaves were collected at 0, 3, 7, 10, and 28 d after treatment for NOR and TRANS samples and 3 and 7 d after treatment for PHOTO samples.

Total radioactive residues (TRRs) were determined by the radiochemical analysis of solvent rinses, acetonitrile extracts, and extracted leaf tissues. Initial TRRs were approximately 217 and 89 ppm for spinosyns A and D NOR samples, respectively. For both treatments, initial organic solvent rinses contained 98% of the TRRs. In subsequent samples, residues in the rinses decreased to approximately 60% of the TRRs at 28 d after treatment. This was accompanied by increases in radioactivity extracted with acetonitrile and nonextractable tissue residues. Radioactivity removed by solvent rinses of samples, covered after treatment (PHOTO), remained at approximately 97% of the TRRs during the sampling period.

Even though TRANS samples were not directly exposed to a radioactive test compound, residues in these samples increased steadily during the 28-d sampling period to a concentration of 0.8 ppm. Analysis of rinses from NOR samples indicated that both spinosyn A and spinosyn D dissipated rapidly.

By 7 d after treatment, spinosyn A represented approximately 10% of the TRRs, and spinosyn D residues were not detected. This was in contrast to PHOTO samples that contained 78% and 82% of the TRRs as spinosyns A and D, respectively. These data demonstrated that photolysis was important in the dissipation of these compounds.

Extensive characterization of radioactivity in solvent rinses, acetonitrile extractable residues, and selected NOR samples following mild acid extraction, from other than 0 d after treatment spinosyns A and D leaves, demonstrated the presence of primarily polar multicomponent residues. The analysis of NOR and TRANS samples demonstrated that

radioactivity was incorporated into natural plant constituents. Based on the position of the radioactive label throughout the macrolide ring system in spinosyns A and D, it was apparent that these materials were extensively degraded to low molecular weight materials capable of being translocated throughout the plant. The characterization of radioactivity in all samples indicated that residue concentrations in leaves treated with spinosyns A and D were similar.

Results obtained using acidic hydrolysis to cleave the forosamine portion of the molecule and produce the pseudoaglycone demonstrated that initial metabolites contained modifications only in the forosamine portion. However, hydrolysis of rinses and acetonitrile extracts 28 d after treatment demonstrated that no metabolites containing the unaltered macrolide and rhamnose portions of the molecule were present. These data indicate that modification in the forosamine portion may be an initial step in the metabolism of spinosyns A and D and that modifications of the rhamnose and macrolide portions to form polar metabolites occur later in the degradative pathway.

Due to the transitory nature of degradates and the formation of polar materials, compounds were isolated only from the spinosyns A and D samples 3 d after treatment for mass spectral analysis. In addition to the confirmation of spinosyns A and D, spectra were obtained for compounds related to spinosyns A and D which had molecular weights16 mass units above those of the respective parent spinosyns, consistent with the presence of one additional oxygen atom. The N-demethyl compound, spinosyn B, and other metabolites having molecular weights of spinosyn A plus 32, 48, and 64 amu, consistent with the addition of two to four oxygen atoms, were isolated from the spinosyn A sample.

Based on these results, the following conclusions are made: spinosyns A and D dissipated rapidly; photolysis was the predominant mechanism in the degradation–metabolism of spinosyns A and D, in samples other than 0 d after treatment; the sample radioactivity was primarily composed of polar, multicomponent residues; and radioactive residues were translocated from a treated branch into untreated leaves.

The proposed metabolic pathway for spinosyns A and D involves the initial formation of nonpolar residues, some of which are modified on the forosamine portion of the molecule. With further photolytic degradation, the macrolide portion and possibly the rhamnose portion of the molecule are also modified to form polar and nonextractable residues that are subject to biochemical processes and incorporation into natural plant constituents.

Confined crop rotation

Crop-rotation studies were not required, since crops are not rotated in orchards.

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

Animal metabolism

Goat
The metabolism of spinosad was determined in the tissues and milk of lactating goats fed ^{14}C spinosyn A or ^{14}C spinosyn D. The data showed that the radioactive residues were transferred to all tissues and milk. The highest residues were in fat, liver, and kidneys, and the lowest in muscle and milk. The goat metabolized spinosyns A and D by two major pathways: N-demethylation of the forosamine moiety, and hydroxylation of the macrolide at several different locations. Several other metabolites resulted from both demethylation and hydroxylation on the macrolide portion of spinosyns A and D. The results from the goat metabolism study show that residues of spinosad concentrated in tissues and milk. The transfer of spinosad residues tends to be higher in fattier tissues (fat and liver). Most of the radioactivity was readily extractable and was not extensively conjugated. The parent compound (spinosyn A or spinosyn D) was the major ^{14}C residue found in tissues (fat, muscle, kidneys, and liver) and milk from goats fed either spinosyn A or spinosyn D.

In summary, the metabolism of both spinosyn A and spinosyn D involved either the loss of a single methyl group from the N-methyl moiety on the forosamine sugar or the hydroxylation of the macrolide at several different positions.

Poultry
Metabolism studies of spinosad in poultry are not required because no poultry feed items are associated with the proposed uses on apples.

Livestock feeding study
Apple pomace is a significant feed item for beef and dairy cattle and may be fed to horses, goats, and sheep. Sixteen dairy cows were orally dosed once daily with spinosad at levels equivalent to 1 ppm (1×), 3 ppm (3×), and 10 ppm (10×) via a gelatin capsule using a balling gun. An additional three cows were not treated and served as control animals. The anticipated maximum dietary burden was calculated to be 0.47 ppm based on a beef cattle diet consisting of 40% wet apple pomace.

The cattle were milked twice daily (a.m. and p.m.) and were fed a daily ration of alfalfa hay cubes and baled hay at each milking with water ad libitum. A composite milk sample from the morning and evening collection was prepared proportional to the amount of milk produced at the morning and evening milkings. In addition, on days 14 and 28 of the study, the composited milk collections were separated into cream and skim milk.

All but four animals, from the 10 ppm dose group, were sacrificed after 28 d of dosing; sacrifice occurred within 24 h of the final dose. The remaining cows received no more doses of spinosad and were sacrificed 8, 15, 29, and 57 d after the final dose. Samples of

fat (peritoneal, omental, and somatic), muscle (composite of flank, loin, and leg), liver, and kidneys were collected and stored frozen (–20°C).

The milk and tissue samples were analyzed for residues of spinosyns A and D using HPLC method GRM 95.03. Spinosyns A and D residues appeared to reach a plateau in milk between days 7 and 10 of dosing for all dose groups. In the 10 ppm dose group, residues increased on day 14 of dosing and then returned to the plateau level in subsequent sampling intervals. The maximum residues of spinosyns A and D at the 1 ppm dosing level were 0.01 ppm (skim milk), 0.06 ppm (whole milk), 0.25 ppm (cream), 0.06 ppm (kidneys), 0.12 ppm (liver), 0.02 ppm (muscle), and 0.6 ppm (fat). Based on the calculated anticipated dietary burden, MRLs will be established to cover residues of spinosad in milk, meat, and meat by-products derived from animals fed with treated apples.

Poultry feeding study
Since apple pomace is not recommended as a poultry feed, a poultry feeding study was not required.

Storage stability
The storage stability data for apples and apple juice were adequate. The data indicate that spiked residues of spinosyns A and D were relatively stable under frozen storage conditions for 6 months in or on apples and 3 months in apple juice. The storage data submitted support the storage stability of samples from the plant metabolism, residue field trial, and processing studies.

The storage stability data for animal commodities were adequate. Residues of spinosyns A and D were stable in milk during frozen storage for up to 136 d (4.5 months). Radioactive residues of spinosyns A and D were also found to be stable in milk, fat, kidneys, liver, and muscle for at least 1.6 years of frozen storage. The storage data submitted support the storage stability of samples from the animal metabolism, residue field trial, and processing studies.

4.2 Residues relevant to consumer safety

Supervised residue trials studies
The proposed Canadian use pattern for Success™ 480SC on apples consists of three foliar broadcast applications per season, each at 87 g a.i./ha, for a total of 261 g a.i./ha per season, a 7-d preharvest interval, and repeat applications at 7–10 d after the initial application. A minimum of 12 trials are proposed for the establishment of an MRL for apples using the Residue Chemistry Guidelines (Regulatory Directive Dir98-02). The petitioner submitted 16 trials, of which 13 were representative of the Canadian crop field trial regions (zones 1, 5, and 11). Data were not provided for the St. Lawrence Valley region (zone 5B) and the Atlantic region (zone 1A).

The level of combined residues of spinosyns A and D in or on apples, treated five times sequentially for a total of 500 g a.i./ha per season, was 0.089 ± 0.014 ppm (mean ± SD of 64 samples). All studies were conducted at 1× the U.S. label rate for apples and approximately 2× the proposed Canadian GAP. Although the residue data were generated at maximum per season rates above the Canadian proposed GAP rate (i.e., >20% as per Dir98-02), an MRL of 0.1 ppm is recommended. No significant difference was observed between the dilute and concentrated applications of NAF-127. The petitioner submitted bridging trial data conducted in tomatoes, spinach, and lettuce which showed no difference in residues between NAF-127 and NAF-85.

Residue decline

Residue data for spinosad-treated apples included an assessment of the effect of varying preharvest interval [PHI or post-treatment interval (PTI)] and the magnitude of the residue (MOR). The residue decline studies indicated that spinosad residues declined at 3- and 7-d PTIs, with no statistical significant decrease beyond 7 d.

Processing study

Apples were treated with spinosad at 2.5 kg a.i./ha per season (~10× the proposed Canadian seasonal rate). Processing of the treated apples showed that total residues of spinosyns A and D concentrated 5.3× in wet pomace. No concentration of residues was observed in juice processed from treated apples. Therefore, the proposed MRL of 0.1 ppm is adequate to cover residues in apple juice for spinosyns A and D.

Dietary risk assessment

For the chronic dietary risk assessment, the potential daily intake (PDI) was determined using the proposed MRLs on plant and animal commodities and the dietary exposure evaluation model (DEEM™) software (version 7.6.2 customized for Canada). The assessment was conducted using the 1994–1998 Continuing Surveys of Food Intake for Individuals (CFSII). The PDI, including an expected environmental concentration (EEC) value of 0.01 ppm, was 80, 60, and <55% of the acceptable daily intake (ADI = 0.009 mg/kg bw/d) for children 1–6 years, children 7–12 years, and the remainder of the total population, including infants and seniors, respectively.

4.3 Residues relevant to worker safety

The question of residues relevant to worker safety has been addressed in Section 3.5.3.

4.4 Proposed MRLs and existing domestic and international MRLs

4.4.1 Compliance with existing MRLs in Canada

Since this active ingredient is a new chemical, there are no existing MRLs.

4.4.2 Proposed Canadian MRLs

The following MRLs are proposed as a result of treating apples with spinosad: apples (0.1 ppm); whole milk (0.1 ppm); meat and meat by-products of cattle, sheep, goat, horse, and hog (0.01 ppm); kidneys (0.03 ppm); liver (0.05 ppm); fat of cattle, sheep, goat, horse, and hog (0.3 ppm).

4.5 Proposed import tolerances

No import tolerances have been petitioned or proposed.

4.6 Established international MRLs

Codex has not established MRLs for residues of spinosad in or on plant or animal commodities. The U.S. EPA has established the following tolerances: apples (0.2 ppm), milk fat (5.0 ppm), whole milk (0.5 ppm), meat (0.15 ppm), meat by-products (1.0 ppm), and fat (3.5 ppm).

5.0 Fate and behaviour in the environment

5.1 Physical and chemical properties relevant to the environment

The solubility of spinosad factors A and D in water decreases with increasing pH. Factor A is very soluble to soluble (90 and 6 mg/L at pH 5 and pH 9, respectively), and factor D is soluble to practically insoluble (28.7 and 0.053 mg/L at pH 5 and pH 9, respectively). Factors A and D are relatively nonvolatile (vapour pressure 3.0×10^{-11} and 2.0×10^{-11} kPa, respectively), and the values for Henry's Law Constant (9.2×10^{-13} and 4.4×10^{-10} atm m^3/mol, respectively) indicate a low potential for volatilization from water and moist soil. The octanol–water partition coefficients (log K_{ow}) of spinosad factors A and D are 4.0 and 4.5, respectively, at pH 7, and 5.2 and 5.2, respectively, at pH 9. These values indicate a potential for bioconcentration–bioaccumulation in nontarget organisms. Factors A and D (pK_a 8.1 and 7.9, respectively) will predominate in their nondissociated forms at environmentally relevant pH. The maximum absorption of UV and visible light for spinosad factors A and D is less than 290 nm, although a smaller absorption peak is present at 323–340 nm, therefore phototransformation may be a route of transformation of spinosad in the environment.

5.2 Summary of fate and behaviour in the terrestrial environment

5.2.1 Abiotic transformation

See Appendix III, Tables 1–7.

Spinosad is stable to hydrolysis at pH 5 and pH 7 and has a half-life of 200–259 d at pH 9. The half-life for phototransformation on soil for factors A and D is 82 and 44 d, respectively. Consequently, these transformation processes will not be the principal routes of transformation of factors A and D in the terrestrial environment.

5.2.2 Biotransformation

Aerobic biotransformation of factors A and D is a principal route of transformation in soil. Factors A and D will be nonpersistent in soil under aerobic conditions at 25°C (DT_{50} values of 9–17 d, where DT_{50} is the time required for non-first order 50% dissipation) and will be moderately persistent at 20°C (DT_{50} values of 24–69 d). The major transformation products detected in aerobic biotransformation studies in soil were factor B and factor B of D. As the estimated half-lives range from 100 to $200 d, factor B and factor B of D are more persistent than their respective parent compounds.

5.2.3 Mobility

The results from adsorption–desorption studies indicate that factors A and D and the transformation product factor B will be of low mobility to immobile in soils and will not leach. Adsorption was not correlated with the organic carbon content of the soil.

5.2.4 Dissipation and accumulation under field conditions

Factors A and D were nonpersistent under field conditions at a forest plantation in Ontario and a forest region in New Brunswick. The DT_{50} values for factors A and D were 2–12 and 3–6 d, respectively. Thus, spinosad is classified as nonpersistent in forest soil and litter. Factor B and factor B of D were detected at both study sites. Results of these studies indicated that the persistence of these transformation products is expected to be similar to that of the parent compounds. The parent compounds and the transformation products, factor B and factor B of D, were not detected below the top 5 cm depth in soil, indicating that these compounds will not leach in soil. There was good agreement regarding persistence and mobility between the results from the laboratory and field studies.

5.3 Summary of fate and behaviour in the aquatic environment

5.3.1 Abiotic transformation

Spinosad factors A and D are stable to hydrolysis. Both factors A and D phototransformed rapidly in buffered aqueous solutions, with half-lives of 1–2 d. In the water column, factors A and D would be considered nonpersistent based on the rate of phototransformation. The only major transformation product was the beta isomer of 13,14-dihydro of the pseudoaglycone of factor A.

5.3.2 Biotransformation

Under anaerobic conditions in a sediment–water system, factors A and D were moderately persistent to persistent, with half-lives for the total system of 161 and 250 d, respectively. Significant amounts of factors A and D partitioned from water to sediment within the first 7 d. Under anaerobic aquatic conditions, the transformation products accumulated over time. The major transformation products (total for water and sediment) for factor A were identified as “unknown” compounds 1 and 2 and ketoreversepseudoaglycone (compound 814426). These transformation products accumulated over time and were primarily detected in the sediment. For factor D, compound 5 was the only major transformation product. Although data were not provided, it is expected that factors A and D will not biotransform under aerobic aquatic conditions. Therefore, biotransformation in aquatic environments is not an important route of spinosad transformation.

In an aquatic microcosm field study, the DT_{50} for spinosad was 2–3 d. Based on laboratory results, aquatic phototransformation was likely the primary route of dissipation. Factors A and D will be nonpersistent in shallow waters. As the attenuation of light increases with depth in water, however, spinosad may be more persistent in deep water and it will persist in sediment. As the results from adsorption studies indicated that factor A will bind to soil, transportation in runoff via spinosad bound to soil, in addition to surface runoff and spray drift, could also be a potential route of entry of spinosad into aquatic systems.

5.4 Bioconcentration

Bioconcentration of spinosad was studied using rainbow trout (*Oncorhynchus mykiss*). The steady state bioconcentration factors (BCFs) for spinosad factor A in whole fish, muscle tissue, and viscera were 19, 6, and 19, respectively. The corresponding values for factor D were 110, 40, and 130, respectively. Clearance time for whole fish was less than 1 week. Therefore, there is a low potential for bioconcentration of spinosad in fish tissue.

5.5 Expected environmental concentrations (EECs)

5.5.1 Soil

Assuming a soil bulk density of 1.5 g/cm^3, application at the proposed maximum cumulative rate of 261 g a.i./ha (222 g factor A/ha and 39 g factor D/ha) to bare soil with no interception by foliage (consisting of three consecutive sprays a minimum of 7 d apart and considering transformation half-lives on soil) and uniform mixing in soil over a depth of 15 cm, the EEC of spinosad in soil is 0.105 mg a.i./kg soil dry weight.

5.5.2 Aquatic systems

The concentration of spinosad, resulting from a direct overspray of the proposed maximum cumulative application rate (261 g total a.i./ha) in a 30 cm depth of water, is 0.087 mg a.i./L.

5.5.3 Vegetation and other food sources

Concentrations of spinosad on vegetation were estimated using a nomogram developed by the U.S. EPA. The maximum proposed seasonal application rate of 261 g total a.i./ha for spinosad was used. A fresh weight to dry weight conversion was also calculated. The EECs in a typical diet of wild birds and small mammals when exposed to maximum application rates and frequencies of application are shown in Table 5 of Appendix III.

6.0 Effects on nontarget species

The end points for nontarget species are provided in Appendix III.

6.1 Effects on terrestrial organisms

Spinosad is practically nontoxic to bobwhite quail and mallard on an acute basis and on a short-term dietary basis. Spinosad is practically nontoxic to mammals on an acute basis. The short- and long-term no observable effect concentration (NOEC) for the rat is 50 mg a.i./kg dw diet/d. The acute toxicity of spinosad to earthworms and reproduction of bobwhite quail and mallard is low. Spinosad is very toxic to honey bees and predators and parasites (beneficial insects).

6.2 Effects on aquatic organisms

Spinosad is slightly toxic to daphnids, grass shrimp, and rainbow trout, moderately toxic to bluegill sunfish and sheepshead minnow, and very highly toxic to eastern oyster on an acute basis. Spinosad is toxic to midge and daphnids and toxic to mysid shrimp on a chronic basis. The NOEC values for algae and aquatic vascular plants range from 0.049 to 4.3 mg a.i./L.

6.3 Risk assessment

6.3.1 Environmental fate and behaviour

Spinosad will not hydrolyze in the laboratory, however, factors A and D will be nonpersistent in shallow waters because of phototransformation. In deeper water or under turbid conditions, however, spinosad may persist in the water column. Spinosad will be persistent in aquatic sediment. Under anaerobic aquatic conditions, transformation products accumulated over time. Two major transformation products, factor J and ketoreversepseudoaglycone, were detected in the sediment fraction and may be persistent under anaerobic conditions. Factors A and D will be nonpersistent to moderately persistent in soil. Spinosad (factors A and D), and the transformation product factor B, will be of low mobility to immobile in soils and will not pose a risk of leaching. There is a low potential for bioconcentration of spinosad in fish tissue.

6.3.2 Terrestrial organisms

The risk to terrestrial organisms is summarized in Table 6 of Appendix III. Spinosad will not pose a risk to bobwhite quail, mallard, or earthworms. Spinosad will pose a moderate risk to mammals on an acute basis based on the estimated NOEL. According to plant metabolism studies, spinosad has a short half-live on plants (approximately 10% applied radiolabel by day 7) due to phototransformation. Therefore, spinosad will not pose a short- or long-term dietary risk to mammals.

Spinosad will pose a moderate to high risk to honeybees and a low to moderate risk to predators and parasites (beneficial insects). Spinosad will pose a low risk to terrestrial plants.

6.3.3 Aquatic organisms

The risk to aquatic organisms is summarized in Table 7 of Appendix III. Spinosad will pose a low acute risk to aquatic invertebrates and will not pose a risk to fish on an acute or chronic basis. Spinosad will not pose a risk to duckweed or the algal species *Selenastrum capricornutum* and *Anabaena flos-aquae*, but will pose a low risk to the algal species *Skeletonema costatum*.

Spinosad will, however, pose a moderate acute risk to the algal species *Navicula pelliculosa* and a high chronic risk to aquatic invertebrates (midge and daphnids).

6.4 Risk mitigation

Buffer zones can be used to protect sensitive nontarget terrestrial and aquatic organisms.

Terrestrial
No buffer zones are required for nontarget terrestrial habitats.

Aquatic
To protect sensitive nontarget aquatic organisms, buffer zones are required between the last spray swath and the edge of aquatic habitats. To protect sensitive nontarget organisms from harmful exposure to spinosad, the following buffer zones are required between the last spray swath and the edge of sensitive aquatic areas, such as wetlands, ponds, lakes, streams, and rivers: no buffer zone is required for Conserve™ 480SC (turf and ornamentals); 2-m (early season) or 1-m (late season) buffer zones are required for Success™ 480SC (orchards).

7.0 Efficacy data and information

7.1 Effectiveness

7.1.1 Intended uses

Dow AgroSciences Canada Inc. (DWE) of Calgary has applied for the registration of two commercial class end-use products with the trade names Conserve™ 480SC and Success™ 480SC. These products are identical formulations and contain 480 g/L of the new active ingredient spinosad technical insecticide.

Conserve™ 480SC also is proposed for use on outdoor ornamentals and turf. The label makes claims for control of the following pests: leaf beetles (such as elm leaf beetle and willow leaf beetle), western flower thrips, tent caterpillar (such as eastern tent caterpillar), sawfly larvae, gypsy moth, and sod webworm. The proposed rate of application is 52–208 mL product/ha (25–100 g spinosad/ha) for elm leaf beetle and willow leaf beetle and eastern tent caterpillar, 104–208 mL product/ha (50–100 g spinosad/ha) for western flower thrips and sawfly larvae, 52–104 mL product/ha (25–50 g spinosad/ha) for gypsy moth, and 73–208 mL product/ha (35–100 g spinosad/ha) for sod webworm, to a maximum of 600 mL product/ha/year.

Success™ 480SC is proposed for use on apples for control of leafrollers and leafminers. The proposed rates of application are 182–312 mL product/ha (87–150 g spinosad/ha) for control of leafrollers and 150–312 mL product/ha (72–150 g spinosad/ha) for control of leafminers, to a maximum of 600 mL product/ha/year. For leafrollers, no more than three applications per season should be used.

7.1.2 Mode of action

Spinosad causes persistent activation of nicotinic acetylcholine (ACT) receptors. Because ACT and spinosad act on receptors simultaneously, they likely operate at different target sites; this apparently is unique among nicotinic agonists, which normally compete directly with ACT for binding sites (Salado *et al.* 1997). Under certain conditions, spinosad also has effects on "-amino butyric receptors, but the contribution of this activity to its insecticidal properties has not been established.

7.1.3 Crops

Success™ 480SC and Conserve™ 480SC are proposed for use on pests of apples, outdoor ornamentals, and turf.

7.1.4 Effectiveness against pests

USC 14: terrestrial food crops (Submission No. 1997-0777)

Obliquebanded leafroller (OBLR)
Results were submitted from 15 studies assessing control by spinosad of obliquebanded leafroller (OBLR) on apple trees. Thirteen of the studies were done to assess control by spinosad of the summer generation of OBLR, and two studies were done to assess control by spinosad of the overwintering population of OBLR.

For summer-generation OBLR larvae, two applications at 46.5–350 g spinosad/ha of Success™ 480SC significantly reduced populations of OBLR on apple trees and also reduced damage to apples caused by this pest. A single application at 93 or 183 g spinosad/ha of Success™ 480SC in May was sufficient to control overwintering OBLR, whereas an April application showed lower efficacy, presumably because larvae still in their hibernacula were afforded some protection from the insecticide. The efficacy of Success™ 480SC was similar to or better than the efficacy of azinphos methyl, chlorpyrifos, cypermethrin, phosmet, and tebufenozide.

The efficacy data support temporary registration of Success™ 480SC for the control of OBLR at the low proposed rate of 182 mL product/ha (87 g spinosad/ha) to a maximum of 546 mL product/ha/year (261 g spinosad/ha/year). To receive full registration, data are required that establish the lowest effective dose of Success™ 480SC for the control of overwintering and summer-generation OBLR larvae.

Spotted tentiform leafminer (STLM)
Three studies were done in apple orchards during the summers of 1996 and 1997 in Ontario to determine whether a single application of Success™ 480SC would control spotted tentiform leafminer (STLM). One study used 62–350 g spinosad/ha to control tissue feeding larvae, with poor results; two other studies used 46.5–350 g spinosad/ha to control sap mining larvae, also with poor results. These results were not sufficient to support the use of Success™ 480SC for the control of STLM.

USC 27: ornamentals outdoor

Elm leaf beetle
Three field trials using a single application of Conserve™ 480SC (spinosad 480 g/L) or related formulations to control elm leaf beetle larvae on elm trees were submitted for review. Rates of application were between 0.4 and 50 ppm spinosad, with the12-ppm spinosad treatments providing excellent control of larvae in all studies. Lower rates of

application sometimes showed good control, though results were not as consistent as those for the 12-ppm spinosad treatment.

Sufficient data have been submitted to support the full registration of Conserve™ 480SC at 12 ppm spinosad (25 mL product/1000 L of spray) for the control of elm leaf beetle, to a maximum of 200 mL product/ha/year (96 g spinosad/ha/year).

Sawfly
Six studies assessing control of sawfly infestations of red pine, scotch pine, or black spruce by a single application of spinosad were submitted for review. In four of six studies, all of the larvae on treated branches were dead by 2 weeks post-application, sometimes at concentrations as low as 1.5 ppm spinosad. One of the remaining studies showed 80+% control at 1.5, 12, or 50 ppm spinosad, but not at lower rates, and the other study showed up to 100% control after 1 week, without a rate effect, but with poor control after 2 weeks.

The efficacy data are sufficient to support full registration of Conserve™ 480SC for the control of sawfly at the rate of 12 ppm spinosad (25 mL product/1000 L of spray) to a maximum of 200 mL product/ha/year.

Gypsy moth
A single application of spinosad at 3+ ppm to birch or white oak showed good control of gypsy moth. Results for the lowest rates (0.04–0.75 ppm) of spinosad were mixed, sometimes showing good control and sometimes relatively poor control. On birch trees, the 95% lethal doses (LD_{95}) were 7.41 and 2.0 ppm, respectively, for 3- and 7-d exposures to spinosad.

These data are sufficient to support full registration of Conserve™ 480SC applied at 12 ppm for the control of gypsy moth to a maximum of 200 mL product/ha/year.

Eastern tent caterpillar (ETC)
Four studies assessing control of eastern tent caterpillar (ETC) by spinosad were submitted for review. Only two of the studies used sprays that contained more than 4 ppm spinosad. Control of ETC was 100% for 6, 12, 14, or 50 ppm spinosad in these studies and was generally as good as or better than control by acephate. Control by lower rates of spinosad was good in one study, variable in another study, and poor in a final study. Because of the inconsistent results for the lower rates, registration of Conserve™ 480SC for the control of ETC should be at 12 ppm (25 mL product/1000 L of spray) to a maximum of 200 mL product/ha/year.

These data are sufficient to support full registration of Conserve™ 480SC applied at 12 ppm for the control of eastern tent caterpillar to a maximum of 200 mL product/ha/year.

Western flower thrips
Because the six field trials included with the original application yielded inconsistent and often poor control, four additional field trials were submitted using Conserve™ 480SC (spinosad 480 g/L) or related formulations to control western flower thrips on ornamental flowers. Trials were carried out in Brazil and California in 1998 and in Brazil in 1997. Efficacy was assessed by comparing the number of thrips on spinosad-treated flowers or leaves to the number of thrips on untreated and commercial standard (acephate, dichlorvos, endosulfan, and imidacloprid) treated flowers or leaves.

Results from submitted supplementary trials indicate that Conserve™ 480SC was as effective as commercial standards (acephate, dichlorvos, endosulfan, and imidacloprid) in controlling western flower thrips on ornamental flowers. A rate of 25 ppm of spinosad performed better than 12 ppm and achieved the same level of control as 50 or 100 ppm of spinosad, suggesting that the lowest effective rate is approximately 25 ppm of spinosad.

These data are sufficient to support full registration of Conserve™ 480SC applied at the rate of 26 g a.i./1000 L (50 mL product/1000 L) for the control of western flower thrips in outdoor ornamentals. A maximum of three applications per crop per year are allowable.

USC 30: turf

Sod webworm
Four field trials using Conserve™ 480SC (spinosad 480 g/L) to control sod webworm infestations were submitted for review. Larval mortality of 90+% was evident on turf treated with Conserve™ 480SC at 6.3–89.9 g spinosad/ha. Control by 6.3 and 12.6 g spinosad/ha decreased at high sod webworm densities, whereas rates of 24.5 and 49 g spinosad/ha achieved good control for at least 14 d, regardless of sod webworm density or time of year (spring, summer, or fall). Based on these data, full registration of Conserve™ 480SC for use against sod webworm infestations of turf can be supported at 24.5–49 g spinosad/ha (51–102 mL product/ha) to a maximum of 400 mL product/ha /year.

7.1.5 Total spray volume

Spray volumes and dilution rates are site specific and have been discussed earlier in this Regulatory Note.

7.2 Phytotoxicity to target plants (including different cultivars) or target plant products

Phytotoxicity was not noted in any of the field trials on apples (foliage and fruit). Varieties tested included Rhode Island Green, Red Delicious, McIntosh, Empire, Ida Red, Paula Red, Yorking, and Golden Delicious. Treatment regimes in these trials include one to seven applications of spinosad per season at rates ranging from 5 to 350 g spinosad/ha. Adverse phytotoxic effects were not reported on trees or other plants sprayed with a single or several applications of spinosad at 0.05–400 ppm to control sawfly, western

flower thrips, eastern tent caterpillar, or gypsy moth, or on sod treated with spinosad at 1–90 g a.i./ha to control sod webworm. Also included with the submission were summary results from 112 crop-tolerance studies conducted on ornamentals and turf in the United States. No adverse phytotoxic effects were observed in these studies.

7.3 Observations on undesirable or unintended side effects, e.g., on beneficial and other nontarget organisms, succeeding crops, other plants, or parts of treated plants used for propagating purposes (e.g., seed, cutting, runners)

7.3.1 Impact on succeeding crops

7.3.2 Impact on adjacent crops

7.4 Economics

7.5 Sustainability

7.5.1 Survey of alternatives

The number and type of available alternative insecticide products differ for the various pests proposed for Success™ 480SC and Conserve™ 480SC. The major insecticide active ingredients currently used for control of the proposed pests include, but are not necessarily limited to, those listed in Table 7.5.1.

Table 7.5.1 Alternative insecticide products

Pest	Available alternative active ingredients
Obliquebanded leafroller	Organophosphates (azinphos-methyl, phosmet), carbamates (methomyl), *Bacillus thuringiensis*, insect growth regulators (tebufenozide), synthetic pyrethroids (cypermethrin, deltamethrin)
Spotted tentiform leafminer	Organophosphates (diazinon, phosmet), carbamates (carbaryl, methomyl), synthetic pyrethroids (cypermethrin, deltamethrin), chloro nicotinyl (imidacloprid)
Leaf beetle	Organophosphates (acephate), carbamates (carbaryl)
Sawfly	Organophosphates (acephate, malathion), carbamates (carbaryl), synthetic pyrethroids (permethrin)
Gypsy moth	Organophosphates (acephate, chlorpyrifos, phosmet), carbamates (carbaryl), *Bacillus thuringiensis*, synthetic pyrethroids (permethrin, deltamethrin)
Tent caterpillar	Organophosphates (acephate, chlorpyrifos, phosmet), carbamates (carbaryl), synthetic pyrethroids (D-phenothrin, D-trans allethrin, permethrin)

Pest	Available alternative active ingredients
Western flower thrips	Organophosphates (azinphos-methyl, chlorpyrifos, diazinon, malathion), carbamates (carbaryl), synthetic pyrethroids (permethrin, pyrethrins)
Sod webworm	Organophosphates (chlorpyrifos, diazinon), carbamates (carbaryl)

7.5.1.1 Nonchemical control practices

7.5.1.2 Chemical control practices

The proposed labels for Success™ 480SC and Conserve™ 480SC state that the application rate of 52–312 mL product (25–150 g spinosad/ha) does not significantly impact beneficials (parasites and predators), including ladybird beetles, lacewings, predatory mites, big-eyed bugs, damsel bugs, minute pirate bugs, assassin bugs, flower bugs, *Stethorus*, and spiders.

7.5.2 Contribution to risk reduction

Spinosad is potentially an alternative to organophosphate insecticides for control of obliquebanded leafroller, larval fleas, leaf beetles, conifer sawfly, gypsy moth, tent caterpillar, sod webworm, and western flower thrips. Organophosphate insecticides have been identified as a priority for reevaluation by the PMRA and the U.S. EPA.

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

Spinosad is a new chemistry with a novel mode of action reported to be different from any other insecticide presently commercialized. Spinosad causes persistent activation of nicotinic acetylcholine (ACT) receptors. Because ACT and spinosad act on receptors simultaneously, they likely operate at different target sites, which apparently is unique among nicotinic agonists (e.g., imidacloprid, thiamethoxam).

The applicant has stated that there are no known reports of cross-resistance between spinosad and other insecticide chemistries. Because of its novel chemistry, spinosad is potentially a valuable tool for use in rotation with currently registered chemistries in the management of insect resistance. To mitigate against the development of resistance by obliquebanded leafroller, the maximum number of applications of Success™ 480SC is limited to three. Also, the label recommends that application of spinosad to apple and ornamental pests should be based on pest monitoring, incorporation of cultural and biological control practices, alternation of an active class of insecticides on succeeding generations, and targeting the most susceptible life stage as strategies for mitigating against the development of resistance to spinosad.

According to Regulatory Directive DIR99-06, Voluntary Pesticide Resistance Management Labelling Based on Target Site/Mode of Action, the following statements should be incorporated on the labels for the end-use products.

Resistance management recommendations
For resistance management, please note that Success™ 480SC (Conserve™ 480SC) contains a group 5 insecticide. Any insect population may contain individuals naturally resistant to Success™ 480SC (Conserve™ 480SC) and other group insecticides. The resistant individuals may dominate the insect population if this insecticide is used repeatedly in the same fields. Other resistance mechanisms that are not linked to site of action but are specific for individual chemicals, such as enhanced metabolism, may also exist. Appropriate resistance-management strategies should be followed.

To delay insecticide resistance,

- where possible, rotate the use of Success™ 480SC (Conserve™ 480SC) with different groups that control the same pests in a field;
- use tank mixtures with insecticides from a different group when such use is permitted;
- insecticide use should be based on an integrated pest management (IPM) program that includes scouting and record keeping and considers cultural, biological, and other chemical control practices;
- monitor treated pest populations for resistance development;
- contact the local extension specialist or certified crop advisors for any additional pesticide resistance-management and IPM recommendations for the specific site and pest problems in the area; and
- for further information or to report suspected resistance, contact (company representatives) at (toll free number) or at (Internet site).

7.6 Conclusions

Adequate efficacy and value data have been provided to support the label claims for control of obliquebanded leafroller in apple, leaf beetle, conifer sawfly, gypsy moth, tent caterpillar, sod webworm, and western flower thrips in outdoor ornamentals and turf.

Insufficient efficacy data have been provided to support the proposed use claims for control of spotted tentiform leafminer.

8.0 Overall conclusions

8.1 Chemistry

The product chemistry data for spinosad technical in the end-use products Success™ 480SC and Conserve™ 480SC are complete. The technical material was fully characterized and the specifications were supported by the analysis of five production batches of the product for active ingredient and impurities using specific validated methods of analysis.

8.2 Toxicology

Technical spinosad was rapidly absorbed, completely metabolized, and rapidly eliminated. The compound was considered to be of low acute toxicity by the oral, dermal, and inhalation routes. It was nonirritating to the skin and minimally irritating to the eyes of rabbits.

Spinosad is not considered to be neurotoxic, genotoxic, oncogenic, or teratogenic. In the long-term studies and high doses, it appears to have effects on the reproductive system. Target organ sensitivity was not entirely consistent across species, with the following organs identified as the major targets: thyroid (rat - follicular epithelium and dog - parafollicur cells), kidneys, lymphoid organs (spleen and lymph nodes), liver (rat and dog), bone marrow, and the reproductive organs (ovaries - mice, testes - dogs and rats, uterus - rats and mice, vagina - rats and mice, epididymides, prostate - rats).

For dietary exposure to food residue, the ADI was based on a 1-year study in dogs. An additional (3×) uncertainty factor (beyond the usual safety factor of 100 for inter- and intra-species variation) was deemed necessary based on the concern for: the steepness of the dose-response curve, reproductive effects noted in the rat multigeneration reproduction study and effects on thyroid, lymphoid, and hematopoietic tissue in several species (mice, rats, and dogs). The recommended ADI for spinosad is 0.009 mg/kg bw/day.

8.3 Occupational exposure

Adequate margins of exposure were obtained for mixers, loaders, and applicators for all proposed uses of spinosad, for bystanders in contact with treated ornamentals or turf; for workers hand thinning, summer pruning, or harvesting apples 14 d post-application; for hand weeding, transplanting, or harvesting turf 2 d post-application; and for pruning outdoor ornamentals on the day of application.

Based on these findings, it is concluded that the use of spinosad on ornamentals, turf, and apples results in an acceptable risk provided that labels are modified to include a 14-d restricted-entry interval for hand thinning, summer pruning, or harvesting apples and a 2-d restricted-entry interval for hand weeding, transplanting, or harvesting turf.

Additional studies to refine the post-application exposure assessment for the proposed use on apples would be required.

8.4 Residue

The qualitative nature of the residue is adequately understood based on metabolism studies conducted on apples. Spinosad (spinosyns A and D) was the major ^{14}C residue identified in early harvest samples (e.g., 0–3 d after treatment) of apple fruits and leaves. Minor metabolites identified include spinosyn B, N-demethyl spinosyn D, spinosyn K, and N-formyl spinosyn B.

In samples collected at subsequent intervals, the residue levels of spinosyns A and D declined significantly. The decline in residue levels of the parents was accompanied by incremental increases in nonextractable and polar ^{14}C residues. Extensive fractionation and characterization of nonextractable and polar ^{14}C residues in selected raw agricultural commodity (RAC) samples indicate that most of the radioactivity was degraded to multicomponent residues of low molecular weight which are subsequently incorporated into natural plant constituents. The petitioner has provided evidence to indicate that photolysis on foliage plays a role in the initial degradation of the parent compounds. The proposed metabolic pathway involves the conversion of the spinosad to metabolites resulting from modifications to the forosamine portion of the molecule, such as spinosyn B and N-demethyl spinosyn D. The rhamnose and macrolide portions are subsequently modified to form polar and nonextractable residues.

The qualitative nature of the residue in ruminants is adequately understood based on evaluated goat metabolism studies. The results of the studies indicate that residues of spinosyns A and D concentrate in tissues and milk, with greater transfer to fat and liver. Spinosyns A and D were the major ^{14}C residues identified in tissues (fat, muscle, kidneys, and liver) and milk.

Metabolism studies in poultry were not required because no poultry feed items are associated with the proposed uses on apples.

The storage stability data for apples and apple juice are adequate. The data indicate that spiked residues of spinosyns A, D, B, and K and N-demethyl spinosyn D are relatively stable under frozen storage conditions for at least 6 months in or on apples and 3 months in apple juice. These data support the storage conditions and intervals of samples from the submitted field trial and processing studies.

The storage stability data for animal commodities are adequate. Residues of spinosyns A, D, and B and N-demethyl spinosyn D are stable in frozen milk for up to 136 d (4.5 months). Radioactive residues of spinosyns A, D, and B and N-demethyl spinosyn D were also found to be stable in milk, fat, kidneys, liver, and muscle for at least 1.6 years of frozen storage. These data support the storage conditions and intervals of samples from the submitted cattle feeding study.

HPLC methods GRM 95.05 and GRM 94.22 are adequate for the purposes of MRL enforcement and collection of residue data for spinosyns A, D, K, and B and N-demethyl spinosyn D in or on apples and its processed commodities. Adequate independent method validation and concurrent method recovery data have been submitted. HPLC method GRM 95.03 is adequate for the purposes of collection of residue data for spinosyns A, D, and B and N-demethyl spinosyn D in animal commodities; adequate independent method validation and concurrent method recovery data have been submitted. The method has also been adequately radiovalidated and appears suitable as an analytical method for enforcement purposes.

The petitioner submitted a description of and validation data for immunoassay method GRM 95.14, a method for the determination of total spinosyn related residues in milk and cattle muscle, liver, and kidneys. Adequate validation data for spinosyn A were submitted.

Data pertaining to multiresidue methods (MRM) testing of spinosyns A and D were submitted and showed that spinosyns A and D were not amenable to quantitation by these MRMs.

The proposed Canadian use pattern for Success™ 480SC on apples consists of three foliar broadcast applications per season, each at 87 g a.i./ha, for a total of 261 g a.i./ha per season, a 7-d preharvest interval, and repeat applications at 7–10 d after the initial application. A minimum of 12 trials are proposed for the establishment of an MRL for apples using the Residue Chemistry Guidelines (Regulatory Directive DIR98-02). The petitioner submitted 16 trials, of which 13 were representative of the Canadian crop field trial regions (zones 1, 5, and 11). Data were not provided for the St. Lawrence Valley region (zone 5B) and the Atlantic region (zone 1A).

The level of combined residues of spinosyns A and D in or on apples, treated five times sequentially for a total of 500 g a.i./ha per season, was 0.089 ± 0.014 ppm (mean ± SD of 64 samples). All studies were conducted at 1× the U.S. label rate for apples and approximately 2× the proposed Canadian GAP rate. Although the residue data were generated at maximum per season rates above the Canadian proposed GAP rate (i.e., >20% as per DIR98-02), an MRL of 0.1 ppm is recommended.

Data from residue-decline studies indicate that spinosad residues decline at 3- and 7-d post-treatment intervals (PTIs), with no further statistical significant decrease beyond 7 d. The data support the label restriction of a 7-d preharvest interval.

The submitted apple-processing data are adequate. The data indicate that total residues of spinosyns A and D concentrated 5.3× in wet pomace processed from treated apples. No concentration of residues was observed in juice processed from treated apples.

The submitted dairy cattle feeding data are adequate. They indicate that MRLs for residues of spinosad are required for milk and the fat, meat, and meat by-products of cattle, goat, hogs, horses, and sheep. Based on the available residue field trial data for apples harvested 7 d following treatment at the maximum proposed seasonal application rate, mean residue concentration is 0.089 ppm. Therefore, the maximum total spinosyns A and D residues expected in apple wet pomace would be 0.47 ppm. Detectable residues of spinosad were observed in the milk, fat, kidneys, and liver of cattle fed spinosad at 1 ppm in their diet (~2× the maximum theoretical dietary burden) for 28 d. There are no poultry feed items associated with this petition. Therefore, data pertaining to the magnitude of spinosyn residues in poultry commodities were not required.

The following MRLs are proposed as a result of treating apples with spinosad: apples (0.1 ppm); milk fat (0.1 ppm); whole milk (0.1 ppm); meat and meat by-products of cattle, sheep, goat, horse, and hog (0.01) ppm); kidneys (0.03 ppm); liver (0.05 ppm); fat of cattle, sheep, goat, horse, and hog (0.3 ppm).

For the chronic dietary risk assessment, the PDI was determined using the proposed MRLs on plant and animal commodities and DEEM™ software (version 7.6.2 customized for Canada). The assessment was conducted using the 1994–1998 Continuing Surveys of Food Intake for Individuals (CFSII). The PDI, including an EEC value of 0.01 ppm, was 80, 60, and <55% of the acceptable daily intake (ADI = 0.009 mg/kg bw) for children 1–6 years, children 7–12 years, and the remainder of the total population, including infants and seniors, respectively.

8.5 Environmental assessment

Spinosad will be nonpersistent in shallow waters because of phototransformation but will be persistent in aquatic sediment. The major transformation products, factor J and ketoreversepseudoaglycone, may be persistent in sediment under anaerobic conditions. Spinosad has a low risk for leaching to groundwater and a low potential for bioaccumulation in fish tissue.

Spinosad will not pose a risk to bobwhite quail, mallard ducks, earthworms, fish, duckweed, and the algal species *S. capricornutum* and *A. flos-aquae* and will not pose a short- or long-term dietary risk to mammals, although there is a moderate risk to mammals on an acute oral basis. There is a high risk to the sediment-dwelling midge *Chironomus riparius*, a moderate to high risk to honey bees, and low and moderate risks to the algal species *S. costatum* and *N. pelliculosa*, respectively. There is a low acute risk to aquatic invertebrates. Buffer zones around aquatic environments will mitigate the risk to aquatic species.

8.6 Value assessment

Adequate efficacy and value data have been provided to support the label claims for control of obliquebanded leafroller in apple, conifer sawfly, gypsy moth, tent caterpillar, sod webworm, and western flower thrips in outdoor ornamentals and turf.

Insufficient efficacy data have been provided to support the proposed use claims for control of spotted tentiform leafminer.

8.7 Toxic substance management policy considerations

During the review of spinosad technical, Success™ 480SC, and Conserve™ 480SC, the PMRA has considered the implications of the Federal Toxic Substances Management Policy and the PMRA Regulatory Directive DIR99-03 and has concluded the following.

Spinosad factors A and D do not meet the criteria for persistence. The half-life of spinosad factors A and D in water was 2–3 d based on phototransformation. This value is below the TSMP Track-1 cutoff criteria for water ($\geq$182 d). The half-life of spinosad in soil (9–69 d) is below the TSMP Track-1 cutoff for soil ($\geq$182 d). A half-life in sediment was not determined, however, the half-lives in a water–sediment system were 161 and 250 d. These values are below the TSMP Track-1 cutoff criteria for sediment ($\geq$365 d). Although a half-life in air was not determined, spinosad is nonvolatile from water and moist soil, and thus a phototransformation study in air was not triggered.

The octanol–water partition coefficients (log K_{ow}) of spinosad factors A and D are 4.0 and 4.5, respectively, at pH 7, and 5.2 and 5.2, respectively, at pH 9. At pH 9, octanol–water coefficients are greater than the TSMP Track-1 cutoff value of $\geq$5.0. However, steady state BCFs for spinosad factors A and D in rainbow trout (6–19 and 40–130, respectively) are below the TSMP Track-1 cutoff value for BCF ($\geq$5000).

Spinosad (factors A and D) does not meet the criteria for a TSMP Track-1 substance.

Based on the elution profile in the reverse-phase HPLC analysis in the environmental fate studies, the transformation products are estimated to have K_{ow} values that are less than those of the parent compounds, factors A and D. Therefore, the transformation products do not meet the criteria for a TSMP Track-1 substance.

Spinosad does not contain any by-products or microcontaminants that meet the TSMP Track-1 criteria. Impurities of toxicological concern are not expected to be present in the raw materials, nor are they expected to be generated during the manufacturing process.

The formulated product does not contain any formulants that are known to contain TSMP Track-1 substances.

9.0 Regulatory decision

The active ingredient spinosad and associated end-use products Success™ 480SC Naturalyte Insect Control Product and Conserve™ 480SC Naturalyte Insect Control Product for the control of insect pests on apples, outdoor ornamentals, and turf have been granted temporary registration under Section 17 of the Pest Control Products Regulations, subject to the following conditions:

- additional studies must be conducted to refine the post-application exposure assessment;

- additional efficacy studies must be conducted to establish the lowest effective dose for the control of overwintering and summer-generation obliquebanded leafroller larvae on apples; and

- additional residue studies must be conducted in apples at or near approved Canadian maximum per season use rate.

List of abbreviations

ACT	acetylcholine
AD	administered dose
ADI	acceptable daily intake
A/G	albumin/globulin ratio
a.i.	active ingredient
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AP	alkaline phosphatase
AR	applied radiolabel
ARfD	acute reference dose
AST	aspartate aminotransferase
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
bwg	body-weight gain
C_{max}	peak concentration in blood
CAS	Chemical Abstract Services
CFSII	Continuing Surveys of Food Intake for Individuals
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DRA	dietary risk assessment
DT_{50}	time required for non-first-order 50% dissipation
DT_{90}	time required for non-first-order 90% dissipation
DWE	Dow AgroScience Canada Inc.
EEC	expected environmental concentration
ELS	early life stage
EPA	Environmental Protection Agency (U.S.)
ETC	eastern tent caterpillar
EUP	end-use product
FOB	functional observational battery
F_0	parental animals
F_1	first-generation offspring
F_2	second-generation offspring
GENEEC	Generic Expected Environmental Concentration
GGT	gamma glutamyl transferase
GIT	gastrointestinal tract
GOT	aspartate aminotransferase
GPT	alkaline aminotransferase
H, K	Henry’s law constants
HCT	hematocrit
HGB	hemoglobin
HPLC	high-performance liquid chromatography
ILV	independent laboratory validation
IPM	integrated pest management

IUPAC	International Union of Pure and Applied Chemistry
K_{oc}	normalized organic carbon adsorption coefficient
K_{ow}	*n*-octanol–water partition coefficient
LC_{50}	lethal concentration 50%
LC–MS	liquid chromatography – mass spectrometry
LD_{50}	lethal dose 50%
LD_{95}	lethal dose 95%
LOAEL	lowest observed adverse effect level
LOQ	limit of quantification
MAS	maximum average score (at 24, 48, and 72 h)
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MOE	margin of exposure
MOR	magnitude of residue
MRL	maximum residue limit
MRM	multiresidue methods
MTD	maximum tolerated dose
NOAEL	no observed adverse effect level
NOEC	no observable effect concentration
NZW	New Zealand White
OBLR	obliquebanded leafroller
P_1	first parental generation
PDI	potential daily intake
PHED	Pesticide Handlers Exposure Database
PHI	preharvest interval
PIS	primary irritation scar
PTI	post-treatment interval
RBC	red blood cells
ROC	residue of concern
SF	safety factor
SOP	standard operating procedure
STLM	spotted tentiform leafminer
$t_{½}$	excretion half-life
T_4	thyroxine
TG	technical grade
TGAI	technical grade active ingredient
TRR	total radioactive residue
TSMP	toxic substance management policy
UDS	unscheduled deoxyribonucleic acid synthesis
UV	ultraviolet
WBC	white blood cell
8_{max}	adsorption maximum

Appendix I Toxicology summary

Metabolism

Spinosad technical (XDE-105) contains two components, factor A and factor D, in a ratio of approximately 5:1; toxicokinetic studies were carried out with both components

Absorption

Factor A

• rapid absorption of -70–80% of the AD; blood peak concentrations were reached 1 h after single or repeat low doses (10 mg/kg bw) in both sexes or 2 h and 6 h after high-dose administration (100.0 mg/kg bw) for females and males, respectively

• no significant differences between sexes (dose level 10, 100, or repeated 10 mg/kg bw)

Factor D

• -60 % of the AD was absorbed (100 mg/kg bw dose only)

Excretion

Factor A

• low dose: -68–80% of the AD was excreted in 24 h and >91% in 7 d

• high dose: -68% of the AD was excreted in 24 h and >91% in 7 d

• eliminated in the feces (-82–87% fecal activity was eliminated in 7 d), 7–10% via urine and <0.6 (low) to 3% (high) remaining in tissues

• about 20% (factor A) of the dose was eliminated unabsorbed in the feces

• no significant differences in mass balance between sexes, dose level, or duration

• fecal excretion half-life at high dose: $t_{½}$" = 13.64 h for males and 28.74 h for females; at multiple and single low dose: $t_{½}$" = -9 h for males and 10 h for females

Factor D

• 35% (factor D) of the dose was eliminated unabsorbed in the feces

• feces as major route: 84–92% of the AD; bile contains approximately 36% of radioactivity

• urine: 3–5%

• biphasic elimination: data show lipid concentration

Distribution

Factor A

• wide distribution in tissue in 6–12 h; the highest concentrations were found in the liver, kidneys, adrenal glands, lungs, thyroid, lymph nodes, fat, and GIT

• -21% of the AD was found in tissues and carcass after 24 h; <3% of the radiolabel was found in the tissue at 168 h after the single high dose; <0.6% after the low dose (single or repeat)

• no significant difference in tissue distribution between sexes, dose levels (10, 100, or repeated 10 mg/kg bw), and single or multiple doses

Factor D

• high-dose administration only, wide distribution; <1.0% remaining in tissues and carcass at 168 h after dosing

• tissue levels lower than those for factor A; not quantifiable in thyroid

Metabolites

• – and O-demethylation (factors A or D); glutathione conjugation of parent and – and O-demethylated factors A and D

• no sex differences noted

• cysteine conjugate of XDE-105 (factors A and D) and a cysteine-conjugated O-demethylated XDE-105 (factor A) were reported in the feces and were possibly attributed to metabolic action by intestinal microflora, since cysteine conjugates were not detected in urine or bile

Overall comparisons between factor A and factor D

• the absorption, distribution, metabolism, and elimination of ^{14}C-XDE-105 were similar for factors A and D; marginally lower absorption of factor D was observed (60% versus 70% for factor A)

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
Acute studies			
Oral	Rat, Fisher 344; 5000 mg/kg Mouse, CD-1; 6000 mg/kg	Rat: LD_{50} > 5000 mg/kg (**&**) Mouse: LD_{50} > 6000 mg/kg (**%**/**&**)	Four males and 1 female rat died; 1 mouse per sex died; not a guideline study, however, XDE-105 was of **low acute toxicity** to mice
Oral	Rat, Fisher 344; 2000 mg/kg; 5 per sex	LD_{50} > 2000 mg/kg (**%**/**&**)	No mortality noted; clinical signs up to day 4 include poor grooming, hypoactivity; **low acute toxicity**
Dermal	Rabbit, NZW; 5 per sex	LD_{50} > 2000 mg/kg (**%**/**&**)	No clinical signs or mortality noted; **low acute toxicity**
Inhalation (4h; nose only)	Rat, Fisher 344; 10 per sex per group; 0.90 and 5.18 mg/L	LC_{50} (4-h) > 5.18 mg/L	Two deaths (1 **&** at 0.9 and 5.18 mg/L); clinical signs include poor grooming and chromodacryorrhea; **low acute toxicity**
Eye irritation	Rabbit, NZW; 0.1-g dose; unwashed, 3 per sex	Maximum average score (MAS) = 6.0/110	**Minimally irritating**; slight to moderate conjunctival irritation at 1 h; cleared by 48 h post-instillation
Skin irritation	Rabbit, NZW; 3 per sex; 0.5-g dose	PIS (24 h) = 0	**Non-irritating**
Skin sensitization (Buehler method)	Guinea pig, Hartley albino; 10 males per group Test material: 0.4 g 100% XDE-105 Positive control: 0.4 mL 10% DER 331	Negative	No effect noted with XDE-105; 9 positive responses in 10 with positive control; **not a skin sensitizer**
Acute studies: NAF-85			
Oral	Rat, Fisher 344; 5000 mg/kg	LD_{50} > 5000 mg/kg bw	**Low acute toxicity**
Dermal	Rabbit, NZW; 5 per sex	LD_{50} > 2000 mg/kg bw	**Low acute toxicity**
Inhalation (4-h nose only)	Rat, Fisher 344; 5 per sex; 5.0 mg/L	LC_{50} > 8.7 mg/L (nominal); LC_{50} > 5.0 mg/L (actual)	**Low acute toxicity**
Skin irritation	Rabbit, NZW; 3 per sex; 0.5-mL dose	PIS = 1/8.0	**Minimally irritating**
Eye irritation	Rabbit, NZW; 0.1-g dose; unwashed, 3 per sex	MAS (24-h) = 1.3/110	**Minimally irritating**
Skin sensitization (Buehler method)	Guinea pig, Hartley albino; 10 males per group Test material: 0.4 g NAF-85 Positive control: 0.4 mL 10% DER 331	Negative	**Not a skin sensitizer**

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
SHORT TERM			
21-d dermal	Rabbit, NZW; 5 per sex per dose; 0, 100, 500, or 1000 mg/kg bw/d; 6 h/d, 5 d/week for 15 applications	NOAEL = 1000 mg/kg bw/d; no LOAEL was established	No systemic toxicity at any dose tested
90-d dietary	Mouse, CD-1; 10 per sex per dose; 0, 0.005, 0.015, 0.045, or 0.12 % (equivalent to 0, 7.5, 22.5, 67.5, or 180 mg/kg bw/d)	NOAEL = 7.5 mg/kg bw/d (**%**/**&**); LOAEL = 22.5 mg/kg bw/d (**%**/**&**)	22.5 mg/kg bw/d: ♀ bw g (**%**), clinical signs of toxicity (perineal–ventral soiling and rough–oily hair **%**); ♂ spleen weights (**&**); mild vacuolation in lymphoid cells [spleen (**%**), lymph nodes (**&**)]; renal tubular degeneration; hepatocellular hypertrophy; gastric glandular dilation; bone marrow necrosis; histiocytosis of lymphoid organs **67.5 mg/kg/d:** ♀ bw, bwg (**%**); ♀ HGB (**%**), HCT (**%**), MCV (**&**/**%**), MCH (**&**/**%**); ♂ neutrophils; ♂ ALT, AST, ALP; ♀ albumin; ♂ kidneys (**%**/**&**), liver (**%**/**&**), and spleen weight (**&**); moderate vacuolation in lymphoid cells, liver (hepatocytes, Kupffer cells), kidneys (renal tubules), pancreas (acini), tongue, reproductive organs (**%**/**&**), adrenal cortex (**%** only); hepatocellular hypertrophy; renal tubular degeneration; foamy lung alveolar macrophages; myopathy of tongue; splenic hematopoiesis; gastric dilation; bone marrow necrosis; histiocytosis of lymphoid organs, stomach, uterus **180 mg/kg/d:** discontinued after day 44 due to mortality (3 **%**, 2 **&**) caused by hepatic necrosis and cachexia; clinical signs include hypoactivity, poor grooming, thinness, rapid respiration; significant ♀ bw, bwg, cytoplasmic vacuolation in multiple organs of both sexes, including kidneys, liver, heart, stomach, lymphoid organs (spleen, thymus, lymph nodes), reproductive organs (ovaries, uterus, cervix, vagina, epididymis), and inflammatory cell infiltration (histiocytosis) microcytic, hypochromic anemia and hepatobiliary disruption (♂ AP, ALP, AST, globulin; ♀ albumin, glucose, BUN, total

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
90-d dietary (continued)	Mouse, CD-1; 10 per sex per dose; 0, 0.005, 0.015, 0.045, or 0.12 % (equivalent to 0, 7.5, 22.5, 67.5, or 180 mg/kg bw/d)	NOAEL = 7.5 mg/kg bw/d (**%**/**&**); LOAEL = 22.5 mg/kg bw/d (**%**/**&**)	bilirubin, both sexes, 9 cholesterol and TG, **%** only); no organ weight data available; multifocal necrosis of the spleen, liver, lymph nodes; necrosis of bone marrow and skeletal myopathy
90-d dietary	Rat, Fisher 344; 10 per sex per group; 0, 0.05, 0.1, 0.2, or 0.4% (0, 33.9, 68.5, 133.5, or 273.1 mg/kg bw/d for **%**; 0, 38.8, 78.1, 151.6, or 308.2 mg/kg bw/d for **&**)	NOAEL not established; LOAEL = 33.9 and 38.8 mg/kg bw/d (**%**/**&**)	**33.9 and 38.8 mg/kg bw/d (%/&):** thyroid cell vacuolation, lymph node histiocytosis **68.5 and 78.1 mg/kg bw/d (%/&):** 8 blood urea nitrogen (BUN) and inorganic phosphorus and 9 urinary pH in females; 8 absolute and relative spleen weights (**&**); multifocal granulomatous vacuolation of Kupffer cells; mild cardiomyopathy (**%**); lymph node enlargement and skeletal muscle multifocal degeneration–regeneration (**&**); splenic and lymph node histiocytosis and thyroid cell vacuolation (**%**/**&**) **133.5 and 151.6 mg/kg bw/d (%/&):** significantly 9 bw, bwg, and feeding efficiency in both sexes; significantly 9 food consumption (**&**); hematological and clinical pathology parameters affected: [9 HGB (**%**/**&**), HCT(**%**), MCV (**&**/**%**), MCH (**&**/**%**); and 8 reticulocytes and leukocytes (**&**/**%**), AST (**%**/**&**), BUN (**&**), and inorganic phosphorus (**&**); 9 triglycerides (**%**) and urinary pH (**%**/**&**)]; 8 absolute and relative weights of heart, kidneys, spleen, adrenals, thyroid–parathyroid (**%**/**&**);8 liver and uterus weights (**&**); 8 prostate weight (**%**); increased incidence and severity of vacuolization in liver (Kupffer cells), kidneys, and thyroid (**%**/**&**); vacuolar change in lymph nodes, oviduct, uterus, and adrenals (**&**); moderate to marked histiocytosis in spleen and lymph nodes; multifocal granuloma in the liver; hyperkeratosis in the stomach (**%**/**&**); foamy alveolar macrophages (**&**)

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
90-d dietary (continued)	Rat, Fisher 344; 10 per sex per group; 0, 0.05, 0.1, 0.2, or 0.4% (0, 33.9, 68.5, 133.5, or 273.1 mg/kg bw/d for **%**; 0, 38.8, 78.1, 151.6, or 308.2 mg/kg bw/d for **&**)	NOAEL not established; LOAEL = 33.9 and 38.8 mg/kg bw/d (**%**/**&**)	**273.1 and 308.2 mg/kg bw/d (%/&):** 5 per sex died or killed moribund after 5-6 weeks on test; all animals terminated on test day 44; clinical signs include deep, rapid, or labored breathing, hypothermia, thinness, chromorhinorrhea, piloerection, and distended penis; ↓ bw, bwg, food consumption, feed efficiency; anemia as evidenced by ↓ MCV and MCHC, ↑ nucleated RBC, anisocytosis, polychromasia, erythroblastosis, and neutrophilia; ↑ ALT, AST, AP, and GGT in both sexes and BUN and inorganic phosphorus in females only; ↑ cytoplasmic vacuolation in multiple organs in both sexes [kidneys, liver, heart, stomach, lymphoid organs (spleen, thymus, lymph nodes), reproductive organs (ovaries, uterus, cervix, vagina, epididymis)] and inflammatory cell infiltration (histiocytosis) in several organs; less prominent vacuolation effects were noted in the heart, pancreas, prostate and adrenal cortex; bone marrow hypocellularity (**%**/**&**) and hypospermatogenesis in **%**
90-d dietary (with 4-week recovery)	Rat, Fisher 344; 10 per sex per group plus 10 per sex in recovery control and high dose; 0, 0.003, 0.006, 0.012, or 0.06% (0, 2.2, 4.3, 8.6, or 42.7 mg/kg bw/d for **%**; 0, 2.6, 5.2, 10.4, or 52.1 mg/kg bw/d for **&**)	NOAEL = 8.6 and 10.4 mg/kg bw/d (**%**/**&**); LOAEL = 42.7 and 52.1 mg/kg bw/d (**%**/**&**)	**42.7 and 52.1 mg/kg/d (%/&):** very slight to slight thyroid follicle epithelial cell vacuolation; severity and incidence slightly reversible after 4-week recovery period. No effect of thyroid (T_4) levels noted.
90-d dietary	Dog, beagle; 4 per sex per dose (0, 150, 300, and 1350/900 ppm for **%** or 900 ppm for **&**) (0, 4.89, 9.73, or 33.4 mg/kg bw/d for **%**; 0, 5.38, 10.47, or 29.9 mg/kg bw/d for **&**)	NOAEL = 4.89 and 5.38 mg/kg bw/d (**%**/**&**); LOAEL = 9.73 and 10.47 mg/kg bw/d (**%**/**&**)	**9.73 and 10.47 mg/kg bw/d (%/&):** ↑ cytoplasmic vacuolation – vacuolated cell aggregation in lymph nodes, faucial tonsil, lungs, pancreas, and lymphoid tissue in GIT (**%**/**&**); atrophic stomach mucosa (**&**) **33.4 and 29.9 mg/kg bw/d (%/&):** test material concn. ↓ on day 38 due to mortality in one dog, decreased mean bw and food consumption, anemia; ↑ spleen, thyroid, pancreas and liver weights (**%**/**&**), liver enzymes; ↓ A/G

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
90-d dietary (continued)	Dog, beagle; 4 per sex per dose (0, 150, 300, and 1350/900 ppm for **%** or 900 ppm for **&**) (0, 4.89, 9.73, or 33.4 mg/kg bw/d for **%**; 0, 5.38, 10.47, or 29.9 mg/kg bw/d for **&**)	NOAEL = 4.89 and 5.38 mg/kg bw/d (**%**/**&**); LOAEL = 9.73 and 10.47 mg/kg bw/d (**%**/**&**)	ratio; 8 globulin, total cholesterol, AST, ALT, ALP, arteritis, focal necrosis – cellular depletion in bone marrow, thymic atrophy, atrophic white pulp of spleen, Kupffer cell proliferation in the liver (**%**/**&**), cytoplasmic vacuolation – vacuolated cell aggregation [spleen, lymph nodes, faucial tonsil, lungs,pancreas, liver, parathyroid and lymphoid tissue in GIT, nerve tissue, testes, adrenal cortex (**%**/**&**)]; 9 spermatogenesis, arteritis of epididymis and testes, vacuolated testis seminiferous epithelial cells; 8 spermatid giant cells in testes
12-month dietary (1996)	Dog, beagle; 4 per sex per dose; 0, 50/60, 100/120, or 300/360 ppm; after 4 weeks, the amount of food was reduced to prevent the animals from becoming obese [0, 1.33, 2.72, or 8.22 mg/kg bw/d (**%**); 0, 1.44, 2.68, or 8.46 mg/kg bw/d (**&**)]	NOAEL = 2.7 mg/kg bw/d (**%**/**&**); LOAEL = 8.2 and 8.5 mg/kg bw/d (**%**/**&**)	**8.2 and 8.5 mg/kg bw/day (%/&):** 8 serum ALT, AST, and triglycerides levels in **%**; 8 incidence of vacuolated cells aggregates in the various lynphoid tissues (spleen, faucial tonsil, lymph nodes, and intestine) in **%** and **&**; two **%** had glandular cell vacuolation of the parathyroid; 8 (statistically significant) absolute and relative thyroid weight in 3 of 4 **&**
Chronic toxicity–oncogenicity			

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
80-week dietary (1995)	Mouse, CD-1; 70 per sex per dose (10 per sex per dose for interim sacrifice at 3 and 12 months); 0, 0.0025, 0.08, or 0.036% [0, 3.4, 11.4, or 50.9 mg/kg bw/d (**%**); 0, 4.2, 13.8, or 67.0 mg/kg bw/d (**&**)]	NOAEL = 11.4 and 13.8 mg/kg bw/d (**%**/**&**); LOAEL = 50.9 and 66.0 mg/kg bw/d (**%**/**&**); no oncogenic effect	**50.9 and 66.0 mg/kg bw/d (%/&):** 9 bw, bwg, and corresponded to 9 in amount of fat seen at necropsy in **%** and **&**; 8 increased mortality (**%**/**&**); hematologic effects [9 Hg, Ht, at 3 and 12 months in **%** and at 3 months in **&**; 8 WBC count at 12 months (**%**/**&**); and 8 hypochromasia of RBC at 18 months in **%**]; 8 AST in **%**; 8 absolute and relative spleen weights (**%**/**&**) was correlated with 8 incidence of extramedullary hematopoiesis; 8 incidence of thickening of the gastric mucosa (**%**/**&**) seen at necropsy; 8 incidence and severity of mucosal inflammation; 8 incidence of hyperplasia of the glandular gastric mucosa (**%**); 8incidence of slight vacuolation and degeneration–inflammation of multiple organs (kidneys, lungs, lymph nodes, pancreas, parathyroid glands, skeletal muscle, tongue, epididymides, ovaries, and uterus) in **%**/**&**
80-week dietary (1995) (continued)	Mouse, CD-1; 70 per sex per dose (10 per sex per dose for interim sacrifice at 3 and 12 months); 0, 0.0025, 0.08, or 0.036% [0, 3.4, 11.4, or 50.9 mg/kg bw/d (**%**); 0, 4.2, 13.8, or 67.0 mg/kg bw/d (**&**)]	NOAEL = 11.4 and 13.8 mg/kg bw/d (**%**/**&**); LOAEL = 50.9 and 66.0 mg/kg bw/d (**%**/**&**); no oncogenic effect	No treatment-related increase in tumor incidence, tumor spectrum; or latency when compared with controls

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
80-week dietary (1997); this study was submitted to upgrade a previous study and was started during the time of the previous study, but most of the data included in the report are related to the high-dose females	Mouse, CD-1; 50 per sex per dose (10 per sex per dose for interim sacrifice at 12 months); 0, 0.0008, or 0.024 % [1.1 or 32.7 mg/kg bw/day (**%**); 0, 1.3, or 41.5 mg/kg bw/day (**&**)]	NOAEL = 11.4 and 13.8 mg/kg bw/d (**%**/**&**); LOAEL = 32.7 and 41.5 mg/kg bw/d (**%**/**&**); no oncogenic effect	**1.1 and 1.3 mg/kg bw/d (%/&):** no effects **32.7 and 41.5 mg/kg bw/d (%/&):** ↓ bw, bwg in **%**; ↑ WBC count in **%**/**&** at 12 and 18 months was associated with a chronic inflammation of the glandular mucosa of the stomach; histopathological evaluations were only conducted on the control and high-dose female mice; histopathological effects consisted of aggregates of alveolar macrophages in the lungs, sinus histiocytosis of lymph nodes, vacuolation of parathyroid glands, skeletal myopathy (of the face and tongue), and hyperkeratosis, hyperplasia, and inflammation of stomach; there was no increase relative to controls in the incidence of any type of tumor
2-year dietary (1995)	Rat, Fischer 344; 65 per sex per group (15 per sex per group were killed after 12 months); 0, 0.005, 0.02, 0.05, or 0.1% [(0, 2.4, 9.5, 24.1, or 49.4 mg/kg bw/d (**%**); 0, 3.0, 12.0, 30.3, or 62.8 mg/kg bw/d (**&**)]	NOAEL = 2.4 and 3.0 mg/kg bw/d (**%**/**&**); LOAEL = 9.5 and 12 mg/kg bw/d (**%**/**&**)	**9.5 and 12 mg/kg bw/d (%/&):** ↑ incidence of slight vacuolation of the follicular epithelial cells of the thyroid in **%**/**&** **24.1 and 30.3 mg/kg bw/d (%/&):** ↑ incidence of slight to moderate vacuolation of the follicular epithelial cells of the thyroid in **%**/**&**; very slight to moderate necrosis and inflammation of the thyroid gland in **&**; ↑ in absolute and relative thyroid weights in **&**; the severity of treatment-related alterations in the thyroid progressed with time; ↑ incidence of slight inflammation of the lungs in **%** and **&** **49.4 and 62.8 mg/kg bw/d (%/&):** ↑ mortality (**%**/**&**); the group was terminated on days 714 and 611 (**%**/**&**); ↓ bw, bwg; ↑ perineal soiling; ↑ WBC count in **&**; ↑ AP, AST, and BUN in **%**/**&**; at 12 months ↑ incidence in **%** and **&** of decreased body fat reserves, degenerative and inflammatory lesions in the heart, hydrothorax, inflammatory

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
2-year dietary (1995) (continued)	Rat, Fischer 344; 65 per sex per group (15 per sex per group were killed after 12 months); 0, 0.005, 0.02, 0.05, or 0.1% [(0, 2.4, 9.5, 24.1, or 49.4 mg/kg bw/d (**%**); 0, 3.0, 12.0, 30.3, or 62.8 mg/kg bw/d (**&**)]	NOAEL = 2.4 and 3.0 mg/kg bw/d (**%**/**&**); LOAEL = 9.5 and 12 mg/kg bw/d (**%**/**&**)	changes in the lungs, vacuolation of the tubular epithelial cells of the kidneys, degenerative and inflammatory changes of the skeletal muscles, aggregates of reticuloendothelial cells in the liver, spleen, and mesenteric lymph nodes, degenerative and inflammatory changes in the glandular mucosa of the stomach, and follicular epithelial vacuolation and inflammation of the thyroid gland; no histopathological examinations at termination **No carcinogenic potential at the MTD of 0.05%**
Reproduction and developmental toxicity			
Multigeneration (2 generations, 2 litters in F_1 and 1 litter in F_2)	Rat, Sprague-Dawley; 30 per sex per dose per generation; 0, 0.005, 0.02, or 0.2%; 0, 50, 200, or 2000 ppm (equivalent to 0, 3, 10, or 100 mg/kg bw/d)	NOAEL (systemic toxicity) = 10 mg/kg bw/d; NOAEL (reproductive–offspring toxicity) = 10 mg/kg bw/d; LOAEL (systemic toxicity) = 100 mg/kg bw/d; LOAEL (reproductive–offspring toxicity) = 100 mg/kg bw/d	**100 mg/kg bw/d, systemic toxicity:** decreased body weight in P_1 dams during F_{1a} and F_{1b} gestation, increased mortality of dams; 8 heart, kidney, spleen, liver, and thyroid weights with corroborative pathology: **spleen and mesenteric lymph nodes,** sinus histiocytosis; **thyroid,** diffuse cytoplasmic vacuolation of the follicular epithelial cells with associated chronic active inflammation and necrosis; **lungs,**8 incidence of multifocal subacute to chronic inflammation of the interalveolar septae with multifocal aggregates of alveolar macrophages; **males only:** degeneration of the myocardium with or without inflammation, tubular degeneration in the kidneys, and chronic active inflammation of the prostate; **females only:** dilation of the glandular crypts with cellular debris in the pyloric region of the stomach **100 mg/kg bw/d, reproductive–offspring toxicity:** decreased litter size, pup survival (F_{1a} and F_{1b}), and pup weights ($F_{1a,}$ F_{1b}, and F_2 days 14 and 21); increased incidence of dystocia and vaginal bleeding postpartum with associated increased mortality of dams

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
Developmental toxicity and teratogenicity	Rabbit, NZW; 20 mated per dose; 0, 2.5, 10, or 50 mg/kg bw/d	NOAEL (maternal toxicity) = 10 mg/kg bw/d; NOAEL (developmental toxicity) = 50 mg/kg bw/d; LOAEL (maternal toxicity) = 50 mg/kg bw/d; LOAEL (developmental toxicity) was not determined	**50 mg/kg/d, maternal toxicity:** 9 bwg and food consumption and fecal output during the gestational dosing period; no developmental or teratological effects were noted up to and including the high dose of 50 mg/kg bw/d
Developmental toxicity and teratogenicity	Rat, Sprague-Dawley; 30 mated per dose; 0, 10, 50, or 200 mg/kg bw/d	NOAEL (maternal toxicity) = 50 mg/kg bw/d; NOAEL (developmental toxicity) = 10 mg/kg bw/d; LOAEL (maternal toxicity) = 200 mg/kg bw/d; LOAEL (developmental toxicity) = 50 mg/kg bw/d	**200 mg/kg/d, maternal toxicity:** 9 bwg during the gestational dosing period **50 mg/kg bw/d, developmental toxicity:** 8 delayed ossification, sternebrae; no teratological effects were noted up to and including the high dose of 200 mg/kg bw/d
Genotoxicity			
Study	**Species–strain or cell type**	**Doses employed**	**Effects**
Salmonella Typhimurium, *E. coli* (Ames test) (1996)	TA98, TA100, TA1535, TA1537, TA1538m, WP2uvrA	100, 250, 500, 1000, 2500, 5000 Fg/plate with TA98, TA100, TA1535, WP2uvr2 with–without S9 mix, and with TA1537 with S9 mix; 25, 50, 100, 250, 500, 1000, 2000 Fg/plate with TA1537 without S9 mix	Negative
Mammalian cytogenetics (in vitro) (1992)	Mouse lymphoma cells	0 (DMSO), 15, 20, 25, 30, 35, 40, 45, 50 Fg/mL with S9 mix; 0 (DMSO), 1, 5, 10, 15, 20, 25 Fg/mL without S9 mix	Negative
Mammalian chromosomal aberration (in vitro) (1992)	Chinese hamster ovary cells	0 (DMSO), 100, 250, 500 Fg/mL with S9 mix; 0 (DMSO), 20, 26, 35 Fg/mL without S9 mix	Negative
Micronucleus assay (in vivo) (1992)	ICR mice	0, 500, 1000, 2000 mg/kg bw	Negative
Unscheduled deoxyribonucleic acid synthesis (UDS) in vitvo (1992)	Rat hepatocyte cultures	0.01, 0.05, 0.1, 0.5, 1,5 Fg/mL	Negative

Study	Species, strain, and dose	NOEL–NOAEL and LOAEL (mg/kg bw/d)	Target organ, significant effects, and comments
Special studies (if applicable)			
Acute neurotoxicity (1994)	Rat, Fisher 344; 10 per sex per dose; 0, 200, 630, 2000 mg/kg bw, single dose; observation time 15 d	NOAEL = 2000 mg/kg bw; LOAEL > 2000 mg/kg bw	No evidence of neurotoxicity at any dose level; 630 and 2000 mg/kg bw, transient decrease in body weight was seen on day after dosing in both sexes
Subchronic neurotoxicity (1993)	Rat, Fisher 344; 10 per sex per dose; 0, 0.003, 0.006, 0.012, or 0.06% [0, 2.2, 4.3, 8.6, or 42.7 mg/kg bw/d (**%**); 0, 2.6, 5.2, 10.4, or 52.1 mg/kg bw/d (**&**)]	NOAEL = 42.7 and 51.2 mg/kg bw/d (**%**/**&**); LOAEL > 42.7 and 51.2 mg/kg bw/d (**%**/**&**)	No evidence of neurotoxicity at any dose level
Chronic neurotoxicity (1995); combined with a 2-year chronic toxicity–carcino genicity study	Rat, Fisher; 10 per sex per dose underwent neurobehavioural testing at 0, 3, 6, 9, and 12 months; 5 per sex control and 0.1% rats were assessed for neuropathology at 12 months; 0, 0.005, 0.02, 0.05, or 0.1% [0, 4.6, 9.2, 23, or 46 mg/kg bw/d (**%**); 0, 5.7, 11.4, 28.5, or 57 mg/kg bw/d (**&**)]	NOAEL = 46 and 57 mg/kg bw/ d (**%**/**&**); LOAEL > 46 and 57 mg/kg bw/d (**%**/**&**)	No evidence of neurotoxicity; no effect on FOB or motor activity; no histopathological changes of the central or peripheral nervous system

Appendix II Summary of integrated food residue chemistry

Parameter	Pertinent information
Chemical	spinosad (spinosyn A + spinosyn D)
Formulation	Success™ 480SC
Crop	Apples
Type of application	Foliar broadcast
Number of applications	Three per season
Application rate	87 g a.i./ha
Maximum seasonal application rate	261 g a.i./ha
Preharvest interval (PHI)	7 d
Label restrictions	Repeat applications 7–10 d after the initial application
Nature of the residue in animals	
Lactating goat	In the goat metabolism studies, spinosad (spinosyns A and D) was not extensively metabolized; some identified metabolites occurred as a result of demethylation, hydrolysis, and conjugation; spinosyns A and D and related metabolites were transferred to milk, fat, muscle, kidneys, and liver; these residues ranged from 0.11 to 3.57 ppm; spinosyn A related residues in milk and tissues were 2–3× higher than that observed from spinosyn D dosing; TRRs in all samples were readily extractable; spinosyns A and D were metabolized through N-demethylation of the forosamine, hydroxylation of the macrolide at several locations, and a combination of these reactions which produced several isomeric metabolites
Radiolabelling positions	Spinosyns A and D
Proposed metabolic pathway	Involves either the loss of a single methyl group from the N-methyl moiety on the forosamine sugar or the hydroxylation of the macrolide at several different positions
Residue of concern (ROC)	Spinosyns A and D

Parameter	Pertinent information
Nature of the residue in plants	
Crop	Apple: spinosad (spinosyns A and D) is readily metabolized in apples: the major metabolites detected in apples were adequately tested in the rat and were found not to be of toxicological concern; incorporation of radioactivity into the general carbon pool and endogenous biochemicals was demonstrated
Radiolabelling positions	Spinosyns A and D
Proposed metabolic pathway	Involves the initial formation of nonpolar residues, some of which are modified on the forosamine portion of the molecule; with further photolytic degradation, the macrolide portion and possibly the rhamnose portion of the molecule are also modified to form polar and nonextractable residues that are subject to biochemical processes and incorporation into natural plant constituents
Residue of concern (ROC)	Spinosyns A and D
Residue analytical method: plant	
(ROC)	Spinosyns A and D
Data gathering method	HPLC with UV detection (250 nm); 85–101% for recovery of spinosyns A and D in apple tissues; LOQ 0.01 ppm; LOD 0.003 ppm
Confirmation method	LC–MS can detect and quantitate the analytes of interest
Enforcement method	The enforcement method is equivalent to data gathering method
Independent laboratory validation (ILV)	ILV method indicated good reliability and reproducibility
Residue analytical method: animal	
(ROC)	Spinosyns A and D
Data gathering methods	HPLC with UV detection (250 nm); 76–120% for recovery of spinosyns A + D in meat and milk; LOQ 0.01 ppm; LOD 0.003 ppm in milk and tissues Immunoassay (RaPID Assay Kit by Ohmicron) determined combined residues of spinosyns A and D in tissues and milk at an absorbance of 450 nm; recoveries were 77–101% in milk and tissues; LOQ 0.01 ppm; LOD 0.003 ppm; statistical comparison of HPLC and immunoassay method indicated correlation coefficient of 0.95
Confirmatory method	LC–MS can detect and quantitate the analytes of interest
Enforcement method	The enforcement method is equivalent to the data gathering method

Parameter	Pertinent information
Independent laboratory validation (ILV)	ILV method indicated good reliability and reproducibility
Multiresidue method	Protocols from existing multiresidue methods were not suitable for the determination of spinosyns A and B residues in apples or animal commodities
Storage stability data	
Plant matrices	Up to 6 months (apples) and up to 3 months (juice) at –20°C
Animal matrices	1.6 years in milk, fat, kidneys, liver, and muscle at –20°C
Crop field trials	Thirteen residue trials on apples were conducted in three of the five zones required for a Canadian registration (zones 1, 5, and 11); the trials were conducted at the U.S. maximum seasonal rate (~2× the proposed Canadian rate); the highest field trial value was 0.089 ppm.
Residue decline	The residue decline studies indicated that spinosad residues declined at 3 and 7 d post-treatment (PTI), with no statistically significant decrease beyond 7 d
Processed food–feed	Apples were treated at 5× the proposed maximum seasonal rate and harvested 7 d following the last of five foliar applications; total (spinosyn A + spinosyn D) concentration: 0.1× in apple juice; 5.3× in wet pomace
Livestock feeding	Dairy cattle were fed orally with 1, 3, and 10 ppm of spinosad for 28 d; at 1 ppm feeding, highest residues of spinosad (spinosyn A + spinosyn D) were 0.012 ppm (skim milk), 0.057 ppm (whole milk), 0.249 ppm (cream), 0.06 ppm (kidneys), 0.115 ppm (liver), 0.023 ppm (muscle), and 0.56 ppm (fat); the anticipated residues in milk and tissues were based on a dietary burden of 0.47 ppm
Confined accumulation in rotational crops	Not applicable
Field accumulation in rotational crops	Not applicable
Proposed MRLs	Apples (0.1 ppm), whole milk (0.1 ppm); cattle, sheep, goat, horse, hog meat, and meat by-products (0.01 ppm); kidneys (0.03 ppm); liver (0.05 ppm); cattle, sheep, goat, horse, hog fat (0.3 ppm)
Proposed import tolerances	No import tolerances have been petitioned
U.S. tolerances	Apples (0.2 ppm), milk fat (5.0 ppm), whole milk (0.5 ppm), meat (0.15 ppm), meat by-products (1.0 ppm), fat (3.5 ppm)
Codex MRLs	None established

Parameter	Pertinent information
Dietary risk assessment (DRA), DEEM™ version 7.6.2; 1994–1998 Continuing Surveys of Food Intake for Individuals	The potential daily intake (PDI), including water allocation (EEC value of 0.01 ppm), was 80, 60, and <55% of the acceptable daily intake (ADI = 0.009 mg/kg bw) for children 1–6 years, children 7–12 years, and the remainder of the total population, including infants and seniors, respectively

Appendix III Environmental assessment

Table 1 Summary of transformation, mobility, and fate of spinosad

Study		Factor A	Factor D	Interpretation
Technical grade active ingredient (TGAI)				
Hydrolysis (25°C)		No transformation at acidic and neutral pH; $t_{½}$ = 200 d at pH 9	No transformation at acidic and neutral pH; $t_{½}$ = 259 d at pH 9	Not an important route of transformation
Phototransformation on soil		$t_{½}$ = 82 d	$t_{½}$ = 44 d	Not an important route of transformation
Aerobic soil biotransformation[1]	25°C	DT_{50} = 9–17 d; DT_{90} < 56 d	DT_{50} = 15 d; DT_{90} < 56 d	Important route of transformation; more persistent at lower temperatures
	20°C	DT_{50} = 24–43 d; DT_{90} = 143–220 d	DT_{50} = 15–69 d; DT_{90} = 144–227 d	
Adsorption–desorption (K_{oc})		2862 (sand); 844 (loamy sand); 4310 (sandy loam); 140 434 (silt loam); 24 397 (clay loam)	A study was not conducted	Low mobility in loamy sand soil; slightly mobile in sand; immobile in sandy loam, silt loam, and clay loam soils
		Factor B: 2138 (sand), 672 (loamy sand), 2931 (sandy loam), 77 826 (silt loam)		Low mobility in loamy sand soil; slightly mobile in sand and sandy loam soils; immobile in silt loam soil
End-use product (EUP)				
Canadian field dissipation–accumulation (microplot)		DT_{50} = 2–12 d; DT_{90} = -40–50 d	DT_{50} = 3–6 d; DT_{90} < 35 d	NAF-85[2]; parent compound not detected below 5 cm; nonpersistent

[1] DT_{50}, time required for non-first-order 50% dissipation; DT_{90}, time required for non-first-order 90% dissipation.
[2] Former name of Success™ 480SC.

Table 2 Summary of transformation products formed in terrestrial fate studies

Fate process	Major transformation products (maximum percentage of applied factors A and D)	Minor transformation products (maximum percentage of applied factors A and D)
Phototransformation on soil	No major transformation products	Unidentified (2.1–5.6%); factor B (No. 210984) (6.8%); factor B of D (No. 202149) (4.2%)
Aerobic soil biotransformation	Factor B (No. 210984) (61%); factor B of D (No. 202149) (68%)	Unidentified (2.3–8.1%)
Terrestrial field dissipation	Factor B of D (No. 202149) (33.6%)	Factor B (No. 210984) (5%)

Table 3 Summary of aquatic transformation and fate of spinosad

Study	Factor A	Factor D	Interpretation
Technical grade active ingredient (TGAI)			
Hydrolysis (25°C)	Stable at pH 5 and pH 7; $t_{½}$ = 200 d, pH 9	Stable at pH 5 and pH 7; $t_{½}$ = 259 d, pH 9	Stable; not an important route of transformation
Phototransformation in water	$t_{½}$ = 2 d	$t_{½}$ = 1 d	Important route of transformation
Anaerobic sediment–water biotransformation (25°C)	DT_{50} = 161 d; DT_{90} > 365 d	DT_{50} = 250 d; DT_{90} > 365 d	Moderately persistent to persistent; not an important route of transformation
End-use product (EUP)			
U.S. aquatic field dissipation	DT_{50} = 2–3 d; DT_{90} < 4 d		Applied NAF-85 (480 g Spinosad/L); nonpersistent

Table 4 Summary of transformation products formed in aquatic fate studies

Fate process	Major transformation products (maximum percentage of applied factors A and D)	Minor transformation products (maximum percentage of applied factors A and D)
Hydrolysis	No major transformation products	Factor B (No. 210984); deforosamine derivative (No. 235477); 16,17-dehydropseudoaglycone (No. 809779); 17,18-dehydropseudoaglycone (No. 809780); pseudoaglycone of factor A; factor B of D (No. 202149)
Phototransformation in water	13,14-Dihydropseudo-aglycone of factor A, beta isomer (20–25% AR)	Unidentified compounds (4.2–9.3% AR)
Anaerobic aquatic biotransformation	Factor J (loss of CH_2 from methylrhamnose sugar) (12.2% AR); factor J minus a further CH_2 from methylrhamnose sugar (14.9% AR); ketoreversepseudoaglycone (No. 814426) (14.4% AR); unknown 5 (11.9% AR)	Factor B (No. 210984) (9.1% AR); reversepseudoaglycone (No. 806643) (isomer 1, 3.2%; isomer 2, 3.7%); factor B of D (No. 202149) (7.1%); reversepseudoaglycone of D (No.806643) (isomer 1, 2.3%; isomer 2, 2.2%); unidentified compounds (3.3–7.6)
U.S. aquatic field dissipation	Factor B (No. 210984); factor B of D (No. 202149)	No minor transformation products

Table 5 The maximum expected environmental concentrations (EECs) of spinosad (factors A and D) on vegetation and other food sources immediately following application at the proposed maximum seasonal application rate of 261 g total a.i./ha

Environmental compartment	EEC fresh weight (mg total a.i./kg)	Ratio of fresh weight to dry weight	EEC dry weight (mg a.i./kg)		
			Factor A	Factor D	Total
Short range grass	55.9	3.32	156.7	27.6	184.3
Leaves and leafy crops	29.2	11.02	273.4	48.2	321.6
Long grass	25.6	4.42	95.6	16.9	112.5
Forage crops	13.6	5.42	62.3	11	73.3
Small insects	13.6	3.83	43.9	7.7	51.6
Large insects	2.3	3.83	7.5	1.3	8.8
Grain and seeds	2.3	3.83	7.5	1.3	8.8

Table 6 Summary of risk to nontarget terrestrial organisms

Organism	Study	NOEC–NOEL (or LD_{50} where indicated)	EEC (total a.i.)	Margin of Eexposure	Risk
Bobwhite quail	Acute oral	50 mg a.i./kg bw	31.3 mg a.i./kg diet	16	None
	8-d dietary	656 mg a.i./kg diet		21	
	Reproduction	550 mg a.i./kg diet		18	
Mallard	Acute oral	200 mg a.i./kg bw	8.8 mg a.i./kg diet	218[1]	None
	8-d dietary	302 mg a.i./kg diet		34	
	Reproduction	550 mg a.i./kg diet		63	
Rat	Acute oral (XDE-105)	LD_{50} > 2000 mg a.i./kg bw (% + &)	131.7 mg a.i./kg diet	89[2]	None
		One-tenth of LD_{50} = 200 mg a.i./kg bw		0.375	Moderate
	2-year dietary	50 mg a.i./kg dw diet		0.4	None[3]
Mouse	90-d dietary	50 mg a.i./kg dw diet	130.9 mg a.i./kg diet	0.4	None[3]
Earthworm	14-d acute	970 mg a.i./kg substrate	0.105 mg a.i./kg soil	9240	None
Honey bees	48-h contact	LD_{50} = 0.0029 Fg a.i./bee (/3.25 g a.i./ha)[4]	87 g a.i./ha single application	0.037	High
	48-h contact	LD_{50} = 0.045 Fg a.i./bee (/50.4 g a.i./ha)[4]		0.58	Moderate
	48-h oral	LD_{50} = 0.060 Fg a.i./bee (/67.2 g a.i./ha)[4]		0.77	Moderate
Predators and parasites	24-h contact	LD_{50} = 29.1 mg a.i./L	150 mg a.i./L in spray tank	0.33	Moderate
	24-h oral	LD_{50} > 200 mg a.i./L		>2.3	Low
Terrestrial plants	Seedling emergence	No significant effects at 560 g a.i./ha	261 g a.i./ha[5]	2	Low
	Vegetative vigour	No significant effects at 560 g a.i./ha		2	Low

[1] Estimate of the number of days required for a bird or mammal to consume a dose of spinosad, from contaminated food, that would be equivalent to the dose administered by gavage which had no observable effect on the laboratory population. Based on average body weight and food consumption of control animals from the reproduction study (birds) or standard body weight and food consumption.
[2] Estimate of the number of days required for a mammal to consume a dose of spinosad, from contaminated food, that would be equivalent to the dose administered by gavage which killed 50% of the individuals in the laboratory population. Based on standard body weight and food consumption of animals.
[3] Based on a refined assessment using plant metabolism data, spinosad will dissipate rapidly from plant foliage and should not pose a risk to mammals through consumption of contaminated diet.
[4] According to Atkins.
[5] Maximum seasonal application rate with no transformation.

Table 7 Summary of risk to aquatic nontarget organisms

Organism	Study	NOEC (mg a.i./L)	EEC (mg a.i./L)	Margin of Exposure	Risk
Daphnia magna	48-h	0.3	0.087[1]	3	Low
	21-d	0.00062	0.018[2]	0.034	High
Midge, *Chironomus riparius*	25-d	0.0014	0.018	0.078	High
Grass shrimp	96-h	1.66	0.087	19	None
Mysid shrimp	28-d	0.084	0.018	5	Low
Eastern oyster	96-h shell deposition	0.11	0.087	1.3	Low
Bluegill sunfish	96-h	2.1	0.087	24	None
Rainbow trout	96-h	5.2	0.087	60	None
	32-d ELS[3]	0.5	0.018	28	None
Sheepshead minnow	96-h	1.8	0.087	21	None
	32-d ELS	1.15	0.018	64	None
Anabaena flos-aquae	120-h	3.9	0.087	45	None
Selenastrum capricornutum	7-d	4.3	0.087	49	None
Navicula pelliculosa	120-h	0.049	0.087	0.56	Moderate
Skeletonema costatum	120-h	0.17	0.087	2	Low
Duckweed, *Lemna gibba*	14-d	1.86	0.087	21	None

[1] Maximum EEC in water was calculated for a direct overspray.
[2] EEC in water was calculated for a 21-d average concentration from runoff (GENEEC).
[3] ELS, early life stage.

Appendix IV Value summary

Table 1 USC 14: terrestrial food crops (control of obliquebanded leafroller)

Site	Apples (USC 14)
Product	Success™ 480SC
Rate of application	182 mL product/ha (87 g spinosad/ha) to a maximum 546 mL product/ha/year (261 g spinosad/ha/year)
Number of applications	Not more than three per year
Application timing	To determine whether treatment or retreatment is required, infestation densities of the larval stages should be monitored; in addition, moth flights for the summer generation can be monitored
Pest controlled	Obliquebanded leafroller

Table 2 USC 27: ornamentals outdoor (control of larvae of conifer sawfly, gypsy moth, tent caterpillar, and leaf beetle)

Site	Ornamentals outdoor (USC 27)
Product	Conserve™ 480SC
Rate of application	25 mL product/1000 L of spray (12–24 g a.i./ha),[1] to a maximum 200 mL product/ha/year (96 g spinosad/ha/year)
Number of applications	Not more than once every 7 d
Application timing	To be applied when surveys indicate pests are a problem
Pest controlled	Larvae of conifer sawfly, gypsy moth, tent caterpillar (such as eastern tent), and leaf beetle (such as elm and willow leaf beetle)

[1] Based on spray volume of 1000–2000 L/ha.

Table 3 USC 27: ornamentals outdoor (control of western flower thrips)

Site	Ornamentals outdoor (USC 27)
Product	Conserve™ 480SC
Rate of application	50 mL product/1000 L of spray (26 g a.i./1000 L)
Number of applications	Not more than once every 7 d; maximum of three applications per crop per year
Application timing	Monitor for thrips, and apply when populations reach damaging levels; consult provincial guidelines and local extension experts; for best results, apply at the floral stage of development
Pest controlled	Western flower thrips

Table 4 USC 30: Turf

Site	Turf (USC 30)
Product	Conserve™ 480SC
Rate of application	51–102 mL product/ha (24.5–49 g a.i./ha), to a maximum 400 mL product/ha (192 g a.i./ha)
Number of applications	Not more than once every 7 d
Application timing	To be applied when surveys indicate pests are a problem.
Pest controlled	Larvae of sod webworm