SPINETORAM

First draft prepared by
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Explanation

Spinetoram, also known as XDE-175 or XR-175, is a fermentation product derived from the actinomycete bacterium *Saccharopolyspora spinosa,* which has been slightly modified by chemical reaction. Spinetoram is a macrocyclic lactone insecticide. It acts by causing persistent activation of insect nicotinic acetylcholine receptors.

Spinetoram is composed of numerous spinosyns, known as "factors", which differ slightly from each other. Each spinosyn consists of a large complex hydrophobic ring, a basic amine group, and two sugar moieties. The insecticidal activity of spinetoram is attributed to two spinosyns, identified

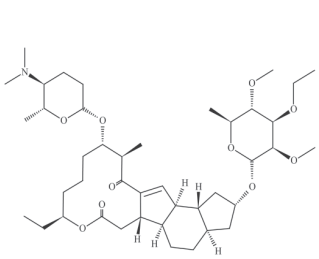
as XDE-175-J ("factor J") and XDE-175-L ("factor L"), which comprise the overwhelming majority of the technical material (Figure 1). The ratio of factor J to factor L ranges from 70 : 30 to 90 : 10. Unless otherwise stated, the studies of toxicity described in the present monograph were conducted with factor J and factor L in a ratio equal to 75 : 25. Some studies were repeated with factor J and factor L in the ratio of 85 : 15; this was done to demonstrate that the 85 : 15 ratio produces a toxicity profile that is essentially the same as that seen with a ratio of 75 : 25 ratio.]

The remaining components of spinetoram comprise a number of additional spinosyns (that have minor substitutions at various locations in the spinosyn molecule) and other impurities consisting of inorganic salts, carbohydrates and proteinaceous material that would be expected to be produced during a fermentation process.

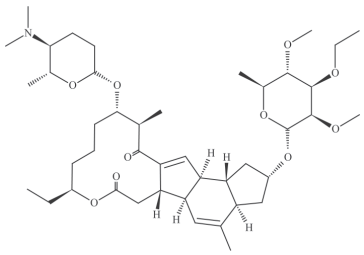
Spinetoram has not been evaluated previously by the JMPR and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide residues (CCPR).

All the pivotal studies met the basic requirements of the relevant (Organization for Economic Co-operation and Development) OECD or national test guidelines and included certificates of compliance with good laboratory practice (GLP).

Figure 1. Chemical structures of the two principal spinosyns (factors J and L) contained in spinetoram



XDE-175-J (factor J)



XDE-175-L (factor L)

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

The two insecticidally active factors in spinetoram, identified as spinosyns XDE-175-J and XDE-175-L (factors J and L), were evaluated separately in a series of studies of absorption, distribution and metabolism. Groups of four male and female Fischer 344 rats were given a single dose of macrolide-ring ^{14}C-labeled factor at 10 or 100 mg/kg bw by oral gavage; unlabelled compound as repeated oral doses at 10 mg/kg bw per day for 14 days, followed by a single radiolabelled dose; or an intravenous dose of 10 mg/kg bw of each of the two active factors.

After dosing by oral gavage, both factor J and factor L were rapidly absorbed without any apparent lag time. With factor J, peak plasma concentrations of radiolabel were attained within approximately 2 h in males and females at both doses, while with factor L, peak plasma concentrations of radiolabel were seen at 2 h in females and 4 h in males, at both doses. Plasma elimination half-lives of radiolabel ranged from 4 h to 24 h depending on dose and factor, with the radiolabel clearing slightly more slowly with factor L than with factor J. Comparison of plasma area-under-the-curve of concentration–time (AUCs) of radiolabel from rats dosed orally with those from rats dosed intra-

venously indicated that a minimum of 26–29% of factor J and 39–57% of factor L was systemically available after the lower dose (10 mg/kg bw), with higher amounts (37–36% factor J and 73–92% factor L) systemically available after a higher dose (100 mg/kg bw). Based on data on excretion of metabolites in the urine and faeces (Tables 1 and 2), it was estimated that at least 70% of an oral dose was absorbed, possibly with some pre-systemic metabolism. However, no studies were conducted in bile-duct cannulated rats. AUCs after oral administration were slightly greater than proportional to dose: 14-fold for ZDE-175-J and 20-fold for factor J. The reason for this was not clear.

The radiolabel remaining in the tissues and carcass 7 days after dosing was approximately 0.6–1.4% of the administered dose of factor J regardless of dose, and approximately 3% of factor L at the lower dose (10 mg/kg bw) and 7% at the higher dose (100 mg/kg bw). At termination (168 h after dosing), there were no differences in tissue concentrations of radiolabel between rats given a single oral dose of radiolabelled material or repeated doses of unlabelled material followed by a single radiolabelled dose of either factor J or factor L.

With factor J, the concentration of ^{14}C residues in tissues collected at T_{max} generally decreased in relative order from the gastrointestinal tract > lymph nodes > liver > lungs > adrenals > spleen. The rank order of residue concentrations in tissues of rats killed at $t = ½T_{max}$ remained approximately the same for the gastrointestinal tract, lymph nodes, lungs, adrenals, and spleen; however, the rank order of liver residues was lower, while the rank order for residues in fat and bone marrow was higher. Concentrations of factor J and related metabolites were observed in the liver, kidney, plasma, and thyroid tissue extracts of male and female rats given this compound orally. The parent compound was detected in all four tissues chemically analysed from the T_{max} groups and in all except plasma from the $t = ½T_{max}$ groups. Parent factor J was most abundant in the liver, with concentrations at T_{max} ranging from 1.4% to 3.1% of the administered radiolabel.

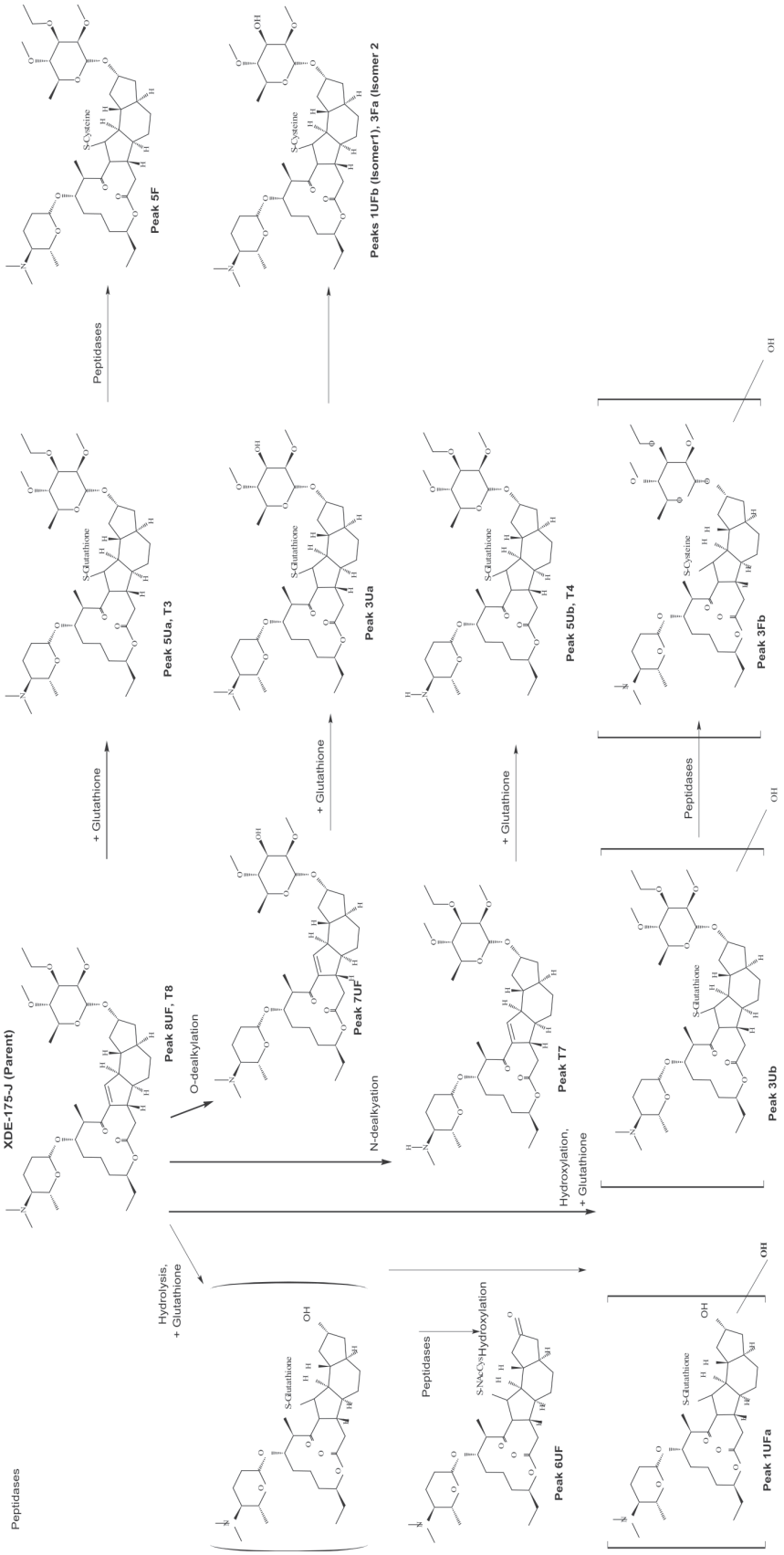
With factor L, the concentration of ^{14}C residues in tissues collected at T_{max} generally decreased in relative order from the gastrointestinal tract > lymph nodes > liver > lungs > adrenals > spleen. The rank order of residue concentrations in tissues of rats killed at $t = ½T_{max}$ remained approximately the same for the gastrointestinal tract, lymph nodes, adrenals, and lungs; however, the rank order of liver residues was lower, while the rank order of residues in fat and bone marrow was higher. Concentrations of factor L test material and related metabolites were observed in the liver, kidney, plasma, and thyroid tissue extracts of male and female rats given this compound orally. Parent compound was detected in all four tissues chemically analysed from the T_{max} group and the $t = ½T_{max}$ group. Parent factor L was most abundant in the liver, with concentrations at T_{max} ranging from 3.4% to 6.0% of the administered radiolabel.

Although tissue concentrations indicated very low residues of spinetoram, studies of repeated dietary exposure revealed a toxicity profile that was consistent with the slow accumulation of very small quantities of spinetoram during prolonged exposure. This toxicity profile is consistent with trapping of a cationic amphiphilic compound within the lysosomes. These effects have been shown to be reversible upon cessation of exposure.

Both factor J and factor L were highly metabolized in male and female rats given these compounds orally or intravenously. Parent compound accounted for 7–40% (factor J) and 7–26% (factor L) of the total radiolabel eliminated in the faeces. Most of the radiolabel in the urine and in faecal extracts was present as seven or nine metabolites (for factor J or factor L respectively). One major metabolite (the cysteine conjugate of factor J and factor L) was present in all urine and faecal extracts and accounted for 31–61% and 51–66% of the administered dose (for factor J or factor L, respectively).

The major metabolic pathway was via glutathione conjugation of the parent, and glutathione conjugation of metabolites arising from *N*-demethylation and *O*-deethylation of each factor, as well as hydroxylation and deglycosylation of parent factor J. In addition, small amounts of the sulfate and glucuronide conjugates of the aglycone of factor L were detected. The proposed pathways for metabolism of factor J and factor L are shown in Figures 2 and 3.

Figure 2. Proposed pathway for metabolism of factor J



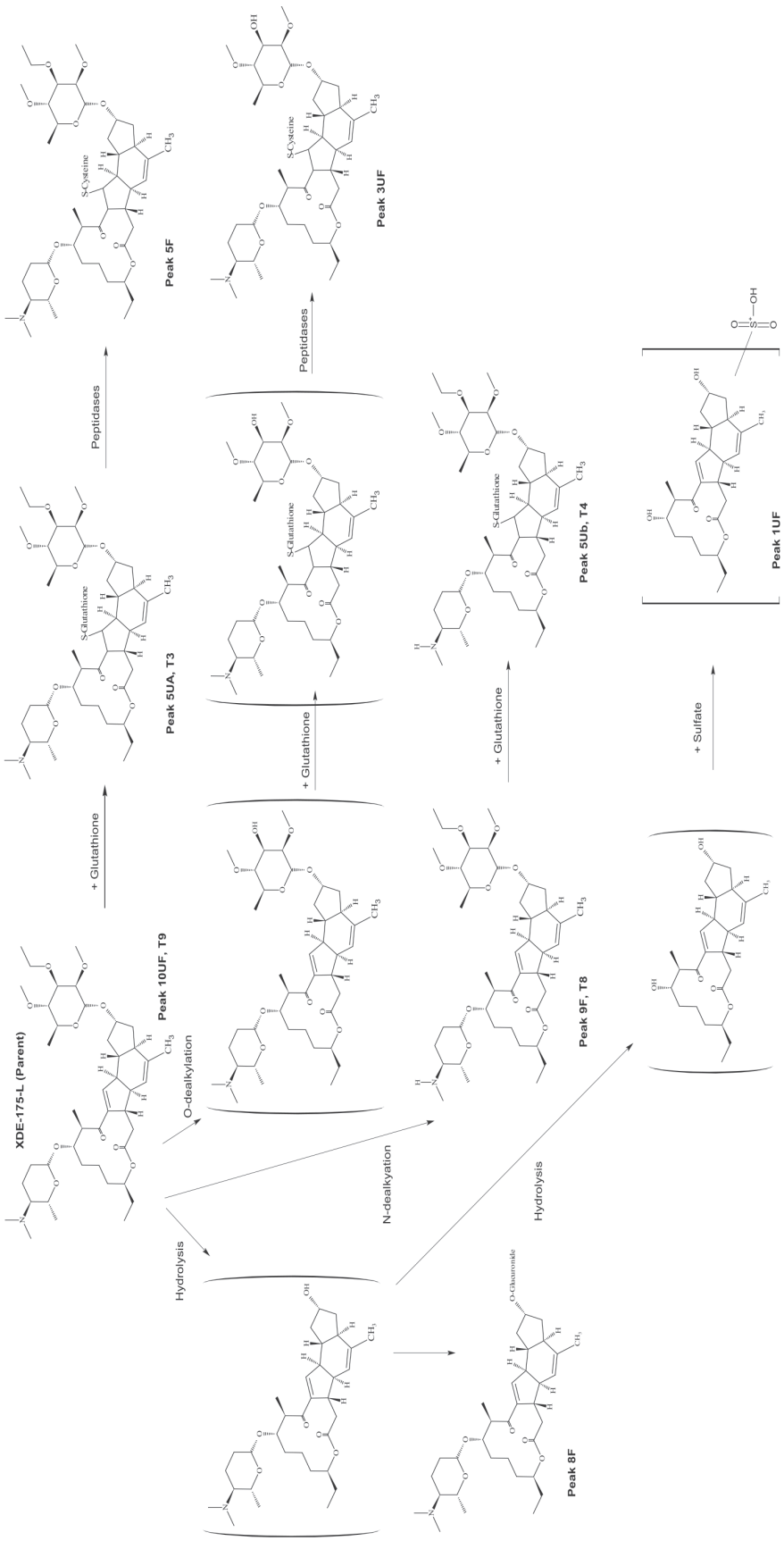


Figure 3. Proposed pathway for metabolism of factor L

Table 1. Recovery of radiolabel in tissues and excreta of rats given radiolabelled spinosyn factor J

Sample	Recovery of radiolabel (% of administered dose)							
	Single dose at 10 mg/kg bw, by gavage		Single dose at 100 mg/kg bw, by gavage		Pre-treatment followed by single dose at 10 mg/kg bw, by gavage[a]		Single dose at 10 mg/kg bw, intravenous	
	Males	Females	Males	Females	Males	Females	Males	Females
Expired air	NQ	NQ	NA	NA	NA	NA	NA	NA
Tissues	0.30	0.38	0.44	0.81	0.28	0.32	0.89	0.84
Carcass	0.19	0.40	0.55	1.010.17	0.17	0.41	0.61	1.30
Cage wash	0.73	0.01	0.11	0.11	0.42	0.04	0.14	0.25
Urine:								
0–12 h	2.90	3.48	2.53	2.73	2.23	2.71	6.43	6.29
12–24 h	0.50	0.58	0.75	0.79	0.41	0.65	0.93	1.12
24–48 h	0.25	0.28	0.34	0.56	0.28	0.40	0.88	0.85
48–72 h	0.16	0.05	0.22	0.21	0.15	0.12	0.34	0.55
72–96 h	0.13	0.07	0.13	0.24	0.11	0.07	0.18	0.41
96–120 h	0.04	0.05	0.06	0.09	0.06	0.05	0.11	0.17
120–144 h	0.03	0.03	0.04	0.06	0.03	0.05	0.08	0.11
144–168 h	0.02	0.03	0.11	0.05	0.02	0.02	0.05	0.09
Faeces:								
0–24 h	78.39	69.32	70.16	57.75	76.94	74.96	52.49	59.31
24–48 h	5.27	11.01	8.51	18.57	6.00	10.03	14.46	13.52
48–72 h	1.72	2.24	2.24	3.43	1.55	2.32	5.87	5.34
72–96 h	0.73	0.93	1.24	1.89	0.67	1.00	2.21	3.00
96–120 h	0.38	0.53	0.63	1.04	0.30	0.64	1.16	2.02
120–144 h	0.22	0.33	0.47	0.72	0.21	0.38	0.74	1.08
144–168 h	0.13	0.21	0.48	0.54	0.17	0.25	0.51	0.80
Total	92.10	89.93	89.01	90.60	90.00	94.42	88.08	97.05

From Rick et al. (2005a)

NA, not analysed; NQ, not quantifiable; radiolabel was not detected in tissues at a concentration exceeding the limit of quantitation (LOQ).

[a] Unlabelled compound as repeated oral doses at 10 mg/kg bw per day for 14 days, followed by a single radiolabelled dose.

Rates of faecal and urinary elimination of factor J and factor L were similar, regardless of dose, sex, number of doses, or route of administration. Faeces represented the primary route of excretion. On average, approximately 85% of the administered dose was excreted in the faeces, with most being excreted in the first 24 h. Urine accounted for approximately 3–4% of the administered dose. The half-lives of faecal excretion were approximately 24 h, with urinary excretion half-lives of approximately 24–30 h (Rick et al., 2005a, 2005b, 2007a, 2007b).

1.2 Bioavailability

Mice

In a study of toxicity (also described in section 2.2) in CD-1 mice given diets containing spinetoram (purity, 95%; ratio, 64% factor J and 31% factor L) at doses ranging from approximately 8

Table 2. Recovery of radiolabel in tissues and excreta of rats given radiolabelled spinosyn factor L

Sample	Recovery of radiolabel (% of administered dose)							
	Single dose at 10 mg/kg bw, by gavage		Single dose at 100 mg/kg bw, by gavage		Pre-treatment followed by single dose at 10 mg/kg bw, by gavage [a]		Single dose at 10 mg/kg bw, intravenous	
	Males	Females	Males	Females	Males	Females	Males	Females
Expired air	NQ	NQ	NA	NA	NA	NA	NA	NA
Tissues	1.23	2.22	4.39	3.63	1.98	1.17	3.49	7.08
Carcass	1.46	1.70	2.74	3.15	1.32	1.65	3.69	5.75
Cage wash	0.46	0.08	0.06	0.19	0.41	0.04	0.58	0.08
Urine:								
0–12 h	1.71	1.75	1.54	1.55	1.59	1.47	2.43	2.19
12–24 h	0.47	0.46	0.77	0.53	0.38	0.35	0.43	0.44
24–48 h	0.24	0.28	0.48	0.62	0.22	0.24	0.39	0.37
48–72 h	0.10	0.11	0.22	0.22	0.13	0.09	0.19	0.18
72–96 h	0.07	0.07	0.12	0.15	0.06	0.05	0.13	0.12
96–120 h	0.04	0.05	0.08	0.11	0.04	0.03	0.09	0.11
120–144 h	0.03	0.04	0.05	0.08	0.03	0.04	0.07	0.07
144–168 h	0.03	0.02	0.08	0.06	0.02	0.02	0.05	0.05
Faeces:								
0–24 h	65.52	55.82	48.33	43.27	68.66	63.18	48.36	46.75
24–48 h	10.56	18.70	17.86	20.73	10.57	13.65	14.86	13.04
48–72 h	4.13	4.51	7.45	10.13	3.08	4.44	6.59	7.47
72–96 h	1.61	2.12	3.93	3.57	2.04	1.75	4.21	4.20
96–120 h	1.22	1.33	2.14	2.51	1.04	1.11	2.88	3.13
120–144 h	0.88	0.77	1.46	1.75	0.66	1.62	2.08	2.27
144–168 h	0.63	0.72	1.30	1.36	0.67	0.61	1.74	1.63
Total	90.39	90.75	93.00	93.61	92.90	91.51	92.26	94.93

From Rick et al. (2005b)

NA, not analysed; NQ, not quantifiable; radiolabel was not detected in tissues at a concentration exceeding the limit of quantitation (LOQ).

[a] Unlabelled compound as repeated oral doses at 10 mg/kg bw per day for 14 days, followed by a single radiolabelled dose.

to 226 mg/kg bw per day for 28 days, systemic bioavailability of factor L was 4–44% greater than that of factor J in all groups except females at 1200 ppm. In the latter, bioavailability of factor L was 16% lower, suggesting preferential absorption of factor L or faster elimination/first-pass metabolism of factor J. Although females were exposed to a relatively higher dietary concentration of spinetoram than males, owing to higher food consumption relative to body weight, serum concentrations of the two spinosyns in females were mostly lower than in males, suggesting lower systemic bioavailability, especially for factor L. Systemic bioavailability became nonlinear at the highest dose suggesting non-linear absorption (saturation), which was pronounced for factor L in females (Wilson et al., 2005a).

In a study of toxicity (also described in section 2.2), Fischer 344 rats were given diets containing spinetoram (purity, 95%; ratio, 64% factor J and 31% factor L)at doses ranging from 11 to 185 mg/kg bw per day for 28 days. Serum collected at termination indicated that recovery was greater than was proportional to the administered dose, this being more pronounced with factor J than with factor L.

This was an indication of saturation of elimination at high doses or more efficient first-pass elimination at lower doses, but absorption from the gastrointestinal tract appeared to be unaffected. A similar trend was observed in steady-state AUCs at 24 h, determined from three blood samples collected at 05:00, 10:00 and 17:00, determined 24 days after initiation of the feeding study. Plasma elimination half-lives increased with increasing dose; plasma elimination half-lives were 7 h and 9 h, 10 h and 12 h, and 32 h and 16 h for factor J and factor L at the lowest, intermediate and highest doses, respectively. The half-life of parent in this study was approximately 7 h at the lowest dose (Yano et al., 2004).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with spinetoram are summarized in Table 3.

Spinetoram was of low acute oral and dermal toxicity in rats, giving oral and dermal median lethal dose (LD_{50}) values of > 5000 mg/kg bw.

The median lethal concentration (LC_{50}) for spinetoram administered by inhalation was > 5.44 mg/l, the highest aerosol concentration tested.

After oral dosing, clinical signs were observed only at doses of 2500 mg/kg bw and greater. Clinical signs after dosing orally and by inhalation included watery faeces, perineal soiling, perioral soiling and/or soiling of the coat (Carney et al., 2005a; Durando, 2007a).

(b) Dermal and ocular irritation and sensitization

The results of studies of irritation and sensitization with spinetoram are given in Table 4.

Studies of dermal and ocular irritation in New Zealand White (NZW) rabbits showed no irritation after dermal application and only transient ocular irritation immediately after instillation, which cleared within 24 h (Brooks & Golden, 2005a, 2005b; Durando, 2007c, 2007d).

In a local lymph node assay (LLNA) with BALB/c mice, spinetoram was shown to be a moderate skin sensitizer, while in a second LLNA with CBA/J mice (the recommended species for this assay, according to OPPTS 870.2600, OECD TG 429, and EC B.42 guidelines), spinetoram did not

Table 3. Acute toxicity of spinetoram

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Reference
Rat	F344/DuCrl	Females	Oral (gavage)	85.8% (64.6% factor J and 21.2% factor L)	> 5000	—	Carney et al. (2005a)
Rat	Fischer 344	Females	Oral (gavage)	86.3% (73.0% factor J and 13.3% factor L)	> 5000	—	Durando (2007a)
Rat	F344/DuCrl	Males and females	Dermal/topical	85.8% (64.6% factor J and 21.2% factor L)	> 5000	—	Carney et al. (2005b)
Rat	Fischer 344	Males and females	Dermal/topical	86.3% (sum of factor J and factor L)	> 5000	—	Durando (2007b)
Rat	F344/DuCrl	Males and females	Inhalation (nose only; 4 h)	85.8% (64.6% factor J and 21.2% factor L)	—	> 5.50	Hotchkiss et al. (2005)
Rat	Fischer 344	Males and females	Inhalation (nose only; 4 h)	84.5% (71.7% factor J and 12.9% factor L)	—	> 5.44	Krieger et al. (2007)

Table 4. Results of studies of sensitization and irritation with spinetoram

Species	Strain	Sex	Route	Purity (%)	Result	Reference
Rabbit	NZW	M & F	Dermal/topical	85.8% (64.6% factor J and 21.2% factor L)	No irritation	Brooks & Golden (2005a)
Rabbit	NZW	M & F	Dermal/topical	86.3% (73.0% factor J and 13.3% factor L)	Slight irritation	Durando (2007c)
Rabbit	NZW	M & F	Ocular (instillation)	85.8% (64.6% factor J and 21.2% factor L)	Transient irritation	Brooks & Golden (2005b)
Rabbit	NZW	M & F	Ocular (instillation)	86.3% (sum of factor J and factor L)	Transient irritation	Durando (2007d)
Mouse	BALB/c	F	Dermal/topical (local lymph node assay)	85.8% (64.6% factor J and 21.2% factor L)	Moderate sensitization	Woolhiser & Wiescinski (2006)
Mouse	CBA/J	F	Dermal/topical (local lymph node assay)	86.3% as the sum of (73.0% factor J and 13.3% factor L)	No sensitization	Wiescinski & Sosinski (2007)

F, female; M, male; NZW, New Zealand White

elicit a stimulation index that met the 3× threshold (i.e. a response that was three times greater than than elicited by the vehicle control), thus indicating a lack of dermal sensitization potential in mice in this assay (Woolhiser & Wiescinski, 2006; Wiescinski & Sosinski, 2007).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a short-term study of systemic toxicity, groups of five male and five female CD-1 mice were given diets containing spinetoram (purity, 95% active ingredient; 64% factor J and 31% factor L) at a concentration of 0, 50, 150, 450 or 1200 ppm (equal to 0, 8.28, 24.5, 75.1, and 183 mg/kg bw per day for males; and 0, 10.6, 31.3, 96.3, and 226 mg/kg bw per day for females) for 28 days. Steady-state serum concentrations of factor J and factor L were determined after 28 days for three males and three females per group. The homogeneity or stability of the diet was not determined.

Body weight was measured before exposure, twice during the first week and weekly during the remainder of the study. Food consumption and intake of spinetoram was determined twice during the first week and at least weekly thereafter for all mice. Ophthalmoscopic examinations were conducted before exposure and during necropsy. Haematology and clinical chemistry investigations were carried out at termination. Measurement of organ weights and examination for gross pathology were performed on all mice at termination. Histopathology examinations were carried out on mice in the control group and in the group at the highest dose at termination.

Terminal body-weight decreases of 10.4% for males and 3% for females, relative to controls, were statistically significant in mice at 1200 ppm, the highest dose. The body-weight gain of mice at 1200ppm was only 50% (males) and 83% (females) that of mice in the control groups. Treatment-related decreases in food consumption occurred in males and females at 1200 ppm.

The primary treatment-related effect was cytoplasmic vacuolation of the parenchymal cells, epithelial cells, macrophages (with increased numbers), and fibroblasts of various organs in mice at 1200 ppm, with more subtle effects of a similar nature in mice at 450 ppm. Other treatment-related effects included hyperplasia of the glandular mucosa of the stomach in females at 1200 ppm and in males at a dietary concentration of 450 ppm or greater, degeneration with regeneration of skeletal

muscle fibers in mice at 450 ppm or greater, and very slight hypertrophy of the zona fasciculata in adrenal glands of males at 1200 ppm.

Administration of spinetoram at a dietary concentration of 1200 ppm caused increases in absolute and relative liver and spleen weights. Treatment-related increases in absolute and relative adrenal weights (relative adrenal weights increased by 75%) occurred in males at 450 ppm or greater.

Although there was no decrease in the erythrocyte count, mice receiving spinetoram at 1200 ppm had slight microcytic anaemia, as shown by decreases in eythrocyte cell volume, haemoglobin (10%), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV). Similar but more subtle changes in erythrocyte cell volume and haemoglobin (11%) occurred in male mice at 450 ppm. All males and one of five females at 1200 ppm had splenic extramedullary haematopoiesis. Mice at 450 ppm or greater had an increase in the percentage of monocytes, which correlated with the macrophage response that occurred in many tissues. Males at 450 ppm or greater also had an increase in neutrophil numbers consistent with the inflammatory response that occurred. The results of haematology analyses are shown in Table 5.

At a dietary concentration of 1200 ppm, spinetoram caused significant elevation of hepatobiliary enzyme activity in serum (alkaline phosphatase: males, +37%; and aspartate aminotransferase (AST): males, +195%; females, +148%). Male and female mice at 1200 ppm had a slight statistically significant (for males and females combined) treatment-related decrease in albumin concentration that was interpreted to be the result of treatment-related decrements in food consumption at this dietary concentration.

Table 5. Selected haematology findings in mice given diets containing spinetoram for 28 days

Dietary concentration (ppm)	Parameter					
	Erythrocyte count (R6/µl)	Haemoglobin (g/dl)	Haematocrit[c] (%)	Mean corpuscular volume (fl)	Mean corpuscular haemoglobin (pg)	Mean corpuscular haemoglobin concentration (g/dl)
Males						
0	9.57	15.8	49.9	52.2	16.5	31.6
50	9.54	15.3	47.9	50.2	16.1	32.0
150	9.39	15.3	48.3	51.5	16.2	31.6
450	8.62*	14.1*	45.0*	52.1	16.4	31.4
1200	9.55	14.2	45.7*	47.9	14.9	31.1
Range for historical controls[a]	8.73–9.48	14.5–15.9	47.0–49.0	52.4–54.3	15.8–17.0	29.8–32.0
Females						
0	9.28	15.7	48.9	52.7	16.9	32.1
50	9.65	16.0	50.3	52.3	16.7	31.8
150	9.67	15.6	50.2	51.9	16.2	31.2
450	9.30*	15.1*	48.0	51.6	16.3	31.5
1200	9.40*	14.1*	45.7*	48.8	15.1	30.9
Range for historical controls[a]	0.00–9.54[b]	15.1–16.0[b]	45.5–51.4	51.3–53.0	15.0–17.1	29.0–33.4

From Wilson et al. (2005a)

[a] Values for historical control groups from the same laboratory for six studies of dietary toxicity in CD-1 mice during the past 5 years.

[b] Values for historical control groups from the same laboratory for five studies of dietary toxicity in CD-1 mice during the past 5 years.

[c] Erythrocyte volume fraction = haematocrit/100

*Dunnett test statistically identified at alpha = 0.05.

The no-observed-adverse-effect level (NOAEL) for CD-1 mice given diets containing spinetoram for 28 days was 150 ppm , equal to 24.5 mg/kg bw per day and 31.3 mg/kg bw per day for males and females, respectively, on the basis of haematological changes in males and cytoplasmic vacuolation of the parenchymal cells, epithelial cells, macrophages (with increased numbers), and fibroblasts of various organs in mice at 450 ppm (Wilson et al., 2005a).

In a short-term study of toxicity, groups of ten male and ten female CD-1 mice were given diets containing spinetoram (purity, 83.0%; 62% factor J and 21.0% factor L) at a concentration of 0, 50, 150 or 450 ppm (equal to 0, 7.5, 22.8, and 70.5 mg/kg bw per day for males and 0, 10.2, 29.6, and 89.9 mg/kg bw per day for females) for at least 90 days. Parameters evaluated were daily observations, detailed clinical observations, ophthalmological examinations, body weight, food consumption, haematology, clinical chemistry, weights of selected organs, and gross and histopathology examinations. Diet homogeneity and stability were not determined.

Males at 450 ppm had treatment-related decrements in food consumption of up to 11% and decreases in body weights of up to 8%, relative to controls. The body-weight gains of males at this dose were substantially lower than those of the controls throughout the study, with a 24% reduction by study termination.

Administration of spinetoram at dietary concentration of 450 ppm caused slight treatment-related microcytic hypochromic anaemia, as shown by a decrease (approximately 5%) in the erythrocyte count, which did not reach statistical significance, and by statistically significant decreases in haemoglobin concentration (≤ 10%) and erythrocyte volume fraction, decreased mean corpuscular volume, and increased reticulocyte counts (statistically significant in males only). Leukocyte count was statistically significantly elevated in females at 150 and 450 ppm. Compared with controls, males and females at 450 ppm had treatment-related increases in the activity of serum AST and females at 450 ppm showed increases in the activity of alanine aminotransferase (ALT).

Males and females at 450 ppm had treatment-related increases in mean absolute (31–38%) and relative spleen weights (40%) that were statistically significant. The increase in spleen weight was attributed to splenic extramedullary haematopoiesis. Males and females at 450 ppm had treatment-related increases in mean absolute (11–17%) and relative (18–19%) liver weights. Only the change in relative liver weight was statistically significant.

The primary treatment-related histological change was cytoplasmic vacuolation of parenchymal cells, epithelial cells, macrophages and fibroblasts in numerous organs in mice at 450 ppm. Other treatment-related histological changes included hyperplasia of the glandular mucosa of the stomach, multifocal degeneration and regeneration of skeletal muscle fibers and renal tubular epithelium, and a slight increase in splenic extramedullary haematopoiesis in males and females at 450 ppm. The only treatment-related histological change in mice at 150 ppm was subtle vacuolation of the tubules of the caput epididymis in males, slight splenic extramedullary haematopoiesis and very slight dilatation of the glandular stomach in females. Histopathology findings are summarized in Table 6.

The NOAEL was 50 ppm, equivalent to 7.5 mg/kg bw per day for males and 10.2 mg/kg bw per day for females, on the basis of slight splenic extramedullary haematopoiesis in females and slight vacuolation of the tubules of the caput epididymis of males at 150 ppm (Wilson et al., 2005b).

Rats

In a 28-day study of toxicity, groups of five male and five female Fischer 344 rats were given diets containing spinetoram (purity, 95% active ingredient; 64% factor J and 31% factor L) at a concentration of 0, 120, 500, 1500 ppm (females only) or 2000 ppm (males only) for at least 28 days. These concentrations corresponded to intakes of 0, 11.4, 48.4 and 185 mg/kg bw per day for males and 0, 11.7, 48.2 and 142 mg/kg bw per day for females. Body weight, food consumption, compound

Table 6. Selected histopathology findings in mice given diets containing spinetoram for up to 90 days

Finding	Dietary concentration (ppm)							
	Males (n = 10 per dose)				Females (n = 10 per dose)			
	0	50	150	450	0	50	150	450
Epididymides								
Vacuolization, epithelium, multifocal:								
Very slight	0	0	6	0	—	—	—	—
Slight	0	0	0	10	—	—	—	—
Kidneys								
Degeneration with regeneration, tubule, multifocal: slight	0	0	0	6	0	0	0	3
Liver								
Vacuolization, macrophages, perivascular, multifocal: very slight	0	0	0	0	0	0	0	6
Lymph node, mediastinal								
Vacuolization, macrophages: very slight	0	0	0	10	0	0	0	8
Lymph node, mesenteric								
Vacuolization, macrophages: very slight	0	0	0	10	0	0	0	9
Sinus histiocytosis, increased: slight	0	0	0	2	0	0	0	3
Skeletal muscle								
Degeneration with regeneration, muscle fiber, multifocal:								
Very slight	0	0	0	10	0	0	0	0
Slight	0	0	0	0	0	0	0	9
Spleen								
Extramedullary haematopoeisis, increased: slight	0	0	0	7	1	1	3	7
Vacuolization, macrophages: very slight	0	0	0	7	1	0	0	6
Stomach								
Dilatation, glandular, glandular mucosa, multifocal: very slight	1	0	0	8	0	0	2	7
Hyperplasia, glandular mucosa, diffuse:								
Very slight	0	0	0	3	0	0	0	2
Slight	0	0	0	7	0	0	0	7
Inflammation, chronic active, glandular mucosa, multifocal:								
Very slight	1	0	0	4	0	0	0	4
Slight	0	0	0	0	0	0	0	2

From Wilson et al. (2005b)

intake, ophthalmoscopic examination, haematology, clinical chemistry, urine analysis, necropsy and histopathology were evaluated.

Analyses to confirm all doses administered were determined before exposure. The homogeneity of the diet containing spinetoram at the lowest and highest concentrations was determined concurrently. A study of toxicity previously conducted with a structurally similar compound had shown that test material was stable in the diet for up to 40 days. The stability of spinetoram was assumed to be similar, and was therefore not investigated further. Samples of all prepared diets were retained and stored frozen at approximately –20 °C for possible future analyses.

There were no treatment-related effects in clinical observations, ophthalmic observations, haematology, coagulation, clinical chemistry, or urine-analysis parameters.

Males at the highest dose had decreases in body weight relative to controls (4.6%), and body-weight gain (8.6%) at the end of the study that were consistent with the slightly lower food consumption in this group. Males at 2000 ppm had higher relative weights of heart, spleen (both statistically significant) and kidney. Females at 1500 ppm had higher absolute and relative heart, spleen, liver and kidney weights that were statistically significantly different from values for the controls. Spleen weights (19% greater than values for controls) were interpreted to be treatment-related as they were outside the range of values for historical controls and because treatment-related histopathological effects were also observed in the spleen.

Treatment-related histopathological effects occurred in the thyroid and kidneys of males and females in the groups receiving the highest dose and consisted of a slight vacuolation of the follicular epithelial cells of the thyroid and a very slight vacuolation of the renal tubular epithelial cells. Two females at 500 ppm also had a very slight vacuolation of the renal tubular epithelium. Males and females at the highest dose had slight accumulation of macrophages/histiocytes within the white pulp of the spleen and females had an increase in spleen weight. Slight splenic histiocytosis also occurred in one female at 500 ppm. In addition, males and females at the highest dose had very slight or slight accumulation of macrophages/histiocytes in the cortex of mesenteric lymph nodes. Histopathology findings are summarized in Table 7.

The NOAEL was 500 ppm, equal to 48 mg/kg bw per day, on the basis of minor histopathological changes in several organs observed in rats at the highest dietary concentration. The low incidence, and minimal severity of the vacuolation in the kidney and histiocytosis in the spleen observed in a few females at 48 mg/kg bw per day were not considered to be adverse effects (Yano et al., 2004).

Table 7. Selected histopathology findings in rats given diets containing spinetoram for 28 days

Finding	Dietary concentration (ppm)							
	Males (n = 5 per group)				Females (n = 5 per group)			
	0	120	500	2000	0	120	500	1500
Thyroid								
Vacuolation, follicular cells, cytoplasmic:								
Very slight	1	0	1	0	0	0	1	0
Slight	0	0	0	5	0	0	0	5
Spleen								
Aggregates of macrophages-histiocytes, periarteriolar lymphoid sheath:								
Focal, very slight	1	1	1	0	0	0	0	0
Multifocal, very slight	0	0	0	4	0	0	1	4
Lymph node, mesenteric								
Aggregates of macrophages-histiocytes, cortex:								
Focal, very slight	1	0	0	4	0	0	0	5
Multifocal, slight	0	0	0	1	0	0	0	0
Kidney								
Vacuolation increased, tubular epithelium: very slight	0	0	0	5	0	0	2	5

From Yano et al. (2004)

In a 90-day study to evaluate potential systemic toxicity, groups of 10 male and 10 female Fischer 344 rats were given diets formulated to supply spinetoram (purity, 83.0%; 62.0% factor J and 21.0% factor L) at a concentration of 0, 120, 500, 1000, 2000 ppm, or 4000 ppm (females only) for at least 90 days. These dietary concentrations were equal to doses of 0, 8.49, 34.7, 70.6, and 137 mg/kg bw per day for males and 0, 10.1, 42.4, 85.0, 170, and 332 mg/kg bw per day for females. Additional groups of 10 males and 10 females were given spinetoram at 0 or 1000 ppm for 90 days, and were then maintained on control diet for an additional 4 weeks to assess the potential reversibility of treatment-related effects. Parameters evaluated included cage-side observations, detailed clinical observations, ophthalmological exams, body weight, food consumption, haematology, clinical chemistry (including thyroid-hormone analysis), urine analysis, selected organ weights, gross and histopathological examinations, and electron microscopy.

The homogeneity of the diet containing spinetoram at the lowest and highest doses for females was determined before exposure, near the middle, and at the end of the study. Stability was established for 62 days in diet containing spinetoram at concentrations ranging from 0.0005% to 4%. The concentrations of spinetoram were confirmed by LC/MS/MS analysis in samples of diet containing spinetoram at all doses (plus control diet and pre-mix) analysed before exposure, near the middle, and at the end of the study.

Males at 2000 ppm showed a decrease in body-weight gain of 10.2% at the end of the dosing phase, while females at 2000 or 4000 ppm had body-weight gain decreases of 7.9% and 14.8%, respectively. Food consumption of males at 2000 ppm and females at 4000 ppm was also less than that of the controls.

Females at 2000 or 4000 ppm showed decreases in erythrocyte parameters (haemoglobin concentration: 2000 ppm, –8%; 4000 ppm, –11%, erythrocyte volume fraction, MCV and MCHC) and higher reticulocyte counts (2000 ppm, +29%; 4000 ppm, +41%). Changes in these parameters were also observed in females at 1000 ppm, reaching statistical significance for haemoglobin and erythrocyte volume fraction. At this dose, the reticulocyte count was 17% higher than that in the controls. There were no decreases in erythrocyte parameters in the males. The leukocyte counts of females at 1000 ppm or greater were also higher than those of the controls (+30%, +53% and +80%, respectively, statistically significant at the two higher doses). Males at 2000 ppm and females at 2000 ppm or greater had higher activities of serum liver enzymes (ALT in males; AST in males and females). The triglyceride concentrations of females at 500 ppm or greater were statistically significantly lower than those of the controls and showed a dose-dependent relationship, while cholesterol concentrations were reduced at 2000 ppm or greater. Females at 4000 ppm also had a slightly higher alkaline phosphatase activity relative to that of controls.

Concentrations of triiodothyronine (T3) were slightly, statistically significantly, reduced in females at 2000 and 4000 ppm. Concentrations of thyroxin (T4) were slightly, statistically significantly, reduced in females at dietary concentrations of 500 ppm, 1000 ppm and 2000 ppm, but not at the highest concentration, 4000 ppm, where concentrations were not dissimilar to values for the controls. There were no changes in concentrations of thyroid-stimulating hormone (TSH).

Microscopic treatment-related effects consisting of the presence of aggregates of macrophages/histiocytes occurred in numerous lymphoid tissues, including the spleen, lymph nodes, Peyer patches of the jejunum or ileum, and thymus, and the liver and bone marrow. Vacuolation of parenchymal cells occurred in the thyroid gland and kidney, and there was skeletal muscle degeneration involving multiple muscles. Females were more affected than males, with effects on the thyroid, spleen, bone marrow, jejunum, liver, kidney and mesenteric and mediastinal lymph nodes at the lowest observed-effect level (LOEL). Histopathology findings are summarized in Tables 8 and 9.

Evaluation of the kidneys by electron microscopy indicated that females at 4000 ppm had vacuoles within tubular epithelial cells that contained a flocculent material or membranous whorls.

Table 8. Selected histopathology findings in female rats given diets containing spinetoram for 90 days

Finding	Dietary concentration (ppm)					
	0	120	500	1000	2000	4000
Lymph node, mediastinal (No. examined)	10	10	9	9	10	10
Aggregates of macrophages-histiocytes, multifocal:						
Very slight	2	0	1	5	8	8
Slight	0	0	0	0	2	1
Lymph node, mesenteric (No. examined)	10	10	10	10	10	10
Aggregates of macrophages-histiocytes						
Focal, very slight	2	3	1	0	0	0
Multifocal, moderate	0	0	0	4	8	10
Spleen (No. examined)	10	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:						
Slight	0	0	0	2	5	4
Moderate	0	0	0	0	3	3
Bone marrow (No. examined)	10	10	10	10	10	10
Aggregates of macrophages-histiocytes:						
Hindlimb, multifocal, very slight	0	0	3	6	5	4
Hindlimb, multifocal, slight	0	0	0	1	4	6
Sternum, multifocal, very slight	0	0	2	4	9	8
Vertebrae, focal, very slight	0	0	2	3	3	1
Vertebrae, multifocal, very slight	0	0	1	1	3	6
Kidney (No. examined)	10	10	10	10	10	10
Vacuolization, tubules, slight	0	0	0	0	6	8
Thyroid (No. examined)	10	10	10	10	10	10
Vacuolization, follicle, epithelial cell, slight	0	0	0	7	10	10
Depletion, with altered tinctorial properties, colloid, very slight	0	0	0	4	4	3

From Yano et al. (2005)

These effects were consistent with those observed in animals given agents known to be cationic amphiphilic drugs and establish spinetoram as a cationic amphiphilic compound.

The potential to recover from the effects induced by spinetoram was investigated in male and female rats given spinetoram at a dietary concentration of 1000 ppm for 90 days, followed by control food for 28 days. Variable degrees of recovery occurred during the recovery phase. Complete recovery was noted for a number of effects including changes in erythrocyte parameters, lower concentrations of triglycerides, higher relative liver weights, higher relative heart weight, and microscopic effects involving the ileum (males), jejunum (males), kidney (females), liver (males), spleen (males), skeletal muscle and thymus. Partial recovery occurred for relative spleen weights and microscopic effects involving the kidneys (males), spleen (females), jejunum (females), liver (females) and thyroid glands. In the recovery phase, histopathological findings related to mesenteric lymph nodes were almost un-changed from those in rats not allowed a recovery phase while microscopic effects in ileum in females were more severe than those in rats without a recovery phase. ALT activity in males at 1000 ppm remained statistically significantly elevated at the end of the recovery phase, at levels similar to those found at the end of the dosing period.

Table 9. Selected histopathology findings in male rats given diets containing spinetoram for 90 days

Finding	Dietary concentration (ppm)				
	0	120	500	1000	2000
Lymph node, mesenteric (No. examined)	9	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Very slight	3	2	5	6	0
Slight	0	0	0	2	8
Liver (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Very slight	0	0	0	0	4
Slight	0	0	0	0	3
Thymus (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes:					
Focal, very slight	3	0	1	3	3
Multifocal, very slight	0	0	0	2	5
Kidney (No. examined)	10	0	10	10	10
Hyaline droplet formation, decreased, proximal convoluted tubule:					
Slight	2	—	1	4	4
Moderate	0	—	0	1	6
Thyroid (No. examined)	10	10	10	10	10
Vacuolization, follicle, epithelial cell, slight	0	0	0	1	6
Depletion, with altered tinctorial properties, colloid, very slight	0	0	0	1	5

From Yano et al. (2005)

The NOAEL was 500 ppm for males and 120 ppm for females (equivalent to 34.7 mg/kg bw per day for males and 10.1 mg/kg bw per day for females) on the basis of changes in haematological parameters (reticulocyte and leukocyte counts) and histopathological findings in the bone marrow (Yano et al., 2005).

In a short-term dietary study of toxicity, groups of eight male and eight female CD (Sprague-Dawley) rats were fed diets providing spinetoram (purity, 83.0% active ingredient; 62.0% factor J and 21.0% factor L) at a dose of 0, 10, 50, 100, or 150 mg/kg bw per day for 90 days. This study was designed as a pilot study for dose selection for the two-generation study of reproductive toxicity (Carney et al., 2006) and was not designed to satisfy all regulatory requirements for a short-term dietary study of oral toxicity in rats. Body weight, food consumption, compound intake, clinical chemistry, urine analysis, necropsy and histopathology were evaluated.

The homogeneity of the diets determined during the third week of study. Stability was established for 62 days in diet containing spinetoram at concentrations ranging from 0.0005% to 4%.

There were no treatment-related clinical signs of toxicity at any dose. Treatment-related increases in organ weights were observed in males at 150 mg/kg bw per day and females at 100 or 150 mg/kg bw per day. In males, there were statistically significant increases in relative weights of the heart and spleen. Absolute spleen weight was increased non-significantly in males and females. In females at 150 mg/kg bw per day, relative weights of the heart and kidney, and absolute and relative weights of the liver and spleen were increased. The changes in spleen weight did not reach statistical significance. At 100 mg/kg bw per day, relative weights of the heart and absolute and relative

weights of the liver were increased, although the change in absolute liver weight was not statistically significant.

Treatment-related histological alterations were limited to the thyroid, spleen, and kidneys. Thyroid follicular epithelial cells in most males and females at 150 mg/kg bw per day were enlarged and distended with fine cytoplasmic vacuoles. This histopathological effect was also present with moderate severity in two males and three females at 100 mg/kg bw per day. Treatment-related, diffuse vacuolation of a lesser degree (slight) was present in the thyroid of males and females at 50 or 100 mg/kg bw per day. Thyroid-hormone analysis indicated differences from control values in concentrations of TSH (increased), T4 and T3 (decreased) for males at 10, 50 or 100 mg/kg bw per day. However, the biological significance of the decrease in T_4 was equivocal as there was no dose–response pattern. In females, there were no changes in TSH concentrations, but there was a statistically significant decrease in T4 concentrations in rats receiving spinetoram at 100 mg/kg bw per day or greater.

The NOAEL was 10 mg/kg bw per day on the basis of slight vacuolation in the thyroid of males and females receiving spinetoram at 50 mg/kg bw per day (Wilson et al., 2005a).

In a short-term study of potential systemic toxicity, groups of 10 male and 10 female Fischer 344 rats were given diets containing spinetoram (purity, 86.3%; 73.0% factor J and 13.3% factor L; 85 : 15 ratio of J:L) at a concentration of 0, 120, 500, 1000 or 2000 ppm for 90 days. These dietary concentrations corresponded to doses of 0, 8, 35, 69 or 137 mg/kg bw per day for males and 0, 9, 35, 71, or 142 mg/kg bw per day for females. Body weight, food consumption, compound intake, ophthalmoscopic examination, haematology, clinical chemistry, urine anaalysis, necropsy and histopathology were evaluated. A previously conducted study with spinetoram (75 : 25 ratio) indicated that the test material was stable in the diet for at least 62 days at concentrations ranging from 0.0005% to 4%. The doses received and homogeneity of the diets administered were confirmed analytically.

There were slight reductions in mean body weights and body-weight gains in males at 2000 ppm (from day 50), but these differences were not statistically significant. Food consumption was not consistently affected by treatment.

Males at 2000 ppm had treatment-related changes in various erythrocyte parameters: mean leukocyte count and mean reticulocyte counts increased by 21%, and 14%, respectively. Females at 1000 or 2000 ppm had treatment-related decreases in mean platelet counts. Treatment-related alterations in leukocyte parameters for females consisted of a higher mean total leukocyte count at 2000 ppm (+26%), a lower percentage of neutrophils at 1000 or 2000 ppm, and a higher percentage of basophils and large unstained cells and increases in mean reticulocyte counts (+52%) at 2000 ppm.

Males at 1000 or 2000 ppm had treatment-related increases in mean serum alanine and AST activities. Treatment-related increases in alkaline phosphatase activity were seen in males at 2000 ppm. Females at 2000 ppm had a treatment-related increase in mean AST activity. Alkaline phosphatase activity was also slightly elevated in this group.

Microscopic treatment-related effects consisting of the presence of aggregates of macrophages-histiocytes occurred in lymphoid tissues, including the spleen, lymph nodes, Peyer patches of the jejunum or ileum, and thymus, and the liver and bone marrow. Vacuolization of parenchymal cells occurred in the thyroid gland and kidney, and muscle degeneration was noted in the heart and skeletal muscle of the larynx. Females were more affected than males, with thyroid, bone marrow, liver, thymus, and mesenteric and mediastinal lymph node effects at the lowest-observed-effect level (LOEL) of 500 ppm. Histopathology findings are summarized in Tables 10 and 11.

The NOAEL for spinetoram (85:15 ratio) was 500 ppm in males and 120 ppm in females (equivalent to 35 mg/kg bw per day for males and 9 mg/kg bw per day for females) on the basis of histopathological findings in lymph nodes and bone marrow at higher doses (Stebbins & Card, 2007).

Table 10. Selected histopathology findings in male rats given diets containing spinetoram for 90 days

Finding	Dietary concentration (ppm)				
	0	120	500	1000	2000
Lymph node, mediastinal (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Very slight	3	1	1	6	8
Slight	0	0	0	0	2
Lymph node, mesenteric (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal, slight	0	0	0	5	10
Spleen (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Slight	0	0	0	0	2
Moderate	0	0	0	0	6
Thymus (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal, very slight	0	0	0	1	8
Bone marrow (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes:					
Hindlimb, multifocal, very slight	0	0	0	0	7
Hindlimb, multifocal, moderate	0	0	0	0	1
Sternum, multifocal, very slight	0	0	0	0	2

From Stebbins & Card (2007)

Dogs

Groups of two male and two female beagle dogs were fed diets containing spinetoram (purity, 83.0% active ingredient; 62.0% factor J and 21.0% factor L) at a concentration of 0, 200, 900, or 2000 ppm (equal to 0, 5.9, 30.9, and 65.0 mg/kg bw per day in males, and 0, 8.1, 35.1, and 62.3 mg/kg bw per day in females) for 28 days. Parameters evaluated included daily observations, detailed clinical observations, ophthalmic examinations, body weight, food consumption, prothrombin time, clinical chemistry, haematology, urine analysis, selected organ weights, and gross and histopathological examinations. The homogeneity and stability of the diet and doses administered were confirmed analytically.

There were no treatment-related effects on daily observations, detailed clinical observations, ophthalmic examinations, prothrombin time, urine analysis, or gross pathology examinations.

Treatment-related changes in numerous parameters were noted at 900 and 2000 ppm. Dogs at these doses had lower body weight (6% for females and 8% for males) and body-weight gain, relative to controls. One female at 900 ppm and two females at 2000 ppm lost body weight over the duration of the study (starting from day 4) and food consumption in these dogs was reduced. Food consumption in males was unaffected.

Alterations in erythrocyte, leukocyte, and platelet parameters reflective of a non-regenerative anaemia (erythrocytes, –15%; haemoglobin, –22%; erythrocyte volume fraction, –17%; leukocytes, –52%; and platelets, –73%) at 2000 ppm in males. Similar changes were observed at 2000 ppm in females, and slightly less marked changes were observed at 900 ppm in females. Increases in serum ALT (+41%) and asparate aminotransferase (+61%) activities occurred in males at 2000 ppm, with similar changes in asparate aminotransferase in females at 900 or 2000 ppm.

Table 11. Selected histopathology findings in female rats given diets containing spinetoram for 90 days

Finding	Dietary concentration (ppm)				
	0	120	500	1000	2000
Lymph node, mediastinal (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Very slight	1	1	7	7	6
Slight	0	0	0	1	4
Lymph node, mesenteric (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal:					
Slight	2	0	6	10	8
Moderate	0	0	0	0	2
Spleen (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal					
Slight	0	0	0	3	7
Moderate	0	0	0	0	2
Thymus (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes, multifocal, very slight	0	0	1	5	8
Bone marrow (No. examined)	10	10	10	10	10
Aggregates of macrophages-histiocytes:					
Hindlimb, multifocal, slight	0	0	0	1	8
Sternum, multifocal, very slight	0	0	2	4	9
Vertebra, multifocal, very slight	0	0	0	1	2

From Stebbins & Card (2007)

Treatment-related increases in absolute and/or relative liver weights were seen at 900 and 2000 ppm (relative liver weight, +45% in males at 2000 ppm). One male and one female at 900 ppm, and one female at 2000 ppm had treatment-related decreases in absolute and relative weight of the thymus. The lower weights of the thymus corresponded to atrophy of the thymic cortex observed microscopically in the two affected females.

Histologically, treatment-related vacuolization (very slight or slight severity) of macrophages within lymphoid tissue occurred in the caecum, gallbladder, ileum, larynx, lymph nodes, nasal tissue, rectum, spleen, stomach, thymus, and tonsils of males and females at 900 or 2000 ppm. In addition, very slight to slight necrosis and moderate diffuse hyperplasia of mononuclear cells were noted in the bone marrow at 900 and 2000 ppm. Extramedullary haematopoiesis of the spleen, noted in one female at 900 ppm and one male and one female at 2000 ppm, was interpreted to be a response to the bone-marrow necrosis and anaemia at these doses. Hyperplasia and hypertrophy (very slight or slight severity) of Kupffer cells in the liver occurred in all dogs at 900 or 2000 ppm. Some of the Kupffer cells of dogs at 2000 ppm had treatment-related cytoplasmic vacuolization. All dogs at 2000 ppm had treatment-related aggregates of alveolar macrophages in the lungs. Histopathology findings are summarized in Table 12.

The NOAEL for systemic toxicity in beagle dogs given diets containing spinetoram for 28 days was 200 ppm, which corresponded to 5.9 mg/kg bw per day for males and 8.1 mg/kg bw per day for females, on the basis of haematological, biochemistry and histopathological findings at higher doses (Stebbins & Brooks, 2004).

Table 12. Selected histopathology findings in dogs given diets containing spinetoram for 28 days

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	200	900	2000	0	200	900	2000
Bone marrow								
Hyperplasia, mononuclear cell, diffuse, moderate	0	0	0	2	0	0	1	2
Necrosis, multifocal:								
Very slight	0	0	1	1	0	0	0	0
Slight	0	0	0	1	0	0	2	2
Vacuolization, macrophages:								
Very slight	0	0	1	0	0	0	0	0
Slight	0	0	0	2	0	0	2	2
Liver								
Hyperplasia and hypertrophy, Kupffer cell:								
Very slight	0	0	2	0	0	0	2	0
Slight	0	0	0	2	0	0	0	2
Vacuolization, Kupffer cell, very slight	0	0	0	2	0	0	0	2
Spleen								
Extramedullary haematopoiesis:								
Very slight	0	0	0	0	0	0	0	1
Slight	0	0	0	1	0	0	1	1
Vacuolization, macrophages, white pulp: very slight	0	0	0	2	0	0	0	2

From Stebbins & Brooks (2004)

Groups of four male and four female beagle dogs were fed diets containing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at a concentration of 0, 150, 300, or 900 ppm (equal to 0, 5.73, 9.82, and 27.1 mg/kg bw per day in males, and 0, 4.97, 10.2, and 31.0 mg/kg bw per day in females) for 90 days. The homogeneity and stability of the diet and actual doses administered were confirmed analytically.

Males at 300 or 900 ppm, and females at 900 ppm had treatment-related lower mean body weights compared with controls (males, –14% and –17%, respectively) and body-weight gains during most of the study (males, –87%; females, –24%). There was appreciable inter-individual variation in the magnitude of these changes, particularly in males, and they did not achieve statistical significance. Food consumption was not affected by treatment.

Decreases in mean leukocyte count, erythrocyte count (males, –18%; females, –7%; at 90 days), haemoglobin concentration (males, –20%; females, –15%; at 90 days), erythrocyte volume fraction, and platelet count were reported in males and females at 900 ppm associated with a non significant increase of reticulocytes (males, 36%; females, 105%; at 90 days). Only the change in haemoglobin concentration was statistically significant.

Clinical-chemistry alterations consisted of a statistically non-significant increase in alkaline phosphatase activity in males given 300 or 900 ppm. AST activity was slightly, although statistically significantly, increased in males and females at 900 ppm.

Treatment-related increases in absolute and relative weights of the liver were noted in males (+56%) and females (+23%) at 900 ppm. In males and females, absolute and relative weights of the thymus were statistically significantly reduced at 900 ppm, and absolute weights were statisti-

cally significantly reduced at 300 ppm, to below the limit of the range for historical controls for the laboratory.

Histologically, treatment-related vacuolization (very slight or slight severity) of macrophages within lymphoid tissue occurred in the caecum, colon, duodenum, ileum, jejunum, lungs, lymph nodes, nasal tissue, rectum, spleen, stomach, and tonsil of males and females at 300 or 900 ppm (Table 13). Very slight vacuolization of macrophages within lymphoid tissue also occurred in the ileum, jejunum, lymph nodes, nasal tissues, and rectum of some males at 150 ppm. Treatment-related arteritis or perivascular inflammation (very slight, slight, or moderate severity) occurred in numerous tissues of some males and females at 300 or 900 ppm. The more severe arteritis was frequently accompanied by necrosis of the arterial walls, with occasional associated haemorrhage. Very slight to moderate bone-marrow necrosis was present in some males and females at 300 or 900 ppm. Extramedullary haematopoiesis of the spleen and liver in some females at 300 or 900 ppm was interpreted to be a response to the bone-marrow necrosis and/or aanemia at these doses. Hyperplasia and hypertrophy (very slight or slight severity) of Kupffer cells, and vacuolization of Kupffer cells occurred in the liver of some males and females at 300 or 900 ppm.

Table 13. Incidence of vacuolation of macrophages in dogs given diets containing spinetoram for 90 days

Tissue or organ[a]	Severity	Dietary concentration (ppm)							
		Males (n = 4 per group)				Females (n = 4 per group)			
		0	150	300	900	0	150	300	900
Bone marrow	Very slight	0	0	0	0	0	0	0	1
	Slight	0	0	2	3	0	0	1	3
Caecum	Very slight	0	0	3	3	0	0	2	1
Colon	Very slight	0	0	2	1	0	0	2	1
Duodenum	Very slight	0	0	0	0	0	0	0	2
Ileum	Very slight	0	2	3	3	0	0	2	2
	Slight	0	0	0	1	0	0	1	2
Jejunum	Very slight	0	1	2	0	0	0	0	0
	Slight	0	0	0	1	0	0	0	1
Larynx	Very slight	0	0	1	0	0	0	0	1
Lungs	Very slight	0	0	0	3	0	0	0	1
Lymph node, mediastinal	Very slight	0	0	3	3	0	0	1	2
	Slight	0	0	0	1	0	0	0	0
Lymph node, mesenteric	Very slight	0	1	4	2	0	0	2	4
	Slight	0	0	0	2	0	0	0	0
Nasal tissue	Very slight	0	1	3	1	0	0	1	3
	Slight	0	0	0	2	0	0	0	1
Rectum	Very slight	0	2	3	4	0	0	1	3
Spleen	Very slight	0	0	1	4	0	0	2	0
Stomach	Very slight	0	0	1	2	0	0	0	1
Tonsil: vacuolation	Very slight	0	0	3	0	0	0	3	1
	Slight	0	0	0	4	0	0	0	3

From Stebbins & Brooks (2005)

[a] Vacuolated macrophages in the bone marrow were present adjacent to sites of necrosis. All other vacuolated macrophages were present in lymphoid tissue of the affected organs and tissues.

The NOAEL was 150 ppm, equivalent to 5.73 mg/kg bw per day in males, and 4.97 mg/kg bw per day in females, on the basis of histopathological findings (vacuolization, arteritis or perivascular inflammation) and extramedullary haematopoiesis at 300 ppm. The occurrence of very slight vacuolization of macrophages in lymphoid tissues of a few male dogs at 150 ppm was not considered to be adverse (Stebbins & Brooks, 2005).

Groups of four male and four female beagle dogs were fed diets containing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at a concentration of 0, 50, 100, or 200 ppm for 1 year. These concentrations were equal to doses of approximately 0, 1.6, 3.0, and 5.4 mg/kg bw per day in males, and 0, 1.3, 2.5, and 5.8 mg/kg bw per day in females. Parameters evaluated included daily cage-side observations, weekly detailed clinical observations, ophthalmology, body weights, food consumption, clinical chemistry, haematology, prothrombin time, urine analysis, selected organ weights, and gross and histopathology examinations. The homogeneity and stability of the diet and actual doses administered were confirmed analytically.

There were no treatment-related effects on daily observations, detailed clinical observations, ophthalmic examinations, body weights, food consumption, haematology, clinical pathology parameters, or gross pathology examinations. The mean absolute and relative weights of the liver of males at 200 ppm were 17.7% and 19.4% higher than those of the controls, respectively. These increases were not statistically significant, but were considered most likely to be treatment-related as the liver weights were above the range for historical controls for the laboratory. There were no associated clinical pathology or microscopic changes.

Arteritis in one male and one female at 200 ppm was the only potentially treatment-related histopathological effect noted (Table 14). Arteritis occurred bilaterally in the epididymides of one male at 200 ppm, and in the thymus, thyroid, larynx, and urinary bladder of one female at 200 ppm. Although no arteritis was observed in the controls in this study, the incidence in the treated groups was within the range for historical controls. The arteritis was accompanied by necrosis of the arterial walls in the affected dogs. No treatment-related vacuolation of macrophages was observed in this study.

The NOAEL was 100 ppm, approximately 3.0 mg/kg bw per day for males and 2.5 mg/kg bw per day for females, on the basis of histopathological findings (arteritis) at 200 ppm (Stebbins & Brooks, 2006).

Table 14. Incidence of chronic arterial inflammation in dogs given diets containing spinetoram for 1 year

Organ or tissue	Severity	Dietary concentration (ppm)							
		Males (n = 4 per group)				Females (n = 4 per group)			
		0	50	100	200	0	50	100	200
Epididymis	Very slight	1	0	0	0	—	—	—	—
Epididymisa	Slight	0	0	1	0	—	—	—	—
	Moderate	0	0	0	1	—	—	—	—
Larynx	Very slight	0	0	0	0	0	0	0	1
Thymus	Slight	0	0	0	0	0	0	0	1
Thyroid	Very slight	0	0	0	0	0	0	0	1
Urinary bladder	Very slight	0	0	0	0	0	0	0	1

From Stebbins & Brooks (2006)

[a] Chronic-active inflammation.

(b) Dermal administration

Rats

Groups of ten male and ten female Fischer 344 rats were exposed dermally to spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at a dose of 0, 100, 500, or 1000 mg/kg bw per day at a semi-occluded skin test site for 6 h per day, 7 days per week, for 28 consecutive days. The test substance or vehicle (0.5% methylcellulose) was applied to an area of not less than 10% of the total body surface area on the back of the rat (from the scapulae to the hipbone and half way down the flank), which was clipped free of hair at least 24 h before initiation of dosing and on an as-needed basis during the study (approximately weekly). The exposure site was semi-occluded with gauze dressing and non-absorbent cotton. The rat was wrapped in an elastic bandage to hold the test material, gauze dressing and cotton in place.

The only treatment-related change observed was a minimal, localized, microscopic skin effect at the site of application, consisting of very slight or slight epidermal hyperplasia variably accompanied by very slight hyperkeratosis in the majority of males and females at 500 or 1000 mg/kg bw per day and in some males and females at 100 mg/kg bw per day. The Meeting considered that this was an adaptive response.

The NOAEL for systemic effects was 1000 mg/kg bw per day in males and females, the highest dose tested. The NOAEL for local effects on the skin was 1000 mg/kg bw per day, the highest dose tested (Thomas et al., 2005).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female Crl:CD1(ICR) mice were given diets containing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L at a concentration of 0 (controls), 25, 80, 150 or 300 ppm (equal to 0, 3.0, 10.0, 18.8, and 37.5 mg/kg bw per day for males, and 0, 4.0, 12.8, 23.9, and 46.6 mg/kg bw per day for females) for up to 18 months. Doses were confirmed analytically before exposure and after approximately 4, 8, 12, and 16 months. A previous study of toxicity had shown spinetoram to be stable for at least 62 days at concentrations ranging from 0.0005% to 4%. Body weight, food consumption and compound intake (before exposure, weekly during the first 13 weeks of the study and then at approximately monthly intervals until study termination), ophthalmoscopic examination (at necropsy), haematology (at 12 months and 18 months) gross pathology and histopathology were evaluated.

Survival overall was ≥ 74% (in all groups receiving spinetoram and in the control group) and treatment had no effect on mortality.

On days 232–546, females at 300 ppm had decreases in mean body weights and mean body-weight gains that ranged from 0.3–5.4% and 4.3–15.4%, respectively, when compared with those of the controls. At study termination, the mean body weight and mean body-weight gain of females at 300 ppm were 1.3% and 5.8% lower than those of the controls, respectively. These differences were not statistically significant. There were no treatment-related effects on body weights or body-weight gains of females at 25, 80, or 150 ppm, or of males at any dose.

Males at 300 ppm had treatment-related increases in mean absolute (11.2%) and relative (12.1%) liver weights that were outside the range for historical controls in 18-month studies in mice recently conducted at this laboratory, but that were not statistically significant. There were no histopathological correlates to the increased liver weights. Treatment-related histopathological alterations occurred in the stomach, lungs, and epididymides of mice at 300 ppm (Table 15). The treatment-related alterations in the stomach consisted of an increase in the incidence and severity of hyperplasia of the glandular mucosa, with associated dilatation of mucosal glands and chronic

Table 15. Selected histopathology findings in mice fed diets containing spinetoram for 18 months

Finding	Severity	Dietary concentration (ppm)									
		Males					Females				
		0	25	80	150	300	0	25	80	150	300
Stomach											
Dilatation, mucosal gland, multifocal	Very slight	12	15	13	18	23*	14	18	14	15	26
	Slight	0	0	0	1	1	0	0	0	1	3
	Any severity	12	15	13	19	24*	14	18	14	16	29*
Hyperplasia, glandular mucosa, diffuse	Moderate	0	0	0	0	1	0	0	0	0	0
Hyperplasia, glandular mucosa, multifocal	Very slight	11	10	6	9	6	6	7	6	9	8
	Slight	3	4	3	2	9	3	5	3	4	8
	Moderate	0	0	0	0	0	0	0	0	0	3
	Any severity	14	14	9	11	15	9	12	9	13	19
Inflammation, chronic, glandular, submucosa, multifocal	Very slight	4	7	5	3	15*	9	8	7	4	12
	Slight	0	0	0	1	1	0	0	0	2	6*
	Any severity	4	7	5	4	16*	9	8	7	6	18
Lungs											
Aggregates of alveolar macrophages	Very slight	6	4	1	8	5	9	6	7	11	22*
	Slight	0	0	0	0	4	0	1	0	1	4
	Any severity	6	4	1	8	9	9	7	7	12	26*
Epididymides											
Vacuolization, epithelium, head	Very slight	17	14	18	17	23	—	—	—	—	—
	Slight	0	0	0	0	19*	—	—	—	—	—
	Any severity	17	14	18	17	42*	—	—	—	—	—

From Stebbins & Dryzga (2007)
*Statistically significant by the Yates chi-square test, alpha = 0.05, two-sided.

inflammation of the glandular submucosa. In general, the stomach alterations were most prominent in the region of the glandular mucosa near the limiting ridge, and lessened in the pyloric area. Females at 300 ppm had a treatment-related increase in the incidence of very slight or slight aggregates of alveolar macrophages in the lungs. In addition, four males at 300 ppm had slight aggregates of alveolar macrophages that were interpreted by the study authors to be treatment-related. Males at 300 ppm had a treatment-related increase in the incidence and severity of cytoplasmic vacuolization of epithelial cells lining the ducts in the head of the epididymides.

No significant increase in the incidence of tumours was observed in either male or female mice at any dose, indicating that spinetoram did not have carcinogenic potential under the conditions of this study.

The NOAEL was 150 ppm, equivalent to 18.8 mg/kg bw per day for males, and 23.9 mg/kg bw per day for females) on the basis of histopathological alterations at 300 ppm (Stebbins & Dryzga, 2007).

Rats

Groups of 65 male and 65 female Fischer 344 rats were fed diets containing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor Lat a concentration of 0 (controls), 50,

250, 500, or 750 ppm for up to 2 years. The doses selected were based on the results of the 90-day study with spinetoram (Yano et al., 2005) in comparison with data from 90-day and long-term studies with a structural analogue (Bond et al., 1995). The time-weighted average doses ingested, based upon mean food consumption and mean body weight data were 0, 2.12, 10.8, 21.6, and 32.9 mg/kg bw per day for males and 0, 2.63, 13.2, 26.6, and 40.0 mg/kg bw per day for females, respectively. After 1 year of treatment, necropsy was performed for the assessment of toxicity in ten males and ten females per group, for the assessment of neuropathology in five males and five females per group, while the remaining 50 males and females per group were fed the respective diets for up to 2 years. The long-term study of neurotoxicity is reported separately in section 2.6.

Body weight, food consumption and compound intake (before exposure, weekly during the first 13 weeks of the study and then at approximately monthly intervals until study termination), ophthalmoscopic examination (at necropsy), haematology and clinical chemistry (at 12, 18 and 24 months), urine analysis (at 3, 6, 12, 18 and 24 months) gross pathology and histopathology (at 12 and 24 months) were evaluated.

Premixes were mixed periodically throughout the study based on stability data. Dietary concentrations were not adjusted for purity. Diets were prepared as a fixed percent of test material in rodent chow. All doses were confirmed analytically before exposure, and at approximately 4, 8, 12, 18, and 22 months. The homogeneity of the diets containing spinetoram at the lowest and highest concentrations was determined concurrently with dose confirmation. A previous study of toxicity had shown spinetoram to be stable for at least 62 days at concentrations ranging from 0.0005% to 4%.

Survival overall for males and females in all groups combined after 2 years was ≥ 54% and there was no treatment-related effect on mortality.

Effects attributed to ingestion of spinetoram for up to 2 years consisted of a marginal, but statistically significant reduction in body weight for males at 500 or 750 ppm (≤ 5%), statistically significant, increased relative and absolute heart weights in females at ≥ 250 ppm (approximately 11% at 750 ppm) and in relative heart weights in males at ≥ 500 ppm, in absolute and relative liver weights in females at ≥ 250 ppm, reaching statistical significance only at some doses. In terms of histopathology (Table 16), treatment-related effects were also noted as thyroid follicular-cell vacuolation in males and females at 500 or 750 ppm, and aggregates of macrophages-histiocytes in the mesenteric lymph nodes of males at 750 ppm and females at 500 or 750 ppm. Females at 500 or 750 ppm also had an increased incidence and/or severity of aggregates of macrophages-histiocytes in the mediastinal lymph nodes and Peyer patches of the ileum and the spleen, and decreased numbers of basophilic foci of altered hepatocytes. Furthermore, females at 750 ppm had an increased incidence of aggregates of alveolar macrophages in the lung, and retinal degeneration/vacuolation.

No treatment-related effects were observed in males or females given spinetoram at a dietary concentration of 50 or 250 ppm.

No significant increase in the incidence of tumours was observed in male or female rats at any dose, indicating that spinetoram did not have carcinogenic potential under the conditions of this study.

The NOAEL was 250 ppm, equivalent to 10.8 mg/kg bw per day for males and 13.2 mg/kg bw per day for females, on the basis of histopathological findings in the thyroid at higher doses (Yano et al., 2007).

2.4 Genotoxicity

An adequate range of studies was used to test spinetoram containing two different ratios of factor J to factor L for genotoxicity in vitro and in vivo. The results of these studies are summarized in Table 17.

In two different assays for reverse mutation in *Salmonella typhimurium* and *Escherichia coli* in vitro, spinetoram did not cause a positive increase in the mean number of revertants per plate with

Table 16. Selected histopathology findings in rats fed diets containing spinetoram for 2 years

Finding	Severity	Dietary concentration (ppm)									
		Male					Female				
		0	50	250	500	750	0	50	250	500	750
Thyroid gland (No. examined)		48	49	48	49	49	50	50	50	48	49
Vacuolation, follicular cell	Very slight	3	2	4	28*	8	0	0	0	40*	14*
	Slight	0	0	0	6*	29*	0	0	0	3	27*
Lymph node, mesenteric (No. examined)		50	50	49	50	50	50	50	50	50	50
Aggregates of macrophages-histiocytes, multifocal	Very slight	21	36*	35*	32*	5*	15	15	12	2*	3*
	Slight	21	9	9	15	15	28	32	29	28	16*
	Moderate	1	1	1	0	26*	5	3	5	19*	27*
Lymph node, mediastinal (No. examined)		48	50	49	49	50	48	50	48	50	50
Aggregates of macrophages-histiocytes, multifocal	Very slight	0	2	1	0	3	7	9	5	13	17*
Spleen (No. examined)		50	31	30	24	50	50	50	50	50	50
Aggregates of macrophages-histiocytes, white pulp; multifocal	Very slight	0	0	0	0	0	7	7	9	14	17*
Ileum (No. examined)		50	50	49	48	50	50	50	50	50	50
Aggregates of macrophages-histiocytes, Peyer patches	Focal, very slight	3	5	3	5	8	3	4	7	10	5
	Multifocal, very slight	1	3	3	1	3	2	2	1	8	7
Lung (No. examined)		49	50	50	50	50	50	50	50	50	50
Alveolar aggregates of macrophages-histiocytes, multifocal	Very slight	0	0	1	0	0	10	4	3	6	26*
Eye (No. examined)		49	28	19	20	50	50	50	50	50	50
Degeneration; retina; bilateral	Very slight	16	2	4	3	16	14	9	7	11	22
	Slight	0	0	0	0	1	0	1	0	0	12*
	Moderate	0	0	0	0	0	0	0	0	0	2
Vacuolation; retina; bilateral; multifocal	Very slight	0	0	0	0	0	0	0	0	0	9*

From Yano et al. (2007)
*Statistical difference from values for the controls by the Yates chi-squared test, alpha = 0.05, two sided.

any tester strain either in the presence or absence of metabolic activation (S9 fraction prepared from the liver of rats induced with Aroclor™ 1254). Spinetoram gave negative results in two assays for chromosomal aberration in rat lymphocytes in vitro. In two assays for forward gene mutation at the *Hgprt* locus in Chinese hamster ovary cells in vitro, spinetoram was not-mutagenic.

The genotoxic potential of spinetoram in vivo was evaluated by examining the incidence of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow of mice. There were no statistically significant increases in the frequencies of MN-PCE in groups treated with spinetoram

Table 17. Results of studies of genotoxicity with spinetoram

End-point	Test system	Concentration or dose	Result	Reference
In vitro				
Reverse mutation	S. typhimurium strains TA98, TA100, TA1535 & TA1537	3.33–5000 µg/plate (+S9); 1.0–1000 µg/plate (–S9); in ethanol	Negative	Mecchi (2005)
	E. coli WP2urvA	33.3–5000 µg/plate (±S9); in ethanol		
Reverse mutation	S. typhimurium strains TA98, TA100, TA1535 & TA1537	10–2500 µg/plate (+S9); 1.0–1000 µg/plate (–S9); in ethanol	Negative	Mecchi (2007a)
	E. coli WP2uvvA	33.3–5000 µg/plate (±S9); in ethanol		
Chromosomal aberration	Rat lymphocytes	2.5–100 µg/ml (–S9); 5–100 µg/ml (+S9); in 1% DMSO	Negative	Charles et al. (2005a)
Chromosomal aberration	Rat lymphocytes	5–370 µg/ml –S9; 10–370 µg/ml +S9; in 1% DMSO	Negative	Schisler et al. (2007)
Forward mutation	Chinese hamster ovary cells (Hgprt locus)	5–200 µg/ml (±S9) Confirmatory assay: 10–100 µg/ml (–S9); 20–400 µg/ml (+S9); in 1% DMSO	Negative	Siedel et al. (2005)
Forward mutation	Chinese hamster ovary cells (Hgprt locus)	10–320 µg/ml (±S9) Confirmatory assay: 10–160 µg/ml (–S9); 20–320 µg/ml (+S9); in 1% DMSO	Negative	Schisler & Kleinert (2007)
In vivo				
Micronucleus formation	Male mouse bone-marrow polychromatic erythrocytes	500, 1000, 2000 mg/kg bw per day by oral gavage (two doses, 24 h interval); in 0.5% Methocel	Negative	Charles et al. (2005b)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from livers of male rats.

when compared with the negative controls. There were no statistically significant differences in the percentage of PCE in groups treated with spinetoram.

2.5 Reproductive toxicity

(a) Multigeneration study

Groups of 27 male and 27 female Crl:CD(SD) rats were fed diets providing spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at a dose of 0, 3, 10, or 75°mg/kg bw per day for approximately 10 weeks before breeding, and continuing through breeding, gestation, and lactation for two generations. Statements of compliance with GLP and QA were provided. In-life parameters measured included clinical observations, food consumption, body weights, estrous cyclicity, thyroid-hormone analyses, reproductive performance, pup survival, pup body weights, and puberty onset. In addition, evaluations carried out post mortem included gross pathology, histopathology, organ weights, oocyte quantitation and sperm count, motility and morphology in adults, and gross pathology and organ weights in weanlings.

The overall actual mean concentrations of spinetoram in the diets fed to the rats during the study were 97.1%, 95.3%, and 93.9% of the target concentrations for males and 107%, 103%, and 92.0% of the target concentrations for females at 3, 10, and 75 mg/kg bw per day, respectively.

At a dose of 75°mg/kg bw per day, very slight to slight cytoplasmic vacuolation of the thyroid follicular epithelial cells was observed in adult males and females of both generations (Table 18).

However, these changes were not accompanied by any consistent, treatment-related changes in thyroid hormone levels (T_3, T_4 or TSH), although there were significant differences in some dose groups of either the parental (F_0) or the F_1 generation in one or other of the hormones. Absolute and relative liver weights were statistically significantly increased in the F_1 adult males and females at the highest dose, but there were no corresponding histopathological changes. In the kidneys of some parental (F_0) or F_1 females and occasional F_1 males at 75 mg/kg bw per day, there was a minor, subtle, treatment-related change that consisted of a very slight increase in the amounts of a light yellow-brown, pigmented material (probable lipofuscin-like substance) usually within a vacuole, in the proximal tubular epithelial cells at occasional foci. Neither the changes in liver weight nor the histopathological changes in the kidney were considered to be adverse.

Among females at 75 mg/kg bw per day, three F_0 and three F_1 females had complications of parturition (dystocia), in most cases the protracted delivery of pups over several days. These

Table 18. Incidence of histopathology findings in the parental generation (F0) in a study of reproductive toxicity in rats fed diets containing spinetoram

Finding	Dose (mg/kg bw per day)							
	Male				Female			
	0	3	10	75	0	3	10	75
Thyroid gland (No. examined)	27	26	26	27	27	27	27	27
Vacuolization, cytoplasmic, follicular cell:								
Very slight	0	0	0	4	0	0	0	10
Slight	0	0	0	22	0	0	0	14
Kidney (No. examined)	27	3	1	27	27	27	27	27
Pigment, increased, proximal tubule, multifocal:								
Very slight	0	0	0	0	0	0	0	9

From Carney et al. (2006)

Table 19. Incidence of histopathology findings in the F1 generation in a study of reproductive toxicity in rats fed diets containing spinetoram

Finding	Dose (mg/kg bw per day)							
	Male				Female			
	0	3	10	75	0	3	10	75
Thyroid gland (No. examined)	26	27	27	27	27	27	27	27
Vacuolization, cytoplasmic, follicular cell, diffuse:								
Slight	0	0	0	22	0	0	0	18
Kidney (No. examined)	27	27	27	27	27	27	27	27
Pigment, increased, proximal tubule, multifocal:								
Very slight	0	0	0	2	1	0	0	11

From Carney et al. (2006)

females also exhibited clinical signs (e.g. postpartum vulvar discharge, pale skin/mucous membranes, perinasal/perineal soiling), had reduced body weights and food consumption during lactation, and associated decreases in the survival and body weight of their pups. Two of these females were killed in a moribund condition as a secondary consequence of dystocia. Effects in the remaining litters of dams at the highest dose were limited to slightly decreased survival during gestation and an associated slight increase in post-implantation loss, although only the reduction in survival during gestation in the F_1 generation reached statistical significance. Overall, the effects at 75 mg/kg bw per day appeared to be maternally-mediated and restricted to the process of parturition. There were no effects on any parameter of reproductive performance or offspring growth and survival at 3 and 10 mg/kg bw per day, nor were there any reproductive effects in males at any dose.

The NOAEL for parental, reproductive and offspring toxicity was 10 mg/kg bw per day on the basis of slight thyroid vacuolation in adult males and females, dystocia in females at 75 mg/kg bw per day and decreased survival during gestation in pups at this dose (Carney et al., 2006).

(b) Developmental toxicity

Rats

In a preliminary evaluation of the maternal toxicity and embryo/fetal lethality potential of spinetoram in rats, groups of seven or eight time-mated CD rats were given spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) at targeted doses of 0, 50, 150 or 300 mg/kg bw per day by gavage in aqueous 0.5% methylcellulose (dose volume, 4 ml/kg bw; adjusted daily on the basis of individual body weights) on days 6 to 20 of gestation. In-life parameters evaluated for all groups included clinical observations, body weight, body-weight gain, and food consumption. On day 21 of gestation, all surviving rats were killed and examined for gross pathological alterations. Liver and kidney weights were recorded, as were the number of corpora lutea, implantations, resorptions, and live/dead fetuses.

At 300 mg/kg bw per day, spinetoram caused slight decreases in maternal body-weight gain early in the treatment period, although this was not statistically significant. There were no effects on any other parameters of maternal toxicity at this or lower doses. There were no treatment-related observations relating to gross pathology, nor any effects on reproductive parameters. There were no effects on any measure of embryo/fetal toxicity at any dose (Carney et al., 2005c).

The preliminary study was followed by the main study in which groups of 26 time-mated female CD rats were given spinetoram (suspended in 0.5% methylcellulose) at targeted doses of 0, 30, 100, or 300 mg/kg bw per day by oral gavage on days 6 to 20 of gestation. In-life maternal study parameters measured included clinical observations, body weight, body-weight gain, and food consumption. On day 21 of gestation, all rats were killed and examined for alterations in gross pathology. Liver, kidneys, and weights of the gravid uterine were recorded, together with the number of corpora lutea, uterine implantations, resorptions, and live/dead fetuses. All fetuses were weighed, sexed, and examined for external alterations. Approximately half of the fetuses were examined for visceral alterations while skeletal examinations were conducted on the remaining fetuses.

There was a statistically significant reduction in maternal body-weight gain and food consumption at 300 mg/kg bw per day (body weight gain was 43.5% less than that of the controls during days 6–9 of gestation). There was also a slight, though not statistically significant, decrease in body-weight gain during days 9–12 of gestation. As a result, body-weight gain over the dosing period (days 6–20 of gestation) in females receiving spinetoram at a dose of 300 mg/kg bw per day was decreased non-significantly by approximately 8% when compared with that of the controls.

No treatment-related embryo/fetal toxicity or teratogenicity was observed at doses of up to and including 300 mg/kg bw per day, There were no treatment-related fetal skeletal malformations identified in any of the treated groups when compared with the controls. At 300 mg/kg bw per day there was one litter with extra thoracic vertebrae, thoracic centra, thoracic rib, and sternebrae (three fetuses with the same anomalies), and one fetus with class II wavy ribs. The fetal findings of extra thoracic vertebrae, thoracic centra, thoracic rib, and sternebrae that occurred in the three fetuses are most likely to be due to a genetic effect, as they were limited to one litter, and no associated axial skeleton segmentation anomalies were observed.

The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of decreased maternal body-weight gain, and the NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested (Carney et al., 2005a).

Rabbits

In a preliminary evaluation of the maternal toxicity and embryonal/fetal lethality of spinetoram in rabbits, groups of seven time-mated female New Zealand White rabbits were given spinetoram purity, 83.0%; 62% factor J and 21.0% factor L) at targeted doses of 0, 6.4, 15.7, 30 or 64 mg/kg bw per day by gavage in aqueous 0.5% methylcellulose (dose volume, 4 ml/kg bw; adjusted daily on the basis of individual body weights) on days 7 to 27 of gestation. Additional groups receiving spinetoram at 0, 100 or 150 mg/kg bw per day were subsequently evaluated in order to establish a better estimate of the maximum tolerated dose.

At 150 and 100 mg/kg bw per day, excessive maternal toxicity was evident as decreased food consumption, decreased faecal output, and decreased body weight during the treatment period. Owing to severe inanition and subsequent weight loss, all rabbits from these groups were killed by day 15 of gestation with no further data collection. At 64 mg/kg bw per day, food consumption was decreased, though this was rarely statistically significant. There was a decrease in faecal output in some of the rabbits at the highest dose. There were no effects on organ weights, gross pathology, or reproductive parameters in any of the rabbits surviving to scheduled necropsy (Carney et al., 2005b).

The preliminary study was followed by a main study studying which groups of 25–26 time-mated female New Zealand White rabbits were given spinetoram at targeted doses of 0, 2.5, 10, or 60 mg/kg bw per day by gavage on days 7 to 27 of gestation. In-life parameters evaluated for all groups included clinical observations, body weight, body-weight gain, and food consumption. On day 28 of gestation, all surviving rabbits were killed and examined for alterations in gross pathology and changes in weights of the liver, kidney, and gravid uterine. The number of corpora lutea, uterine implantations, resorptions and live/dead fetuses were determined. All fetuses were weighed, sexed and examined for external, visceral and skeletal alterations. The internal structures of the head were examined by serial sectioning of approximately one-half of the fetuses in each litter.

Treatment-related decreases in food consumption, and body weight gain, particularly during the earlier part of gestation (Table 20), faecal output, and statistically significant increases in mean absolute and relative liver weights were observed at rabbits at 60 mg/kg bw per day (Table 21).

In addition, one dam at 60 mg/kg bw per day was killed on day 21 of gestation due to inanition and subsequent weight loss that were interpreted by the study authors to be treatment-related. There were no maternal effects at the lower doses, and no signs of developmental toxicity at any dose.

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of decreased food consumption, faecal output, and body-weight gain in females at 60 mg/kg bw per day. The NOAEL for developmental toxicity was 60 mg/kg bw per day, the highest dose tested (Carney et al., 2005d).

Table 20. Body-weight gains during gestation in a study of developmental toxicity in female rabbits given spinetoram by gavage

Time-point (days of gestation)	Mean body-weight gain (g)			
	Dose (mg/kg bw per day)			
	0	2.5	10	60
Days 7–10	20.9	16.6	34.3	–17.7[a]
Days 10–13	50.0	59.9	47.8	30.2
Days 13–16	82.2	71.7	79.9	11.5[a]
Days 16–20	29.7	46.7	31.7	14.2
Days 7–28	294.3	327.4	355.7	205.6[b]

From Carney et al. (2005d)

[a] Statistically different from mean for controls by Wilcoxon's test, alpha = 0.05.

[b] Statistically different from mean for controls by Dunnett's test, alpha = 0.05.

Table 21. Liver weights in a study of developmental toxicity in female rabbits given spinetoram by gavage

Weight	Dose (mg/kg bw per day)			
	0	2.5	10	60
Terminal body weight (g)	3478	3438	3540	3388
Liver (g)	75.27	76.14	81.24	83.25*
Liver (g/100)	2.164	2.218	2.299	2.459*

From Carney et al. (2005d)

* Statistically different from mean for controls by Dunnett's test, alpha = 0.05.

2.6 Special studies

(a) Acute neurotoxicity

Rats

In a study of acute neurotoxicity, groups of 10 male and 10 female F344/DuCrl rats were given spinetoram (purity, 85.8% active ingredient; 64.6% factor J and 21.2% factor L) as a single oral dose at 0, 200, 630 or 2000 mg/kg bw by gavage. Body weights were recorded and a functional observational battery (FOB) and test for motor activity were conducted before exposure (baseline), on the day of dosing (day 1), and on day 8, and day 15. The FOB included hand-held and open-field observations as well as measurements of rectal temperature, grip performance, and landing-foot splay. Clinical observations were conducted on days 2, 3, and 4. At the end of the study, five males and females from the control group and from the group at the highest dose were perfused for histopathological evaluation of the central and peripheral nervous systems.

There were no treatment-related effects seen on body weight, FOB, motor activity, or on neuropathological evaluation at any time.

The NOAEL for acute neurotoxicity in male and female F344/DuCrl rats was 2000 mg/kg bw, the highest dose tested (Maurissen, 2005).

(b) Short-term study of neurotoxicity

As part of a long-term study toxicity and carcinogenicity study, a 1-year study of neurotoxicity was conducted in groups of 10 male and 10 female Fischer 344 rats given diets containing spinetoram at a concentration of 0, 50, 250, 500, or 750 ppm (equal to 0, 2.4, 12.0, 24.4, and 36.7 mg/kg bw per day in males and 0, 2.9, 14.7, 29.6, and 44.3 mg/kg bw per day in females). An automated test of motor activity, a FOB, determinations of grip performance, rectal temperature, and landing-foot splay evaluations were carried out before exposure, and after 1, 3, 6, 9, and 12 months. After 12 months (1 year) of exposure, five males and five females per group were perfused, and tissues from the central and peripheral nervous system of rats in the control group and rats at the highest dose were submitted for neuropathological examination.

No treatment-related effects were seen on grip performance, landing-foot splay, rectal temperature, or motor activity at any time. For the ranked and categorical FOB, there were no observations that could be attributed to treatment. There were no treatment-related findings after gross or histopathological examination of the central or peripheral nervous system after 12 months of dietary exposure.

In summary, there were no effects of spinetoram on any parameter that would suggest a neurotoxic effect, and the NOAEL for neurotoxicity in male and female Fischer 344 rats was 750 ppm, equal to 36.7 mg/kg bw per day, the highest dose tested (Maurissen, 2007).

(c) Studies on metabolites

The metabolites of spinetoram are not predicted to be present in groundwater at concentrations of greater than 0.1 µg/l and are therefore not considered to be toxicologically relevant. Although most metabolites of spinetoram have not therefore been tested individually for toxicity in mammals, the plant metabolites *N*-formyl-factor J and *N*-formyl-factor L have been evaluated in a study of acute oral toxicity and an Ames test. In addition, the metabolism of *N*-formyl-factor J was evaluated in the rat.

(i) Metabolism of metabolites of spinetoram

The plant metabolite *N*-formyl-factor J was extensively metabolized in the F344 rat. Based on the faecal metabolite profile seen with this material in rats, it is estimated that 21–28% of the administered dose is converted to metabolites that may be common to those formed from the parent factor J (Rick et al., 2005).

(ii) Acute toxicity of metabolites of spinetoram

Both *N*-formyl-factor J and *N*-formyl-factor L were of low acute toxicity (LD_{50} > 5000 mg/kg bw) and were found to lack mutagenic potential. These findings are consistent with the toxicity profile observed with parent spinetoram (Lowe, 2007a and 2007b).

3. Observations in humans

The period of development of spinetoram as a commercial product has been too short for any information from medical surveillance of manufacturing plant personnel to become available. There have been no exposure incidents involving laboratory or field personnel working with spinetoram. There are no medical reports of alleged human health effects associated with spinetoram.

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[1] Most of the studies of toxicity were conducted with factor J and factor L in a ratio equal to 75 : 25. Some studies were repeated with factor J and factor L in the ratio of 85 : 15; this was done to demonstrate that the 85 : 15 ratio produces a toxicity profile that is essentially the same as that seen with the 75 : 25 ratio.

Comments

Biochemical aspects

The toxicokinetics and metabolism of the two insecticidally active factors in spinetoram, factor J and factor L, are quite similar. In rats, the factors were rapidly and extensively (≥ 70%) absorbed. Peak plasma concentrations of radiolabel were achieved within 2–4 h. Systemic bioavailability was at least 26–29% for factor J and 39–57% for factor L. The factors were extensively distributed in the tissues, with highest concentrations in the gastrointestinal tract, fat, carcass and the liver. Excretion was primarily via the faeces (85%), mainly as metabolites, with only 3–4% of the administered dose excreted in the urine. Most of the administered dose was recovered within 24 h. The plasma half-lives of radiolabelled factor J and factor L were 4–11 h and 8–24 h, respectively. Very little radiolabel remained in the carcass after 7 days: 0.6–1.4% with factor J and 3–7% with factor L. Pre-treatment of rats with a low dose of either factor for 14 days did not affect the subsequent absorption and excretion of the respective factor.

Both factor J and factor L were extensively metabolized. The major metabolic pathway was glutathione conjugation, either of the parent, or of the products of *N*-demethylation, *O*-deethylation and deglycosylation of each factor, as well as hydroxylation of parent factor J. The aglycone of factor L was also subject to sulfate and glucuronide conjugation. The major metabolite was the cysteine conjugate of the parent factor.

Toxicological aspects[1]

Spinetoram was of low acute toxicity in rats: oral LD_{50} > 5000 mg/kg bw; dermal LD_{50} > 5000 mg/kg bw; and 4-h inhalational LC_{50} > 4.44 mg/l. There was no mortality at limit doses of 5000 mg/kg bw and 4.4 mg/l, respectively. Spinetoram is not a skin or eye irritan**t.**

In a local lymph node assay in BALB/c mice, spinetoram was shown to be a moderate skin sensitizer, while in a second assay in CBA/J mice (the recommended strain for this assay according to OECD TG 429 guidelines), spinetoram was not a skin sensitizer.

A range of effects was observed in short- and long-term studies with repeated dosing, and the effects were broadly similar in mice, rats and dogs. In short-term studies in mice, rats and dogs, cytoplasmic vacuolation of parenchymal cells, epithelial cells, macrophages and fibroblasts of a variety of tissues was observed, with some degeneration of muscle. There was also an increase in the incidence and/or severity of aggregates of macrophages/histiocytes in the lymphoid structures of numerous tissues. In mice, the NOAEL was 150 ppm, equal to 24.5 mg/kg bw per day, in a 28-day study. The NOAEL was 50 ppm, equal to 7.5 mg/kg bw per day, in a 90-day study in which there was also slight splenic extramedullary haematopoiesis in females at the lowest-observed-adverse-effect level (LOAEL). In rats, the NOAEL was 500 ppm, equal to 48 mg/kg bw per day, in a 28-day study in which there was vacuolation of the thyroid follicular epithelium and the renal tubular epithelium at the LOAEL. In three 90-day studies in which rats were exposed to spinetoram at two different ratios of factor J to factor L (75 : 25 and 85 : 15), the overall NOAEL was 10 mg/kg bw per day, the factor ratio having little effect on sensitivity. There was also an increase in reticulocyte and leukocyte counts at the LOAEL in one of these studies. In beagle dogs, the NOAEL was 200 ppm, equal to 5.9 mg/kg bw per day, in a 28-day study. In addition to vacuolation of numerous tissues, there was extramedullary splenic haematopoiesis at the LOAEL. In a 90-day study, the NOAEL was 150 ppm, equal to 5.0 mg/kg bw per day. Arteritis or perivascular inflammation and extramedullary haematopoiesis were also observed at the LOAEL in this study. The NOAEL in a 1-year study was 100 ppm, equal to 2.5 mg/kg bw per day, on the basis of arteritis, accompanied by necrosis of the arterial walls at the LOAEL of 200 ppm. The incidence of arteritis in the group receiving spinetoram at 200 ppm was low (one out of four males and one out of four females), and may have reflected the normal background incidence of lesions often seen in beagle

dogs; however, the fact that more severe effects that were considered to be treatment-related were noted in dogs given spinetoram at 300 or 900 ppm for 90 days suggested that these changes in the 1-year study may be treatment-related. The overall NOAEL was 5 mg/kg bw per day in dogs.

In long-term studies in rats and mice, tissue vacuolation was again commonly observed at doses at and above the LOAEL. In an 18-month study in mice, the NOAEL was 150 ppm, equal to 18.8 mg/kg bw per day, on the basis of histopathological changes in the stomach, lungs and epididymides at the LOAEL. In addition to cytoplasmic vacuolation of the epithelium of the ducts lining the head of the epididymides and aggregates of alveolar macrophages in the lungs, hyperplasia and inflammation of the glandular mucosa of the stomach, with dilatation of the mucosal glands were also observed. In a 2-year study in rats, the NOAEL was 250 ppm, equivalent to 10.8 mg/kg bw per day.

Selected tissues from short-term studies of toxicity with spinetoram and with the structurally related compound spinosad in rats (both compounds) and in mice (spinosad only) were examined by electron microscopy. Vacuolation was shown to be associated with cytoplasmic lamellar inclusion bodies, reflecting dysregulation of lysosomal storage (i.e. phospholipidosis). While such effects may arise through a variety of mechanisms that prevent degradation of cell constituents usually processed in the lysosomes, it is most likely that spinetoram acts through a physicochemical mechanism associated with its cationic amphiphilic structure, in common with other such compounds.

In long-term studies of toxicity and carcinogenicity, there was no evidence of treatment-related tumourigenicity in rats or mice. The Meeting concluded that spinetoram was not carcinogenic.

Spinetoram gave negative results in an adequate range of studies of genotoxicity in vitro and in vivo. The Meeting concluded that spinetoram had no genotoxic potential.

On the basis of the absence of carcinogenicity and genotoxicity, the Meeting concluded that spinetoram is unlikely to pose a carcinogenic risk to humans

The reproductive effects of spinetoram have been investigated in a two-generation study in rats. Cytoplasmic vacuolation of thyroid follicular epithelial cells was observed in adults of both generations at the highest dose (75 mg/kg bw per day). Among females at this dose, three parental (F_0) and three F_1 females had complications of parturition (dystocia), in most cases evidenced by the protracted delivery of pups over several days. These females also exhibited clinical signs (e.g., postpartum vulvar discharge, pale skin/mucous membranes, perinasal/perineal soiling), had reduced body weights and food consumption during lactation, and associated decreases in survival and body weight of their pups. The dystocia occurred in a few females (about 13%) at the highest dose of 75 mg/kg bw per day. A similar effect (in up to about 24% of litters) was seen with spinosad at a higher dose of 100 mg/kg bw per day. For both substances, the NOAEL for this effect was 10 mg/kg bw per day, which was also the NOAEL for maternal toxicity, For females at the highest dose without dystocia, gestational survival was slightly decreased, with an associated increase in postimplantation loss. No other measures of reproductive performance were affected in either males or females. The NOAELs for parental, reproductive and offspring toxicity were 10 mg/kg bw per day on the basis of slight thyroid vacuolation in adult males and females, dystocia in females and decreased gestation survival in pups at 75 mg/kg bw per day

The developmental toxicity of spinetoram had been investigated in rats and rabbits. In rats, maternal body weight and food consumption were reduced at 300 mg/kg bw per day, with a NOAEL of 100 mg/kg bw per day. There was no treatment-related embryo/fetal toxicity or teratogenicity at doses up to and including 300 mg/kg bw per day. The NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested.

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[2] Marginal differences out of concurrent controls but within the range for historical controls.

In a preliminary study of developmental toxicity in rabbits, dams given doses of 150 or 100 mg/kg bw per day showed decreased food consumption, decreased faecal output, and decreased body-weight gain from the beginning of the treatment period. No other clinical findings were present in these two groups. The effect on body weight and faecal output, which were associated with a marked and consistent decrease in food consumption, were most likely a consequence of local irritation of the gastrointestinal tract. Owing to severe inanition and subsequent weight loss, all rabbits from these groups were killed by day 15 of gestation with no further data collection.

In the main study of developmental toxicity in rabbits, treatment with spinetoram resulted in decreases in food consumption, faecal output, and body-weight gain, and increased mean absolute and relative liver weights at a dose of 60 mg/kg bw per day. In addition, one dam at 60 mg/kg bw per day was killed on day 21 of gestation owing to inanition and subsequent weight loss, considered to be treatment-related. There were no signs of developmental toxicity at any dose. The NOAEL for maternal toxicity was 10 mg/kg bw per day. The NOAEL for developmental toxicity was 60 mg/kg bw per day, the highest dose tested.

The Meeting concluded that the existing database on spinetoram was adequate to characterize the potential hazards to fetuses, infants and children.

Neurotoxicity was investigated in rats given single doses of up to 2000 mg/kg bw, or repeated doses of up to 750 ppm (36.7 mg/kg bw per day) for 12 months. Comprehensive behavioural and histopathological investigations revealed no evidence of neurotoxicity.

The plant metabolites *N*-formyl-factor J and *N*-formyl-XDE-1175-L were evaluated in a test for acute oral toxicity and in an Ames test for genotoxicity. Both metabolites were of low acute oral toxicity (LD_{50} > 5000 mg/kg bw) and gave negative results in the Ames test.

The development of spinetoram as a commercial product had been too short for any information from medical surveillance of manufacturing-plant personnel to be available. There were no documented cases of intoxication or of any other clinical effects associated with its use.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) 0–0.05 mg/kg bw based on an overall NOAEL of 5.0 mg/kg bw per day, identified on the basis of arteritis, accompanied by necrosis of the arterial walls in the affected organ(s), in studies of toxicity in dogs, and with a safety factor of 100. Although arteritis was observed only in some dogs, at an incidence that was within the range for historical controls, the incidence of arteritis at the LOAEL was greater in the concurrent controls and clear effects were found at higher doses in another study. Additionally, the structurally related compound spinosad had also been observed to cause arteritis in dogs given spinosad for 1 year, at doses not dissimilar to the LOAEL for the present study. Hence, the Meeting concluded that while there was some uncertainty as to the toxicological significance of the finding of arteritis at the LOAEL for spinetoram, use of the overall NOAEL from studies of toxicity in dogs as a basis for establishing the ADI was scientifically justified.

The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for spinetoram on the basis of its low acute toxicity, the absence of neurotoxic potential and of developmental or any other effects of relevance for acute exposure in studies of longer duration. Effects on gestational survival of pups observed in the multigeneration study in rats were most likely to be secondary to maternal toxicity, which was not a consequence of acute exposure.

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[3] Recommended strain.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month combined toxicity and carcinogenicity[a]	Toxicity	150 ppm, equal to 18.8 mg/kg bw per day	300 ppm, equal to 37.5 mg/kg bw per day[c]
		Carcinogenicity	300 ppm, equal to 37.5 mg/kg bw per day[c]	—
Rat	2-year combined study of toxicity and carcinogenicity[a]	Toxicity	250 ppm, 10.8 mg/kg bw per day	500 ppm, equal to 21.6 mg/kg bw per day
		Carcinogenicity	750 ppm, equal to 32.9 mg/kg bw per day[c]	—
	Two-generation study[a]	Parental	10 mg/kg bw per day	75 mg/kg bw per day[c]
		Offspring toxicity	10 mg/kg bw per day	75 mg/kg bw per day[c]
		Reproductive toxicity	10 mg/kg bw per day	75 mg/kg bw per day[c]
	Developmental toxicity[b]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day[c]
		Foetotoxicity	300 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	60 mg/kg bw per day[c]
		Foetotoxicity	60 mg/kg bw per day[c]	—
Dog	Oral 90-day and 1-year studies	Toxicity	150 ppm, equal to 5.0 mg/kg bw per day	200 ppm, equal to 5.4 mg/kg bw per day)[2]

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.05 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful for continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to spinetoram

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid (tmax 2–4 h) and extensive (> 70%). Systemic bioavailability of factor J (26–29%) < factor L (39–57%)
Distribution	Rapidly and extensive. Highest concentrations of radioactivity in the gastrointestinal tract, followed by fat, carcass and liver
Potential for accumulation	Tissue and carcass concentrations low after 7 days (0.6–1.4% of administered dose).

Rate and extent of excretion	Rapidly excreted, plasma half-lives 4–24 h; 85% of dose in faeces, mainly as metabolites; 3–4% in urine, mostly in first 24 h
Metabolism in animals	Extensively metabolized, primarily by glutathione conjugation of parent and products of phase-one metabolism. Some sulfate and glucuronide conjugation of aglycone of factor L
Toxicologically significant compounds (animals, plants and environment)	Spinetoram, comprising factors J and L

Acute toxicity

Rat, LD50, oral	> 5000 mg/kg bw
Rat, LD50, dermal	> 5000 mg/kg bw
Rat, LC50, inhalation	> 5.44 mg/l for 4 h (nose only)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Transient irritation
Mouse, dermal sensitization	Not sensitizing (local lymph node assay in CBA/J mice)[3]

Short-term studies of toxicity

Target/critical effect	Mice, rats, dogs: vacuolation of macrophages in a wide range of lymphoid tissues within numerous organs and aggregates of macrophages/histiocytes in a number of tissues, non-regenerative anaemia, arteritis (dogs)
Lowest relevant oral NOAEL	5.0 mg/kg bw per day (90-day and 1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (28-day study in rats, highest dose tested)
Lowest relevant inhalation NOAEL	No data

Genotoxicity

	Negative in vitro and in vivo

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Mice, rats: vacuolation of cells (thyroid in rats; epididymes in mice) and increases in aggregates of macrophages/histiocytes in lymphoid tissues in numerous organs, hyperplasia of the glandular mucosa of the stomach and inflammation of the glandular submucosa (mice)
Lowest relevant NOAEL	2-year study, rat: 10.8 mg/kg bw per day
Carcinogenicity	Not carcinogenic

Reproductive toxicity

Reproduction target/critical effect	Dystocia (difficulty in delivery), decrease in gestation survival of pups.
Lowest relevant reproductive NOAEL	10 mg/kg bw per day (rats)
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	60 mg/kg bw per day (rabbit; highest dose tested)

Neurotoxicity/delayed neurotoxicity

Acute neurotoxicity and short-term studies of neurotoxicity	No indications of neurotoxicity in single- or repeat-dose studies

Medical data

	No data available on manufacturing-plant personnel (production-scale manufacturing has yet to start). No reports of adverse health effects in exposed subjects.

Summary

	Value	Study	Safety factor
ADI	0–0.05 kg bw	Dog, 90-day and 1-year study	100
ARfD	Unnecessary	—	—

Factor J, XDE-175-J
Factor L, XDE-175-L

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