

KRESOXIM-METHYL

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Explanation

Kresoxim-methyl, methyl-(E)-2-methoxyimino-2-[2-(2-methylphenoxy)methyl]phenyl acetate, is a broad-spectrum fungicide and a member of the strobilurin family, a new class of biologically active compounds structurally related to strobilurin A, a natural product of the wood-decaying fungus *Strobilurus tenacellus*. It is intended for use as an agricultural spray in the control and treatment of fungal infections on crops and fruits. Strobilurins are known to bind to the bcl complex (complex III), one of the oxide reductase proteins of the electron transport chain in mitochondria. The ester linkage in kresoxim-methyl is essential for its activity. Kresoxim-methyl was evaluated for the first time by the present Meeting.

Evaluation for Acceptable Daily Intake**1. Biochemical aspects***(a) Absorption, distribution, and excretion*

Kresoxim-methyl labelled with ^{14}C on the phenyl A ring (phenoxy; radiochemical purity, > 98%) or B ring (phenyl; radiochemical purity, > 98%) or with ^{13}C on the carbon side-chain was administered to rats by gavage as a suspension in 0.5% carboxymethyl cellulose (CMC) or

intravenously as a 0.9% saline solution. The design of the study conformed to good laboratory practice. In groups of five male and five female rats given [^{14}C -B ring]kresoxim-methyl by gavage at 50 or 500 mg/kg bw, with or without pretreatment with unlabelled kresoxim-methyl, or [^{14}C -A ring]kresoxim-methyl at a dose of 500 mg/kg bw, the compound was excreted predominantly in faeces. At the low dose of [^{14}C -B ring]-labelled compound, faecal excretion represented 65–67% of the administered dose and urinary excretion, 20–28% of the dose within 48 h; less than 1% of the radiolabel was recovered in urine and faeces at this time. Pretreatment with unlabelled kresoxim-methyl at the low dose for 14 days did not change the excretion pattern. At the high dose, faecal excretion represented 80–81% of the dose and urinary excretion, 8–13% within 48 h. The total radiolabel recovered within 120 h was 97% of the [^{14}C -A ring] and 90–96% of the [^{14}C -B ring], with 62–78% of the A ring and 81% of the B ring excreted in faeces and 17–33% of the A ring and 9–13% of the B ring in urine. No radiolabel was detected in exhaled air.

In the groups given the [^{14}C -B ring]-labelled material, peak concentrations of radiolabel in plasma were reached 0.5–1 h after dosing at the low dose and 8 h after dosing at the high dose. The plasma level then declined, with a terminal half-life of 17–19 h at the low dose and 22–30 h at the high dose. The ratios of the area under the curve for the high:low dose (10:1) were 2.3 for males and 2.1 for females. Radiolabel concentrations were determined in tissues 0.5, 8, 24, 96, and 120 h after dosing. Except for the gastrointestinal tract, the highest residual concentration was found in the liver (0.1 g/g at 120 h and 0.3–1.4 g/g at 24 h after dosing at 50 mg/kg bw). The residual concentrations in other tissues were less than 0.1 g/g tissue at 120 h after dosing at 50 mg/kg bw. The concentrations of radiolabel in the tissues were comparable in males and females, indicating a similar pattern of wide distribution and elimination.

Groups of five male and five female rats given [^{14}C -B ring]kresoxim-methyl intravenously as a single dose of 5 mg/kg bw excreted 49–66% of the radiolabel in urine and 23–48% in faeces within 120 h.

Groups of four male and four female rats with cannulated bile ducts were given the [^{14}C -B ring]-labelled material as a single oral dose of 50 or 500 mg/kg bw. Biliary excretion accounted for 35–43% of the radiolabel at the low dose and 14–15% at the high dose within 48 h. Urinary excretion represented 20–28% at the low dose and 8–13% at the high dose, and faecal excretion represented 65–67% at the low dose and 80–81% at the high dose within 48 h. Excretion of the [^{14}C -A ring]-labelled material in bile was not examined (Gans, 1994).

(b) Biotransformation

The samples collected in the experiments described above (Gans, 1994) were analysed for metabolites of kresoxim-methyl, in a study that conformed to good laboratory practice. After oral administration, high proportions of parent compound were found in the faeces (Table 1), but none was detected in the bile or in tissues (plasma, liver, and kidney) sampled about 4 h after administration of the low or high dose (Table 2). A total of 34 metabolites, including conjugates, was identified by nuclear magnetic resonance spectroscopy and mass spectrometry in rat excreta, with 20 in urine, eight in faeces, and 17 in bile. The major metabolites identified in urine and faeces were M1, a hydrolytic product of the acetyl ester; M2, an oxidative metabolite of the aryl-methyl moiety of M1; and M9, a hydroxylated metabolite of the phenoxy ring of M1. M1 and M9 were the major metabolites identified in tissues. Glucuronated conjugates were detected in notable quantities in the bile. There was no evidence that the metabolic pathways were induced by pretreatment with kresoxim-methyl. A small difference in the metabolite pattern in urine and bile was observed between males and females, the percentages of M1 and M9 in urine from females being greater than in urine from males. In summary, the metabolic pathways of kresoxim-methyl consisted of hydrolytic cleavages of the ester, the oxime ether, and the benzyl ether bonds; hydroxylation at the *para* position of the phenoxy ring; oxidation of the aryl-methyl group to benzyl alcohol and its subsequent oxidation to the corresponding carboxylic acid; and conjugation of the resulting hydroxy groups with glucuronate and sulfate (Kohl, 1994). The proposed metabolic pathway for kresoxim-methyl in rats is shown in Figure 1.

The major metabolites identified in plants were a hydrolytic product of the acetyl ester (M1), an oxidative metabolite of the aryl-methyl moiety (M2), a hydroxylated metabolite of *para*- or

Table 1. Percents of a single oral dose of kresoxim-methyl found as parent compound and metabolites in rat excreta and tissues

Substance	Faeces				Urine			
	50 mg/kg bw		500 mg/kg bw		50 mg/kg bw		500 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
Parent	49.5	47.1	74.9	39.5				
M1	2.1		0.1	7.1	0.4	2.7	2.8	2.2
M2	2.7	0.5	0.5	5.8	2.0	3.4	1.5	2.0
M4	1.1	0.5	0.3	2.5	mix1	mix1	mix1	mix1
M6					2.8	1.1	1.9	0.5
M8					0.1	0.4		mix3
M9	5.2	6.0	0.9	13.3	5.5	11.0	2.7	4.9
M11					mix2		mix2	mix3
M12					mix2		mix2	mix3
M14					mix1	mix1	mix1	mix1
M15	1.3	2.7	0.1	3.4				
M16							0.3	mix3
M20					mix1	mix1	mix1	mix1
M24			0.1				0.1	0.4
M26					mix2		mix2	mix3
mix1					1.4	1.6	0.9	1.1
mix2					0.9		0.8	
mix3								1.4
UK1	1.3	0.6	0.4	0.1		0.1		0.2
UK2		0.2	0.1	1.4				
UK3			0.1	1.8				
UK4			0.3					
UK5			0.2					
UK5				0.1				
Recovery	83.3	86.7	84.1	86.1	99.2	97.4	100.1	99.4

mix1, mixture of M4 + M14 + M20; mix2, M11 + M12 + M26; mix3, M8 + M11 + M12 + M16 + M26; UK, unknown compound

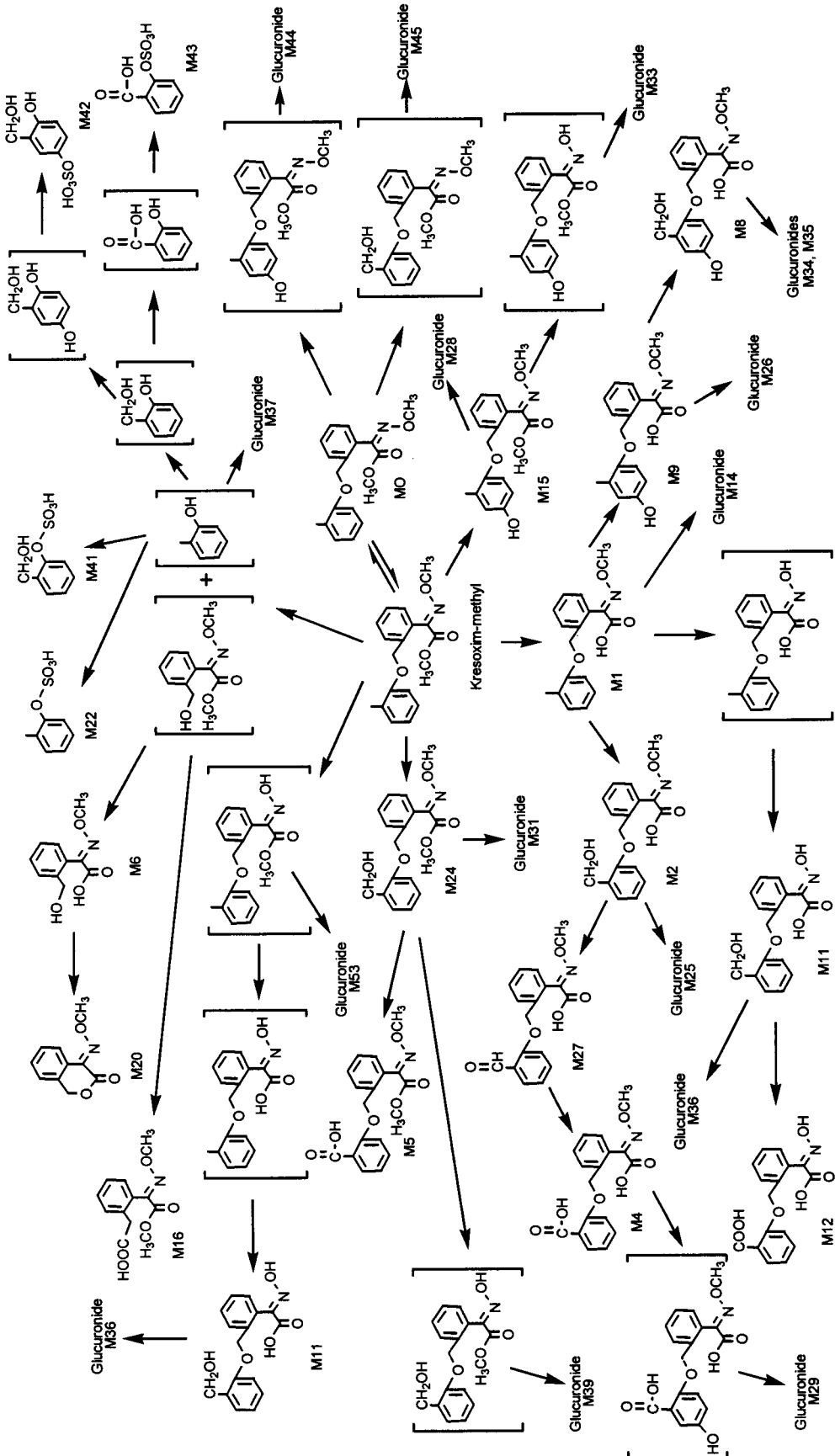
Table 2. Percents of a single oral dose of kresoxim-methyl found as parent compound and metabolites in rat bile and tissues

Substance	Bile		Plasma		Liver			
	50 mg/kg bw		50 mg/kg bw		50 mg/kg bw		500 mg/kg bw	
	Male	Female	Male	Female	Male	Female	Male	Female
Parent	0	0	0	0	0	0	0	0
M1	1.7	1.9	0.386	0.304	0.13	0.07	0.07	0.12
M2	mix5	mix5	0.095	mix8	0.08	0.04	0.04	0.04
M4			0.041	mix8	0.03	0.02	0.04	0.02
M6			0.027	0.006				
M9	1.1	1.3	0.173	0.164	0.17	0.07	0.06	0.09
M11			0.002		mix4	mix4	mix4	mix4
mix4			mix4		mix4	mix4	mix4	mix4
M28	0.7	2.9						
M31	0.5	1.1						
M35	1.7	0.7						
M44	0.4	0.3						
M45	mix5	mix5						
mix4			0.115		0.08	0.02	0.02	0.01
mix5	1.1	1.2						
mix6	6.3	3.6						
mix7	0.4	0.2						
mix8				0.169				
UK1					0.02			
UK2				0.024	0.02	0.01		
Recovery	100.0	100.1			84.8	82.5	87.0	96.8

mix4, M11 + M12 + M16 + M26; mix5, mixture of M2 + M45; mix6, M25+M26+M29+M33+M39; mix7, M34+M36+M37; mix8, M2 + M4

The tissue samples were collected 3.5–4 h after a single oral administration. The values in plasma are expressed as microgram equivalent per ml.

Figure 1. Proposed metabolic pathways of kresoxim-methyl in rats



meta-hydroxylated metabolites of the phenoxy ring of the first metabolite (M9 or M54), and their conjugates (Grosshans, 1994a,b; Nelsen et al., 1995).

2. Toxicological studies

(a) Acute toxicity

Studies of the acute toxicity of kresoxim-methyl are summarized in Table 3. Oral administration of 5000 mg/kg bw kresoxim-methyl as a suspension of 0.5% CMC produced no deaths or abnormal clinical signs in mice or rats, and no abnormal changes in organs were seen at necropsy. Dermal application of 2000 mg/kg bw in a suspension of 0.5% CMC caused no deaths or signs of clinical toxicity, except for a slight but definite erythema at the site of application in some animals. Groups of five male and five female Wistar rats were exposed to a dust aerosol of kresoxim-methyl at concentrations of 2 and 5.6 mg/L through a head–nose inhalation system. The mass median aerodynamic diameter of the dust aerosol particles was 1.8–2.4 μm . No deaths occurred; during exposure to either concentration, nonspecific clinical signs such as accelerated and intermittent respiration, urine-smearing, reddish nose, eye discharge, and reddish eyelid crust, were observed. These signs disappeared one day after exposure.

In a study conducted according to good laboratory practice, white Vienna rabbits of each sex received 4-h dermal applications of a single dose of 0.5 g kresoxim-methyl (purity, 93.7%) as a fine powder moistened with distilled water. The skin was examined 1, 24, 48, and 72 h after removal of the compound: little or no erythema was observed (Rossbacher & Kirsch, 1992a).

In another study conducted according to good laboratory practice, a single dose of 39 mg kresoxim-methyl (purity, 93.7%) in a volume of 0.1 ml was administered to the right eye of white Vienna rabbits of each sex. The eyes were examined 1, 24, 48, and 72 h after application, without washing. Some conjunctival redness (score, 0.1–4) was observed at 1, 24, and 48 h but had disappeared by 72 h after application (Rossbacher & Kirsch, 1992b).

In a further study conducted according to good laboratory practice, the skin sensitizing potential of kresoxim-methyl (purity, 93.7%) was tested in female Dunkin Hartley guinea-pigs by the maximization method. For induction, a 5% suspension of kresoxim-methyl in 0.5% CMC was applied intradermally, followed by topical application of a 50% suspension. At challenge, 50% kresoxim-methyl (20 rabbits) or the vehicle (10 rabbits) was applied dermally. No dermal reaction was observed in the rabbits challenged with kresoxim-methyl (Rossbacher & Kirsch, 1993).

(b) Short-term toxicity

Mice

In a range-finding study conducted according to good laboratory practice, groups of five male and five female B6C3F₁(Cr) mice were given diets containing kresoxim-methyl (purity, 96.6%)

Table 3. Acute toxicity of kresoxim-methyl

Species	Strain	Sex	Route	LD ₅₀ or LC ₅₀ (mg/kg bw or mg/L air)	Purity (%)	Reference
Mouse	ICR	M/F	Oral	> 5000	94.3	Yamamoto (1994)
Rat	Chbb Wistar	M/F	Oral	> 5000	93.7	Kirsch & Hildebrand (1993a)
Rat	Chbb Wistar	M/F	Dermal	> 2000	93.7	Kirsch & Hildebrand (1993b)
Rat	Chbb Wistar	M/F	Inhalation	> 5.6	96.6	Gamer & Kirsch (1992)

These studies were conducted in accordance with good laboratory practice.

at concentrations of 0, 500, 2000, or 8000 ppm for 28 days, equal to 0, 110, 480, and 2100 mg/kg bw per day for males and 0, 180, 800, and 3800 mg/kg bw per day for females. The animals were observed for clinical signs, deaths, food consumption, body weight, and clinical chemical, haematological, and pathological end-points. There were no deaths or signs of clinical toxicity. At the highest dose, significantly reduced serum concentrations of triglyceride and cholesterol were observed in males, and significantly increased relative liver weights ($p < 0.05$) were observed in animals of each sex. No compound-related lesions were observed on histopathological examination. The NOAEL was 8000 ppm, equal to 2100 mg/kg bw per day, as the increased relative liver weights were not accompanied by histopathological changes (Schilling & Hildebrand, 1992b).

Groups of 10 male and 10 female C57Bl/6N(Cr) mice were given diets containing kresoxim-methyl (purity, 98.7%) at concentrations of 0, 250, 1000, 4000, or 8000 ppm, equal to 0, 57, 230, 910, and 1900 mg/kg bw per day for males and 0, 80, 330, 1300, and 2600 mg/kg bw per day for females, for three months. The study was carried out according to good laboratory practice. The animals were observed for clinical signs, deaths, food consumption, body weight, clinical chemical parameters including the activities of serum alanine (ALAT) and aspartate aminotransferases (ASAT), alkaline phosphatase (AP), and γ -glutamyl transferase (GGT), and haematological and pathological end-points. There were no deaths, signs of clinical toxicity, or changes in haematological or clinical chemical parameters. Dose-dependent reductions in terminal body weight, by 4% at 4000 and 7% at 8000 ppm, and body-weight gain, by 11% at 4000 and 24% at 8000 ppm, were seen in males; however, these reductions were not significant. Significant increases in relative liver weight were observed in males at 4000 and 8000 ppm, but no compound-related lesions were observed on histopathological examination. The NOAEL was 8000 ppm, equal to 1900 mg/kg bw per day, on the basis of the absence of toxicologically significant changes (Mellert & Hildebrand, 1994b)

Rats

In a range-finding study that conformed to good laboratory practice, groups of five male and five female Wistar (Cr) rats were given diets containing kresoxim-methyl (purity, 96.55%) at a concentration of 0, 1000, 4000, or 16 000 ppm for 28 days, equal to 0, 91, 360, and 1400 mg/kg bw per day for males and 0, 95, 380, and 1500 mg/kg bw per day for females. The rats were observed for clinical signs, deaths, food consumption, body weight, clinical chemical parameters including the activities of serum ALAT, ASAT, AP, and GGT, and haematological and pathological end-points. There were no deaths, signs of clinical toxicity, or changes in haematological parameters. The terminal body weights were slightly reduced in animals of each sex at 4000 ppm (by 4% in males and 10% in females) and at 16 000 ppm (by 7% in males and 6% in females), and the absolute liver weights were slightly increased in males (by 8%) and females (9%) at 16 000 ppm; however, these changes were not statistically significant. Significant increases in relative liver weights were observed in females at 16 000 ppm, and significantly increased serum GGT activity and albumin concentration were observed in males at this dose. No compound-related lesions were observed on histopathological examination. The NOAEL was 4000 ppm, equal to 360 mg/kg bw per day, on the basis of increased serum enzyme activity in males and increased relative liver weight in females (Schilling & Hildebrand, 1992a).

Groups of 10 male and 10 female Wistar (Chbb) rats were given diets containing kresoxim-methyl (purity, 98.7%) at concentrations of 0, 500, 2000, 8000, or 16 000 ppm, equal to 0, 36, 150, 580, and 1200 mg/kg bw per day in males and 0, 43, 170, 670, and 1400 mg/kg bw per day in females, for 90 days. The rats were observed for clinical signs, deaths, food consumption, body weight, clinical chemical parameters including the activities of serum ALAT, ASAT, AP, and GGT, and haematological and pathological end-points. Food consumption and body weights were determined once a week, and enzyme activities were determined after six weeks and at the end of the study.

There were no deaths, signs of clinical toxicity, changes in food consumption, or compound-related changes in haematological parameters. Slight but significant decreases in terminal body weight (7–8% at 8000 and 11–13% at 16 000 ppm) and body-weight gain (7–10% at 8000 and 13–15% at 16 000 ppm) were observed in males. Significant increases in relative liver weight were observed in males at 16 000 ppm (10%) and in females at 2000 ppm and higher (10% at 2000, 7% at 8000, and 12% at 16 000 ppm). Significant increases in relative kidney weight were also observed in males, but the absolute weights were not increased. No compound-related histopathological lesions were observed in these or other organs in treated groups. Dose-dependent, statistically significantly increased activities of GGT were observed in males at 8000 ppm and higher, and significantly decreased activities of AP and ALAT were observed in males at all doses and in females at 2000 ppm and higher. These reductions in enzyme activity were considered to be related to the slight decrease in food consumption on the basis of mechanistic studies on percent reductions in intestinal and hepatic isozymes per total serum AP activity (Moss, 1994; Mellert et al., 1997a). The NOAEL was 2000 ppm, equal to 150 mg/kg bw per day, on the basis of decreased body weight and body-weight gain and increased GGT activity in males at higher doses (Mellert & Hildebrand, 1994a).

Groups of five male and five female Wistar (Chbb) rats received dermal applications of kresoxim-methyl (purity, 94.3%) suspended in 0.5% CMC at a dose of 0 or 1000 mg/kg bw per day under a semi-occlusive dressing (four layers of absorbent gauze and an elastic dressing) for 6 h/day for 21 days. The study design corresponded to good laboratory practice. The rats were observed for clinical signs, deaths, food consumption, body weight, clinical chemical parameters including the activities of serum ALAT, ASAT, AP, and GGT, haematological parameters including clotting times, and pathological end-points. Blood samples for haematological and clotting analysis and for clinical chemistry were collected at termination. There were no compound-related effects on mortality rates, clinical signs, haematological parameters, clotting times, or clinical chemical parameters, including serum enzyme activities. There were no significant changes in body-weight gain or food consumption in the treated group, and no signs of irritation were observed on treated skin of test or control animals. No effect on organ weights was observed, and histopathological examination revealed no treatment-related alterations in the liver or in any other tissue examined. The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Kirsch & Hildebrand, 1994c).

Dogs

Groups of six male and six female beagles, six to nine months old, were given diets containing kresoxim-methyl (purity, 94–95.9%) at concentrations of 0, 1000, 5000, or 25 000 ppm, equal to 0, 28, 140, and 740 mg/kg bw per day for males and 0, 32, 160, and 800 mg/kg bw per day for females, for three months. The study was conducted according to the principles of good laboratory practice. The animals were observed for clinical signs, deaths, food consumption, body weight, clinical chemical parameters including the activities of serum ALAT, ASAT, AP, and GGT, and haematological and pathological end-points. Blood samples for haematological and clinical chemical analysis were collected during weeks 4 and 13 of treatment.

No deaths or ophthalmological abnormalities were observed. During the first three weeks, diarrhoea and vomiting were observed frequently in most animals at 25 000 ppm, and a slight but significant reduction in body-weight gain was observed in females at this dose throughout the study. There were no treatment-related changes in haematological or urinary parameters; slight but significant decreases in the concentration of total protein were observed in males at 25 000 ppm, and significant decreases in the concentration of albumin were observed in females at 5000 ppm and animals at 25 000 ppm. These changes were observed during week 4 of treatment but had disappeared by week 13. The changes in albumin and total protein concentration might not be related to treatment, because they were slight and transient, and may have been a result of the vomiting and diarrhoea that occurred during the first weeks of the study. Dose-dependent increases in the absolute and relative weights of the liver were observed but were not significant. Histopathological examination revealed no compound-related lesions in tissues, including the

liver. The NOAEL was 5000 ppm, equal to 140 mg/kg bw per day, on the basis of vomiting and diarrhoea in animals of each sex and reduced body-weight gain in females (Mellert & Hildebrand, 1994c).

Groups of six male and six female beagles, six to nine months old, were given diets containing kresoxim-methyl (purity, 93.7%) at a concentration of 0, 1000, 5000, or 25 000 ppm, equal to 0, 27, 140, and 710 mg/kg bw per day in males and 0, 30, 150, and 760 mg/kg bw per day in females, for 12 months. The study conformed to good laboratory practice. The animals were observed for clinical signs, deaths, food consumption, body weight, ophthalmological end-points, clinical chemical parameters including the activities of serum ALAT, ASAT, AP, and GGT, haematological parameters including clotting time, and pathological end-points. Blood samples were collected for haematological and clotting analysis and clinical chemistry after 3, 6, and 12 months of treatment.

No deaths or ophthalmological abnormalities were observed. Diarrhoea and vomiting occurred infrequently in animals of each sex at 25 000 ppm, and the body weights of males at this dose were significantly reduced at study termination. There was no reduction in body-weight gain or food consumption at any dose. Significant increases in the number of platelets were observed in males at all doses; the values for males at 25 000 ppm were within the range in historical controls, except for the mean value at the third month. There were no compound-related changes in clotting time. There were no compound-related changes in urinary or clinical chemical parameters or in the activities of serum enzymes. Significant increases in relative liver weights were observed in males at 5000 ppm, but the absolute liver weights were not significantly increased. Histopathological examination revealed no treatment-related alterations in the liver or in any other tissue examined. The NOAEL was 5000 ppm, equal to 140 mg/kg per day, on the basis of reduced body weight in males (Hellwig & Hildebrand, 1994b).

(c) *Long-term studies of toxicity and carcinogenicity*

Mice

Groups of 50 male and 50 female C57Bl/6N (Cr) mice were given diets containing kresoxim-methyl (mean purity, 96.3% during the first 12 months and 93.2% during the following six months) at concentrations of 0, 400, 2000, or 8000 ppm for 18 months. Satellite groups of 10 mice of each sex were treated concurrently for 12 months. The doses were equivalent to 0, 60, 300, and 1300 mg/kg bw per day in males and 0, 81, 400, and 1700 mg/kg bw per day in females in the main groups, and 0, 61, 320, and 1400 mg/kg bw per day in males and 0, 84, 410, and 1900 mg/kg bw per day in females in the satellite groups. The animals were observed for clinical signs, deaths, food consumption, body weight, and haematological and pathological end-points. Blood samples for haematology were collected during months 12 and 18 of treatment. The study conformed to good laboratory practice.

No compound-related effects were observed with respect to mortality rates, clinical signs, food consumption, or haematological parameters throughout the study. Statistically significant decreases in terminal body weights and body-weight gains were observed in the main groups in males at 8000 ppm and in females at 2000 and 8000 ppm during the final six months. Increased relative liver weights were observed in females in the satellite group examined at 12 months and in the main groups examined at 18 months at 8000 ppm. Increased relative adrenal weights were observed in males at 12 and 18 months and in females at 18 months. Histopathological examination at 12 months revealed no compound-related lesions in any group treated for 12 months, but examination at 18 months revealed significantly increased incidences of centrilobular fatty infiltration (1/50 at 0 and 16/50 at 8000 ppm) in the liver, a significantly increased incidence and a greater degree of severity of hepatic amyloidosis (6/50 at 0 and 16/50 at 8000 ppm), and increased incidences of lymphoid infiltration (16/50 at 0 and 27/50 at 8000 ppm) and papillary necrosis of the kidney (2/50 at 0 and 13/50 at 8000 ppm) in females. There was no treatment-related increase in the incidence of neoplastic lesions. The NOAEL was 400 ppm, equal to 81 mg/kg bw per day, on the basis of reductions in body weight and body-weight gain in females (Mellert & Hildebrand, 1994e).

Rats

In a study of toxicity, groups of 20 male and 20 female Wistar rats were given diets containing kresoxim-methyl (purity, 92.7–96.6%) at concentrations of 0, 200, 800, 8000, or 16 000 ppm, equal to 0, 9, 36, 370, and 750 mg/kg bw per day in males and 0, 12, 46, 500, and 1000 mg/kg bw per day in females, for 24 months. The animals were observed for clinical signs, deaths, food consumption, body weight, ophthalmological end-points, clinical chemical parameters including the activities of serum ALAT, ASAT, AP, and GGT, and haematological, urinary, and histopathological end-points. Blood samples for haematology and clinical chemistry were collected at 3, 6, 12, 18, and 24 months of the treatment. The design of the study conformed to good laboratory practice.

There were no treatment-related effects on mortality rates, clinical signs, or ophthalmoscopic parameters. The terminal body weight and body-weight gain were slightly reduced in males at 16 000 ppm (by 4%) and significantly reduced in females at 8000 ppm (by 9 and 13%, respectively) and 16 000 ppm (by 6 and 10%, respectively). No significant change in food consumption was observed. Slight but significant reductions in mean corpuscular volume and mean corpuscular haemoglobin were observed in males at 16 000 ppm and in females at ≥ 200 ppm; however, these changes were within the background range and were not clearly dose-dependent. The activity of serum ALAT was significantly decreased in animals of each sex at 8000 and 16 000 ppm and that of serum AP in animals of each sex at ≥ 200 ppm. The author suggested that these reductions in enzyme activities are not toxicologically relevant, which is reasonable (Moss, 1994; Mellert et al., 1997a). The relative liver weights were significantly increased in males at 8000 and 16 000 ppm, and the absolute liver weights were significantly increased in males at the highest dose. Significant, dose-related increases in GGT activity were also observed in males at ≥ 8000 ppm.

Microscopic examination revealed evidence of neoplasia in the liver. Increased incidences of hepatocellular carcinoma were observed in animals of each sex at 8000 and 16 000 ppm (in males, 0/20 at 0, 1/20 at 200 ppm, 1/20 at 800 ppm, 3/20 at 8000 ppm, and 8/20 at 16 000 ppm; in females, 0/20 at 0, 0/20 at 200 ppm, 2/20 at 800 ppm, 6/20 at 8000 ppm, and 6/20 at 16 000 ppm). No hepatocellular adenomas were observed. The incidence and severity of hepatocellular hypertrophy were dose-dependent and increased in animals of each sex (males, 0/20 at 0, 3/20 at 800 ppm, 4/20 at 8000 ppm, and 7/20 at 16 000 ppm; females, 1/20 at 0 and 8/20 at 16 000 ppm); however, statistical significance was achieved only at 16 000 ppm in animals of each sex. Significant increases in the incidence and severity of eosinophilic foci (0/20 at 0, 6/20 at 8000 ppm, and 8/20 at 16 000 ppm) and mixed-cell foci (0/20 at 0, 4/20 at 8000 ppm, and 5/20 at 16 000 ppm) were observed in males. Evidence of a proliferative response in bile-duct cells was associated with increased incidences of biliary cysts in males at 16 000 ppm (0/20 in controls versus 4/20) and in females at 8000 and 16 000 ppm (3/20 in controls versus 7/20 and 7/20), bile-duct proliferation in females at 8000 and 16 000 ppm (5/20 in controls versus 8/20 and 11/20), and pericholangitis of the liver in males at 16 000 ppm (1/20 in controls versus 4/20). Significantly increased incidences of tubular casts of the kidneys (2/20 in controls versus 10/20) and tubular atrophy of the kidney (4/20 in controls versus 12/20) were seen in females at 16 000 ppm. Increased incidences of lesions in other tissues were age-related or independent of dose and were not considered to be toxicologically significant.

The NOAEL for non-neoplastic alterations was 800 ppm, equal to 36 mg/kg bw per day, on the basis of increased activity of serum GGT, increased relative liver weight, and increased incidence and degree of severity of eosinophilic foci in males. The NOAEL for neoplasia was also 800 ppm on the basis of an increased incidence of hepatocellular carcinoma in animals of each sex (Mellert & Hildebrand, 1994d).

In a study of carcinogenicity, groups of 50 male and 50 female Wistar rats were fed diets containing kresoxim-methyl (purity, 92.7–96.6%) at concentrations of 0, 200, 800, 8000, or 16 000 ppm, equal to 0, 9, 36, 380, and 770 mg/kg bw per day for males and 0, 12, 47, 500, and 1000 mg/kg bw per day for females, for 24 months. The animals were observed for clinical signs, deaths, food consumption, body weight, and haematological and histopathological end-points.

Blood samples for haematology were collected at the end of the study. The study was carried according to the principles of good laboratory practice.

There were no treatment related effects on mortality rates or clinical signs. The terminal body weights and body-weight gains were significantly reduced in animals of each sex at 8000 ppm (9 and 13% in males and 13 and 20% in females, respectively) and 16 000 ppm (9 and 12% in males and 14 and 21% in females, respectively). No significant change was observed in food consumption. Significantly increased relative liver weights were observed in males at 16 000 ppm. Microscopic examination revealed hepatic neoplasia: increased incidences of hepatocellular carcinoma were observed in animals of each sex at 8000 and 16 000 ppm (males, 7/50 at 0, 5/50 at 200 ppm, 2/50 at 800 ppm, 18/50 at 8000 ppm, and 11/50 at 16 000 ppm; females, 1/50 at 0, 1/50 at 200 ppm, 2/50 at 800 ppm, 13/50 at 8000 ppm, and 16/50 at 16 000 ppm). The numbers of animals with adenoma plus carcinoma in the liver were significantly increased among males at 8000 ppm (8/50 at 0, 19/50 at 8000 ppm, and 13/50 at 16 000 ppm) and among females at 8000 and 16 000 ppm (1/50 in controls versus 15/50 and 17/50). The incidence of hepatocellular hypertrophy was increased in males at 8000 and 16 000 ppm and in females at 16 000 ppm but reached statistical significance only in males at 16 000 ppm (males, 3/50 at 0, 5/50 at 8000 ppm, and 10/50 at 16 000 ppm; females, 5/50 at 0, and 7/50 at 16 000 ppm). There were dose-dependent increases in the incidences of eosinophilic foci (males, 1/50 at 0, 5/50 at 8000 ppm, and 11/50 at 16 000 ppm; females, 3/50 at 0, 8/50 at 8000 ppm, and 5/50 at 16 000 ppm) and mixed-cell foci in animals of each sex (males, 4/50 at 0, 9/50 at 8000 ppm, and 12/50 at 16 000 ppm; females, 0/50 at 0 and 5/50 at 16 000 ppm); however, significant results were observed only at 16 000 ppm. There was evidence of alterations in bile-duct cells, including an increased incidence of bile-duct proliferation in females at 16 000 ppm (10/50 in controls versus 28/50), cholangiofibrosis in females at 16 000 ppm (1/50 in controls versus 7/50), and biliary cysts in males at 8000 ppm (males, 1/50 at 0, 7/50 at 8000 ppm, and 6/50 at 16 000 ppm; females, 8/50 at 0, 12/50 at 8000 ppm, and 15/50 at 16 000 ppm). Other non-neoplastic lesions included tubular mineralization of the kidneys in males at 16 000 ppm (6/50 in controls versus 18/50), and round-cell infiltration of the adrenal cortex in males at 8000 ppm (5/50 at 0, 13/50 at 8000 ppm, and 5/50 at 16 000 ppm). The tubular mineralization was dose-related and considered to be related to treatment. The lesions observed in other tissues were considered to be independent of dose and age-related.

The NOAEL for non-neoplastic alterations was 800 ppm, equal to 36 mg/kg bw per day, on the basis of reduced body weight and body-weight gain and hepatic alterations. The NOAEL for neoplasia was also 800 ppm on the basis of increased incidences of hepatocellular carcinoma (Mellert & Hildebrand, 1994f).

A histopathological re-evaluation on the hepatocellular tumour incidence in the two studies in rats was conducted by a pathology working group. The results are shown in Table 4. Concurrent reassessment revealed similar dose-response relationships in the occurrence of hepatocellular carcinoma, and the statistically significant results with the combined data clearly indicate the hepatic carcinogenic potential of kresoxim-methyl in rats (van Ravenzwaay, 1996).

(d) Genotoxicity

The results of assays for the genotoxicity of kresoxim-methyl are summarized in Table 5. No point mutations were observed *in vitro* in bacterial or mammalian cells. A significantly increased frequency of chromosomal damage was observed in Chinese hamster lung cells with an exogenous metabolic activation system treated with kresoxim-methyl (purity, 93.7%) at ≥ 100 $\mu\text{g/ml}$; however, crystals were observed in medium cultured at 100 $\mu\text{g/ml}$ for 6 h. No chromosomal damage was observed in human lymphocytes *in vitro*. Assays for DNA repair and damage in rat hepatocytes showed marked cytotoxicity, characterized by altered cell morphology and reduced numbers of live cells at ≥ 10 $\mu\text{g/ml}$. Kresoxim-methyl at these doses also increased extracellular lactic dehydrogenase activity. The percent of cells in repair was slightly increased at ≥ 1 $\mu\text{g/ml}$ (by 1–2% in comparison with 52% in the positive control), but the authors considered these percentages to be below their evaluation criteria (net grain, $> 5\%$). Kresoxim-methyl did not cause DNA damage or repair *ex vivo* in hepatocytes isolated from treated rats. It did not induce micronucleus formation in mice or rats treated *in vivo*.

Table 4. Incidences of hepatocellular carcinomas and other parameters in rats in the studies of Mellert & Hildebrand (1994e,f)

Parameter	Dose (ppm)				
	0	200	800	8000	16 000
<i>Study of toxicity</i>					
<i>Males</i>					
Incidence	0/20	1/20	1/20	3/20	8/20*
% incidence	0	5	5	15	40
Absolute body weight (% of control)	100	106	109	94	96
Body-weight gain (% of control)	100	109	112	91	96
<i>Females</i>					
Incidence	1/20	0/20	2/20	6/20	6/20
% incidence	5	0	10	30	30
Absolute body weight (% of control)	100	105	88	91(*)	94(*)
Body-weight gain (% of control)	100	107	81	87(*)	90(*)
<i>Study of carcinogenicity</i>					
<i>Males</i>					
Incidence	7/50	5/50	2/50	18/50*	13/50**
% incidence	14	10	4	36	26
Absolute body weight (% of control)	100	101	98	91 (*)	91 (*)
Body-weight gain (% of control)	100	102	98	87 (*)	79 (*)
<i>Females</i>					
No of incidence	1/50	1/50	2/50	13/50*	16/50*
% of incidence	2	2	4	26	32
Absolute body weight (% of control)	100	99	98	87(*)	86(*)
Body-weight gain (% of control)	100	98	97	80(*)	79(*)

Terminal absolute body weight and body-weight gain were expressed as percent of control, but the statistical significance was calculated on the basis of weight.

* Statistically significantly different from control.

* Includes two animals with hepatocholangiocarcinomas

(e) *Reproductive toxicity*

(i) *Multigeneration reproductive toxicity*

Rats

In a two-generation study of reproductive toxicity, which conformed to good laboratory practice, groups of 25 male and 25 female Wistar rats were fed diets containing kresoxim-methyl (purity, > 93.7%) at concentrations of 0, 50, 1000, 4000, or 16 000 ppm. The F₀ generation was exposed directly, the F_{1a} and F_{1b} generations directly and indirectly, and F₂ generation indirectly. The mean daily intakes of kresoxim-methyl by the F₀ generation were 5, 100, 410, and 1600 mg/kg bw per day for males and 6, 120, 480, and 2300 mg/kg bw per day for females. Female intakes were 6, 110, 440, and 1700 mg/kg bw per day during pre-mating; and 4, 87, 360, and 1400 mg/kg bw per day during gestation and 7, 150, 600, and 2400 mg/kg bw per day during lactation for the F_{1a} and F_{1b} generations. The mean daily intakes by the F₁ generation were 4, 88, 360, and 1500 mg/kg bw per day for males and 5, 110, 440, and 1800 mg/kg bw per day for females. Female intakes were 5, 100, 420, and 1700 mg/kg bw per day during pre-mating; 4, 85, 350, and 1300 mg/kg bw per day during gestation for F_{2a}; and 7, 140, 560, and 2300 mg/kg bw per day during lactation for F_{2a}.

The parental rats were observed for clinical signs, deaths, food consumption, body weight, and clinical chemical, histopathological, and reproductive parameters including mating, fertility, gestation, and live-birth indices. The litters and pups were observed for viability, lactation, behaviour, and developmental indices that included pinna unfolding and opening of the auditory canal and eyes. The functional tests included grip strength, startle reflex, and pupillary reflex. Reproductive organs and the pituitary, liver, and kidney were examined histopathologically. The clinical chemical end-points included assays for serum ALAT, ASAT, AP, and GGT activity.

No compound-related clinical signs or deaths were observed in the F₀, F₁, or F₂ generation throughout the study. F₀ and F₁ parental animals showed no effects on mating, fertility, gestation,

Table 5. Results of assays for the genotoxicity of kresoxim-methyl

End-point	Test object	Concentration	Purity (%)	Result	Reference
<i>In vitro</i> Reverse mutation ^{a,b}	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 <i>uvrA</i>	20–5000 µg/plate	93.7	Negative	Engelhardt & Hoffmann (1993a)
Reverse mutation ^{a,b}	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 <i>uvrA</i>	20–5000 µg/plate	94.3	Negative	Engelhardt & Hildebrandt (1994)
Reverse mutation ^{a,b}	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 <i>uvrA</i>	20–5000 µg/plate	90.2	Negative	Engelhardt (1996)
Reverse mutation ^{b,c}	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 <i>uvrA</i>	51–5000 µg/plate	98.6	Negative	Nakajima (1997)
DNA repair ^d	<i>B. subtilis</i> rec M45 ⁺ , H17 ⁻	191–6100 µg/plate (–S9) 95–3050 µg/plate (+S9)	98.6	Negative Negative	Nakajima (1997)
Gene mutation ^e	Chinese hamster ovary cells, <i>hprt</i> locus	0.01–100 µg/ml (–S9) 0.1–100 µg/ml (+S9)	94.3	Negative	Polloth & Hoffman (1994a)
Chromosomal aberration ^{b,f}	Human lymphocytes	10–40 µg/ml	98.7	Negative	Engelhardt & Hoffmann (1993b)
Chromosomal aberration ^g	Chinese hamster lung cells	0.45–55 µg/ml (–S9) 50–200 µg/ml (+S9)	93.7	Negative Positive	Akanuma et al. (1997)
DNA damage and repair ^h	Wistar rat hepatocytes	0.33–10 µg/ml	94.3	Negative	Polloth & Hoffman (1994b)
<i>In vivo</i> DNA damage and repair ⁱ	Wistar rat hepatocytes	Single oral gavage, 18 h 0, 20, 200, 1000 mg/kg bw	94.3	Negative	Polloth & Hildebrandt (1994c)
DNA damage and repair ^j	Wistar rat hepatocytes	3-week feeding 0, 200, 16 000 ppm	94.3	Negative	Polloth & Hoffman (1994b)
Micronucleus formation ^k	NMRI mouse bone marrow	Single i.p., 16 and 48 h, 0, 500, 1000, 2000 mg/kg bw	93.7	Negative	Engelhardt & Hoffmann (1993c)
Micronucleus formation ^l	Wistar rat bone marrow	Single i.p., 24 and 48 h, 0, 500, 1000, 2000 mg/kg bw	94.9	Negative	Engelhardt & Hoffmann (1997)

S9, microsomal fraction of rat hepatocytes; i.p., intraperitoneal

All of the tests were carried out according to good laboratory practice, and all of the positive controls produced the expected results.

^a In dimethyl sulfoxide; the positive controls were 2-aminoanthracene, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine, 9-aminoacridine chloride, and 4-nitro-*ortho*-phenylenediamine.

^b In the presence and absence of S9

^c In dimethyl sulfoxide; the positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide for TA98, TA100, and WP2*uvrA*; sodium azide for TA1535; and 9-aminoacridine chloride for TA1537 –S9 and 2-aminoanthracene + S9.

^d Positive controls were mitomycin C –S9 and try-P-1 +S9; negative control was kanamycin –S9.

^e Positive controls were ethylmethanesulfonate –S9 and 3-methylcholanthrene +S9.

^f Positive controls were mitomycin C –S9 and cyclophosphamide +S9.

^g Positive controls were mitomycin C –S9 and benzo[*a*]pyrene.

^h Positive control was 2-acetylaminofluorene.

ⁱ Positive control was 2-acetylaminofluorene at a single oral dose of 50 mg/kg bw.

^j Positive controls were cyclophosphamide at a single i.p. dose of 20 mg/kg bw and vincristine at a single i.p. dose of 0.15 mg/kg bw.

^k Positive control was cyclophosphamide at a single i.p. dose of 20 mg/kg bw.

or live-birth indices, but significant reductions in food consumption were observed in F₀ and F₁ males during treatment and in F₀ and F₁ females during gestation and lactation at 16 000 ppm. Significant reductions in body weight were seen at doses of 4000 ppm and higher in F₀ and F₁ males and in F₀ and F₁ females during gestation and lactation of the F_{1a} and F_{1b} generations. Significant reductions in body-weight gain were also observed in F₀ and F₁ males at these doses and in F₀ females at 16 000 ppm during the premating period before the first gestation. Significant reductions

in the activities of ALAT and AP were observed in F₀ and F₁ parents of each sex, although these reductions may not be toxicologically relevant (Moss, 1994; Mellert & Hildebrand, 1995). The activity of GGT was significantly increased in F₀ males at 4000 ppm and higher and in F₁ animals of each sex at 16 000 ppm. Significantly decreased numbers of fat storage cells were observed in the livers of F₀ and F₁ males at 4000 ppm and higher; however, this change may have occurred as a result of the reduced food consumption at higher doses. Significant increases in relative kidney weights were observed in F₀ males at 16 000 ppm and in F₀ females and F₁ males at 4000 ppm and higher. No treatment-related morphological lesions were observed in the liver or kidney.

No compound-related changes in clinical signs, sex ratio, viability index, or lactation index were seen in pups of the F_{1a}, F_{1b}, and F_{2a} generations. Body weights and body-weight gain during lactation were significantly decreased in F_{1a}, F_{1b}, and F_{2a} pups at 4000 ppm and higher. A significantly lower percentage of F_{1b} pups at these doses had pinna unfolding; significant retardations in opening of the auditory canal and eyes were also observed in F_{1b} and F_{2a} pups at 4000 ppm, but were not dose-dependent. There were no differences in the results of reflex tests between controls and treated animals in any generation. Necropsy of pups revealed no external abnormality.

The NOAEL for parental toxicity was 1000 ppm, equal to 100 mg/kg bw per day in F₀ males and 88 mg/kg bw per day in F₁ males, on the basis of reduced body weight and body-weight gain, increased serum GGT activity, and increased relative kidney weights. The NOAEL for reproductive toxicity was 1000 ppm, equal to an overall mean intake of 100 mg/kg bw per day for F₁ and F₂ pups, on the basis of reduced body weight and body-weight gain (Hellwig & Hildebrand, 1994a).

(ii) *Developmental toxicity*

Rats

Groups of 25 female Wistar rats were given kresoxim-methyl (purity, > 93.7%) suspended in 0.5% CMC by gavage at a dose of 0, 100, 400, or 1000 mg/kg bw per day on days 6–15 of gestation. The study was conducted in accordance with good laboratory practice. No treatment-related changes in clinical signs, mortality rates, body weight, or food consumption were observed in maternal animals. There were no differences in conception rate, mean number of corpora lutea, total implantations, resorptions, pre- or post-implantation loss, or number of live fetuses. No significant differences in fetal sex ratio, placental weight, or fetal body weight were observed between control and treated groups. External examination revealed three fetuses with external malformations: one fetus at 100 mg/kg bw per day had anasarca and a cleft palate, one fetus at 400 mg/kg bw per day was acaudate, and one fetus at 1000 mg/kg bw per day had meningocele and unilateral microphthalmia; however, the incidence of these malformations was within the range for historical controls. One fetus at 1000 mg/kg bw per day had hydrocephalus, but this incidence was also within the historical control range. A significantly increased incidence of incompletely ossified thoracic vertebral bodies was seen in 23% of all fetuses and 58% of litters at 1000 mg/kg bw per day; the mean historical control values were 8% (0–49%) of all fetuses and 23% (0–100%) of litters. The NOAEL for maternal toxicity was 1000 mg/kg bw per day, and that for embryo and fetal toxicity was 400 mg/kg bw per day on the basis of a slight increase in variations in fetuses at 1000 mg/kg bw per day. There was no evidence of teratogenicity at doses ≤ 1000 mg/kg bw per day (Hellwig, 1994).

Rabbits

Groups of 15 female Himalayan rabbits were given kresoxim-methyl (purity, 96.6%) suspended in 0.5% CMC by gavage at a dose of 0, 100, 400, or 1000 mg/kg bw on days 7–19 of gestation. The study was conducted in accordance with good laboratory practice. No compound-related changes in clinical signs, deaths, body weight, or food consumption were observed in maternal animals, and there were no compound-related changes in conception rate, mean numbers of corpora lutea, total implantations, resorptions, pre- or post-implantation loss, or live fetuses. No significant differences in fetal sex ratio, placental weight, or fetal body weight were observed

between control and treated groups. External examination revealed one fetus with microcephaly and brachygnathia at 100 mg/kg bw per day, but the incidence was within that of historical controls. Eight fetuses (0/15 at 0, 2/15 at 100, 2/15 at 400, and 3/15 at 1000 mg/kg bw per day) had soft-tissue malformations: one at 100 mg/kg bw per day had a septal defect and one had agnesis of the gall-bladder (2.5% incidence); two at 400 mg/kg bw per day had a septal defect (1.9%); at 1000 mg/kg bw per day, one had a septal defect, dilatation of the aortic arch, and a descending aortic, one had hydrocephaly, and one had agnesis of the gall-bladder (4.1%). The percent of soft-tissue malformations in historical controls was 2.2–3.1%. The incidences of ventricular septal defects in the treated groups were comparable to historical values. Increased incidences of fused sternebrae were observed in 3/15 controls, 11/15 at 100 mg/kg bw per day, 7/15 at 400 mg/kg bw per day, and 9/15 at 1000 mg/kg bw per day; the increase at 100 mg/kg bw per day was significant but was within the historical control range. Increased total numbers of fetal malformations were also observed in treated groups but again at incidence rates comparable to those of historical controls (0% at 0, 4.9% at 100, 3.8% at 400, and 4.1% at 1000 mg/kg bw per day versus 2.9–3.5% for historical controls). The NOAEL for both maternal and developmental toxicity was thus 1000 mg/kg bw per day, the highest dose tested (Hellwig & Hildebrand, 1993, GLP)

(f) *Special studies*

(i) *Tumour initiating potential*

Groups of 10 Wistar rats of each sex were subjected to a partial hepatectomy and 14 h later received a single dose of 2388 mg/kg bw technical-grade kresoxim-methyl (purity, 92.7–94.3%) suspended in 0.5% CMC by gavage to rats. For promotion, phenobarbital was incorporated in the diet at a concentration of 500 ppm for eight weeks. Liver slices were examined histologically on slides stained with haematoxylin and eosin (H&E) or stained immunochemically for the placental form of glutathione *S*-transferase (GST-P). The study conformed to good laboratory practice. The incidences of hepatocellular alteration (foci) and of GST-P-positive foci were used to estimate initiating potential. *N*-Nitrosomorpholine was used as the positive control. Hepatocellular hypertrophy was found in almost all of the phenobarbital-treated animals, and GST-positive foci and foci of hepatocellular alteration were found in nearly all animals treated with the positive control. The number of animals with GST-P-positive foci in groups treated with kresoxim-methyl was comparable to that of vehicle controls. The numbers of foci per liver in promoted animals were 0–3 in those given kresoxim-methyl, 0–10 in vehicle controls, and 3–100 in positive controls. The results suggest that kresoxim-methyl does not have tumour initiating potential in rats in this test (Gamer & Hildebrand, 1995).

(ii) *Tumour promoting potential*

In a medium-term study of promotion, which did not conform to good laboratory practice, male Fischer rats were initiated with a single intraperitoneal injection of *N*-nitrosodiethylamine at a dose of 299 mg/kg bw. The animals were then maintained on basal diet *ad libitum* for 14 days. Five groups of 16 male rats were fed diets containing 0, 200, 800, 8000, or 16 000 ppm kresoxim-methyl (purity, 95.4%) for six weeks, with average intakes of 0, 11, 42, 430, and 890 mg/kg bw per day (not adjusted for purity). The remaining 16 male rats were fed a diet containing 500 ppm phenobarbital (28 mg/kg bw per day) as a positive control for six weeks. The animals were subjected to a two-thirds partial hepatectomy after the first week of feeding with kresoxim-methyl or phenobarbital and were observed for clinical signs, deaths, food consumption, and body weight. The liver was examined grossly and histopathologically.

There were no compound-related deaths or clinical signs of toxicity. Body weight and food consumption in groups given kresoxim-methyl were comparable to those of controls. Significant increases in the absolute and relative weights of the liver were observed in groups given kresoxim-methyl at 800 ppm and higher. Treatment with phenobarbital caused significant increases in body weight, food consumption, and relative liver weight. Quantification of hepatic foci with a computer-assisted image analyser revealed significant, dose-related increases in the number and

area of GST-P-positive hepatocellular foci in groups given kresoxim-methyl at ≥ 8000 ppm, as well as in the phenobarbital-treated positive controls. The NOAEL for promotion was 800 ppm (Harada et al., 1997).

(iii) *Hepatic-cell proliferation*

A series of studies was conducted to investigate the effect of kresoxim-methyl on hepatic-cell proliferation in rats, by measuring S-phase DNA synthesis, an indicator of cell proliferation. Incorporation of bromodeoxyuridine (BrdU) into DNA was measured by immunohistochemical staining.

In the first study, groups of five young male Wistar rats, 64 days old, were given diets containing kresoxim-methyl (purity, 94.3%) at concentrations of 0, 200, or 16 000 ppm, equal to 0, 15, and 1100 mg/kg bw per day, for three weeks. Osmotic minipumps filled with BrdU were implanted subcutaneously one week before necropsy. The animals were observed for clinical signs, deaths, food consumption, and body weight. The livers were examined grossly and immunohisto-pathologically. Samples of the hepatic lobule and the jejunum were taken as positive tissues for proliferation and were stained with H&E and immunochemically with an antibody against BrdU. Immunopositive and H&E-counterstained hepatocyte nuclei from 11 fields for each of three lobes were counted. No treatment-related changes in body weight, food consumption, or clinical signs were seen. A slight increase in liver weights was observed at 16 000 ppm, but no treatment-related gross lesions or histopathological changes were observed in the livers of treated rats. A statistically significant increase in the number of hepatocytes in which BrdU was incorporated into the DNA of S-phase cells was observed in the periportal zone (zone 1) and the intermediate zone (zone 2) of the hepatic lobule in the group at 16 000 ppm. No significant increase in cell proliferation was observed in the group at 200 ppm (Polloth & Hildebrand, 1994a).

In a supplementary study with a similar design, groups of five young male Wistar rats received kresoxim-methyl (purity, 94.9%) in the diet at a concentration of 0, 800, or 8000 ppm, equal to 0, 61, and 600 mg/kg bw per day, for three weeks. Results similar to those observed at 16 000 ppm in the first study were observed at 8000 ppm. Statistically significant increases in cell proliferation were observed in zones 1 and 2 of the hepatic lobule at 8000 ppm, but not at 800 ppm (Mellert et al., 1997a).

The NOAEL from the combined results of these two studies for hepatic cell proliferation was 800 ppm, equal to 61 mg/kg bw per day.

In a study of the hepatic proliferating activity of kresoxim-methyl in the livers of older rats, groups of five male Wistar (Chbb) rats aged 16 months were given diets containing kresoxim-methyl (purity, 94.3%) at a concentration of 0, 200, or 16 000 ppm for three weeks. The design of the study was similar to those described above. No compound-related changes were seen in clinical signs or body weight, and no compound-related lesions in the liver were observed by microscopic examination with H&E staining. A statistically significant increase in cell proliferation was observed in zone 1 of the hepatic lobule at 16 000 ppm, which was comparable to that observed in the young rats (Polloth & Hildebrand, 1994d).

In a study of the hepatic proliferating activity of kresoxim-methyl in the livers of rats treated for various periods, groups of five male Wistar (Chbb) rats, 42 days old, were given diets containing kresoxim-methyl (purity, 92.7%) at a concentration of 0 or 16 000 ppm for 1, 6, or 13 weeks. Groups were allowed to recover for two or three weeks. Significant increases in cell proliferation were observed in the treated groups after one week (zones 1, 2, and 3) and after six weeks (zone 1). The increase in zone 1 in the group treated for one week was greater than that in the group treated for six weeks. This compound-related enhancement of cell proliferation was significantly reversed in the groups allowed to recover. The zonal distribution of increased cell proliferation revealed a selective effect of kresoxim-methyl on hepatocytes in zone 1 (Mellert et al., 1996a).

In a study of unscheduled DNA synthesis and S-phase response in rat hepatocytes, groups of three male Wistar (Chbb) rats received a single oral dose of 0, 20, 200, or 1000 mg/kg bw kresoxim-methyl (purity, 94.3%) by gavage. 2-Acetylaminofluorene was used as a positive control, at a dose of 50 mg/kg bw in the assay of unscheduled DNA synthesis and at 1000 mg/kg bw in the assay of S-phase response. Hepatocytes were prepared by in-situ hepatic perfusion 18 h after treatment. The isolated hepatocytes were cultured with ³H-thymidine for 18 h, and S-phase response and unscheduled DNA synthesis were evaluated autoradiographically in the labelled cells. Exposure of rats to kresoxim-methyl *in vivo* was not cytotoxic to liver cells. Slight but dose-dependent increases in the number of cells in S-phase were observed in all treated groups, with 1% at 0, 1.37% at 20, 2.78% at 200, and 2.58% at 1000 mg/kg bw, as well as in the positive control group (5.87%). The results suggest that kresoxim-methyl induced a moderate increase in S-phase DNA synthesis at 200 mg/kg bw and has a weak potential for enhancing hepatic cell proliferation (Polloth & Hildebrand, 1994c).

(iv) *Morphology of hepatic proliferation*

Groups of three female Wistar (Chbb) rats, 12 weeks old, received diets containing kresoxim-methyl (purity, 94.3%) at concentrations of 0, 200, or 16 000 ppm, equal to 0, 15, and 1200 mg/kg bw per day, for three weeks. At termination, the livers were fixed *in situ* by perfusion, and the peroxisomes in the liver were examined by light and electron microscopy after staining with diaminobenzidine to detect catalase activity. There were no compound-related changes in clinical signs, body weight, or food consumption; reduced body-weight gain was observed at 16 000 ppm. No compound-related lesions were observed in the liver, and no difference was seen between treated and control animals in the numbers of peroxisomes (Mellert et al., 1995a).

Groups of three female Wistar (Chbb) rats, 15 months old, received diets containing kresoxim-methyl (purity, 94.3%) at concentrations of 0, 200, or 16 000 ppm for three weeks and were then fixed *in situ* by perfusion. Liver samples were examined by light and electron microscopy. There were no compound-related changes in clinical signs or body weight, and no compound-related lesions were observed in the liver on light microscopic examination. Electron microscopy showed that the amount, shape, and size of hepatocyte mitochondria in the treated group were comparable to those in controls. (Mellert et al., 1995b).

(v) *Induction of hepatic metabolic enzyme activities*

Groups of 10 male and 10 female Wistar rats were fed diets containing kresoxim-methyl at concentrations of 0, 200, or 16 000 ppm for three weeks, equal to 0, 13, and 1000 mg/kg bw per day for males and 0, 15, and 1200 mg/kg bw per day for females. The animals were observed for clinical signs, deaths, body weight, and food consumption. Indicators of hepatic enzymes were measured, including the activities of GGT and drug metabolizing enzymes, the concentration of glutathione in liver homogenates, and the content of cytochrome P450 in microsomes. Significant increases in the activities of GGT and pentoxoresorufin deethylase and in P450 content were observed in males at 16 000 ppm. The pattern of induction of drug metabolizing enzyme activities resembled that of phenobarbital. In females, only a tendency towards induction was observed (Mellert et al., 1996b).

(vi) *Mechanism of decreased serum enzyme activities*

As marked reductions in the activities of serum AP and ALAT were reported in short- and long-term studies of toxicity, a series of experiments was conducted in which groups of five males and five females were fed diets containing kresoxim-methyl at a concentration of 8000 ppm for two weeks. These studies did not conform to good laboratory practice. In the first experiment, AP activity was determined in serum samples and extracts of liver and small intestine. The intestinal activity of AP was not changed by treatment, the estimated ratio of intestinal and hepatic or bone AP isozyme activities in the serum being 38.5%. The author indicated the reduction in serum AP

activity observed in the kresoxim-methyl treated groups was mostly due to a reduction in intestinal AP activity. In the second experiment, serum AP activity was markedly reduced after fasting and was increased by feeding a diet supplemented with olive oil. In the third experiment, addition of sera collected from treated animals to sera collected from untreated animals did not suppress AP activity, indicating the absence of an inhibitor. The observed reductions in serum AP and ALAT activities was therefore probably due to a slight alteration in food absorption in treated rats. (Moss, 1994).

In a second study to investigate the reduced enzyme activities, groups of 10 male and 10 female Wistar rats were fed diets containing kresoxim-methyl at a concentration of 0 or 16 000 ppm for two weeks, equal to 910 mg/kg bw per day for males and 1100 mg/kg bw per day for females. The animals were observed for clinical signs, deaths, body weight, and food consumption. ALAT and AP activities in serum and urine were assayed at the end of the study. There were no compound-related changes in clinical signs or mortality rates. Significantly decreased food consumption was observed in treated animals of each sex. A slight but significant decrease in body weight was observed in treated males. Significantly reduced activities of ALAT and AP in serum were observed in animals of each sex, but no change in the activities of either enzyme was observed in urine. No change in urinary creatinine or urinary volume was observed in treated animals, indicating no change in renal function. Thus, the reduced enzyme activity observed in sera of kresoxim-methyl-treated rats was not caused by a change in renal excretion of the enzymes (Mellert et al., 1997b).

(g) *Studies on metabolites*

(i) *Acute toxicity*

Metabolites M1, M2, and M9 were given orally to rats in a suspension of 0.5% CMC. M1 (purity, 98.5%) produced a variety of abnormal clinical changes including dyspnoea, staggering gait, and tremor at doses of 2000 mg/kg bw and higher. M2 (purity, 97.7%) caused no deaths or abnormal symptoms at 5000 mg/kg bw. M9 (purity, 99.6%) caused dyspnoea and exhaustion in animals of each sex at 5000 mg/kg bw but resulted in no change in general appearance at 3000 mg/kg bw (Kirsch & Hildebrand, 1994a,b,c, 1995).

(ii) *Genotoxicity*

M1, M2, and M9 of the same purities described above did not induce reverse mutation in bacteria at a concentration of 5000 µg/plate, whereas the positive controls used gave the expected positive responses (Hoffman & Engelhardt, 1995a,b,c)

Comments

About 60% of an oral dose of 50 mg/kg bw and 25% of a dose of 500 mg/kg bw kresoxim-methyl was absorbed. It was excreted mainly in the faeces (70% of the low dose and 80% of the high dose), predominantly via the bile (about 40% of the low dose and 15% of the high dose within 48 h), with lesser amounts in urine (about 20% of the low dose and 10% of the high dose). Peak levels of the radiolabel in plasma were reached 0.5–1 h after the low dose and 8 h after the high dose. The plasma half-life was 17–19 h at the low dose and 22–31 h at the high dose. The highest residual concentrations were found in the liver, but the concentrations in all tissues, including the liver, were less than 0.1 g equivalent/g tissue after 120 h of treatment at the low dose.

After oral administration of kresoxim-methyl, a high proportion of the parent compound was found in the faeces, but none was detected in tissues or bile examined 4 h after dosing. In rats, 34 metabolites of kresoxim-methyl were identified. The proposed metabolic pathways are hydrolytic cleavage of the ester, the oxime ether, and the benzyl ether bonds, hydroxylation at the *para* position of the phenoxy ring, hydroxylation of the aryl-methyl group and its subsequent

oxidation to form the corresponding carboxylic acid, and conjugation of the resulting hydroxy groups with glucuronate or sulfate. The major metabolites identified in both rats and plants were the free acid, code number 490M1 {(E)-methoxyimino[α -(*ortho*-tolylloxy)-*ortho*-tolyl]acetic acid}, the hydroxy derivative of this, 490M2 [α -(*ortho*-hydroxymethylphenoxy)-*ortho*-tolyl(methoxyimino)acetic acid] formed by hydroxylation of the aryl-methyl group, the *para*-hydroxytolylloxy product 490M9 [α -(*para*-hydroxy-*ortho*-tolylloxy)-*ortho*-tolyl(methoxyimino)acetic acid], and their conjugates. 490M1, 490M2, and 490M9 all had low acute toxicity and were not mutagenic.

WHO has not classified kresoxim-methyl for acute toxicity.

In a range-finding study in B6C3F₁ mice, kresoxim-methyl was administered in the diet at concentrations of 0, 500, 2000, or 8000 ppm for 28 days. The NOAEL was 8000 ppm, equal to 2100 mg/kg bw per day. In a three-month study, C57Bl/6N mice received kresoxim-methyl in the diet at concentrations of 0, 250, 1000, 4000, or 8000 ppm. The NOAEL was 8000 ppm, equal to 1900 mg/kg bw per day.

In a range-finding study in rats, kresoxim-methyl was administered in the diet at concentrations of 0, 1000, 4000, or 16 000 ppm for 28 days. The NOAEL was 4000 ppm in males, equal to 370 mg/kg bw per day, on the basis of increased activities of serum γ -glutamyl transferase at 16 000 ppm, equal to 1500 mg/kg bw per day. In a three-week study of toxicity in rats, kresoxim-methyl was administered in the diet at concentrations of 0, 10, 50, or 8000 ppm. The NOAEL was 50 ppm, equal to 3 mg/kg bw per day, on the basis of increased hepatic γ -glutamyl transferase activity in males at 8000 ppm. In a 90-day study of toxicity in rats, kresoxim-methyl (purity, 98.7%) was administered in the diet at concentrations of 0, 500, 2000, 8000, or 16 000 ppm. The NOAEL was 500 ppm in females, equal to 43 mg/kg bw per day, based on increased relative liver weight at 2000 ppm and above, and 2000 ppm in males, equal to 150 mg/kg bw per day, based on decreased body-weight gain and increased activity of serum γ -glutamyl transferase at 8000 ppm and above.

In a three-month study of toxicity in dogs, kresoxim-methyl was administered at dietary concentrations of 0, 1000, 5000, or 25 000 ppm. The NOAEL was 5000 ppm, equal to 140 mg/kg bw per day, on the basis of vomiting, diarrhoea, and reduced body-weight gain in animals of each sex at 25 000 ppm. In a 12-month study in dogs, kresoxim-methyl was administered at dietary concentrations of 0, 1000, 5000, or 25 000 ppm. The NOAEL was 5000 ppm, equal to 140 mg/kg bw per day, on the basis of a reduction in body weight in males at 25 000 ppm. No compound-related toxicity was observed in females.

In an assay for carcinogenicity in mice, kresoxim-methyl was administered at dietary concentrations of 0, 400, 2000, or 8000 ppm for 18 months. The NOAEL was 400 ppm, equal to 81 mg/kg bw per day, in females on the basis of reduction in body weight at 2000 ppm. The NOAEL in males was 2000 ppm, equal to 300 mg/kg bw per day, on the basis of decreased body weight and increased relative adrenal weight at 8000 ppm. At this dose, increased incidences of renal papillary necrosis and hepatic amyloidosis were observed in females. There was no evidence of carcinogenicity.

In a two-year study of toxicity in rats, kresoxim-methyl was administered at dietary concentrations of 0, 200, 800, 8000, or 16 000 ppm. The NOAEL was 800 ppm, equal to 36 mg/kg bw per day, on the basis of an increased incidence of hepatocellular carcinoma in animals of each sex, increased serum γ -glutamyl transferase activity, increased relative liver weight, an increased incidence and degree of severity of eosinophilic foci and mixed-cell foci in males, and a decrease in terminal body weight and body-weight gain in females at 8000 ppm and 16 000 ppm. There was also an increased incidence of biliary cysts and bile-duct proliferation.

In a study of carcinogenicity in rats, kresoxim-methyl was administered at dietary concentrations of 0, 200, 800, 8000, or 16 000 ppm for 24 months. Evidence of biliary alterations included increased incidences of biliary cysts and cholangiofibrosis in females at 16 000 ppm. At this dose, increased relative liver weights and an increased incidence of hepatocellular hypertrophy were observed in males. The NOAEL was 800 ppm, equal to 36 mg/kg bw per day, on the basis of increased incidences of hepatocellular carcinoma, reductions in body weight and body-weight

gain, and an increased incidence of eosinophilic foci and mixed-cell foci in animals of each sex at 8000 ppm and above. The overall NOAEL for neoplastic and non-neoplastic effects was 800 ppm, equal to 36 mg/kg bw per day.

It is generally recognized that the process of carcinogenesis is divided into three stages: initiation, promotion, and progression. A series of mechanistic studies was conducted with kresoxim-methyl, including tests for tumour initiating and promoting potential. In a study on tumour initiating activity, kresoxim-methyl did not increase the number of liver-cell foci in rats at a single dose of 2400 mg/kg bw. In a study on the promoting potential of kresoxim-methyl, rats received an initiating dose of *N*-nitrosodiethylamine and then a diet containing 0, 200, 800, 8000, or 16 000 ppm kresoxim-methyl for six weeks. Quantitative analysis of hepatic foci with a computer-assisted image analyser revealed significant, dose-dependent increases in the number and area of placental-type glutathione *S*-transferase-positive hepatocellular foci, indicating a promoting effect of kresoxim-methyl on hepatocarcinogenesis at doses of 8000 ppm and above. The NOAEL for the promoting effect was 800 ppm, equal to 43 mg/kg bw per day.

Four studies were conducted to investigate the effect of kresoxim-methyl on hepatic-cell proliferation in rat liver by measuring bromodeoxyuridine incorporation into hepatocyte DNA during S-phase DNA synthesis. The results showed a selective cell proliferation effect of kresoxim-methyl on hepatocytes in the periportal zone. The NOAEL was 800 ppm, equal to 61 mg/kg bw per day, while animals treated with 8000 ppm and above showed a statistically significant increase in cell proliferation. There was no difference in the sensitivity of young and old rats.

The genotoxic potential of kresoxim-methyl was investigated in a series of tests, including assays for gene mutation in bacteria and mammalian cells, unscheduled DNA synthesis, and cytogenicity *in vitro*, an assay for micronucleus formation *in vivo*, and an assay for unscheduled DNA synthesis *ex vivo*. Kresoxim-methyl had moderate potential to induce chromosomal aberrations *in vitro* with exogenous metabolic activation, but positive effects were not observed in any other test, including the assay for micronuclei in rat bone marrow. The Meeting concluded that kresoxim-methyl is not genotoxic. The three major metabolites in rats did not induce reverse mutation in *Salmonella typhimurium in vitro*.

The increased incidence of liver tumours observed in rats at 8000 ppm and above was considered to be associated with increased cell proliferation. The mechanistic studies indicated that kresoxim-methyl has tumour promoting potential at 8000 ppm, which coincides with the lowest level at which increased liver-cell proliferation was observed. These results indicate a threshold for the neoplastic mode of action. The Meeting concluded that a level of 800 ppm kresoxim-methyl has no carcinogenic potential.

In a two-generation study of reproductive toxicity in rats, the NOAEL values were 1000 ppm for parental animals of each sex, 100 mg/kg bw per day for F₀ offspring, and 88 mg/kg bw per day for F₁ offspring; these were based on reductions in body weight and body-weight gain and increased serum γ -glutamyl transferase activity and relative kidney weight at 4000 ppm and above. The NOAEL for pups was 1000 ppm, equal to 110 mg/kg bw per day for F₁ pups and 97 mg/kg bw per day for F₂ pups, on the basis of reductions in body weight and body-weight gain at 4000 ppm and above.

The NOAEL for embryo- and fetotoxicity in a study of developmental toxicity in rats was 400 mg/kg bw per day. No maternal toxicity or teratogenic effects were observed at doses up to and including the highest one of 1000 mg/kg bw per day. Kresoxim-methyl did not induce toxicity in a study of developmental toxicity in rabbits up to and including the highest dose of 1000 mg/kg bw per day.

An ADI of 0–0.4 mg/kg bw was established on the basis of the NOAEL of 800 ppm, equal to 36 mg/kg bw per day, in the 24-month study of toxicity and carcinogenicity in rats, and a 100-fold safety factor.

An acute RfD was not allocated because kresoxim-methyl has low acute toxicity and did not exhibit developmental toxicity. The Meeting concluded that the acute intake of residues is unlikely to present a risk to consumers.

Toxicological evaluation

Levels that cause no toxic effect

Mouse:	400 ppm, equal to 81 mg/kg bw per day (18-month study of toxicity)
Rat:	800 ppm, equal to 36 mg/kg bw per day (two-year study of toxicity and carcinogenicity) 1000 ppm, equal to 88 mg/kg bw per day (two-generation study of reproductive toxicity) 400 mg/kg bw per day (study of developmental toxicity)
Rabbit:	1000 mg/kg bw per day (developmental toxicity; highest dose tested)
Dog:	5000 ppm, equal to 140 mg/kg bw per day (12-month study of toxicity)

Estimate of acceptable daily intake for humans

0–0.4 mg/kg bw

Estimate of acute reference dose

Not allocated (unnecessary)

Studies that would provide information useful for continued evaluation of the compound

Observations in humans

List of end-points relevant for setting guidance values for dietary and non-dietary exposure

Absorption, distribution, excretion, and metabolism in mammals

Rate and extent of oral absorption	Rapid, 25–60% absorbed
Dermal absorption	No data
Distribution	Minimum, highest levels in liver
Potential for accumulation	Very little
Rate and extent of excretion	Rapid/complete, 87–93% within 48 h
Metabolism in animals	Extensive. No parent compound in urine, bile, or tissues; 34 metabolites identified.
Toxicologically significant compounds (animals, plants and environment)	Parent compound in rat; three major metabolites in plants

Acute toxicity

Rat: LD ₅₀ oral	> 5000 mg/kg bw
Rat: LD ₅₀ dermal	> 2000 mg/kg bw
Rat: LC ₅₀ inhalation	> 5.6 mg/L
Skin irritation	Not irritating
Eye irritation	Not irritating
Skin sensitization	Not sensitizing

Short-term toxicity

Target/critical effect	Liver: increased relative liver weight (mouse, rat)
Lowest relevant oral NOAEL	Rat: 28-day, 43 mg/kg bw per day
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEL	No data

Genotoxicity

Not genotoxic

Long-term toxicity and carcinogenicity

Target/critical effect:	Hepatocellular carcinoma
Lowest relevant NOAEL	Rat: 2-year, 36 mg/kg bw per day, diet
Carcinogenicity	Non-genotoxic carcinogen, tumour promoter

Reproductive toxicity

Reproduction target/critical effect	Reduction in F ₀ body weight at parenterally toxic dose
Lowest relevant reproductive NOAEL	Rat: 97 mg/kg bw per day, diet
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	Rat: 1000 mg/kg bw per day, highest dose tested

Neurotoxicity/Delayed neurotoxicity

No data

Other toxicological studies

No data

Medical data

No data

<i>Summary</i>	<i>Value</i>	<i>Study</i>	<i>Safety factor</i>
ADI	0–0.4 mg/kg bw	2-year study of toxicity and carcinogenicity, rat	100
Acute reference dose	Not allocated (unnecessary)		

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